Synthesis and Analysis of Libraries of Potential Flavour Compounds

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by

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Science is organised knowledge. Wisdom is organised life.

Immanuel Kant

(1724 - 1804)

Abstract

The goal of this project was to synthesise potential flavour compounds combinatorially and identify key components for further investigation as flavourants in dairy products. This thesis describes the design and synthesis of libraries of ketones and γ-lactones that will be evaluated for flavour potential. Gas chromatography-mass spectrometry (GC-MS), the Fox, and gas chromatography-olfactometry (GC-O) were used throughout this study.

Ketones were synthesised individually *via* a two-step sequence: a Grignard reaction followed by the oxidation of the resulting alcohol in Chapter 2. Some compounds selected from the Fox analysis were assessed by GC-O. The analysis gave promising results for aromatic and cyclopropyl ketones and a library of cyclopropyl ketones was prepared. Individual racemic lactones were synthesised *via* a two-step sequence: the Linstead modification of the Knoevenagel reaction and subsequent lactonisation in Chapter 3. Libraries of racemic γ-lactones (C_8-C_{12}), including α-substituted γ-lactones, were produced combinatorially. Further, synthesis of a library of γ-thionolactones was achieved by treatment of a library of γ-lactones with Lawesson's reagent. The libraries were analysed by GC-O. A (*R*)-dodecalactone was synthesised from *L*-glutamic acid and the (*S*)-enantiomer was synthesised by the same sequence from D-glutamic acid in Chapter 4. Asymmetric syntheses of both enantiomeric series of γ-lactones utilizing the Sharpless asymmetric dihydroxylation reaction was employed to give the libraries in Chapter 5. Libraries of α-substituted and β-substituted γ-lactones were synthesised combinatorially and analysed by GC-O.

Acknowledgements

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Abbreviations

AD	asymmetric dihydroxylation
AEDA	aroma extract dilution analysis
AIBN	Azo*bis*isobutyronitrile
aq.	aqueous
b.p.	boiling point
Boc	butyloxycarbonyl
CBS	Corey-Bakshi-Shibata
CoA	coenzyme A
DDC	*N,N*'-dicyclohexyldicarbodiimide
DDQ	2,3-dichloro-5,6-dicyanobenzoquinone
$(DHQ)_2PHAL$	dihydroquinidine derivative of the phthalazine class of ligands
$(DHQD)_2PHAL$	dihydroquinine derivative of the phthalazine class of ligands
DMAP	4-(dimethylamino)pyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
E1	elimination, first order
E2	elimination, second order
EN	electronic nose
EtOAc	ethyl acetate
FD	factor of dilution
FID	flame ionisation detector
FMDV	foot and mouth disease virus
g	gram
GC-MS	gas chromatography-mass spectrometry
GC-O	gas chromatography-olfactometry
h	hour
hex	hexane
HLE	horse liver esterase
HRMS	high resolution mass spectrometry
IR	infra-red
L	litre

LAB	lactic acid bacteria
M^+	molecular ion
m.p.	melting point
M.S.	molecular sieve
mg	milligram
min	minute
mL	milliliter
MsCl	methanesulfonyl chloride
μL	microlitre
N	normal
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser effect spectroscopy
PC	principal component
PCA	principal component analysis
PDC	pyridinium dichromate
pKa	acid dissociation constant
PLE	pig liver esterase
ppm	parts per million
R_f	retention factor
RT	room temperature
R_T	retention time
s	second
sat'd	saturated
TBDPS	*tert*-butyldiphenylsilyl
TFAA	trifluoroperacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane

Chapter 1

Chapter 1: Background and introduction: food flavour and combinatorial chemistry

1.1 Background

The flavour industry has grown dramatically in recent years because of the high demand of consumers *vis-à-vis* taste and flavour. There is a huge diversity of compounds that comprise flavours in nature. While many of these compounds in isolation do not have flavour or aroma attributes similar to the food from which they were isolated, it is a goal of flavour chemists to identify a group of flavour compounds, often referred to as character impact compounds, which have the distinct character of the natural food from which they were derived. Important flavour compounds have been isolated from many food products including fruits, vegetables, breads, meats, beverages and dairy products. This project has been focussed on the flavour of dairy products, due to the experience and interests of our collaborators at Fonterra, and the potential economic importance of this industry to New Zealand.

1.2 Cheese flavour

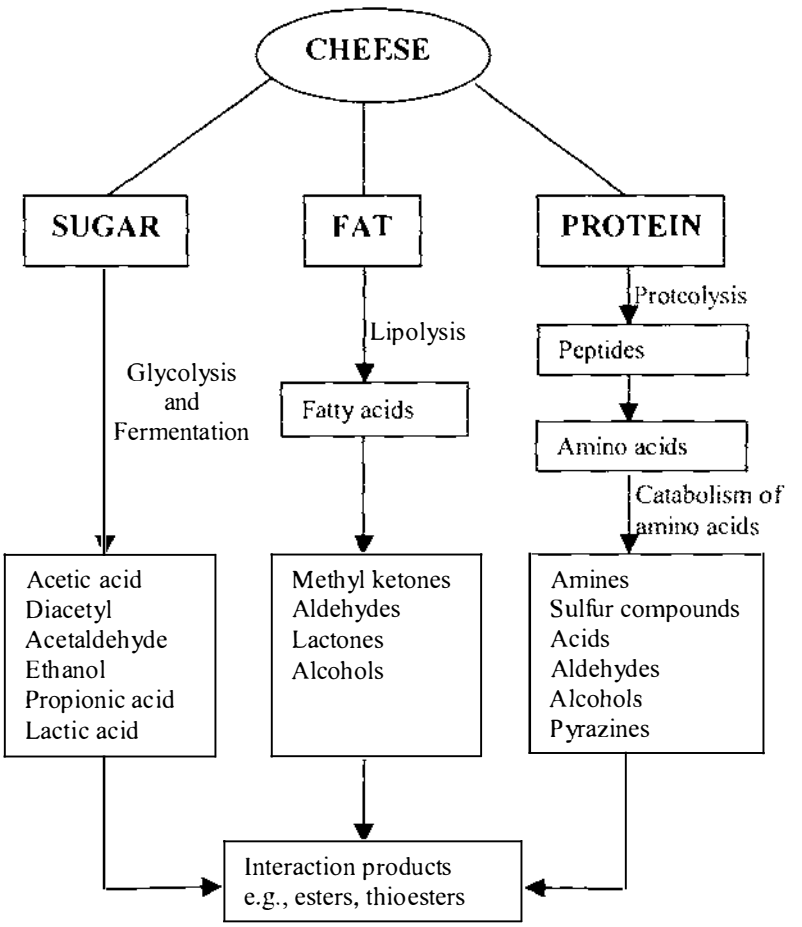
Dairy products originate from milk, their flavour components tend to be similar and the secret of their varied and unique characters is in the balance of those components. In particular, cheese flavour is one of the most complicated and interesting subjects in the flavour industry because of the variation of cheese flavours among varied types of cheese. There is not a single compound or class of compounds which is responsible for the full flavour of cheese. The correct balance of a mixture of volatile components contributes to

the flavour of cheese and this is known as the "component balance theory".[1] The flavour of cheese is derived from degradation of milk protein (*e.g.*, casein), fat, and carbohydrate (*i.e.*, lactose) generating the complex, balanced flavour of aged cheese. The compounds identified in the flavour of cheeses are fatty acids, methyl ketones, alcohols, phenolic compounds, lactones, esters, aldehydes, sulfur compounds, pyrazines and amines. Some cheese flavour compounds, identified in selected cheeses, are listed in Table 1.1.

Table 1.1: Important flavour compounds in selected cheeses.[2]

Cheese	**Compounds**
Cheddar	Methanethiol, dimethyldisulfide, diacetyl, 3-methylbutan-1-ol, acetic acid, butyric acid
Camembert	Nonan-2-one, oct-1-ene-3-ol, *N*-isobutylacetamide, 2-phenylethanol, 2-phenylethylacetate, heptan-2-ol, nonan-2-ol, ammonia, isovaleric acid, isobutyric acid, hydroxybenzoic acid, hydroxyphenylacetic acid
Romano	Butanoic acid, hexanoic acid, octanoic acid
Parmesan	Butanoic acid, hexanoic acid, octanoic acid, ethyl butyrate, ethyl hexanoate, ethyl acetate, ethyl octanoate, ethyl decanoate, methyl hexanoate
Provolone	Butanoic acid, hexanoic acid, octanoic acid
Surface ripened cheese	4-Methyloctanoic acid, 4-ethyloctanoic acid, *p*-cresol, *m*-cresol, 3,4-dimethylphenol
Muenster	Dimethyl disulfide, isobutyric acid, 3-methylvaleric acid, isovaleric acid, benzoic acid, phenylacetic acid
Blue cheeses	Heptan-2-one, nonan-2-one, methyl esters of $C_{4,6,8,10,12}$ acids, ethyl esters of $C_{1,2,4,6,8,10}$ acids
Brie	Isobutyric acid, isovaleric acid, methyl ketones, sulfur compounds, oct-1-en-3-ol
Mozzarella	Ethyl isobutanoate, ethyl 3-methylbutanoate

Many of the flavour molecules in cheese arise from the transformation of compounds in milk by microorganisms, enzymes and chemical reactions. Flavour generation in cheese involves a complex series of reactions and interactions of lactose, fatty acids and amino acids. These reactions are either biochemical or chemical in nature. The numerous compounds involved in cheese aroma are mainly derived from three major metabolic pathways: glycolysis and fermentation, lipolysis and proteolysis (Scheme 1.1).[3] Each of these will be discussed in more detail in the following sections.



Scheme 1.1: Flavour development in cheese.[3]

1.2.1 Glycolysis (Lactose into lactic acids)

Most of the lactose in milk is removed in the whey as lactose or lactic acid, and that retained in the cheese curd is metabolised to lactate, partly during curd manufacture and partly during the early stages of ripening, normally by the starter culture. The pathway for lactose metabolism is driven by starter bacteria (*i.e.*, *Lactococcus lactis ssp. lactis* and *Lactococcus lactis ssp. cremoris*).[4] The conversion of lactose into lactic acid makes a major contribution to the flavour of acid-coagulated cheeses and contributes significantly to the flavour of young, rennet-coagulated cheeses.

1.2.2 Lipolysis (Milk fat into fatty acids)

The fat fraction of cheese has a major effect on cheese texture and is important for the perception and development of cheese flavour. The fat in cheese is converted into fatty acids by hydrolysis in the presence of lipases. Lipases in cheese originate from milk, starter, adjunct starter or non-starter bacteria and mammalian enzymes (*e.g.*, calf, kid and lamb pregastric esterases).[5]

1.2.3 Proteolysis (Milk protein into amino acids)

Proteolysis is the most complex, and the most extensive, series of biochemical reactions during the maturation of most cheese varieties. Proteolysis is mainly responsible for softening of the texture of cheese during the early stages of ripening and helps the development of cheese flavour *via* the formation of amino acids and peptides. Proteolytic enzymes involved in the various stages of protein and peptide degradation include the milk-clotting enzyme (from calf rennet), natural milk proteinases and proteolytic enzymes from lactic acid bacteria (*i.e.*, *Lactococcus lactis ssp. lactis and Lactococcus lactis ssp. cremoris*) used as starter organisms.[6] Proteolysis is the pathway by which milk proteins

are hydrolysed to produce free amino acids and peptides in cheese. Free amino acids, the final products of proteolysis, have been used as indices of ripening in cheese for many years.[7]

1.2.4 Catabolism of amino acids

Amino acid catabolism is a major process for flavour formation in cheese. The conversion of amino acids to aroma compounds by microorganisms, especially by lactic acid bacteria and *Brevibacterium linens*, is the main pathway in many cheeses.[8] The identification of the key aroma compounds of various cheeses shows that amino acid degradation is a major process for aroma formation in cheese and that aromatic amino acids (**1.1-1.3**), branched-chain amino acids (**1.4-1.6**) and methionine (**1.7**) are the major precursors of these aroma compounds (Figure 1.1).

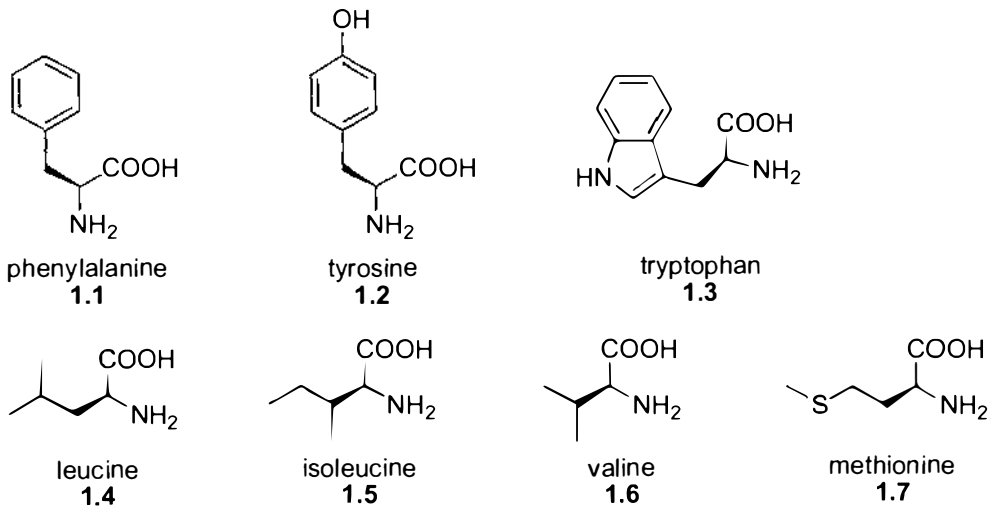
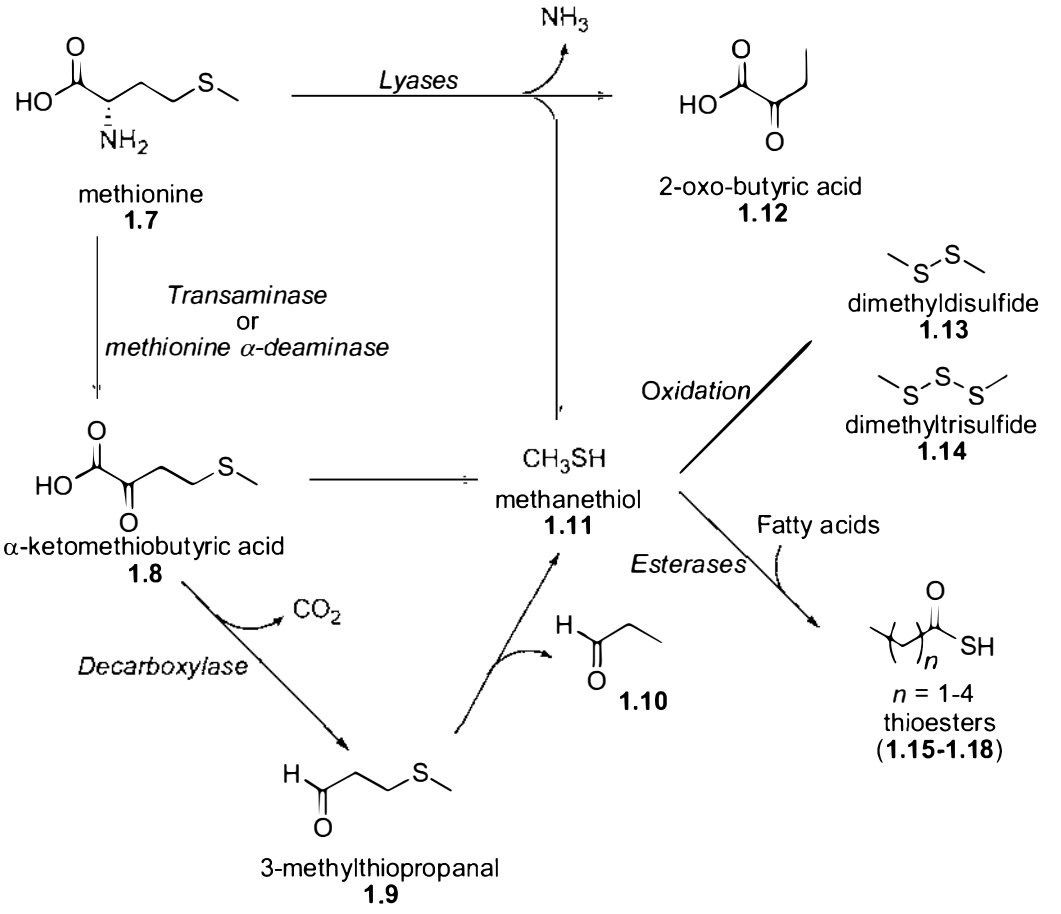


Figure 1.1: The major amino acids for aroma formation in cheese.

The conversion of amino acids to aroma compounds proceeds by two different pathways. The first is initiated by elimination reactions catalysed by amino acid lyases that cleave the side chain of amino acids, especially for aromatic amino acids and methionine (**1.7**).

The second pathway goes through α-keto acid intermediate **1.8** and is mainly initiated by a transamination reaction catalysed by amino acid aminotransferases or by α-deaminase (liberating ammonia in the latter case) and has been observed for aromatic amino acids, branched-chain amino acids and methionine (**1.7**). α-Keto acid **1.8** is further degraded to various compounds either by enzymatic reactions or by chemical reactions (Scheme 1.2).[9]



Scheme 1.2: Conversion of methionine (**1.7**) to volatile sulfur compounds.

Controlling amino acid catabolism by cheese microorganisms is carried out by selecting strains of microorganisms with interesting catabolic activities.

1.3 Biosynthesis of cheese flavour compounds

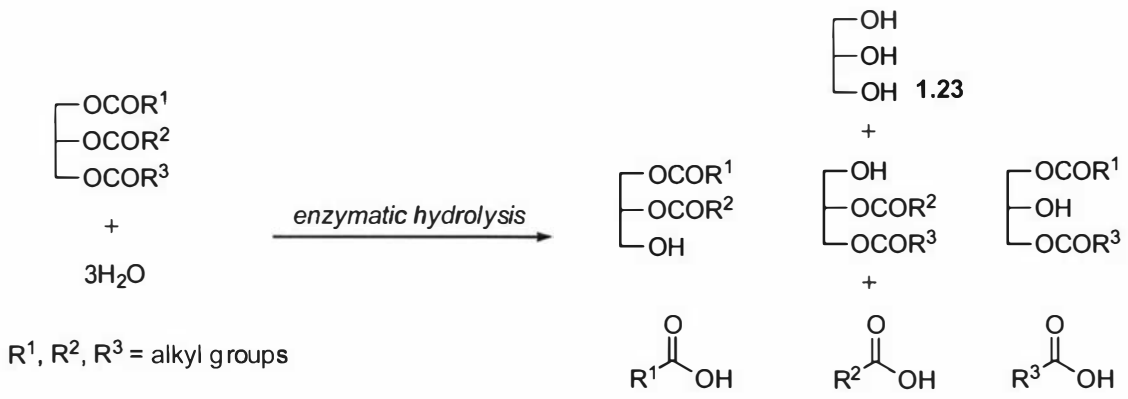
1.3.1 Fatty acids

Fatty acids are important as flavour compounds in the aroma of cheeses *e.g.*, mold-ripened cheese, Italian style cheeses and hard cheese. They are also precursors of methyl ketones, alcohols, lactones, aldehydes and esters. The hydrolysis of fat in cheese has been widely described and is important in various cheeses. The even-numbered carbon free fatty acids, ranging from acetic (**1.19**) to octadecanoic acid (**1.20**), isobutyric (**1.21**) and isovaleric acid (**1.22**) are present (Figure 1.2). [10]

1.19 **1.20** **1.21** **1.22**

Figure 1.2: Fatty acids in cheese flavour.

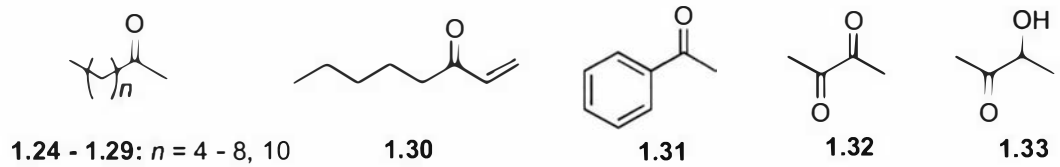
Milk fat contains neutral lipids, from which the hydrolysis by lipases produces free fatty acids in cheeses. These lipases hydrolyse triglycerides to form diglycerides, monoglycerides, glycerol (**1.23**), and free fatty acids (Scheme 1.3).[11] A controlled enzymatic hydrolysis using lipases gives a fatty acid composition dependent upon the specificity of the lipases used. Certain lipases *e.g.*, mamalian pregastric esterases, contribute a high degree of specificity towards the short chain fatty acids.[12] Other lipases preferentially liberate long chain fatty acids and some do not display any particular preference.[13] Lipolysed milk fat products have found wide applications, for example for the enhancement of butter-like flavours[14] and flavour development in milk chocolate.[15]



Scheme 1.3: Enzymatic hydrolysis of triglyceride.

1.3.2 Ketones

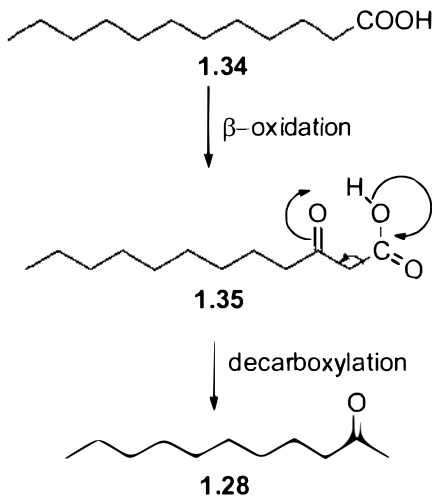
The homologous series of odd-chain methyl ketones, from C_3 to C_{17}, are some of the most important compounds in the aroma of blue cheese and surface mold-ripened cheese.[16] The two major odour impact methyl ketones are heptan-2-one (**1.24**) and nonan-2-one (**1.26**). Eleven other methyl ketones (*i.e.*, all alkan-2-ones from C_4 to C_{13} as well as octan-3-one), have been isolated from camembert cheeses by vacuum distillation. Each ketone presents a characteristic odour and can be described as a note: octan-2-one (**1.25**, floral), nonan-2-one (**1.26**, fruity), decan-2-one (**1.27**, fruity), undecan-2-one (**1.28**, floral) and tridecan-2-one (**1.29**, musty). Oct-1-en-3-one (**1.30**) has a mushroom note in an aqueous layer and a metallic note in a lipid layer. Acetophenone (**1.31**) has an orange blossom note. Diacetyl (**1.32**) and acetoin (**1.33**) are well known for their buttery notes. The mushroom, musty and buttery notes are extremely important in camembert cheese.[10a,16b] Ketones are also present in cheddar, gruyère, and French cantal cheese (Figure 1.3).[17]



1.24 - 1.29: *n* = 4 - 8, 10 **1.30** **1.31** **1.32** **1.33**

Figure 1.3: Ketones in cheese flavour.

Methyl ketones are produced by the oxidation of free fatty acids and glyceride esters, followed by decarboxylation of the resulting β-keto acids (*e.g.*, **1.35**, Scheme 1.4). Another possible mechanism is that β-keto acids in milkfat as glyceride esters are directly decarboxylated. This pathway is important because 60% of the carbonyl compounds produced by enzymes are methyl ketones.[18] Other ketones, especially diacetyl (**1.32**) and acetoin (**1.33**) are obtained from pyruvate, stemming from lactose and citrate metabolism by the activity of lactic acid bacteria.[19]



Scheme 1.4: The biosynthesis of undecan-2-one (**1.28**) from dodecanoic acid (**1.34**).

1.3.3 Alcohols

Primary and secondary alcohols, along with ketones, are important aroma compounds in cheese. Oct-1-en-3-ol (**1.36**) has a mushroom note and 3-methylbutan-1-ol (**1.37**) gives an alcoholic, floral note. 2-Phenylethanol (**1.38**) has a rose floral note and octa-1,5*E*-dien-3-ol (**1.39**) has been found to have a celluloid taste (Figure 1.4).[20]

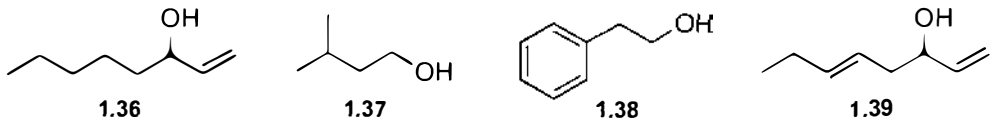
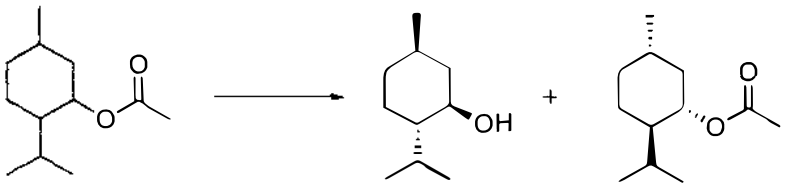


Figure 1.4: Alcohols in cheese flavour.

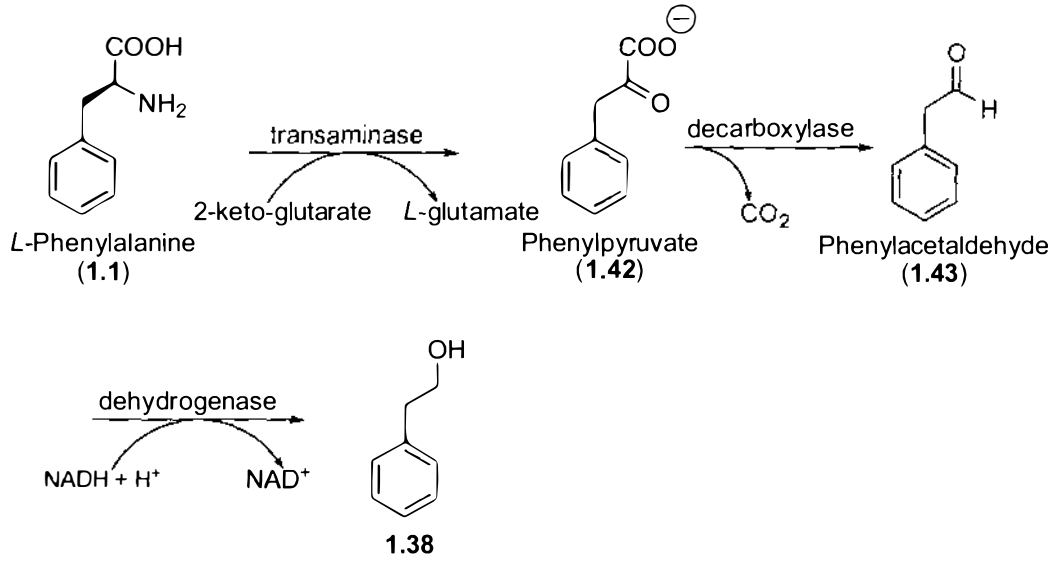
Lipases have been used to perform enantiospecific hydrolyses to yield one enantiomer of aliphatic and terpene alcohols. An example is *l*-menthol (***l*-1.41**), which is one of the most important terpene alcohols in the fragrance and flavour industry. It is the main component of peppermint oil. Many microbial lipases can resolve racemic menthyl esters (*e.g.*, ***d,l*-1.40**) *via* the selective hydrolysis of the *l*-ester (Scheme 1.5).[21]



***d,l*-1.40** ***l*-1.41** ***d*-1.40**

Scheme 1.5: Enantioselective hydrolysis of (*d,l*)-menthyl acetate.

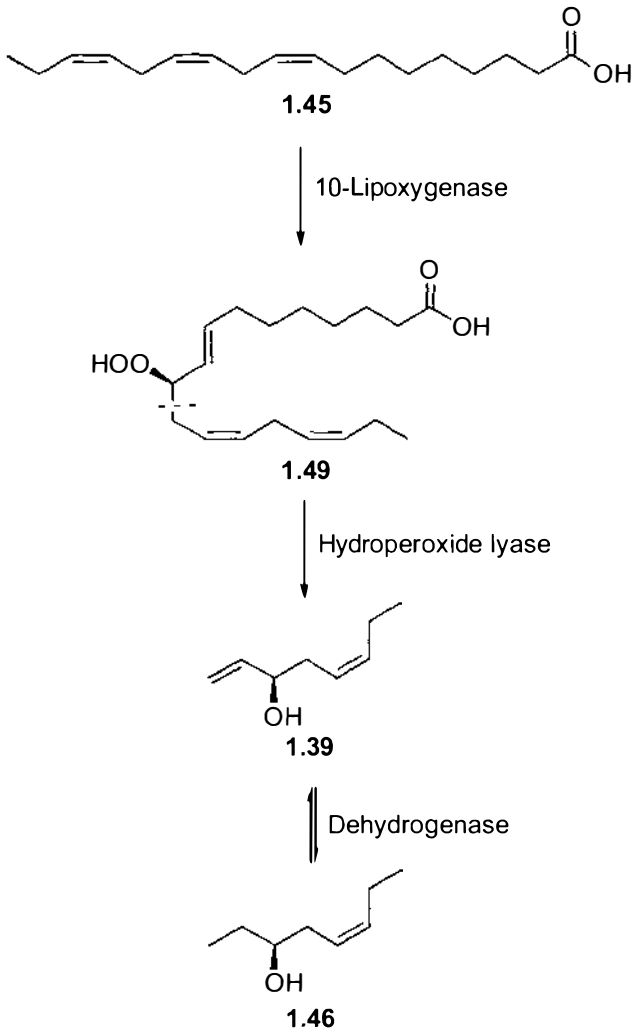
Some alcohols are derived from the Ehrlich pathway[22] of amino acid metabolism (Scheme 1.6) or from aldehyde degradation.



Scheme 1.6: Ehrlich pathway for 2-phenylethanol (**1.38**) synthesis.

Linoleic acid (**1.44**) and linolenic acid (**1.45**) are precursors of some alcohols, particularly 3*S*,5*Z*-oct-5-en-3-ol (**1.46**),[23] oct-2-en-1-ol (**1.47**), 5*E*-octa-1,5-dien-3-ol (**1.39**) and 5*Z*-

octa-1,5-dien-3-ol (**1.48**). The principal enzymes implicated in this alcohol synthesis are lipoxygenase and a hydroperoxide lyase found in molds (Scheme 1.7).



Scheme 1.7: Lipoxygenase-mediated generation of 3*S*,5*Z*-oct-5-en-3-ol (**1.46**) from α-linolenic acid (**1.45**).

1.3.4 Lactones

Lactones which occur in cheese include dihydro-5-hexyl-2(3*H*)-furanone (**1.50**), tetrahydro-6-pentyl-(2*H*)-pyran-2-one (**1.51**), dihydro-5-octyl-2(3*H*)-furanone (**1.52**) and tetrahydro-6-heptyl-(2*H*)-pyran-2-one (**1.53**) (Figure 1.5). The nature and levels of these

compounds are dependent on the diet of the animal producing the milk. Lactones are generally characterised by fruity notes (*i.e.*, peach, apricot and coconut).[16b]

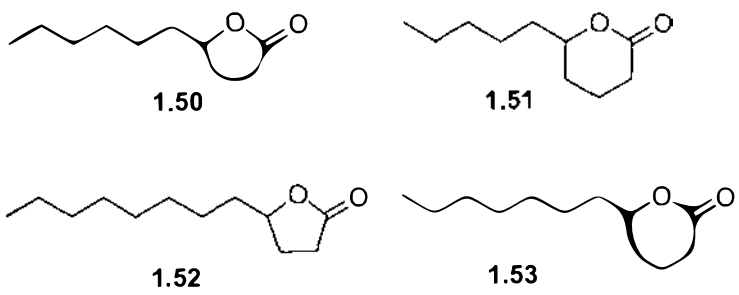


Figure 1.5: Lactones in cheese flavour.

Hydroxylated fatty acids are the precursors of lactones. The closing of the ring occurs by the action of pH, microorganisms, or both. Hydroxyacids can be present as triglycerides in milk and lipases can liberate them. The fatty acids are then cyclised. Dihydro-5-octyl-2(3*H*)-furanone (**1.52**) can be formed, from long-chain saturated fatty acids (linoleic acid, **1.44** and linolenic acid, **1.45**).[24] More details are described in Chapter 3.

1.3.5 Esters

Esters are common volatile components of cheeses. Ethyl esters of the straight-chain fatty acids [*e.g.*, ethyl butanoate (**1.54**) and ethyl hexanoate (**1.55**) and 2-phenylethyl acetate (**1.56**) are frequently found in cheese (Figure 1.6)]. Most esters encountered in cheeses are described as having fruity, floral notes.[25]

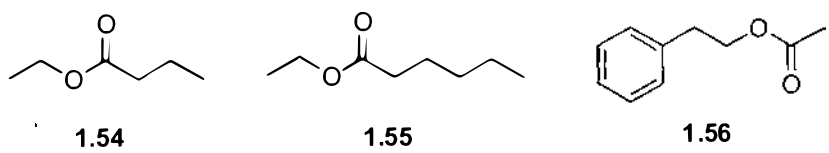
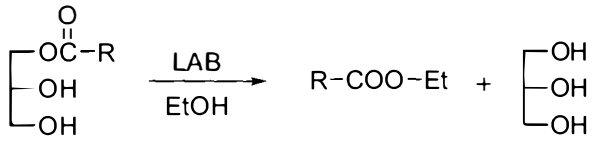


Figure 1.6: Esters in cheese flavour.

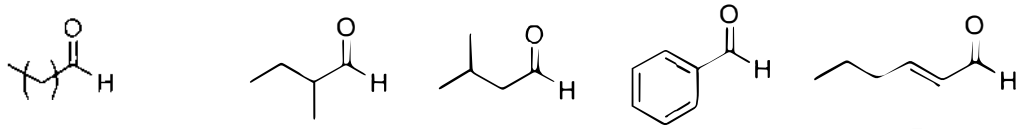
A wide range of enzymes and mechanisms are involved in ester-forming reations.[26] An example involves the alcoholysis of milk fat glycerides by lactic acid bacteria (LAB) in a transferase reaction (Scheme 1.8).[27]



Scheme 1.8: The alcoholysis of a milk fat monoglyceride and ethanol by LAB.

1.3.6 Aldehydes

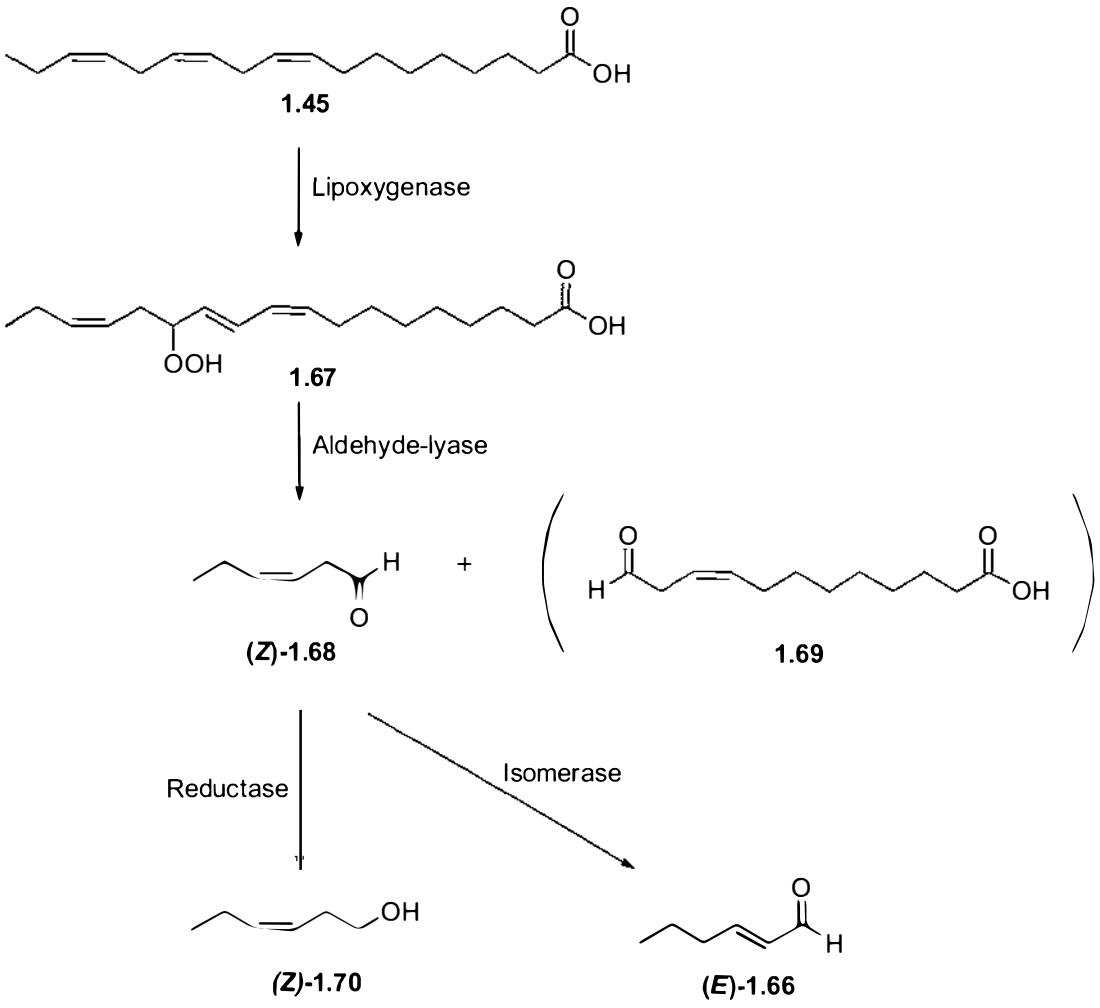
The main aldehydes encountered in camembert cheese and brie are hexanal (**1.57**), heptanal (**1.58**), nonanal (**1.60**), 2-methylbutanal (**1.63**), 3-methylbutanal (**1.64**) and benzaldehyde (**1.65**). Hexanal (**1.57**) and 2*E*-hexenal (***E*-1.66**) give the green note of immature fruit. Octanal (**1.59**), nonanal (**1.60**), decanal (**1.61**), and dodecanal (**1.62**) are described as having an orange note and benzaldehyde (**1.65**) is described as having an aromatic note of bitter almond (Figure 1.7).[28]



1.57-1.62: *n* = 4 - 8, 10 **1.63** **1.64** **1.65** ***E*-1.66**

Figure 1.7: Aldehydes in cheese flavour.

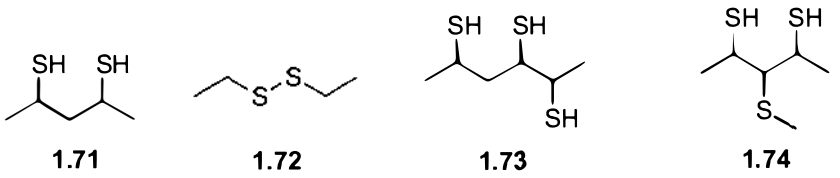
3*Z*-Hexenal (***Z*-1.68**) arises through the action of lipoxygenase on the unsaturated fatty acid, linolenic acid (**1.45**) followed by the action of hydroperoxide lyase. Further enzymatic transformation gives rise to 2*E*-hexenal (**(*E*)-1.66**).[29] Aldehyde ***Z*-1.68** can be reduced to the corresponding alcohol (**1.70**, Scheme 1.9).



Scheme 1.9: Lipoxygenase-mediated generation of 3*Z*-hexenal (***Z*-1.68**) from α-linolenic acid (**1.45**).

1.3.7 Sulfur compounds

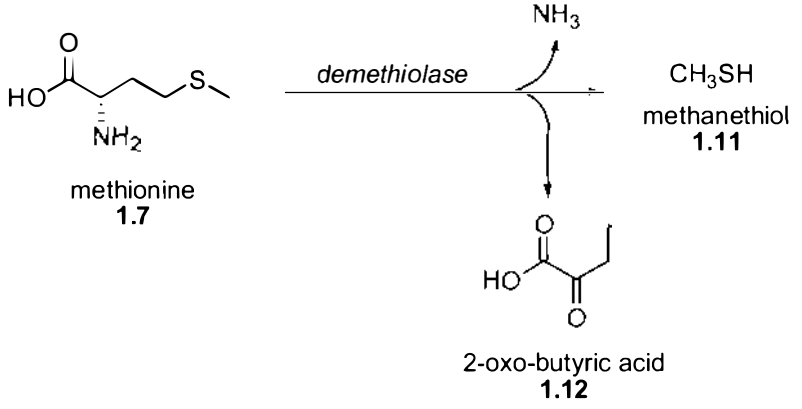
Four sulfur compounds that form a garlic note fraction have been isolated from camembert and brie cheeses: 2,4-dithiopentane (**1.71**), diethyldisulfide (**1.72**), 2,3,5-trithiohexane (**1.73**), and 2,4-dithio-3-methylthiopentane (**1.74**) (Figure 1.8). Other sulfur compounds are also encountered in cheeses and they are described as having a strong garlic, very ripe cheese odour.[10a]



1.71 **1.72** **1.73** **1.74**

Figure 1.8: Sulfur compounds in cheese flavour.

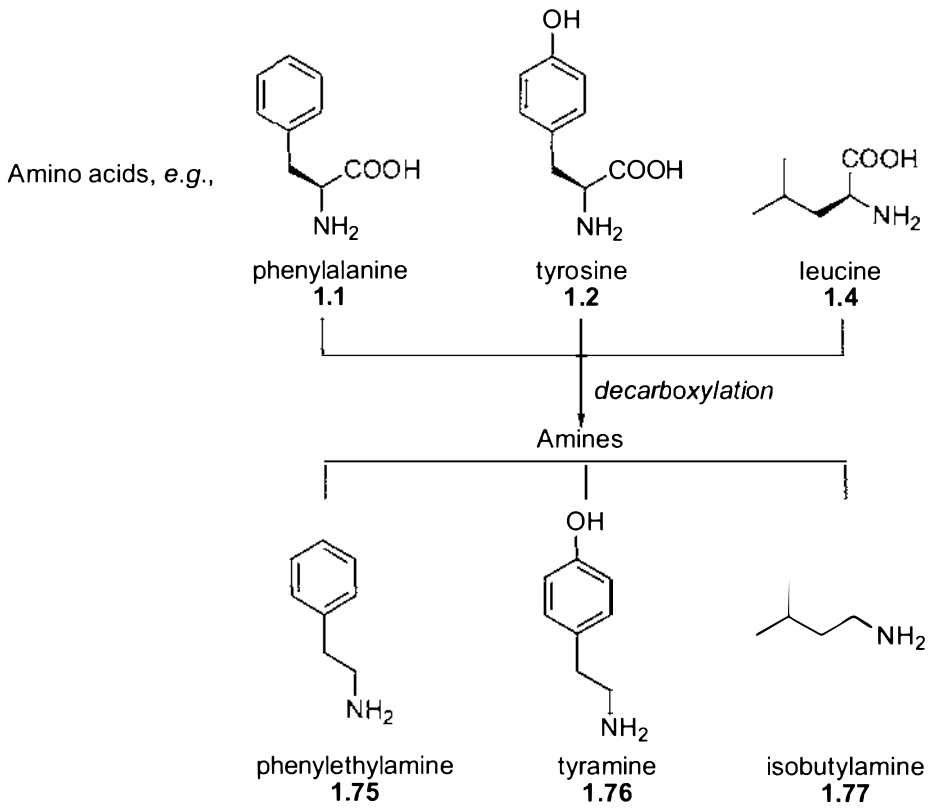
Many microorganisms are able to produce sulfur compounds in cheeses. Methionine is an amino acid which is a precursor to methanethiol. Methionine demethiolase cleaves a C-S bond (Scheme 1.10).[30]



Scheme 1.10: The formation of methanethiol (**1.11**).

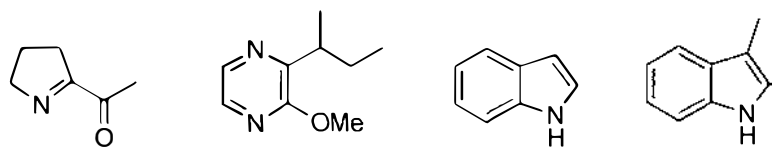
1.3.8 Nitrogen-containing compounds

Amino acid decarboxylation leads to carbon dioxide and free amine (Scheme 1.11). This reaction needs pyridoxal-phosphate as a coenzyme. Phenylalanine (**1.1**) thus gives phenylethylamine (**1.75**), tyrosine (**1.2**) gives tyramine (**1.76**), and leucine (**1.4**) gives isobutylamine (**1.77**).[31] Numerous volatile amines have been identified in the headspace of a cheese; some cause bitterness.[32]



Scheme 1.11: Decarboxylation of amino acids.

2-Acetyl-1-pyrroline (**1.78**, in Figure 1.9) has been identified in a variety of processed and cooked food, including dry and fresh milk, camembert and Swiss gruyère cheese, rennet casein, and liquid cheddar whey.[33] Compound **1.78** and pyrazines (*e.g.*, 2-*sec*-butyl-3-methoxy pyrazine, **1.79**, in Figure 1.9) are formed by the Maillard reaction between carbohydrate fragments and amino acid residues. Indole (**1.80**) and 3-methyl indole (**1.81**, skatole) are considered to be important flavour compounds in water buffalo mozzarella and emmental, respectively.



1.78 **1.79** **1.80** **1.81**

Figure 1.9: Nitrogen-containing compounds in cheese.

1.4 Flavour compounds in other foods

Many of the flavour compounds in dairy food occur in other types of food as well. The ultimate objective of this programme is to utilise lead flavour compounds in foods of all types (*i.e.*, not restricted to dairy products). A brief survey of flavour compounds in other types of food is therefore included.

1.4.1 Cereal flavours[34]

Flavours in raw cereals originate from the grain. Cereal products obtain their flavour enzymatically, mainly in treatments like dough-kneading and fermentation, and non-enzymatically, in processing for instance by different types of heat treatment. Special attention has been paid to the formation of alkyl pyrazines that are specially produced from the yeast metabolites in extrusion and roasting processes.[35] Off-flavour in cereal and cereal products is caused by advanced lipid oxidation and growth of microorganisms on the grain.

1.4.2 Meat flavour[34]

There are over 1000 volatile compounds found in cooked meats. Meat flavour develops during cooking from the complex interaction of precursors derived from both the fat and lean components of meat. Heterocyclic compounds, such as pyrazines and thiazoles contribute to roast and grilled aromas, while certain aliphatic and heterocyclic sulfur compounds provide some of the characters of boiled meat. Sulfur compounds are extremely important in meat flavour and certain furanthiols and furan disulfides possess the meaty aroma.

1.4.3 Beer flavour[34]

Beer contains numerous taste and aroma compounds. Some of them are derived from the raw materials, malt and hops, but by far the majority are formed during the brewing process. Hops contribute significantly to beer bitterness and beer flavour. The main compounds in hops are bitter resins called α-acids or humulones (**1.82-1.84**) and β-acids or lupulones (**1.85-1.87**) (Figure 1.10). After bottling, the beer flavour is unstable and changes considerably with time.

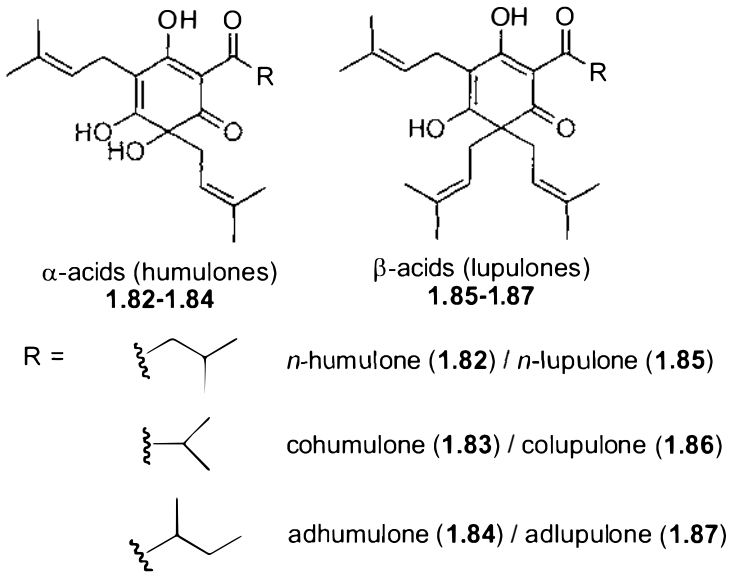
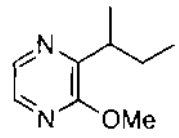


Figure 1.10: Chemical structures of hop bitter acids.

1.4.4 Wine flavours[34]

Wine flavour is affected by an enormous number of variables. The grape variety and factors affecting wine development and berry composition exert major influences on distinctive flavours.[36] There are two classes of compounds, terpenes and pyrazines (*e.g.*, the bell pepper pyrazine, 2-*sec*-butyl-3-methoxy pyrazine, **1.79**, Figure 1.11), identified in grapes that are flavour impact compounds. Terpenes contribute to the distinctive floral

aromas and the bell pepper pyrazine **1.79** has an aroma described as herbaceous or vegetative.



1.79

Figure 1.11: Chemical structure of the bell pepper pyrazine (**1.79**).

1.4.5 Cocoa flavour[34]

Cocoa makes a unique contribution to the flavour of chocolate. The following 13 flavour compounds contribute to the overall chocolate flavour (Figure 1.12).

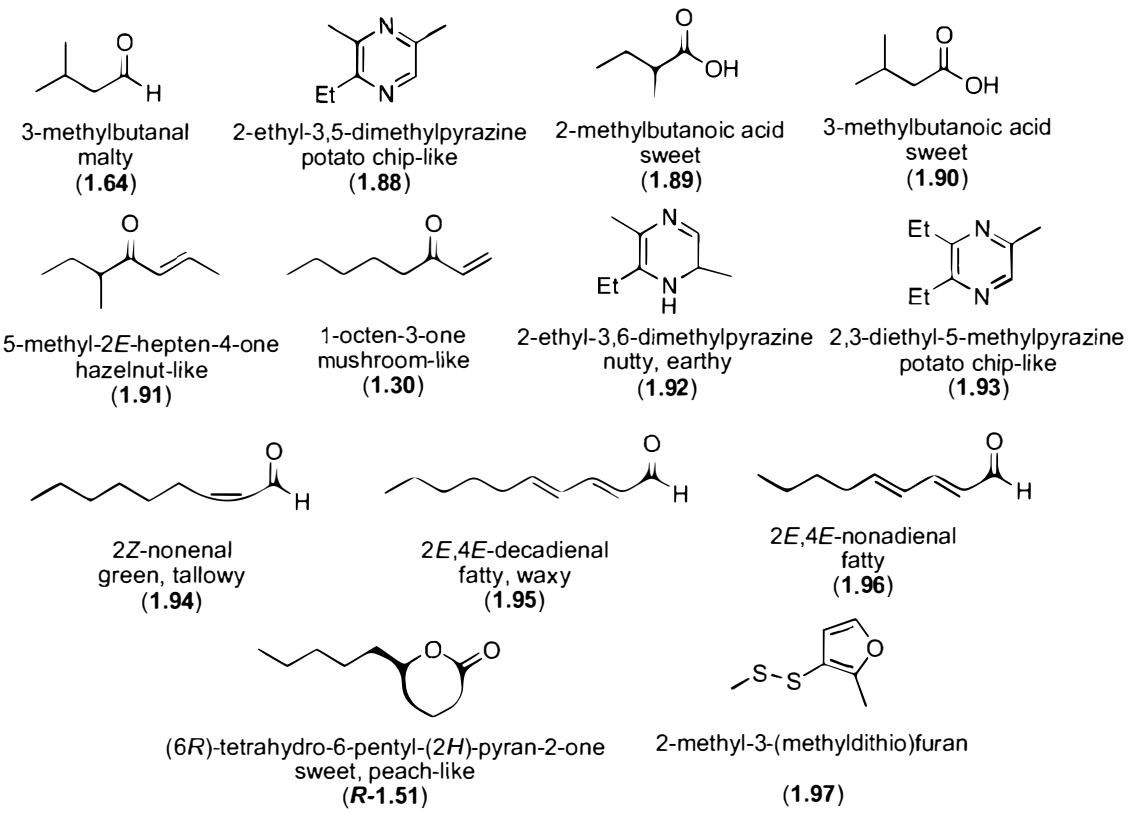


Figure 1.12: Flavour compounds in chocolate.

1.5 Instrumental analysis of food flavours

There are indications that only a small fraction of the complex mixture of volatiles occurring in foods causes the overall odour. The determination of odour-active compounds from volatiles having little or no odour is the task to be solved in flavour analysis. The odour detection threshold is the lowest concentration of a certain odour compound that is perceivable by the human sense of smell.[37]

1.5.1 GC-O, GC-MS and EN

The chemical analysis of flavour in dairy products is complicated since significant levels of lipids, proteins and carbohydrates in milk make it difficult to separate flavour-active chemicals. GC-MS is an excellent tool to separate the volatile compounds by gas chromatography (GC) and then identify and analyse the components by mass spectrometry (MS). However, GC-MS is an indirect method to identify the odour-active compounds in dairy products, since the unknown volatiles separated by gas chromatography are identified by mass spectrometry rather than odour impact.[38]

GC-O is the combination of techniques that combine olfactometry, or the use of human detectors, to assess odour activity in defined air streams with the gas chromatographic separation of volatiles. GC-O is used to determine odour thresholds (*e.g.*, from Aroma extract dilution analysis (AEDA)[39] and Charm analysis[40]) in various food including meat, breads, vegetables, beverages and dairy products.[41] The application of GC-O to dairy products has included the analysis of fresh and heated milk, cheeses, yogurt, and milk chocolate.[42] Some of the aroma compounds identified in dairy products using GC-O are listed in Table 1.2.

Table 1.2: Odour descriptions of compounds found in dairy products.

Compound	**Odour description**
ethyl butanoate	banana, pineapple, sweet
ethyl hexanoate	fruity, pineapple
heptanal	green, sweet
1-octen-3-ol	mushroom-like
dimethyl sulfide	intense, boiled cabbage, sulphurous
2-heptanone	fruity, spicy, cinnamon
2-undecanone	floral, rose-like
tetrahydro-6-heptyl-(2*H*)-pyran-2-one	coconut
diacetyl	buttery

In addition to classical studies on correlation models based on physico-chemical properties there are an increasing number of studies using an electronic nose (EN) system.[43] The electronic nose (EN) is an analytical instrument that compares the odours of samples with those of standards. It has been applied extensively in the chemical, cosmetic and food and beverage industries. There are a number of different technologies available and EN analyses the complete headspace above a product by passing it over a series of sensors. These sensors are semi-conductors and they react with volatile chemicals in the sample headspace. The FOX 4000 is a commercial EN and it has been used to characterise odourants in food, particulary in the wine industry.[44]

1.6 Combinatorial chemistry

1.6.1 Background

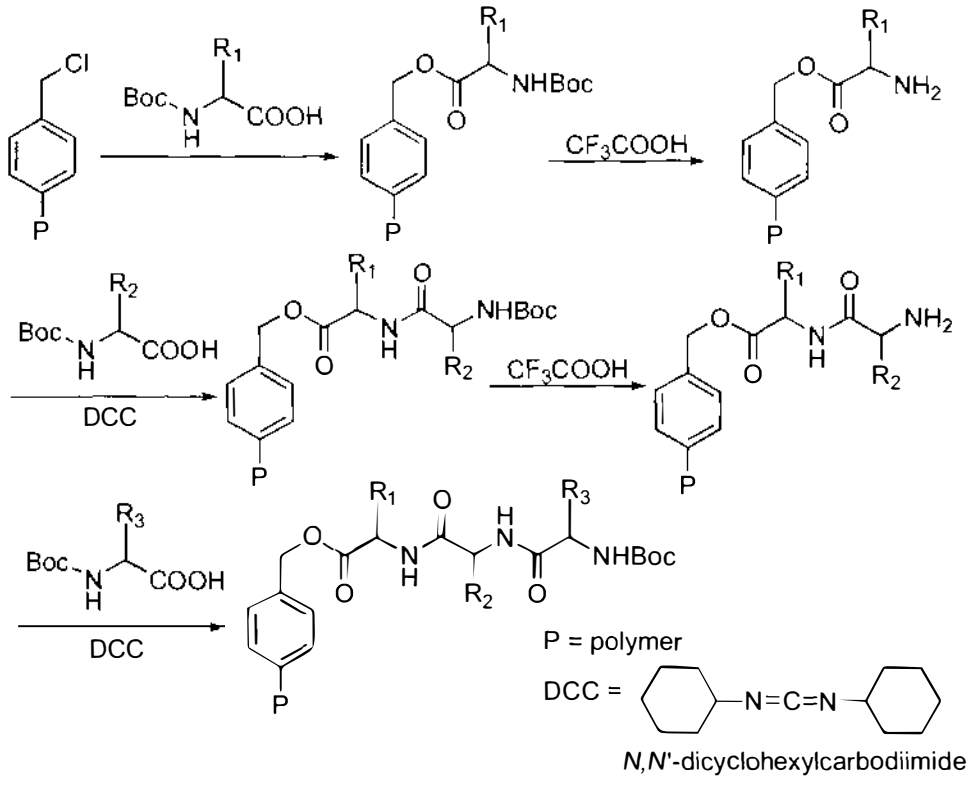
Combinatorial chemistry is a relatively new way of generating a large number of compounds rapidly in a format that is described as a library. Combinatorial chemistry was first reported by Mario Geyson and his colleagues in 1984.[45] He was interested in VP1, an immunologically important, 213 residue, coat protein of the foot and mouth disease virus (FMDV). A library of hexapeptides corresponding to the 208 possible overlapping hexapeptides of the viral protein was screened for binding to the antibodies generated in response to the intact FMDV. This proved which regions of the FMDV protein (epitopes) were important in terms of recognition of the antibodies. Residues 146 to 152 were identified as being crucial for binding.

Combinatorial chemistry can be divided into two challenges: making the library and finding the active compound. The big advantage of combinatorial chemistry is that a library is generated exponentially and then screened for its biological activity. The chemical library is then scrutinised to find the active compound. Robotic instruments and computational tools have enabled the identification of biologically active compounds by screening a mixture of thousands of compounds in libraries.

1.6.2 Generating a chemical library

1.6.2.1 Solid-phase organic synthesis

Since the first report of solid-phase organic synthesis by Merrifield[46] in 1963, the general field of solid-phase organic synthesis has grown enormously. The fundamental technique is based on polymeric resin beads to which a reactive functional group is bonded covalently. This immobilized group serves as the starting unit for the assembly of additional covalently bonded units. When the desired array of molecular building blocks is assembled, the original covalent bond to the resin is cleaved, to generate the new molecule as a separate entity. The Merrifield approach to solid–phase peptide synthesis is shown in Scheme 1.12.

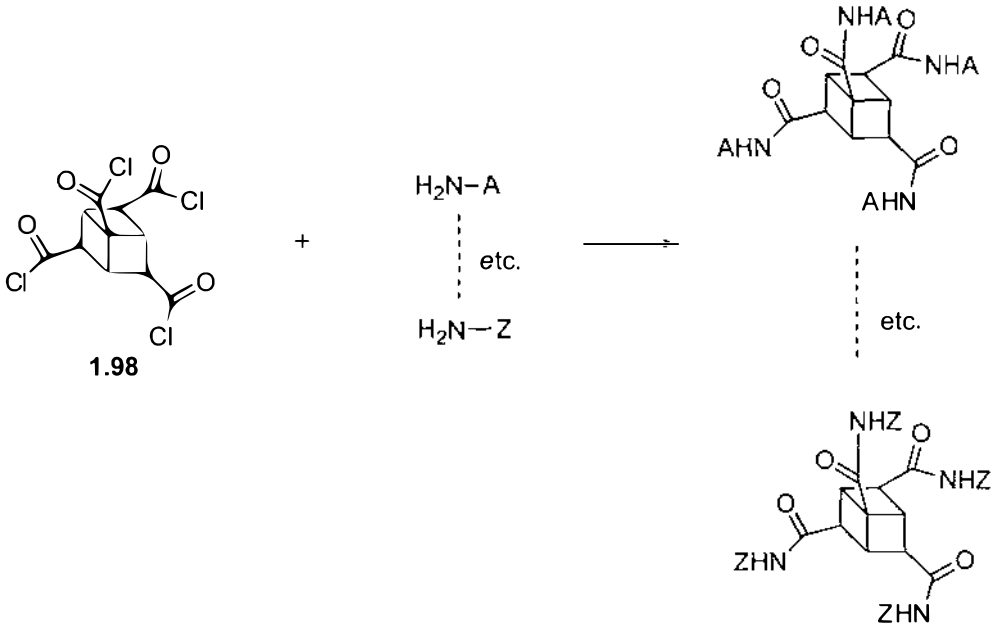


Scheme 1.12: Solid-phase peptide synthesis according to Merrifield.[48]

The advantage of solid-phase organic synthesis is that the growing product is always chemically bound to the resin bead. Thus reactions can be driven to completion by using excess reagents, and waste products can be separated readily and repeatedly from reactants by simple filtration and extensive washing. The rapid production of libraries of molecules by solid phase synthesis has led to modifications related to miniaturisation, automation, and robotics.

1.6.2.2 Solution-phase organic synthesis

Although solid-phase organic synthesis has been widely developed, particularly in the peptide industry, solution-phase organic synthesis is also used for producing small, drug-related molecules in the field of combinatorial chemistry. The concept of generating libraries of small organic molecules with a drug-related structure is that a rigid core molecule supporting multiple reactive sites is combined with a mixture of building blocks to produce a random mixture of polyfunctionalised structures. For example, cubane tetra acid chloride (**1.98**) (Scheme 1.13) could be combined with four molar equivalents of an equimolar mixture of amines A-Z to produce tetra-substituted cubane compounds A, A, A, A, through to Z, Z, Z, Z. This method of library generation has several advantages. First it is a powerful method of generating molecular diversity in a single combinatorial step. Reactions are pushed to completion by the use of excess quantities of the reactive reagent, and products are isolated by solvent-solvent extraction without further purification.[47]



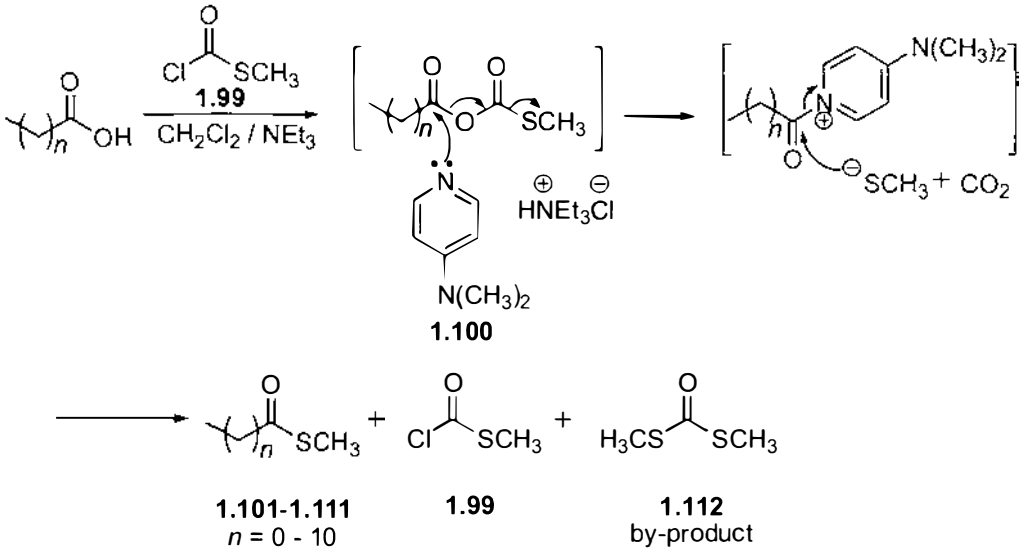
Scheme 1.13: Synthesis of a combinatorial library from cubane tetraacid chloride (**1.98**) and amines.

The advantage of solution synthesis relative to solid phase synthesis is that the compounds are in a free form to be assessed for activity. Solution-phase organic synthesis is a reliable way to apply traditional organic reactions (*e.g.*, Diels-Alder, aldol, Mitsunobu, Stille, Suzuki, Heck, Grignard, Wittig).[48]

1.6.3 Combinatorial chemistry in food flavour

The application of combinatorial chemistry to food flavour is an emerging area that has the potential to dramatically reduce the time and cost associated with the synthesis and evaluation of potential flavour compounds. The introduction of combinatorial chemistry to food flavour has been pioneered by two research groups.

Volfson's group prepared a library of *S*-methyl thioesters and screened it for sensory unique components.[49] Thioesters have been identified as one of the known classes of compounds which are formed in maturing cheeses and contribute to their characteristic aroma with very low thresholds. Synthesis of a combinatorial library of eleven *S*-methyl thioesters was effected by reaction between methyl chlorothiolformate (**1.99**) and eleven carboxylic acids with various carbon chain lengths in the presence of 4-(dimethylamino)pyridine (DMAP, **1.100**) (Scheme 1.14).[50] The library of synthetic *S*-methyl thioesters was screened using GC-O and identified *S*-methyl thiopropionate (**1.102**, $n = 1$) as having a possible characteristic aroma of camembert cheese.[51]

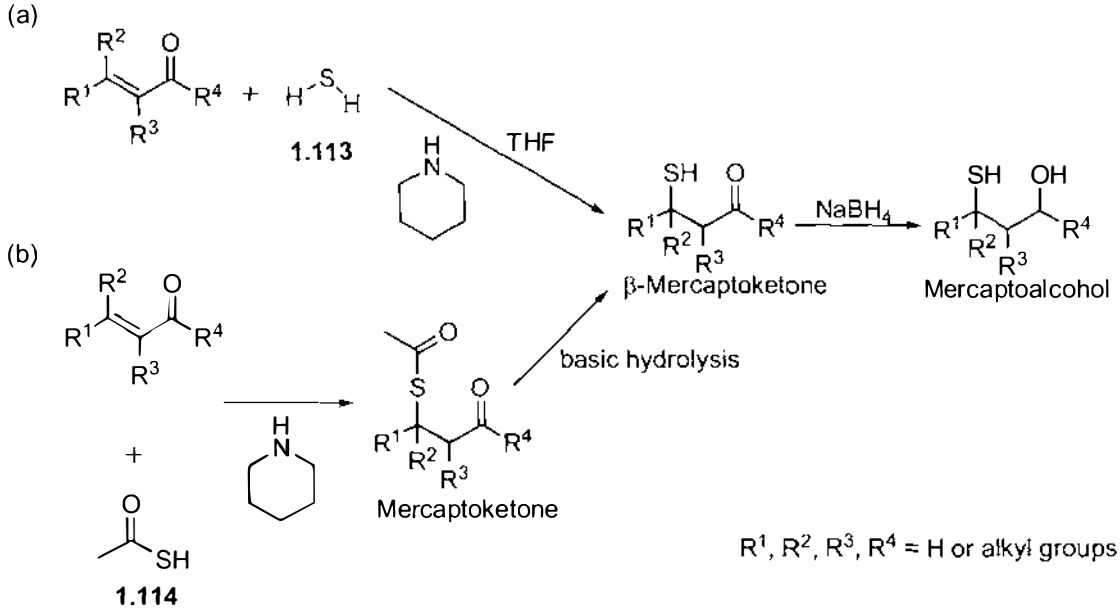


Scheme 1.14: Synthesis of *S*-methyl thioesters from carboxylic acids and methyl chlorothiolformate (**1.99**).

Collins' group studied sensorial properties and syntheses of mercaptoketones,[52] mercaptoaldehydes,[53] and mercaptoesters.[54] Libraries of β-mercaptoketones were synthesised *via* two synthetic pathways by conjugate addition of (a) hydrogen sulfide

(**1.113**) or (b) thioacetic acid (**1.114**) to α,β-unsaturated ketones with various substituents. Subsequent reduction of the mixture of ketones led to the formation of mercaptoalcohols (Scheme 1.15).[55]

Each intermediate step was monitored by thin layer chromatography (TLC) and the composition of the libraries was identified by GC-MS. The sensorial analysis of libraries was completed by GC-O to provide the odour descriptions and threshold values.



Scheme 1.15: Synthetic pathways for production of mercaptoalcohols from α,β-unsaturated ketones.

5-Methyl 4-mercaptohexan-2-one (**1.115**) and one diastereoisomer of 5-methyl 4-mercaptohexan-2-ol (**1.116**) were the most pleasant odours perceived at the sniffing port. 4-Mercapto-4-methylpentan-2-one (**1.117**) and 4-mercapto-3-methylpentan-2-ol (**1.118**) were identified with very low detection limits of 0.004 and 0.012 ng L^{-1}, respectively.

Combinatorial synthesis of mercaptoaldehydes was achieved *via* a similar approach starting with α,β-unsaturated aldehydes. Three 3-mercapto-2-methylaldehydes (**1.119-1.121**) were identified to possess meat aroma (Figure 1.13).

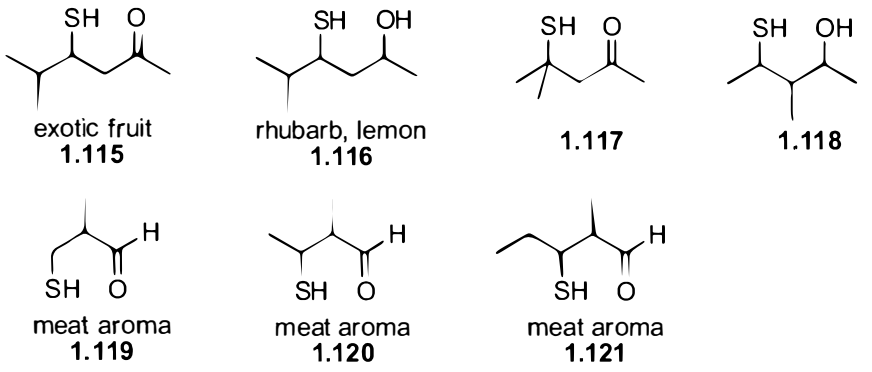
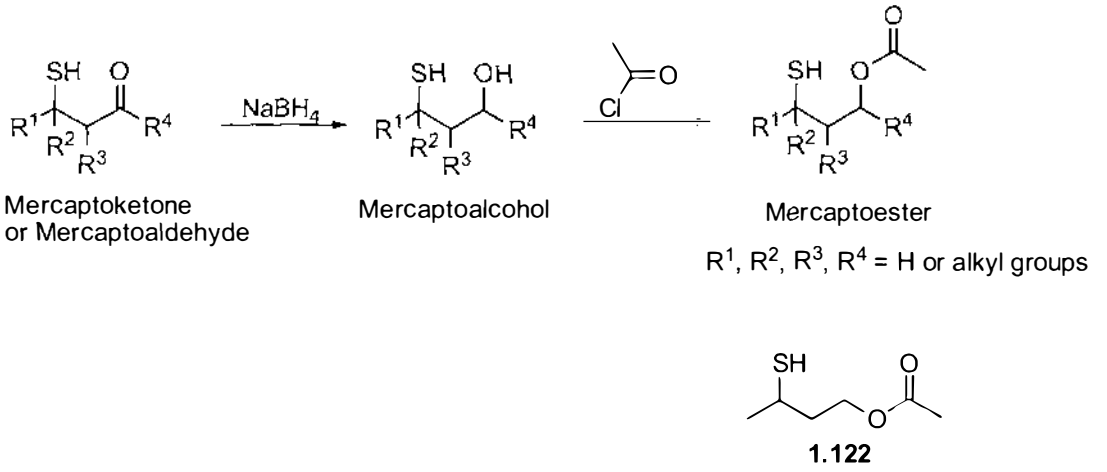


Figure 1.13: Mercaptoalcohols, mercaptoketones and mercaptoaldehydes with desirable properties.

Mercaptoesters were synthesised combinatorially from thioacetic acid and the mercaptoalcohols described above (Scheme 1.16). Mercaptoesters are known flavour compounds in foods and beverages. For example, 3-mercapto-3,3-methylbutylformate (**1.122**, R^1 = Me, R^2 = H, R^3 = H, R^4 = H) is characterised by an exceptionally low odour threshold (2–5 ppt) and has been found in coffee and beer.



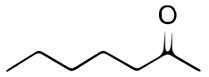
Scheme 1.16: Synthesis of mercaptoesters.

1.7 Aims and objectives of this project

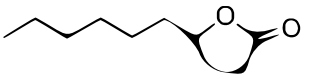
The area of flavour and fragrance is huge and is still an area of development *vis-à-vis* contributions to the food industry. Specifically, the flavour of dairy products arises from complex mixtures of flavour compounds. This project was initiated to synthesise potential flavour compounds combinatorially and identify key components for further investigation as flavourants in dairy products.

Solution-phase combinatorial synthesis is a good way to generate potential flavour compounds rapidly to investigate further structure-activity relationships in food flavour.

Previous work in this area by researchers at Fonterra Research Centre Limited illustrated the potential of ketones and lactones in food products (Figure 1.14). Further investigation of these classes of compounds, particularly with regard to unsaturated and stereochemically pure derivatives, was considered worthwhile.



blue cheese like
1.24



fruity, peach
1.50

Figure 1.14: Potential flavour compounds.

The generation and screening of a library of ketones is discussed in Chapter 2. Libraries of racemic γ-lactones were investigated in Chapter 3 and approaches to enantiomerically enriched γ-lactones are described in Chapters 4 and 5.

Chapter 2

Chapter 2: Synthesis of a library of ketones as potential flavour compounds

2.1 Introduction

Ketones, and most notably methyl ketones, are key compounds in the flavour of mold-ripened cheeses.[56] In camembert and blue veined cheeses, high concentrations of methyl ketones (Table 2.1) and 2-hydroxyalkanes (*i.e.*, CH_3CHOHR) are found, including some unsaturated methyl ketones such as octa-1,5-dien-3-one (**2.8**), and oct-1-en-3-one (**1.30**) and non-3-en-2-one (**2.9**) (Table 2.2).[57]

Table 2.1. Flavour notes of straight chain methyl ketones in cheese.[11]

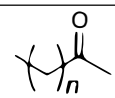
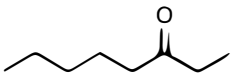
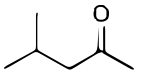
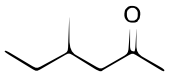
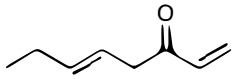
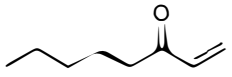
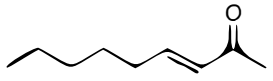
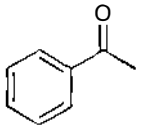
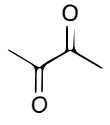
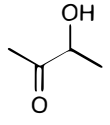
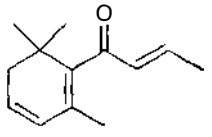
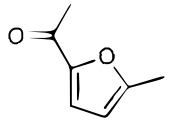
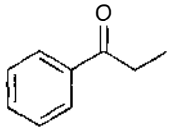
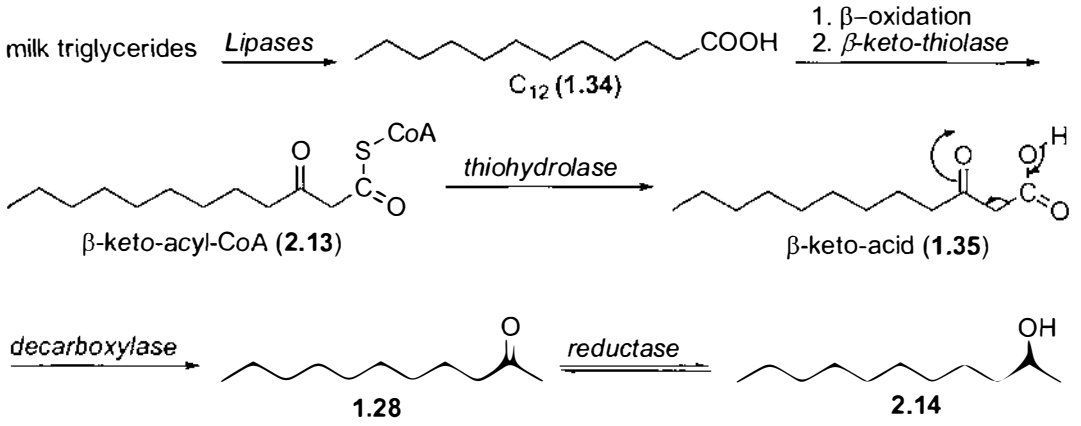
Name	**Structure**	**Flavour note**
		
Propan-2-one (**2.1**)	$n = 0$	Acetone
Butan-2-one (**2.2**)	$n = 1$	Acetone
Pentan-2-one (**2.3**)	$n = 2$	Fruity, acetone
Hexan-2-one (**2.4**)	$n = 3$	Floral, fruity
Heptan-2-one (**1.24**)	$n = 4$	Blue cheese, Roquefort cheese
Octan-2-one (**1.25**)	$n = 5$	Fruity, musty
Nonan-2-one (**1.26**)	$n = 6$	Fruity, musty
Decan-2-one (**1.27**)	$n = 7$	Fruity, musty
Undecan-2-one (**1.28**)	$n = 8$	Floral, herbaceous
Tridecan-2-one (**1.29**)	$n = 10$	Fruity, green mushroom, fruity

Table 2.2: Flavour notes of other ketones in cheese.

Name	Structure	Flavour note
Octan-3-one (**2.5**)		Fruity, green mushroom, fruity
4-Methyl-pentan-2-one (**2.6**)		Fruity, green mushroom, fruity
4-Methyl-hexane-2-one (**2.7**)		Geranium leaf, soil
Octa-1,5-dien-3-one (**2.8**)		Geranium leaf, soil
Oct-1-en-3-one (**1.30**)		Mushroom
Non-3-en-2-one (**2.9**)		Mushroom
Acetophenone (**1.31**)		Orange blossom
Diacetyl (**1.32**)		Buttery
Acetoin (**1.33**)		Buttery
Damascenone (**2.10**)		Woody
Methylfurylacetone (**2.11**)		Woody
Propiophenone (**2.12**)		Woody

Methyl ketones are formed by enzymatic oxidative decarboxylation of fatty acids[58] as described briefly in Chapter 1, and by the spores of *Penicillium roqueforti, mycelium*, and *Penicillium camemberti.*[18b, 16c, 59] The metabolic pathway for their formation is shown in Scheme 2.1. The free fatty acids are oxidised to β-ketoacids, followed by decarboxylation to give methyl ketones. The methyl ketones can be metabolised further to secondary alcohols by *Penicillium roqueforti.*



Scheme 2.1: The metabolic pathway for the formation of methyl ketone.

The chemical synthesis of ketones was the first step in our efforts to produce compounds that may have application to cheese and fresh dairy flavours. The library design was based on the generic structure shown in Figure 2.1 with two points of diversity, R^1 and R^2. R^1 represents long alkyl chains with unsaturation in some cases and variation in the position and orientation of double bonds. R^2 is a relatively small alkyl group. The set of target molecules is outlined in Table 2.3.

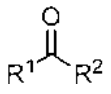
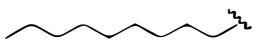
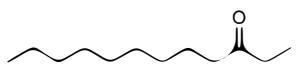
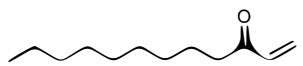
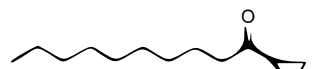
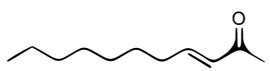
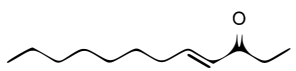
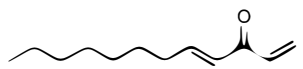
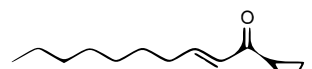
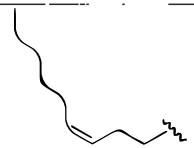
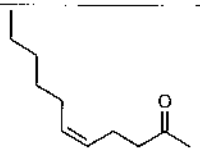
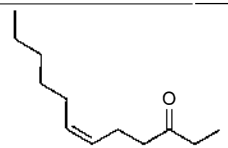
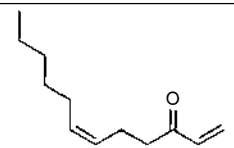
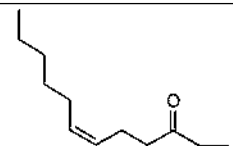
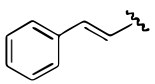
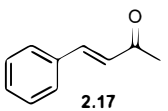
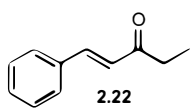
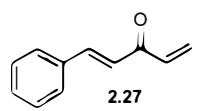
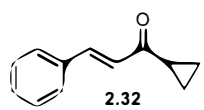
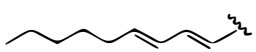
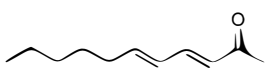
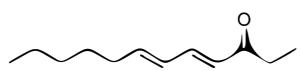
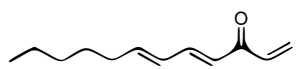
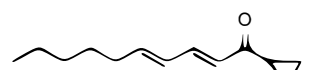


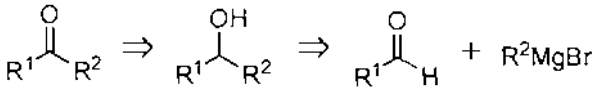
Figure 2.1: The general structure of ketone targets.

Table 2.3: The first library of 20 ketones as potential flavour compounds.

R^1 \ R^2				
	 1.28	 2.19	 2.24	 2.29
	 2.15	 2.20	 2.25	 2.30
	 2.16	 2.21	 2.26	 2.31
	 2.17	 2.22	 2.27	 2.32
	 2.18	 2.23	 2.28	 2.33

2.2 Strategy for the chemical synthesis of the library of ketones

A wide variety of chemical reactions can be called upon to produce ketones. The synthesis of a library of ketones as potential flavour compounds was envisaged to arise *via* the oxidation of secondary alcohols. The alcohols were to come from the Grignard reaction between commercially available aldehydes and Grignard reagents. This was an appealing approach since the Grignard reaction is widely used and a number of reagents are commercially available (Scheme 2.2).



Scheme 2.2: Retrosynthetic analysis for ketone library.

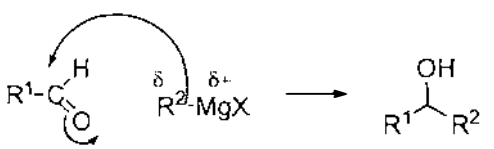
The synthesis of each ketone was initially performed individually to identify and solve the problems associated with their synthesis.

2.3 Synthesis

2.3.1 Grignard reaction

The Grignard reaction is one of the most widely used reactions in synthetic organic chemistry.[60] It is especially useful as a means of forming new carbon-carbon bonds. Synthesising new compounds (*e.g.,* pharmaceuticals, food additives, pesticides) depends on the ability to build up the carbon skeleton of target molecules.

The Grignard reaction involves the nucleophilic attack of a carbanion-like species (the Grignard reagent, RMgX, X=Cl, Br, or I) at a carbonyl carbon. The C=O double bond is broken and the carbonyl compound becomes an alcohol. In the process, a new carbon-carbon bond is formed between the Grignard reagent and the carbonyl carbon, now the alcohol carbon (Scheme 2.3).



Scheme 2.3: The general mechanism for the Grignard reaction.[61]

Five commercially available aldehydes and four commercially available solutions of Grignard reagents were used in the synthesis of a library of twenty alcohols (Figure 2.2).

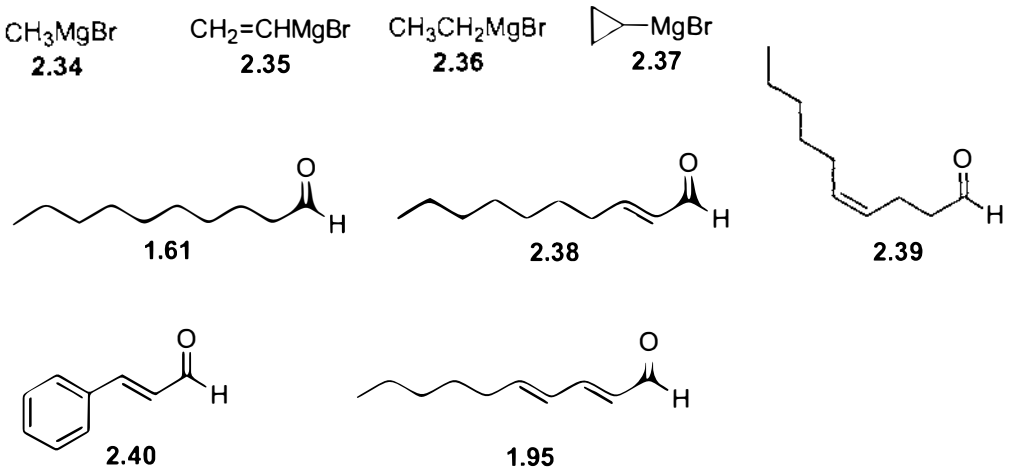
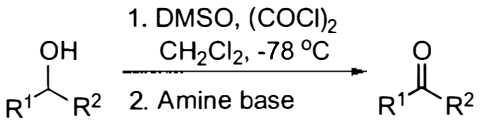


Figure 2.2: Commercially available aldehydes and solutions of Grignard reagents being used in the synthesis of ketones.

The Grignard reactions were performed in THF at 0 °C. In each case, after an hour, TLC indicated the formation of a new, more polar compound. After standard workup and purification by flash chromatography, the 20 alcohols were obtained. One compound from the library of alcohols will be discussed to illustrate the salient features of the NMR spectra. The ^{1}H NMR spectrum of undecan-2-ol (**2.14**) exhibited a three-proton doublet at δ 1.23 that was assigned to the methyl protons in the RCHOHC<u>H</u>$_3$ group. The ^{13}C NMR spectrum of undecan-2-ol (**2.14**) included signals at δ 25.7 (RCHOH<u>C</u>H$_3$) and δ 68.1 (R<u>C</u>HOHCH$_3$), confirming the presence of the secondary alcohol.

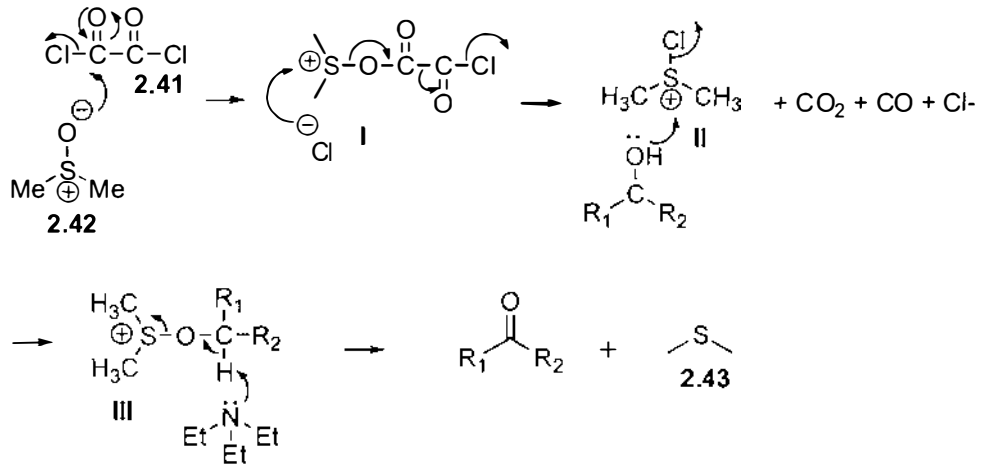
2.3.2 Swern oxidation

The Swern oxidation is a mild method for the oxidation of primary and secondary alcohols to the corresponding aldehydes and ketones, respectively (Scheme 2.4).[62]



Scheme 2.4: The overall transformation of the Swern oxidation.

The first step of the Swern oxidation involves the interaction between oxalyl chloride (**2.41**) and dimethyl sulfoxide (DMSO) (**2.42**) at –78 °C to afford chlorosulfonium chloride (**II**) (Scheme 2.5). Reaction of this species with the alcohol affords an alkoxysulfonuim salt (**III**) and forms the desired carbonyl species and dimethyl sulfide (**2.43**) (Scheme. 2.5).[63]

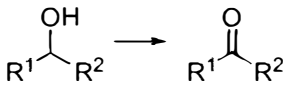


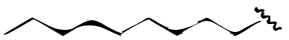
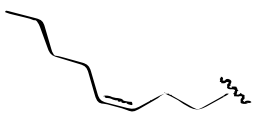
Scheme 2.5: Mechanism of the Swern oxidation.

Both aldehydes and ketones may be made by the Swern oxidation. Double bonds and activated C-H bonds are unreactive under the mild and specific conditions of the Swern oxidation. A disadvantage of the Swern oxidation is that strictly anhydrous conditions are required due to the moisture-sensitivity of oxalyl chloride. Moreover, DMSO (**2.42**) and oxalyl chloride (**2.41**) explode when combined at room temperature and therefore low temperatures are necessary.

The oxidation of secondary alcohols with saturated R^1 and R^2 groups occurred smoothly *via* the Swern protocol. Those compounds with double bonds remote to the alcohol functional group were also successfully oxidised (Table 2.4). A three-proton singlet was observed at δ 2.13 in the ^{1}H NMR spectrum of undecan-2-one (**1.28**) that was assigned to the methyl group located next to the carbonyl group of the ketone. The resonance at δ 209.1 in the ^{13}C NMR spectrum was attributed to the newly formed carbonyl group.

Table 2.4: Ketones prepared *via* Swern Oxidation of Secondary Alcohols.



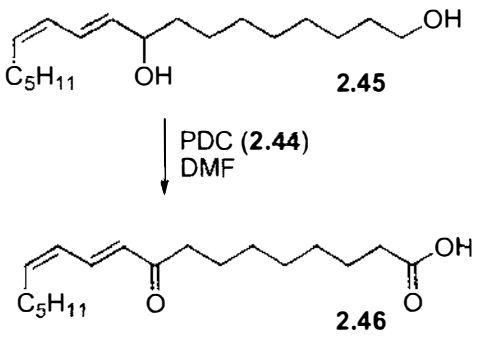
R^1 \ R^2	CH_3-	CH_3CH_2-	CH_2=CH-	
	**1.28** 71 %	**2.19** 47 %	**2.24** 60 %	**2.29** 53 %
	**2.15** 66 %	**2.20** 96 %	**2.25** 6 %	**2.30** 30 %
	**2.16** 47 %	**2.21** 94 %	**2.26** 38 %	**2.31** 73 %

Unfortunately, allylic and/or benzylic secondary alcohols gave, at best, low yields of ketones *via* the Swern protocol. Alternative oxidation methods were therefore investigated to get good yields of these ketones.

2.3.3 Oxidation with PDC

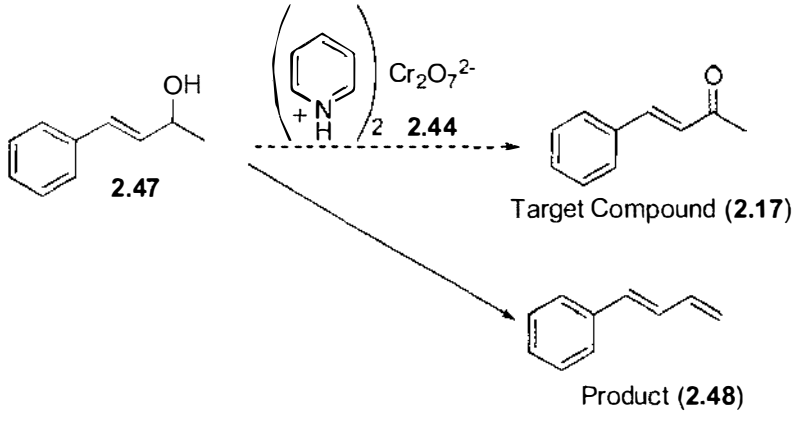
In 1979, Corey and Schmidt[64] introduced pyridinium dichromate, $(C_5H_5NH^+)_2Cr_2O_7^{2-}$ (PDC, **2.44**), for the oxidation of secondary and allylic alcohols. Compound **2.44** is

reported to be a more useful oxidant for allylic alcohols than for their saturated analogs. The oxidation of (10*E*,12*Z*)-10,12-octadecadiene-1,-9-diol (**2.45**) with PDC (**2.44**) in *N,N*-dimethylformamide (DMF) gave rise to the keto acid **2.46** (Scheme 2.6).[65]



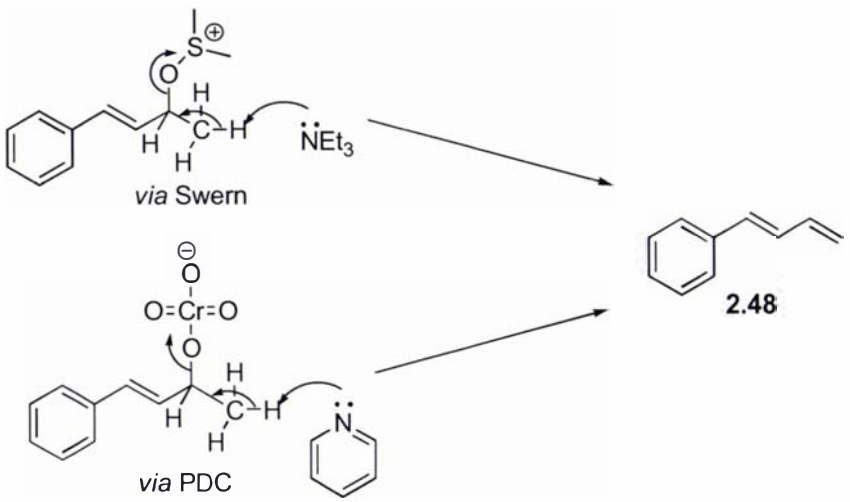
Scheme 2.6: The oxidation of (10*E*,12*Z*)-10,12-octadecadiene-1,9-diol (**2.45**) with PDC (**2.44**).[66]

When this procedure was applied to the oxidation of 4-phenyl-but-3-en-2-ol (**2.47**), the compound isolated was analysed by NMR. The ^{1}H NMR spectrum exhibited two new resonances in the δ 6.1-6.6 ppm region that were assigned to olefinic protons of the conjugated diene **2.48**. There was no singlet in the δ 2.0-2.5 ppm region as would be expected for the methyl group of the desired methyl ketone (**2.17**) (Scheme 2.7).

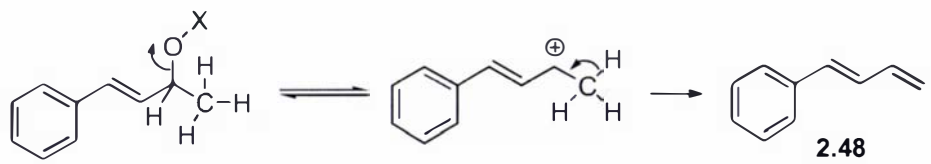


Scheme 2.7: The attempted oxidation of allylic alcohol **2.47** with PDC (**2.44**).

Both the Swern and PDC oxidations failed to form the desired ketone from allylic alcohols. Our mechanistic explanation for this is based on the observed product from the PDC oxidation. Once the alkoxysulfonium salt (*via* the Swern oxidation), or the alkoxychromium salt (*via* the PDC oxidation) is formed, β-elimination would give rise to a diene (**2.48**) *via* an E1 or E2 mechanism (Scheme 2.8). An E1 mechanism would procede *via* a carbocation that is stabilised by the adjacent π-bond system (Scheme 2.9).



Scheme 2.8: The mechanistic possibility of E2.



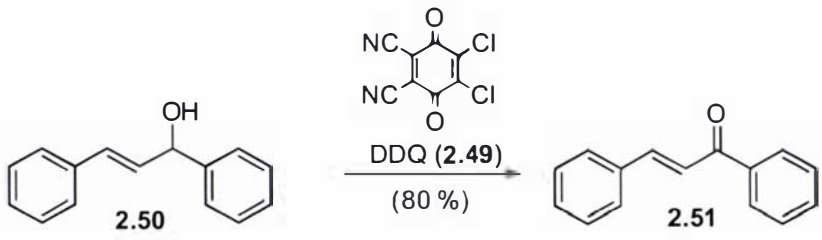
X = +SMe_2 (*via* Swern)

X = CrO_3^- (*via* PDC)

Scheme 2.9: The mechanistic possibility of E1.

2.3.4 Oxidation with DDQ (2.49)

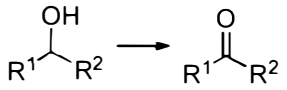
2,3-Dichloro-5,6-dicyanobenzoquinone (DDQ, **2.49**) is a potential quinone that selectively oxidises allylic alcohols.[66] Compound **2.49** is generally used under anhydrous conditions and often in large excess. The oxidation of allylic alcohols using a catalytic amount of DDQ in a biphasic, slightly acidic benzene/water system, in the presence of periodic acid was carried out at room temperature overnight. The oxidation of 1,3-diphenyl-3-hydroxy-1-propene (**2.50**) was reported as a typical example (Scheme 2.10).[67]

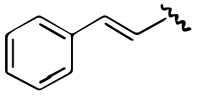
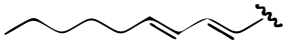


Scheme 2.10: The oxidation of 1,3-diphenyl-3-hydroxy-1-propene (**2.50**) by DDQ (**2.49**).[70]

The example in Scheme 2.10 provided good precedent for the oxidation of alcohols that had undergone elimination rather, than oxidation, under other conditions. Indeed, after workup and purification by flash chromatography, the allylic ketones were obtained with reasonably high yields (Table 2.5).

Table 2.5: A library of the ketones produced by DDQ Oxidation.



R^1 \ R^2	CH_3-	CH_3CH_2-	CH_2=CH-	
	**2.17** 59 %	**2.22** 56 %	**2.27** 60%	**2.32** 81%
	**2.18** 68 %	**2.23** 40 %	**2.28** 72 %	**2.33** 45 %

2.4 Screening of the first ketone library

For the first library of twenty ketones, each compound was synthesised individually and characterised by ^{1}H and ^{13}C NMR spectroscopy. Sixteen ketones were suitable for screening by the Fox 4000[68] and GC-O. Four ketones (**2.25**, **2.28**, **2.30**, and **2.33**, Figure 2.3) appeared to be unstable; it seems oxidative decomposition occurred while the samples were in storage. These ketones were therefore not available for analysis.

The respective odour characteristics of the 16 remaining ketones were analysed by the Fox 4000, an instrument that compares the odour of compounds and that of standards (*e.g.*, cheeses such as cheddar, gouda, parmesan and camembert).

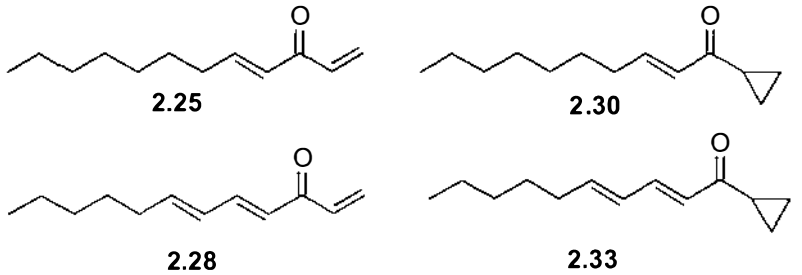


Figure 2.3: The ketones that were not sufficiently stable for the Fox analysis.

2.4.1 The FOX 4000

The electronic nose (EN) has emerged as an exciting new analytical tool having broad application. In flavour chemistry, the electronic nose has the potential to reproducibly monitor flavours, thus maintaining product consistency and quality. In a headspace analysis, volatile molecules are injected into the EN and they react with the sensor array. The chemical nature and concentration of the analyte affect the response characteristics. Once the gas-phase molecules are at the sensors, they need to be able to react with the sensors to produce a response. In this study, an Alpha M.O.S. Fox 4000 EN was equipped with 18 metal-oxide sensors to analyse pure chemical samples. The sensors react to the presence of organic compounds by changes in their electrical resistance. The raw data of a single analysis run consists of 18 numbers, representing the maximum relative change in resistance experienced by the sensors. The visual result from principal components analysis (PCA) was performed by the computer software and the clusters of data points represented the aroma of samples. The distance between two clusters represented the difference between the aromas of the two respective samples.

The individual ketones were evaluated at low and high concentrations by comparison with each cheese and a blank reference. For the data analysis, the overall PCA was used to show the relationships between each ketone and the four cheeses (Figure 2.4).[21] Two principal components, PC1 and PC2 gave a measure of how close the aroma of each ketone was to those of each cheese. The relationship of each ketone with the four cheeses was quantified as a vector. The calculated angles between zero-cheese (vector 1) and zero-ketone (vector 2) are summarised in Table 2.6.

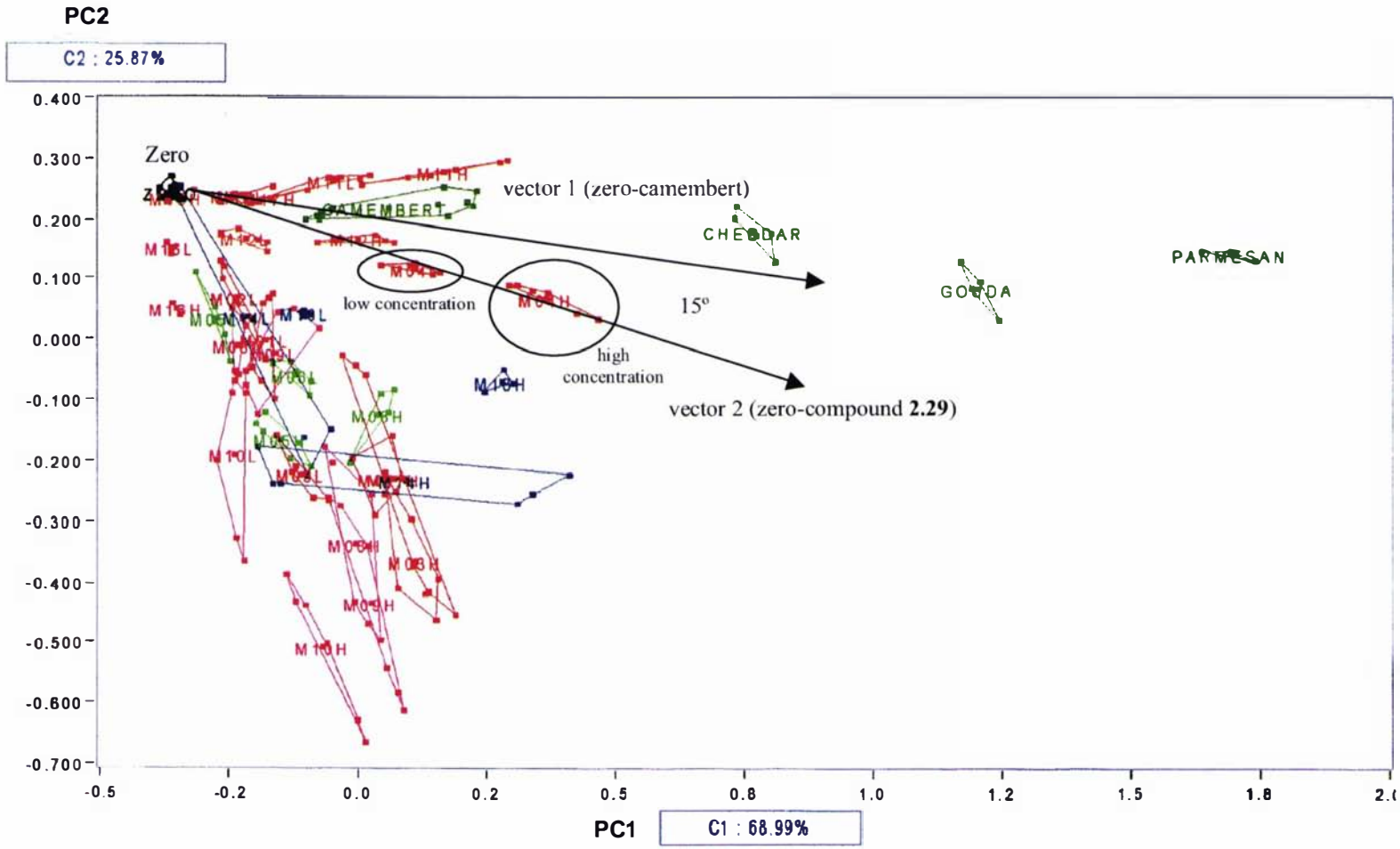
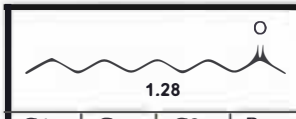
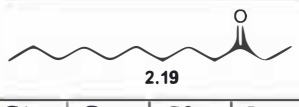
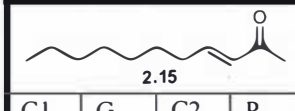
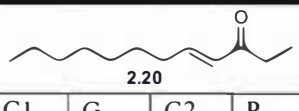
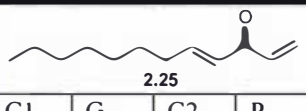
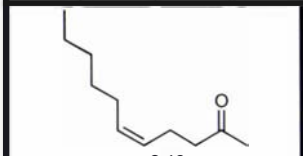
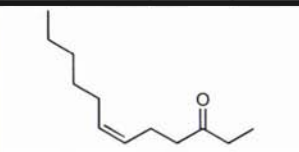
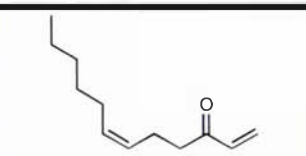
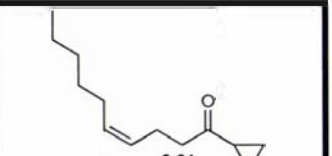
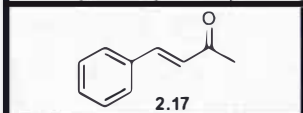
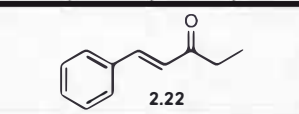
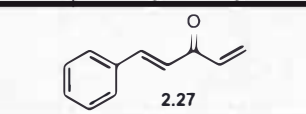
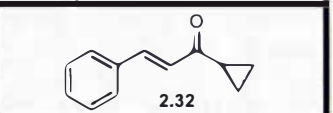
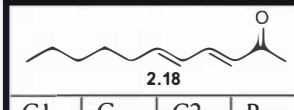
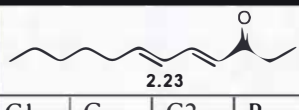
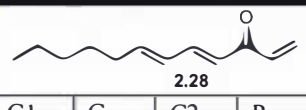


Figure 2.3: PCA map for the blank, 4 cheeses and 16 ketones, with illustration of angle determination for compound **2.29**.

Table 2.6: FOX results which quantify the relationship between each ketone and four cheeses (C1 = camembert; G = gouda; C2 = cheddar and P = parmesan). Angles are those determined in accordance with Figure 2.3.

 1.28				 2.19				 2.24				 2.29			
C1	G	C2	P	C1	G	C2	P	C1	G	C2	P	C1	G	C2	P
35°	57°	59°	62°	39°	52°	54°	57°	50°	52°	54°	57°	15°	23°	22°	27°
 2.15				 2.20				 2.25				 2.30			
C1	G	C2	P	C1	G	C2	P	C1	G	C2	P	C1	G	C2	P
60°	61°	63°	66°	41°	42°	43°	48°	unstable				unstable			
 2.16				 2.21				 2.26				 2.31			
C1	G	C2	P	C1	G	C2	P	C1	G	C2	P	C1	G	C2	P
54°	65°	67°	69°	45°	61°	64°	66°	69°	70°	73°	74°	83°	not determined		
 2.17				 2.22				 2.27				 2.32			
C1	G	C2	P	C1	G	C2	P	C1	G	C2	P	C1	G	C2	P
11°	22°	17°	26°	23°	27°	26°	33°	12°	not determined			13°	not determined		
 2.18				 2.23				 2.28				 2.33			
C1	G	C2	P	C1	G	C2	P	C1	G	C2	P	C1	G	C2	P
36°	64°	67°	69°	22°	not determined			unstable				unstable			

These results revealed six compounds that gave relatively small angles and thus were promising *vis-à-vis* the aroma of cheeses, especially camembert cheese. The cells for these compounds have been shaded in Table 2.6.

2.4.2 GC-O and GC-MS

Six ketones identified from Fox 4000 analysis were further analysed by GC-O for the sensory aspect and this GC-O was conducted by the author only. The odour description of each ketone is shown in Figure 2.3 and compound **2.23** gave no odour. The purity of each individual ketone was good by GC-MS.

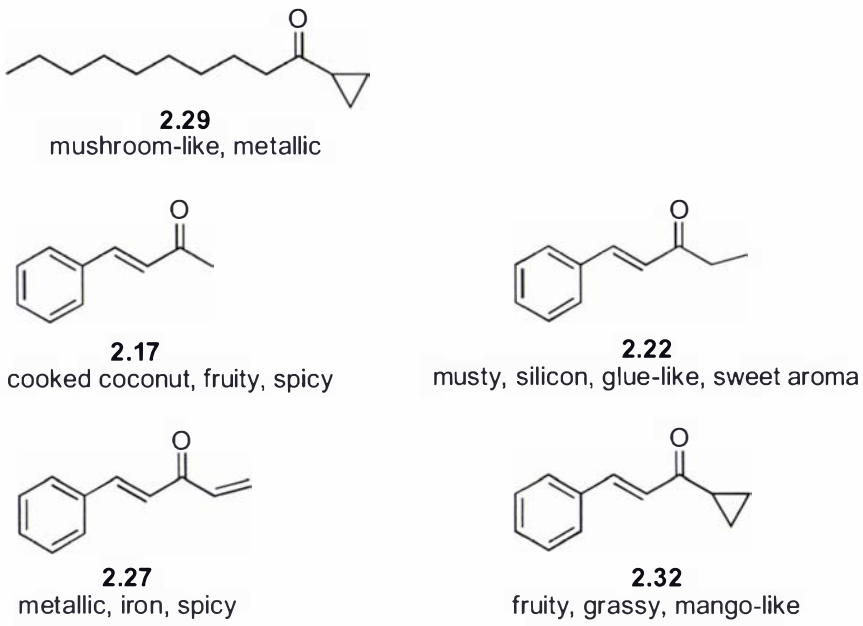


Figure 2.3: The structure and odour description of lead ketones from the first library.

2.5 Combinatorial chemistry

In the previous work, the synthesis of each ketone was performed individually *via* a 2-step sequence. The synthesis of this initial batch of compounds was essential for identifying and solving the problems associated with the oxidation of secondary alcohols in highly conjugated systems. Sixteen ketones were screened by the FOX 4000 and selected compounds by GC-O for sensory aspects of each compound. The ketones with

cyclopropyl and phenyl groups showed potential. Therefore, it was desirable to make more of these compounds in a combinatorial way to reduce time.

2.5.1 A Library of ketones containing a cyclopropane

Cyclopropane-containing compounds (Figure 2.6) have been isolated from the fermentation broth of *Streptoverticillium fervens*.[69] The biosynthetic pathway and metabolism of cyclopropane-containing natural products has been studied.[70] The most likely mechanism for cyclopropane ring formation involves electrophilic attack of a carbenium ion on a homoconjugated double bond of an unsaturated fatty acid. This mechanism has been derived from an observed peroxide fragmentation in the female gametes of *Ectocarpus siliculosus* (Scheme 2.11).[71]

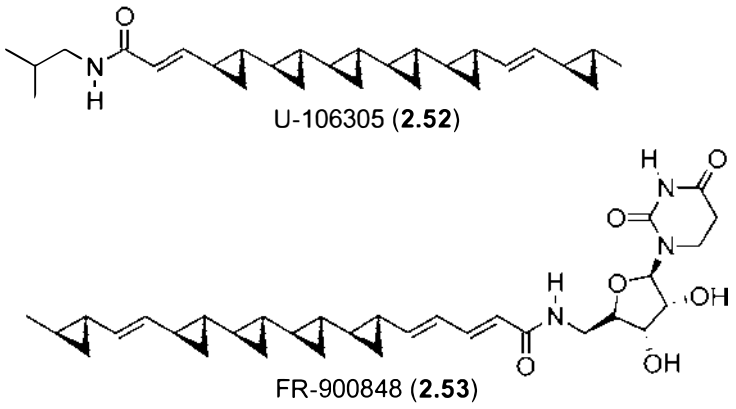
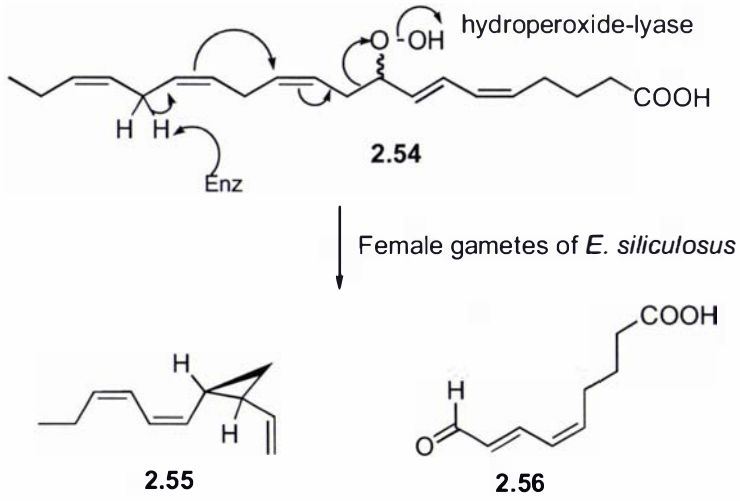
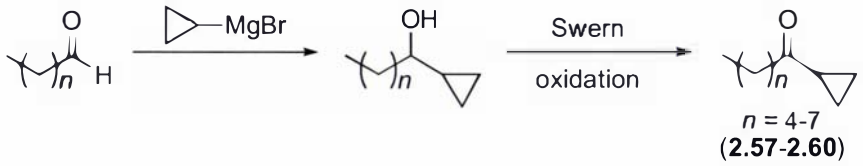


Figure 2.6: Oligocyclopropane-containing compounds.[72]



Scheme 2.11: Mechanism for cyclopropane formation in nature.

A library of four cyclopropyl ketones was synthesised in a combinatorial manner, involving the Grignard reaction of four commercially available aldehydes with cyclopropyl magnesium bromide, followed by Swern oxidation (Scheme 2.12). We focussed our attention on smaller molecules than those in the original library, since they were deemed to be more volatile and odiferous.



Scheme 2.12: Combinatorial synthesis of a library of four cyclopropyl ketones.

This library of cyclopropyl ketones was tested against camembert cheese only in the FOX 4000 analysis, since cyclopropyl ketones were shown earlier to be most compatible with camembert cheese (Table 2.6). The PCA map is shown in Figure 2.7. The relationship between the odour from our library of ketones and camembert cheese was still good, since the angle was calculated to be 29°.

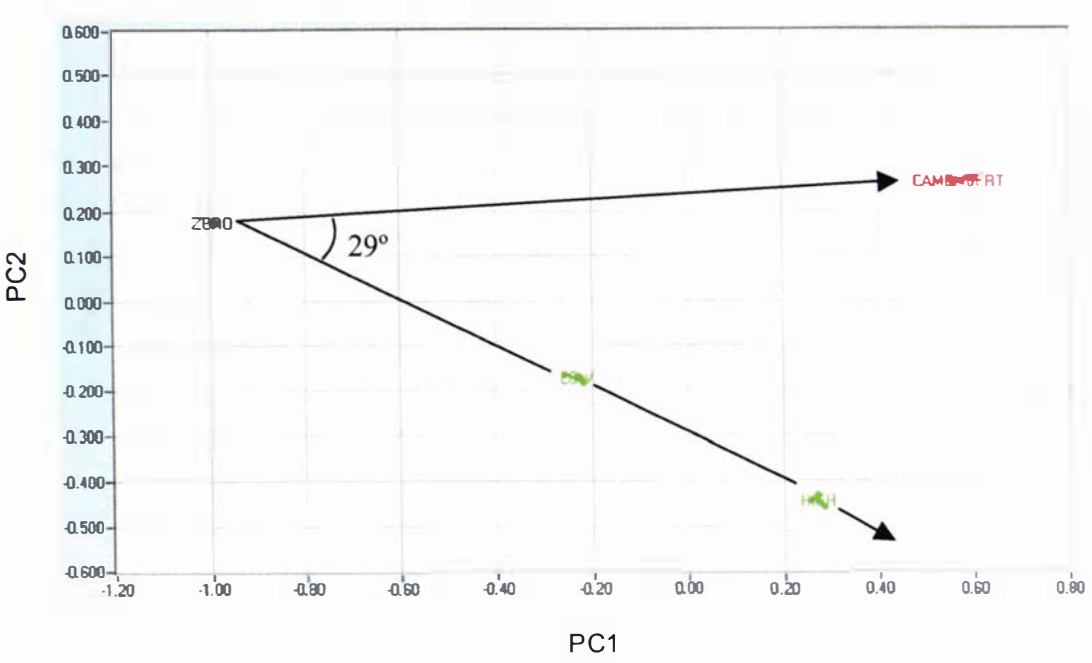


Figure 2.7: PCA map for the blank, camembert and the library of four ketones (high and low concentrations) (**2.57-2.60**).

The library of cyclopropyl ketones was also analysed by GC and the compounds eluted in the order of increasing mass. The library was also assessed by GC-O (Figure 2.8).

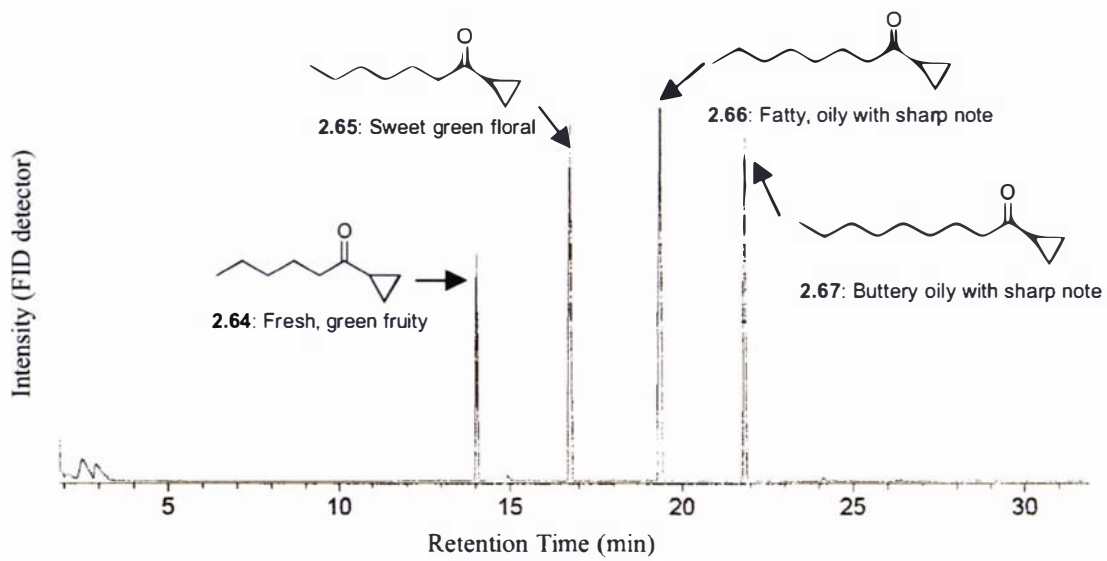


Figure 2.8: GC trace for the library of cyclopropyl ketones and their odour descriptions.

2.6 Summary

Synthesis and analysis of ketones were the focus of this chapter. Ketones were synthesized individually *via* a two-step sequence. The Grignard reaction was the first step to produce an alcohol from a commercially available aldehyde, using 1-3 carbon Grignard reagents which were also commercially available. The oxidation of the secondary alcohol ensued, to produce a ketone. Different reaction conditions were required for the oxidation: Swern oxidation was effective for the oxidation of saturated, long-chain alcohols, while oxidation with DDQ was superior for alcohols in highly conjugated systems. Sixteen ketones were synthesised individually and each ketone was screened by the Fox 4000. Some compounds selected from the Fox analysis were assessed by GC-O. The analysis gave promising results for aromatic and cyclopropyl ketones. The first library of cyclopropyl ketones was synthesised and screened.

2.7 Experimental procedure

2.7.1 General procedure

General methods. ^{1}H NMR spectra were recorded at 270 MHz, and ^{13}C NMR spectra were recorded at 67.5 MHz. All chemical shifts are reported relative to residual TMS as an internal reference. Mass spectra were recorded on a Shimadzu GCMS-QP5000 mass spectrometer. The MS was operated in electron impact mode with an ionization potential of 70 eV and a scanning rate at 0.5 sec/scan over a mass range of *m/z* 29-350. Silica gel flash column chromatography was performed with Scharlau Silica gel 60, 0.4~0.6 mm, 230-400 mesh. Experiments requiring anhydrous conditions were performed under a nitrogen atmosphere. Analytical thin layer chromatography (TLC) was performed with Merck pre-coated TLC plates, silica gel 60 F_{254}, thickness 0.25 mm. Tetrahydrofuran (THF) was distilled under a nitrogen atmosphere from sodium/benzophenone. Dichloromethane (CH_2Cl_2) was distilled under a nitrogen atmosphere from calcium hydride. Dimethyl sulfoxide (DMSO) and triethylamine (Et_3N) were distilled under a nitrogen atmosphere from calcium hydride and were stored over potassium hydroxide.

Grignard reaction. A solution of the Grignard reagent (commercially available solution in THF, 1.2 equiv.) was added dropwise to a solution of the aldehyde (250 mg, 1.0 equiv.) in THF (5 mL) at 0 °C. After stirring for 1 h at 0 °C, water (10 mL) was added and the mixture was extracted with Et_2O (3 x 20 mL). The combined extracts were washed with brine (10 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The crude oil was purified by chromatography (hex-EtOAc, 5:1) and the secondary alcohol (Table 2.7) was used directly in the next step.

Table 2.7: The properties of secondary alcohols from the Grignard reaction.

Grignard	Aldehyde	Scale (mmol)	R_f (5:1 hex/EtOAc)	Physical Property
MeMgBr **(2.34)**	Decanal	1.6	0.21	Colourless oil
	2-*trans* Decenal	1.6	0.45	Colourless oil
	4-*cis* Decenal	1.6	0.52	Colourless oil
	Cinnamaldehyde	1.8	0.22	Yellow oil
	2,4-*trans*, *trans* Decadienal	1.2	0.47	Yellow oil
EtMgBr **(2.35)**	Decanal	1.6	0.61	Colourless oil
	2-*trans* Decenal	1.6	0.59	Colourless oil
	4-*cis* Decenal	1.6	0.58	Colourless oil
	Cinnamaldehyde	1.8	0.50	Yellow oil
	2,4-*trans*, *trans* Decadienal	1.2	0.45	Yellow oil
$(CH_2=CH)MgBr$ **(2.36)**	Decanal	1.6	0.30	Colourless oil
	2-*trans* Decenal	1.6	0.41	Colourless oil
	4-*cis* Decenal	1.6	0.40	Colourless oil
	Cinnamaldehyde	1.8	0.33	Yellow oil
	2,4-*trans*, *trans* Decadienal	1.2	0.40	Yellow oil
 **(2.37)**	Decanal	1.6	0.52	Colourless oil
	2-*trans* Decenal	1.6	0.45	Colourless oil
	4-*cis* Decenal	1.6	0.40	Colourless oil
	Cinnamaldehyde	1.8	0.29	Yellow oil
	2,4-*trans*, *trans* Decadienal	1.2	0.40	Yellow oil

General procedure for Swern oxidation. To a cooled (-73 °C), stirred solution of oxalyl chloride (1.5 equiv.) in CH_2Cl_2 (7 mL) was added DMSO (2 equiv.) dropwise. The

mixture was stirred at –73 °C for 30 min, and a solution of the alcohol (0.25 g, 1 equiv.) in CH_2Cl_2 (1 mL) was added dropwise over 5 min. After 30 min, Et_3N (5 equiv.) was added, and the mixture was then allowed to warm to room temperature. Water (10 mL) was added, and the layers were separated. The aqueous layer was extracted with $CHCl_3$ (3 x 30 mL). The combined organic layers were washed with water (3 x 10 mL) and brine (20 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The resulting oil was redissolved in Et_2O (40 mL) and washed with water (3 x 10 mL) and brine (20 mL). After drying ($MgSO_4$), the ethereal solution was concentrated *in vacuo* to provide the ketone.

General procedure for oxidation with DDQ. To a stirred solution of allylic alcohol (0.25 g, 1 equiv.) and DDQ (0.3 equiv.) in benzene (7 mL) was added periodic acid (0.9 equiv.) in 0.1 N hydrochloric acid (30 mL). The resultant mixture was stirred at room temperature for 16 h, then poured into saturated aqueous sodium carbonate solution (50 mL), and extracted with dichloromethane. The extracts were washed with water, dried with magnesium sulfate, and concentrated *in vacuo* to provide the ketone.

2.7.2 Experimental data

The chemical structure of each compound is given in Table 2.2.

Undecan-2-one (1.28) prepared *via* Swern oxidation. The residue was purified by chromatography (10:1 hex-EtOAc) to give **1.28** (190.4 mg, 86 %) as a yellow oil: R_f = 0.40 (10:1 hex-EtOAc); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.88 (t, J = 6.1 Hz, 3H, C<u>H</u>$_3$CH$_2$-), 1.22-1.27 (m, 12H, 6 x –CH$_2$-), 1.57 (p, J = 7.3 Hz, 2H, -C<u>H</u>$_2$CH$_2$CO-), 2.13 (s, 3H,

CH_3CO-), 2.42 (t, J = 7.3 Hz, 2H, -CH_2CO-); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.1, 22.7, 23.9, 29.2, 29.3, 29.4, 29.8, 31.9, 43.8, 209.1; MS obsd. for $C_{11}H_{22}O$ (M^+): 170.

3*E*-Undecen-2-one (2.15) prepared *via* Swern oxidation. The residue was purified by chromatography (10:1 hex-EtOAc) to give **2.15** (175.8 mg, 66%) as a yellow oil: R_f = 0.55 (10:1 hex-EtOAc); ^1H NMR (270 MHz, $CDCl_3$) δ 0.95 (t, J = 7.0 Hz, 3H, $C\underline{H}_3CH_2$-), 1.30-1.35 (m, 6H, 3 x –CH_2-), 1.49-1.53 (m, 6H, 2 x –CH_2-), 2.31 (s, 3H, CH_3CO-), 6.13 (d, J = 15.9 Hz, 1H, -CH=C$\underline{H}$CO-), 6.88 (dt, J = 15.9 Hz, 6.8 Hz, 1H, -CH_2-C$\underline{H}$=CH-); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.1, 22.6, 26.8, 28.1, 29.0, 29.1, 31.7, 32.5, 131.1, 148.4, 198.4; MS obsd. for $C_{11}H_{20}O$ (M^+): 168.

5*Z*-Undecen-2-one (2.16) prepared *via* Swern oxidation. The residue was purified by chromatography (10:1 hex-EtOAc) to give **2.16** (117.5 mg, 47%) as a yellow oil: R_f = 0.43 (10:1 hex-EtOAc); ^1H NMR (270 MHz, $CDCl_3$) δ 0.89 (t, J = 7.2 Hz, 3H, $C\underline{H}_3CH_2$-), 1.28-1.38 (m, 6H, 3 x –CH_2-), 2.03 (q, J = 6.6 Hz, 2H, –CH_2-), 2.15 (s, 3H, CH_3CO-), 2.32 (q, J = 6.8 Hz, 2H, –CH_2-), 2.48 (t, J = 7.1 Hz, 2H, –CH_2-), 5.28-5.42 (m, 2H, -C$\underline{H}$=C$\underline{H}$-); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.1, 21.7, 22.6, 27.1, 29.3, 29.9, 31.5, 43.6, 127.4, 131.1, 208.2; MS obsd. for $C_{11}H_{20}O$ (M^+): 168.

4-Phenyl-3*E*-buten-2-one (2.17) prepared *via* DDQ oxidation. The residue was purified by chromatography (5:1 hex-EtOAc) to give **2.15** (147.5 mg, 59%) as a dark yellow oil: R_f = 0.53 (5:1 hex-EtOAc); ^1H NMR (270 MHz, $CDCl_3$) δ 2.39 (s, 3H, CH_3CO-), 6.70 (d, J = 16.2 Hz, 1H, =CH-Ph), 7.38–7.45 (m, 3H, ArH), 7.48-7.50 (m, 1H, =CHCO-), 7.48–

7.53 (m, 2H, ArH); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 27.5, 127.0, 128.1, 128.8, 130.4, 134.2, 143.3, 198.2; MS obsd. for $C_{10}H_{10}O$ (M^+): 146.

3*E*,5*E*-Undecadien-2-one (2.18) prepared *via* DDQ oxidation. The residue was purified by chromatography (5:1 hex-EtOAc) to give **2.18** (129.7 mg, 68%) as a dark yellow oil: R_f = 0.45 (5:1 hex-EtOAc); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.79 (t, J = 6.8 Hz, 3H, $C\underline{H}_3CH_2$-), 1.22-1.38 (m, 4H, $CH_3C\underline{H}_2CH_2C\underline{H}_2CH_2CH$=), 1.44 (p, J = 7.0 Hz, 2H, $CH_3CH_2C\underline{H}_2CH_2CH_2CH$=), 2.14-1.22 (m, 2H, $CH_3CH_2CH_2CH_2C\underline{H}_2CH$=), 2.26 (s, 3H, $C\underline{H}_3CO$-), 6.04 (d, J = 5.6 Hz, 1H, -CH=C<u>H</u>-CO-), 6.17-6.20 (m, 2H, -CH_2-C<u>H</u>=C<u>H</u>-), 7.06–7.15 (m, 1H, -CH=CH-C<u>H</u>=CH-CO-); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 13.8, 22.3, 26.9, 28.2, 31.2, 32.9, 128.4, 128.5, 143.7, 145.4, 198.1; MS obsd. for $C_{11}H_{18}O$ (M^+): 166

Dodecan-3-one (2.19) prepared *via* Swern oxidation. The residue was purified by chromatography (5:1 hex-EtOAc) to give **2.19** (322.7 mg, 47 %) as a yellow oil: R_f = 0.36 (5:1 hex-EtOAc); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.88 (t, J = 6.6 Hz, 3H, $C\underline{H}_3CH_2$-), 1.04 (t, J = 7.4 Hz, 3H, $C\underline{H}_3CH_2CO$-), 1.25-1.29 (m, 12H, 6 x -CH_2-), 1.56 (q, J = 6.8 Hz, 2H, -$C\underline{H}_2CH_2CO$-), 2.35–2.44 (m, 4H,-$C\underline{H}_2COC\underline{H}_2$-); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 7.5, 13.8, 22.5, 23.7, 29.1, 29.3, 31.7, 35.5, 42.1, 210.7; MS obsd. for $C_{12}H_{24}O$ (M^+): 184.

4*E*-Dodecen-3-one (2.20) prepared *via* Swern oxidation. The residue was purified by chromatography (5:1 hex-EtOAc) to give **2.20** (239.8 mg, 96%) as a yellow oil: R_f = 0.75 (5:1 hex-EtOAc); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.88 (t, J = 7.1 Hz, 3H, $C\underline{H}_3CH_2$-), 1.10 (t, J = 7.2 Hz, 3H, $C\underline{H}_3CH_2CO$-), 1.23-1.30 (m, 8H, 4 x –CH_2-), 1.46 (p, J = 7.1 Hz, 2H, -

$C\underline{H}_2CH_2CH=CH$-), 2.20 (q, J = 6.8 Hz, 2H, -$C\underline{H}_2CH=CH$-), 2.56 (q, J = 7.2 Hz, 2H, -$COC\underline{H}_2CH_3$), 6.10 (dt, J = 5.8 Hz, 1.3 Hz, 1H, -CH=C$\underline{H}$CO-), 6.89–6.78 (dt, J = 5.8 Hz, 6.8 Hz, 1H, -C$\underline{H}$=CHCO-); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 8.1, 14.1, 22.6, 28.1, 29.0, 29.1, 31.7, 32.4, 33.1, 129.8, 147.0, 200.9; MS obsd. for $C_{12}H_{22}O$ (M^+): 182.

6*Z*-Dodecen-3-one (2.21) prepared *via* Swern oxidation. The residue was purified by chromatography (5:1 hex-EtOAc) to give **2.21** (235.1 mg, 94%) as a yellow oil: R_f = 0.76 (5:1 hex-EtOAc) ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.89 (t, J = 7.1 Hz, 3H, $C\underline{H}_3CH_2$-), 1.05 (t, J = 7.2 Hz, 3H, $C\underline{H}_3CH_2CO$-), 1.22-1.39 (m, 6H, 3 x –CH_2-), 2.02 (q, J = 7.0 Hz, 2H, $C\underline{H}_2CH=CH$-), 2.22–2.35 (m, 2H, –CH=CH$C\underline{H}_2$-), 2.39–2.47 (m, 4H, –$C\underline{H}_2COC\underline{H}_2$-), 5.28–5.44 (m, 2H, -C$\underline{H}$=C$\underline{H}$-); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 7.9, 14.1, 21.8, 22.6, 27.2, 29.4, 31.5, 36.0, 42.3, 127.6, 131.1, 210.9; MS obsd. for $C_{12}H_{22}O$ (M^+): 182.

1-Phenyl-2*E*-penten-3-one (2.22) prepared *via* DDQ oxidation. The residue was purified by chromatography (5:1 hex-EtOAc) to give **2.22** (141.2 mg, 56%) as a dark yellow solid: R_f = 0.83 (5:1 hex-EtOAc); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.16 (t, J = 7.5 Hz, 3H, $C\underline{H}_3CH_2CO$-), 2.67 (q, J = 7.2 Hz, 2H, -$COC\underline{H}_2CH_3$), 6.73 (d, J = 16.3 Hz, 1H, -CH=C$\underline{H}$CO-), 7.36-7.38 (m, 5H, ArH), 7.52–7.58 (m, 1H, PhC$\underline{H}$=CH-); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 8.1, 33.9, 125.8, 128.0, 128.7, 130.1, 134.3, 141.9, 200.5; MS obsd. for $C_{11}H_{12}O$ (M^+): 160.

4*E*,6*E*-Dodecadien-3-one: (2.23) prepared *via* DDQ oxidation. The residue was purified by chromatography (5:1 hex-EtOAc) to give **2.23** (101.5 mg, 40%) as a yellow oil: R_f =

0.67 (5:1 hex-EtOAc); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.89 (t, J = 7.0 Hz, 3H, $C\underline{H}_3CH_2$-), 1.11 (t, J = 7.4 Hz, 3H, $C\underline{H}_3CH_2CO$-), 1.25–1.38 (m, 4H, $CH_3C\underline{H}_2CH_2C\underline{H}_2CH_2CH$=), 1.44 (p, J = 6.4 Hz, 2H, $CH_3CH_2C\underline{H}_2CH_2CH_2CH$=), 2.15–2.21 (m, 2H, $CH_3CH_2CH_2CH_2C\underline{H}_2CH$=), 2.56 (q, J = 7.2 Hz, 2H, $CH_3C\underline{H}_2CO$-), 6.08 (d, J=15.4 Hz, 1H, -CH=C<u>H</u>-CO-), 6.15–6.19 (m, 2H, -CH_2-C<u>H</u>=C<u>H</u>-), 7.10–7.30 (m, 1H, -CH=CH-C<u>H</u>=CH-CO-); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 8.3, 14.0, 22.4, 28.4, 31.3, 33.0, 33.5, 127.4, 128.7, 142.6, 145.3, 201.0; MS obsd. for $C_{12}H_{20}O$ (M^+): 180.

Dodec-1-en-2-one (2.24) prepared *via* Swern oxidation. The residue was purified by chromatography (10:1 hex-EtOAc) to give **2.24** (150 mg, 60%) as a yellow oil: R_f = 0.66 (10:1 hex-EtOAc); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.88 (t, J = 7.0 Hz, 3H, $C\underline{H}_3CH_2$-), 1.24-1.29 (m, 12H, 6 x -CH_2-), 1.62 (p, J = 7.0 Hz, 2H, -$C\underline{H}_2CH_2C$●-), 2.58 (t, J = 7.4 Hz, 2H, -$C\underline{H}_2CO$-), 5.81 (dd, J = 10.4 Hz, 1.5 Hz, 1H, -COCH=C<u>H</u>$_2$), 6.20 (dd, J = 17.7 Hz, 1.4 Hz, 1H, -COCH=C<u>H</u>$_2$), 6.36 (dd, J = 17.7 Hz, 10.2 Hz, 1H, -COC<u>H</u>=CH_2); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.1, 22.7, 24.1, 29.3, 29.5, 31.9, 39.7, 127.7, 136.5, 200.8; MS obsd. for $C_{12}H_{22}O$ (M^+): 182.

1,4*E*-Dodecdien-2-one (2.25) prepared *via* Swern oxidation. The residue was purified by chromatography (5:1 hex-EtOAc) to give **2.25** (11.5 mg, 6%) as an orange oil: R_f = 0.49 (5:1 hex-EtOAc); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.88 (t, J = 6.8 Hz, 3H, $C\underline{H}_3CH_2$-), 1.26-2.31 (m, 1●H, 5 x -CH_2-), 1.48 (p, J = 7.4 Hz, 2H, -$C\underline{H}_2CH_2CH$=CH-), 2.25 (qd, J = 7.1 Hz, 1.5 Hz, 2H, -$C\underline{H}_2CH$=CH-), 5.80 (dd, J = 10.5 Hz, 1.3 Hz, 1H, -CH=C<u>H</u>$_2$), 6.28 (dt, J = 7.3 Hz, 1.3 Hz, 1H, -CH=C<u>H</u>CO-), 6.36 (dt, J = 15.6 Hz, 1.6 Hz, 1H, -CH=C<u>H</u>$_2$), 6.67

(dd, J = 7.6 Hz, 0.6 Hz, 1H, -COC<u>H</u>=CH_2), 6.95 (dt, J = 15.9 Hz, 7.2 Hz, 1H, -CH_2C<u>H</u>=CHCO-); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.1, 22.7, 28.1, 29.1, 29.2, 31.8, 32.8, 128.0, 128.1, 134.8, 149.1, 189.6.

1,6*Z*-Dodecdien-2-one (2.26) prepared *via* Swern oxidation. The residue was purified by chromatography (10:1 hex-EtOAc) to give **2.26** (56.7 mg, 38%) as a yellow oil: R_f = 0.52 (5:1 hex-EtOAc); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.89 (t, J= 6.8 Hz, 3H, CH_3CH_2-), 1.25-1.37 (m, 6H, 3 x –CH_2-), 2.03 (q, J = 6.8 Hz, 2H, -C<u>H</u>$_2$CH=CH-), 2.36 (q, J = 6.5 Hz, 2H, -CH=CHC<u>H</u>$_2$-), 2.64 (t, J = 7.8 Hz, 2H, -CH_2CO-), 5.31-5.44 (m, 2H, -CH_2C<u>H</u>=C<u>H</u>CH_2-), 5.83 (dd, J = 10.1 Hz, 1.6 Hz, 1H, C<u>H</u>$_2$=CHCO-), 6.21 (dd, J = 7.8 Hz, 1.6 Hz, 1H, C<u>H</u>$_2$=CHCO-), 6.35 (dd, J = 7.7 Hz, 10.2 Hz, 1H, CH_2=C<u>H</u>CO-); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.1, 21.8, 22.6, 27.2, 29.3, 31.5, 39.6, 127.5, 127.9, 131.2, 136.4, 200.0; MS obsd. for $C_{12}H_{20}O$ (M^+): 180.

1-Phenyl-1,3*E*-pentadien-3-one (2.27) prepared *via* DDQ oxidation. The residue was purified by chromatography (5:1 hex-EtOAc) to give **2.27** (150 mg, 60%) as an orange oil: R_f = 0.34 (5:1 hex-EtOAc); ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.90 (dd, J = 10.5 Hz, 1H, -COCH=C<u>H</u>$_2$), 6.37 (d, J = 16.1 Hz, 1H, -COCH=C<u>H</u>$_2$), 6.70 (t, J = 10.6 Hz, 1H, -COC<u>H</u>=CH_2), 7.03 (d, J = 16.1 Hz, 1H, PhCH=C<u>H</u>CO-), 7.38-7.44 (m, 5H, ArH), 7.68 (d, J = 16 Hz, 1H, PhC<u>H</u>=CHCO-); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 123.9, 128.2, 128.5, 128.8, 130.5, 134.5, 135.3, 143.8, 189.3; MS obsd. for $C_{11}H_{10}O$ (M^+): 158.

1,4*E*,6*E*-Undecatrien-3-one: (2.28) prepared *via* DDQ oxidation. The residue was purified by chromatography (5:1 hex-EtOAc) to give **2.28** (180.2 mg, 72%) as a yellow oil: R_f = 0.65 (5:1 hex-EtOAc); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.89 (t, *J* = 7.0 Hz, 3H, $C\underline{H}_3CH_2$-), 1.26-1.36 (m, 4H, $CH_3C\underline{H}_2CH_2C\underline{H}_2CH_2CH$=), 1.44 (p, *J* = 7.5 Hz, 2H, $CH_3CH_2C\underline{H}_2CH_2CH_2CH$=), 2.15-2.23 (m, 2H, $CH_3CH_2CH_2CH_2C\underline{H}_2CH$=), 5.79 (dd, *J* = 10.6 Hz, 0.6 Hz, 2H, -COCH=C$\underline{H}_2$), 6.20-6.25 (m, 1H, -CH_2-C$\underline{H}$=CH-), 6.31-6.38 (m, 1H, -CH_2-CH=C$\underline{H}$-CH=CH-CO-), 6.60 (dd, *J* = 17.4 Hz, 10.5 Hz, 2H, =C$\underline{H}$COC$\underline{H}$=), 7.22-7.32 (m, 1H, =CH-C$\underline{H}$=CHCO-); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.0, 22.5, 28.3, 31.4, 33.1, 125.6, 127.7, 128.8, 135.2, 144.4, 146.4, 189.6.

1-Cyclopropyl decan-1-one (2.29) prepared *via* Swern oxidation. The residue was purified by chromatography (10:1 hex-EtOAc) to give **2.29** (129.7 mg, 53%) as a yellow oil: R_f = 0.77 (5:1 hex-EtOAc); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.81-0.99 (m, 4H, cycloH), 1.00 (t, *J* = 7.4 Hz, 3H, $C\underline{H}_3$-), 1.25-1.29 (m, 12H, -CH_2-), 1.55-1.63 (m, 1H, cycloH), 1.84-1.97 (m, 2H, -CH_2CH_2CO-), 2.53 (t, *J* = 7.3 Hz, 2H, -C$\underline{H}_2$-CO-); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 6.6, 10.5, 19.3, 20.3, 22.7, 29.2, 29.3, 29.4, 29.5, 39.0, 43.5, 77.4; MS obsd. for $C_{13}H_{24}O$ (M^+): 196.

1-Cyclopropyl-2*E*-decen-1-one: (2.30) prepared *via* Swern oxidation. The residue was purified by chromatography (10:1 hex-EtOAc) to give **2.30** (75 mg, 30 %) as a yellow oil: R_f = 0.67 (5:1 hex-EtOAc); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.83-0.93 (m, 4H, cycloH), 1.08 (t, *J* = 4.2 Hz, 3H, $C\underline{H}_3$-), 1.27-1.31 (m, 10H, -C$\underline{H}_2$-), 1.43-1.51 (m, 1H, cycloH), 2.01-2.32 (m, 2H, -C$\underline{H}_2$-CH=CH-), 6.22 (d, *J*=15.0 Hz, 1H, -CH=C$\underline{H}$CO-), 6.91

(dt, J = 15.8, 6.9 Hz, 1H, -C<u>H</u>=CHCO-); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 11.0, 14.1, 18.6, 22.6, 28.2, 29.1, 29.2, 31.7, 32.5, 130.3, 146.9, 200.1.

1-Cyclopropyl-4*Z*-decen-1-one: (2.31) prepared *via* Swern oxidation. The residue was purified by chromatography (5:1 hex-EtOAc) to give **2.31** (183.1 mg, 73%) as a yellow oil: R_f = 0.74 (5:1 hex-EtOAc); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.81-0.91 (m, 2H, cycloH), 1.01 (p, J = 4.2 Hz, 2H, cycloH), 1.23-1.39 (m, 5H. C<u>H</u>$_3$C<u>H</u>$_2$-) 1.88-1.97 (m, 1H, cycloH), 2.02 (q, J = 6.6 Hz, 2H, -C<u>H</u>$_2$CH=CH-), 2.33 (q, J = 6.9 Hz, 2H, -C<u>H</u>$_2$CH$_2$CO-), 2.60 (t, J=7.1 Hz, 2H, -C<u>H</u>$_2$CO-), 5.27-5.44 (m, 2H, -C<u>H</u>=C<u>H</u>-); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 10.4, 13.9, 20.2, 21.7, 22.5, 27.0, 29.2, 31.4, 43.2, 127.6, 130.7, 209.8; MS obsd. for $C_{13}H_{22}O$ (M^+): 194.

1-Cyclopropyl-3-phenyl-2*E*-propen-1-one: (2.32) prepared *via* DDQ oxidation. The residue was purified by chromatography (5:1 hex-EtOAc) to give **2.32** (202.4 mg, 81%) as a yellow oil: R_f = 0.57 (5:1 hex-EtOAc); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.91-1.00 (m, 2H, cycloH), 1.12-1.17 (m, 2H, cycloH), 2.18-2.27 (m, 1H, cycloH), 6.85 (dd, J = 16.1 Hz, 2.6 Hz, 1H, -CH=C<u>H</u>-CO-), 7.32-7.36 (m, 3H, ArH), 7.51-7.54 (m, 2H, ArH), 7.59 (d, J = 16.2 Hz, -C<u>H</u>=CH-CO-); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 11.2, 19.4, 126.1, 127.9, 128.6, 130.0, 134.3, 141.6, 199.5; MS obsd. for $C_{12}H_{12}O$ (M^+): 172.

1-Cyclopropyl-2*E*,4*E*-decadien-1-one: (2.33) prepared *via* DDQ oxidation. The residue was purified by chromatography (5:1 hex-EtOAc) to give **2.33** (112.5 mg, 45%) as a yellow oil: R_f = 0.37 (5:1 hex-EtOAc); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.87-0.93 (m, 4H,

cycloH), 1.08 (t, J=4.4 Hz, 3H, C$\underline{H}_3$-), 1.23-1.39 (m, 6H, -C$\underline{H}_2$-), 1.41-1.49 (m, 1H, cycloH), 2.07-2.22 (m, 2H, -C$\underline{H}_2$-CH=CH-), 4.11 (q, J = 7.1 Hz, 1H, -C$\underline{H}$=CH-CH=CHCO-), 6.18-6.26 (m, 2H, -CH=C$\underline{H}$-CH=C$\underline{H}$CO-), 7.16-7.28 (m, 1H, -C$\underline{H}$=CH-C$\underline{H}$=CHCO-); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 10.9, 14.0, 19.2, 22.5, 28.4, 31.4, 33.1, 127.8, 128.7, 142.4, 145.5, 199.6.

Simultaneous synthesis of 1-cyclopropyl ketones (2.64, 2.65, 2.66, and 2.67)

Formation of 1-cyclopropyl alcohols: A solution of the cyclopropyl magnesium bromide (0.5 M in THF, 9.6 mL, 4.8 equiv.) was added dropwise to a solution of hexanal (100 mg, 122 μL, 1.0 mmol, 1.0 equiv.), heptanal (114 mg, 140 μL, 1.0 mmol, 1.0 equiv.), octanal (128 mg, 156 μL, 1.0 mmol, 1.0 equiv.) and nonanal (142 mg, 172 μL, 1.0 mmol, 1.0 equiv.) in THF (20 mL) at 0 °C. After stirring for 1 h at 0 °C, water (20 mL) was added and the mixture was extracted with Et_2O (3 x 20 mL). The combined extracts were washed with brine (10 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The crude oil was purified by chromatography (hex-EtOAc, 5:1) to give a colourless oil (four alcohols: 462 mg, 71 %) which was used directly in the next step.

Swern oxidation: To a cooled (-73 °C), stirred solution of oxalyl chloride (533 mg, 360 mL, 4.2 mmol, 6.0 equiv.) in CH_2Cl_2 (15 mL) was added DMSO (437 mg, 397 mL, 5.6 mmol, 8 equiv.) dropwise. The mixture was stirred at –73 °C for 30 min, and a solution of the four alcohols obtained above (462 mg, ~0.7 mmol each of the four alcohols) in CH_2Cl_2 (5 mL) was added dropwise over 5 min. After 30 min, Et_3N (5 equiv.) was added, and the mixture was then allowed to warm to room temperature. Water (10 mL) was added, and the layers were separated. The aqueous layer was extracted with $CHCl_3$ (3 x

30 mL). The combined organic layers were washed with water (3 x 20 mL) and brine (20 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The resulting oil was redissolved in Et_2O (40 mL) and washed with water (3 x 20 mL) and brine (20 mL). After drying ($MgSO_4$), the ethereal solution was concentrated *in vacuo* to provide the ketones. The residue was purified by flash chromatography eluting with 5:1 Hex/EtOAc to give a mixture of four ketones (**2.64**, **2.65**, **2.66**, and **2.67**) as a yellow oil (281 mg; 62 %); MS obsd. for $C_9H_{19}O$ (M^+): 142; MS obsd. for $C_{10}H_{20}O$ (M^+): 156; MS obsd. for $C_{11}H_{22}O$ (M^+): 170; MS obsd. for $C_{12}H_{24}O$ (M^+): 184.

2.7.3 Experimental procedure for screening with the FOX 4000

The ketone samples were prepared in lower and higher concentrations (Table 2.8) to get a Fox response to compare with four cheeses (*e.g.*, cheddar, gouda, parmesan and camembert cheeses). The amount of each ketone was placed in a suitable beaker (*e.g.*, 2 L and 5 L) along with three FOX vials that could capture the odour. The sealed beaker with the ketone and the vials was heated at 60 °C for 15 min. The vials with vacuum-sealed lids were ready for the FOX analysis.

The cheese samples were prepared by coring and wire-cutting disks to give the same size (1 cm length x 0.5 cm diameter) of each cheese. The cheese sample was placed in a FOX vial with a sealed lid on. The blank was prepared by adding one drop of water with air present in a FOX vial. Five blanks and three duplicates of each ketone were placed in two trays at room temperature, and three duplicates of each cheese were placed in a cooled sample tray (2-4 °C). Then 2.5 mL of headspace was injected into the FOX for analysis.

Table 2.8: The amount of ketone added in a beaker for the sample preparation.

Compound*	High		Low	
	amount (μL)	vol. of beaker (L)	amount (μL)	vol. of beaker (L)
1.28	1	2	1	5
2.19	1.5	2	0.5	2
2.24	3	2	1	2
2.29	10	2	5	2
2.15	0.5	2	0.7	5
2.20	2	2	0.7	2
2.16	1	5	0.4	5
2.21	0.8	2	0.3	2
2.26	0.8	2	0.3	2
2.31	0.8	2	0.3	2
2.17	1.5	2	0.5	2
2.22	33.6 mg	2	14.8 mg	2
2.27	0.8 mg	2	12.0 mg	2
2.32	48.4 mg	2	16.3 mg	2
2.18	1	2	1	5
2.23	5	2	2	2

*The structure of each compound is given in Table 2.2.

The FOX

The FOX 4000 (AlphaMOS, Toulouse, France) electronic nose system equipped with the standard set of 18 metal-oxide sensors, a CTC HS 1000 head space autosampler, and an

ACU 5000 air conditioning unit providing humidified air (20% relative humidity) at 36 °C. The carrier gas was ultra-grade compressed air (79% nitrogen and 21% oxygen). The headspace (2.5 mL) was taken from the vial (10 mL) and was injected into a stream of synthetic air passing at 150 mL/min over metal oxide sensors at 60°C. Acquisition time for each sample vial was 3 min.

2.6.4 Experimental procedure of screening with GC-MS and GC-O

The sample (1 μL) was dissolved in Et_2O (1 mL) and was analysed by GC-MS and GC-O.

GC-MS

GC-MS analyses were carried out using a Shimadzu 6C-17A gas chromatograph coupled to a Shimadzu GCMS-QP5000 mass spectrometer. Samples (1 μL) were injected by split / splitless injection at 250 °C (splitless time: 30 sec). The mass detector was maintained at 250 °C. For separation, an Alltech Econo-Cap™ EC-1000™ (30 m x 0.25 mm i.d. with 0.25 μm film) was employed. Helium was used as the carrier gas at a constant flow-rate of 1.8 mL/min. The column temperature was programmed from 35 °C to 230 °C at a rate of 5 °C/min and then held for 21 min at 230 °C. The MS was operated in electron impact mode with an ionization potential of 70 eV and a scanning rate at 0.5 sec/scan over a mass range of *m/z* 29-350.

GC-O

GC-O analysis was carried out using a Shimadzu GC9A series (Shimadzu corporation, Kyoto, Japan). The chromatograph was equipped with an Alltech Econo-Cap™ EC-1000™ (30 m x 0.25 mm with 0.25 μm film). The oven temperature was programmed

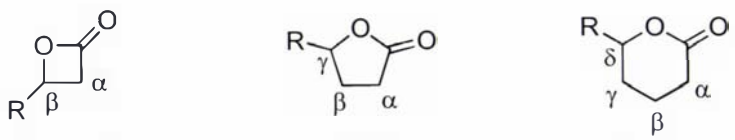
from 35 °C to 230 °C at a rate of 5 °C/min and then held for 21 min. Samples (2 μL) were injected by split / splitless injection at 250 °C. Helium was used as the carrier gas at a flow rate of 1 mL/min. The end of the column was equipped with an effluent splitter connected to two identical lengths of fused silica detectivated capillary tubing, one going to the flame ionisation detector (FID, 250 °C) and the other to the custom built smelling port *via* a heated tube. A stream of humidified air (75 mL/min) was used to sweep the effluent through a glass cone to the sniffer. Time and odour descriptions were recorded when an odour was detected.

Chapter 3

Chapter 3: Synthesis of a library of racemic lactones as potential flavour compounds

3.1 Introduction

A lactone is a cyclic ester (Figure 3.1). It is the intramolecular condensation product of an alcohol and a carboxylic acid. Lactone nomenclature contains a prefix that indicates the ring size: β-lactone (4-membered), γ-lactone (5-membered), δ-lactone (6-membered ring).



β-lactone γ-lactone δ-lactone

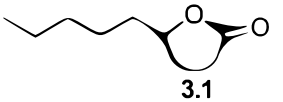
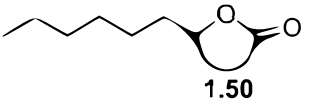
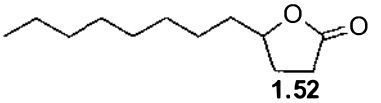
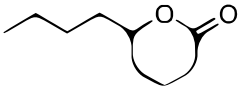
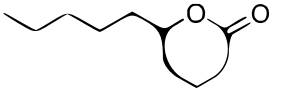
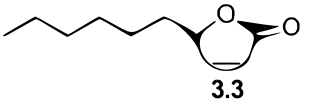
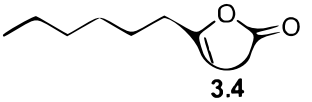
Figure 3.1: General structures of lactones.

Lactones are important flavour components in many food products including fruits, vegetables, breads, meats, beverages and dairy products. Lactones are widely used by the flavour industry because of their characteristic organoleptic properties, including fruity, coconut-like, buttery, sweet, or nut-like.[73] In particular, C_8 to C_{14} γ- and δ-lactones have been identified in low concentrations in various types of cheese, and identified as the principal contributors to flavour and aroma because of their low thresholds.[74]

The flavour of lactones is influenced by the ring size (4, 5 or 6), the length of the lateral carbon chain and the presence of unsaturation (Table 3.1). The nature and intensity of

odour has been reported to vary with the configuration at Cγ and Cδ of the γ- and δ-lactones respectively.[75]

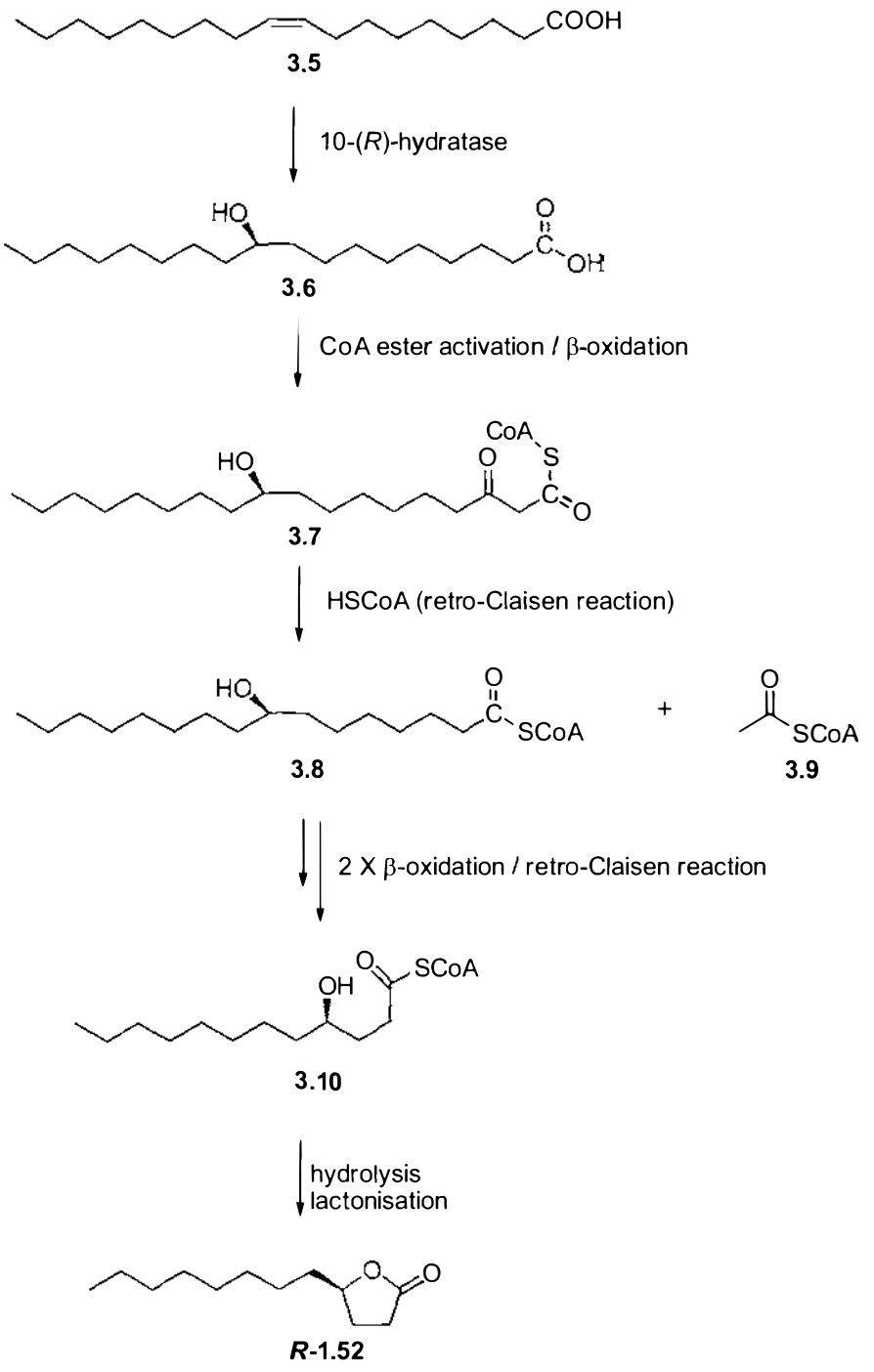
Table 3.1: Structure, name and odourous properties of lactones.[76]

Structure	Name	Odorant notes
 3.1	dihydro-5-pentyl-2(3*H*)-furanone	coconut, fatty fruity, aniseed
 1.50	dihydro-5-hexyl-2(3*H*)-furanone	peach, fatty, fruity
 1.52	dihydro-5-octyl-2(3*H*)-furanone	peach, butter, fatty
 3.2	tetrahydro-6-butyl-(2*H*)-pyran-2-one	fatty, oily, sweet, nutty
 1.51	tetrahydro-6-pentyl-(2*H*)-pyran-2-one	peach, oily, creamy
 1.53	tetrahydro-6-heptyl-(2*H*)-pyran-2-one	peach, buttery, coconut
 3.3	5-hexyl-2(5*H*)-furanone	mushroom
 3.4	5-hexyl-2(3*H*)-furanone	fruity, oily, fatty

3.2 Generation of γ-lactones in nature

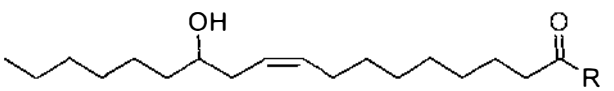
Lactones in cheeses arise biosynthetically from the hydrolysis of lipids to hydroxy acids that undergo β-oxidation cycles, followed by cyclisation to form lactones, during heat treatment of the milk. Another possible mechanism of formation of lactones in cheese supposes a lactonisation of hydroxy fatty acids present in milkfat as glyceride esters. Lactones are also formed by the enzymatic reduction of milk oxy fatty acids known to exist in milk fat to produce the hydroxy acids which then lactonise.[77]

Spores from *Penicillium roqueforti*, isolated from Roquefort cheese, have been used to synthesise dihydro-5-octyl-2(3*H*)-furanone (**1.52**, Scheme 3.1). The precursor fatty acids, released by the action of *Candida cylindracea* lipase on soybean oil, are predominantly the C_{18} fatty acids (*e.g.*, oleic acid and linoleic acid). Oleic acid (**3.5**) was regioselectively hydroxylated at C_{10}. It is possible to obtain either 10-(*R*)-hydroxystearic acid (**3.6**), or a mixture of the two enantiomers, depending on the organism employed. The acid is then activated as its coenzyme A ester (**3.7**) and its C_{18} chain degraded by three β-oxidation cycles to form the resulting 4-hydroxy-dodecanoyl-CoA (**3.10**). (5*R*)-Dihydro-5-octyl-2(3*H*)-furanone (***R*-1.52**) was formed, following hydrolysis and lactonisation (Scheme 3.1).[78]



Scheme 3.1: Possible pathway for dihydro-5-octyl-2(3*H*)-furanone formation by *Penicillium roqueforti.*

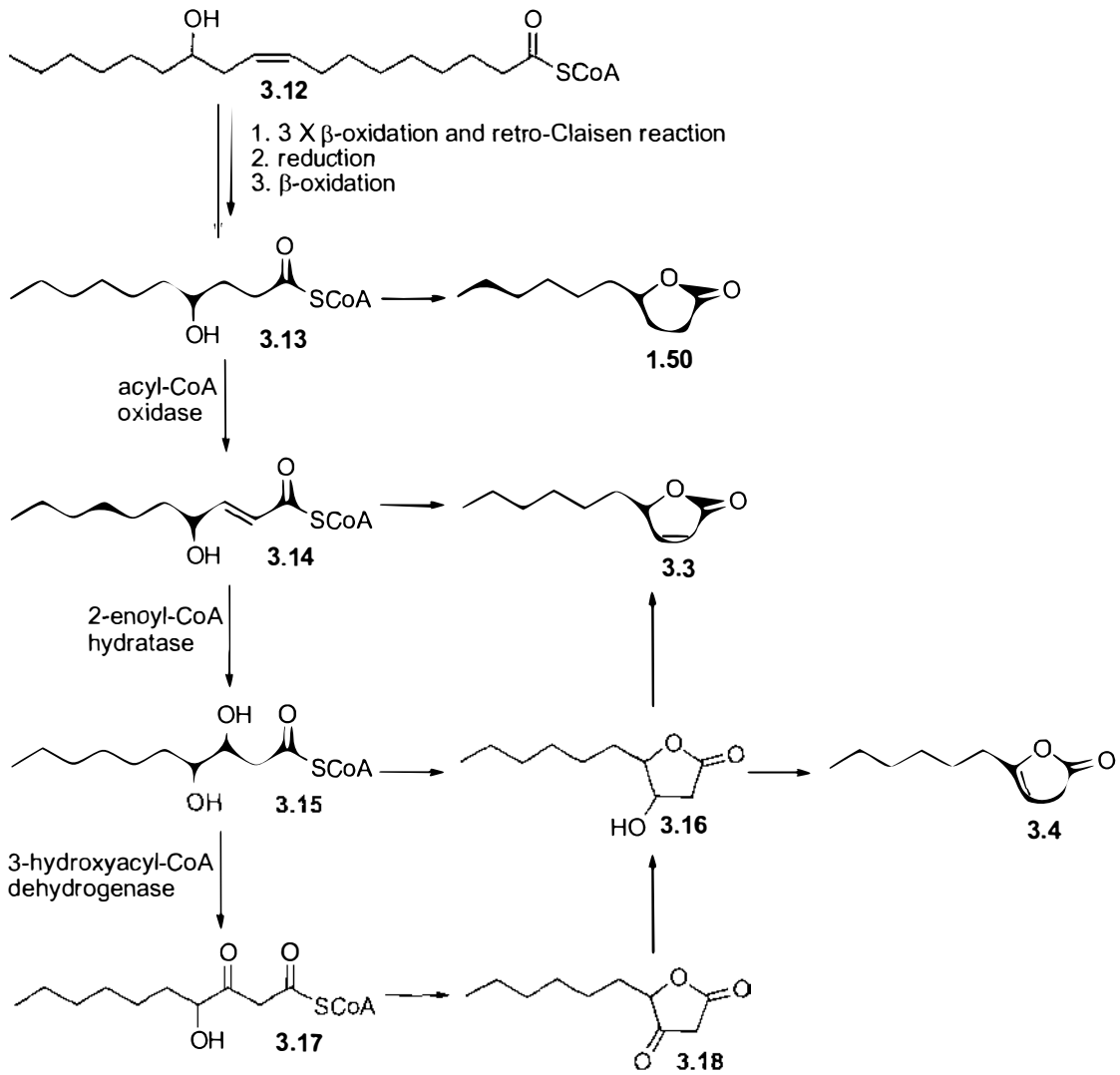
It is possible to produce lactones through biotechnology. Some fungi[79] and yeast species[80] were identified for their ability to produce small amounts of lactones as aroma compounds. *Yarrowia lipolytica* has been employed to produce γ-lactones. The series of biotransformation begins with a long-chain hydroxy fatty acid precursor like ricinoleic acid (**3.11**), a major component of castor oil, which is activated as its coenzyme A ester **3.12** (Figure 3.2).



3.11: R = OH
3.12: R = SCoA

Figure 3.2: Long-chain hydroxy fatty acid precursors.

The accepted pathway from ricinolenyl-CoA (**3.12**) to dihydro-5-hexyl-2(3*H*)-furanone (**1.50**) involves three β-oxidation cycles, followed by reduction. The last β-oxidation produces 4-hydroxy-decanoyl-CoA (**3.13**), which then cyclises to give dihydro-5-hexyl-2(3*H*)-furanone (**1.50**) (Scheme 3.2).[76] Scheme 3.2 illustrates how *Yarrowia lipolytica* produces dihydro-5-hexyl-2(3*H*)-furanone (**1.50**), 5-hexyl-2(5*H*)-furanone (**3.3**), 5-hexyl-2(3*H*)-furanone (**3.4**), dihydro-3-hydroxy-5-hexyl-(3*H*)-furanone (**3.16**) and dihydro-3-keto-5-hexyl-(3*H*)-furanone (**3.18**) under various conditions.



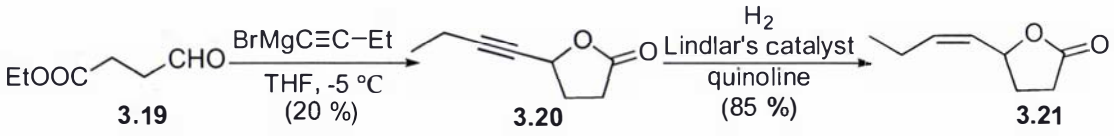
Scheme 3.2: Lactones from ricinolenyl-CoA (**3.12**).

The synthesis of racemic lactones is discussed in this chapter, and the study of stereoisomerically pure lactones is the subject of subsequent chapters of this thesis.

3.3 Previous racemic syntheses of γ-lactones

There are a large number of syntheses of γ-lactones in the literature. The following is a selection of relatively short racemic syntheses that might be applied in a combinatorial approach.

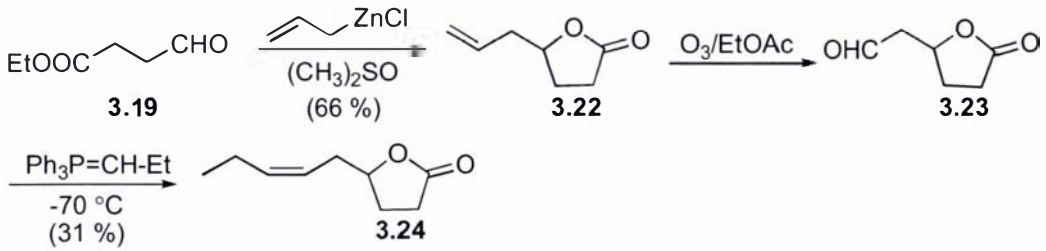
In 1982, Maurer and Hauser[81] reacted acetylenic Grignard reagents with ethyl 4-oxobutanoate (**3.19**). The acetylenic γ-lactone **3.20** was hydrogenated over Lindlar's catalyst to give the corresponding lactone (**3.21**) (Scheme 3.3).



3.19 **3.20** **3.21**

Scheme 3.3: Synthesis of (*Z*)-5-octen-4-olide (**3.21**).

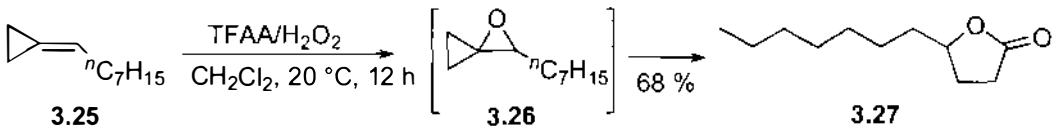
Another approach by Maurer and Hauser[81] involved addition of an allyl zinc species to ethyl 4-oxobutanoate (**3.19**). A Wittig reaction was used to form the lactone (**3.24**) with a *Z*-double bond in its side chain (Scheme 3.4).



3.19 **3.22** **3.23** **3.24**

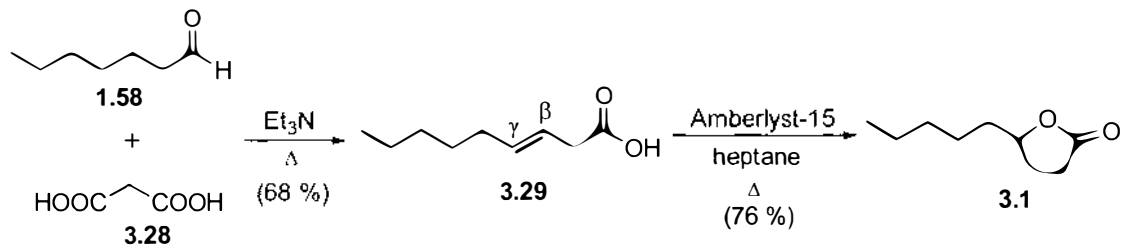
Scheme 3.4: Synthesis of (*Z*)-6-nonen-4-olide (**3.24**).

In 1998, Krief[82] reported the synthesis of γ-lactones in a one-pot transformation from an alkylidenecyclopropane **3.25**. Trifluoroperacetic acid, generated *in situ* from trifluoroacetic anhydride and hydrogen peroxide, was the reagent of choice for the reaction leading to the intermediate epoxide **3.26**, which subsequently underwent expansion to form dihydro-5-heptyl-2(3*H*)-furanone (**3.27**) (Scheme 3.5).



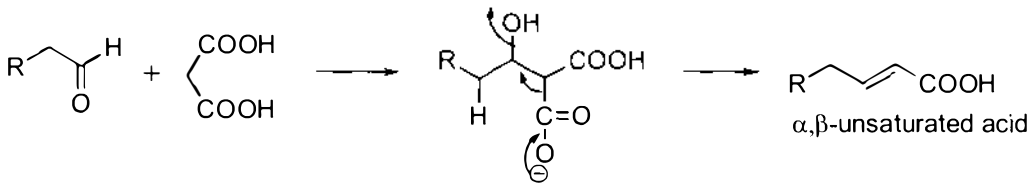
Scheme 3.5: Synthesis of dihydro-5-heptyl-2(3*H*)-furanone (**3.27**).

In 1990, Bunce and Reeves modified a long-standing undergraduate laboratory experiment[83] to make dihydro-5-pentyl-2(3*H*)-furanone (**3.1**). The synthesis begins with the Linstead modification of the Knoevenagel condensation between malonic acid (**3.28**) and heptanal (**1.58**), in the presence of triethylamine. The unsaturated acid **3.29** is the major product with >95 % selectivity for the β,γ-double bond isomer. The unsaturated acid **3.29** is then lactonised using an acidic resin in heptane at reflux to form dihydro-5-pentyl-2(3*H*)-furanone (**3.1**) (Scheme 3.6).[84]



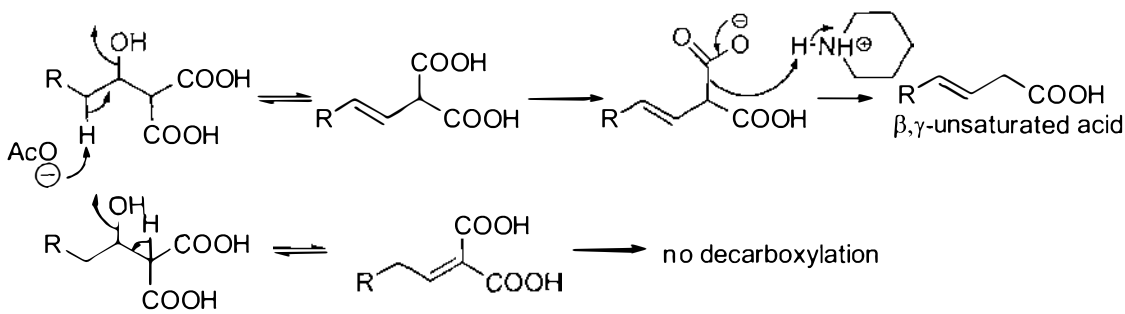
Scheme 3.6: Undergraduate laboratory experiment of making dihydro-5-pentyl-(3*H*)-furanone (**3.1**).

Ragoussis *et al.* have investigated the Linstead modification of the Knoevenagel condensation in detail and have optimized reaction conditions to provide >99.9 % of the β,γ double-bond isomer.[85] The ratio of malonic acid, aldehyde and piperidinium acetate was 2:1:0.2. The classical Knoevenagel reaction is illustrated in Scheme 3.7. The decarboxylative elimination of water from the β-hydroxymalonic acid normally leads to the α,β-unsaturated acid.



Scheme 3.7: Mechanism of the classical Knoevenagel condensation.

In the presence of piperidinium acetate (*i.e.*, a dehydration catalyst rather than a base) the intermediate β-hydroxymalonic acid is dehydrated to give the isomeric unsaturated malonic acids (Scheme 3.8). Only the β,γ-unsaturated decarboxylic acid is isolated since there is reversible hydration-dehydration, and the α,β-unsaturated dicarboxylic acid cannot decarboxylate under the reaction conditions (Scheme 3.8).

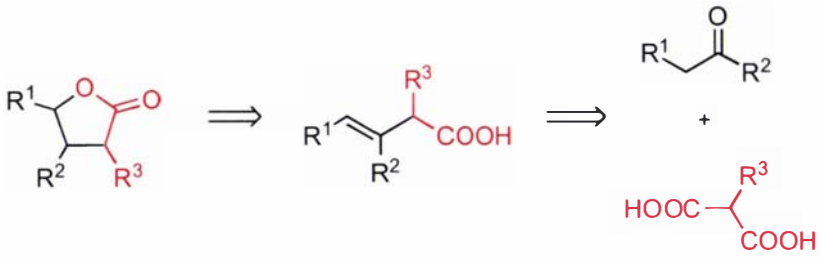


Scheme 3.8: Dehydration and decarboxylation of a β-hydroxy-α-dicarboxylic acid.

3.4 Racemic Lactones

3.4.1 Strategy for the chemical synthesis of a library of γ-lactones

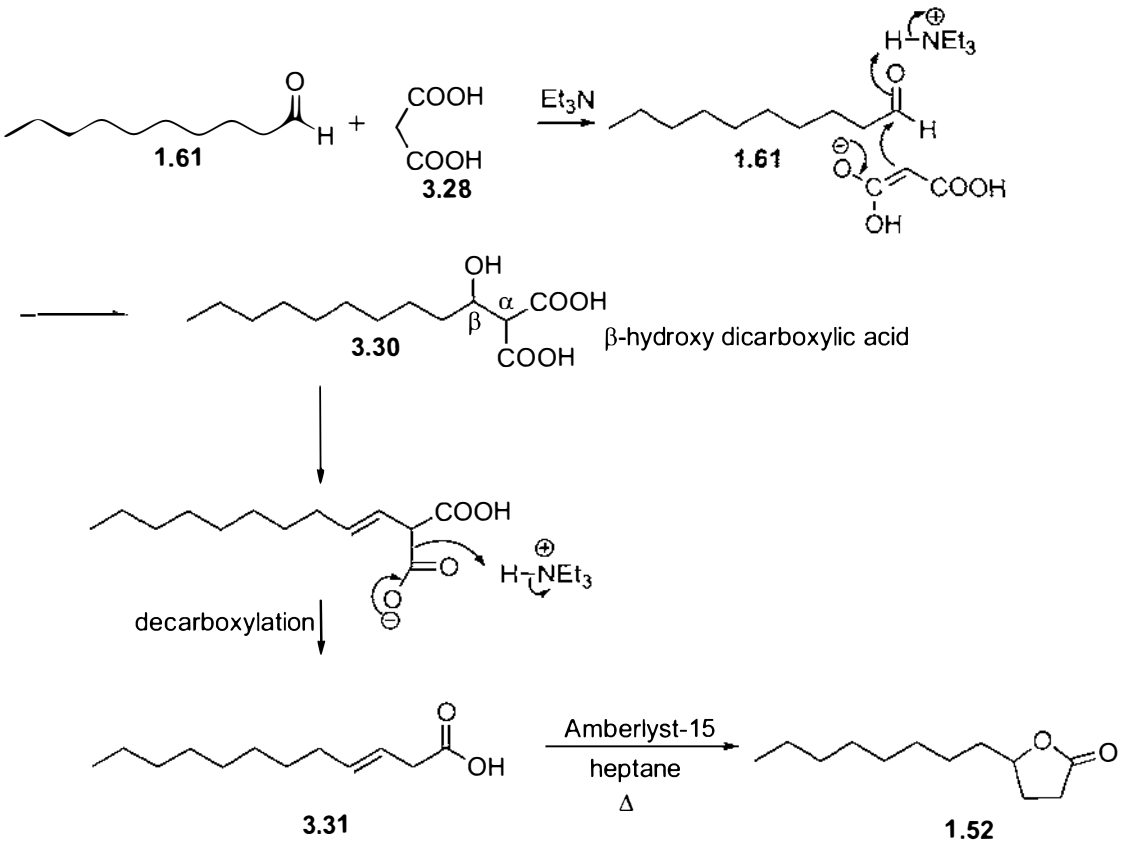
Libraries of racemic lactones can be envisaged to arise *via* the lactonisation of β,γ-unsaturated carboxylic acids (Scheme 3.9). The unsaturated carboxylic acids can in turn be obtained *via* the Linstead modification of the Knoevenagel condensation of alkyl malonic acids with a carbonyl compound (Scheme 3.9).



Scheme 3.9: Retrosynthetic analysis for racemic lactones.

3.4.2 Synthesis of racemic dihydro-5-octyl-2(3*H*)-furanone

The Linstead modification of the Knoevenagel condensation was first applied to the synthesis of dihydro-5-octyl-2(3*H*)-furanone (**1.52**) utilising malonic acid (**3.28**) and decanal (**1.61**) utilising the base as the solvent (*i.e.*, triethylamine, Scheme 3.10).



Scheme 3.10: Mechanism for lactone formation.

A broad singlet was observed at δ 11.30 ppm in the ^{1}H NMR spectrum of 3-dodecenoic acid (**3.31**), which was assigned to the carboxylic acid proton (Figure 3.3a). A multiplet at δ 5.56 ppm corresponding to the two olefinic hydrogens was also observed. Following lactonisation, these signals disappeared and a new signal at δ 4.49 ppm appeared

corresponding to Hγ in the newly formed lactone functionality of compound **1.52** (Figure 3.3b).

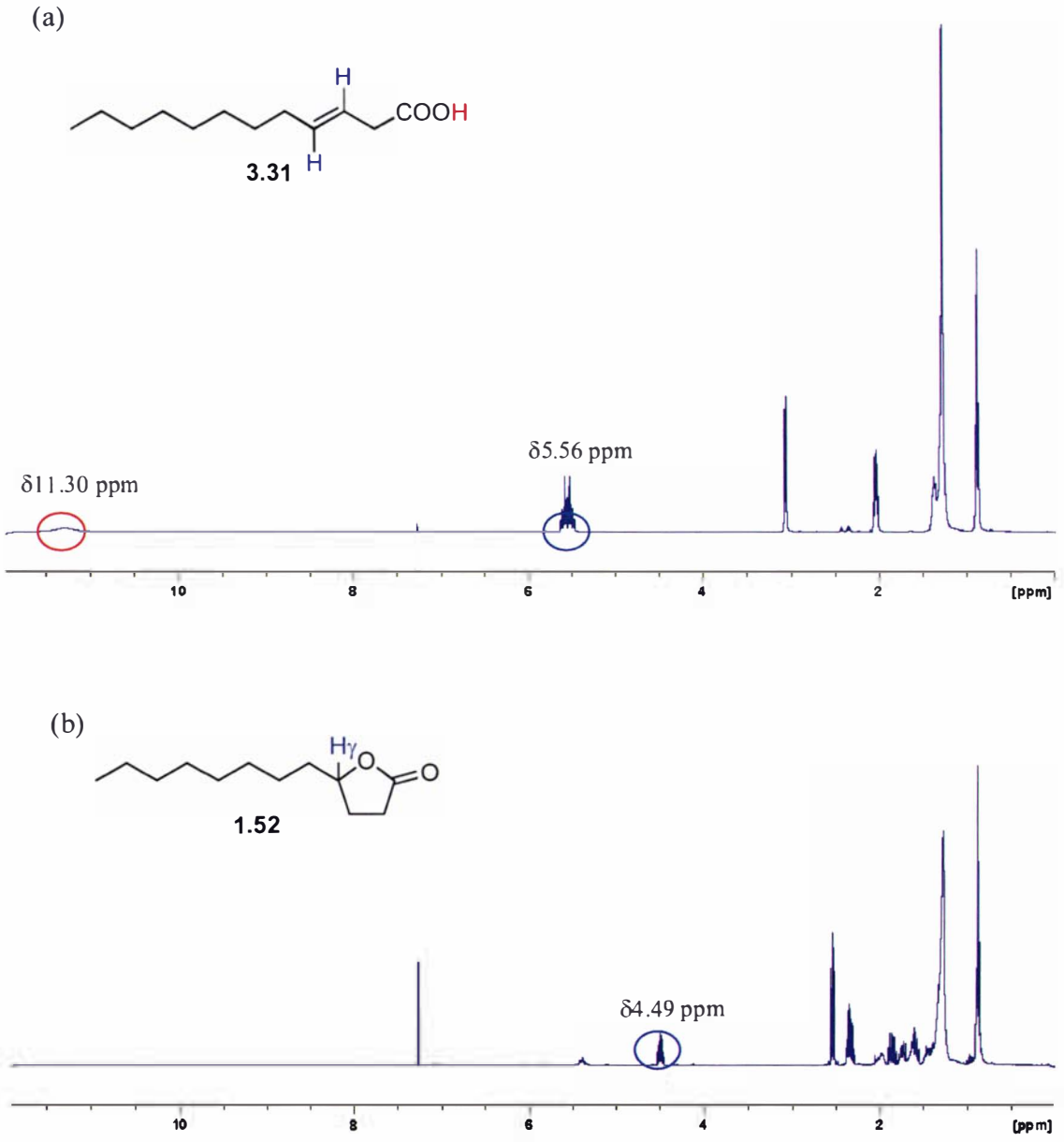


Figure 3.3: ^{1}H NMR spectra (400 MHz, $CDCl_3$) of the crude reaction products: (a) the β,γ-unsaturated carboxylic acid (**3.31**) and (b) the γ-lactone (**1.52**).

The purity and identity of dihydro-5-octyl-2(3*H*)-furanone (**1.52**) were demonstrated by GC-MS. The mass spectrum showed a molecular ion at *m/z* 198. The base peak at *m/z* 85 is a diagnostic feature of the mass spectra of γ-lactones. This results from loss of the side chain as illustrated in Figure 3.4.[86]

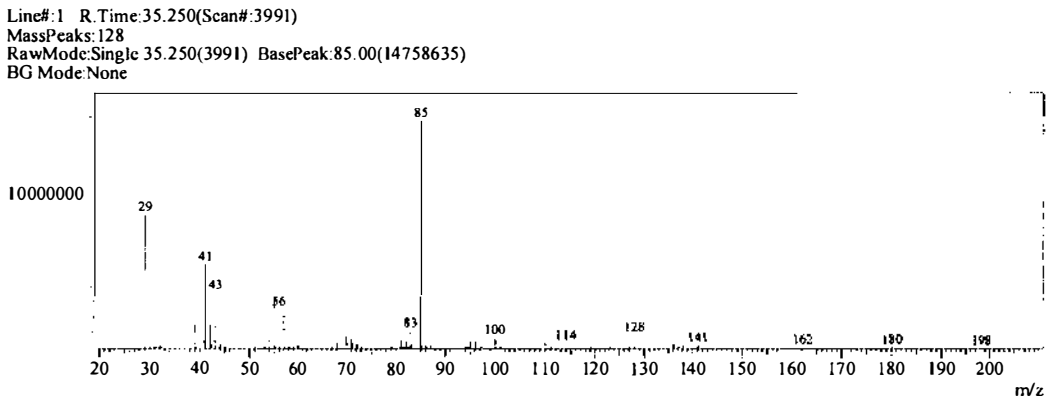
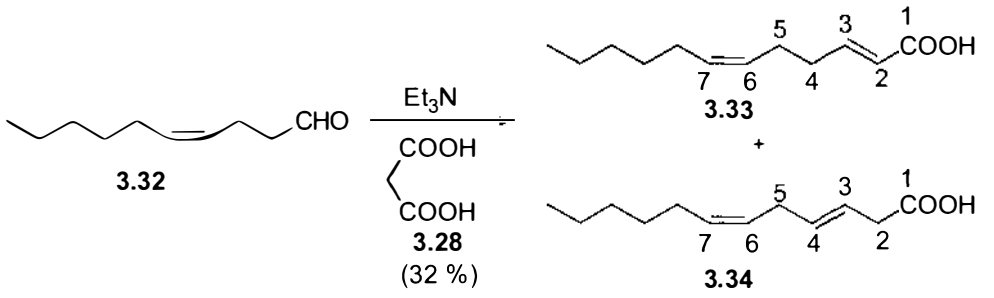


Figure 3.4: Mass spectrum of dihydro-5-octyl-2(3*H*)-furanone (**1.52**).

While the Linstead modification of the Knoevenagel condensation gave >95 % selectivity for the β,γ-double bond isomer when saturated aldehydes were employed, a problem arose when there was unsaturation in the aldehyde. *cis*-4-Decenal (**3.32**) and malonic acid (**3.28**) were heated at reflux in triethylamine (these reaction conditions will be referred to

as Method A) to give the α,β- (**3.33**) and β,γ-double bond (**3.34**) isomers as a mixture (Scheme 3.11).



Scheme 3.11: Reaction applied to an unsaturated aldehyde **3.32** (Method A).

In the ^{1}H NMR of the product mixture (Figure 3.5a), there is a multiplet at δ 5.34-5.45 ppm corresponding to the two olefinic hydrogens at the 6- and 7- positions, as in the spectrum of *cis*-4-decenal. A multiplet at δ 5.56-5.59 ppm was assigned to H-3 and H-4 of the isomer **3.34**. A doublet at δ 5.84 ppm with $J_{2,3}$ = 15.6 Hz was attributed to H-2 of compound **3.33** and a doublet of triplets at δ 7.09 ppm with $J_{2,3}$ = 15.6 Hz and $J_{3,4}$ = 6.7 Hz represented the other olefinic hydrogen, *i.e.*, H-3, for the isomer **3.33**. The ratio of isomers was calculated by integration of the peaks in the GC trace and by integration of NMR signals (Figure 3.5).

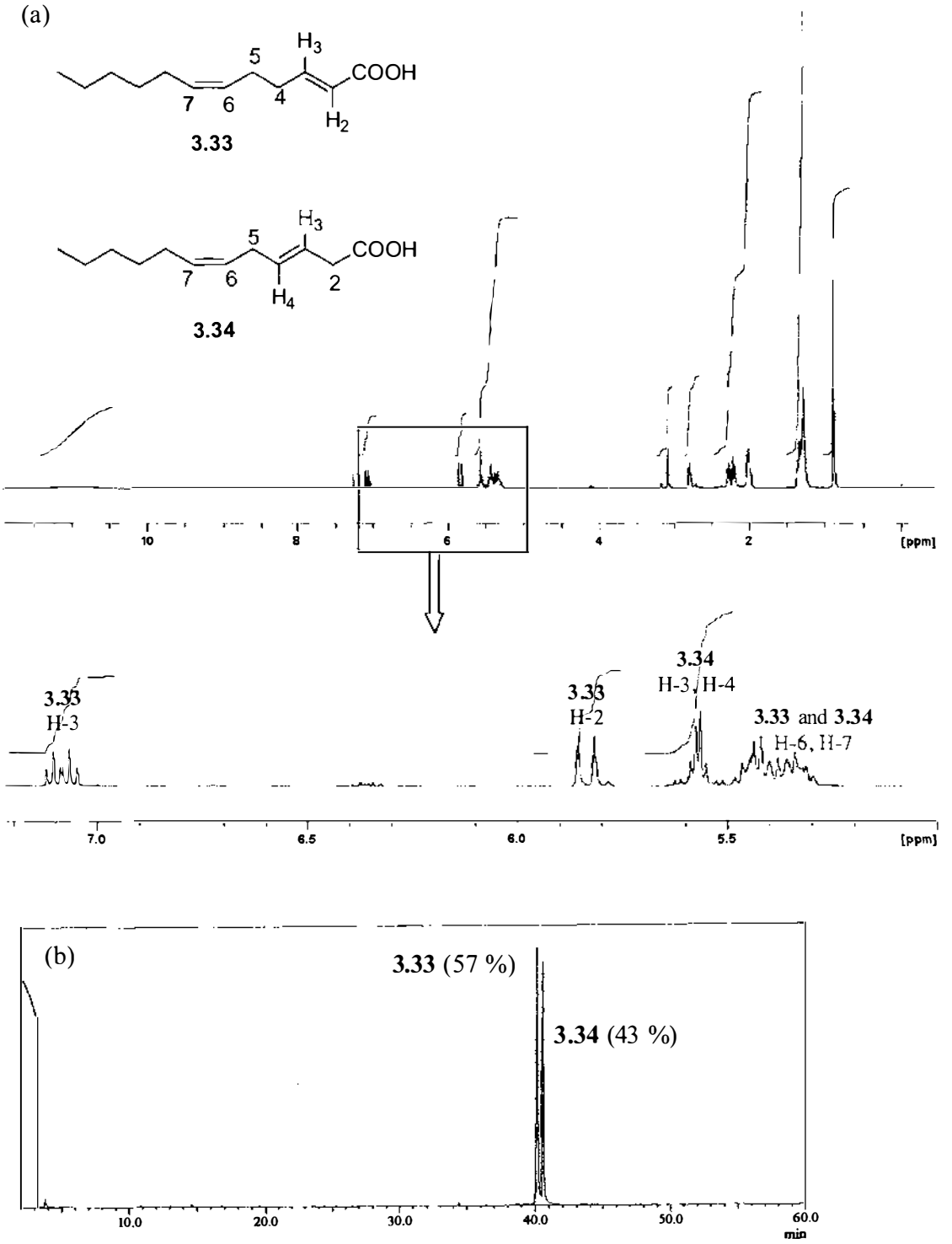
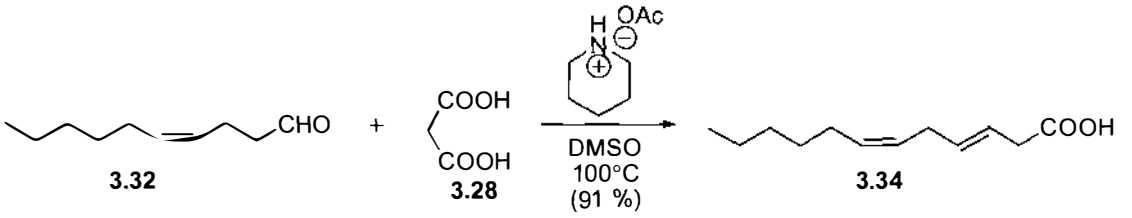


Figure 3.5: (a) NMR data and (b) GC trace of the mixture of **3.33** and **3.34**.

Therefore, *cis*-4-decenal (**3.32**) and malonic acid (**3.28**) were reacted, by what we shall refer to as Method B (Scheme 3.12), as originally described by Ragoussis *et al.*[85] It gave the isomerically pure β,γ-unsaturated carboxylic acid **3.34**.



Scheme 3.12: Reaction applied to an unsaturated aldehyde **3.32** (Method B).

The ^{1}H NMR spectrum of this product (Figure 3.6) included a multiplet at δ 5.32-5.49 ppm corresponding to the olefinic hydrogens on the 6,7-*cis* double bond and a multiplet at δ 5.53-5.59 ppm corresponding to the β,γ-olefinic hydrogens. There was no sign of the α,β-double bond isomer **3.33**. These reaction conditions (Method B) were applied to both saturated and unsaturated aldehydes to compare with the original reaction conditions in Method A. The yields and purity of the products were improved. Table 3.2 summarises the chemical yields and product distribution under the two sets of reaction conditions.

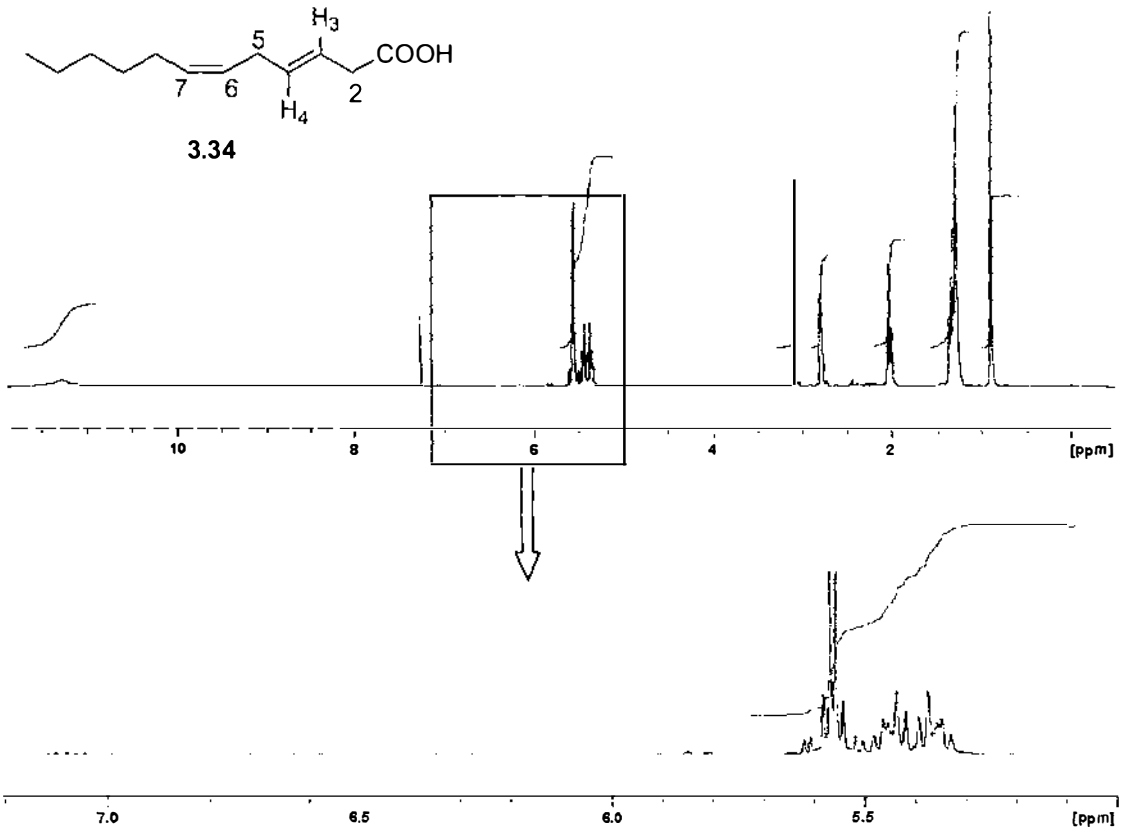
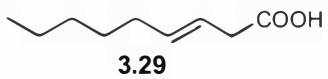
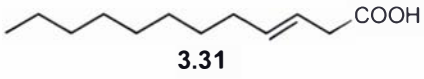
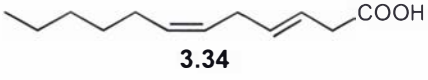
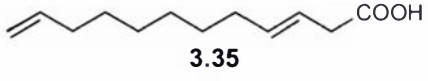
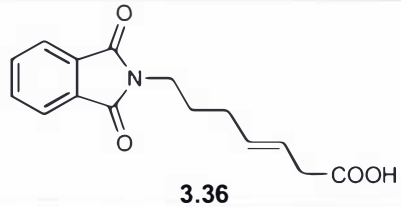


Figure 3.6: ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of the crude reaction mixture containing **3.34** and a trace of **3.33**.

Table 3.2: Forming β,γ-unsaturated carboxylic acids under the two sets of reaction conditions.

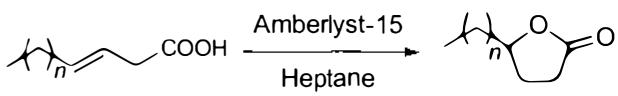
β,γ-unsaturated carboxylic acid	Yield (%)	
	Method A	Method B
 **3.29**	50 %	77 %
 **3.31**	84 %	83 %
 **3.34**	32 %*	91 %
 **3.35**	19 %*	58 %
 **3.36**	Nil	68 %

* As a mixture of α,β- and β,γ-unsaturated carboxylic acids

Method A: malonic acid (1.0 equiv.), aldehyde (1.0 equiv.), Et_3N (solvent), reflux, 1 h; **Method B:** malonic acid (2.0 equiv.), aldehyde (1.0 equiv.), piperidinium acetate (0.2 equiv.), DMSO (solvent), 100 °C, 5 h.

3.4.3 Lactonisation of β,γ-unsaturated acids

There are a number of ways in which β,γ-unsaturated acids might be converted to γ-lactones, including halolactonisation,[87] selenolactonisation[88] and *via* the Sharpless asymmetric dihydroxylation.[89] However, one-step, acid-catalysed lactonisation held obvious appeal (Scheme 3.13). Various acid catalysts have been employed: sulfuric acid,[90] *p*-toluenesulfonic acid,[91] or acidic ion exchange resins (*e.g.*, Amberlyst-15 and Dowex H^+).[92] Amberlyst-15 resin was chosen as the heterogeneous catalyst for the lactonisation of unsaturated carboxylic acids, since it is a porous sulfonated polystyrene resin that serves as an excellent source of strong acid in nonaqueous media. The advantages of using resins are that they require less rigour in handling, react faster, and possess higher loading capacities than the other liquid acidic methods. An additional advantage is that the catalyst can be regenerated and used several times.



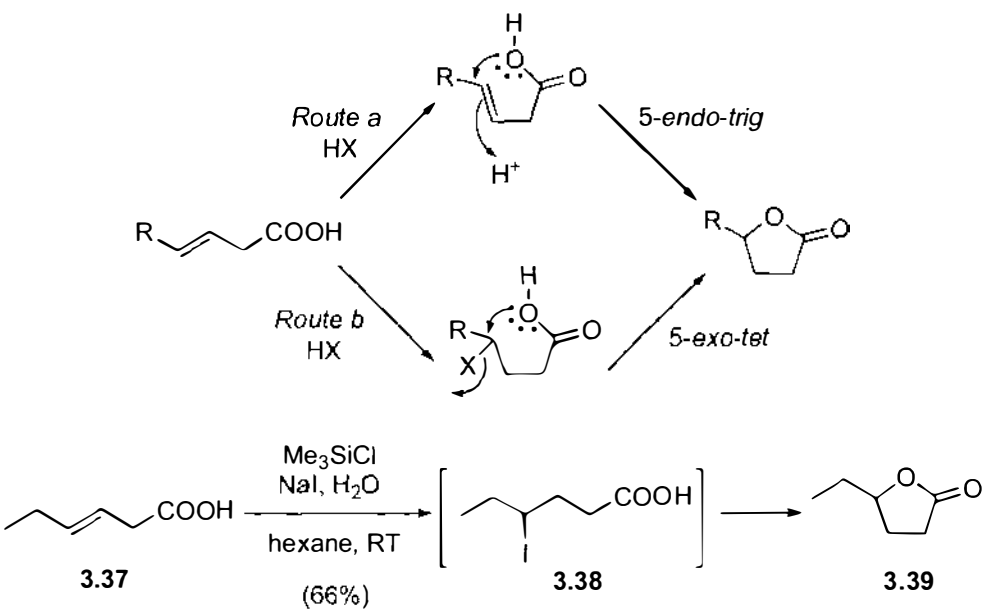
n = 4 (**3.29**)
n = 7 (**3.31**)

n = 4 (**3.1**) ; 84 %
n = 7 (**1.52**); 88 %

Scheme 3.13: Lactonisation of β,γ-unsaturated acids (**3.29** and **3.31**).

Although lactonisation of simple β,γ-unsaturated carboxylic acids was achieved in good yield (Scheme 3.13), we had difficulty forming lactones in substrates with double bonds elsewhere in the carbon skeleton. Unfortunately, only trace amounts of product were obtained when more highly unsaturated acids [*e.g.*, 3(*E*),6(*Z*)-dodeca-3,6-dienoic acid (**3.34**)] were subjected to these conditions. To try to understand this, we needed to

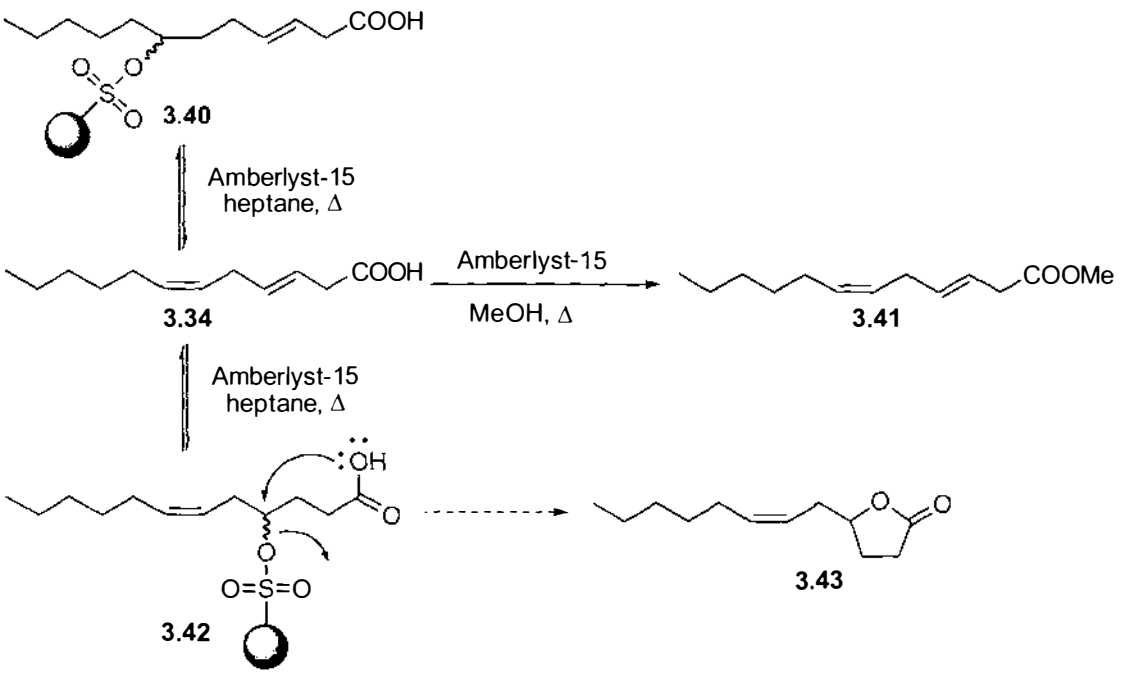
consider the mechanism of the reaction. At first we considered a concerted mechanism for cyclisation in which protonation of the double bond is accompanied by lactonisation (Scheme 3.14, Route a) but this is disfavored according to Baldwin's rules.[93] An alternative, involving regioselective addition of the acid across the double bond, followed by intramolecular substitution seems much more likely. This was also suggested by Sakai *et al.* in 1987 who reported the formation of dihydro-5-ethyl-2(3*H*)-furanone (**3.39**) from hex-3-enoic acid (**3.37**), presumably *via* the addition of hydrogen iodide across the olefin, followed by intramolecular S_N2 displacement of the iodide (Scheme 3.14).[94]



Scheme 3.14: Proposed mechanism of lactonisation.

If the mechanism proposed (Scheme 3.14, Route b) is in operation, then it is possible that either double bond of a diene might interact with the acidic resin (Scheme 3.15). An intermediate γ-sulfate **3.42** might undergo lactonisation *via* intramolecular substitution as outlined above. Clearly this is not a competitive pathway. Attachment of the acid to the

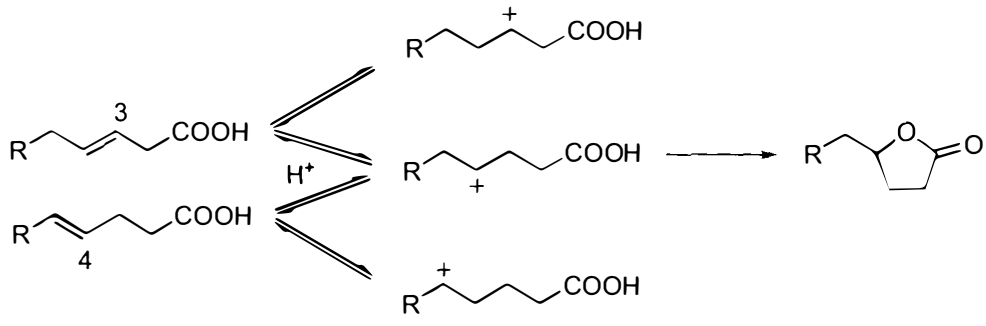
resin *via* a more remote double bond (*e.g.*, formation of **3.40**) cannot lead to γ-lactone **3.43** and indeed formation of a macrolactone would be unlikely for entropic reasons. Interestingly, the formation of a methyl ester (**3.41**) can be performed in the presence of Amberlyst-15 by heating in methanol. Intermolecular Fischer esterification is therefore faster than the intramolecular processes.



Scheme 3.15: Proposed mechanism for the lactonisation of a β,γ-unsaturated carboxylic acid with Amberlyst-15 resin.

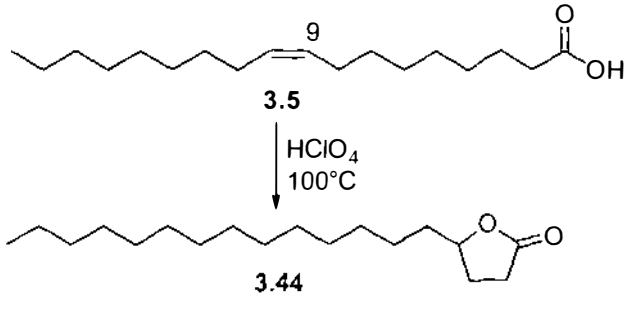
Ansell and Palmer proposed a mechanism for the formation of γ-lactones from unsaturated fatty acids.[95] The lactonisation of short-chain fatty acids was achieved in the presence of large excesses of sulfuric or trifluoroacetic acids at 150 °C. The cyclisation of fatty acids *via* a carbocation derived from a double bond in the 4- or 5- position of the

backbone leads to a γ-lactone. Since only the γ-lactone is produced arising from both 3- and 4- positions of the double bond, carbocation migration is possible (Scheme 3.16).



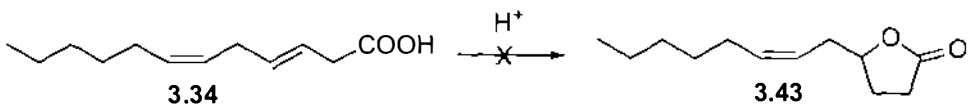
Scheme 3.16: A mechanism for the formation of γ-lactones from unsaturated fatty acids proposed by Ansell and Palmer.[95]

It is possible that the rate-determining step in lactonisation of an olefinic acid is faster when the positive charge is localised at the 4-position that leads directly to the 5-membered ring. This explanation was discussed by Showell and Swern.[96] They developed a method for the perchloric acid-catalysed isomerisation of oleic acid (**3.5**) into γ-stearolactone (**3.44**) (Scheme 3.17). This result provided evidence that the carbocation must migrate along the carbon chain to the 4-position where γ-lactone formation is favoured.



Scheme 3.17: The perchloric acid-catalysed isomerisation of oleic acid (**3.5**) into γ-stearolactone (**3.44**).

The possibility of double bond migration may explain the difficulty in isolating homogeneous products from attempts to lactonise more highly unsaturated acids. The possibility of migration of carbonium ions makes many structures possible. Even though 5-membered ring formation is preferred to give the γ-lactone, the positional integrity of double bonds in the side chain cannot be preserved (Scheme 3.18).

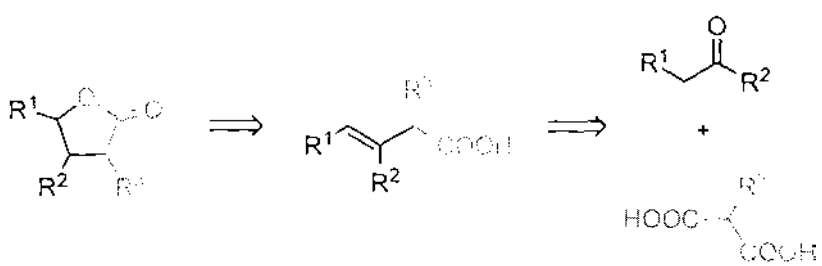


3.34 **3.43**

Scheme 3.18: Unsuccessful pathway for lactonisation of a more highly unsaturated acid **3.34**.

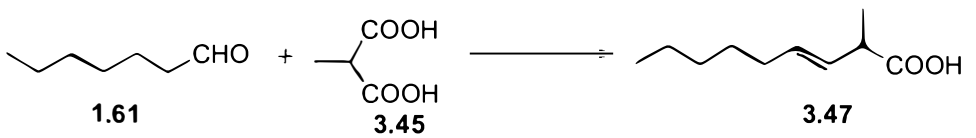
3.4.4 Substituted γ-lactones

If the generation of γ-lactones by condensation and cyclisation were possible, according to the retrosynthetic analysis in Scheme 3.9 (reiterated below from p.75), then we would have an expedient route to variously substituted γ-lactones, albeit as mixtures of stereoisomers. Thus, the use of substituted malonic acids ($R^3 \neq H$) would lead to the incorporation of a substituent at the α-position. Incorporation of a ketone (instead of an aldehyde; $R^2 \neq H$) would introduce a β-substituent. Ketones were unreactive, as observed by Ragoussis,[85] thus making it impossible to introduce a β-substituent *via* this approach.



Scheme 3.9: Retrosynthetic analysis for racemic lactones.

We investigated the scope of the reaction using α-substituted malonic acids, *i.e.*, methyl malonic acid (**3.45**) and ethyl malonic acid (**3.46**) in the presence of triethylamine (Method A). α-Substituents could be incorporated, but the yields were poor and formation of saturated carboxylic acids as by-products resulted. Condensation in the presence of piperidinium acetate (Method B) of α-substituted malonic acids and aldehydes gave no reaction at all. This provided an important clue: for once, Method A gave superior results to Method B. Perhaps basicity, as well as dehydrating capacity, is important in the case of α-substituted malonic acids (Scheme 3.19).



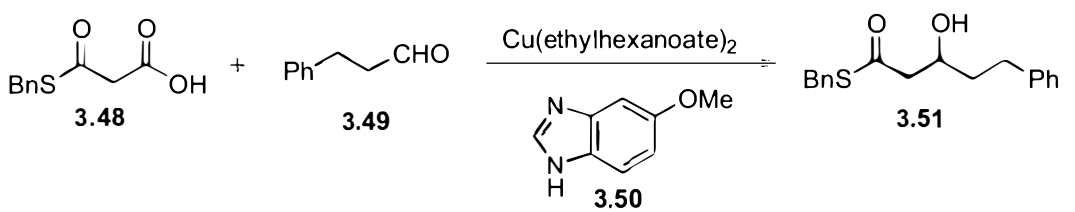
1.61 **3.45** **3.47**

Method A: 11 %

Method B: 0 %

Scheme 3.19: Condensation of α-substituted malonic acid **3.45** and aldehyde **1.61**.

There are examples of condensations between α-substituted malonic acids and aldehydes in the literature. Lalic *et al.* reported the metal-catalysed decarboxylative aldol reaction depicted in Scheme 3.20.[97]



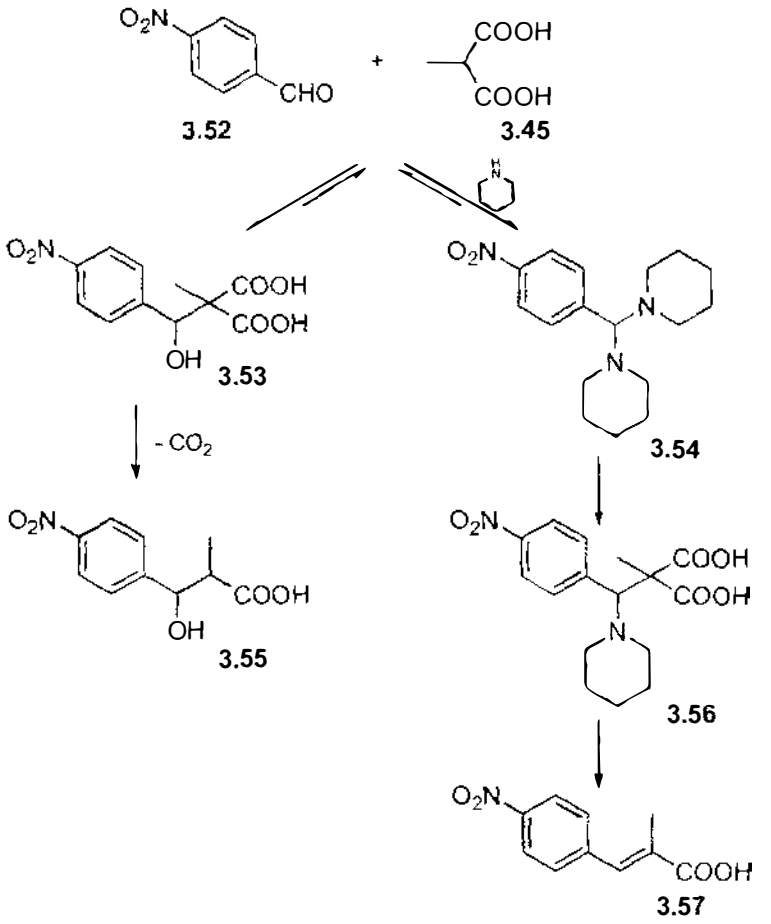
3.48 **3.49** **3.50** **3.51**

THF

(82 %)

Scheme 3.20: The metal-catalysed decarboxylative aldol reaction.

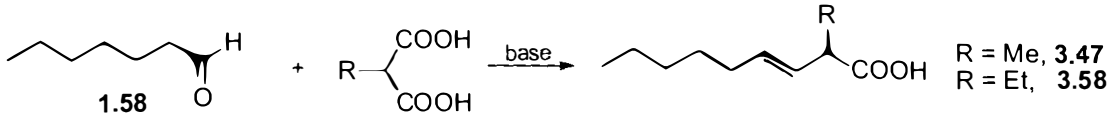
Tanaka *et al.* studied the Knoevenagel reaction of *p*-nitrobenzaldehyde (**3.52**) with methyl malonic acid (**3.45**) (Scheme 3.21). The reaction was monitored by ^{1}H NMR, which demonstrated reversibility between the starting materials and the β-hydroxy intermediate **3.53**. The Knoevenagel reaction in the presence of piperidine proceeded *via* *bis*-piperidide intermediate **3.54**. The *bis*-piperidide intermediate **3.54** is a stronger electrophile and reacts with the malonate anion as shown by NMR experiments. Decarboxylation and elimination ensued to give the unsaturated carboxylic acid **3.57** as the major product.[98]

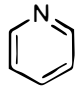
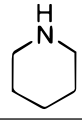
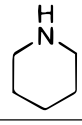


Scheme 3.21: The Knoevenagel reaction of *p*-nitrobenzaldehyde (**3.52**) with methyl malonic acid (**3.45**).

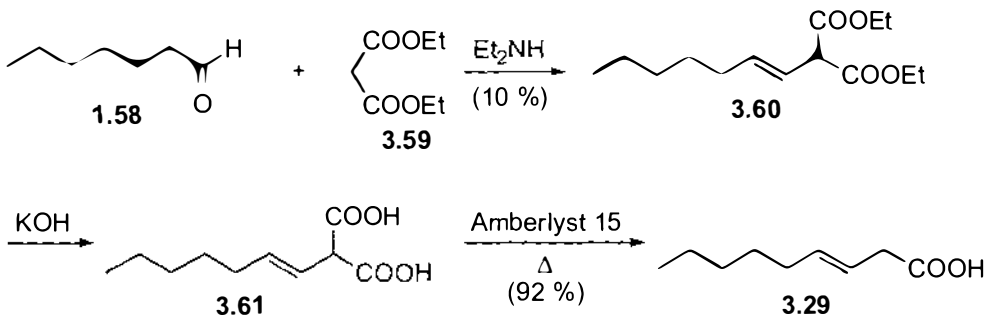
In light of this alternative mechanistic pathway, which was productive for α-substituted malonic acids, we investigated the Knoevenagel reaction with methyl malonic acid (**3.45**) and heptanal (**1.58**) in the presence of different bases (Table 3.3). Tertiary amines, (*i.e.*, triethylamine and pyridine), gave low yields and saturated carboxylic acids were formed as by-products. Secondary amines, (*i.e.*, piperidine and diethylamine) gave greatly improved yields of the desired products. The condensation with ethyl malonic acid (**3.46**) and heptanal (**1.58**) was also investigated and gave similar results (Table 3.3).

Table 3.3: Condensation of α-alkyl malonic acids in the presence of amine bases.



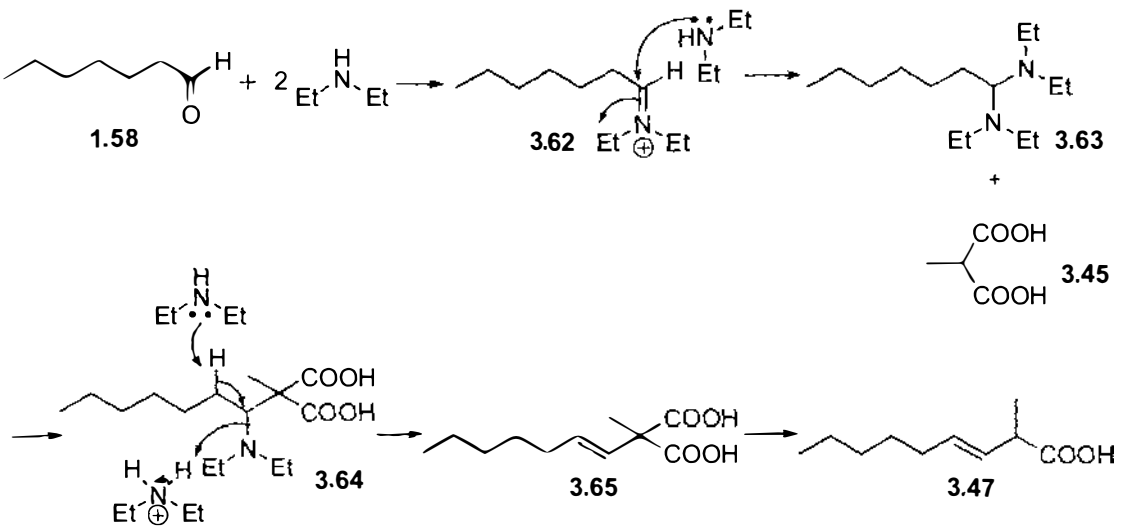
R	Base	p*K*a of Base	Yield
$-CH_3$	NEt_3	11.0	11 %
$-CH_3$	$NHEt_2$	10.5	60 %
$-CH_3$		5.3	11 %
$-CH_3$		11.2	26 %
$-CH_2CH_3$	NEt_3	11.0	0 %
$-CH_2CH_3$		11.2	12 %
$-CH_2CH_3$	$NHEt_2$	10.5	60 %

Dehydration and decarboxylation were key steps in the condensation reaction following attack of the malonate anion on a *bis*-diimide derivative. In an attempt to investigate the order of these events, we set up a simple experiment with a protected malonic acid, *i.e.*, diethyl malonate (**3.59**), which cannot undergo decarboxylation. An unsaturated diester (**3.60**) was isolated and the resonances at δ 128.6 and δ 150.0 ppm in the ^{13}C NMR spectrum were attributed to the carbons at the newly formed double bond in **3.60**, indicating that dehydration could occur before decarboxylation. The unsaturated diester was hydrolysed and then decarboxylation was carried out to give the desired product **3.29** (Scheme 3.22).



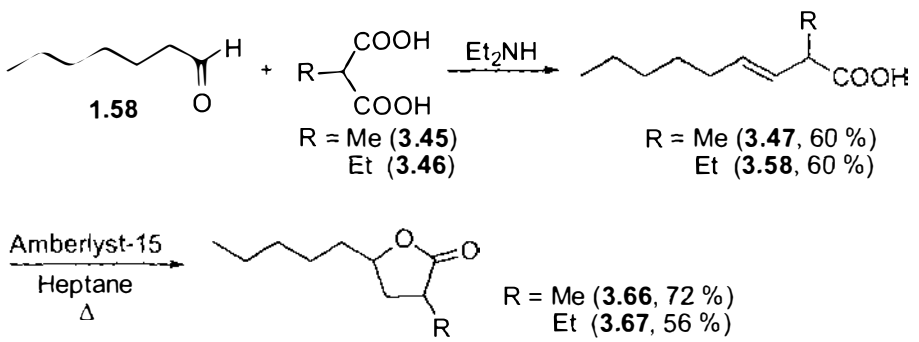
Scheme 3.22: The condensation reaction followed by dehydration and decarboxylation.

Therefore, the choice of base in the reaction is based on steric bulk and basicity. The final choice of base was a secondary amine, diethylamine, which gave a better result than other basic conditions (Scheme 3.23).



Scheme 3.23: The Knoevenagel reaction of heptanal (**3.52**) with methyl malonic acid (**3.45**) in the presence of diethylamine.

Once the α-substituted β,γ-unsaturated carboxylic acids were in hand, lactonisation in the presence of acid catalyst was straightforward (Scheme 3.24).



Scheme 3.24: Synthesis of α-substituted lactones (**3.66** and **3.67**).

There were four isomers expected and the ratio of the two pairs of enantiomers present was calculated by integration of the NMR signals (Figure 3.7). We presume that the major products have the *trans*-orientation of the two substituents since this would be thermodynamically more stable.

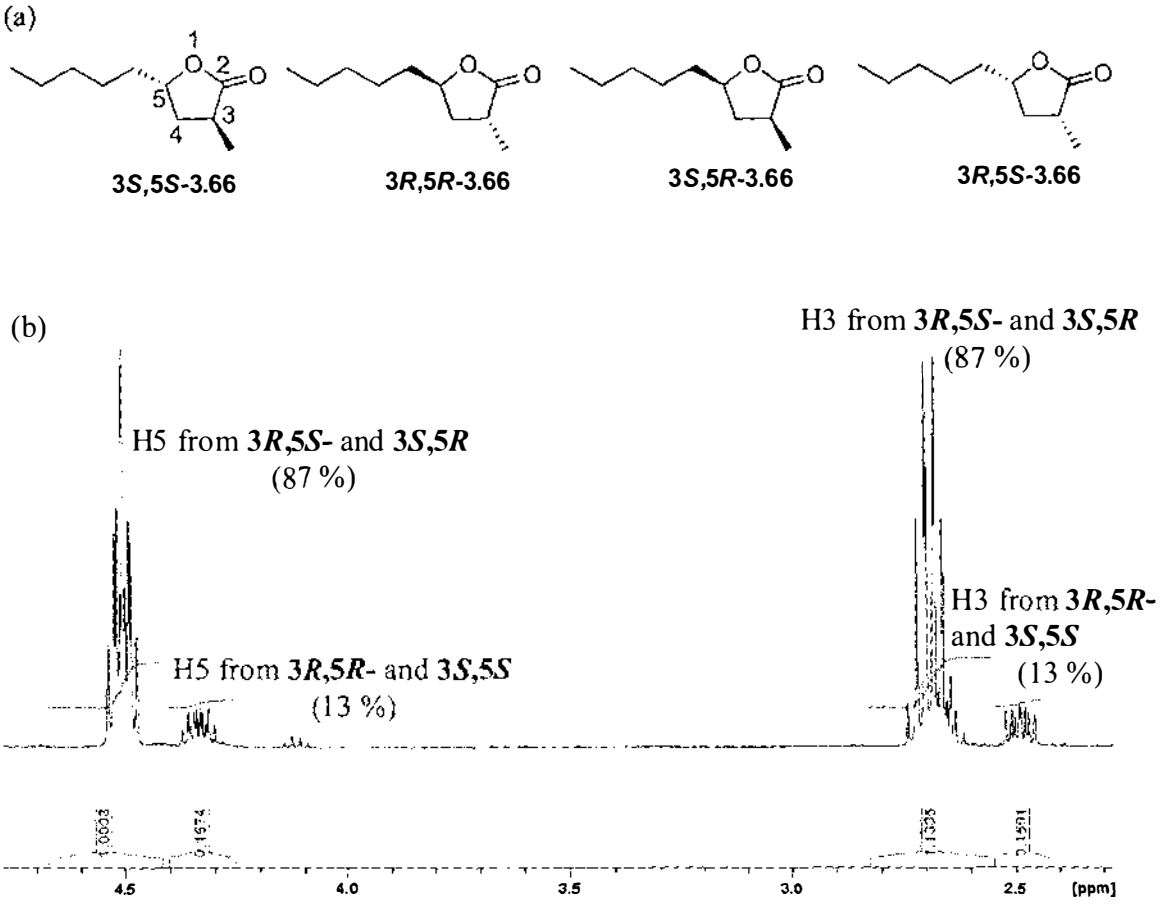
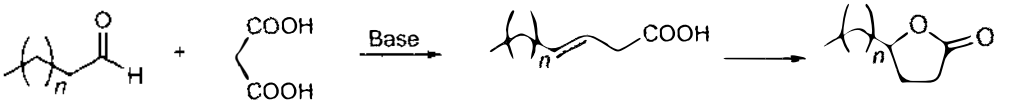


Figure 3.7: (a) Four diastereomers of compound **3.66** and (b) the NMR trace of the mixture.

3.4.5 Synthesis of a library of racemic γ-lactones

The Linstead modification of the Knoevenagel condensation, under the various conditions described, enabled us to prepare β,γ-unsaturated carboxylic acids, which can lactonise to give γ-lactones. We sought to prepare a library of racemic lactones in a combinatorial fashion. Mixtures of aldehydes and malonic acids could be used to produce libraries of γ–lactones. The first library involved five aldehydes in combination with malonic acid (**3.28**) to form β,γ-unsaturated acids and cyclisation of β,γ-unsaturated acids

were followed (Scheme 3.25). The GC traces of libraries of five acids and lactones showed peaks corresponding to five compounds in each library (Figure 3.8). The peaks could be attributed to each compound and identified by the MS.



n = 3; **1.57**	**3.29**	*n* = 3; **3.68**	*n* = 3; **3.71**
n = 4; **1.58**		*n* = 4; **3.29**	*n* = 4; **3.1**
n = 5; **1.59**		*n* = 5; **1.69**	*n* = 5; **1.50**
n = 6; **1.60**		*n* = 6; **3.70**	*n* = 6; **3.27**
n = 7; **1.61**		*n* = 7; **1.31**	*n* = 7; **1.52**

Scheme 3.25: Synthesis of a library of γ-lactones (*n* = 3-7).

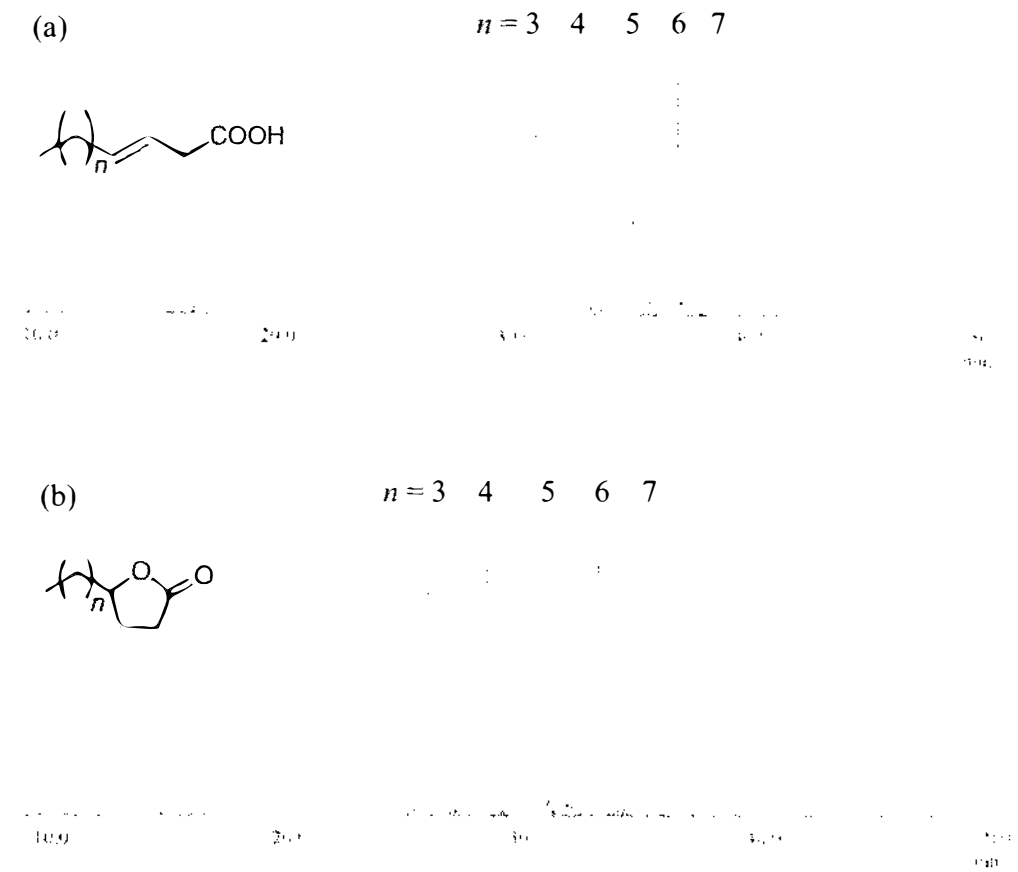


Figure 3.8: GC traces of (a) five carboxylic acids and (b) five racemic lactones, *n* = 3-7.

A mixture of the same five aldehydes was reacted with five equivalents of methyl malonic acid (**3.45**), leading to a library of racemic diastereomers of lactones containing a methyl group at the 3-position. The library was analysed as for the previous library by GC-MS (Figure 3.9) to establish their identities and purities. The experiment with ethyl malonic acid (**3.46**) was also carried out to synthesise five racemic dihydro-3-ethyl-5-alkyl-(3*H*)-furanones and the library was again analysed by GC-MS to establish their identities and purities.

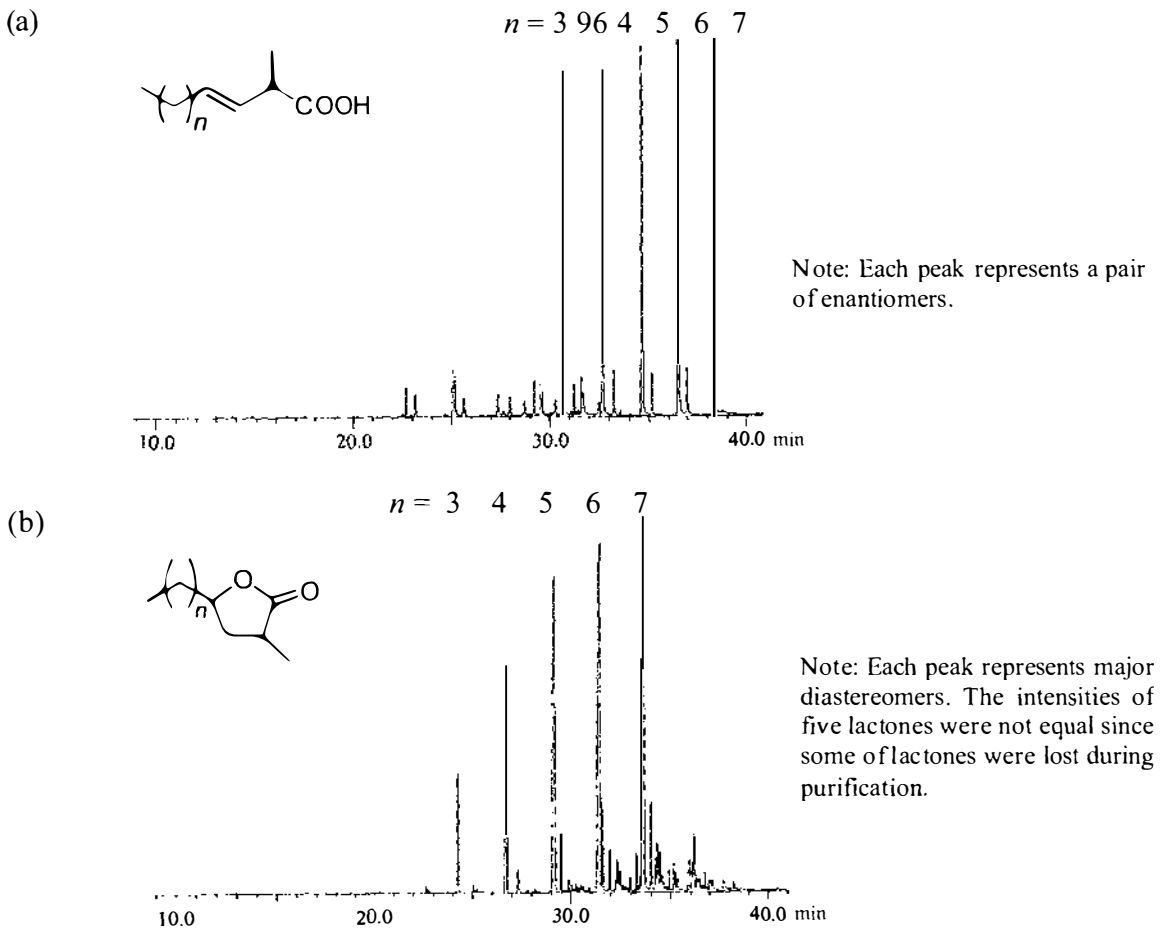
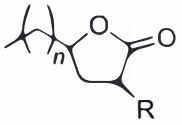


Figure 3.9: GC traces of (a) five carboxylic acids and (b) five dihydro-3-methyl-5-alkyl-(3*H*)-furanones.

3.4.6 Screening of the lactone libraries

The odour descriptions for γ-lactones were obtained from screening by GC-O and their details are shown in Table 3.4.

Table 3.4: The odour description of γ-lactones.



RT (min)	Compounds	Odour Description	Lit. Odour[99]
Library 1 (R = H)			
25.9	dihydro-5-butyl-2(3*H*)-furanone (**3.71**, C_8)	coconut	coconut
28.4	dihydro-5-pentyl-2(3*H*)-furanone (**3.1**, C_9)	apricot, coconut	coconut, fatty fruity, aniseed
30.8	dihydro-5-hexyl-2(3*H*)-furanone (**1.50**, C_{10})	fruity juice, coconut	peach, fatty, fruity
33.1	dihydro-5-heptyl-2(3*H*)-furanone (**3.27**, C_{11})	peach, strawberry	musty, fruity, peach
35.3	dihydro-5-octyl-2(3*H*)-furanone (**1.52**, C_{12})	sweet peach	peach, butter, fatty
Library 2 (R = Me)			
24.2	dihydro-3-methyl-5-butyl-(3*H*)-furanone (**3.72**, C_9)	coconut, sweet, herbaceous	green, coconut
26.8	dihydro-3-methyl-5-pentyl-(3*H*)-furanone (**3.66**, C_{10})	sweet, coconut. milky, fruity	
29.2	dihydro-3-methyl-5-hexyl-(3*H*)-furanone (**3.73**, C_{11})	peach, fruity, sweet	
31.4	dihydro-3-methyl-5-heptyl-(3*H*)-furanone (**3.74**, C_{12})	burning sweet peach	
33.7	dihydro-3-methyl-5-octyl-(3*H*)-furanone (**3.75**, C_{13})	fatty, slightly peach, sweet	
Library 3 (R = Et)			
25.8	dihydro-3-ethyl-5-butyl-(3*H*)-furanone (**3.76**, C_{10})	fatty, coconut	
27.9	dihydro-3-ethyl-5-pentyl-(3*H*)-furanone (**3.67**, C_{11})	fatty, fruity	
30.3	dihydro-3-ethyl-5-hexyl-(3*H*)-furanone (**3.77**, C_{12})	sweet peach	
32.4	dihydro-3-ethyl-5-heptyl-(3*H*)-furanone (**3.78**, C_{13})	sweet, fatty, slightly peach	
34.5	dihydro-3-ethyl-5-octyl-(3*H*)-furanone (**3.79**, C_{14})	fatty, waxy, sweet	

3.5 Thionolactones

3.5.1 Introduction

Sulfur-containing compounds play prominent roles in the flavour of many kinds of fruits, such as passion fruit, blackcurrant and grapefruit.[100] For example, 3(*S*)-sulfanylhexanol (**3.80**)[101] is found in yellow passion fruit. Guava contains 3(*S*)-methylthiohexanol (**3.81**),[102] 2(*R*)-methyl-4(*S*)-propyl-1,3-oxathiane (**3.82**) and 3(*S*)-propyl-γ-sultone (**3.83**) (Figure 3.10). [103] Some of them are identified as the principal contributors to cheese flavour (e.g., methanethiol **1.11** and dimethyldisulfide **1.13** in cheddar).

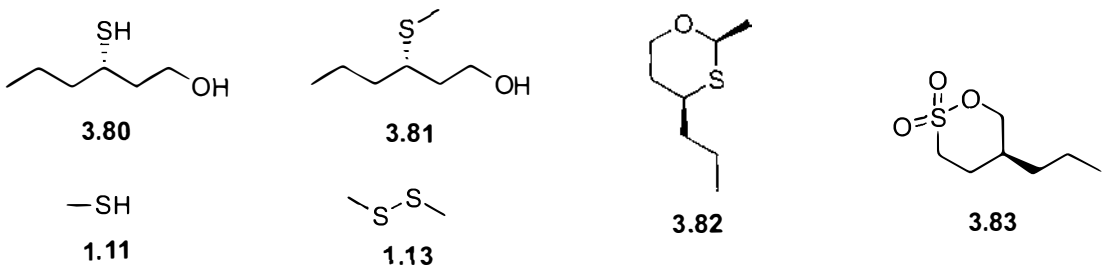


Figure 3.10: The structures of natural flavour compounds in fruits and cheeses.

Thiol-substituted furans, such as 2-methyl-3-furanthiol (**3.84**), 2-furanmethanethiol (**3.85**), and the corresponding disulfides, *bis*(2-methyl-3-furyl)disulfide (**3.86**) and *bis*(2-furylmethyl)disulfide (**3.87**) are found in roasted coffee at low concentrations (Figure 3.11).[104]

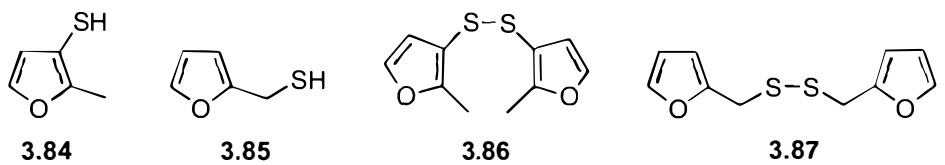


Figure 3.11: Sulfur-containing compounds in roasted coffee.

The importance of sulfur compounds in meat flavour was reported by Brennan and Bernhard in 1964. They identified hydrogen sulfide and methyl, ethyl, propyl and butyl mercaptans in the headspace of canned cooked beef.[105] Liebich identified dimethyl disulfide and dimethyl sulfone in boiled beef.[106]

Since sulfur-containing compounds are identified as key compounds in a variety of food, the conversion of lactones into thionolactones has the potential to produce interesting flavour compounds. Thio- (I), thiono- (II) and dithio-derivatives (III) of lactones have been studied with respect to their unique odours. A number of natural products containing a thiolactone ring have been isolated [*e.g.*, thiolactomycin (**3.88**), thiotetromycin (**3.89**), thiocoumarin (**3.90**)] (Figure 3.12).[107]

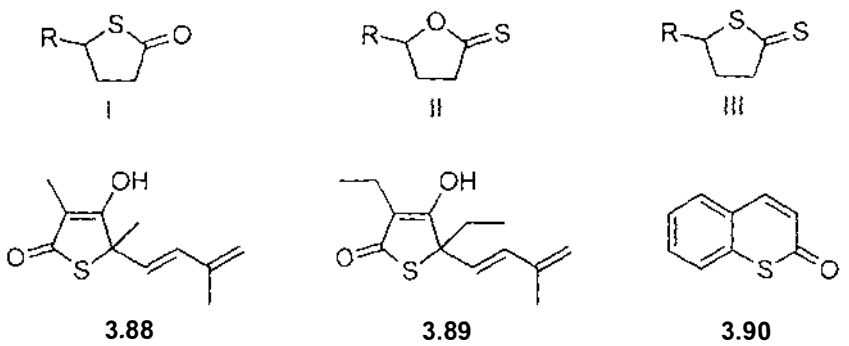
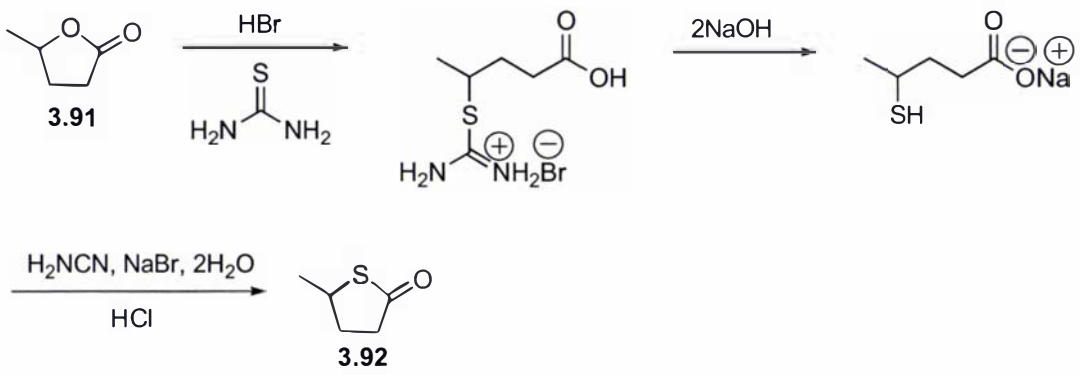


Figure 3.12: Examples of thiolactone rings in natural products.

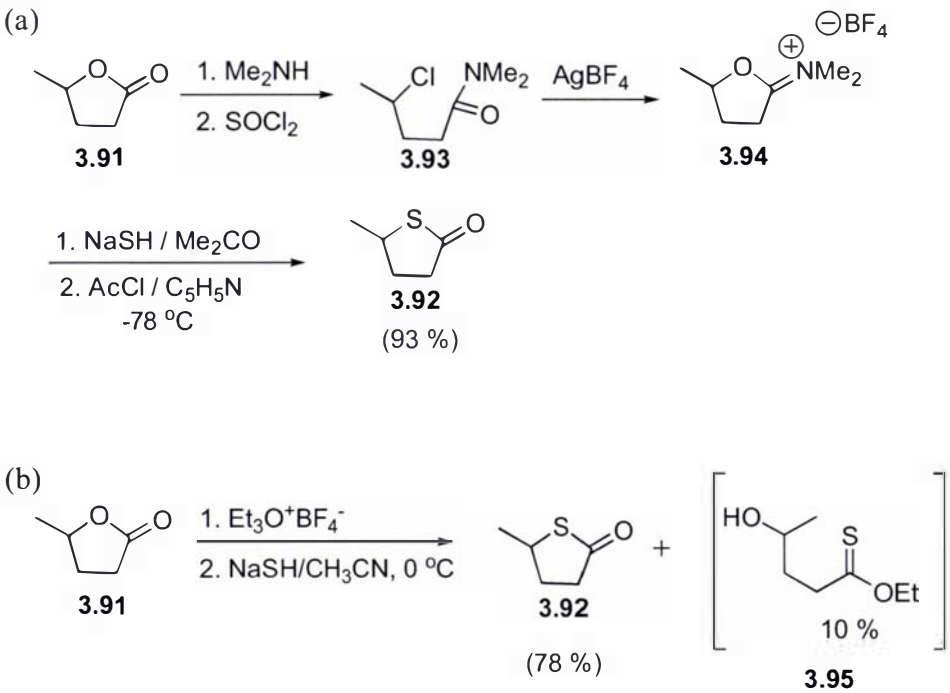
3.5.2 Previous synthetic work

The chemistry of thionoesters has been an area of interest in both organic and flavour chemistry because of their unique behaviour and odour. In 1963, Kharasch and Langford reported the conversion of dihydro-5-methyl-(3*H*)-furanone (**3.91**) into the corresponding thiolactone (**3.92**) *via* isothiouronuim bromides (Scheme 3.26).[108]



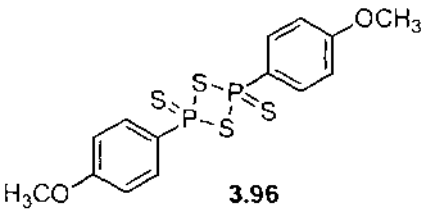
Scheme 3.26: Kharasch and Langford's synthetic route to γ-thiolactone.

In the late 1970's, Kaloustian synthesised thionolactones via a two-step procedure of sulfhydrolysis / acetylation of *N,N*-dimethyliminolactonium salts at low temperature (Scheme 3.27a).[109] In 1981, the same group reported a shorter and more convenient route (Scheme 3.27b),[110] although a by-product (**3.95**) was obtained, reducing the yield of the desired product.



Scheme 3.27: Kaloustian's approach to oxygen-sulfur exchange.

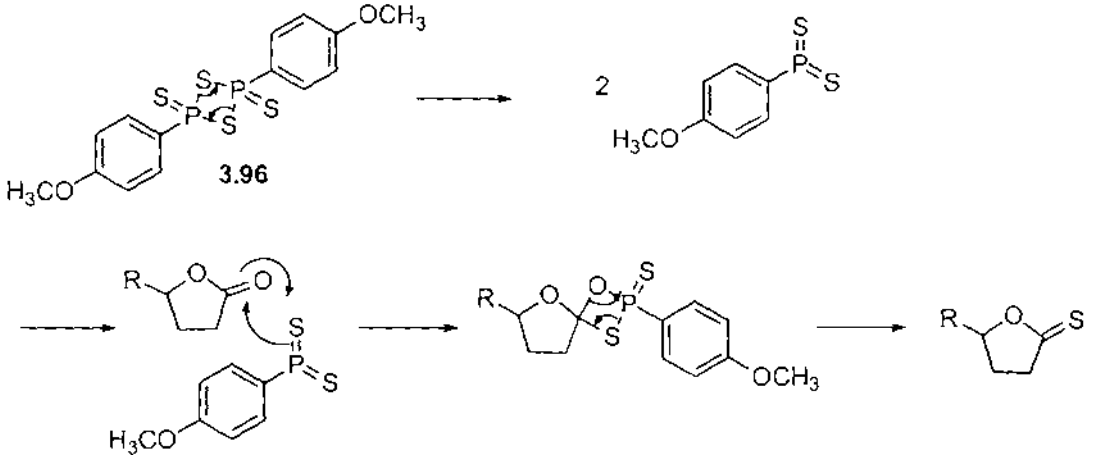
In 1979, Lawesson introduced 2,4-*bis*(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (**3.96**, Lawesson's reagent, Figure 3.13) to synthesise thiono and dithiolactones. Lawesson's group found that thionolactones were generally pale yellow oils or colourless crystals that are stable, whereas the orange-red oily dithiolactones gave a most unpleasant odour and decomposed upon storage.[111]



3.96

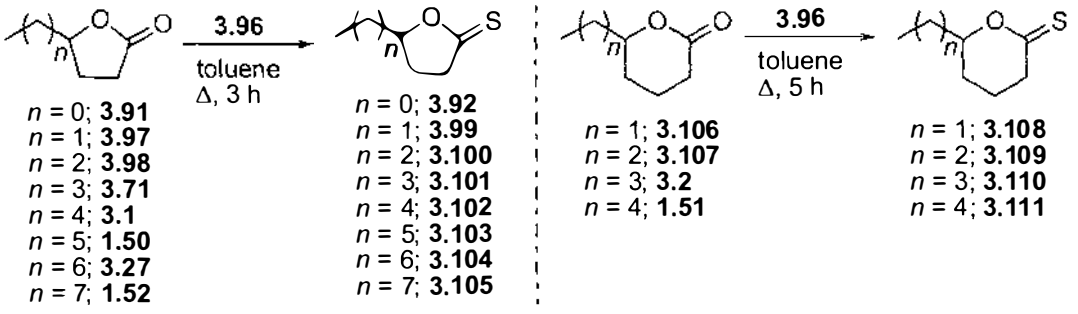
Figure 3.13: 2,4-*bis*(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (Lawesson's reagent, **3.96**).

A mechanistic proposal for the replacement of the oxygen atom of the carbonyl group of the γ-lactone with sulfur is illustrated in Scheme 3.28.



Scheme 3.28: A proposal for the mechanism of thionation of a γ-lactone with Lawesson's reagent.

Since 2,4-*bis*(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (**3.96**) seems to be a convenient thionation reagent, it has been widely adopted. Hayashi's group made γ- and δ-thionolactones individually (Scheme 3.29) and reported physical and chemical properties.[112]



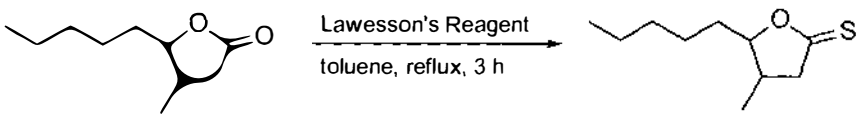
Scheme 3.29: Individual syntheses of γ- and δ-thionolactones.

Also, in 1998, Beck and Mosandl prepared γ– and δ–thionolactones by Lawesson's method and analysed their odour by GC-O utilising a chiral column (Table 3.5).[113]

Table 3.5: Odour description for thionolactones.[113]

Compound	**Absolute configuration**	**Odour description**
γ-C_6 **3.99**	*R*	green, grassy
	S	green, grassy, mint, burnt
γ-C_8 **3.101**	*R*	mushroom-like odour, hay-like odour
	S	mushroom-like odour, hay-like odour, pungent
γ-C_{10} **3.103**	*R*	fruity
	S	sweet, fruity
γ-C_{12} **3.105**	*R*	fruity
	S	fruity
δ-C_6 **3.112**	*R*	maggi-like odour, spicy, burnt
	S	maggi-like odour, sulfurous, burnt
δ-C_8 **3.109**	*R*	mushroom-like odour, grassy, green
	S	mushroom-like odour, grassy, hay like odour, sulfurous
δ-C_{10} **3.111**	*R*	green, slightly sweet, slightly fruity
	S	sweet, fruity
δ-C_{12} **3.113**	*R*	sweet, fruity
	S	sweet, fruity

In 2001, Schmarr, Eiseneich and Engel synthesised thio-, thiono- and dithio-derivatives of whiskey lactone (**3.66**) by Lawesson's method and described their odour (Scheme 3.30).[114]

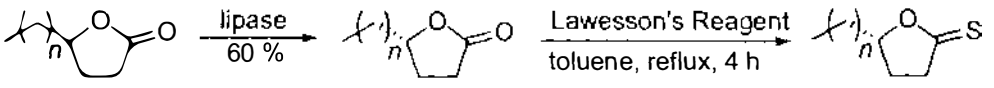


3.66
coconut-like

3.114
mushroom-like

Scheme 3.30: Synthesis of thiono-derivative of whiskey lactone **3.114**.

Finally, in 2002, Lizzani-Cuvelier's group resolved racemic γ–lactones enzymatically and then applied Lawesson's method to exchange oxygen and sulfur (Scheme 3.31).[115]



n = 0; **3.91**
n = 1; **3.97**
n = 2; **3.98**
n = 3; **3.71**
n = 4; **3.1**
n = 5; **1.50**

n = 0; **S-3.91**
n = 1; **S-3.97**
n = 2; **S-3.98**
n = 3; **S-3.71**
n = 4; **S-3.1**
n = 5; **S-1.50**

n = 0; **S-3.92**
n = 1; **S-3.99**
n = 2; **S-3.100**
n = 3; **S-3.101**
n = 4; **S-3.102**
n = 5; **S-3.103**

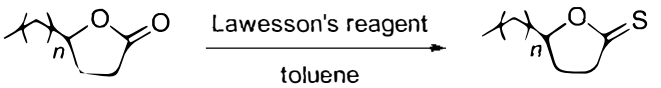
Scheme 3.31: Enzymatic resolution of racemic γ–lactones and thionation of chiral lactones.

3.5.3 Synthesis of a library of racemic thionolactones

We first synthesised the thionolactone derived from commercially available dihydro-5-methyl-(3*H*)-furanone (**3.91**). We investigated the use of Lawesson's reagent in different stoichiometric ratios to optimise the yield (Table 3.6). The main problem faced with this dihydro-5-methyl furan-2-thione (**3.92**) was its volatility; care must be taken not to leave it under vacuum too long. Dihydro-5-octyl furan-2-thione (**3.105**), the highest molecular

weight compound of the proposed library, was also synthesised by the oxygen-sulfur exchange of dihydro-5-octyl-(3*H*)-furanone (**1.52**) (Table 3.6). Both compounds were characterised by NMR, IR and MS and they were in agreement with literature values.[116] Significant spectroscopic evidence confirmed the conversion of the C=O functionality in the lactones to the C=S moiety in the thionolactones. Dihydro-5-octyl-2(3*H*)-furanone has a peak at δ 177.4 ppm in its ^{13}C NMR spectrum attributable to the lactone C=O, the corresponding thionolactone C=S signal appears at δ 222.6 ppm. Changes are also observed in the IR spectrum, with υ(C=O) at 1769 cm^{-1} for dihydro-5-octyl-2(3*H*)-furanone and υ(C=S) at 1273 cm^{-1} for dihydro-5-octyl furan-2-thione. The mass spectrum showed that the molecular ion peak had increased by 16 amu following conversion to the thionolactone. Thus good evidence was provided for formation of the thionolactone.

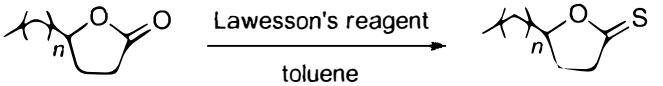
Table 3.6: The thionation of lactones.



Compounds	Lawesson's reagent, equiv.	Yield (%)
n = 0 **3.92**	0.5	37
	1	56
	2	70
n = 7 **3.105**	2	81

The individual syntheses of dihydro-5-methyl furan-2-thione (**3.92**) and dihydro-5-octyl furan-2-thione (**3.105**) gave us confidence to prepare a library of thionolactones using this methodology. A library of five thionolactones was prepared according to Scheme 3.32 and analysed by GC-MS (Figure 3.14). Dihydro-5-methyl furan-2-thione (**3.92**),

dihydro-5-ethyl furan-2-thione (**3.99**) and dihydro-5-propyl furan-2-thione (**3.100**) were synthesised individually and assessed for their odour properties (Table 3.7).



3.71: *n* = 3
3.1: *n* = 4
1.50: *n* = 5
3.27: *n* = 6
1.52: *n* = 7

3.101: *n* = 3
3.102: *n* = 4
3.103: *n* = 5
3.104: *n* = 6
3.105: *n* = 7

Scheme 3.32: Combinatorial synthesis of a library of five thionolactones.

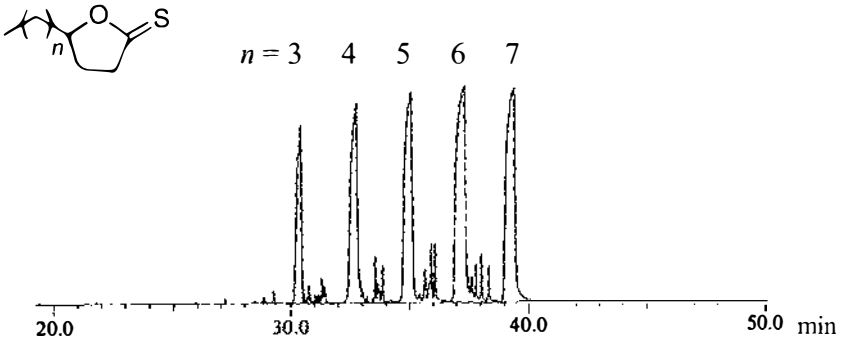
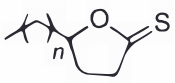


Figure 3.14: GC trace for library of five thionolactones (*n* = 3-7).

The resulting library of eight thionolactones was checked for purity and composition by GC-MS and then screened by GC-O. The odour descriptions of the individual compounds are given in Table 3.7, along with descriptions from the literature where compounds have been reported previously.

Table 3.7: The structure and odour description of thionolactones.



n		Odour Description	Lit. Odour Description[113]
0	**3.92**	sulfury, slightly fruity	
1	**3.99**	green, burnt, mint	sweet, sulfury, burnt
2	**3.100**	sweet, metallic, burnt	
3	**3.101**	metallic, blue cheese like, chives	mushroom homogenate, coconut, sweet, sulfury
4	**3.102**	mushroom, creamy, spring onion	
5	**3.103**	softer note, butter, mushroom	fruity, fatty, rancid
6	**3.104**	pasta, mushroom	
7	**3.105**	sweeter note, peach peel, mushroom	slightly fruity, soapy

3.6 Summary

Syntheses and analyses of racemic lactones were the focus of this chapter. Individual racemic lactones were synthesised *via* a two-step reaction. The Linstead modification of the Knoevenagel condensation to produce a β,γ-unsaturated acid followed by lactonisation were applied to the synthesis of dihydro-5-octyl-2(3*H*)-furanone. Different reaction conditions were required for the Knoevenagel condensation of different substrates. Once the reaction chemistry was optimised, libraries of dihydro-5-alkyl-(3*H*)-furanones (C_8-C_{12}), and 3-substituted dihydro-5-alkyl-(3*H*)-furanones were produced. Further, synthesis of a library of eight γ-thionolactones was achieved from a library of γ-lactones with Lawesson's reagent. The libraries were analysed by GC-MS and GC-O.

3.7 Experimental procedure

3.7.1 General procedure for racemic lactones and thionolactones

General methods. As described in Chapter 2, with the following modifications. ^{1}H NMR spectra were recorded at 400 MHz, and ^{13}C NMR spectra at 100 MHz on a Bruker Avance 400 spectrometer. High resolution mass spectra were recorded on a VG 70SE mass spectrometer, operating at a nominal accelerating voltage of 70 eV. Pyridine, piperidine and diethylamine (Et_2NH) were distilled from calcium hydride and stored over KOH pellets. Piperidinium acetate was freshly prepared for each occasion, by combining piperidine (99 μL, 85 mg, 1 mmol) and acetic acid (57 μL, 60 mg, 1 mmol) in DMSO (1 mL).

General procedure for the Linstead modification of the Knoevenagel reaction.

Method A. A mixture of malonic acid (10 mmol, 1.0 equiv.) and aldehyde (10 mmol, 1.0 equiv.) in Et_3N (2 mL) was heated at reflux for 1 h. The mixture was cooled to RT and transferred to a separatory funnel using Et_2O (20 mL).

Method B. Piperidinium acetate (as prepared above, 0.02 mmol, 0.02 equiv.) in DMSO (20 μL) was treated wth malonic acid (20 mmol, 2.0 equiv.) and aldehyde (10 mmol, 1.0 equiv.) in DMSO (20 mL), and stirred under nitrogen at RT for 20 min. The mixture was then heated at 100 °C for 5 h, cooled to RT and transferred to a separatory funnel using Et_2O (20 mL).

Method C. A mixture of aldehyde (10 mmol, 1.0 equiv.) and Et_2NH (50 mmol, 5.0 equiv.) in CH_2Cl_2 (20 mL) was heated at reflux for 1 h. An α-substituted malonic acid

(20 mmol, 2.0 equiv.) was added into the mixture and heated at reflux overnight. The mixture was cooled to RT and transferred to a separatory funnel using Et_2O (20 mL).

Work-up (same for methods A, B and C). The mixture was washed once with ice cold 10% HCl (10 mL) followed by 5% NaOH (10 mL). The alkaline aqueous extract was washed once with Et_2O (10 mL), acidified by the addition of 10% HCl (10 mL) and extracted with Et_2O (10 mL). The organic layer was washed with brine (20 mL), dried over $MgSO_4$, filtered, and concentrated. The carboxylic acids thus obtained were employed directly in the next step, without purification.

(3*E*)-Non-3-enoic acid (3.29): prepared from heptanal and malonic acid, giving a colourless solid; 0.87 g (50 %, Method A), 1.33 g (77 %, Method B). R_f = 0.48 (5:1 hex-EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3H, CH_3-), 1.24–1.31 (m, 4H, $CH_3C\underline{H}_2C\underline{H}_2$-), 1.37 (p, J = 7.2 Hz, 2H, -$C\underline{H}_2CH_2CH$=CH-), 2.04 (q, J = 7.0 Hz, 2H, -$C\underline{H}_2CH$=CH-), 3.06 (dd, J = 6.6, 0.9 Hz, 2H, -CH=CH-$C\underline{H}_2COOH$), 5.49-5.53 (m, 1H, -CH=C$\underline{H}CH_2COOH$), 5.53-5.59 (m, 1H, -C$\underline{H}$=$CHCH_2COOH$), 11.60 (s, 1H, -COOH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.5, 28.7, 31.3, 32.4, 37.8, 120.6, 135.5, 178.9; HRMS calcd for $C_9H_{16}O_2$ (M^+): 156.11503; obsd: 156.11505.

(3*E*)-Dodec-3-enoic acid (3.31): prepared from decanal and malonic acid, giving a colourless solid; 1.66 g (84 %, Method A), 1.64 g (83 %, Method B). R_f = 0.50 (5:1 hex-EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, J = 6.8 Hz, 3H, CH_3-), 1.23 – 1.31 (m, 10H, 5 x -CH_2-), 1.38 (p, J = 6.9 Hz, 2H, -$C\underline{H}_2CH_2CH$=CH-), 2.03 (q, J = 6.8 Hz, 2H, -$C\underline{H}_2CH$=CH-), 3.06 (d, J = 6.4 Hz, 2H, -CH=CH-$C\underline{H}_2COOH$), 5.47-5.56 (m, 1H, -CH=C$\underline{H}CH_2COOH$), 5.56-5.63 (m, 1H, -C$\underline{H}$=$CHCH_2COOH$), 11.30 (s, 1H, COOH); ^{13}C

NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 29.1, 29.1, 29.2, 29.4, 31.9, 32.5, 37.8, 120.6, 135.5, 180.7; HRMS calcd for $C_{12}H_{22}O_2$ (M^+): 198.16198; obsd: 198.16170.

(3*E*,6*Z*)-Dodeca-3,6-dienoic acid (3.34): prepared from (4*Z*)-dec-4-enal and malonic acid, giving a yellow oil; 0.62 g (32 %, Method A), 1.78 g (91 %, Method B). R_f = 0.17 (5:1 hex-EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, J = 7.0 Hz, 3H, CH_3-), 1.26–1.31 (m, 4H, 2 x -CH_2-), 1.34 (p, J = 7.3 Hz, 2H, -C$\underline{H}_2$$CH_2$CH=CH-), 2.02 (q, J = 7.0 Hz, 2H, -CH_2C$\underline{H}_2$CH=CH-), 2.79 (app. t, J = 6.7 Hz, 2H, -CH=CH-C$\underline{H}_2$-CH=CH-), 3.08 (d, J = 5.0 Hz, 2H, -CH=CH-C$\underline{H}_2$COOH), 5.35-5.46 (m, 2H, *cis*-C$\underline{H}$=C$\underline{H}$-), 5.54-5.58 (m, 2H, *trans*-C$\underline{H}$=C$\underline{H}$-), 11.28 (s, 1H, COOH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.5, 27.1, 29.2, 30.2, 31.4, 37.8, 121.2, 126.4, 131.3, 133.5, 178.8; HRMS calcd for $C_{12}H_{20}O_2$ (M^+): 196.14633; obsd: 196.14633.

(3*E*)-Dodeca-3,11-dienoic acid (3.35): prepared from dec-9-enal and malonic acid, giving a yellow oil; 0.37 g (19 %, Method A), 1.13 g (58 %, Method B). R_f = 0.25 (5:1 hex-EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27–1.37 (m, 8H, 4 x -CH_2-), 2.04 (q, J = 6.7 Hz, 4H, =CHC$\underline{H}_2$$(CH_2)_4C\underline{H}_2$CH=), 3.06 (dd, J = 6.6 Hz, 0.8 Hz 2H, -CH=CH-C$\underline{H}_2$COOH), 4.94 (dq, J = 17.1, 2.0 Hz, 1H, *trans*-C$\underline{H}_2$=CH-), 4.94 (m, 1H, *cis*-C$\underline{H}_2$=CH-), 5.45-5.55 (m, 1H, -CH_2C$\underline{H}$=C$\underline{H}$$CH_2$COOH), 5.55-5.63 (m, 1H, -CH_2CH=C$\underline{H}$$CH_2$COOH), 5.81 (ddt, J = 17.0, 10.3, 6.7 Hz, 1H, CH_2=C$\underline{H}$-), 11.17 (s, 1H, COOH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.8, 28.9, 28.9, 29.0, 32.4, 33.7, 37.8, 114.2, 120.7, 135.4, 139.1, 178.9; HRMS calcd for $C_{12}H_{20}O_2$ (M^+): 196.14633; obsd: 196.14624.

7-Phthalimido-3-heptenoic acid (3.36): prepared from 5-phthalimido pentanal and malonic acid, giving a colourless solid; 1.13 g (68 %, Method B). R_f = 0.25 (5:1 hex-EtOAc). ^{1}H NMR (400 MHz, MeOH) δ 1.72 (p, J = 7.3 Hz, 2H, H-6), 2.16 (q, J = 7.5 Hz, 2H, H-5), 3.03 (d, J = 5.2 Hz, 2H, H-2), 3.35 (t, J = 7.0 Hz, 2H, H-7), 5.61-5.63 (m, 2H, H-3 and H-4), 7.42-7.95 (m, 4H, Ph), 11.17 (s, 1H, COOH); ^{13}C NMR (100 MHz, MeOH) δ 29.4, 30.9, 38.7, 40.4, 124.0, 128.7, 130.5, 130.8, 131.2, 133.0, 134.6, 139.6, 169.5, 172.8; HRMS calcd. for $C_{15}H_{15}NO_4$ (M^+): 273.10011; obsd: 273.10025.

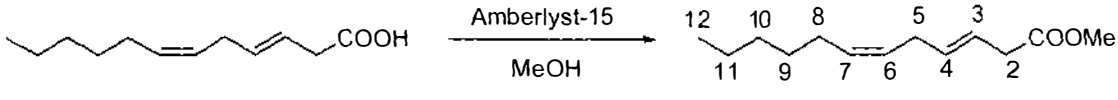
Diethyl (*E*)-hept-1-enylmalonate (3.60): prepared from heptanal and diethyl malonate, giving a colourless oil; 640 mg (25 %, Method C). R_f = 0.56 (3:1 hex-EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (t, J = 6.8 Hz, 3H, CH_3-), 1.15–1.45 (m, 12H, 3 x -CH_2-, 2 x -OCH_2CH_3), 2.25 (q, J = 7.6 Hz, 2H, -CH_2CH=CH-), 4.09-4.18 (m, 1H, -CH(COOEt)$_2$), 4.23 (q, J = 7.1 Hz, 4H, 2 X -OCH_2CH_3), 5.58-5.62 (m, 2H, -CH=CH-); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.5, 28.2, 28.9, 29.7, 31.5, 61.1, 61.2, 128.6, 150.0, 165.6.

(3*E*)-Non-3-en-oic acid (3.29): Diethyl ester **3.60** (128 mg, 0.5 mol) was added into a solution of potassium hydroxide (128 mg) in water (10 mL). The mixture was heated under reflux for 3 h until hydrolysis was complete. The reaction mixture was diluted with water (20 mL) and then the ethanol produced in the reaction, along with water (total volume 20 mL) was removed by distillation. Amberlyst 15 (128 mg) was added into the cooled residue and the mixture was heated under reflux for 2 h. The reaction mixture was extracted with Et_2O (3 x 20 mL), washed with brine (20 mL), dried over $MgSO_4$ and

concentrated to give a colourless solid, **3.29** (80 mg, 92 %). Physical data was in agreement with that described in detail above.

(3*E*)-2-Methyl-non-3-enoic acid (3.47): prepared from heptanal and methyl malonic acid, giving a colourless oil; 1.12 g (66 %, Method C). R_f = 0.41 (3:1 hex-EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, J = 6.8 Hz, 3H, CH_3-), 1.28–1.36 (m, 6H, 3 x -CH_2-), 1.44 (p, J = 7.2 Hz, 2H, -C$\underline{H}_2$CH_2CH=CH-), 1.84 (d, J = 1.0 Hz, 3H, C$\underline{H}_3$CHCOOH), 2.20 (p, J = 7.0 Hz, 1H, -C$\underline{H}$COOH), 6.90-6.94 (m, 2H, -C$\underline{H}$=C$\underline{H}$-), 11.30 (s, 1H, -COOH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9, 14.0, 22.6, 28.4, 28.9, 29.0, 31.6, 126.9, 145.5, 173.8; HRMS calcd. for $C_{10}H_{19}O_2$ (MH^+): 171.13850; obsd: 171.13863.

(3*E*)-2-Ethyl-non-3-enoic acid (3.58): prepared from heptanal and ethyl malonic acid, giving a colourless oil; 1.12 g (61 %, Method C). R_f = 0.48 (3:1 hex-EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.75 – 0.85 (m, 6H, 2 x CH_3-), 1.15–1.76 (m, 8H, 4 x -CH_2-), 1.97 (q, J = 7.2 Hz, 2H, -C$\underline{H}_2$CH=CH-), 2.80 (q, J = 7.7 Hz, 1H, -C$\underline{H}$(CH_2CH_3)COOH), 5.30-5.55 (m, 2H, -C$\underline{H}$=C$\underline{H}$-), 10.09 (s, 1H, -COOH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.6, 14.0, 22.5, 25.6, 28.8, 31.3, 32.4, 50.8, 126.7, 134.3, 181.1; HRMS calcd. for $C_{11}H_{21}O_2$ (MH^+): 184.14633; obsd: 184.14582.



Methyl (3*E*,6*Z*)-Dodeca-3,6-dienoate (3.41): A solution of (3*E*,6*Z*)-dodeca-3,6-dienoic acid (1.96 g, 10.0 mmol) in methanol (50 mL) was heated at reflux in the presence of Amberlyst-15 (2.0 g) for 1 h, then cooled. The Amberlyst-15 was removed by filtration and rinsed with Et_2O (50 mL). The filtrate and washings were concentrated and the residue purified by chromatography (3:1 hex-EtOAc) to give **3.41** as a light yellow oil

(1.91 g, 91 %). R_f = 0.57 (3:1 hex-EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, J = 6.9 Hz, 3H, H-12), 1.22-1.38 (m, 6H, H-9, H-10, H-11), 2.02 (q, J = 6.9 Hz, 2H, H-8), 2.78 (t, J = 7.1 Hz, 2H, H-5), 3.04 (d, J = 4.4 Hz, 2H, H-2), 3.68 (s, 3H, OCH_3), 5.33-5.58 (m, 4H, H-3, H-4, H-6, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.5, 27.1, 29.3, 30.3, 31.5, 37.9, 51.8, 121.8, 126.6, 131.2, 133.0, 172.5; HRMS calcd. for $C_{13}H_{23}O_2$ (MH^+): 211.16980; obsd: 211.17002.

General procedure for lactonisation: A suspension of the carboxylic acid (1.0 g) and Amberlyst-15 (1.0 g) in heptane (2 mL) was heated at reflux for 1 h, then cooled. The Amberlyst-15 was removed by filtration and rinsed with Et_2O (5 mL). The filtrate and washings were concentrated, and the residue purified as specified.

Dihydro-5-pentyl-2(3*H*)-furanone (3.1): on a scale of 8.5 mmol, to give the title compound, purified by distillation, as a pale yellow oil (1.12 g, 84%). R_f = 0.70 (5:1 hex-EtOAc), b.p. 135-145°C/30 mm Hg. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, J = 6.3 Hz, 3H, CH_3-), 1.27-1.89 (m, 8H, 4 x -CH_2-), 2.33 (app. sextet, J = 6.6 Hz, 2H, H_β), 2.54 (dd, J = 9.4, 2.5 Hz, 2H, H_α), 4.49 (p, J = 6.5 Hz, 1H, H_γ); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.7, 22.3, 24.5, 27.6, 28.6, 31.6, 35.3, 80.9, 177.4; HRMS calcd. for $C_9H_{17}O_2$ (MH^+): 157.12285; obsd: 157.12291.

Dihydro-5-octyl-2(3*H*)-furanone (1.52): on a scale of 8.3 mmol, to give the title compound, purified by distillation, as a pale yellow oil; 1.44 g, 88%, R_f = 0.73 (5:1 hex-EtOAc), b.p. 185-195°C/30 mm Hg. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, J = 6.8 Hz, 3H, CH_3-), 1.27-1.88 (m, 14H, 7 x -CH_2-), 2.33 (app. sextet, J = 7.1 Hz, 2H, H_β), 2.54

(dd, J = 6.9, 2.5 Hz, 2H, H_α), 4.49 (p, J=6.9 Hz, 1H, H_γ); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.9, 22.4, 25.1, 27.9, 28.8, 29.1, 29.2, 31.7, 33.9, 35.4, 81.0, 177.4; HRMS calcd. for $C_{12}H_{23}O_2$ (MH^+): 199.16981; obsd: 199.17021.

Dihydro-3-methyl-5-pentyl-(3*H*)-furanone (3.66): on a scale of 1.0 mmol, to give the title compound, purified by chromatography (5:1 hex-EcOAc) to give a colourless oil (134 mg, 72 %). R_f = 0.36 (5:1 hex-EtOAc). ^1H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, J = 6.8 Hz, 3H, CH_3-), 1.28 (d, J = 7.3 Hz, 3H, -CH_3 at α), 1.30-1.75 (m, 8H, 4 x -CH_2-), 2.01 (dt, J = 12.8, 7.5 Hz, 1H, H_β), 2.12 (ddd, J = 12.8, 9.0, 5.0 Hz, 1H, $H_{\beta'}$), 2.69 (m, 1H, H_α), 4.51 (tt, J = 7.9, 5.3 Hz, 1H, H_γ); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 15.8, 22.4, 24.9, 31.4, 33.9, 35.3, 35.4, 78.4, 180.0; HRMS calcd. for $C_{10}H_{19}O_2$ (MH^+): 171.13850; obsd: 171.13910.

Dihydro-3-ethyl-5-pentyl-(3*H*)-furanone (3.67): on a scale of 1.0 mmol, to give the title compound, purified by chromatography (5:1 hex-EcOAc) to give a colourless oil (103 mg, 56 %). R_f = 0.62 (5:1 hex-EtOAc). ^1H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, J = 6.8 Hz, 6H, 2 x CH_3-), 1.22-1.77 (m, 10H, 5 x -CH_2-), 2.00 (dt, J = 12.9, 7.5 Hz, 1H, H_β), 2.13 (ddd, J = 13.1, 8.8, 4.5 Hz, 1H, $H_{\beta'}$), 2.64–2.75 (m, 1H, H_α), 4.46–4.56 (m, 1H, H_γ); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 15.9, 22.6, 25.3, 29.1, 29.4, 31.8, 34.0, 35.4, 78.5, 180.1; HRMS calcd. for $C_{11}H_{21}O_2$ (MH^+): 185.13850; obsd: 185.13910.

General procedure for thionolactone formation: A mixture of the γ-lactone (1.0 mmol, 1.0 equiv.) and Lawesson's reagent (2.0 mmol, 2.0 equiv.) in dry toluene (10 mL) was

heated at reflux for 4 h and, cooled, filtered and concentrated. The thionolactone was isolated by chromatography (5:1 hex-EtOAc).

Dihydro-5-methyl furan-2-thione (3.92): from dihydro-5-methyl-2(3*H*)-furanone, to give a yellow oil (81 mg, 70 %). R_f = 0.37 (5:1 hex-EtOAc). IR (υ max) 1238 (C=S) cm^{-1} (for dihydro-5-methyl-(3*H*)-furanone: IR (υ max) 1764 (C=O) cm^{-1}); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (d, *J* = 6.3 Hz, 3H, CH_3-), 1.88-1.92 (m, 1H, H_β), 2.41-2.44 (m, 1H, $H_{\beta'}$), 3.06 (dt, *J* = 18.8, 9.5 Hz, 1H, H_α), 3.19 (ddd, *J* = 18.9, 8.9, 4.0 Hz, 1H, $H_{\alpha'}$), 5.04 (sextet, *J* = 6.7 Hz, 1H, H_γ); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.3, 31.1, 44.9, 87.1, 222.5; HRMS calcd for C_5H_8OS (M^+): 116.02959; obsd: 116.02962.

Dihydro-5-ethyl furan-2-thione (3.99): from dihydro-5-ethyl-2(3*H*)-furanone, to give a yellow oil (72 mg, 55 %). R_f = 0.38 (5:1 hex-EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, *J* = 6.9 Hz, 3H, CH_3-), 1.27-1.30 (m, 2H, -CH_2-), 1.87-1.92 (m, 1H, H_β), 2.36-2.40 (m, 1H, $H_{\beta'}$), 3.03 (dt, *J* = 18.8, 9.2 Hz, 1H, H_α), 3.17 (ddd, *J* = 18.9, 8.9, 3.9 Hz, 1H, $H_{\alpha'}$), 4.88 (p, *J* = 7.2 Hz, 1H, H_γ); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.3, 31.1, 44.9, 87.1, 222.5; HRMS calcd for $C_6H_{10}OS$ (M^+): 130.04524; obsd: 130.04539.

Dihydro-5-propyl furan-2-thione (3.100): from dihydro-5-propyl-2(3*H*)-furanone, to give a yellow oil (67 mg, 47 %). R_f = 0.42 (5:1 hex-EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.9 Hz, 3H, CH_3-), 1.26-1.34 (m, 4H, -CH_2-), 1.87-1.92 (m, 1H, H_β), 2.38-2.42 (m, 1H, $H_{\beta'}$), 3.03 (dt, *J* = 18.8, 9.2 Hz, 1H, H_α), 3.17 (ddd, *J* = 18.9, 8.9, 3.9 Hz, 1H, $H_{\alpha'}$), 4.88 (p, *J* = 7.2 Hz, 1H, H_γ); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.3, 25.6, 31.0, 44.8, 87.0, 222.5; HRMS calcd for $C_7H_{12}OS$ (M^+): 144.06089; obsd: 144.06099.

Dihydro-5-octyl-furan-2-thione (3.105): from dihydro-5-octyl-2(3*H*)-furanone, to give a yellow oil (173 mg, 81 %). R_f = 0.58 (5:1 hex-EtOAc). IR (υ max) 1273 (C=S) cm^{-1} (for dihydro-5-octyl-2(3*H*)-furanone: IR (υ max) 1769 (C=O) cm^{-1}); ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.9 Hz, 3H, CH_3-), 1.27-1.35 (m, 14H, -CH_2-), 1.87-1.92 (m, 1H, H_β), 2.34-2.37 (m, 1H, $H_{\beta'}$), 3.01 (dt, *J*=18.9, 9.1 Hz, 1H, H_α), 3.16 (ddd, *J* = 18.9, 9.0, 3.8 Hz, 1H, $H_{\alpha'}$), 4.86 (p, *J* = 7.3 Hz, 1H, H_γ); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 25.3, 29.2, 29.3, 29.4, 29.6, 31.8, 34.9, 44.9, 91.1, 222.6; HRMS calcd for $C_{12}H_{22}OS$ (M^+): 214.13914; obsd: 214.13925.

3.7.2 Simultaneous synthesis of γ-lactones and γ-thionolactones

Formation of β,γ-unsaturated acids: Malonic acid (1.18 g, 10 mmol, 10 equiv.) was added to a solution of hexanal (120 μL, 100 mg, 1 mmol, 1 equiv.), heptanal (140 μL, 115 mg, 1 mmol, 1 equiv.), octanal (160 μL 131 mg, 1 mmol, 1 equiv.), nonanal (170 μL, 141 mg, 1 mmol, 1 equiv.), and decanal (190 μL, 158 mg, 1 mmol, 1 equiv.) in DMSO (20 mL). Piperidinium acetate (100 μL of the solution prepared as described above, ~0.1 mmol) was added and the mixture was stirred at room temperature for 20 min, then heated at 100 °C for 5 h. The mixture was cooled, diluted with Et_2O (20 mL) and washed with ice cold 10 % HCl (20 mL). The organic layer was extracted with 5 % NaOH (20 mL), washed with Et_2O (20 mL) and then acidified by the addition of 10 % HCl (20 mL). The mixture of acids was then extracted with Et_2O (2 x 20 mL), dried over $MgSO_4$, filtered and concentrated to give a colourless oil (five β,γ-unsaturated acids: 730 mg, 86 %) that was used without purification in the next step.

Lactonisation: A mixture of the five β,γ-unsaturated carboxylic acids obtained above (610 mg, ~0.7 mmol each of the five β,γ-unsaturated carboxylic acids) was dissolved in heptane (10 mL) and Amberlyst-15 (610 mg) was added. The mixture was heated at reflux for 1 h, cooled, filtered (washing the resin well with Et_2O), and concentrated. The residue was purified by chromatography (5:1 hex-EtOAc) to give a mixture of five γ-lactones as a yellow oil (480 mg; 79 %); MS obsd. for $C_8H_{15}O_2$ (M^+): 142; MS obsd. for $C_9H_{17}O_2$ (M^+): 156; MS obsd. for $C_{10}H_{19}O_2$ (M^+): 170; MS obsd. for $C_{11}H_{21}O_2$ (M^+): 184; MS obsd. for $C_{12}H_{23}O_2$ (M^+): 198.

Thionation: A mixture of the five γ-lactones (339 mg, ~0.36 mmol each, 1 equiv. each) and Lawesson's reagent (1.62 g, 4 mmol, 10 equiv.) in dry toluene (60 mL) was heated at reflux for 4 h. After cooling, filtration and evaporation the residue was purified by chromatography (5:1 hex-EtOAc) to give a mixture of five γ-thionolactones (278 mg, 73 %) as a yellow oil: R_f = 0.68 (5:1 hex-EtOAc); MS obsd. for $C_8H_{14}OS$ (M^+): 158; MS obsd. for $C_9H_{16}OS$ (M^+): 172; MS obsd. for $C_{10}H_{18}OS$ (M^+): 186; MS obsd. for $C_{11}H_{20}OS$ (M^+): 200; MS obsd. for $C_{12}H_{22}OS$ (M^+): 214.

Formation of α-substituted β,γ-unsaturated acids: Et_2NH (25 mmol, 25 equiv.) was added to a solution of hexanal (120 μL, 100 mg, 1 mmol, 1 equiv.), heptanal (140 μL, 115 mg, 1 mmol, 1 equiv.), octanal (160 μL 131 mg, 1 mmol, 1 equiv.), nonanal (170 μL, 141 mg, 1 mmol, 1 equiv.), and decanal (190 μL, 158 mg, 1 mmol, 1 equiv.) in CH_2Cl_2 (20 mL). The mixture was heated at reflux for 1 h, and then an α-substituted malonic acid (10 mmol, 10 equiv., methylmalonic acid or ethylmalonic acid) was added to the mixture. The reaction mixture was heated at reflux overnight. The mixture was cooled, diluted with Et_2O (20 mL) and washed with ice cold 10 % HCl (20 mL). The organic layer was

extracted with 5 % NaOH (20 mL), washed with Et_2O (20 mL) and then acidified by the addition of 10 % HCl (20 mL). The mixture of acids was then extracted with Et_2O (2 x 20 mL), dried over $MgSO_4$, filtered and concentrated to give a colourless oil (five α-methyl β,γ-unsaturated acids: 343 mg, 37 %; five α-ethyl β,γ-unsaturated acids: 370 mg, 37 %) that was used without purification in the next step.

Lactonisation: The mixture of five β,γ-unsaturated carboxylic acids obtained above (343 mg, ~0.4 mmol each of the five α-methyl β,γ-unsaturated carboxylic acids; 370 mg, ~0.4 mmol each of the five α-ethyl β,γ-unsaturated carboxylic acids) was dissolved in heptane (10 mL) and Amberlyst-15 (the same amount by weight as the acid mixture) was added. The mixture was heated at reflux for 1 h, cooled, filtered (washing the resin well with Et_2O), and concentrated. The residue was purified by chromatography (5:1 hex-EtOAc) to give a mixture of five γ-lactones as a yellow oil (the five dihydro-3-methyl-5-alkyl-(3*H*)-furanones: 138 mg, 40 %, MS obsd. for $C_9H_{16}O_2$ (M^+): 156; MS obsd. for $C_{10}H_{18}O_2$ (M^+): 170; MS obsd. for $C_{11}H_{20}O_2$ (M^+): 184; MS obsd. for $C_{12}H_{22}O_2$ (M^+): 198; $C_{13}H_{24}O_2$ (M^+): 212; the five dihydro-3-ethyl-5-alkyl-(3*H*)-furanones: 130 mg, 35 %; MS obsd. for $C_{10}H_{18}O_2$ (M^+): 170; MS obsd. for $C_{11}H_{20}O_2$ (M^+): 184; MS obsd. for $C_{12}H_{22}O_2$ (M^+): 198; $C_{13}H_{24}O_2$ (M^+): 212; MS obsd. for $C_{14}H_{24}O_2$ (M^+): 226).

3.7.3 GC-MS and GC-O analyses

GC-MS: As described in Chapter 2.

GC-O: As described in Chapter 2, with the following modifications. The chromatograph was equipped with an Alltech Econo-Cap™ EC-1000™ (30 m x 0.53 mm with 1.20 μm film).

Chapter 4

Chapter 4: Syntheses of chiral lactones from amino acids

4.1 Introduction

Optically active γ-substituted butanolides [γ-substituted-2(*5H*)-furanones] and butenolides have emerged as important compounds in their own right, and as synthons for the synthesis of complex natural products. Examples include antibiotics, pheromones, antifungal[117] and flavour compounds of fruits, dairy products and fermented foods.[118]

(4*S*,5*S*)-Dihydro-4-methyl-5-butyl-2(*3H*)-furanone, (**4*S*,5*S*-4.1**, widely known as (-)-*cis*-Quercus lactone)[119] is isolated together with a isomer (**4*S*,5*R*-4.1**) from oak wood and aged spirits or wines stored in casks made from oak wood. 5-(*3E,6*-Heptadienyl)dihydro-2(*3H*)-furanone (**4.2**) is a melon-fly pheromone[120] and (-)-methylenolactocin (**4.3**)[121] and (-)-protolichesterinic acid (**4.4**)[122] are antitumour antibiotic lactones (Figure 4.1).

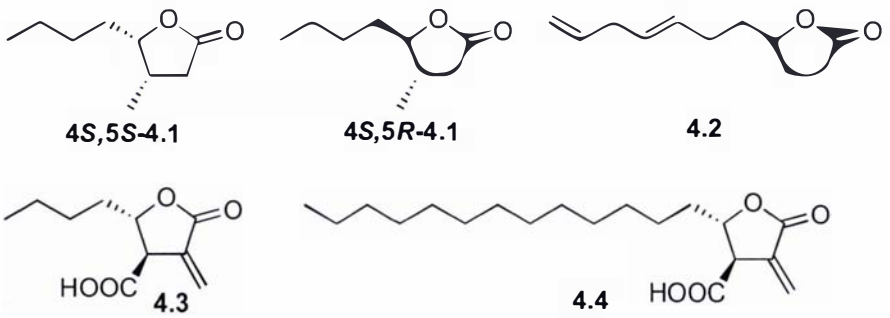
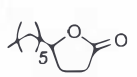
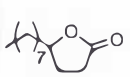
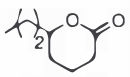
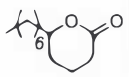


Figure 4.1: Chiral lactones isolated from nature.

The structures and stereochemistry of lactones in various cheeses have been analysed. Enantiomeric ratios for some γ- and δ-lactones (C_8-C_{18}) are listed in Table 4.1.[123] The enantiomeric distributions show an excess of the (*R*)-enantiomer for most lactones. Further investigation is warranted to determine the significance of each enantiomer toward the overall flavour of cheese. The synthesis of chiral γ-lactones is therefore the aim of this chapter.

Table 4.1: γ- and δ-Lactones (C_8-C_{18}) from various cheeses.[123]

Cheese Type	(R):(S) (%) of Lactones						
	γ-C_{10}	γ-C_{12}	δ-C_8	δ-C_{10}	δ-C_{12}	δ-C_{14}	δ-C_{16}
	 **1.50**	 **1.52**	 **3.107**	 **1.51**	 **1.53**	 **4.5**	 **4.6**
Parmesan	N/A	N/A	R>82	R>82	R>87	86:14	Trace
Cheddar	Trace	R>75	17:83	81:19	90:10	85:15	Trace
Limburger	Trace	85:15	78:22	78:22	R>87	83:17	Trace
Emmental	Trace	85:15	85:15	76:24	90:10	83:17	N/A
Blue	Trace	83:17	Trace	80:20	89:11	87:13	Trace

4.2 Previous syntheses of chiral γ-lactones

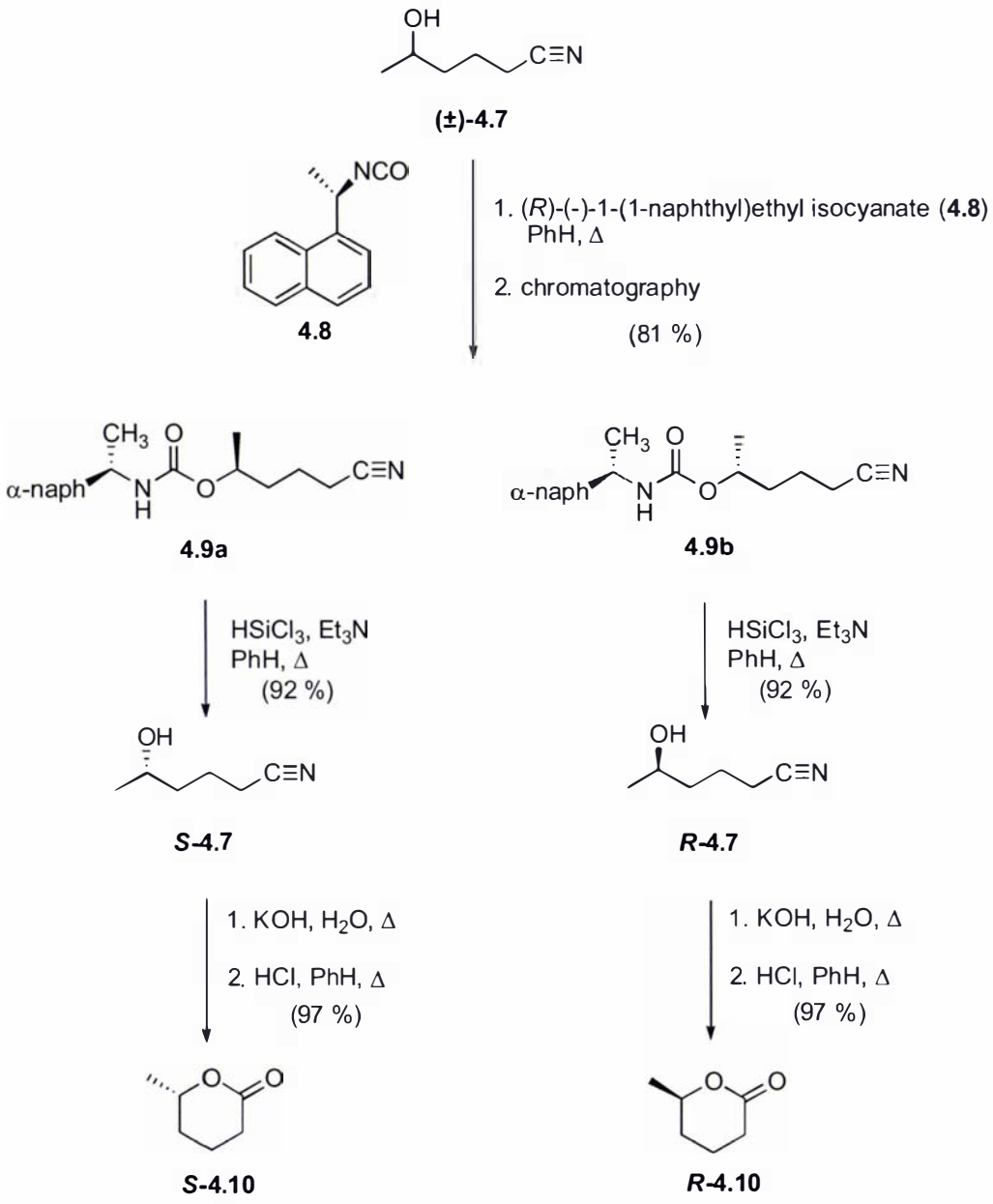
γ-Butyrolactones have been used as intermediates in the production of complex natural products. There have been many approaches to the synthesis of chiral γ-lactones. These syntheses can be classified into three categories, as follows:

- Resolution of racemic compounds;
- Asymmetric synthesis; and
- Synthesis where the stereochemistry is derived form the chiral pool.

4.2.1 Resolution

The classical approach to generating enantiomerically pure compounds is the resolution of racemic compounds by chemical or enzymatic methods. Several resolutions of lactones have been reported.

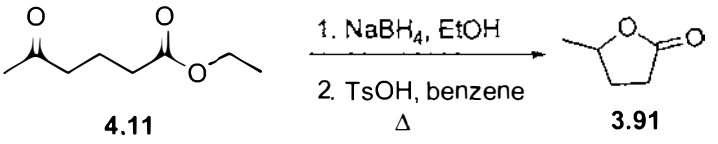
Pirkle and Adams[124] reported the isolation of enantiomerially pure cyano alcohols **4.7**. This was achieved by reaction of racemic cyano alcohol **(±)-4.7** and (*R*)-(-)-1-(1-naphthyl)ethyl isocyanate (**4.8**)[125] to afford diastereomeric cyano carbamates (**4.9a** and **4.9b**). The diastereomers were separated by acidic alumina chromatography and then individually silanolised to generate the enantiomeric cyano alcohols ***S*-4.7** and ***R*-4.7**. The cyanofunctionality of **4.7** was hydrolysed and lactonisation afforded the enantiomerically pure δ-lactones **4.10** (Scheme 4.1).



Scheme 4.1: Chemical resolution of cyano alcohols followed by hydrolysis and lactonisation.

Racemic γ-lactones can be resolved by enzyme-catalysed enantioselective hydrolysis. [126] For example, Fouque and Rousseau prepared a racemic lactone **3.91** from ethyl 5-oxo-hexanoate (**4.11**), by reduction of the carbonyl group and acid-catalysed lactonisation

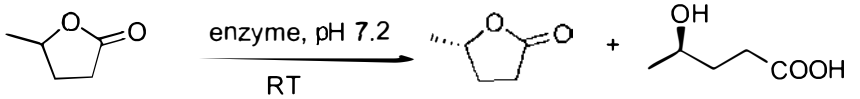
(Scheme 4.2).[127] Enzymatic resolution of the racemic lactone using pig liver esterase (PLE) and horse liver esterase (HLE) at room temperature in a buffered solution of sodium dihydrogenphosphate at pH 7.2 is summarised in Table 4.2.



4.11 **3.91**

Scheme 4.2: Synthesis of a racemic lactone **3.91** from ethyl 5-oxo-hexanoate (**4.11**).

Table 4.2: Enzymatic resolution of racemic lactones.

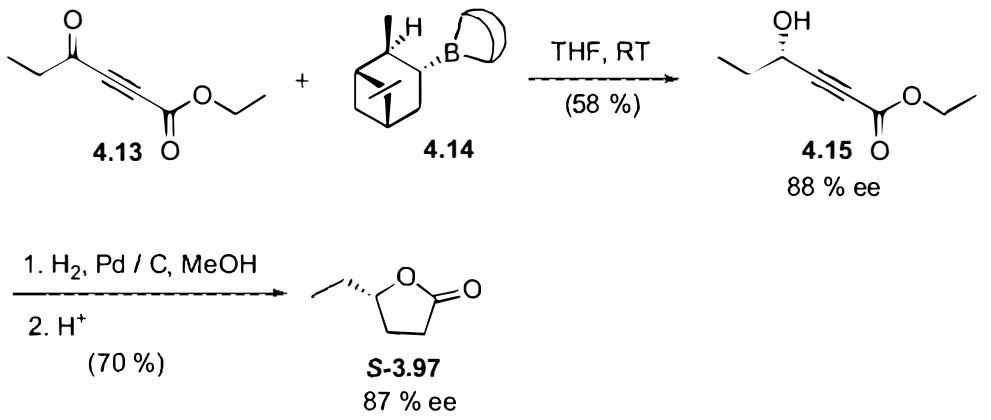


(±)-3.91 ***S*-3.91** ***R*-4.12**

enzyme	conversion (%)	time (h)	%ee of *S*-3.91	%ee of *R*-4.12
PLE	63	1	70	40
HLE	60	0.7	95	64

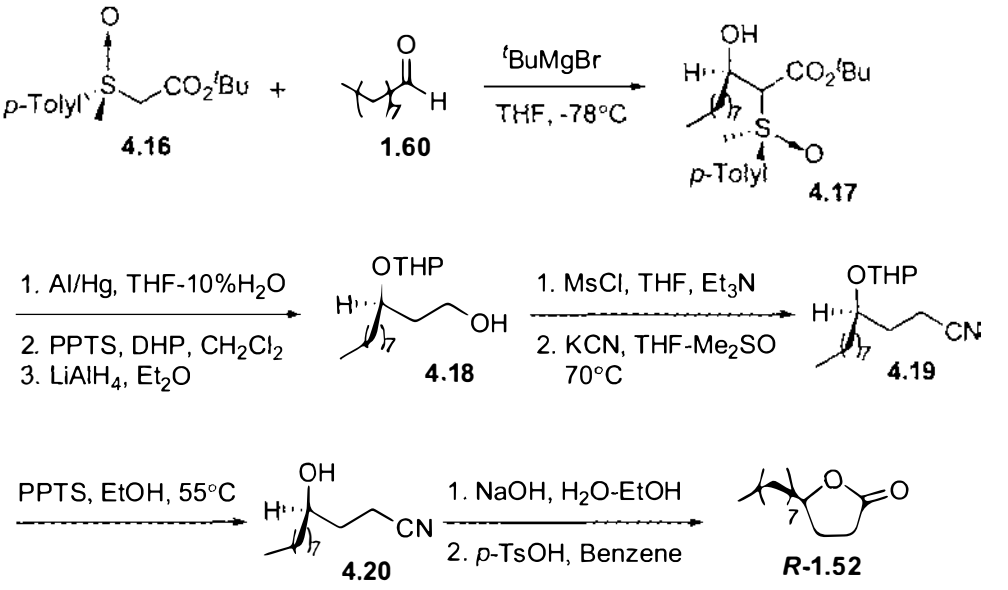
4.2.2 Asymmetric synthesis

Many syntheses of γ-lactones have used asymmetric reagents to introduce the chirality.[128] For example, Midland and Tramontano[129] synthesised an optically active γ-lactone *via* propargylic alcohol **4.15** (88 % ee), prepared by reduction of acetylenic ketone **4.13** in the presence of a chiral reagent, *B*-3-pinanyl-9-BBN (**4.14**).[130] The propargylic alcohol **4.15** was then hydrogenated and lactonised to give (5*S*)-dihydro-5-ethyl-2(3*H*)-furanone (***S*-3.97**) in 41 % overall yield (Scheme 4.3).



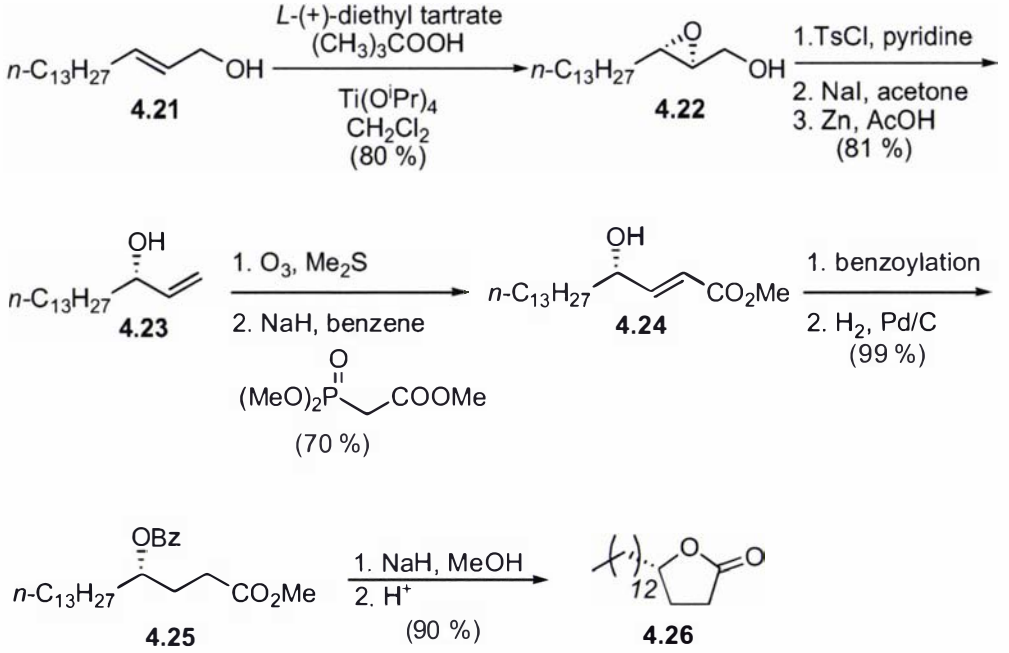
Scheme 4.3: Stereoselective reduction of a ketone in the generation of a chiral lactone.

In 1981, Solladie and Matloubi-Moghadam reported the synthesis of (5*R*)-dihydro-5-octyl-2(3*H*)-furanone (***R*-1.52**, Scheme 4.4) from chiral sulfoxides.[131] The synthesis of the γ-lactone began with the condensation of the anion of (*R*)-(+)-*tert*-butyl (*p*-tolylsulfinyl)acetate (**4.16**) and pelargonaldehyde (**1.60**). The crude adduct **4.17** was desulfurised with aluminium amalgam. Protection of the hydroxyl group with dihydropyran and reduction of the ester function gave a primary alcohol **4.18**. This was converted to the mesylate, and then the mesylate displaced with potassium cyanide to give **4.19**. Finally, after removal of the protecting group, the nitrile **4.20** was hydrolysed to the carboxyl group and the product was cyclised to give (5*R*)-dihydro-5-octyl-2(3*H*)-furanone (***R*-1.52**, Scheme 4.4)



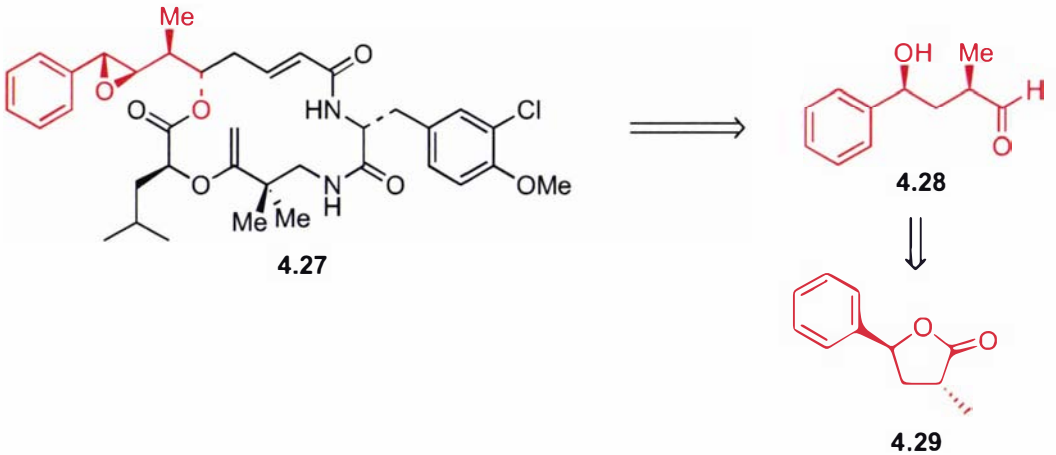
Scheme 4.4: Synthesis of(5*R*)-dihydro-5-octyl-2(3*H*)-furanone (***R*-1.52**).

In 2000, Martin synthesised both enantiomers of γ-lactone **4.26** starting from 2,3-epoxy alcohol **4.22**.[132] The chirality was introduced during the Sharpless asymmetric epoxidation of allylic alcohol **4.21**. This reaction typically leads to compounds with >95 % ee.[133] The epoxy alcohol **4.22** was transformed into the allylic alcohol **4.23** by reductive opening of the corresponding iodide obtained from the epoxy tosylate. The ozonolysis of **4.23** and homologation of the resulting aldehyde with the sodium salt of (trimethylphosphono)acetate yielded the γ-hydroxy α,β-unsaturated ester **4.24** that was benzoylated and hydrogenated to yield the saturated diester **4.25**. Basic hydrolysis, followed by acidification, afforded the desired lactone **4.26** in 40 % overall yield (Scheme 4.5).[134]



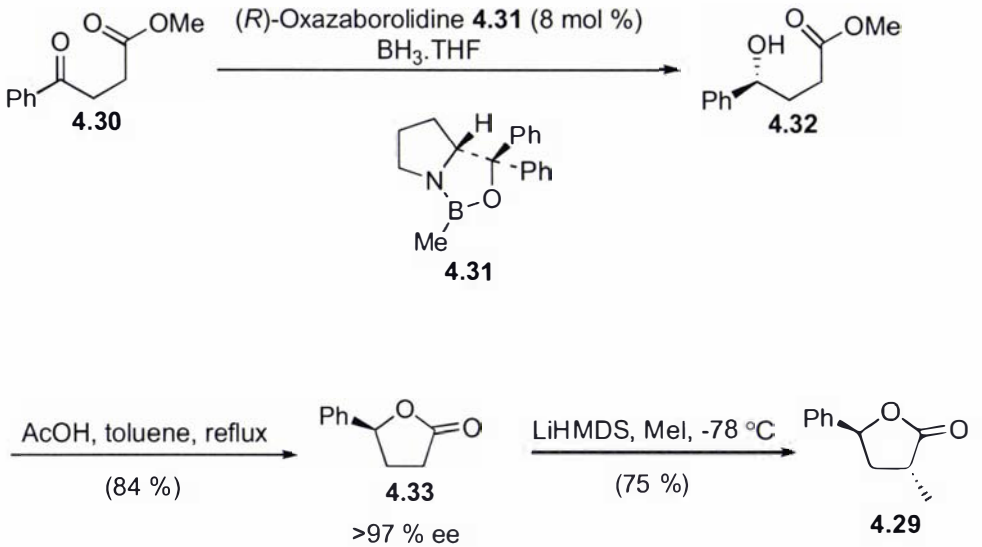
Scheme 4.5: Synthesis of a chiral γ-lactone **4.26** starting from allylic alcohol **4.21**.

In 2003, Gosh reported an enantioselective synthesis of (+)-cryptophytic 52 (**4.27**), a potent antibiotic, antismog agent.[135] One of the fragments in this synthesis (shown in red in Scheme 4.6) was obtained from (3*S*,5*R*)-dihydro-3-methyl-5-phenyl-(3*H*)-furanone (**4.29**).



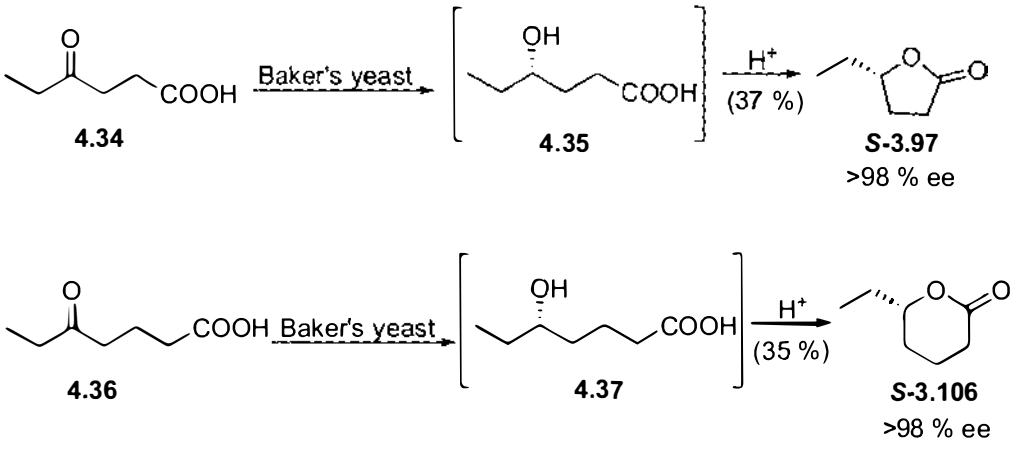
Scheme 4.6: Retrosynthetic analysis of (+)-cryptophytic (**4.27**); the key fragment in red.

The optically active γ-lactone **4.29** was prepared on a milligram scale utilising an enantioselective Corey-Bash-Shibata (CBS) reduction. Enantioselective CBS reduction of **4.30** with 8 mol % oxazaborolidine **4.31**, to produce the corresponding (*S*)-alcohol, was performed as described by Corey.[136] The resulting 4-hydroxyester **4.32** was lactonised by heating under reflux with catalytic acetic acid in toluene to give the lactone **4.33**. The 3-methyl group was introduced by alkylation of the lactone enolate (Scheme 4.7).



Scheme 4.7: Synthesis of chiral γ-lactone **4.29** by utilising an enantioselective Corey-Bakshi-Shibata (CBS) reduction.

Access to chiral γ-butyrolactones *via* reduction with fermenting bakers' yeast[137] has also been reported. The asymmetric reduction of γ- and δ-keto acids using bakers' yeast gave γ- and δ-lactones respectively with >98% ee (Scheme 4.8).



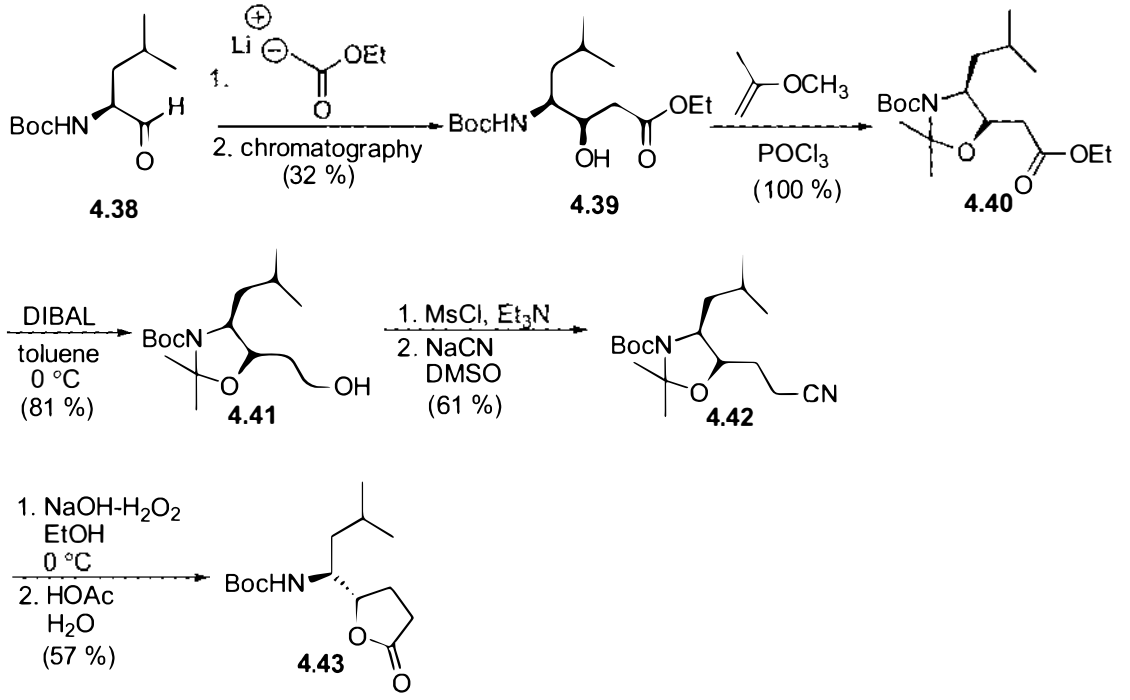
Scheme 4.8: Syntheses of chiral γ-butyrolactones *via* reduction with fermenting bakers' yeast.

4.2.3 The chiral pool as a source of starting materials for lactone synthesis

Chiral γ-lactones have also been synthesised using starting materials from the chiral pool, including such molecules as levoglucosenone,[138] ribonolactone,[139] glucose,[140] xylose,[141] tartaric acid[142] and glutamic acid.[143]

In 2001, Brewer and Rich[117c] synthesised a fully functionalized Phe-Arg hydroxyethylene isostere as a tripeptide derivative for inhibition of the botulinium family of neurotoxins. Enantiomerically pure γ-lactone **4.43** was synthesised as a precursor to the tripeptide derivative (Scheme 4.9).[144] Aldol condensation of α-lithioethyl acetate with *N*-Boc-*L*-leucinal (**4.38**) provided (3*R*,4*S*)-*N*-Boc-statine (48 %) and (3*R*,4*S*)-*N*-Boc-statine (32 %) (**4.39**) as a pair of diastereomers which could be separated by silica gel chromatography.[145] Protection of the hydroxyl and Boc-NH functional groups followed by reduction with diisobutylaluminum hydride, gave alcohol **4.41**. Cyanide displacement

of the mesylate derivative afforded nitrile **4.42** in 61 % yield. Hydrolysis and lactonisation followed, to give lactone **4.43** (Scheme 4.9).



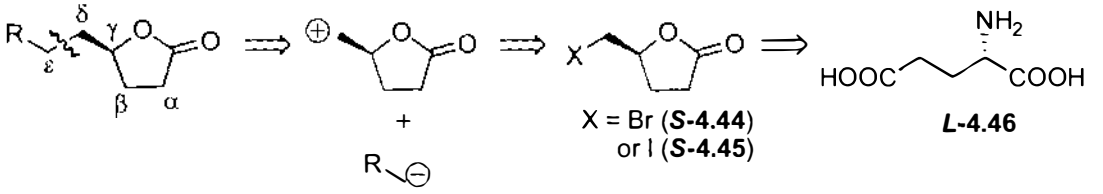
Scheme 4.9: Synthesis of chiral γ-lactone **4.43**, a precursor to a Phe-Arg hydroxyethylene isostere.

4.2.4 Aim of this chapter

The literature examples surveyed above give variable stereoselectivity in their generation of the γ-carbon stereogenic centre. Many examples are specific and the γ-substituent is introduced early in the synthetic sequence. For our purposes, flexibility in the introduction of the γ-substituent was desired, with unambiguous control of stereochemistry. Therefore, the synthesis of optically pure γ-butyrolactones, in a combinatorial fashion, presented a significant challenge.

4.3 Synthesis of chiral γ-lactones

The chemical synthesis of enantiomerically pure γ-lactones was inevitably going to require several more steps than the racemic approach presented in Chapter 3. According to the philosophy of Section 4.2.3, *L*- and *D*-glutamic acids were viewed as a promising source of the required stereogenicity (Scheme 4.10).

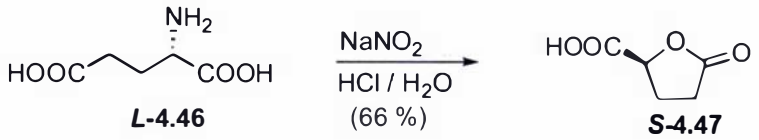


Scheme 4.10: Retrosynthetic analysis of chiral γ-lactones.

For the synthesis of a library of γ-lactones, with variation in the γ-side chain, we envisioned disconnection of the C_δ-C_ε bond (Scheme 4.10). The C_δ-cation synthon is realised by an alkyl halide. Both the bromide (***S*-4.44**) and the iodide (***S*-4.45**) are known compounds, available by well-established routes from glutamic acid. Since both enantiomers of glutamic acid are available at reasonable cost, it was possible to access both *R*- and *S*-lactones. A number of approaches were considered for the attachment of the side chain. These will be discussed in the following sections.

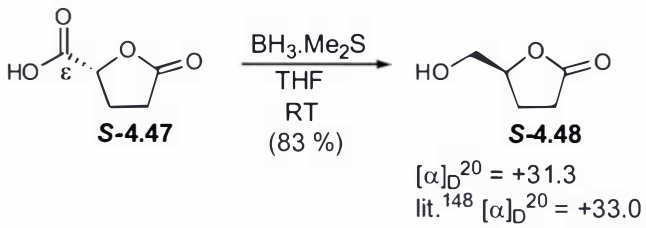
4.3.1 Syntheses of alkyl halides

(5*S*)-Dihydro-5-(hydroxymethyl)-2(3*H*)-furanone (***S*-4.47**) was prepared *via* appropriate modifications of a literature procedure from *L*-glutamic acid (***L*-4.46**).[146] Diazotisation of *L*-glutamic acid (***L*-4.46**) led to (5*S*)-2-oxotetrahydrofuran-5-carboxylic acid (***S*-4.47**) (Scheme 4.11).[147]



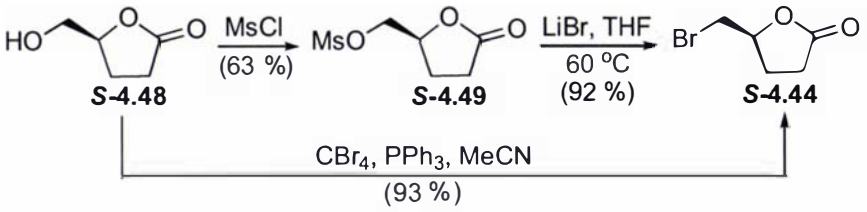
Scheme 4.11: Diazotisation of *L*-glutamic acid (***L*-4.46**).

The carboxylic acid in ***S*-4.47** was reduced with borane-dimethyl sulfide complex to give (5*S*)-dihydro-5-(hydroxymethyl)-2(3*H*)-furanone (***S*-4.48**, Scheme 4.12).[148] The lactone acid is very hygroscopic. It was found that azeotropic distillation with toluene was required, to remove water, in order to get a good yield of the primary alcohol ***S*-4.48**. The ^{1}H NMR spectrum of ***S*-4.48** exhibited two doublet of doublets at δ 3.67 and 3.89 ppm that were assigned to the diastereotopic protons at the δ-carbon. The ^{13}C NMR spectrum of ***S*-4.47** has a resonance at δ 173.4 that is characteristic of the carbonyl group at Cε. This signal is gone in the ^{13}C NMR spectrum of ***S*-4.48** and there is a new resonance at δ 63.7 ppm which is consistent with the $-CH_2OH$ functionality.



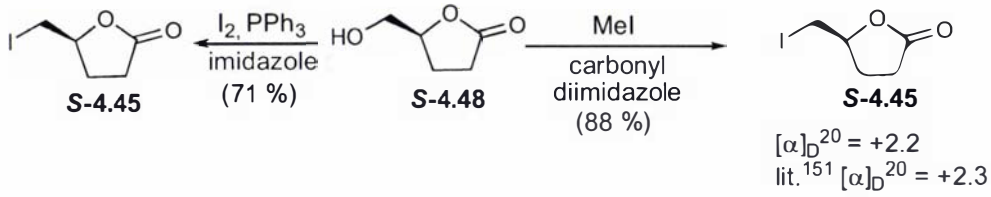
Scheme 4.12: Reduction of (5*R*)-2-oxotetrahydrofuran-5-carboxylic acid (***S*-4.47**).

Conversion of the primary alcohol ***S*-4.48** to the corresponding alkyl bromide ***S*-4.44** was approached in two ways. Displacement of the mesylate ***S*-4.49** with lithium bromide[149] gave (5*S*)-5-(bromomethyl)-2(3*H*)-furanone (***S*-4.44**) in 58 % overall yield. The transformation could be achieved in better yield in a single step, using carbon tetrabromide in combination with triphenylphosphine (Scheme 4.13).[150]



Scheme 4.13: Two routes to (5*S*)-5-(bromomethyl)-2(3*H*)-furanone (***S*-4.44**).

Formation of the analogous primary alkyl iodide ***S*-4.45** was accomplished with iodine, imidazole and triphenylphosphine.[151] Alternative conditions, using methyl iodide and carbonyl diimidazole,[152] gave a higher yield and a product that could be used without further purification (Scheme 4.14).



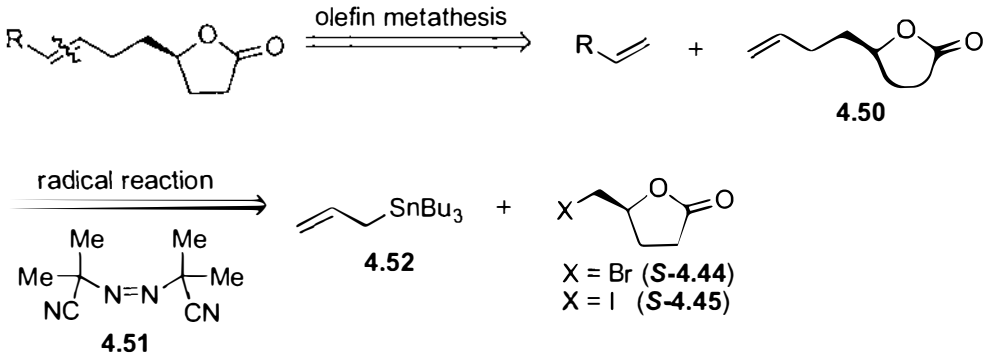
Scheme 4.14: Two routes to (5*S*)-5-(iodomethyl)-2(3*H*)-furanone (***S*-4.45**).

4.3.2 Elaboration of the γ-side chain

Several approaches were considered for attachment of the side chain.

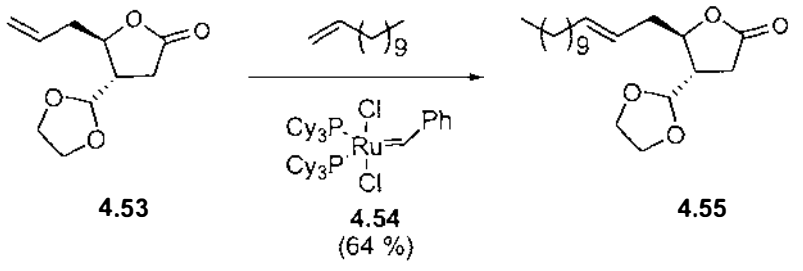
4.3.2.1 Via an olefinic handle

This retrosynthetic analysis of the γ-lactone begins with the olefin metathesis of the double bond to give compound **4.50** which has been reported previously (Scheme 4.15). Compound **4.50** can be prepared by the radical condensation of an alkyl halide and allyltributyl tin (**4.52**) in the presence of a catalytic amount of azobisisobutyronitrile (AIBN, **4.51**).[149] The alkyl halide was available from the previous section (Scheme 4.13 and 4.14 in Section 4.3.1).



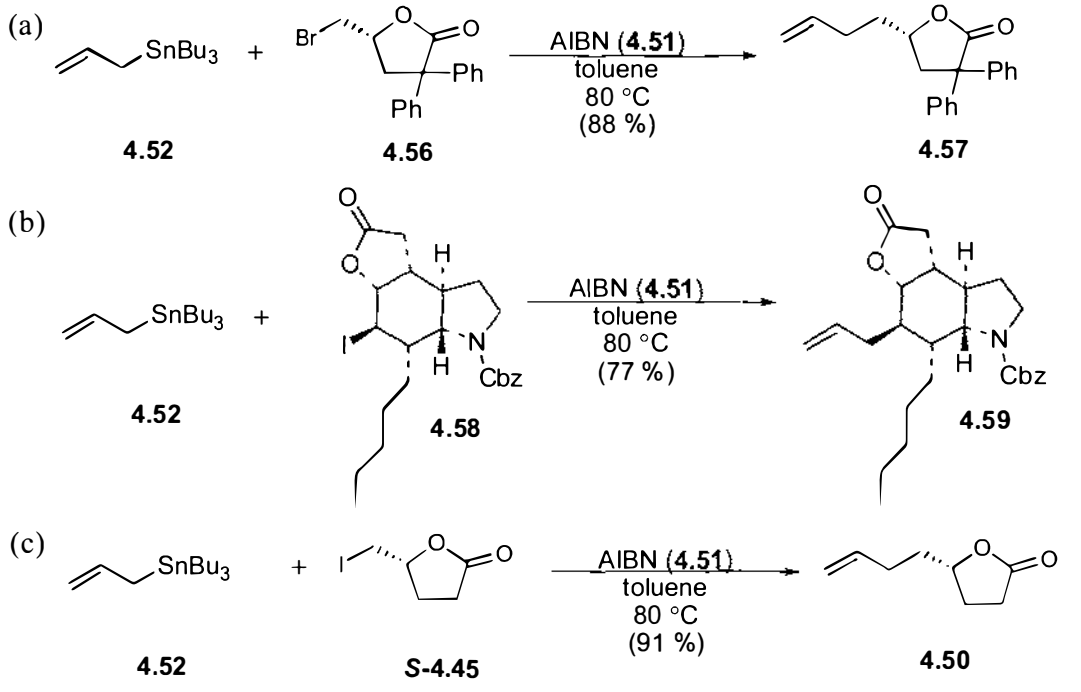
Scheme 4.15: Retrosynthetic analysis of the γ-lactone *via* an olefinic handle.

Precedent for the metathesis step was provided by the work of Reisner's group (Scheme 4.16).[153]



Scheme 4.16: Reisner's extension of the γ-side chain *via* olefin metathesis.

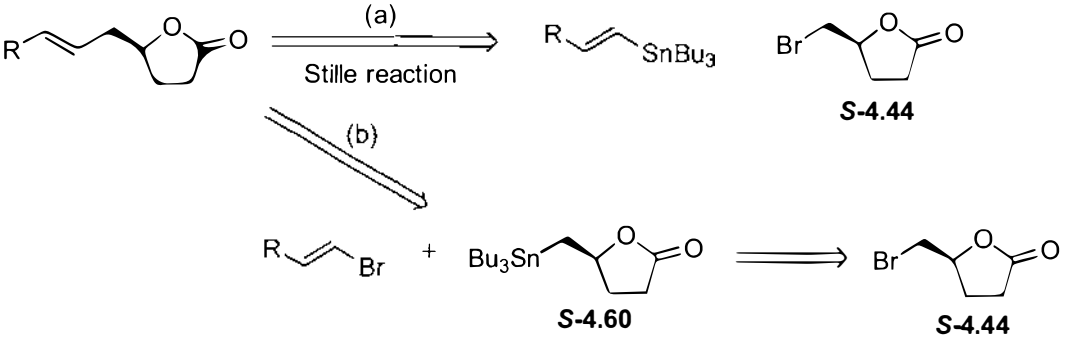
In 1982, Keck[154] reported the condensation of bromide **4.56** and allyltributyl tin in the presence of AIBN (**4.51**) to yield corresponding lactone **4.57** (Scheme 4.17a). Keck's method was applied in some other cases (Scheme 4.17b and 4.17c)[155] providing good precedent for our proposal. Keck's method was attempted with lactone ***S*-4.45** and was unsuccessful, recovering starting materials.



Scheme 4.17: The condensation of halides and allyltributyl tin in the presence of AIBN.

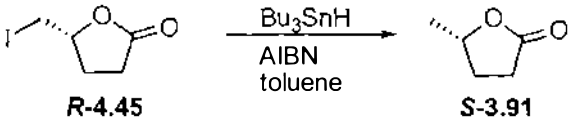
4.3.2.2 A Stille coupling

A retrosynthetic approach to a γ-lactone *via* a Stille coupling has two possible routes. Route (a) is a Stille coupling between a vinyltrialkyl tin reagent and alkyl halide **4.44**. This approach held little promise since the cross-coupling of C_{sp^3}-X electrophiles is not desirable due to slow transmetallation.[156]



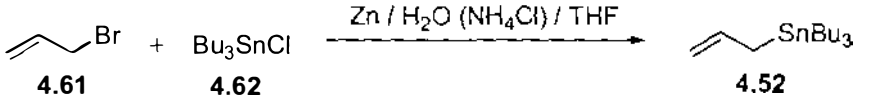
Scheme 4.18: Retrosynthetic approach to a γ-lactone *via* a Stille coupling.

Route (b) reverses the roles, with various vinyl halides and the lactone-based tin reagent ***S*-4.60** (Scheme 4.18). If we could make the lactone-based tin reagent, we could control the extension of the alkyl chain at the γ-position with a variety of commercially available vinyl bromides. SciFinder Scholar revealed one previous report of ***S*-4.60**. However, inspection of this reference revealed that Kosikowski *et al.* did not isolate the trialkyl tin species. It is a presumed intermediate in the reduction of ***S*-4.45** (Scheme 4.19).[157]



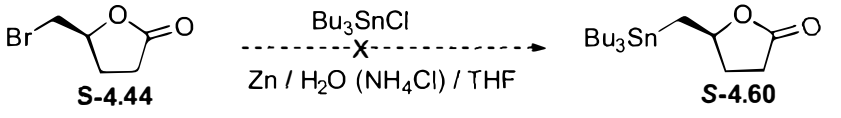
Scheme 4.19: Reduction of ***S*-4.45**.

While allyltributyltinhydride (**4.52**) is commercially available, the zinc-mediated, one-pot Wurtz-type reductive coupling reaction of alkyl halides (*e.g.*, **4.61**) with tributyltin chloride (**4.62**) could be expected to be a useful approach to the synthesis of more complex allylstannanes than **4.52** (Scheme 4.20).[158]



Scheme 4.20: The zinc-mediated one-pot Wurtz-type reductive coupling reaction.

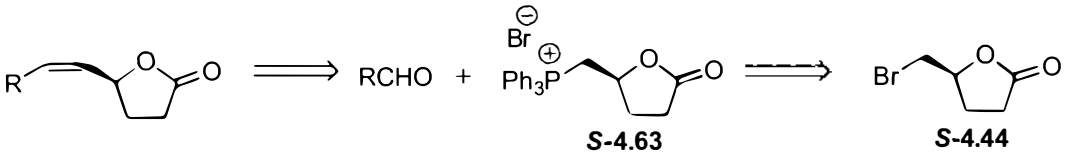
The one-pot synthesis Wurtz-type reductive coupling reaction of alkyl bromide ***S*-4.44** was tried and it was not successful (Scheme 4.21).



Scheme 4.21: Wurtz-type reductive coupling reaction of ***S*-4.44**.

4.3.2.3 A Wittig reaction

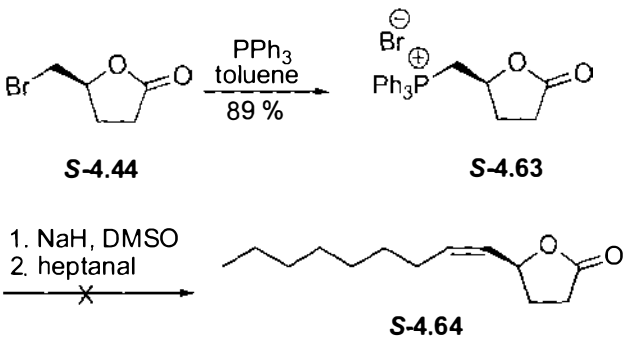
We proposed that γ-lactones could be prepared by a Wittig reaction between various aldehydes and the phosphonium salt which could be formed from the corresponding lactone halide (Scheme 4.22).



***S*-4.63** ***S*-4.44**

Scheme 4.22: Retrosynthetic approach to a γ-lactone *via* a Wittig reaction.

In our synthesis, the reaction of bromide ***S*-4.44** with triphenylphosphine yielded the phosphonium salt ***S*-4.63**. A multiplet was observed at δ 7.25-7.73 ppm in the ^{1}H NMR spectrum of the phosphonium salt ***S*-4.63**, which was assigned to the 15 protons of the triphenylphosphonium group. The phosphonium salt ***S*-4.63** was treated with dimsyl sodium, this choice was made on the basis of bases used previously in Wittig reactions.[159] Heptanal was then introduced to the reaction mixture (Scheme 4.23). The desired compound ***S*-4.64** was not produced. The phosphonium salt ***S*-4.63** was recovered from the aqueous layer.



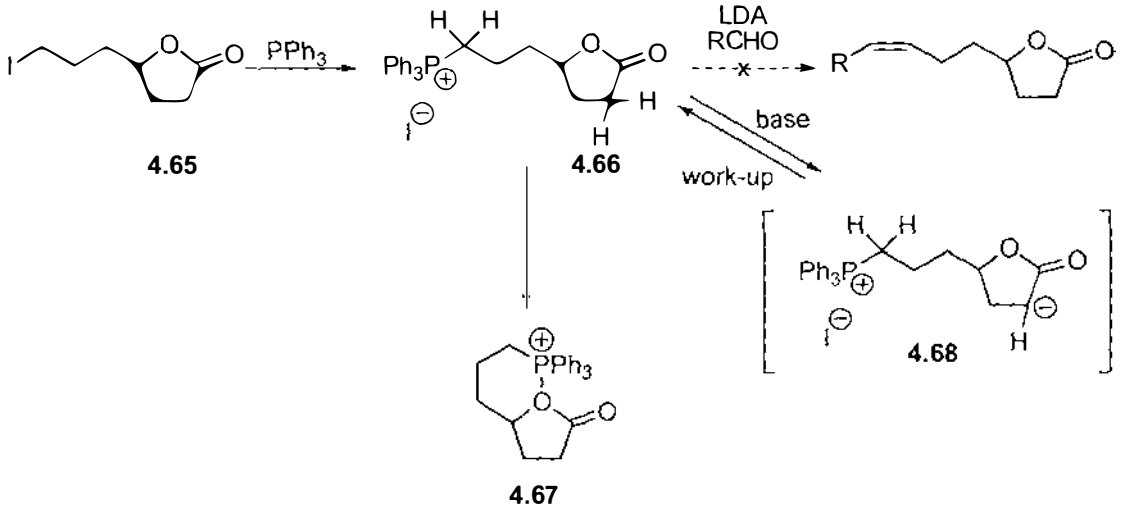
***S*-4.44** ***S*-4.63**

***S*-4.64**

Scheme 4.23: Wittig reaction of ***S*-4.44**.

Some other attempts at using Wittig chemistry in related systems gives insight into the failure of this reaction.

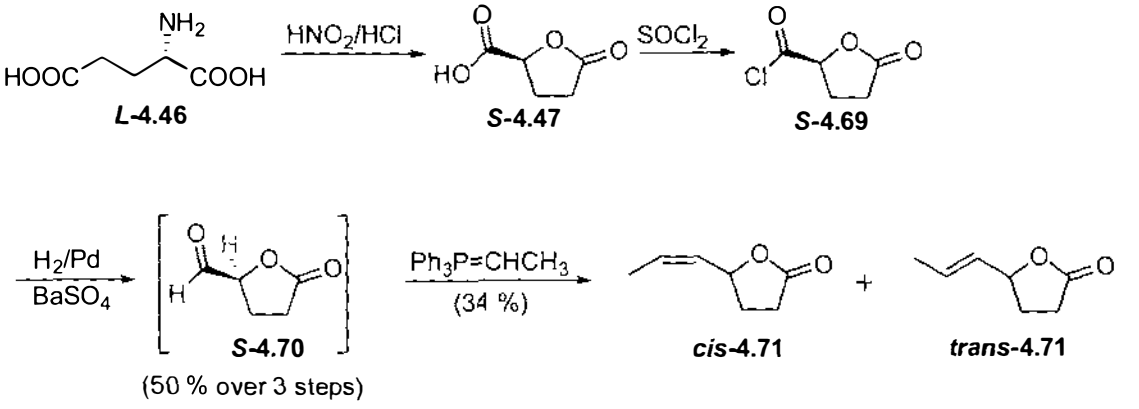
In 2003, Grayson synthesised the phosphonium salt **4.66** from the lactone iodide **4.65** with triphenylphosphine (Scheme 4.24).[160] Their Wittig reagent thus had two additional $-CH_2-$ units relative to compound **4.63** in our synthesis. The phosphonium salt **4.66** was then deprotonated and exposed to an aldehyde. This led to recovery of the phosphonium salt **4.66** from the aqueous phase following work-up of the reaction. They speculated that competitive deprotonation α to the lactone carbonyl function, or the intervention of intramolecular condensation reactions (formation of **4.67**), might account for the failure of the Wittig reaction.[161]



Scheme 4.24: Synthesis and proposed side reactions of phosphonium salt **4.66**.

We considered the possibility of swapping the roles of the lactone and side chain in the Wittig reaction. However, the work of Maurer and Hauser revealed other problems (Scheme 4.25).[81] Aldehyde **4.70** was prepared in 50 % overall yield in three steps from

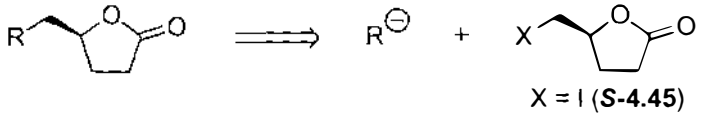
glutamic acid ***L*-4.46**. They had difficulty in characterising the lactone-aldehyde **4.70** because of its instability. The key step in this synthesis was the reaction of aldehyde **4.70** with a Wittig reagent to produce **4.71**. They obtained the final compound **4.71**, as a racemic mixture of 4:1 *cis-trans* isomers. The optical activity was lost *via* enolisation of the unstable aldehyde **4.70**.



Scheme 4.25: Maurer and Hauser's synthetic route to γ-lactones.

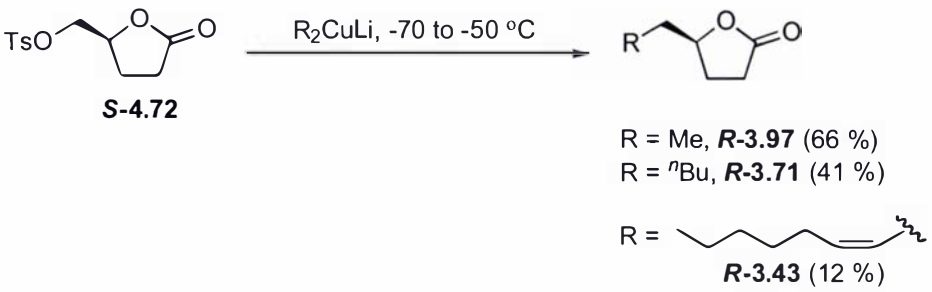
4.3.2.4 A cuprate displacement

The retrosynthetic approach which ultimately gave us a useful result, was a cuprate displacement[162] of lactone halide ***S*-4.45** (Scheme 4.26)



Scheme 4.26: Retrosynthetic approach to a γ-lactone *via* a cuprate displacement.

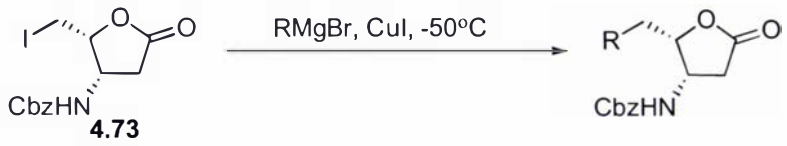
Silverstein and Ravid[163] reported the cuprate displacement of tosylate ***S*-4.72** to afford γ-lactones (***R*-3.97**, ***R*-3.71**, and ***R*-4.43**, Scheme 4.27). The *R*-isomers were also produced.



Scheme 4.27: Silverstein and Ravid's cuprate displacements of tosylate ***S*-4.72**.

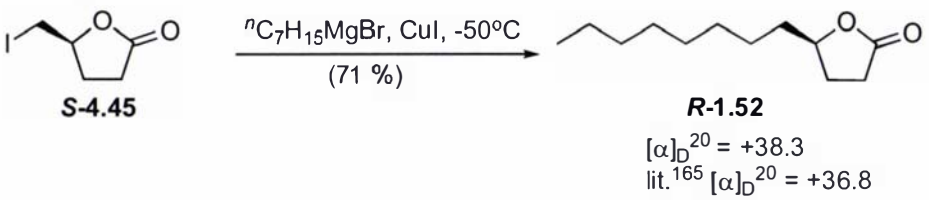
The cuprate displacement was widely applied and recently Monache elongated the γ-side chain of lactone iodide **4.73** using organocopper reagents, generated *in situ* from Grignard reagents, according to Table 4.3.[164]

Table 4.3: Cuprate additions reported by Monache.[164]



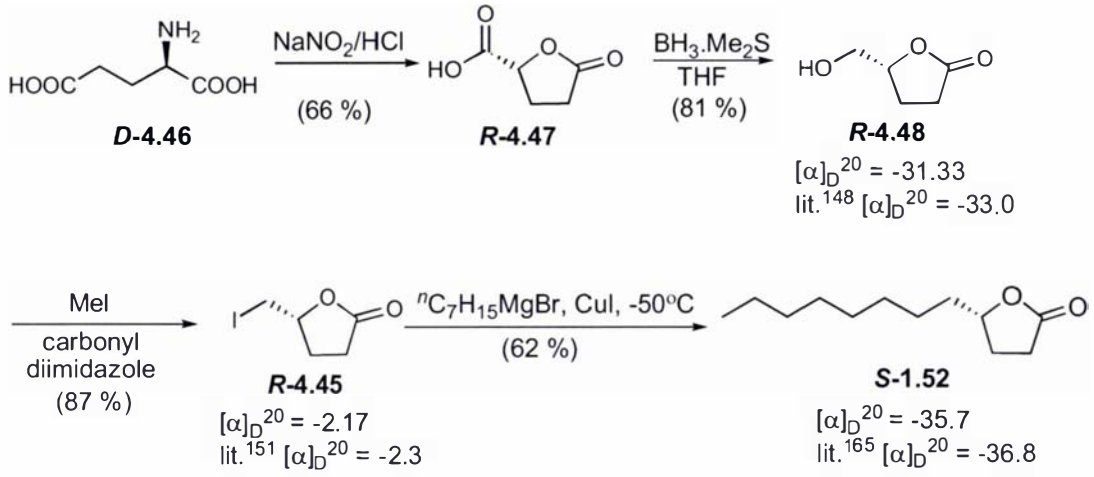
Compound	R	Yield
4.74	Ethyl	69 %
4.75	Dodecyl	72 %
4.76	Isopropyl	61 %
4.77	Cyclohexyl	75 %
4.78	Phenyl	<15 %

Following this precedent, (5*R*)-dihydro-5-octyl-2(3*H*)-furanone (***R*-1.52**) was synthesised according to Scheme 4.28. The cuprate was generated *in situ* from heptylmagnesium bromide. The purification of the copper iodide was essential to the success of this reaction.



Scheme 4.28: Formation of (5*R*)-dihydro-5-octyl-2(3*H*)-furanone (***R*-1.52**).

The ^{1}H NMR spectrum of lactone iodide ***R*-4.45** had a multiplet at δ 3.34 ppm which was assigned to protons of the diastereotopic -CH_2I group. This signal had gone in the spectrum of compound ***R*-1.52** and new upfield resonances at δ 0.88 and 1.27-1.88 ppm were consistent with the replacement of the electron-withdrawing halide by extension of the alkyl chain. High resolution mass spectrometry also supported the formation of compound ***R*-1.52**. The route from *L*-glutamic acid was thus successfully completed in an overall yield of 33 %. The analogous conversion of *D*-glutamic acid (***D*-4.46**) to furanone ***S*-1.52** was successful in an overall yield of 29 %. Each compound was analysed by NMR and its optical rotation measured (Scheme 4.29).

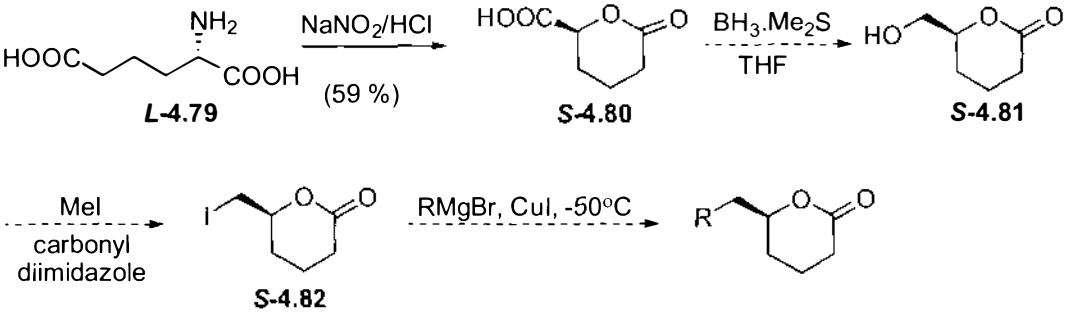


Scheme 4.29: Synthesis of (5*S*)-dihydro-5-octyl-2(3*H*)-furanone (***S*-1.52**) from *D*-glutamic acid (***D*-4.46**).

4.4 Attempted synthesis of chiral δ-lactones

We hoped we might extend this synthetic route to the synthesis of δ-lactones starting with *L*-α-aminoadipic acid (***L*-4.79**) which has an additional -CH_2- unit relative to glutamic acid. *L*-α-Aminoadipic acid is commercially available, although expensive. *D*-α-Aminoadipic acid (***D*-4.79**), required for (*R*)-δ-lactones, is not commercially available.

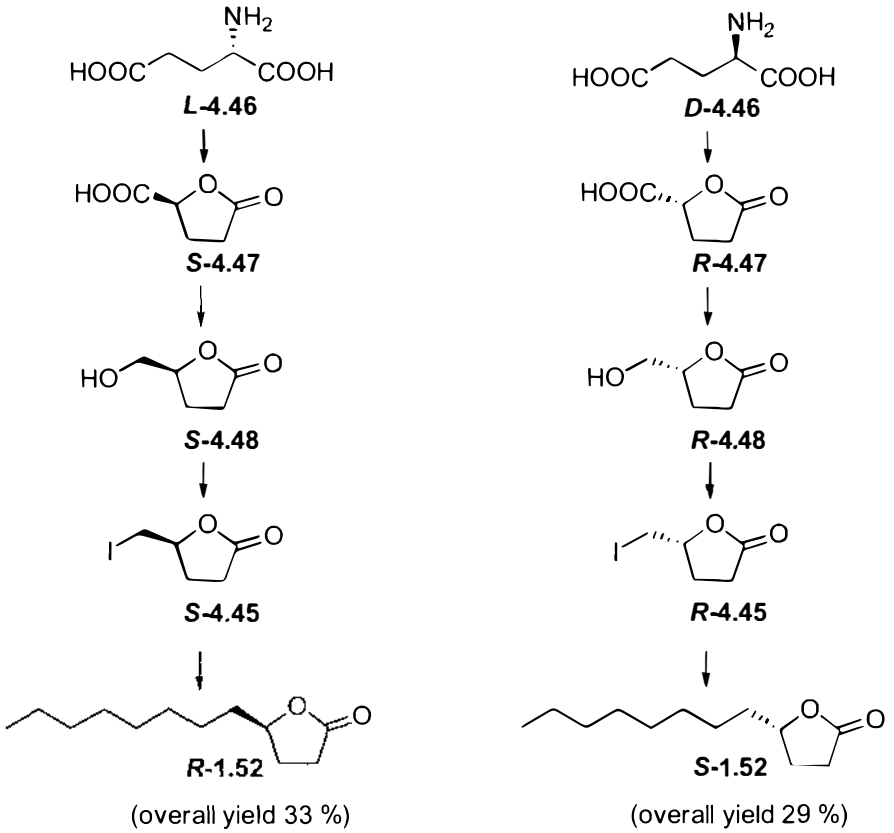
(6*S*)-(+)-Oxo-tetrahydro-*2H*-pyran-2-carboxylic acid (***S*-4.80**) was synthesised by diazotisation of *L*-α-aminoadipic acid. The reduction of the carboxylic acid ***S*-4.80** with borane-dimethyl sulfide complex did not give (*6S*)-(+)-6-hydroxymethyl-tetrahydro-*2H*-pyran ***S*-4.81** (Scheme 4.30). This synthetic route needs further investigation if we are to pursue the synthesis of chiral δ-lactones.



Scheme 4.30: Proposed synthetic route for the formation of chiral δ-lactones.

4.5 Summary

(5*R*)-Dihydro-5-octyl-2(3*H*)-furanone (***R*-1.52**) was synthesised from *L*-glutamic acid (***L*-4.46**) in four steps in 33 % overall yield (Scheme 4.31). The formation of the alkyl halide followed literature procedures, with each step being modified to optimise the yield. The last step, to attach the γ-side chain, was a big challenge as many reaction conditions were unsuccessful. The final choice was a cuprate displacement. The (*S*)-enantiomer (***S*-1.52**) was also synthesised by following the same sequence from *D*-glutamic acid (***D*-4.46**) in 29 % overall yield. Both series of compounds were characterised by ^{1}H and ^{13}C NMR spectroscopy, and optical rotation.



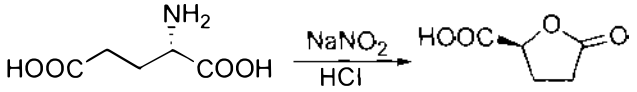
Scheme 4.31: Syntheses of both enantiomers of dihydro-5-octyl-2(3*H*)-furanone (***R*-1.52** and ***S*-1.52**).

4.6 Experimental procedures

4.6.1 General procedure

General methods as described earlier with the following exceptions. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. Acetonitrile (CH_3CN) was distilled from calcium hydride. Toluene was dried and distilled from sodium. Methanesulfonyl chloride (MsCl) was dried and distilled from phosphorous pentoxide. Copper iodide (CuI) was freshly purified by dissolving CuI in boiling saturated aqueous NaI over a period of 30 min. The mixture was cooled, diluted with water, filtered and washed sequentially with water, EtOH, EtOAc, Et_2O, and hexane and dried *in vacuo* for 24 h.

4.6.2 Experimental procedures and data

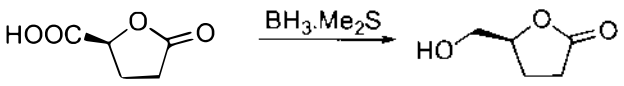


***L*-4.46** **S-4.47**

(5*S*)-2-Oxotetrahydrofuran-5-carboxylic acid (*S*-4.47): Hydrochloric acid (2N, 120 mL) was added to a stirred solution of *L*-glutamic acid (***L*-4.46**) (29.4 g, 0.2 mol, 1.0 equiv.) in water (200 mL). The resultant clear solution was cooled to 0 °C and a solution of sodium nitrite (16.6 g, 0.24 mol, 1.2 equiv.) in water (120 mL) was added dropwise with stirring over 15 min while carefully maintaining the solution at 0 °C. The pale yellow solution was stirred overnight at room temperature. Water was removed by distillation at 45 °C under reduced pressure. The residue was dissolved in ethyl acetate (250 mL) and anhydrous magnesium sulfate (30 g) added. The mixture was stirred for 2 h, filtered and concentrated to yield a clear yellow oil which solidified overnight. The solid was dissolved in ether (120 mL) and stirred at room temperature for 30 min, cooled

to –20 °C and stirred for 5 h. A yellow crystalline solid ***S*-4.47** (16.7 g, 64 %) was isolated by filtration: m.p. 70-72 °C (Lit.[147] m.p. 71–73 °C); R_f = 0.40 (10:1 CH_2Cl_2-MeOH); $[\alpha]_D^{20}$ = +15.8° (*c* 2.00, EtOH), Lit.[147] $[\alpha]_D^{20}$ = +15.6° (*c* 2.00, EtOH); ^{1}H NMR (400 MHz, CD_3OD) δ 2.33 (m, 2H, H-3), 2.61 (m, 2H, H-4), 5.04 (t, *J* = 6.1 Hz, 1H, H-5); ^{13}C NMR (100 MHz, CD_3OD) δ 26.8, 27.7, 77.4, 173.4, 179.1.

(5*R*)-2-Oxotetrahydrofuran-5-carboxylic acid (*R*-4.47) was prepared in an analogous fashion on a scale of 0.2 mol to give ***D*-4.46** as a yellow crystalline solid (17.2 g, 66 %): m.p. 71-73 °C (Lit.[147], m.p. 71–73 °C); R_f = 0.40 (10:1 CH_2Cl_2-MeOH); $[\alpha]_D^{20}$ = -15.7° (*c* 2.00, EtOH), Lit.[147] $[\alpha]_D^{20}$ = -15.6° (*c* 2.00, EtOH).



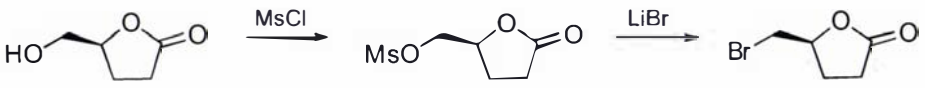
***S*-4.47** ***S*-4.48**

(5*S*)-Dihydro-5-(hydroxymethyl)-2(3*H*)-furanone (*S*-4.48): Borane-dimethyl sulfide complex (6 mL, 2.0 M in THF, 1.2 mol, 1.2 equiv.) was added dropwise, over a period of 30 min, to a stirred solution of ***S*-4.47** (dried by azeotropic distillation with toluene, 1.30 g, 10 mmol, 1.0 equiv.) in dry THF (50 mL) at room temperature. After stirring for 3 h, the reaction mixture was quenched by the cautious addition of anhydrous methanol (50 mL). The mixture was concentrated to give the crude product which was purified by chromatography (1:1 hex-EtOAc) to give ***S*-4.48** as a colourless oil (960 mg, 83 %): R_f = 0.27 (1:1 hex-EtOAc); $[\alpha]_D^{20}$ = +31.3° (*c* 3.00, EtOH), Lit.[148] $[\alpha]_D^{20}$ = +33.0° (*c* 3.00, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.11-2.20 (m, 1H, H-3), 2.24-2.34 (m, 1H, H-3'), 2.50-2.68 (m, 2H, H-4), 3.67 (dd, *J* = 12.5, 4.7 Hz, 1H, H-6), 3.89 (dd, *J* = 12.5, 2.9 Hz,

1H, H-6'), 4.63-4.68 (m, 1H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.0, 28.5, 63.7, 80.9, 178.2.

(5*R*)-Dihydro-5-(hydroxymethyl)-2(3*H*)-furanone (*R*-4.48) was prepared in an analogous fashion on a scale of 10 mmol to give ***R*-4.48** as a colourless oil (941 mg, 81 %). R_f = 0.27 (3:1 hex-EtOAc); $[\alpha]_D^{20}$ = -31.3° (*c* 3.00, EtOH), Lit.[148] $[\alpha]_D^{20}$ = -33.0° (*c* 3.00, EtOH).

(5*S*)-5-(Bromomethyl)dihydro-2(3*H*)-furanone (*S*-4.44):



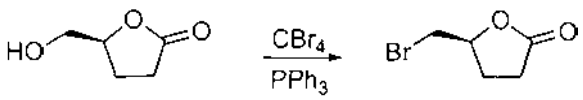
***S*-4.48** ***S*-4.49** ***S*-4.44**

Method A (*via* mesylate).

(5*S*)-5-(Mesyloxymethyl)dihydro-2(3*H*)-furanone (*S*-4.49): Methanesulfonyl chloride (155 μL, 229 mg, 2 mmol, 2 equiv.) was added to a stirred solution of ***S*-4.48** (116 mg, 1 mmol, 1 equiv.) and Et_3N (558 μL, 405 mg, 4 mmol, 4 equiv.) in CH_2Cl_2 (10 mL) at -30°C. The mixture was stirred at this temperature for 30 min, quenched with water (10 mL), and extracted with CH_2Cl_2 (3 x 20 mL). The organic layer was washed with brine (20 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by chromatography (1:3 hex-EtOAc) to give ***S*-4.49** as a colourless oil (122 mg, 63 %). R_f = 0.40 (1:3 hex-EtOAc). $[\alpha]_D^{20}$ = +32.5° (*c* 1.00, $CHCl_3$), Lit.[150] $[\alpha]_D^{20}$ = +33.3° (*c* 1.00, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 2.10-2.15 (m, 1H, H-3), 2.18-2.25 (m, 1H, H-3'), 2.49-2.54 (m, 2H, H-4), 3.01 (s, 3H, CH_3SO_2-), 4.23 (dd, *J* = 12.4, 4.5 Hz, 1H, H-6), 4.37

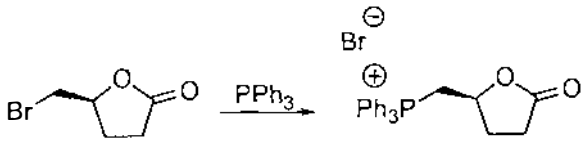
(dd, J = 12.5, 2.8 Hz, 1H, H-6'), 4.70-4.77 (m, 1H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 23.9, 29.2, 29.3, 29.4, 29.8, 31.9, 43.8, 209.1.

(5*S*)-5-(Bromomethyl)dihydro-2(3*H*)-furanone (*S*-4.44): Lithium bromide (109 mg, 1.2 mmol, 1.5 equiv.) was added to a stirred solution of ***S*-4.49** (122 mg, 0.6 mmol, 1 equiv.) in THF (3 mL). The solution was stirred at 60 °C overnight. The reaction was quenched by the addition of water (10 mL) and extracted with EtOAc (3 x 20 mL). The organic layer was washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated to give a residue which was purified by chromatography (1:3 hex-EtOAc) to give e ***S*-4.44** as a colourless oil (104 mg, 92 %). R_f = 0.40 (1:3 hex-EtOAc); $[\alpha]_D^{20}$ = +1.7° (*c* 2.70, $CHCl_3$), Lit.[150] $[\alpha]_D^{20}$ = +2.0° (*c* 2.70, $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.15 (m, 1H, H-3), 2.45 (m, 1H, H-3'), 2.62 (m, 2H, H-4), 3.52-3.61 (m, 2H, H-6), 4.73-4.80 (m, 1H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.1, 28.3, 34.1, 77.8, 176.2.



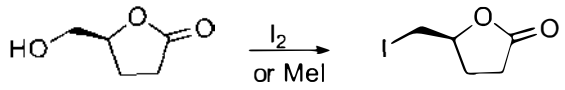
***S*-4.48** ***S*-4.44**

Method B. Triphenylphosphine (525 mg, 2 mmol, 2 equiv.) was added in portions to a stirred solution of ***S*-4.48** (116 mg, 1 mmol, 1 equiv.), K_2CO_3 (690 mg, 5 mmol, 5 equiv.) and CBr_4 (662 mg, 2 mmol, 2 equiv.) in CH_3CN (5 mL) at 0 °C. The mixture was stirred overnight at room temperature and concentrated. The residue was taken up in ether (10 mL) and filtered. The filtrate was evaporated, and the residue was purified by chromatography (1:3 hex-EtOAc) to give ***S*-4.44** as a yellow oil (167 mg, 93 %). Data as above.



***S*-4.44** ***S*-4.63**

[(5*S*)-5-(Methyl)dihydro-2(3*H*)-furyl]triphenylphosphonium bromide (*S*-4.63): The bromolactone ***S*-4.44** (180 mg, 1 mmol, 1 equiv.) was heated at reflux in toluene (5 mL) with PPh_3 (288 mg, 1.1 mmol, 1.1 equiv.) for 5 h. The solvent was decanted from the solid product that was then washed with warm toluene (2 x 10 mL). The solid was dried to give the crude phosphonium salt ***S*-4.63** as a pale yellow solid (393 mg, 89 %). A sample recrystallised from ethyl acetate had m.p. 138-142 °C: R_f = 0.40 (10:1 hex-EtOAc); $[\alpha]_D^{20}$ = +31.3° (*c* 1.00, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.05-2.15 (m, 1H, H-4), 2.37-2.47 (m, 1H, H-4'), 2.50-2.70 (m, 2H, H-3), 3.48-3.58 (m, 2H, H-6), 4.69-4.76 (m, 1H, H-5), 7.25-7.73 (m, 15H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.1, 28.3, 34.0, 77.8, 128.4, 128.6, 133.5, 133.7, 176.1; HRMS calcd. for $C_{23}H_{23}BrO_2P$ (MH^+): 441.06136; obsd: 441.07475.



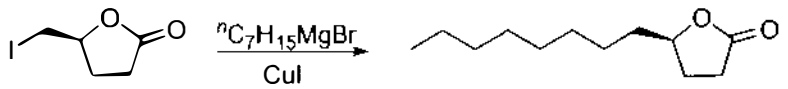
***S*-4.48** ***S*-4.45**

(5*S*)-5-(Iodomethyl)dihydro-2(3*H*)-furanone (*S*-4.45): Method A. Triphenylphosphine (524 mg, 2 mmol, 2 equiv.) was added to a stirred solution of ***S*-4.48** (116 mg, 1 mmol, 1 equiv.), iodine (508 mg, 2 mmol, 2 equiv.) and imidazole (136 mg, 2 mmol, 2 equiv.) in CH_3CN (10 mL) at 0 °C. The mixture was heated at reflux overnight. The reaction was cooled and the mixture was extracted with Et_2O (3 x 20 mL). The combined extracts were

washed with water (20 mL) and brine (20 mL), dried over $MgSO_4$, filtered and concentrated to give the crude product which was purified by chromatography (3:1 hex-EtOAc) to give ***S*-4.45** as a yellow oil (160 mg, 71 %): R_f = 0.33 (3:1 hex-EtOAc); $[\alpha]_D^{20}$ = +2.1° (*c* 2.4, CH_2Cl_2), Lit.[151] $[\alpha]_D^{20}$ = +2.3° (*c* 2.4, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 1.89-1.99 (m, 1H, H-3), 2.38-2.52 (m, 1H, H-3'), 2.53-2.65 (m, 2H, H-4), 3.28-3.38 (m, 2H, H-6), 4.47-4.54 (m, 1H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.9, 27.7, 28.6, 78.1, 176.0.

Method B. A stirred solution of ***S*-4.48** (116 mg, 1 mmol, 1 equiv.) in dry CH_3CN (5 mL) was treated with carbonyl diimidazole (324 mg, 2 mmol, 2 equiv.). After a clear solution was obtained, methyl iodide (0.31 mL, 5 mmol, 5 equiv.) was added. The mixture was stirred at room temperature for 30 min followed by heating under reflux for 2 h. The reaction was cooled and water (10 ml) was added. The mixture was extracted with EtOAc (3 x 20 mL). The combined extractions were washed with saturated aq. $Na_2S_2O_3$ (2 x 10 mL) and dried over $MgSO_4$. The mixture was filtered and concentrated *in vacuo* to give the crude product that was purified by chromatography (5:1 hex-EtOAc) to give ***R*-4.45** as a yellow oil (199 mg, 88 %). Data as above.

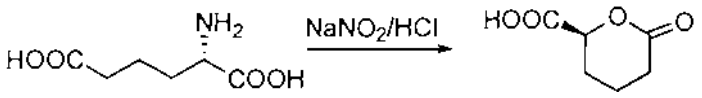
(5*R*)-5-(Iodomethyl)dihydro-2(3*H*)-furanone (*R*-4.45) was prepared in an analogous fashion on a scale of 1 mmol to give ***R*-4.45** as a yellow oil (197 mg, 87 %); R_f = 0.33 (3:1 hex-EtOAc); $[\alpha]_D^{20}$ = -2.2° (*c* 2.4, CH_2Cl_2), Lit.[151] $[\alpha]_D^{20}$ = -2.3° (*c* 2.4, CH_2Cl_2).



***S*-4.45** ***R*-1.52**

(5*R*)-Dihydro-5-octyl-2(3*H*)-furanone (*R*-1.52): Heptylmagnesium bromide (3 mL, 1M in Et_2O, 3 mmol, 3 equiv.) was added over 10 min to a suspension of CuI (286 mg, 1.5 mmol, 1.5 equiv.) in THF (10 mL) at -50 °C. The mixture was stirred at -50 °C for 1 h, then iodolactone ***R*-4.45** (226 mg, 1 mmol, 1 equiv.) in THF (5 mL) was added dropwise over 5 min. The mixture was stirred at -50 °C for 1.5 h. The reaction was quenched by the addition of saturated aq. NH_4Cl (20 mL), stirred for an additional 10 min and extracted with EtOAc (3 x 20 mL). The extracts were washed with brine (20 mL), filtered, dried over $MgSO_4$, and concentrated. The residue was purified by chromatography (3:1 hex-EtOAc) to give ***R*-1.52** as a yellow oil (141 mg, 71 %): R_f = 0.59 (3:1 hex-EtOAc); $[\alpha]_D^{20}$ = +38.3° (*c* 0.30, MeOH), Lit.[135] $[\alpha]_D^{20}$ = +36.8° (*c* 0.30, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3H, CH_3-), 1.27-1.88 (m, 14H, -CH_2-), 2.33 (app. sextet, *J* = 7.1 Hz, 2H, H-4), 2.54 (dd, *J* = 6.9, 2.5 Hz, 2H, H-3), 4.49 (p, *J* = 6.9 Hz, 1H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.9, 22.4, 25.1, 27.9, 28.8, 29.1, 29.2, 31.7, 33.9, 35.4, 81.0, 177.4; HRMS calcd. for $C_{12}H_{23}O_2$ (MH^+): 199.16981; obsd: 199.17021.

(5*S*)-Dihydro-5-octyl-2(3*H*)-furanone (*S*-1.52) was prepared in an analogous fashion on a scale of 1 mmol to give ***S*-1.52** as a yellow oil (123 mg, 62 %): R_f = 0.59 (3:1 hex-EtOAc). $[\alpha]_D^{20}$ = -35.7° (*c* 0.30, MeOH), Lit. [165] $[\alpha]_D^{20}$ = -36.8° (*c* 0.30, MeOH).



***L*-4.79** ***S*-4.80**

(6*S*)-6-Oxo-tetrahydro-*2H*-pyran-2-carboxylic acid (*S*-4.80): Hydrochloric acid (2N, 2.5 mL) was added to a stirred solution of *L*-α-aminoadipic acid (***S*-4.79**, 161 mg, 1 mmol, 1.0 equiv.) in water (16 mL). The resultant clear solution was cooled to 0 °C and a solution of sodium nitrite (110 mg, 1.6 mmol, 1.6 equiv.) in water (12 mL) was added dropwise with stirring over 15 min while carefully maintaining the solution at 0 °C. The pale yellow solution was stirred overnight at room temperature. Water was removed by distillation at 45 °C under reduced pressure. The residue was dissolved in ethyl acetate (20 mL) and anhydrous magnesium sulfate (3 g) added. The mixture was stirred for 2 h, filtered and concentrated to yield a clear yellow oil which solidified overnight. The solid was dissolved in ether (20 mL) and stirred at room temperature for 30 min, cooled to –20 °C and stirred for 5 h. A yellow crystalline solid ***S*-4.80** (85 mg, 59 %) was isolated by filtration: m.p. 102-104 °C; R_f = 0.21 (9:1 EtOAc-MeOH); $[\alpha]_D^{20}$ = +12.3° (*c* 1.00, H_2O); ^{1}H NMR (400 MHz, CD_3OD) δ 0.94–1.10 (m, 2H, H-4), 1.18–1.32 (m, 2H, H-5), 1.45–1.76 (m, 2H, H-3), 2.88 (t, *J* = 7.1 Hz, 1H, H-6), 7.68 (s, 1H, -COO<u>H</u>); ^{13}C NMR (100 MHz, CD_3OD) δ 21.6, 34.2, 34.4, 71.3, 175.6, 176.2; HRMS calcd. for $C_6H_9O_4$ (MH^+): 145.05008; obsd: 145.04959.

Chapter 5

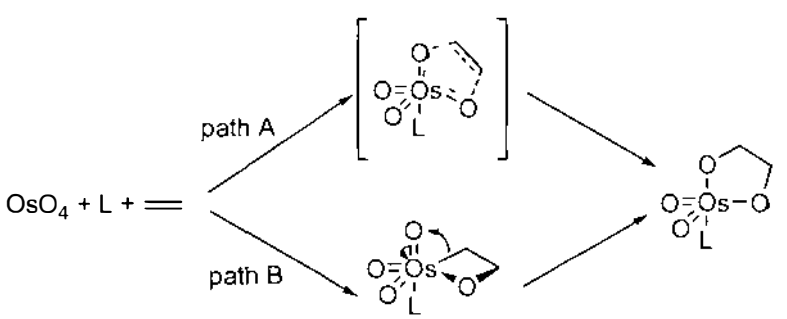
Chapter 5: Asymmetric synthesis of chiral γ-lactones utilizing the Sharpless asymmetric dihydroxylation reaction

5.1 Introduction

5.1.1 Background

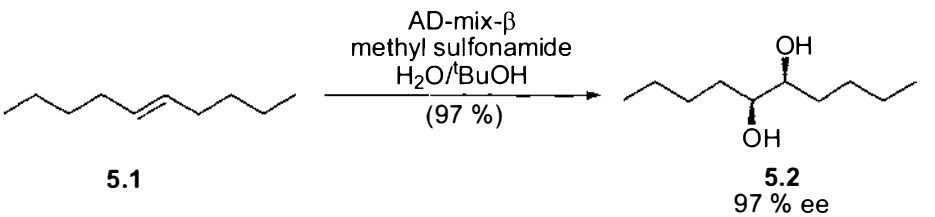
A number of asymmetric reactions have emerged in order to tackle the syntheses of biologically active chiral compounds. Some applications to the synthesis of γ-lactones were summarised in Chapter 4.

The osmium-catalysed dihydroxylation reaction has been investigated and two different mechanisms have been suggested. Böseken and Criegee[166] proposed a concerted [3+2] pathway (Scheme 5.1, path A) while Sharpless' group suggested a stepwise reaction (Scheme 5.1, path B).[167] Path B is initiated by a [2+2]-like addition of the olefin across an Os=O bond followed by rearrangement of the resulting osmaoxetane intermediate.



Scheme 5.1: Two proposed mechanisms for the osmium-catalysed dihydroxylation.

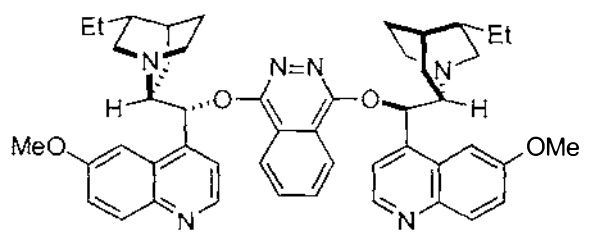
The mild and stereoselective Sharpless' asymmetric dihydroxylation (AD) has been widely adopted in organic synthesis and a typical example is in Scheme 5.2. [168]



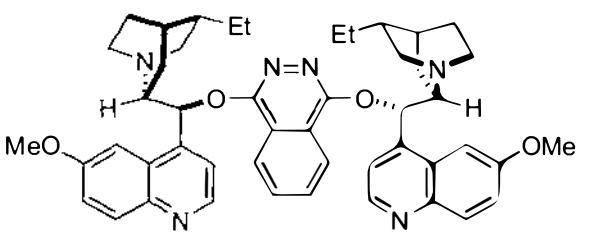
5.1 **5.2**
97 % ee

Scheme 5.2: Sharpless' asymmetric dihydroxylation of olefins.

The AD-mix formulation was optimised by trialling a number of reaction conditions. The key elements are the osmium salt and trace amounts of the phthalazine class of ligands (**5.3α**, **5.3β**) (Figure 5.1).



$(DHQ)_2$-PHAL (**5.3α**)
Ligand used in AD-mix-α

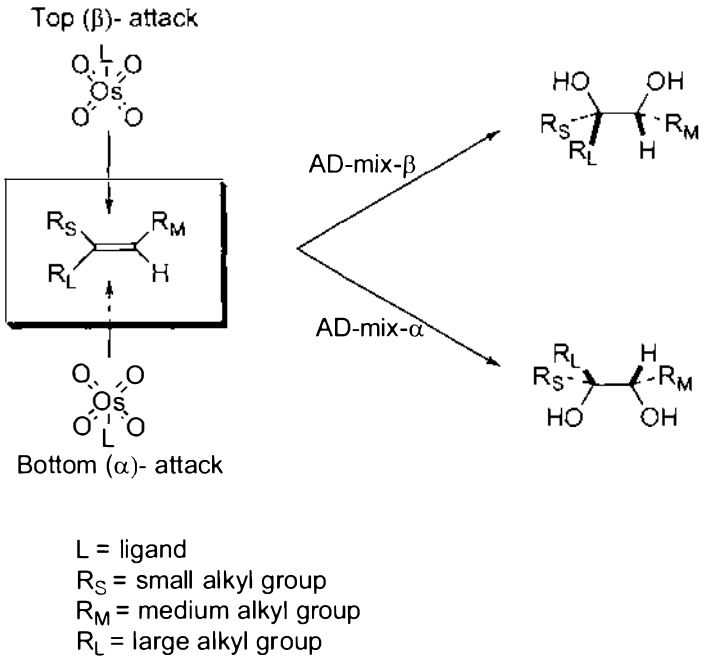


$(DHQD)_2$-PHAL (**5.3β**)
Ligand used in AD-mix-β

Figure 5.1: $(DHQ)_2$PHAL (**5.3α**) and $(DHQD)_2$PHAL (**5.3β**).

The osmium-ligand ensemble can be regarded as a chiral oxygen-donating group. The prochiral double bond can be attacked from either of two faces, with discrimination being provided by the bulky asymmetric ligands. The dihydroquinine derivative $(DHQ)_2$PHAL

(**5.3α**), attacks from the bottom face (*i.e.*, the α-face), and the dihydroquinidine complex containing $(DHQD)_2PHAL$ (**5.3β**), attacks an olefinic group from the top face (*i.e.*, the β-face) (Scheme 5.3).

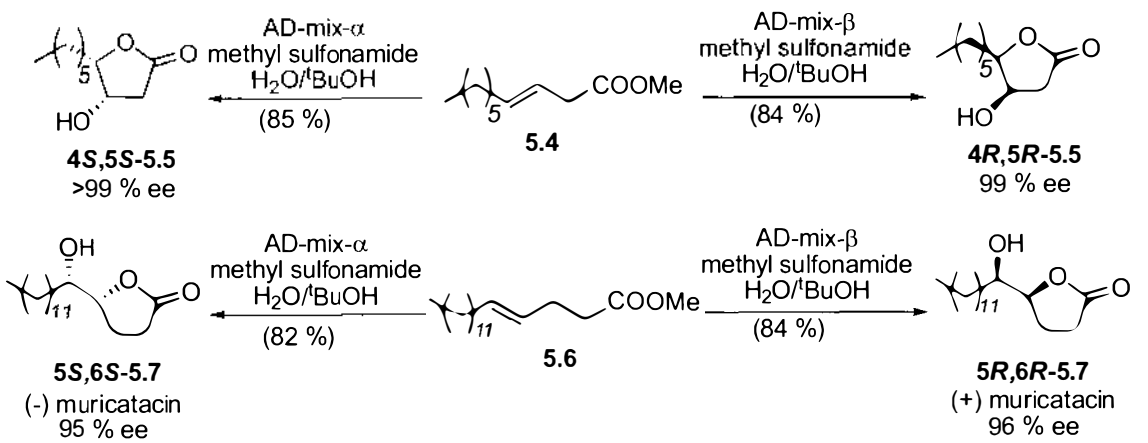


Scheme 5.3: Enantiofacial selectivity of AD reaction.

The AD-mix formulation is prepared with $K_3Fe(CN)_6$ (3.0 equiv.), K_2CO_3 (3.0 equiv.), $K_2OsO_2(OH)_4$ (0.2 mol %) and $(DHQ)_2PHAL$ (1 mol %) for AD-mix-α or $(DHQD)_2PHAL$ (1 mol %) is for AD-mix-β. These reagents are ground together to give a fine powder and kept dry. These two AD-mix formulations are now available from Aldrich.

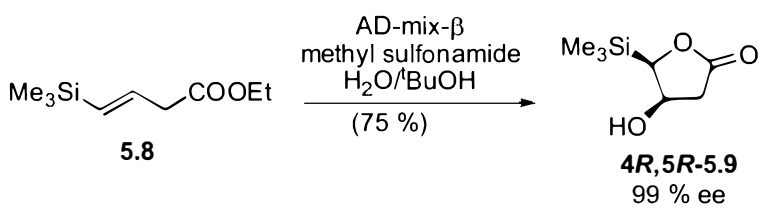
5.1.2 Previous applications of Sharpless' asymmetric dihydroxylation to the synthesis of γ-lactones

In 1992, Sharpless' group reported the asymmetric dihydroxylation of β,γ- and γ,δ-unsaturated esters gave 4-hydroxyl γ-lactones and muricatacins, respectively (Scheme 5.4).[169]



Scheme 5.4: The asymmetric dihydroxylation of β,γ- and γ,δ-unsaturated esters.

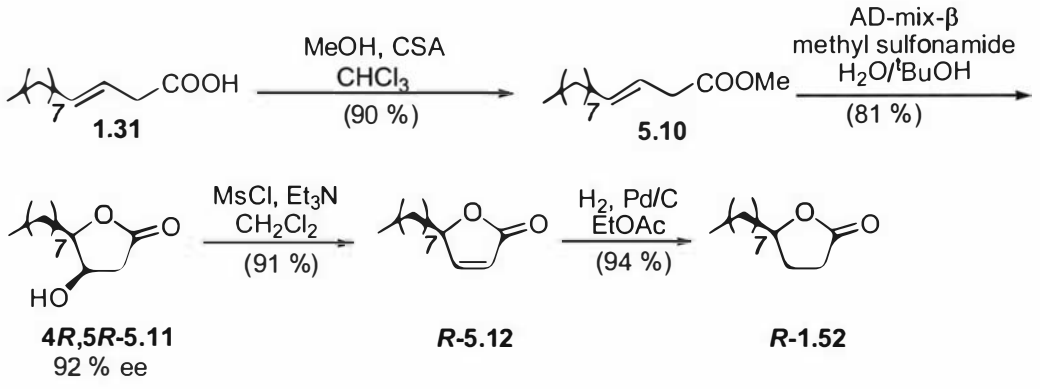
Sato's group reported the synthesis of a chiral building block, (4*R*,5*R*)-4,5-dihydro-4-hydroxy-5-trimethylsilyl-2(3*H*)-furanone (**4*R*,5*R*-5.9**), with AD-mix-β (Scheme 5.5).[170]



Scheme 5.5: Synthesis of a chiral building block **4*R*,5*R*-5.9**.

For the purposes of the current investigation, the most relevant example of the synthesis of enantiopure lactones was reported by Harcken and Bruckner.[171] Their synthesis began with a *trans*-β,γ-unsaturated acid (**1.31**) that was transformed into its methyl ester (**5.10**). Under the conditions of the dihydroxylation, a β-hydroxy-γ-lactone **4*R*,5*R*-5.11** was

obtained. This was dehydrated with mesyl chloride and triethylamine. The saturated γ-lactone ***R*-1.52** was obtained by palladium-catalysed hydrogenation of the double bond (Scheme 5.6).

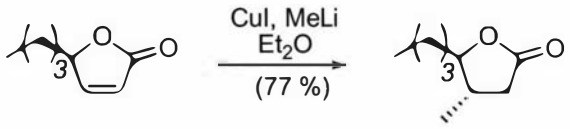


1.31 **5.10**

4*R*,5*R*-5.11 92 % ee **R-5.12** ***R*-1.52**

Scheme 5.6: Synthesis of enantiopure lactone ***R*-1.52**.

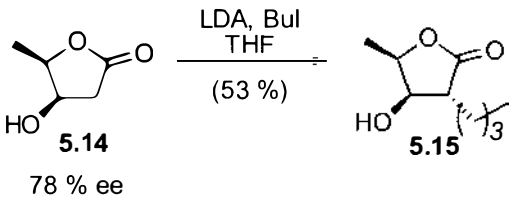
Harcken and Bruckner[147] reported the elaboration of intermediates in Scheme 5.4 to give chiral disubstituted **4*S*,5*R*-4.1**. Lithium dimethyl cuprate added to the butenolide (***R*-5.13**) to give the desired *trans*-configured 1,4-addition product **4*S*,5*R*-4.1** and none of its *cis* isomer (Scheme 5.7).



***R*-5.13** **4*S*,5*R*-4.1**

Scheme 5.7: Cuprate addition of the butenolide ***R*-5.13**.

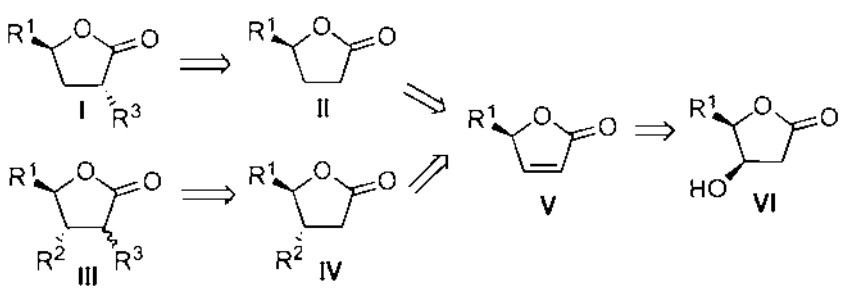
Furthermore, their report described the synthesis of trisubstituted γ-lactones. Compound **5.14** was accessed by analogy to reactions depicted in Scheme 5.4. The α-substituted lactone **5.15** was synthesised by the α-alkylation of dilithiated β-hydroxy-γ-lactone **5.14**. The α-substituent was oriented *trans* to the β-OH group (Scheme 5.8).



Scheme 5.8: The α-alkylation of β-hydroxy-γ-lactone **5.14**.

5.2 Strategy for the chemical synthesis of variously substituted chiral lactones

This chapter is focussed on the utilization of Sharpless' asymmetric dihydroxylation chemistry. The introduction of substituents by Harcken and Bruckner gave us reason to believe that this could be done combinatorially. Our approach is illustrated retrosynthetically in Scheme 5.9.



Scheme 5.9: Retrosynthetic analysis of substituted lactones.

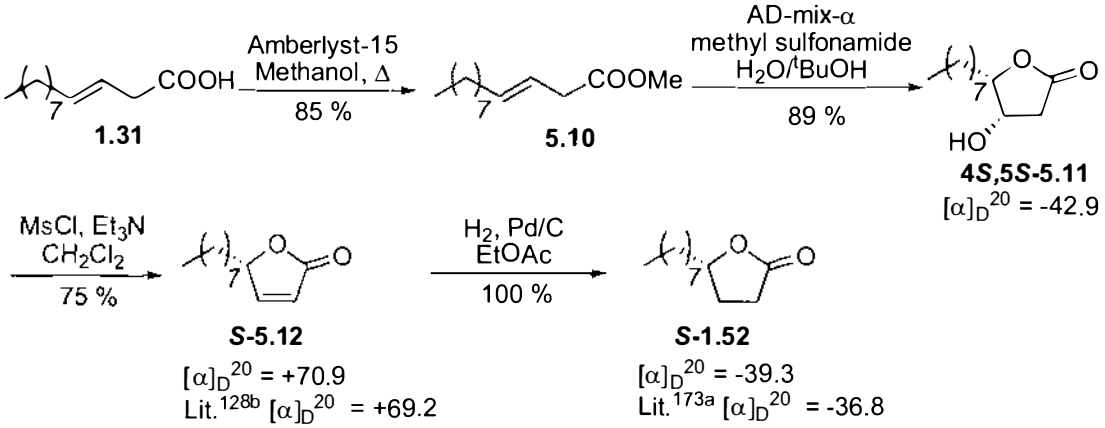
The α-substituted γ-lactone **I** can be accessed *via* alkylation of the enolate of lactone **II**. An α,β,γ-tri-substituted γ-lactone **III** might be synthesised *via* alkylation of the enolate of lactone **IV** that already contains a β-substituent. It is difficult to predict the stereochemical outcome of such a reaction. A β-substituted γ-lactone **IV** can be obtained by conjugate addition of a dialkylcuprate to butenolide **V**, an intermediate in the enantioselective synthesis of γ-lactones (Scheme 5.9). The saturated lactone **II** can be formed by catalytic

hydrogenation of butenolide **V**, which is obtained by dehydration of the lactone **VI**. The enantioenriched β-hydroxy-γ-substituted lactone **VI** is obtained by cyclisation of a β,γ-unsaturated methyl ester employing Sharpless' asymmetric dihydroxylation. This takes advantage of the β,γ-unsaturated acids available from our racemic synthesis *via* the Linstead modification of the Knoevenagel reaction in Chapter 3.

5.3 Synthesis of stereoisomerically pure γ-lactones

5.3.1 Chiral dihydro-5-octyl-2(3*H*)-furanone

Before attempting to generate a library of optically pure γ-lactones in a combinatorial way, we prepared (5*S*)-dihydro-5-octyl-2(3*H*)-furanone (as synthesised in racemic form in Chapter 3) using Sharpless' dihydroxylation methodology (Scheme 5.10).

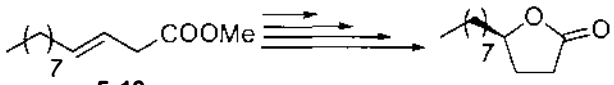


Scheme 5.10: Synthesis of (5*S*)-dihydro-5-octyl-2(3*H*)-furanone.

β,γ-Unsaturated acid **1.31**, available from our racemic synthesis, was esterified. The ^{1}H NMR spectrum of ester **5.10** had a singlet at δ 3.68 ppm that was assigned to protons of the -COOC<u>H</u>$_3$ group. After lactonisation of ester **5.10** in the presence of AD-mix-α, there was no corresponding signal in the spectrum of compound **4*S*,5*S*-5.11**. There was,

however, a multiplet at δ 4.45-4.53 ppm which was assigned to H_γ of the γ-lactone. The hydroxy lactone was converted to butenolide ***S*-5.12** *via* β-elimination of a mesylate. The ^{13}C NMR spectrum of hydroxy lactone **4*S*,5*S*-5.11** had a resonance at δ 69.1 ppm that was assigned to C_β, which bore the –OH group. This signal had gone in the spectrum of compound ***S*-5.12** and there were new resonances at δ 121.3 and 156.4 ppm that were assigned to the carbons of the double bond. Palladium-catalysed hydrogenation afforded the desired product, (5*S*)-dihydro-5-octyl-2(3*H*)-furanone (***S*-1.52**, Scheme 5.10). There were no ^{13}C resonances in the 120-160 ppm region of the spectrum, supporting reduction of the double bond. Mass spectrometry supported the formation of compound ***S*-1.52** by exhibiting a molecular ion at *m/z* 198 in the mass spectrum, consistent with the desired molecular formula $C_{12}H_{22}O_2$.

The *R*-enantiomer was synthesised by the same sequence of reactions utilizing AD-mix-β in the stereoselective lactonisation step (Scheme 5.11).



5.10 **R-1.52**

$[\alpha]_D^{20}$ = +40.0
Lit.[173a] $[\alpha]_D^{20}$ = +36.8

Scheme 5.11: Synthesis of (5*R*)-dihydro-5-octyl-2(3*H*)-furanone.

5.3.2 Generating libraries of chiral γ-lactones

A library of five (*S*)-γ-lactones was synthesised in a combinatorial fashion *via* the reaction of a mixture of five esters with AD-mix-α and each step was analysed by GC-MS. All compounds in this series were of the 5*S*-configuration. The 4-hydroxy intermediates were of the 4*S*-configuration (Figure 5.2).

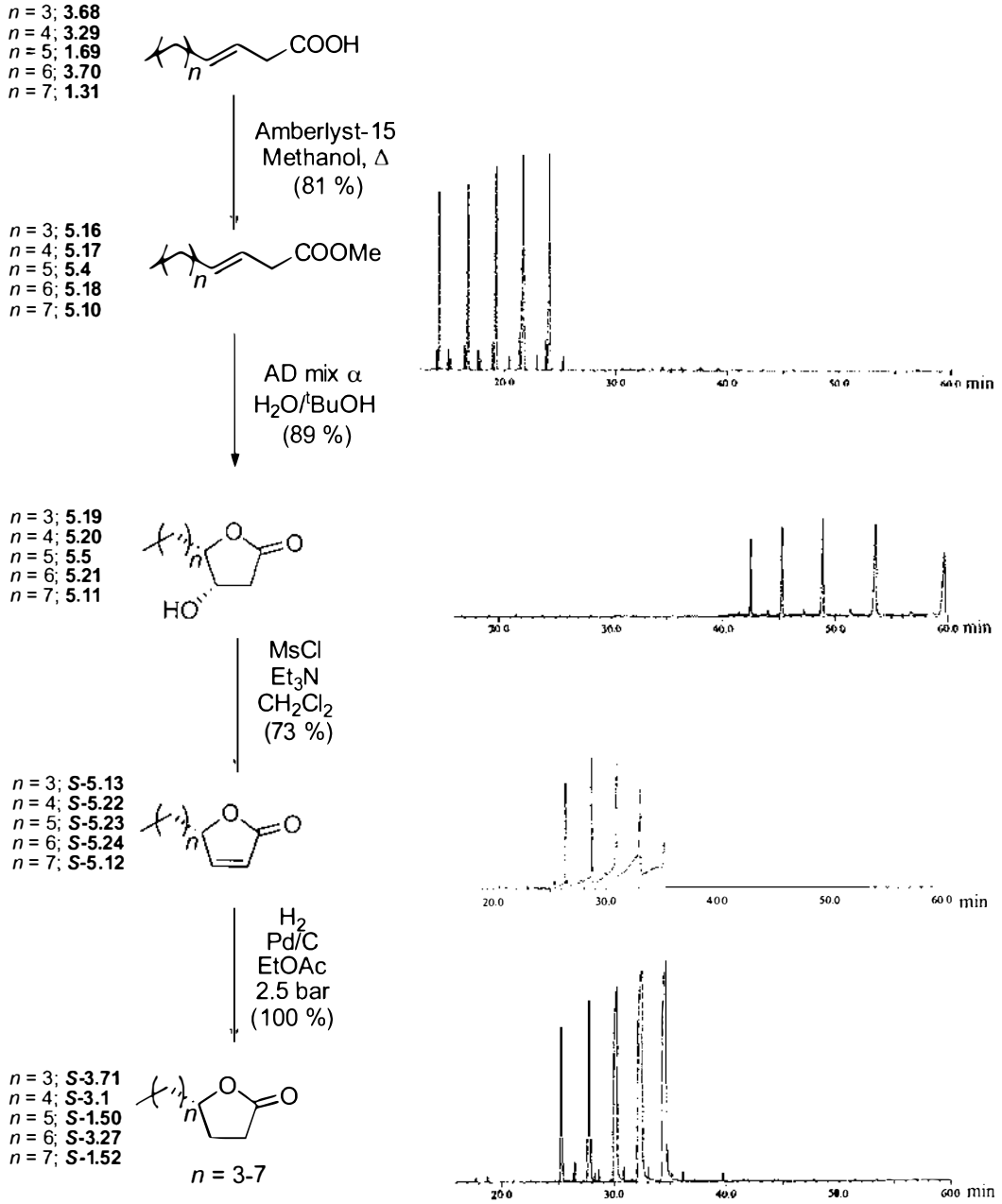
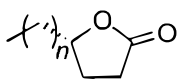
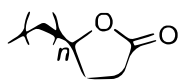


Figure 5.2: Synthesis of libraries of γ-substituted lactones with GC trace at each step.

A library of *R*-enantiomers was synthesised by the same sequence of reactions utilising AD-mix-β in the stereoselective lactonisation step. The odour descriptions of each compound were assessed by GC-O and details are shown in Table 5.1.

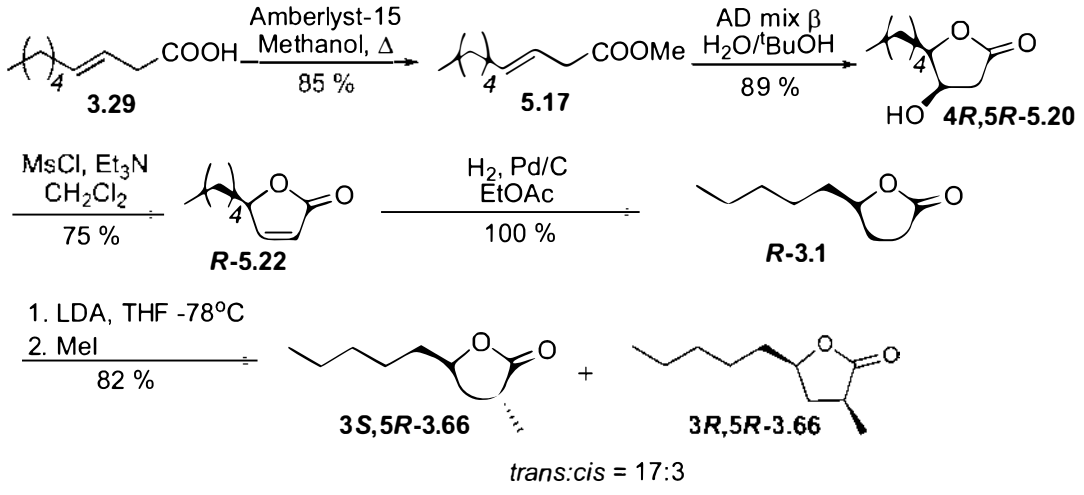
Table 5.1: Odour descriptions for enantiomerically optical γ-lactones.

Structure	Odour Description	Lit. Odour Description[172]
		
n = 1; ***S*-3.71**	coconut, faint	creamy coconut
n = 2; ***S*-3.1**	milky, white chocolate	fatty
n = 3; ***S*-1.50**	milky, apricot	fatty milky
n = 4; ***S*-3.27**	buttery, apricot	soft fatty fruity,
n = 5; ***S*-1.52**	soapy, apricot	sweet, fatty
		
n = 1; ***R*-3.71**	coconut, mandarin peel, coconut	sweet coconut
n = 2; ***R*-3.1**	apricot with skin on, coconut	sweet, spicy, coconut
n = 3; ***R*-1.50**	fermented apple, fruity juice, coconut	fatty, weak coconut
n = 4; ***R*-3.27**	burnt peach, strawberry jam	strong fatty fruity
n = 5; ***R*-1.52**	sweet peach	peach

5.3.3 3-Substituted (5*R*)-dihydro-5-pentyl-2(3*H*)-furanones

To investigate the influence of α-substitution in γ-lactones, we chose to focus on compounds derived from (5*R*)-dihydro-5-pentyl-2(3*H*)-furanone. The size of lactones has an impact on flavour and compounds bigger than dihydro-5-octyl-2(3*H*)-furanone produced little interest from the flavour aspect.[172] As described in Chapter 4, the (*R*)-enantiomer is the major isomer in nature and the more potent. We therefore elected to pursue the 5*R* series of dihydro-5-pentyl-2(3*H*)-furanone.

(5*R*)-Dihydro-5-pentyl-2(3*H*)-furanone ***R*-3.1** was synthesised by the same sequence of reactions described in section 5.3.1 starting from β,γ-unsaturated acid **3.29**. An α-methyl group was then introduced *via* alkylation of the lactone enolate ***R*-3.1** to give (3*S*,5*R*)-dihydro-3-methyl-5-pentyl-(3*H*)-furanone ***3S,5R-3.66*** as the major product (*trans*:*cis*=17:3) in Scheme 5.12. The *trans*:*cis* ratio was calculated by integration of the NMR signals. The alkylation of the lactone enolate followed a typical procedure from the literature.[173] Previous reports indicated that the stereoselectivity of the alkylation led to a *trans*:*cis* ratio of 9:1.[172c,d]



Scheme 5.12: Synthesis of dihydro-3-methyl-5-pentyl-(3*H*)-furanone.

MS analysis supported the incorporation of a methyl group with a weak molecular ion at *m/z* 170 and a base peak at m/z 99. The base peak arises from loss of the side chain as illustrated in Figure 5.3.

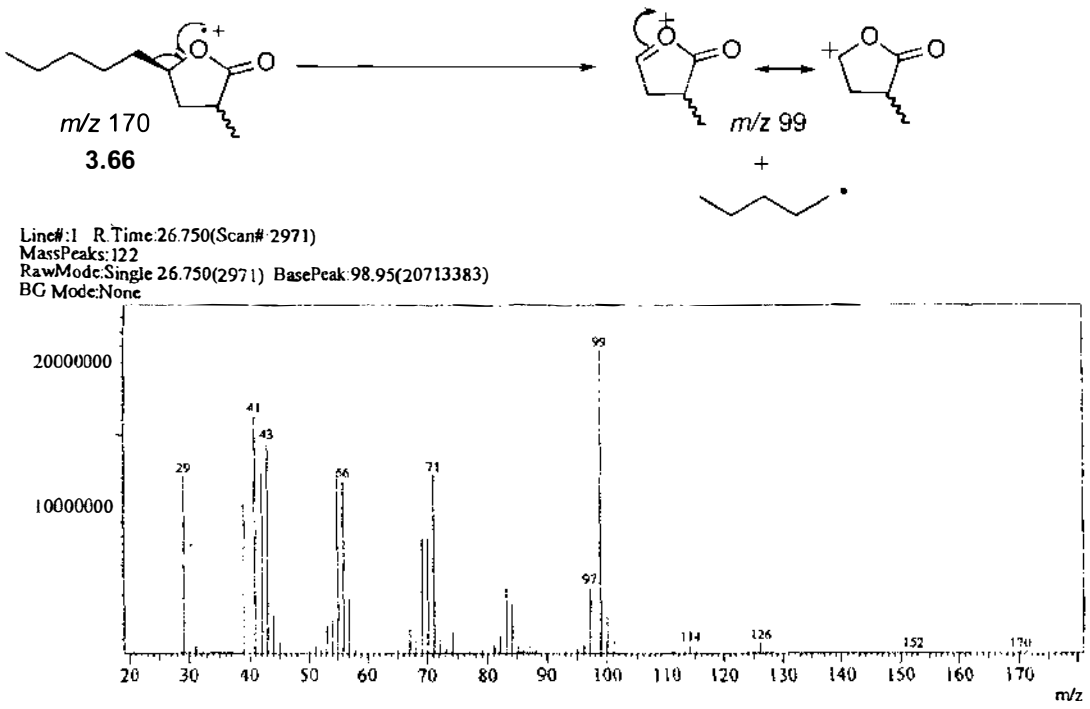


Figure 5.3: Mass spectrum for the mixture of **3*S*,5*R*-3.66** and **3*R*,5*R*-3.66**.

The newly incorporated methyl group gave rise to a doublet at δ1.25 ppm in the ^{1}H NMR spectrum. Nuclear Overhauser effect spectroscopy (NOESY) provided evidence for the *trans* relative stereochemistry of the major product ***3S,5R-3.66***. In the ^{1}H NOESY spectrum, there was no correlation between Hα and Hγ since they were on opposite faces of the ring. There was a correlation between Hγ and the methyl group at Cα, providing evidence that they were on the same face of the lactone ring (Figure 5.4).

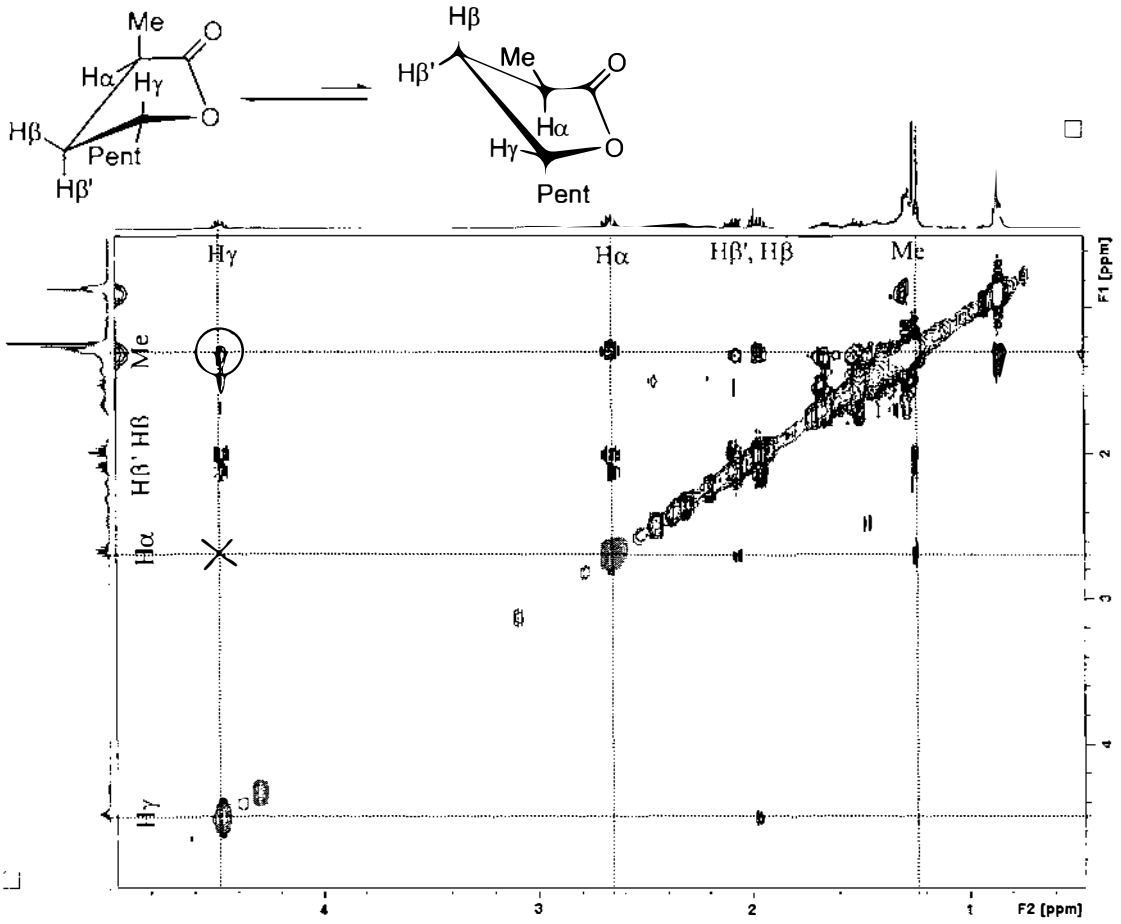
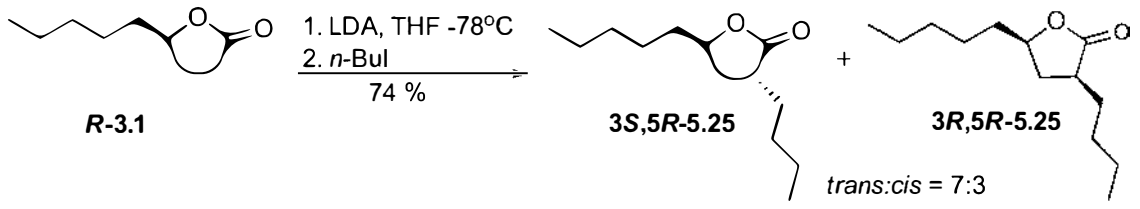


Figure 5.4: NOESY spectrum of lactone ***3S,5R-3.66*** ($CDCl_3$, 500 MHz).

To test the viability of introducing larger substituents, a butyl group was added *via* alkylation of the lactone enolate of ***R*-3.1** to give a *trans*:*cis* mixture of ***3S,5R-5.25*** and ***3R,5R-5.25*** (Scheme 5.13). GC-MS analysis supported the incorporation of a butyl group with a base peak at m/z 141, arising from loss of the γ-side chain.



Scheme 5.13: Introducing a butyl group into the lactone ***R*-3.1**.

5.3.4 Generating a library of 3-substituted dihydro-5-pentyl-2(3*H*)-furanones

A library of four 3-substituted dihydro-5-pentyl-2(3*H*)-furanones was synthesised in a combinatorial fashion *via* alkylation of the lactone enolate with four alkyl iodides. The library was analysed by GC-MS with the compounds eluting in order of increasing size. The mass spectrum corresponding to each peak in the GC trace indicated a base peak at *m/z* 99, 113, 127 and 141, respectively, reflecting an additional -CH_2- unit for each successive member in the series (R = Me, Et, *n*-Pr and *n*-Bu). The intensities of the five lactones were not equal due to partial losses of the smaller lactones, more volatile during work-up and purification. The odour descriptions of each compound were assessed by GC-O (Figure 5.5).

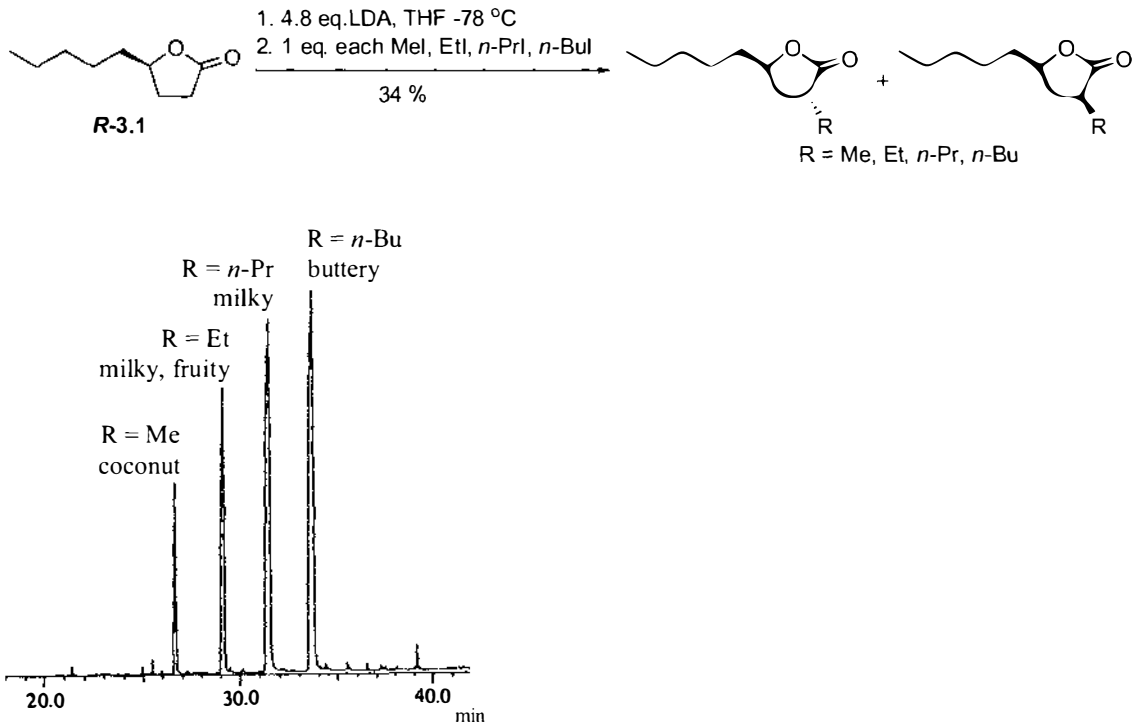
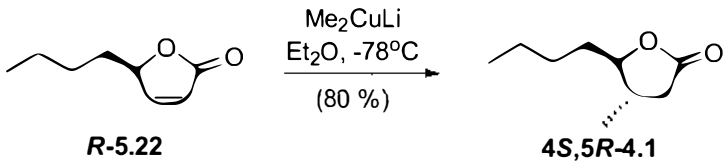


Figure 5.5: A library of four 3-substituted (5*R*)-dihydro-5-pentyl-2(3*H*)-furanones with the GC trace and the odour description.

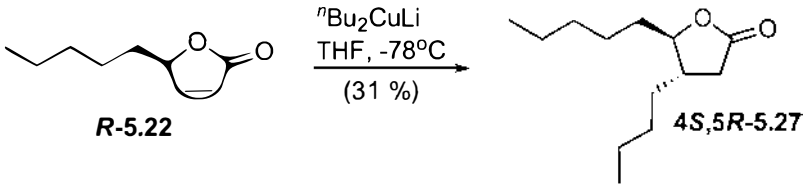
5.3.5 4-Substituted (5*R*)-dihydro-5-pentyl-2(3*H*)-furanones

A 4-substituent can be introduced onto a butenolide *via* conjugate addition of an alkyl cuprate reagent, *i.e.*, dialkyl copper lithium and dialkyl copper magnesium bromide. For example, whisky lactone **4*S*,5*R*-4.1** was obtained by a stereospecific 1,4-addition of lithium dimethylcuprate to butenolide ***R*-5.22** (Scheme 5.14).[174]



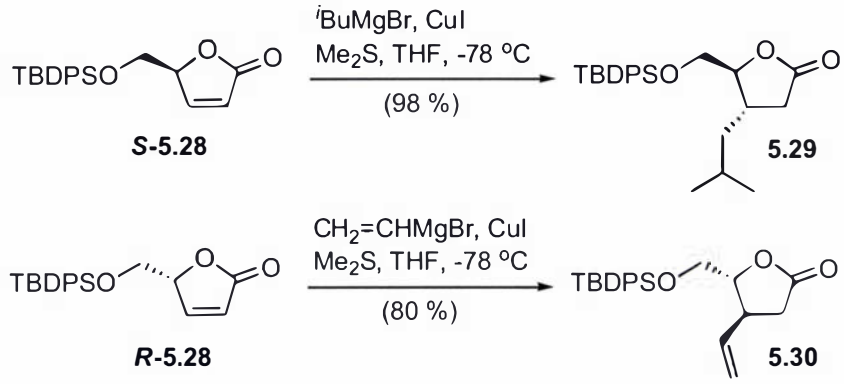
Scheme 5.14: Cuprate addition of the butenolide ***R*-5.22**.

In an analogous fashion, we investigated the conjugate addition of $^{n}Bu_2CuLi$ (generated *in situ*) in our synthesis. The butyl group attacked the butenolide exclusively from the less hindered face, and only the *trans*-configured 1,4-addition product was isolated (Scheme 5.15). Mass spectroscopy analysis supported the incorporation of a butyl group with a base peak at *m/z* 141 that arises from loss of the γ-side chain.



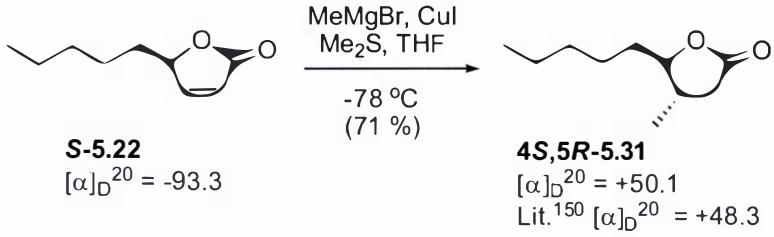
Scheme 5.15: Formation of γ-lactone **4*S*,5*R*-5.27**.

Grignard reagents can also be employed as precursors to cuprate reagents. Some examples by Hanessian's group are given in Scheme 5.16 that shows excellent yields and diastereoselectivity.[175] They looked at both enantiomeric series with a range of Grignard reagents.



Scheme 5.16: Cuprate addition of butenolide.

A cuprate addition with methyl magnesium bromide as the precursor gave the *trans*-configured 1,4-addition product **4*S*,5*R*-5.31** (Scheme 5.17).



Scheme 5.17: Cuprate addition for the formation of γ-lactone **4*S*,5*R*-5.31**.

Analysis by GC-MS supported the incorporation of a methyl group with a base peak at *m/z* 99. The newly incorporated methyl group gave rise to a doublet at δ1.07 ppm in the ^{1}H NMR spectrum. Nuclear Overhauser effect spectroscopy (NOESY) supported the *trans*-orientation of the β-substituent relative to the pre-existing γ-substituent. In the ^{1}H NOESY spectrum, there are no correlations between Hβ and Hγ since *trans*-configured protons give no space connectivity while there is a correlation between the CH_3 group at the β-position and Hγ, since they are on the same face of the lactone ring (Figure 5.6).

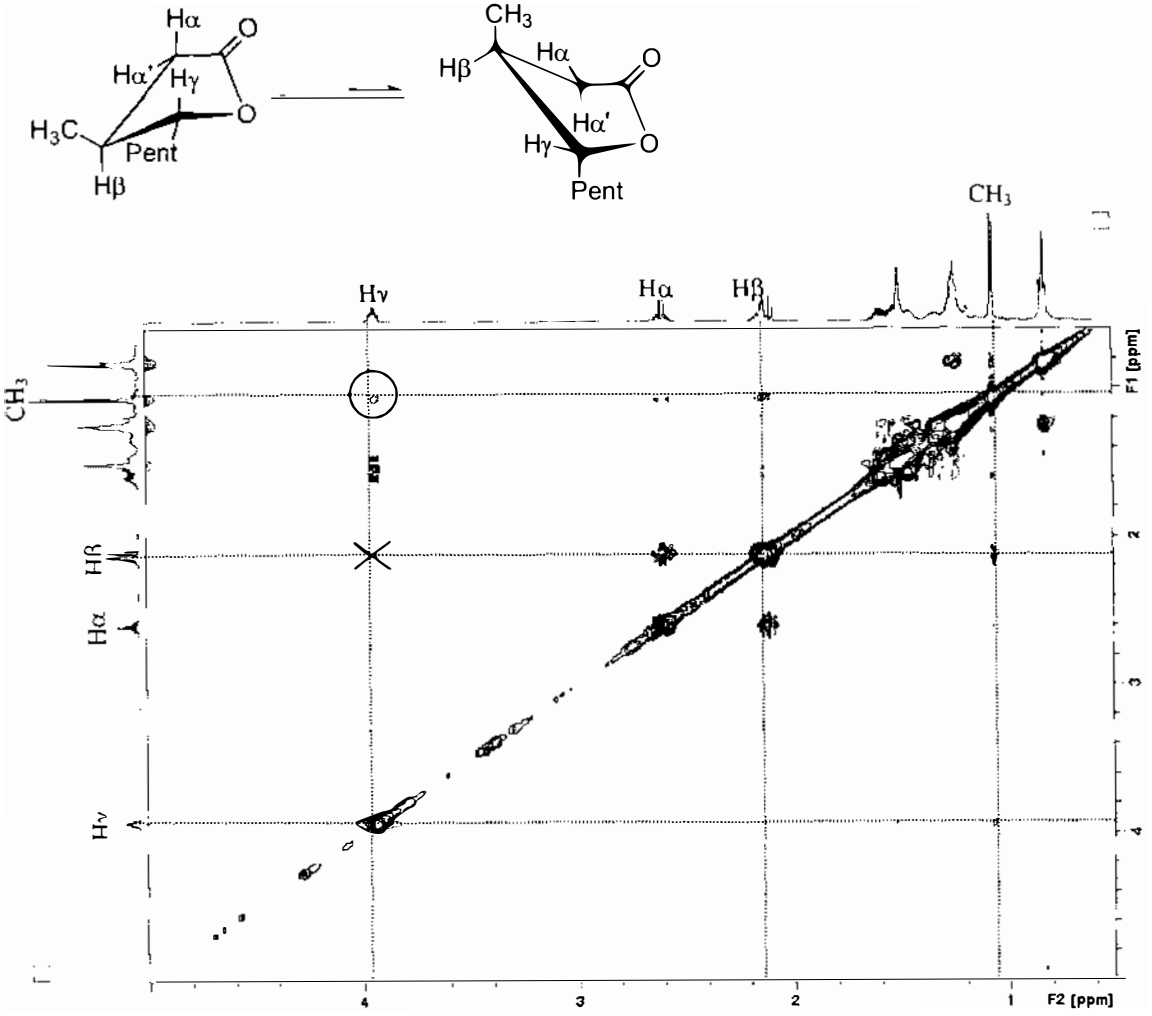
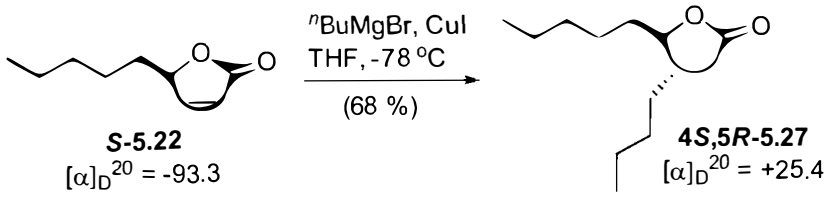


Figure 5.6: NOESY spectrum of lactone **(+)-*trans*-5.31** ($CDCl_3$, 500 MHz).

A cuprate addition with butyl magnesium bromide was attempted and the *trans*-configured 1,4-addition product **4*S*,5*R*-5.27** was synthesised with a better yield than the conjugate addition of $^{n}Bu_2CuLi$ (Scheme 5.18).



Scheme 5.18: Cuprate addition for the formation of γ-lactone **4*S*,5*R*-5.27**.

5.3.6 Generating a library of 4-substituted (5*R*)-dihydro-5-pentyl-2(3*H*)-furanones

A library of three 4-substituted (5*R*)-dihydro-5-pentyl-2(3*H*)-furanones was synthesised in a combinatorial fashion with three Grignard reagents *via* a cuprate addition to the butenolide. The mass spectrum corresponding to each peak in the GC trace indicated a base peak at *m/z* 99, 113 and 141, respectively, reflecting an additional -CH_2- unit for each successive member in the series (R = Me, Et and ^{n}Bu). The odour descriptions of each compound were assessed by GC-O (Figure 5.7).

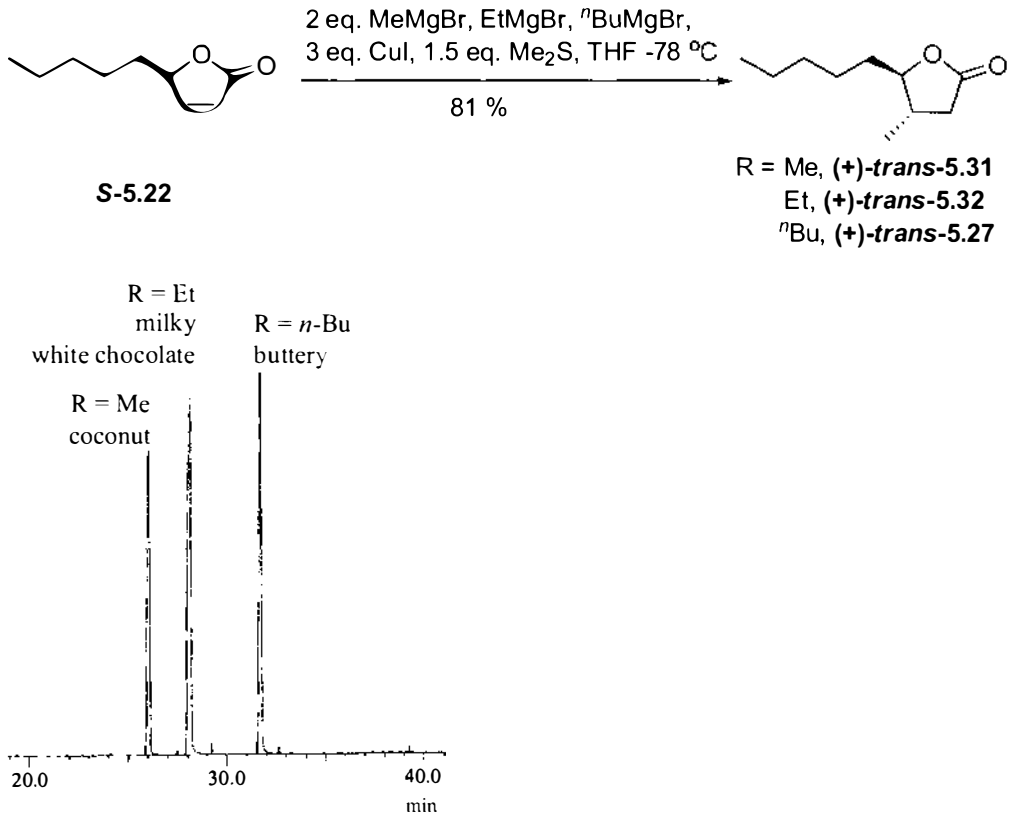
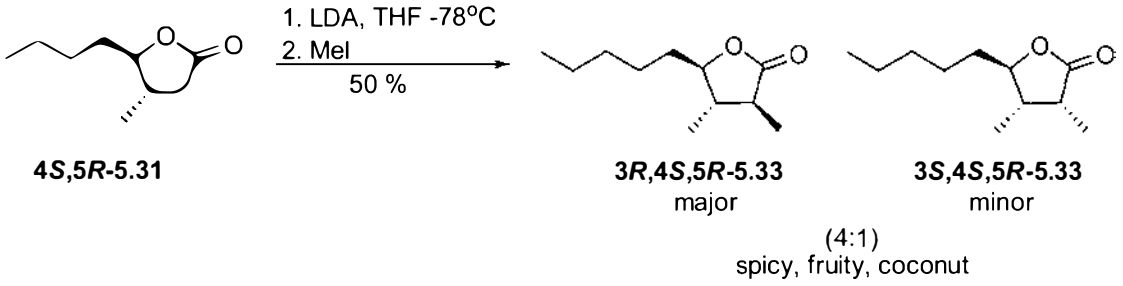


Figure 5.7: A library of three 4-substituted (5*R*)-dihydro-5-pentyl-2(3*H*)-furanones with the GC trace and the odour description.

5.3.7 An 3,4,5-tri-alkyl γ-lactone

An 3,4,5-tri-substituted dihydro-5-pentyl-2(3*H*)-furanone **5.33** can be synthesised *via* alkylation of the enolate of lactone **4S,5*R*-5.31** that already contains a 4-substituent to give (3*R*,4*S*,5*R*)-dihydro-3-methyl-4-methyl-5-pentyl-(3*H*)-furanone **3*R*,4*S*,5*R*-5.33** as a major product (major:minor = 4:1, Scheme 5.19). The ratio was calculated by integration of the NMR signals (see experimental section). The odour of compound **5.33** was assessed by GC-O as described in Scheme 5.19.



Scheme 5.19: Formation of 3,4,5-tri-substituted dihydro-5-pentyl-2(3*H*)-furanone **5.33**.

5.4 Syntheses of optically active γ-lactones with unsaturated γ-side chains

5.4.1 Introduction

(5*R*,1*Z*)-5-(1-Decenyl)dihydro-2(3*H*)-furanone (**5.34**) was isolated from a female Japanese beetle (*Popillia japonica*); only this isomer attracts males of the species in field bioassays. The unsaturation of compound **5.34** is important for bioactivity since male insects showed no response to a saturated analogue **5.35** in Figure 5.8.

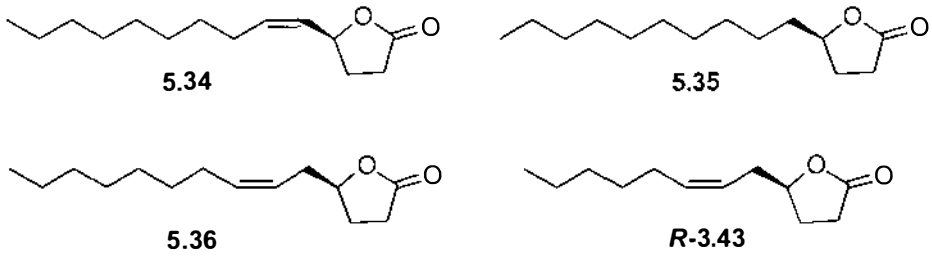


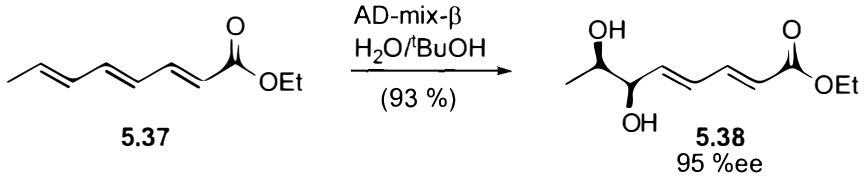
Figure 5.8: Insect pheromones.

Insect pheromones are often recovered from flower fragrances and they play a role in the fragrance industry, *e.g.*, (5*R*,1*Z*)-5-(1-decenyl)dihydro-2(*3H*)-furanone (**5.34**), (5*R*,2*Z*)-5-(2-decenyl)dihydro-2(*3H*)-furanone (**5.36**) and (5*R*,2*Z*)-5-(2-octenyl)dihydro-2(*3H*)-furanone (***R*-3.43**) in Figure 5.8.[176] Pheromones isolated from nature are optical active,[177] and early syntheses were only racemic.[178] Optically active lactones have been obtained *via* resolution of an intermediate or final product. [179] As discussed in Chapter 4, chemical and enzymatic asymmetric syntheses have been reported.[180]

To introduce the unsaturation into an optically active γ-lactone is the focus of this section since chirality and unsaturation of a target compound are important in a bioactive form and its aroma.

5.4.2 The regioselectivity of Sharpless' asymmetric dihydroxylation

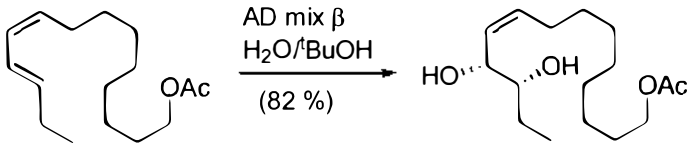
Regioselectivity in the dihydroxylation of a polyene is determined by both electronic and steric effects. Recently, it was shown that rate constants for the dihydroxylation of isolated double bonds are much larger with *trans*-1,2-disubstituted and trisubstituted olefins than with *cis*-1,2-disubstituted and terminal alkenes. Electronic factors influence the regioselectivity, and the osmylation of unsymmetrical polyenes preferentially occurs at the most electron-rich double bond (Scheme 5.20).[181]



5.37 **5.38**
95 %ee

Scheme 5.20: Regioselective dihydroxyation.

Steric effects may play a decisive role in systems with electronically very similar double bonds. Generally, the sterically most accessible site is osmylated preferentially. The *cis*-double bond of a *cis,trans*-polyene will not be attacked to an appreciable extent during asymmetric dihydroxylation of the *trans*-double bond with these ligands (Scheme 5.21).[182]

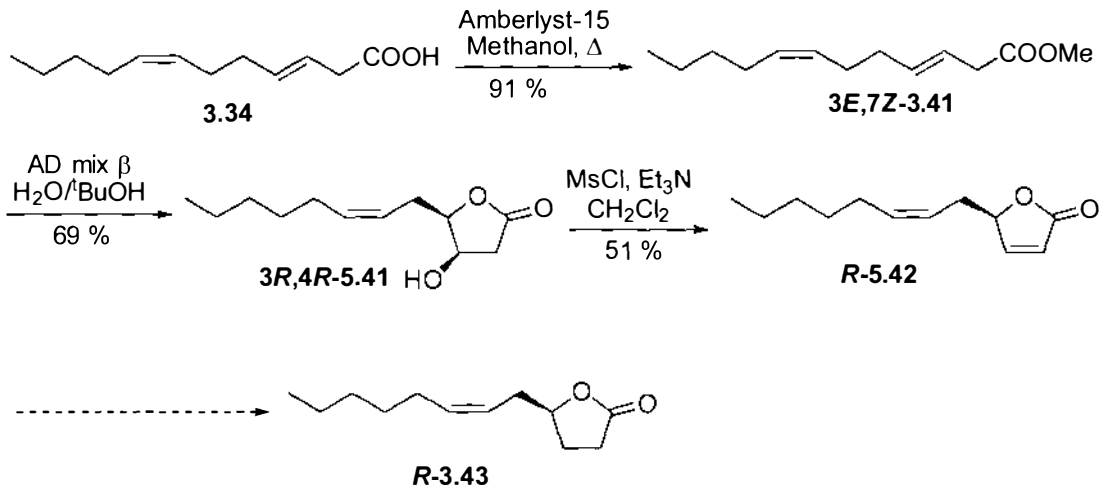


5.39 **5.40** 93 %ee

Scheme 5.21: Steric effects of dihydroxyation.

5.4.3 Synthesis of a γ-lactone with an unsaturated γ-substituent

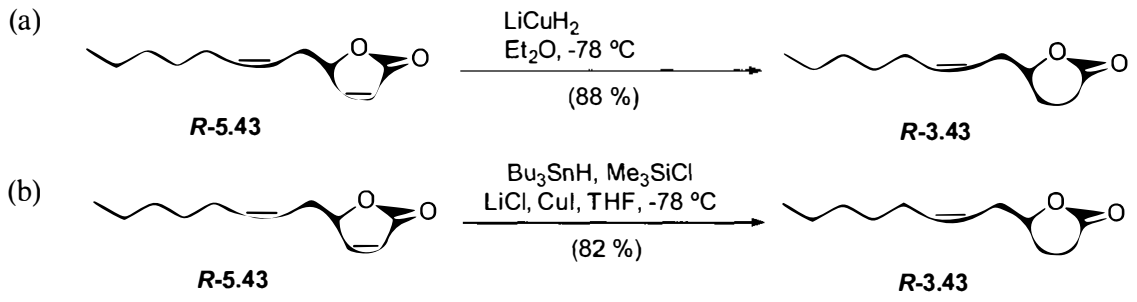
We attempted the synthesis of (5*R*,2*Z*)-5-(2-octenyl)dihydro-2(*3H*)-furanone (***R*-3.43**) by the same sequence of reactions described in section 5.3.1. The β,γ-unsaturated dodecadienoic acid **3.34** (available from Chapter 3) was esterified and lactonised with AD-mix-β (Scheme 5.22).



Scheme 5.22: Possible synthetic pathway for the production of compound ***R*-3.43**.

The ^{1}H NMR spectrum of ester **3*E*,7*Z*-3.41** displayed a singlet at δ3.68 ppm which was assigned to protons of the -COOC$\underline{H}_3$ group. There was no corresponding signal in the spectrum of compound **3*R*,4*R*-5.41**, following lactonisation. There was, however, a triplet at δ 4.46 ppm that was assigned to H_γ of the lactone. The ^{13}C NMR spectrum of hydroxyl lactone **3*R*,4*R*-5.41** has a resonance at δ 68.6 ppm that was assigned to C_β, which bore the –OH group. The hydroxy lactone **3*R*,4*R*-5.41** was converted to the corresponding mesylate and *in situ* β-elimination gave butenolide ***R*-5.42**. The CHOH signal had gone in the ^{13}C NMR spectrum of compound ***R*-5.42** and there were new resonances at δ 121.1 and 155.8 ppm that were assigned to the carbons of the double bond in the lactone ring. The final step to complete the synthesis of (*R,Z*)-5-(2-octenyl)dihydro-2(*3H*)-furanone (***R*-3.43**) was the regioselective reduction of the C_α-C_β double bond in the lactone ring (Scheme 5.22).

The regioselective hydrogenation of the C_α-C_β double bond in the lactone ring was reported previously by Midland[129] with copper hydride[183] (Scheme 5.23a). Koseki's group more recently reported hydrogenation with tributyltin hydride and trimethylsilyl chloride in the presence of copper iodide and lithium chloride in 82 % yield (Scheme 5.23b).[184] Koseki's reaction conditions were attempted in butenolide ***R*-5.42** to give ***R*-3.43** and the starting material was recovered. In the future, this step needs more attention in order to complete this synthesis.



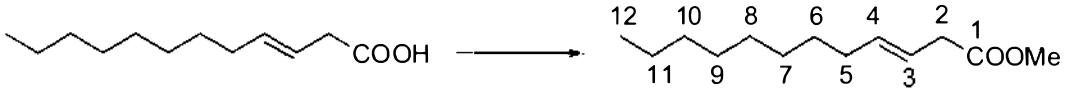
Scheme 5.23: Hydrogenation of the C_{α}-C_{β} double bond in the lactone ring.

5.5 Summary

Syntheses and analyses for libraries of chiral lactones were the focus of this chapter. Both enantiomers of γ-substituted lactones (C8-C12) were synthesised *via* a four-step reaction sequence including the Sharpless asymmetric dihydroxylation. Libraries of α-substituted γ-lactones and ▪ -substituted γ-lactones were produced and analysed by GC-MS and GC-O. Further, synthesis of a γ-lactone with an unsaturated γ-substituent was attempted.

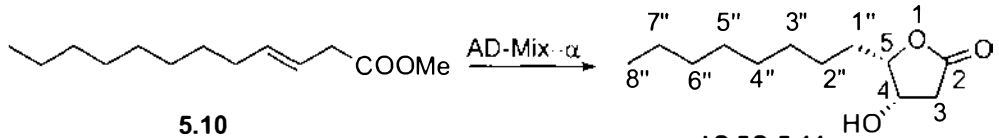
5.6 Experimental procedures

General method: as described earlier.



1.31 **5.10**

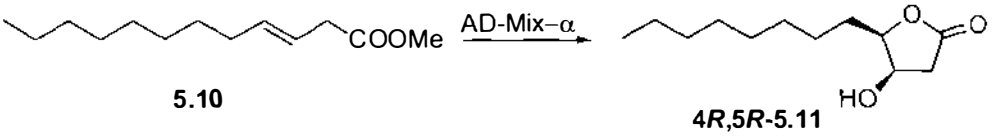
(3*E*)-Methyl 3-dodecanoate (5.10): A solution of 3*E*-dodec-3-enoic acid (1.0 g, 5.0 mmol) in methanol (50 mL) was heated at reflux in the presence of Amberlyst-15 (1.0 g) for 1 h. The mixture was cooled, the Amberlyst-15 removed by filtration and rinsed with Et_2O (20 mL). The filtrate and washings were concentrated and the residue purified by chromatography (5:1 = hex-EtOAc) to give **5.10** as a light yellow oil (900 mg, 85 %). R_f = 0.83 (3:1 hex-EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, J = 6.8 Hz, 3H, H-12), 1.32 (m, 12H, H-6, H-7, H-8, H-9, H-10, H-11), 2.02 (q, J = 6.6 Hz, 2H, H-5), 3.03 (d, J = 5.6 Hz, 2H, H-2), 3.68 (s, 3H, OCH_3), 5.47-5.60 (m, 2H, H-3, H-4); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.6, 29.1, 29.2, 29.4, 31.8, 32.4, 37.9, 51.7, 121.3, 134.9, 172.6.



5.10 **4*S*,5*S*-5.11**

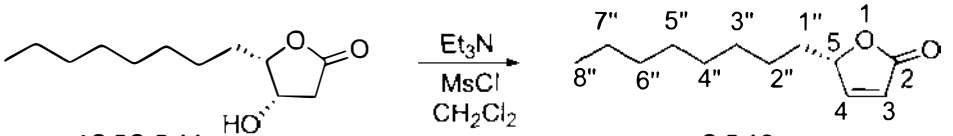
(*4S,5S*)-4,5-Dihydro-4-hydroxy-5-octyl-2(3H)-furanone, 4*S*,5*S*-5.11: AD-mix-α (1.4 g) and the ester **5.10** (212 mg, 1.0 mmol, 1.0 equiv.) were added to a mixture of ^{t}BuOH (20 mL) and H_2O (20 mL) at 0 °C. The mixture was stirred for 48 h, then quenched by the addition of sat'd aq. Na_2SO_3 (20 mL). The mixture was extracted with CH_2Cl_2 (3 x 20 mL), dried over $MgSO_4$, filtered and concentrated. The residue was purified by

chromatography (5:1 = hex-EtOAc → 1:5 = hex-EtOAc) to give **4*S*,5*S*-5.11** as a colourless oil (191 mg, 89 %). R_f = 0.14 (5:1 hex-EtOAc); $[\alpha]_D^{20}$ = -42.9° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (t, *J* = 6.9 Hz, 3H, H-8"), 1.21-2.01 (m, 14H, H-1", H-2", H-3", H-4", H-5", H-6", H-7"), 2.50 (dd, *J* = 17.7, 0.8 Hz, 1H, H-3), 2.77 (dd, *J* = 17.7, 5.5 Hz, 1H, H-3'), 4.30-4.37 (m, 1H, H-4), 4.44-4.48 (m, 1H, H-5), 5.30 (s, 1H, -OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 25.5, 28.2, 29.2, 29.4, 29.5, 31.8, 39.4, 69.1, 84.7, 175.5.



5.10 **4*R*,5*R*-5.11**

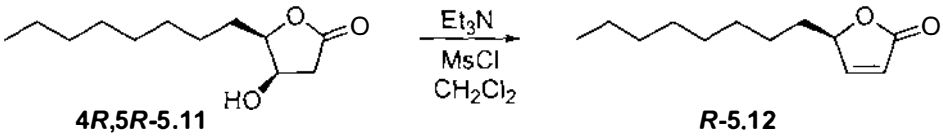
(*4R,5R*)-4,5-Dihydro-4-hydroxyl-5-octyl-2(3H)-furanone, *4R,5R*-5.11 was prepared with AD-mix-β (1.4 g, 1.4 g/1 mmol of ester) and the ester **5.10** (212 mg, 1.0 mmol, 1.0 equiv.) as above to give a colourless oil (191 mg, 89 %); $[\alpha]_D^{20}$ = +43.5° (*c* 1.0, $CHCl_3$).



4*S*,5*S*-5.11 ***S*-5.12**

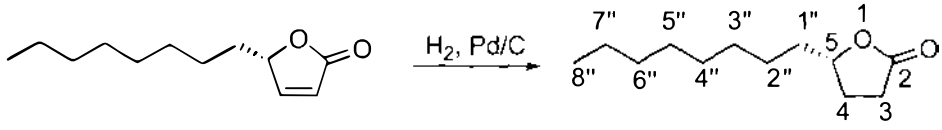
(5*S*)-5-Octyl-2(5H)-furenone (*S*-5.12): Triethylamine (146 μL, 106 mg, 1.05 mmol, 2.1 equiv.) and methanesulfonyl chloride (43 μL, 63 mg, 0.55 mmol, 1.1 equiv.) were added to a solution of lactone **4*S*,5*S*-5.11** (107 mg, 0.5 mmol, 1.0 equiv.) in CH_2Cl_2 (10 mL) at 0 °C. After 1 h the reaction was quenched by adding sat'd aq. NH_4Cl solution (10 mL) and water (20 mL). The mixture was extracted with CH_2Cl_2 (3 x 20 mL), dried over $MgSO_4$, filtered and concentrated. The residue was purified by chromatography (3:1 = hex-EtOAc) to give ***S*-5.12** as a colourless oil (74 mg, 75 %). R_f = 0.47 (3:1 hex-EtOAc); $[\alpha]_D^{20}$ =

+70.9° (*c* 2.0, dioxane), lit.[128b] $[\alpha]_D^{20}$ = +69.2° (*c* 2.10, dioxane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.9 Hz, 3H, H-8"), 1.21-1.47 (m, 12H, H-2", H-3", H-4", H-5", H-6", H-7"), 1.63-1.82 (m, 2H, H-1"), 5.03-5.07 (m, 1H, H-5), 6.10 (dd, *J* = 5.7, 2.0 Hz, 1H, H-4), 7.48 (dd, *J* = 5.7, 1.5 Hz, 1H, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.5, 24.8, 29.0, 29.1, 29.2, 31.7, 33.0, 83.4, 121.3, 156.4, 173.1.



4*R*,5*R*-5.11 **R-5.12**

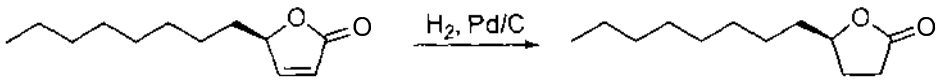
(*5R*)-5-Octyl-2(5H)-furenone (*R*-5.12) was prepared in an analogous fashion on a scale of 1 mmol to give ***R*-5.11** as a colourless oil (74 mg, 75 %); $[\alpha]_D^{20}$ = -73.8° (*c* 2.10, dioxane), lit.[128b] $[\alpha]_D^{20}$ = -69.2° (*c* 2.10, dioxane).



***S*-5.12** ***S*-1.52**

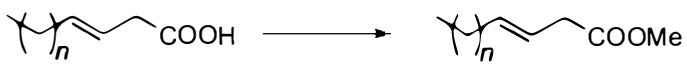
(*5S*)-Dihydro-5-octyl-2(3*H*)-furanone (*S*-1.52): Pd/C (18 mg, 10 % Pd on charcoal, 0.02 mmol, 0.05 equiv.) was added to a solution of lactone ***S*-5.12** (74 mg, 0.4 mmol, 1.0 equiv.) in EtOAc (2 mL). The mixture was shaken at RT overnight under H_2 (2.5 bar). The mixture was filtered through Celite, which was then washed well with EtOAc (20 mL). The filtrate was concentrated to give ***S*-1.52** as a colourless oil (74 mg, 100 %). R_f = 0.46 (3:1 hex-EtOAc); $[\alpha]_D^{20}$ = -39.3° (*c* 0.30, MeOH), lit.[173a] $[\alpha]_D^{20}$ = -36.8° (*c* 0.30, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.9 Hz, 3H, H-8"), 1.26-1.34 (m, 12H, H-2", H-3", H-4", H-5", H-6", H-7"), 1.60-1.74 (m, 2H, H-1"), 1.82-1.89 (m, 1H, H-4), 2.33 (sext, *J* = 6.6 Hz, 1H, H-4'), 2.53 (dd, *J* = 9.7, 6.9 Hz, 2H, H-3), 4.45-4.53 (m, 1H, H-5); ^{13}C

NMR (100 MHz, $CDCl_3$) δ 14.0, 22.6, 25.1, 27.9, 28.8, 29.1, 29.2, 29.3, 31.7, 35.5, 81.0, 177.3.



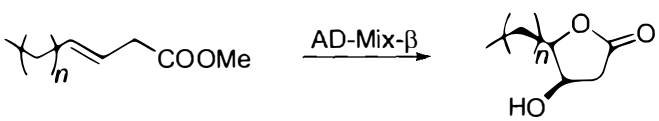
***R*-5.12** ***R*-1.52**

(*5R*)-Dihydro-5-octyl-2(*3H*)-furanone (*R*-1.52) was prepared in an analogous fashion on a scale of 1 mmol to give ***R*-1.52** as a yellow oil (112 mg, 100 %); $[\alpha]_D^{20}$ = +40.0° (*c* 0.30, MeOH), lit.[173a] $[\alpha]_D^{20}$ = +36.8° (*c* 0.30, MeOH).



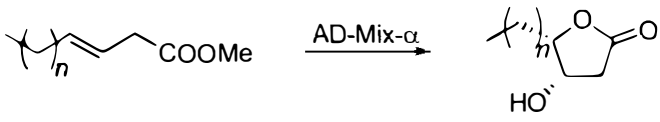
n = 3,4,5,6,7

Esterification. A mixture of the five β,γ-unsaturated carboxylic acids obtained from Chapter 3 (426 mg, ~0.5 mmol each) were dissolved in methanol (10 mL) and Amberlyst-15 (426 mg, the same amount by weight as the acid mixture) was added. The mixture was heated at reflux for 1 h, cooled, filtered (washing the resin well with Et_2O), and concentrated. The residue was purified by chromatography (5:1 hex-EtOAc) to give a mixture of five esters as a light yellow oil (374 mg; 81 %); GC-MS: R_T 14.2 min (*n* = 3, $C_9H_{16}O_2$, M^+ obsd *m/z* 156); R_T 16.8 min (*n* = 4, $C_{10}H_{18}O_2$, M^+ obsd *m/z* 170); R_T 19.3 min (*n* = 5, $C_{11}H_{20}O_2$, M^+ obsd *m/z* 184); R_T 21.6 min (*n* = 6, $C_{12}H_{22}O_2$, M^+ obsd *m/z* 198); R_T 24.0 min (*n* = 7, $C_{13}H_{24}O_2$, M^+ obsd *m/z* 212).



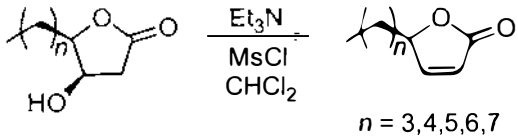
n = 3,4,5,6,7

Asymmetric lactonisation: AD-mix-β (1.4 g) and the five esters (187 mg, ~0.2 mmol each of the five esters) were added to a mixture of ^{t}BuOH (20 mL) and H_2O (20 mL) at 0 °C. The mixture was stirred for 48 h, then quenched by the addition of sat'd aq. Na_2SO_3 (20 mL). The mixture was extracted with CH_2Cl_2 (3 x 20 mL), dried over $MgSO_4$, filtered and concentrated. The residue was purified by chromatography (5:1 = hex-EtOAc → 15:1 = CH_2Cl_2-MeOH) to give a colourless oil (165 mg, 89 %); GC-MS: R_T 42.4 min (*n* = 3, $C_8H_{14}O_3$, M^+ obsd *m/z* 158); R_T 45.2 min (*n* = 4, $C_9H_{16}O_3$, M^+ obsd *m/z* 172); R_T 48.9 min (*n* = 5, $C_{10}H_{18}O_3$, M^+ obsd *m/z* 186); R_T 53.6 min (*n* = 6, $C_{11}H_{20}O_3$, M^+ obsd *m/z* 200); R_T 59.7 min (*n* = 7, $C_{12}H_{22}O_3$, M^+ obsd *m/z* 214).



n = 3,4,5,6,7

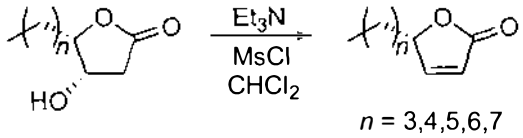
***(4S,5S)*-series** were prepared in an analogous fashion on a scale of esters (187 mg, ~0.2 mmol each of the five esters) as a colourless oil (163 mg, 88 %); GC-MS: R_T 42.1 min (*n* = 3, $C_8H_{14}O_3$, M^+ obsd *m/z* 158); R_T 45.0 min (*n* = 4, $C_9H_{16}O_3$, M^+ obsd *m/z* 172); R_T 48.6 min (*n* = 5, $C_{10}H_{18}O_3$, M^+ obsd *m/z* 186); R_T 53.3 min (*n* = 6, $C_{11}H_{20}O_3$, M^+ obsd *m/z* 200); R_T 59.5 min (*n* = 7, $C_{12}H_{22}O_3$, M^+ obsd *m/z* 214).



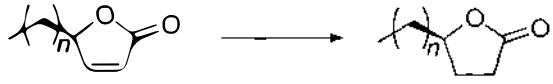
n = 3,4,5,6,7

Elimination: Triethylamine (209 μL, 152 mg, 1.5 mmol, 10.5 equiv.) and methanesulfonyl chloride (60 μL, 89 mg, 0.78 mmol, 5.5 equiv.) were added to a solution of the five lactones (133 mg, 0.14 mmol each, 1 equiv.) in CH_2Cl_2 (10 mL) at 0 °C. After

1 h the reaction was quenched by the addition of sat'd aq. NH_4Cl solution (10 mL) and water (20 mL). The mixture was extracted with CH_2Cl_2 (3 x 20 mL), dried over $MgSO_4$, filtered and concentrated. The residue was purified by chromatography (3:1 = hex-EtOAc) to give a colourless oil (50 mg, 42 %); GC-MS: R_T 26.2 min (*n* = 3, $C_8H_{12}O_2$, M^+ obsd *m/z* 140); R_T 28.7 min (*n* = 4, $C_9H_{14}O_2$, M^+ obsd *m/z* 154); R_T 30.9 min (*n* = 5, $C_{10}H_{16}O_2$, M^+ obsd *m/z* 168); R_T 32.9 min (*n* = 6, $C_{11}H_{18}O_2$, M^+ obsd *m/z* 182); R_T 35.0 min (*n* = 7, $C_{12}H_{20}O_2$, M^+ obsd *m/z* 196).

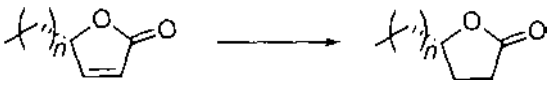


(*5S*)-series were prepared in an analogous fashion as a colourless oil (63 mg, 53 %); GC-MS: R_T 26.0 min (*n* = 3, $C_8H_{12}O_2$, M^+ obsd *m/z* 140); R_T 28.5 min (*n* = 4, $C_9H_{14}O_2$, M^+ obsd *m/z* 154); R_T 30.7 min (*n* = 5, $C_{10}H_{16}O_2$, M^+ obsd *m/z* 168); R_T 32.7 min (*n* = 6, $C_{11}H_{18}O_2$, M^+ obsd *m/z* 182); R_T 34.8 min (*n* = 7, $C_{12}H_{20}O_2$, M^+ obsd *m/z* 196).



Hydrogenation: Pd/C (16 mg, 10 % Pd on charcoal, 5 mol%) was added to a solution of lactones (50 mg, 0.06 mmol each) in EtOAc (2 mL). The mixture was shaken at RT overnight under H_2 (2.5 bar). The mixture was filtered through Celite, which was then washed well with EtOAc (20 mL). The filtrate was concentrated to give a colourless oil (50 mg, 100 %); GC-MS: R_T 25.2 min (*n* = 3, $C_8H_{14}O_2$, M^+ obsd *m/z* 142); R_T 27.6 min (*n* = 4, $C_9H_{16}O_2$, M^+ obsd *m/z* 156); R_T 30.0 min (*n* = 5, $C_{10}H_{18}O_2$, M^+ obsd *m/z* 170); R_T

32.2 min (n = 6, $C_{11}H_{20}O_2$, M^+ obsd m/z 184); R_T 34.4 min (n = 7, $C_{12}H_{22}O_2$, M^+ obsd m/z 198).

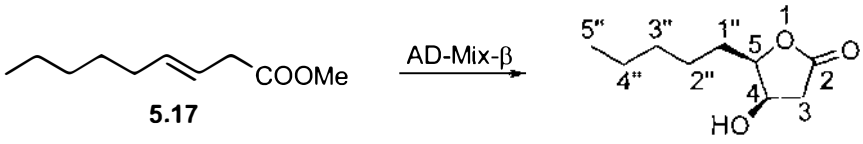


n = 3,4,5,6,7

Five (5*R*)-series were prepared in an analogous fashion to give lactones as a yellow oil (63 mg, 100 %); GC-MS: R_T 25.4 min (n = 3, $C_8H_{14}O_2$, M^+ obsd m/z 142); R_T 27.8 min (n = 4, $C_9H_{16}O_2$, M^+ obsd m/z 156); R_T 30.2 min (n = 5, $C_{10}H_{18}O_2$, M^+ obsd m/z 170); R_T 32.6 min (n = 6, $C_{11}H_{20}O_2$, M^+ obsd m/z 184); R_T 34.6 min (n = 7, $C_{12}H_{22}O_2$, M^+ obsd m/z 198).

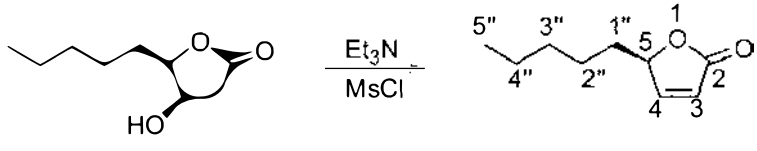
3.29 **5.17**

(3*E*)-Methyl 3-nonenoate (5.17): A solution of 3-nonenoic acid (3.46 g, 5.0 mmol) in methanol (50 mL) was heated at reflux in the presence of Amberlyst-15 (3.46 g) for 1 h. The mixture was cooled, the Amberlyst-15 was removed by filtration and rinsed with Et_2O (20 mL). The filtrate and washings were concentrated and the residue purified by chromatography (5:1 = hex-EtOAc) to give **5.17** as a light yellow oil (2.69 g, 89 %). R_f = 0.78 (3:1 hex-EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, J = 7.0 Hz, 3H, H-9), 1.32 (m, 6H, H-6, H-7, H-8), 2.02 (q, J = 6.6 Hz, 2H, H-5), 3.03 (d, J = 5.5 Hz, 2H, H-2), 3.68 (s, 3H, -OCH_3), 5.47-5.61 (m, 2H, H-3, H-4); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.9, 22.4, 28.7, 31.2, 32.3, 37.8, 51.5, 121.3, 134.8, 172.4.



5.17 **4*R*,5*R*-5.20**

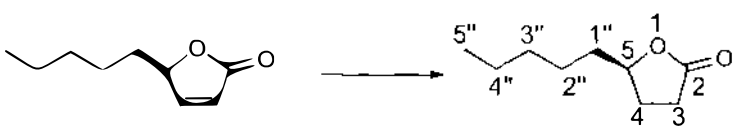
(*4R,5R*)-4,5-Dihydro-4-hydroxy-5-pentyl-2(3H)-furanone (*4R,5R*-5.20): AD-mix-β (1.4 g) and the ester **5.17** (187 mg, 1.0 mmol, 1.0 equiv.) were added to a mixture of ^{t}BuOH (20 mL) and H_2O (20 mL) at 0 °C. The mixture was stirred for 48 h, then quenched by the addition of sat'd aq. Na_2SO_3 (20 mL). The mixture was extracted with CH_2Cl_2 (3 x 20 mL), dried over $MgSO_4$, filtered and concentrated. The residue was purified by chromatography (5:1 = hex-EtOAc → 15:1 = CH_2Cl_2-MeOH) to give ***4R,5R*-5.20** as a colourless oil (185 mg, 98 %). R_f = 0.80 (15:1 CH_2Cl_2-MeOH); $[\alpha]_D^{20}$ = +57.8° (*c* 1.0, $CHCl_3$), lit.[117a] $[\alpha]_D^{20}$ = +62.9° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (t, *J* = 6.9 Hz, 3H, H-5"), 1.67-1.89 (m, 8H, H-1", H-2", H-3", H-4"), 2.54 (dd, *J* = 17.8, 0.8 Hz, 1H, H-3), 2.80 (dd, *J* = 17.8, 5.5 Hz, 1H, H-3'), 4.35-4.42 (m, 1H, H-4), 4.44-4,48 (m, 1H, H-5), 5.38 (s, 1H, -OH),; ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 22.4, 25.1, 28.1, 31.5, 39.4, 68.6, 85.5, 176.9.



4*R*,5*R*-5.20 ***R*-5.22**

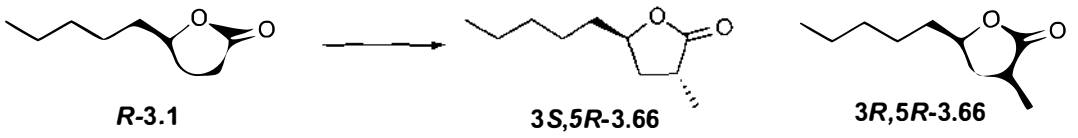
(*5R*)-5-Pentyl-2(5H)-furanone (*R*-5.22): Triethylamine (293 μL, 213 mg, 2.1 mmol, 2.1 equiv.) and methanesulfonyl chloride (85 μL, 126 mg, 1.1 mmol, 1.1 equiv.) were added to a solution of lactone ***4R,5R*-5.20** (185 mg, 1.0 mmol, 1.0 equiv.) in CH_2Cl_2 (10 mL) at 0 °C. After 1 h the reaction was quenched by the addition of sat'd aq. NH_4Cl solution (10

mL). The mixture was extracted with CH_2Cl_2 (3 x 20 mL), dried over $MgSO_4$, filtered and concentrated. The residue was purified by chromatography (3:1 = hex-EtOAc) to give ***R*-5.22** as a colourless oil (129 mg, 75 %). R_f = 0.41 (3:1 hex-EtOAc); $[\alpha]_D^{20}$ = -93.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, *J* = 7.1 Hz, 3H, H-5"), 1.27-1.52 (m, 6H, H-2", H-3", H-4"), 1.63-1.82 (m, 2H, H-1"), 5.02-5.08 (m, 1H, H-5), 6.10 (dd, *J* = 5.7, 2.0 Hz, 1H, H-4), 7.48 (dd, *J* = 5.7, 1.5 Hz, 1H, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 22.3, 24.5, 31.3, 33.0, 83.4, 121.3, 156.4, 173.2.



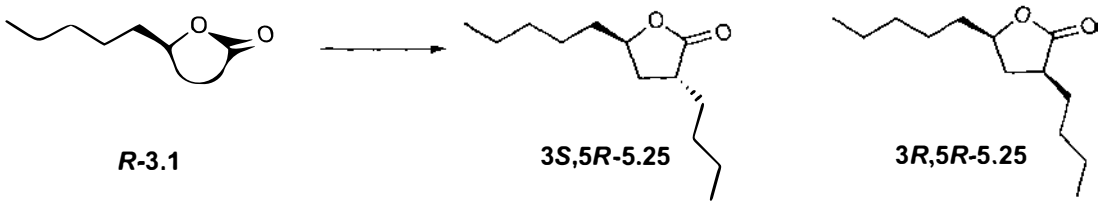
***R*-5.22** ***R*-3.1**

(*5R*)-Dihydro-5-pentyl-2(3H)-furanone (*R*-3.1): Pd/C (18 mg, 10 % Pd on charcoal, 0.02 mmol, 0.05 equiv.) was added to a solution of lactone ***R*-5.22** (74 mg, 0.4 mmol) in EtOAc (2 mL). The mixture was shaken at RT overnight under H_2 (2.5 bar). The mixture was filtered through Celite, washing well with EtOAc (20 mL). The filtrate was concentrated to give ***R*-3.1** as a colourless oil (74 mg, 100 %). R_f = 0.41 (3:1 hex-EtOAc); $[\alpha]_D^{20}$ = +50.1° (*c* 3.0, MeOH), lit.[148] $[\alpha]_D^{20}$ = +50.4° (*c* 3.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, *J* = 6.5 Hz, 3H, H-5"), 1.24-1.91 (m, 8H, H-1", H-2", H-3", H-4"), 2.28-2.37 (m, 2H, H-4), 2.54 (dd, *J* = 9.4, 7.0 Hz, 2H, H-3), 4.45-4.54 (m, 1H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 22.4, 24.8, 27.9, 28.8, 31.4, 35.4, 81.0, 177.3.



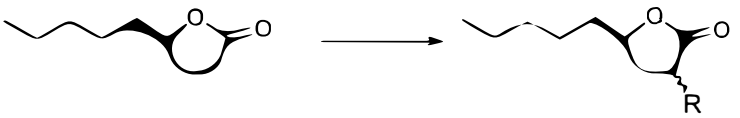
trans:*cis* = 17:3

(3*S*,5*R*)-Dihydro-3-methyl-5-pentyl-(3H)-furanone (3*S*,5*R*-3.66) and (3*R*,5*R*)-Dihydro-3-methyl-5-pentyl-(3H)-furanone (3*R*,5*R*-3.66): ^{n}BuLi (2.5 M in hexane, 480 μL, 1.2 mmol, 1.2 equiv.) was added to a solution of $^{i}Pr_2NH$ (121 mg, 169 μL, 1.2 mmol, 1.2 equiv.) in THF (10 mL) at 0 °C. The mixture was cooled to -78 °C and stirred for 30 min. A solution of lactone ***R*-3.1** (173 mg, 1.0 mmol, 1.0 equiv.) in THF (10 mL) was added to the mixture dropwise and stirring continued for 2 h at this temperature. Methyl iodide (142 mg, 62 μL, 1.0 mmol, 1.0 equiv.) was added and stirred for 3 h at -78 °C. The reaction was quenched by adding MeOH (10 mL) and sat'd NH_4Cl (20 mL). The aqueous layer was extracted with Et_2O (3 x 20 mL) and the combined organic layers were washed with brine (20 mL), dried over $MgSO_4$ filtered and concentrated. The residue was purified by chromatography (3:1 = hex-EtOAc) to give a mixture of **3*S*,5*R*-3.66** and **3*R*,5*R*-3.66** as a colourless oil (153 mg, 82 %). R_f = 0.60 (3:1 hex-EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, J = 6.6 Hz, 3H, $C\underline{H}_3CH_2$-), 1.25 (d, J = 7.3 Hz, 3H, -CH_3 at C-3), 1.27-1.72 (m, 8H, -CH_2-), 1.97 (dt, J = 12.8, 7.5 Hz, 1H, H-4), 2.09 (ddd, J = 12.8, 9.0, 5.0 Hz, 1H, H-4'), 2.43-2.51 (m, 0.15H, *cis* H-3), 2.60-2.72 (m, 0.85H, *trans* H-3), 4.24-4.35 (m, 0.15H, *cis* H-5), 4.51 (tt, J = 7.7, 5.3 Hz, 0.85H, *trans* H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 15.8, 22.4, 24.9, 31.4, 33.9, 35.3, 35.4, 78.6, 180.0; HRMS calcd. for $C_{10}H_{19}O_2$ (MH^+): 171.13850; obsd: 171.13825.



trans:cis = 7:3

(3*S*,5*R*)-Dihydro-3-butyl-5-pentyl-(3H)-furanone (3*S*,5*R*-5.25) and (3*R*,5*R*)-Dihydro-3-butyl-5-pentyl-(3H)-furanone (3*R*,5*R*-5.25): ^{n}BuLi (2.5 M in hexane, 480 μL, 1.2 mmol, 1.2 equiv.) was added to a solution of $^{i}Pr_2NH$ (121 mg, 169 μL, 1.2 mmol, 1.2 equiv.) in THF (20 mL) at 0 °C. The mixture was cooled to -78 °C and stirred for 30 min. A solution of lactone ***R*-3.1** (173 mg, 1.0 mmol, 1.0 equiv.) in THF (5 mL) was added to the mixture dropwise and stirring continued for 2 h. Butyl iodide (184 mg, 114 μL, 1.0 mmol, 1.0 equiv.) was added, and stirred for 3 h at -78 °C. The reaction was quenched by the addition of MeOH (10 mL) and sat'd aq. NH_4Cl (20 mL). The aqueous layer was extracted with Et_2O (3 x 20 mL) and the combined organic layers were washed with brine (20 mL), dried over $MgSO_4$ filtered and concentrated. The residue was purified by chromatography (3:1 = hex-EtOAc) to give a mixture of **3*S*,5*R*-5.25** and **3*R*,5*R*-5.25** as a colourless oil (156 mg, 74 %). R_f = 0.62 (5:1 hex-EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (t, J = 7.2 Hz, 6H, CH_3-), 1.19-1.82 (m, 14H, -CH_2-), 1.97 (dd, J = 8.1, 6.4 Hz, 2H, H-4), 2.36-2.42 (m, 0.3H, *cis* H-3), 2.46-2.58 (m, 0.7H, *trans* H-3), 4.21-4.31 (m, 0.3H, *cis* H-5), 4.40-4.45 (m, 0.7H, *trans* H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.9, 13.9, 22.4, 22.5, 25.0, 29.5, 30.6, 31.5, 33.5, 35.6, 39.3, 78.8, 179.6; HRMS calcd for $C_{13}H_{25}O_2$ (MH^+): 213.18546; obsd: 213.18543.

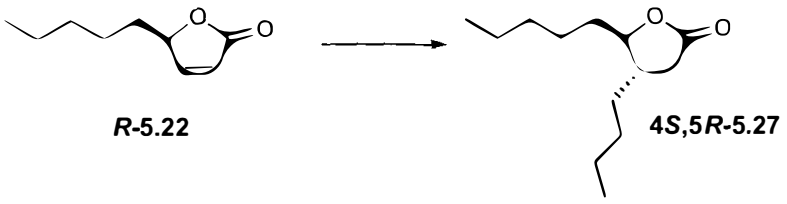


***R*-3.1** R = Me, Et, ^{n}Pr, ^{n}Bu

Four (3,5*R*)-Dihydro-3-alkyl-5-pentyl-(3*H*)-furanones: ^{n}BuLi (2.5 M in hexane, 960 μL, 2.4 mmol, 4.8 equiv.) was added to a solution of $^{i}Pr_2NH$ (243 mg, 337 μL, 2.4 mmol, 4.8 equiv.) in THF (20 mL) at 0 °C. The mixture was cooled to -78 °C and stirred for 30 min. A solution of lactone ***R*-3.1** (346 mg, 2.0 mmol, 4.0 equiv.) in THF (10 mL) was added to the mixture dropwise and stirring continued for 2 h at this temperature. Methyl iodide (142 mg, 62 μL, 1.0 mmol, 1.0 equiv.), ethyl iodide (156 mg, 81 μL, 1.0 mmol, 1.0 equiv.), *n*-propyl iodide (170 mg, 98 μL, 1.0 mmol, 1.0 equiv.), and *n*-butyl iodide (184 mg, 114 μL, 1.0 mmol, 1.0 equiv.) were dissolved in THF (5 mL) and the solution was added dropwise to the reaction mixture at -78 °C, and stirred for 3 h at this temperature. The reaction was quenched by addition of MeOH (10 mL) and sat'd NH_4Cl (20 mL). The aqueous layer was extracted with Et_2O (3 x 20 mL) and the combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated. The residue was purified by chromatography (3:1 = hex-EtOAc) to give a colourless oil (145 mg, 34 %); GC-MS: R_T 26.8 min (R = Me, $C_{10}H_{18}O_2$, M^+ obsd. *m/z* 170); R_T 29.2 min (R = Et, $C_{11}H_{20}O_2$, M^+ obsd. *m/z* 184); R_T 31.5 min (R = ^{n}Pr, $C_{12}H_{22}O_2$, M^+ obsd. *m/z* 198); R_T 33.8 min (R = ^{n}Bu, $C_{13}H_{24}O_2$, M^+ obsd. *m/z* 212).

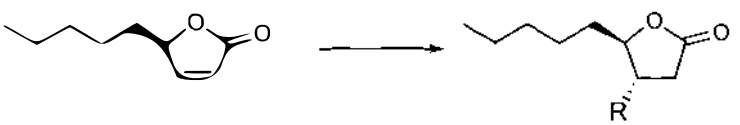
R-5.22 **4*S*,5*R*-5.31**

(4*S*,5*R*)-Dihydro-4-methyl-5-pentyl-(3H)-furanone (4*S*,5*R*-5.31): Methyl magnesium bromide (2.7 mL, 3M in Et_2O, 8.0 mmol, 8.0 equiv.) was added over 10 min to a suspension of CuI (762 mg, 4.0 mmol, 4.0 equiv.) in THF (20 mL) at -78°C. After 10 min, dimethylsulfide (124 mg, 147 μL, 2.0 mmol, 2.0 equiv.) was added and the mixture was stirred at the same temperature for 2 h. Butenolide ***R*-5.22** (171 mg, 1.0 mmol, 1.0 equiv.) in THF (5 mL) was added dropwise over 5 min at -78 °C. The mixture was warmed to 0 °C and stirred for 3 h. The reaction was quenched by the addition of sat'd aq. NH_4Cl (20 mL), stirred for an additional 10 min, concentrated to remove to bulk of the THF and extracted with Et_2O (3 x 20 mL). The extracts were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated. The residue was purified by chromatography (3:1 hex-EtOAc) to give **4*S*,5*R*-5.31** as a colourless oil (120 mg, 64 %). R_f = 0.56 (3:1 hex-EtOAc); $[\alpha]_D^{20}$ = +51.4° (*c* 0.80, CH_2Cl_2), lit.[150] $[\alpha]_D^{20}$ = +48.3° (*c* 0.79, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (t, *J* = 6.6 Hz, 3H, CH_3-), 1.07 (d, *J* = 6.5 Hz, 3H, -CH_3 at C-4), 1.22-1.66 (m, 8H, -CH_2-), 2.08-2.20 (m, 2H, H-3), 2.54-2.65 (m, 1H, H-4), 3.90-3.97 (m, 1H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 17.5, 22.5, 25.4, 31.6, 34.0, 36.1, 37.1, 87.5, 176.6; HRMS calcd for $C_{10}H_{19}O_2$ (MH^+): 171.13850; obsd: 171.13831.



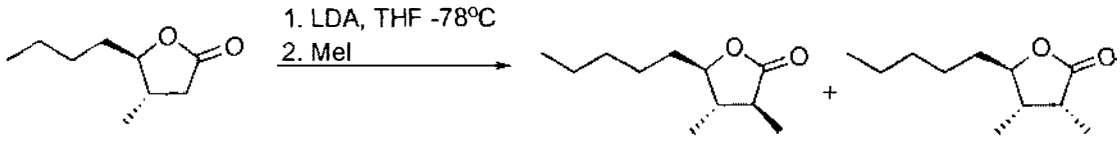
R-5.22 **4*S*,5*R*-5.27**

(4*S*,5*R*)-Dihydro-4-butyl-5-pentyl-(3H)-furanone (4*S*,5*R*-5.27): *n*-Butyl magnesium bromide (4.68 mL, 1.71 M in 1:1 THF/toluene, 8.0 mmol, 8.0 equiv.) was added over 10 min to a suspension of CuI (762 mg, 4.0 mmol, 4.0 equiv.) in THF (20 mL) at -78°C. After 10 min, dimethylsulfide (124 mg, 147 μL, 2.0 mmol, 2.0 equiv.) was added and the mixture was stirred at the same temperature for 2 h and then butenolide ***R*-5.22** (171 mg, 1.0 mmol, 1.0 equiv.) in THF (5 mL) was added dropwise over 5 min at -78 °C. The mixture was stirred at 0 °C for 3 h. The reaction was quenched by the addition of sat'd aq. NH_4Cl (20 mL), stirred for an additional 10 min, concentrated to remove the bulk of the THF and extracted with Et_2O (3 x 20 mL). The extracts were washed with brine (20 mL), dried over $MgSO_4$, filtered, concentrated and the residue purified by chromatography (3:1 hex-EtOAc) to give **4*S*,5*R*-5.27** as a colourless oil (74 mg, 41 %). R_f = 0.56 (5:1 hex-EtOAc); $[\alpha]_D^{20}$ = +24.5° (*c* 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93 (t, *J* = 6.5 Hz, 6H, CH_3-), 1.25-1.38 (m, 12H, -CH_2-), 1.50-1.69 (m, 2H, OCHC$\underline{H}_2$-), 2.05-2.24 (m, 2H, H-3, H-4), 2.66 (dd, *J* = 17.3, 8.3 Hz, 1H, H-3'), 4.03-4.12 (m, 1H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.9, 14.0, 22.5, 22.6, 25.4, 29.7, 31.5, 32.8, 34.6, 35.3, 41.2, 86.2, 176.8; HRMS calcd for $C_{13}H_{25}O_2$ (MH^+): 213.18546; obsd: 213.18548.



***R*-5.15** R = Me, Et, ^{n}Bu

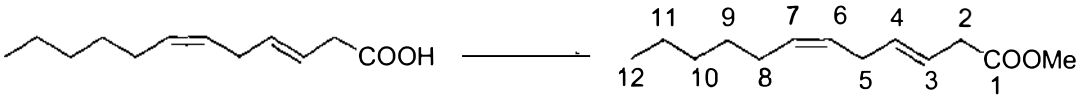
Three (4*S*,5*R*)-Dihydro-4-alkyl-5-pentyl-(3*H*)-furanones: Methyl magnesium bromide (667 μL, 3 M in Et_2O, 2.0 mmol, 2.0 equiv.), ethyl magnesium bromide (667 μL, 3 M in Et_2O, 2.0 mmol, 2.0 equiv.), and *n*-butyl magnesium bromide (1.17 mL, 1.71 M in 1:1 THF/toluene, 2.0 mmol, 2.0 equiv.) were added over 10 min to a suspension of CuI (571 mg, 3.0 mmol, 3.0 equiv.) in THF (20 mL) at -78°C. After 10 min, dimethylsulfide (93 mg, 110 μL, 1.5 mmol, 1.5 equiv.) was added and the mixture was stirred at the same temperature for 2 h. Butenolide ***R*-5.22** (171 mg, 1.0 mmol, 1.0 equiv.) in THF (5 mL) was added dropwise over 5 min. The mixture was stirred at -78 °C for 3 h. The reaction was quenched by the addition of sat'd aq. NH_4Cl (20 mL), stirred for an additional 10 min, concentrated to remove the bulk of the THF and extracted with Et_2O (3 x 20 mL). The extracts were washed with brine (20 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by chromatography (3:1 hex-EtOAc) to give a colourless oil (154 mg, 81 %); GC-MS: R_T 26.0 min (R = Me, $C_{10}H_{18}O_2$, M^+ obsd *m/z* 170); R_T 28.1 min (R = Et, $C_{11}H_{20}O_2$, M^+ obsd *m/z* 184); R_T 31.8 min (R = ^{n}Bu, $C_{13}H_{24}O_2$, M^+ obsd *m/z* 212).



4*S*,5*R*-5.31 **3*R*,4*S*,5*R*-5.33** major **3*S*,4*S*,5*R*-5.33** minor

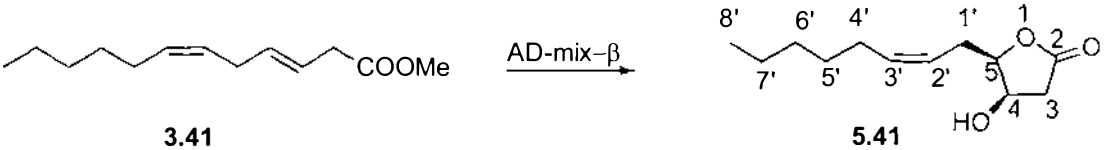
4 : 1

(3*R*,4*S*,5*R*)-Dihydro-3,4-dimethyl-5-pentyl-2(3*H*)-furanone (3*R*,4*S*,5*R*-5.33) and (3*S*,4*S*,5*R*)-dihydro-3,4-dimethyl-5-pentyl-2(3*H*)-furanone (3*R*,4*S*,5*R*-5.33): ^{n}BuLi (2.5 M in hexane, 480 μL, 1.2 mmol, 1.2 equiv.) was added to a solution of $^{i}Pr_2NH$ (121 mg, 169 μL, 1.2 mmol, 1.2 equiv.) in THF (10 mL) at 0 °C. The mixture was cooled to -78 °C and stirred for 30 min. A solution of **4*S*,5*R*-5.32** (170 mg, 1.0 mmol, 1.0 equiv.) in THF (10 mL) was added to the mixture dropwise and stirring continued for 2 h at this temperature. Methyl iodide (142 mg, 62 μL, 1.0 mmol, 1.0 equiv.) was added and stirred for 3 h at -78 °C. The reaction was quenched by the addition of MeOH (10 mL) and sat'd NH_4Cl (20 mL). The aqueous layer was extracted with Et_2O (3 x 20 mL) and the combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated. The residue was purified by chromatography (3:1 = hex-EtOAc) to give a mixture of **3*R*,4*S*,5*R*-5.33** and **3*S*,4*S*,5*R*-5.33** as a yellow oil (93 mg, 50 %). R_f = 0.66 (3:1 hex-EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, J = 6.9 Hz, 3H, CH_3-), 0.99 (d, J = 7.1 Hz, 3H, -CH_3 at C-4), 1.11 (d, J = 6.5 Hz, 3H, -CH_3 at C-3), 1.25-1.73 (m, 8H, -CH_2-), 2.14-2.21 (m, 1H, H-4), 2.58-2.73 (m, 1H, H-3), 3.82-3.94 (m, 0.2H, H-5 as minor), 3.94-4.04 (m, 0.8H, H-5 as major); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 14.0, 17.1, 22.4, 25.3, 31.5, 33.9, 36.0, 37.0, 87.4, 176.5; HRMS calcd for $C_{11}H_{21}O_2$ (MH^+): 185.15415; obsd: 185.15428.



3.34 **3.41**

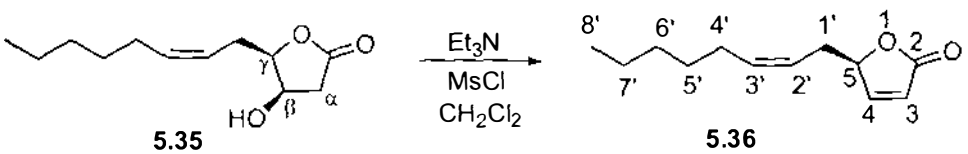
Methyl 3*E*,6*Z*-undecadienoate (3.41): A solution of 3*E*,6*Z*-undecadienoic acid (3.46 g, 5.0 mmol) in methanol (50 mL) was heated at reflux in the presence of Amberlyst-15 (3.46 g) for 1 h. The mixture was cooled, the Amberlyst-15 was removed by filtration and rinsed with Et_2O (20 mL). The filtrate and washings were concentrated and the residue purified by chromatography (5:1 = hex-EtOAc) to give **3.41** as a light yellow oil (2.69 g, 91 %). R_f = 0.78 (3:1 hex-EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, J = 6.9 Hz, 3H, H-12), 1.23-1.38 (m, 6H, H-9, H-10, H-11), 2.03 (q, J = 6.9 Hz, 2H, H-8), 2.76-2.81 (m, 2H, H-5), 3.05 (d, J = 4.4 Hz, 2H, H-2), 3.68 (s, 3H, OCH_3), 5.33-5.57 (m, 4H, H-3, H-4, H-6, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.5, 27.1, 29.3, 30.3, 31.5, 37.9, 51.8, 121.8, 126.6, 131.2, 133.0, 172.5; HRMS calcd for $C_{13}H_{23}O_2$ (MH^+): 211.16980; obsd: 211.17002.



3.41 **5.41**

(*4R,5R*)-4,5-Dihydro-4-hydroxy-5-(2'*Z*)-oct-2-enyl-2(3H)-furanone (5.41): AD-mix-β (1.4 g) and the ester **3.41** (210 mg, 1.0 mmol, 1.0 equiv.) were added to a mixture of ^{t}BuOH (20 mL) and H_2O (20 mL) at 0 °C. The mixture was stirred for 48 h, then quenched by the addition of sat'd aq. Na_2SO_3 (20 mL). The mixture was extracted with CH_2Cl_2 (3 x 20 mL), dried over $MgSO_4$, filtered and concentrated. The residue was purified by chromatography (5:1 = hex-EtOAc → 15:1 = CH_2Cl_2-MeOH) to give **5.41** as a

colourless oil (146 mg, 69 %). R_f = 0.63 (15:1 CH_2Cl_2-MeOH); $[\alpha]_D^{20}$ = +46.1° (*c* 0.65, $CHCl_3$), lit.[183] $[\alpha]_D^{20}$ = +44.5° (*c* 0.65, acetone); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, *J* = 6.9 Hz, 3H, H-8'), 1.23-1.36 (m, 6H, H-5', H-6', H7'), 2.06 (q, *J* = 7.1 Hz, 2H, H-4'), 2.51-2.65 (m, 2H, H-1'), 2.77 (dd, *J* = 17.8, 5.5 Hz, 2H, H-3), 4.32-4.39 (m, 1H, H-4), 4.45-4.49 (m, 1H, H-5), 5.36-5.42 (m, 1H, H-3'), 5.52-5.63 (m, 1H, H-2'); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.5, 26.5, 27.4, 29.1, 31.4, 39.2, 68.6, 84.3, 122.4, 134.1. 176.0; HRMS calcd for $C_{12}H_{20}O_3$ (M^+): 212.14124; obsd: 212.14116.



5.35 **5.36**

(*5R*)-5-Oct-2'*Z*-enyl-2(5H)-furenone (5.42): Triethylamine (146 μL, 106 mg, 1.05 mmol, 2.1 equiv.) and methanesulfonyl chloride (43 μL, 63 mg, 0.55 mmol, 1.1 equiv.) were added to a solution of lactone **5.41** (105 mg, 0.5 mmol, 1.0 equiv.) in CH_2Cl_2 (10 mL) at 0 °C. After 1 h the reaction was quenched by the addition of sat'd aq. NH_4Cl solution (10 mL). The mixture was extracted with CH_2Cl_2 (3 x 20 mL), dried over $MgSO_4$, filtered and concentrated. The residue was purified by chromatography (3:1 = hex-EtOAc) to give **5.42** as a colourless oil (49 mg, 51 %). R_f = 0.38 (3:1 hex-EtOAc); $[\alpha]_D^{20}$ = -134.7° (*c* 1.0, $CHCl_3$), lit.[183] $[\alpha]_D^{20}$ = -134.1° (*c* 0.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, *J* = 6.9 Hz, 3H, H-8'), 1.22-1.35 (m, 6H, H-5', H-6', H-7'), 1.99 (q, *J* = 7.3 Hz, 2H, H4'), 2.50 (p, *J* = 7.9 Hz, 2H, H-1'), 4.98-5.04 (m, 1H, H5), 5.26-5.35 (m, 1H, H-3'), 5.53-5.62 (m, 1H, H-2'), 6.10 (dd, *J* = 5.7, 2.0 Hz, 1H, H4), 7.42 (dd, *J* = 5.7, 1.5 Hz, H-

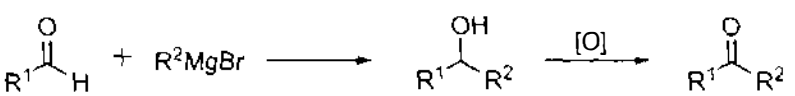
3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.5, 27.3, 29.0, 31.0, 31.4, 82.7, 121.1, 121.9, 134.9, 155.8, 172.9; HRMS calcd. for $C_{12}H_{19}O_2$ (MH^+): 195.13850; obsd: 195.13925.

Chapter 6

Chapter 6: Summary and future work

The goal of this project was to synthesise potential flavour compounds combinatorially and identify key components for further investigation as flavourants in dairy products. This chapter aims to summarise our accomplishments and put them in context.

The synthesis and analysis of ketones were the foci of Chapter 2. Ketones were synthesised individually *via* a two-step sequence. The Grignard reaction was the first step to produce an alcohol and the oxidation of the secondary alcohol ensued, to produce a ketone (Scheme 6.1).



Scheme 6.1: Synthetic route for making ketones in Chapter 2.

Twenty ketones were synthesised individually and sixteen were sufficiently stable to be screened by the Fox 4000. Some compounds selected from the Fox analysis were assessed by GC-O. Results from the preliminary screenings indicated aromatic and cyclopropyl ketones as compounds of interest (Figure 6.1). The first library of cyclopropyl ketones was synthesised and screened (Scheme 6.2).

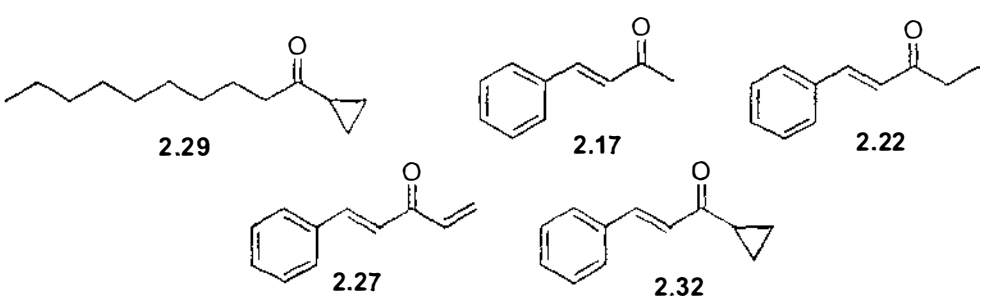
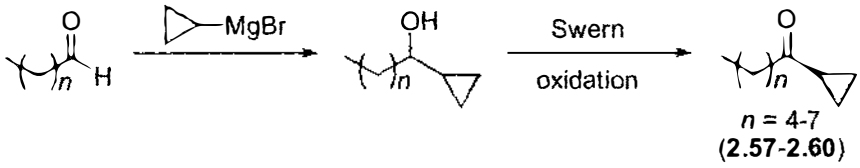


Figure 6.1: The structures of lead ketones from Chapter 2.



Scheme 6.2: Combinatorial synthesis of a library of four cyclopropyl ketones.

Further ketone libraries were produced by a co-worker, David Lun. He synthesised libraries of ketones including ethyl ketones and cyclopropyl ketones. Selected compounds are illustrated in Figure 6.2 and their odour and potency was assessed by the laboratory technician from Fonterra.

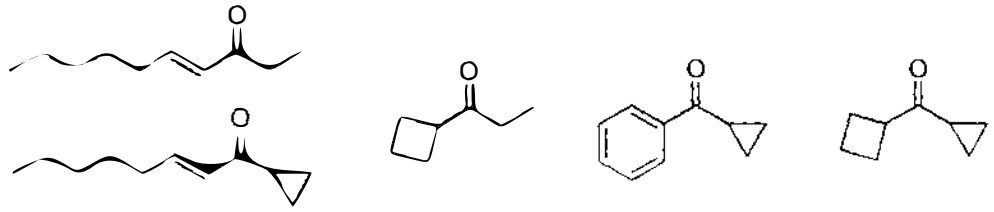
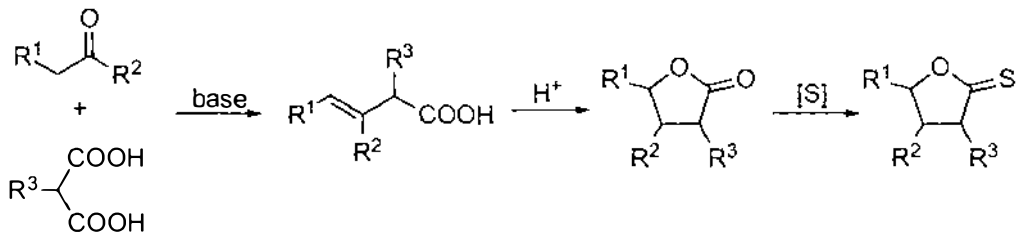


Figure 6.2: Some of the ketones produced in libraries.

The synthesis and analysis of racemic lactones were the foci of Chapter 3. Individual racemic lactones were synthesised *via* a two-step process. Condensation of aldehydes with malonic acids, in the Linstead modification of the Knoevenagel reaction, gave rise to (3*E*)-alk-3-enoic acids. For unsubstituted malonic acid, catalytic piperidinium acetate was the most effective reagent. For 2-alkylmalonic acids, diethylamine was the base of choice. Ketones ($R^2 \neq H$) were unreactive in this condensation. Acid-catalysed cyclisation of these unsaturated acids gave rise to γ-lactones which were converted to γ-thionolactones using Lawesson's reagent. (3*E*)-Alk-3-enoic acids containing additional unsaturation gave

complex product mixtures on attempted cyclisation, due to rearrangements of the intermediate carbocations (Scheme 6.3).



Scheme 6.3: Synthetic route for racemic γ-lactone in Chapter 3.

Libraries of racemic γ-lactones (C_8-C_{12}), and α-substituted γ-lactones were produced combinatorially according to this method. Further, synthesis of a library of γ-thionolactones was achieved from a library of γ-lactones with Lawesson's reagent. The libraries were analysed by GC-O (Figure 6.3). The odour of the lactones was generally characterised by fruity notes (*i.e.*, peach, apricot and coconut).

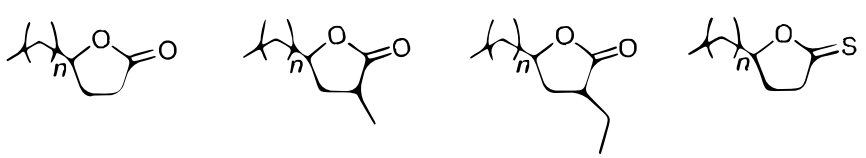
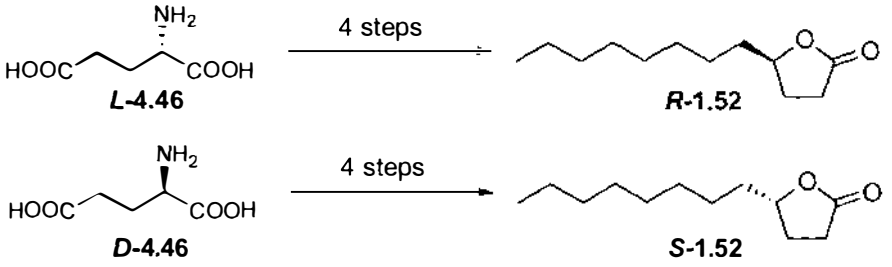


Figure 6.3: Structures of libraries of racemic γ-lactones (n = 3, 4, 5, 6, 7).

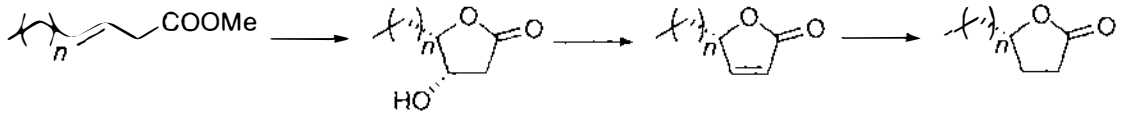
(*5R*)-Dihydro-5-octyl-2(3*H*)-furanone (***R*-1.52**) was synthesised from *L*-glutamic acid (***L*-4.44**) and the (*S*)-enantiomer (***S*-1.52**) was synthesised by analogy from *D*-glutamic acid (***D*-4.44**) in Chapter 4 (Scheme 6.4).



Scheme 6.4: Synthetic route for both enantiomers of **1.52** in Chapter 4.

This route was for the synthesis of chiral γ-lactones individually and more efficient methods are needed for variety of diversities with good overall yields in order to achieve libraries of compounds combinatorially.

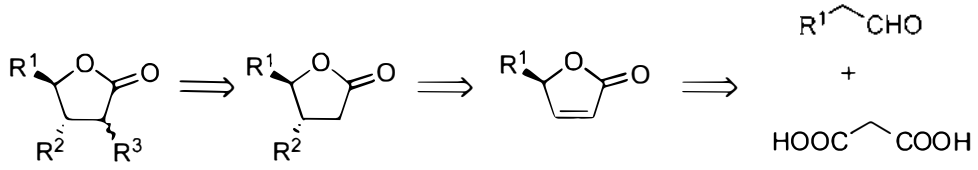
Asymmetric syntheses of both enantiomeric series of γ-lactones utilizing the Sharpless asymmetric dihydroxylation reaction were employed to give libraries (Scheme 6.5).



Scheme 6.5: Synthesis of libraries of enantiomerically pure γ-lactones (n = 3, 4, 5, 6, 7).

According to the retrosynthetic route for optically active γ-lactones in Chapter 5, there are three points of diversity (Scheme 6.6). α-Alkylation of a β,γ-disubstituted γ-lactone has been used to introduce R^3. Cuprate addition to the intermediate butenolide is highly stereoselective, delivering R^2 *trans* relative to R^1. The configuration at Cγ, bearing R^1, is controlled by the Sharpless asymmetric dihydroxylation. We have investigated the introduction of substituents in each position. It was our ultimate goal to produce libraries

in this manner, whereby five aldehydes ($R^1CH_2CH=O$), five alkylmetal species (R^2M), and five alkyl halides (R^3X) would give a library of 125 compounds.



Scheme 6.6: Retrosynthetic analysis of substituted lactones in Chapter 5.

It was a fundamental objective of this project to generate flavour compound libraries using combinatorial chemistry. Understanding the synthetic chemistry required for applying it to the combinatorial approach was the biggest challenge. Introducing modern analytical techniques to the evaluation of our synthetic compounds gave an opportunity to identify new potential flavour compounds. Further assessment of potential flavour compounds is required for rigorous screening to identity their potency.

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Chapter 1

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