γ-Lactones in wine:
Synthesis, quantification and sensory studies

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Doctor of Philosophy

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Abstract

γ-Lactones are found in a wide variety of food and beverage products, in particular grapes and wine. This thesis details the work completed on some γ-lactones in wine: their synthetic preparation, development of quantification methodologies and sensory studies.

Chapter 1 outlines the history of the Australian wine industry from the arrival of the first vines on the First Fleet in 1788 with Captain Arthur Philip. This chapter provides: an overview of Australia's position in the world of grape and wine production; an analysis of the export arm of the industry; and a look at the different wine producing regions around the country. The latter part of the chapter focuses on the different volatile compounds found in wine.

Part A:

Chapter 2 provides an overview on the history of barrel manufacture and the use of oak wood in cooperage, with an emphasis on oak's well known ability to impart desirable characteristics to wine through the extraction of volatile aroma compounds. This chapter provides a summary of these odorants with a particular emphasis on the oak lactones. Previous sensory studies and synthetic work are discussed. Of great importance to this work are the recent advancements in 1,2-dioxine chemistry, highlighted in this chapter.

Chapter 3 details the synthetic work completed for the preparation of all four possible oak lactone stereoisomers. A suitably substituted racemic 1,2-dioxine featured as the common intermediate and enabled preparation of the γ-lactone moiety upon reaction with a chiral malonate diester and separation of the diastereomers by column chromatography. A key step involved the decarboxylation of the ester cleaved γ-lactone diastereomers, which could be directed to give either the *cis*- or *trans*-products. Standard chemical transformations were then utilised to produce the desired stereoisomers of oak lactone.

Chapter 4 describes the results from the sensory studies that were completed on the synthetic oak lactone samples. Odour detection thresholds were measured in both a white and a red wine. The thresholds in the former medium were calculated to be 24 μg/L, 172 μg/L, 132 μg/L and 305 μg/L, while in the latter medium the thresholds were calculated to be 57 μg/L, 380 μg/L, 175 μg/L and 285 μg/L, for (4*S*,5*S*)-*cis*-, (4*S*,5*R*)-*trans*-, (4*R*,5*R*)-*cis*- and (4*R*,5*S*)-*trans*-oak lactone, respectively. Difference testings were completed on the pairs of enantiomers and also on mixtures of the nature-identical isomers: between the *cis*-enantiomers a significant difference was found at the 99% confidence level, while between the *trans*-enantiomers and also the mixtures of *cis*- and *trans*-isomers little difference was observed.

Chapter 5 contains the experimental procedures for ***Part A***.

Part B:

Chapter 6 discusses the sensory properties of some γ- and δ-lactones, with the focus on a series of five-alkyl substituted γ-lactones: γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone. Topics covered in this chapter include chirality, biosynthetic pathways and quantification results in wine from previous studies for these γ-lactones.

Chapter 7 concerns the method development for the quantification of γ-lactones in wine using a stable isotope dilution assay (SIDA). Deuterated analogues were prepared from commercially available racemic γ-lactones for use as internal standards. Initially a head space solid-phase microextraction (HS SPME) method was developed using d_5-standards; however, analysis of bottled wine samples revealed the presence of co-eluting compounds that contained several of the selected ions. Thus an alternative method was developed using d_7-standards, with a specific focus on sample clean-up, *via* solid-phase extraction (SPE). Using this procedure, 44 white and 120 red wines were analysed for their γ-lactone content. The lactones were found to be significantly more common in the red wines, with γ-nonalactone the most abundant lactone in this series.

Chapter 8 deals with the extension of the SIDA method, as developed in Chapter 7, for use with a chiral gas chromatography column. Optically pure standards were prepared, from either L- or D-glutamic acid, and used to determine the order of elution of the enantiomers. A method was developed for the quantification of the individual enantiomers of γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone. The enantiomeric distribution of γ-nonalactone was investigated in 34 red wines; the (*R*)-stereoisomer was found to be dominant with an average of 59%, although there were wines analysed that did contain the (*S*)-stereoisomer in greater amounts.

Chapter 9 describes the results from the sensory studies that were completed on the individual enantiomers of the γ-lactones. Odour detection thresholds were measured in a red wine. The thresholds were calculated to be 238 μg/L, 285 μg/L, 34 μg/L and 8 μg/L for the (*R*)-enantiomers, while the thresholds were calculated to be 135 μg/L, 91 μg/L, 47 μg/L and 39 μg/L for the (*S*)-enantiomers, of γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone, respectively.

Chapter 10 contains the experimental procedures for ***Part B***.

Chapter 11 contains the appendices, followed by the references in **Chapter 12**.

Declaration

'I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.'

Rachel C. Brown
16 November 2007

"I believe that this thesis is properly presented, conforms to the specification for the thesis and is of sufficient standard to be, *prima facie*, worthy of examination"

Gordon M. Elsey
16 November 2007

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'*The important thing is not to stop questioning. Curiosity has its own reason for existing*.' Albert Einstein (1879-1955)

Publications

Refereed journal articles:

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Brown, R.C.; Taylor, D.K.; Elsey, G.M. Utilisation of a 1,2-dioxine for the synthesis of the four possible stereoisomers of oak lactone, *Organic Letters*, **2006**, *8*(3), 463-466.

Refereed conference papers:

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Elsey, G.M.; **Brown, R.C.**; Pardon, K.H.; Capone, D.L.; Sefton, M.A.; Herderich, M.J.; Pretorius, I.S. Chirality and its effect on wine aroma: Recent experiences in aroma research at the AWRI, *Australia and New Zealand Wine Industry Journal*, *in press*.

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Abbreviations and glossary

°C	degrees Celsius
Δ	heat
Å	angstroms
ACCN	1,1'-azo-*bis*-(cyclohexanecarbonitrile)
AcOH	acetic acid (glacial)
Ac_2O	acetic anhydride
AIBN	2,2'-azo-*bis*-(2-methylpropionitrile)
a.k.a	also known as
Al_2O_3	aluminium oxide
app.	apparent
BET	best estimate threshold
$BH_3.Me_2S$	borane-dimethyl sulfide
bpt	boiling point
brine	saturated aqueous sodium chloride solution
c	concentration
cat.	catalytic
CCl_4	carbon tetrachloride
C_6H_6	benzene
$(CH_3)_2CHBr$	*iso*-propyl bromide
CH_2Cl_2	dichloromethane
cm	centimetres
COSY	correlation spectroscopy
$CuSO_4$	copper sulfate
δ	chemical shift (parts per million)
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DCl	deuterium chloride
DIBAL	di-*iso*-butylaluminium hydride
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DME	1,2-dimethoxyethane
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
D_2O	deuterium oxide

ee	enantiomeric excess
EI	electron impact
Et_3N	triethyl amine
EtOAc	ethyl acetate
Et_2O	diethyl ether
EtOH	ethanol
g	grams
GC-MS	gas chromatography-mass spectrometry
HCl	hydrochloric acid
HMBC	heteronuclear multiple bond connectivity
HMQC	heteronuclear multiple quantum coherence
HRMS	high resolution mass spectrometry
hrs	hours
HS SPME	head space solid-phase microextraction
Hz	hertz
J	coupling constant (Hz)
KOH	potassium hydroxide
L	litre
LDA	lithium di-*iso*-propylamide
lit.	literature
LOD	limit of detection
m-CPBA	*m*-chloroperbenozic acid
Me	methyl
MeCN	acetonitrile
MeOH	methanol
mg	milligrams
μg	microgram
$MgSO_4$	magnesium sulfate
MHz	megahertz
mins	minutes
mL	millilitre
mmol	millimole
μm	micrometer
mol	mole

mpt	melting point
m/z	mass to charge ratio
$NaBD_4$	sodium borodeuteride
$NaBH_4$	sodium borohydride
NaH	sodium hydride
$NaHCO_3$	sodium hydrogen carbonate
$NaHSO_4$	sodium hydrogen sulfate
$NaIO_4$	sodium periodate
$NaNO_2$	sodium nitrite
Na_2SO_4	sodium sulfate
NH_4Cl	ammonium chloride
nm	nanometre
NMR	nuclear magnetic resonance
nOe	nuclear Overhauser effect
o/n	overnight
Pd-$BaSO_4$	palladium on barium sulfate
Ph	phenyl
ppm	parts per million
pyr	pyridine
R_f	retention factor
rt	room temperature
$RuCl_3$	ruthenium trichloride
sat.	saturated
SIDA	stable isotope dilution assay/analysis
SIM	selected ion monitoring
SPE	solid-phase extraction
SO_2	sulfur dioxide
TBS	*tert*-butyldimethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMEDA	*N*,*N*,*N*',*N*'-*tetra*-methylethylenediamine
TsCl	*p*-toluenesulfonyl chloride
W	watts

1 Introduction to wine and the Australian perspective

Wine is a complex medium arising from the crushing, fermentation and maturation of grapes. This chapter outlines the history of the Australian wine industry and explores the diversity of the different aroma compounds found in wine.

1.1 The origin of grape vines in Australia

Grape vines arrived in Australia with Captain Arthur Phillip on the First Fleet in January, 1788.[1] The first vines were planted at Farm Cove in Sydney Harbour. Unfortunately, these vines did not prove to be as successful as was first anticipated, due to a combination of the humid weather conditions and failure to identify and control black spot disease.[2] With the need for wine in the new colony, the vines were replanted three years later, in 1791, at Rose Hill (now known as Parramatta) along the Parramatta River.

Captain John Macarthur established the earliest commercial vineyards in 1820. From his travels through France during 1815 and 1816, Macarthur learnt of the cultivation of the vine and returned to Australia in 1817 with cuttings that he planted at Camden Park, 43 miles from Sydney.[3] Wine was produced from these young grapes, but after three years, Macarthur decided to move his vineyard to the Nepean River near Penrith. This 20-acre vineyard produced wine as a practical business and by 1827 output reached 20,000 gallons.[2]

In 1816, the explorer Gregory Blaxland planted a vineyard along the Parramatta River (now known as Ermington). In 1822, Blaxland was the first to export wine (26 gallons) from Australia to England and, in 1823, was awarded the silver medal of the Society for the Encouragement of Arts, Manufacturers and Commerce, now the Royal Society of Arts. Shortly after this time, in 1828, he was awarded the equivalent of a gold medal for his exported wine (400 gallons of red), which was described by the judges as a ‘light but sound wine with much of the odour and

flavour of claret'.[3]

A renowned figure in the establishment of viticultural practices in Australian was James Busby.[4] He emigrated to Australia in 1824 with a strong passion for the cultivation of grape vines. Busby travelled back to Europe in 1831, where he studied viticulture and collected vine stock from some 650 different grape varieties in Spain and France. Of these cuttings, 362 are believed to have survived the journey back to Australia and were planted at the Botanic Gardens in Sydney. Over time these vines were distributed around Australia, with the establishment of a significant collection at the Botanic Gardens in Adelaide, and the founding here of the principal wine state. Due to the importation of over 350 vines, James Busby is considered to be the 'father' of the Australian wine industry.

The Australian wine industry was slow to develop; the initial focus was on the cultivation of table grapes as a supply of fresh fruit for the new colony. By the middle of the 19th century, vineyards for the production of wine had been established in most Australian states.[4] New South Wales, being the first state established with vineyards, saw the emergence of profitable vineyards in the beginning of the 19th century. Early pioneers included George Wyndham and Edward Tyrell. Some of the oldest vineyards were planted in Tasmania in the 1820s. In South Australia, the first grape vines were planted in the 1830s through to the 1860s in Reynella, McLaren Vale and Langhorne Creek. It was due to the work of John Reynell, Dr Alexander Kelly, Thomas Hardy and Frank Potts who planted the initial vines in these areas. Western Australia saw the plantation of its first vines in the 1840s and Queensland in the 1860s.

1.2 The wine industry around the world

Australia features prominently in the world production of grapes and wine (Table 1.1).[5] In 2005, Australia was ranked thirteenth in the world for the largest vineyard area and tenth in the world for total grape production. The top four countries in vineyard area and grape production were France, Italy, Spain and the United States of America. Australia was the sixth highest wine producing country in the world. The four largest wine producing countries, with a combined total of over 50% of the annual total, were France, Italy, Spain and the United States of America. Australia was a significant contributor to the world export market, with nearly half (49.0%) of its wine produced for export, which contributed to over 10% of the world export market value. Wine contributed to 2.2% of Australia's total exports. Nearly 2% of the total world wine consumption occurred in Australia.

Table 1.1 World grape and wine production, exports and consumption for 2005

	vineyard area[a]	grapes[b]	wine made[c]	wine exports[d]	export value[e]	of total exports[f]	wine consumed[g]
Argentina	2.7	3.6	5.4	14.6	1.5	0.8	4.7
Australia	2.0	3.1	5.1	49.0	10.4	2.2	1.9
Austria	0.6	0.5	0.9	28.9	0.5	0.1	1.0
Brazil	1.0	1.8	1.1	0.4	0.0	0.0	1.5
Bulgaria	1.5	0.4	0.7	55.8	0.5	0.9	0.3
Canada	0.1	0.1	0.2	6.1	0.1	0.0	1.5
Chile	2.3	3.4	2.9	52.4	4.3	2.3	1.1
China	6.0	8.7	2.6	0.4	0.0	0.0	2.5
Croatia	0.8	0.5	0.6	1.6	0.0	0.1	0.8
France	11.2	10.3	19.4	27.0	34.0	1.7	14.0
Georgia	0.8	0.3	0.2	59.8	0.3	5.5	0.3
Germany	1.3	1.7	3.2	31.7	3.3	0.1	8.4
Greece	1.7	1.8	1.6	7.8	0.4	0.5	1.3
Hungary	1.3	1.2	1.7	12.6	0.3	0.1	1.4
Italy	11.0	13.0	17.4	32.1	18.0	1.1	11.8
Japan	0.3	0.3	0.3	0.4	0.0	0.0	1.4
Mid. East	6.6	6.9	0.1	22.0	0.1	0.0	0.1
Moldova	1.9	0.9	1.2	91.6	1.4	20.4	0.1
N. Africa	2.7	3.0	0.5	9.5	0.1	0.0	0.5
New Zealand	0.3	0.2	0.4	50.4	1.6	1.7	0.4
Portugal	2.8	1.5	2.0	36.3	2.8	1.6	2.0
Romania	2.9	1.6	2.0	4.6	0.1	0.1	2.5
Russia	0.7	0.5	1.3	0.2	0.0	0.0	4.3
S. Africa	1.6	2.6	3.2	34.3	2.9	1.2	1.5
Spain	15.5	8.9	11.8	43.3	9.3	1.1	6.0
Turkey	7.0	5.5	0.1	16.5	0.0	0.0	0.1
Ukraine	1.1	0.6	0.9	7.4	0.1	0.1	0.7
USA	5.0	9.7	9.0	13.7	3.0	0.1	10.9
Uzbekistan	1.4	0.8	0.2	26.8	0.0	0.2	0.2
Other Asia	1.4	2.7	0.2	5.6	0.9	0.0	0.7
Other Europe	3.6	2.7	2.9	66.1	2.6	0.3	14.4
Other	0.8	1.0	0.9	87.0	0.0	0.1	1.7

Note: 0.0 presumably negligible value; [a] grape vineyard percentage by area; [b] grape production percentage by mass; [c] wine production percentage by volume; [d] wine exports as a percentage of a country's total wine production by volume; [e] exports as a percentage of world wine export value; [f] wine's share as a percentage of all exports by value; [g] share of world beverage wine consumption as a percentage by volume

1.3 The Australian wine industry

1.3.1 Wine exports

Table 1.2 lists the quantities and values of wine exported from Australia.[6] In the 2005-2006 year period, the Australian wine industry exported 722.2 million litres (nearly 50% of the total production) of wine. The principal countries for export included the United Kingdom (36.2% by volume, 34.3% by value) and the United States of America (28.4% by volume, 31.3% by value). Canada (6.8% by volume, 8.9% by value) and Germany (4.2% by volume, 2.3% by value) were the next ranked countries to receive wine from Australia.

Table 1.2 Exports of Australian wine by destination for the year 2005-2006

	quantity		value	
	'000 L	**%**	**$ '000**	**%**
Belgium	17,281	2.4	36,204	1.3
Canada	48,859	6.8	245,715	8.9
Denmark	20,617	2.9	52,235	1.9
France	7,486	1.0	13,781	0.5
Germany	30,049	4.2	63,724	2.3
Hong Kong	3,514	0.5	23,753	0.9
Ireland	12,271	1.7	56,304	2.0
Japan	8,507	1.2	43,767	1.6
Netherlands	22,699	3.1	66,673	2.4
New Zealand	26,700	3.7	96,123	3.5
Singapore	5,103	0.7	40,499	1.5
Sweden	9,058	1.3	42,339	1.5
Switzerland	1,660	0.2	7,605	0.3
UK	261,511	36.2	945,770	34.3
USA	204,907	28.4	864,199	31.3
Other Europe	11,436	1.6	44861	1.7
Other Asia	21,385	2.9	77,707	2.8
Other	9,116	0.3	36,664	0.4
Total	722,159	100	2,757,923	100

1.3.2 Wine making regions

South Australia is justifiably considered to be the most important wine making region in Australia (Table 1.3).[6] In the 2005-2006 vintage, South Australia held the largest area of vineyards (43.3%), followed by New South Wales/Australian Capital Territory/Northern Territory (24.1%), Victoria (23.1%) and Western Australia (7.3%) and the largest percentage of grapes crushed (48.0%), followed by New South Wales/Australian Capital Territory (34.6%), Victoria (13.6%) and Western Australia (3.5%). South Australia Features a diversity of climates, from cool temperatures in the Adelaide Hills to hot weather in the Barossa Valley, and produces a wide variety of white and red wines (50.6% of the total wine produced in Australia). Of the other states in Australia, the key wine producing regions featured in New South Wales/Australian Capital Territory (33.7%), Victoria (12.4%) and Western Australia (3.0%). South Australia featured the highest percentage of total wineries (29.9%), followed by Victoria (24.8%), New South Wales/Australian Capital Territory (21.1%) and Western Australia (18.9%). There are approximately 60 wine producing regions in Australia. These are listed in Table 1.4.[7]

Table 1.3 Around Australia for the 2005-2006 vintage

state	area of vines	grapes crushed	wine production	total wineries
NSW/ACT	24.1[a]	34.6	33.7	21.1
QLD	1.5	0.2	0.1	3.0
SA	43.3	48.0	50.6	29.9
TAS	0.7	0.2	0.1	2.3
VIC	23.1	13.6	12.4	24.8
WA	7.3	3.5	3.0	18.9

Note: all values expressed as percentages; [a] this value also includes NT

Table 1.4 Wine producing regions in Australia

state	wine regions
ACT	Canberra District
NSW	Cowra, Gundagai, Hastings River, Hilltops, Hunter, Mudgee, Orange, Perricoota, Riverina, Shoalhaven Coast, Southern Highlands, Tumbarumba
QLD	Granite Belt , Southern Burnett
SA	Adelaide Hills, Adelaide Plains, Barossa Valley, Clare Valley, Coonawarra, Currency Creek, Eden Valley, Kangaroo Island, Langhorne Creek, McLaren Vale, Mount Benson, Padthaway, Riverland, Southern Fleurieu, Southern Flinders Ranges, Wrattonbully
TAS	Tasmania
VIC	Alpine Valleys, Beechworth, Bendigo, Geelong, Gippsland, Glenrowan, Goulburn Valley, Grampians, Heathcote, Henty, King Valley, Macedon Ranges, Mornington Peninsula, Murray Darling, Pyrenees, Rutherglen, Strathbogie Ranges, Sunbury, Swan Hill, Upper Goulburn, Yarra Valley
WA	Blackwood Valley, Geographe, Great Southern, Manjimup, Margaret River, Peel, Pemberton, Perth Hills, Swan District

1.3.3 Grape varieties

In the 2006 harvest, just over half the Australian wine grapes produced consisted of red varieties (55%), as listed in Table 1.5.[6] Shiraz was the most abundant red grape variety (23.7%), followed by Cabernet Sauvignon (15.4%), Merlot (6.9%) and Pinot Noir (1.9%). Chardonnay was the most abundant white grape variety (22.3%), followed by Semillon (5.4%), Sauvignon Blanc (2.3%) and Riesling (2.2%). Colombard (4.2%) and Muscat Gordo Blanc (2.8%) featured as dominant white grape varieties, although their usage is largely in the production of cask ‘bag in a box’ wine.

Table 1.5 Grape production by variety for the 2006 Australian harvest

white varieties	winemaking (%)	red varieties	winemaking (%)
Chardonnay	22.3	Cabernet Sauvignon	15.4
Chenin Blanc	0.6	Durif	0.2
Colombard	4.2	Greanache	1.3
Muscat Gordo Blanc	2.8	Mataro (Mourvedre)	0.6
Riesling	2.2	Merlot	6.9
Sauvignon Blanc	2.3	Petit Verdot	1.5
Semillon	5.4	Pinot Noir	1.9
Sultana	0.8	Ruby Cabernet	1.5
Traminer	0.6	Sangiovese	0.3
Verdelho	1.1	Shiraz	23.7
Other	2.7	Other	1.7

It is the aroma and flavour compounds derived directly or indirectly (through precursor forms) from the grapes that are responsible for the distinctive characteristics of a particular grape variety. Table 1.6 lists key aroma descriptors for various white and red wine varieties.[4]

Table 1.6 Aroma descriptors for common white and red wine varieties

variety	descriptive characteristics
	white wine
Chardonnay	apple, cucumber, fig, fruit salad, gooseberry, grapefruit, lime, melon, peach, rockmelon, tobacco, tropical fruit
Riesling	apple, citrus, floral, lime, passionfruit, pear, perfumed, tropical fruit
Sauvignon Blanc	asparagus, capsicum, gooseberry, grassy, herbaceous, tropical fruit
Semillon	apple, citrus, gooseberry, grassy, herbaceous, lemon, lime, passionfruit, quince, straw
	red wine
Cabernet Sauvignon	blackcurrant, black olive, capsicum, dusty, herbaceous, leafy, minty, tomato bush
Merlot	beetroot, blackcurrant, cherry, fruit cake, fruity, herbaceous, leafy, perfumed, plum, raspberry, violets
Pinot Noir	cherry, plum, raspberry, stewed plum, strawberry, violets
Shiraz	blackberry, black olive, herbs, jammy, licorice, mulberry, pepper, plum, raspberry, spice

1.4 Volatile compounds in wine

Wine is a complex medium composed of a wide range of different aromas and flavours, all arising from the one plant species *Vitis vinifera*.[8] Several hundred volatile compounds have been identified in grapes and wine and many of these are thought to be important to the aroma and flavour of wine.[9] These odorants are known to originate from several different sources: directly from the grapes, microbiological interactions such as fermentation, through ageing (often in contact with oak wood) and also from chemical reactions or microbiological transformations which occur during storage.

Aroma compounds in wine feature a wide variety of functionalities that can affect the overall perception of wine.[10] The chemical classes include simple aliphatic compounds such as esters, alcohols, acids, acetals and carbonyl compounds, as well as lactones, thiols, benzene derivatives, C_{13}-norisoprenoids, monoterpenes and nitrogen-containing compounds.[9,11] Figure 1.1 shows some examples of the various classes of aroma compounds found in wine.

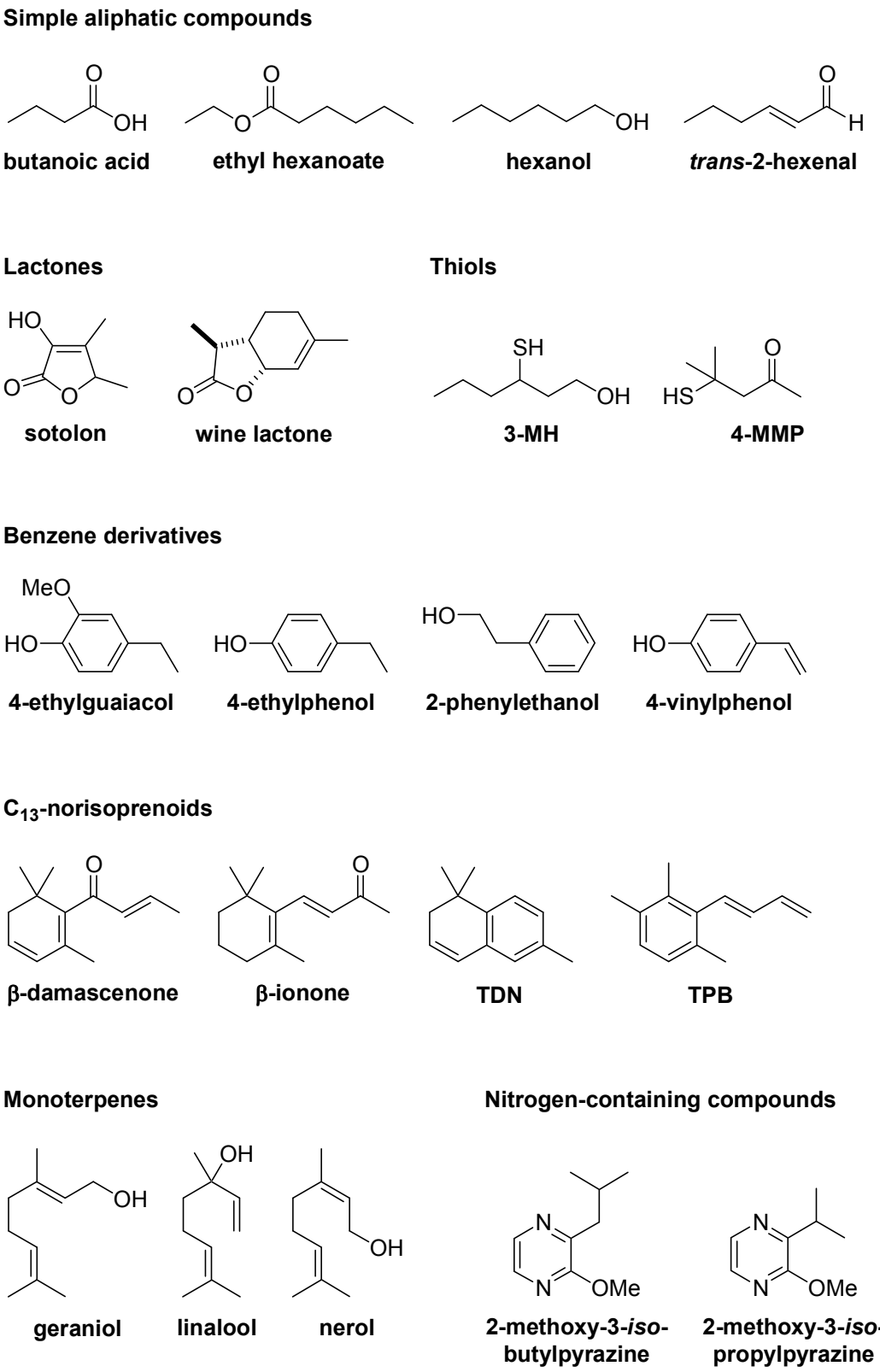


Figure 1.1 Structural examples of aroma compounds in wine

There is a wide variety of aliphatic compounds found in wine. These compounds, which include esters, alcohols, acids, acetals and carbonyl compounds, can be extracted from the grapes but are mainly produced during fermentation. Ethyl esters and simple alcohols are two important groups of compounds in this class.[9,11]

Both γ- and δ-lactones are important aroma compounds which are present in a wide variety of food and beverages. A large number of lactones have been identified in wine and are thought to arise from a range of sources. These include the metabolism of amino and keto acids by yeasts, *Botrytis cinerea* on grapes, aerobic metabolism of flor yeasts on the wine, from precursors extracted from oak wood during ageing and as by-products from the metabolism of pantothenic acid.[11-13] As a result, some lactones are a consequence of the style of wine or the method of storage. Some of these compounds include the five- and six-alkanolides, five-alkoxy and five-acyl lactones. The role of the oak lactones, the most important oak-derived compounds known in wine, is discussed in Chapter 2, while a series of five-alkyl substituted γ-lactones is discussed in detail in Chapter 6.

Thiols are an interesting class of compounds due to their typically low odour detection threshold values.[14] There are five different types of sulfur-containing compounds that have been indentified in wine: sulfur dioxide, mercaptans, thioesters, sulfides and heterocycles. The majority of these compounds are considered to be detrimental to wine aroma and are thought to be formed during fermentation *via* the metabolism of sulfur-containing amino acids.[15,16] Of particular importance to the aroma of white wines are the mercaptans; 4-mercapto-4-methylpentan-2-one (4-MMP), 3-mercapto-hexanol (3-MH) and 3-mercaptohexyl-*O*-acetate (3-MHA). At low concentrations, these mercaptans impart pleasant 'fruity' characteristics but, at high concentrations, they exhibit extremely unpleasant aromas.[17] The last three compounds are formed by yeast from grape-derived cysteine conjugates and are important to the varietal character of Sauvignon Blanc.

Volatile phenols are generally not detected in significant concentrations in grapes and mainly originate from precursor compounds in the grapes, from the wine or from external sources (e.g. barrel maturation).[9] Two different pathways are known for the formation of benzene-derived compounds: as products of shikimate metabolism in

the grape juice or from the degradation of oak wood ligin.[18,19] Such compounds include the ethyl and vinyl phenols, methoxyphenols (guaiacols) and other guaiacol derivatives.

C_{13}-norisoprenoids are a diverse group of aroma compounds that are formed from the degradation of carotenoids and are predominantly derived from glycosidically bound precursors.[20-22] This class of odorants includes the extremely potent compound β-damascenone, the important aged Riesling compound 1,1,6-trimethyl-1,2-dihydronapthalene (TDN)[23] and the recently identified compound (*E*)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB).[24]

Terpenes are generated from the biosynthesis of isoprene units; monoterpenes (C_{10}) from two units and sesquiterpenes (C_{15}) from three units. The most abundant monoterpenes in wine include linalool, geraniol, nerol, α-terpineol and hotrienol.[25] Some terpene compounds present at low concentrations, such as wine lactone, are particularly important to wine aroma.[26] Monoterpenes, as with C_{13}-norisoprenoids, can exist as non-volatile bound precursor compounds, which can be released by enzyme hydrolysis or under the acidic conditions of wine.[27-29]

Various nitrogen-containing compounds have been reported in wine and can be classified as amines, acetamides and heterocyles.[11] With their low odour threshold values,[30] methoxypyrazines are considered to be the most important group of nitrogen-containing molecules. This includes 2-methoxy-3-*iso*-propylpyrazine, 2-methoxy-3-*iso*-butylpyrazine and 2-methoxy-3-*sec*-butylpyrazine. These grape-derived compounds contribute to the herbaceous characteristics of Cabernet Sauvignon, Semillon and Sauvignon Blanc wines.[31]

1.5 Summary

The history of grape growing and wine making in Australia dates back over two hundred years. Since this time when vineyards were first established, Australia has grown to become the tenth-ranked nation in the world for total grape production, and the sixth-ranked nation for wine manufacture. In addition, Australia exports nearly

half of its total wine output. South Australia is the key wine producing region in Australia, with Chardonnay and Shiraz the most dominant grape varieties for white and red wine, respectively.

It is the challenge of the wine researcher to identify and understand the sensory interactions of key aroma compounds that may be derived from the grape berries, from bacterial and yeast interactions through ageing or storage of the wine. The isolation and identification of potent odorants can sometimes be an onerous task. This is further complicated by the need to quantify these compounds and relate these data to their sensorial characteristics in order to gain a thorough understanding of the role of certain odorants in wine.

2 Introduction to oak wood and the oak lactones

Oak has long been known to impart favourable characteristics to alcoholic beverages that have been matured in oak barrels or in contact with oak chips. This chapter outlines the use of oak wood in barrel manufacture, with a particular focus on the oak lactones, their sensory properties and previous work on their synthesis.

2.1 Oak wood

2.1.1 Oak wood in barrel manufacture

The art of barrel making dates back over 2,000 years since its introduction into Roman culture.[13] History has seen a wide variety of products used for the storage, transportation and maturation of alcoholic beverages, including wood, ceramic jars, glass and stainless steel vats.[32] Prior to the advent of stainless steel, wood vessels were found to be the most convenient and robust containers for wine.

Oak was initially chosen for barrel manufacturing because of its availability in Europe, where the production of alcoholic beverages, and thus barrel making, was abundant. Oak wood was found to possess important characteristics, including durability and low leakage rates, which confirmed its suitability for cooperage.[13] Fortuitously, oak wood was also found to play an important role in the development of colour and flavour in alcoholic beverages.[33] The ageing of alcoholic beverages, such as wine and spirits, in oak wood barrels has long been associated with the production of a premium product.[34,35]

There have been many different woods used to manufacture barrels, including red gum, birch, maple, chestnut, locust (false acacia), spruce, cyprus, elm, redwood and fir.[34] In Australia, the wine industry has used jarrah, karri, blackwood, red gum, kauri, redwood and Douglas fir. However, the majority of these woods are unsuitable and lead to problems associated with the cooperage process itself, with leakage, or with the extraction of undesirable flavours and tannins. Only oak wood

has been found to possess all the desirable characteristics necessary to serve as the wood of choice for use in barrels.

Oak wood used in the production of barrels is derived from several species of white oak.[35] The most important American oak species used is *Quercus alba*, followed by *Q. prinus*, *Q. bicolour*, *Q. muehlenbergii*, *Q. stellata*, *Q. macrocarpa*, *Q. lyrata* and *Q. durandii*.[34] The European oak species used are *Q. robur* (a.k.a. *Q. pedunculata*) and *Q. sessilis* (a.k.a. *Q. petraea* or *Q. sessilifora*).[36]

French oak barrels are generally at least two or three times more expensive than barrels made from American oak.[37] This significant difference is largely due to the losses in wood during cooperage, as French oak is more irregular in grain structure and of a higher wood porosity than American oak.[36]

The aroma and flavour characteristics derived from oak are dependent on the type of oak wood that is used. It is difficult to generalise on the subject of the differences in sensory characteristics of wines aged in European and American oak barrels, as there can be significant variations within a particular species of oak wood. European oak generally imparts a soft subtle oak aroma with lemon, citrus and nutty flavours, while American oak is associated with a strong aroma of sweet vanilla notes.[4] It has been reported that an experienced taster can differentiate between wine or brandy aged in American or European oak.[38] Table 2.1 lists typical descriptors used to characterise oak aromas.[4,39]

Table 2.1 Oak wood aroma descriptors

bacon	cigar box	dusty	nutty	smoky
burnt	cinnamon	green	olives	spicy
caramel	citrus	hazelnut	pencil shavings	sweet
cashews	cloves	honey	raisin	toasty
cedar	coconut	lemon	resin	toffee
charred	coffee	malt	sawdust	vanilla

Oak is classified as a hardwood. This is distinguished from a softwood, not by the physical hardness of the wood, but by actual visual differences,[38] since not all softwoods are soft, nor all hardwoods hard.[40] Hardwoods generally have broad or

blade-like leaves, bear their seeds in a flower or fruit structure and are deciduous trees, while softwoods have needle-like leaves, exposed seeds and are evergreen trees.[41] Hardwoods are classified as angiosperms and softwoods as gymnosperms. The general composition of oak wood is that of complex carbohydrates (cellulose (45%) and hemicellulose (25%)), lignin (25%) and extractable compounds (5%).[34] Of interest in wine chemistry is the structure and properties of these extractives. It is these volatile flavour compounds that contribute to the overall aroma of oak-aged alcoholic beverages.[19,33]

2.1.2 Oak wood volatile compounds

Over 200 oak-derived volatile compounds have been identified in alcoholic beverages.[42-51] One such group of compounds is formed from the degradation of oak lignin. This releases a range of phenolic odorants the most important of which are eugenol, guaiacol, 4-methylguaiacol and vanillin. Of all the many volatile compounds in oak wood, it is the oak lactones, in particular the *cis*-isomer, that is considered to be the most important oak-derived compound known in wine.[38] The structural formulae of these compounds are shown in Figure 2.1.

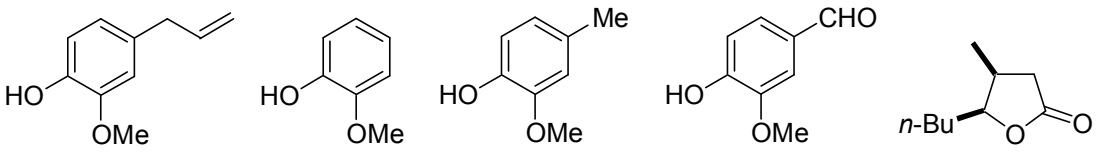


Figure 2.1 Important oak-derived volatile compounds

2.2 The oak lactones

2.2.1 Structural identification

First identified in 1970 by Suomalainen and Nykanen,[52] 'oak lactone' was reported as a single compound in a range of alcoholic beverages that had been matured in oak barrels. In an earlier publication, an unknown compound was identified and tentatively assigned as a branched chain isomer of γ-nonalactone, with a strong

coconut aroma.[51] This particular compound was later identified correctly as the *cis*-isomer of 5-*n*-butyl-4-methyl-2(3H)-furanone (*cis*-oak lactone),[44] with the first reported identification in a Cabernet Sauvignon wine. It was concluded that this compound was of oak origin, as the isomer was not detected in the same wine that had been aged in glass or stainless steel containers. The new compound was named 'whisky lactone', due to its initial isolation in aged whisky. Since this time, various other names have been use for this compound including quercus lactone, quercus lactones a and b, β-methyl-γ-octalactone(s), 4-hydroxy-3-methyloctanoic acid-γ-lactone, 3-methyl-4-hydroxycaprylic acid γ-lactone, 3-methyl-γ-lactone and 3-methyl-4-octanolide.[13]

It was later demonstrated that there were actually two oak lactone structures[45] and not a single compound as had originally been reported. Two diastereomers of oak lactone were isolated and identified from three wood species; *Q. mongolica*, *Q. serrata* and a white oak (from North America, unreported species). These compounds were named quercus lactones a and b and were incorrectly assigned as *cis*- and *trans*-, respectively. This was later corrected in the literature with the appropriate relative and absolute stereochemistry being reported.[46] Otsuka *et al*. also identified the oak lactones directly from wood and reported an increase in concentration of oak lactone with an increase in barrel maturation time.[47]

The structure of oak lactone features two stereocentres and thus there are a total of four possible stereoisomers; two enantiomeric pairs of diastereomers (Figure 2.2). However, it has been established that oak wood contains only the (4*S*,5*S*)-*cis*-**1a** and the (4*S*,5*R*)-*trans*-**2a** isomers of oak lactone.[53-55]

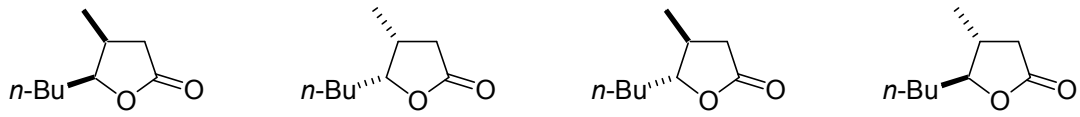


Figure 2.2 The four possible stereoisomers of oak lactone

2.2.2 Reported levels in wine and oak

In an early study on aged distilled liquors, the levels of *cis*- and *trans*-oak lactone were measured in brandy, whisky and rum.[47] The concentration of *cis*-oak lactone was found to be greater (less than 20 μg/L to over 1,000 μg/L) than that of the *trans*-isomer (less than 20 μg/L to over 400 μg/L).

The concentration of total oak lactone was measured in seven white and seven red wines from Australia and Spain that were known to have been aged in oak.[49] The level of oak lactone in white wines ranged from 24 to 538 μg/L, while the range in red wines was from 13 to 479 μg/L. The *cis*-isomer was reported to be present in higher concentrations than the *trans*-isomer in all the wines analysed. The ratio of *cis*-isomer/*trans*-isomer varied between 1.3 and 8.1.

A more recent study on the quantification of oak lactone in 61 red wines, using stable isotope dilution analysis, found that all but one wine (trace level; < 1 μg/L) contained *cis*- and *trans*-oak lactone.[50] This indicated the maturation of these wines in oak barrels or treatment with oak chips during their production. The average concentrations observed were 280 μg/L and 64 μg/L, for *cis*- and *trans*-oak lactone, respectively. The highest level of the *cis*-isomer was recorded at 887 μg/L, while that for the *trans*-counterpart was found to be at 192 μg/L.

American oak is generally considered to contain higher concentrations of oak lactone than French oak wood.[37,42,43,48] The ratio of *cis*- and *trans*-isomers was reported to be characteristic of wines matured in either American or French oak.[36] For American oak (with the exception of Oregon oak), the ratio ranged from 5 to 8, with an average of 6.0 ± 1.3. For European oak, the ratio ranged from 1 to 1.5, with an average of 1.3 ± 0.2. By analysing the ratio of *cis*- and *trans*-isomers, it is possible to determine the source of wood used for barrel maturation. This work has been supported by further results which found American oak to have only approximately 10% of the *trans*-isomer, while French oak was found to have an almost equal amount of the stereoisomers.[48]

2.2.3 Sensory studies

The odour detection thresholds for the oak lactones have been determined in a variety of media (Table 2.2). Sensory studies have been determined on commercially available mixtures of all four stereoisomers[49,55-57] or separate mixtures of either racemic *cis*- or racemic *trans*-oak lactone,[47,53,54] with the exception of one study on enantiomerically enriched *cis*-isomers.[58] The racemic *cis*-oak lactone has consistently been shown to have a lower threshold than its *trans*-counterpart and thus is considered to be the more potent stereoisomer of oak lactone. However, the value of such data obtained on racemic mixtures is questionable as only a single enantiomer of each compound exists in nature.

Table 2.2 Odour detection threshold values for the oak lactones

isomers tested	threshold (μg/L)	medium	reference
all four	51	34% EtOH/H_2O	Salo *et al*. 1972[57]
both *cis*	67	30% EtOH/H_2O	Otsuka *et al*. 1974[47]
both *trans*	790	30% EtOH/H_2O	Otsuka *et al*. 1974[47]
all four	20	H_2O	Boidron *et al*. 1988[56]
all four	15	model wine[a]	Boidron *et al*. 1988[56]
all four	120	white wine	Chatonnet *et al*. 1990[55]
all four	125	red wine	Chatonnet *et al*. 1990[55]
both *cis*	25	model wine[b]	Chatonnet 1991[54]
both *trans*	110	model wine[b]	Chatonnet 1991[54]
both *cis*	92	white wine	Chatonnet 1991[54]
both *cis*	74	red wine	Chatonnet 1991[54]
both *trans*	460	white wine	Chatonnet 1991[54]
both *trans*	320	red wine	Chatonnet 1991[54]
both *cis*	1	GC-O[c]	Abbott *et al.* 1995[53]
both *trans*	20	GC-O[c]	Abbott *et al.* 1995[53]
all four	75	12% EtOH/H_2O	Piggott *et al*. 1995[49]
all four	241	white wine	Piggott *et al*. 1995[49]
all four	853	red wine	Piggott *et al*. 1995[49]
(4*S*,5*S*)-*cis*[d]	23	white wine	Wilkinson *et al*. 2004[58]
(4*S*,5*S*)-*cis*[d]	46	red wine	Wilkinson *et al*. 2004[58]
(4*R*,5*R*)-*cis*[e]	82	white wine	Wilkinson *et al*. 2004[58]

[a] model wine was 12% EtOH/H_2O with 5 g/L tartaric acid, adjusted to pH 3.5 with NaOH, plus 30 mg/L SO_2; [b] model wine in 12% EtOH solution; [c] gas chromatography-olfactometry; [d] mixture comprising 90% (4*S*,5*S*)-isomer, 6% (4*R*,5*S*)-isomer and 4% (4*S*,5*R*)-isomer; [e] mixture comprising 84% (4*R*,5*R*)-isomer, 4% (4*S*,5*S*)-isomer, 7% (4*R*,5*S*)-isomer and 5% (4*S*,5*R*)-isomer

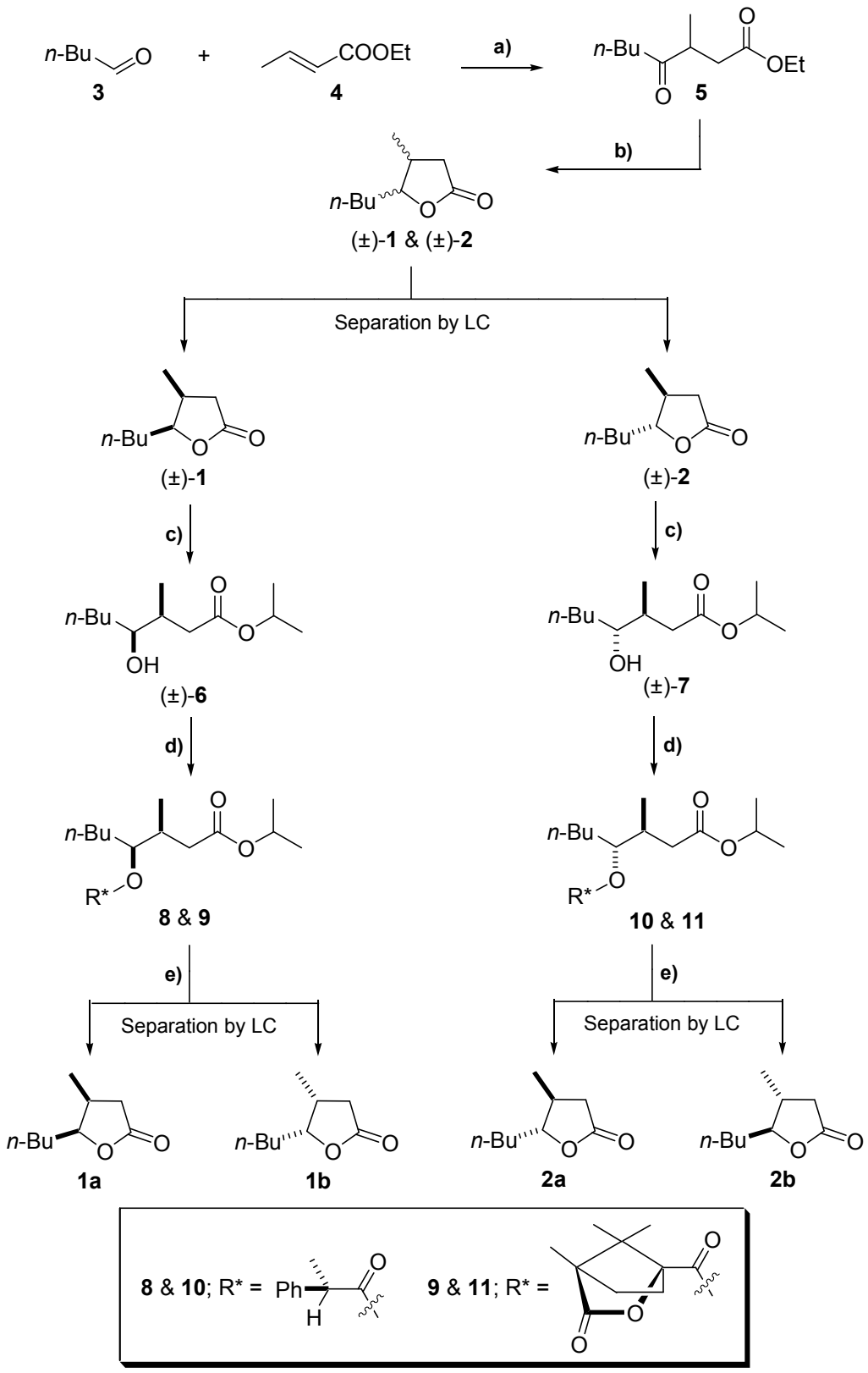
In an earlier study, difference testing between racemic mixtures of the *cis*- and *trans*-isomers was undertaken in a triangle preference test.[47] The concentration used was ten times the odour threshold values, as determined by the authors (*cis*-oak lactone concentration 670 μg/L; *trans*-oak lactone concentration 7,900 μg/L). Of the 36 judges, 24 judges correctly identified a difference in aroma (significant difference at the 95% confidence level) and 22 of these 24 judges preferred the aroma of the *trans*-oak lactone to that of the *cis*-isomer.

As can be seen in Table 2.2, a thorough sensory threshold study on the four individual isomers in white and red wine is lacking from the literature. This is

largely due to the fact that commercially available oak lactone is a mixture of *cis*- and *trans*-isomers, each as a racemate. There is the need to develop a concise synthetic pathway from which all four stereoisomers of oak lactone can be prepared and thus utilised for sensory studies. Chapter 3 describes just such a synthesis, and Chapter 4 covers the resulting sensory investigation.

2.2.4 Previous synthetic work on the oak lactones

Günther and Mosandl[59] reported the separation of the four stereoisomers of the oak lactone from a racemic sample of the lactones (Scheme 2.1). The synthesis began with the production of the racemic *cis*- and *trans*-mixture. Ethyl 3-methyl-4-oxo-octanoate (**5**) was produced from pentanal (**3**) and ethyl crotonate (**4**). Reduction with $NaBH_4$ followed by cyclisation formed the lactone mixture, (±)-**1** and (±)-**2**. The individual racemates of *cis*-**1** and *trans*-**2** oak lactone were separated by liquid chromatography, the ring was opened under basic conditions and trapped with *iso*-propyl bromide as (±)-**6** and (±)-**7**, respectively. The addition of a chiral auxiliary, (*R*)-2-phenylpropionic chloride, which formed **8** and **10** for *cis*- and *trans*-, respectively, or (*S*)-camphanic chloride, which formed **9** and **11** for *cis*- and *trans*-, respectively, resulted in the formation of diastereomers and enabled separation by liquid chromatography. Cleavage of the ester groups by base hydrolysis and final treatment with acid produced the individual stereoisomers of oak lactone (**1a**, **1b**, **2a** and **2b**) upon re-lactonisation in 95% enantiomeric excess. This was the first reported preparation of all four optically pure isomers. There were, however, multiple chromatography steps and only milligram quantities of the final products were produced.



Reagents and conditions: a) $(C_6H_5COO)_2$, 80 °C, 9 hrs, 73%; **b)** i. NaOH, $NaBH_4$, MeOH; ii. HCl, 50 °C, 2 hrs, 96%; **c)** i. KOH, MeOH, rt, 20 hrs; ii. $(CH_3)_2CHBr$, DMF, rt, 24 hrs, 70-82%; **d)** R*-Cl, DMAP, CCl_4, rt, 1-3 hrs, 70%; **e)** i. KOH, MeOH, rt, 24 hrs; ii. HCl (pH 1-2), 50 °C, 24 hrs, 64-70%.

Scheme 2.1

Günther and Mosandl performed some limited sensory work on the four oak lactone isomers (Table 2.3). Odour threshold values were not determined; only qualitative sensory studies were completed. In general, the isomers' odours were described as being reminiscent of 'coconut'; the *cis*-isomers were 'woody' and 'earthy', while the *trans*-isomers resembled 'celery'. This work was carried out on a lactone concentration of 10 mg/L in a sugar solution and a 10% EtOH solution. This very high level is incomparable with the concentrations actually found in wine or spirits.

Table 2.3 Aroma descriptors for the four stereoisomers of oak lactone

stereoisomer	aroma descriptors
(4*S*,5*S*)-*cis*-**1a** *	coconut, earthy, hay, musty
(4*R*,5*R*)-*cis*-**1b**	coconut, sweet, woody
(4*S*,5*R*)-*trans*-**2a***	celery, coconut, green walnut, spicy
(4*R*,5*S*)-*trans*-**2b**	celery, coconut

* naturally occurring oak lactone stereoisomers

In another study, Wilkinson *et al*. obtained milligram quantities of the four stereoisomers of oak lactone from the hydrolysis of their respectively prepared glycosides.[58] Chiral GC-MS analysis of the oak lactone samples revealed the stereoisomers not to be 'pure' but to be highly enriched in their respective isomer (Table 2.4). Due to the significantly higher threshold for racemic *trans*-oak lactone, minor amounts of this isomer in the *cis*-enriched solution would be expected to have very little impact on the overall sensory properties. However, contamination of the *trans*-isomer by the *cis*-isomer would have a significant effect. Thus, sensory studies were limited to only the *cis*-enriched solutions. The odour detection thresholds were determined to be 23 μg/L and 82 μg/L for the (4*R*,5*R*)- and (4*S*,5*S*)-*cis*-isomers respectively in a white wine. The (4*R*,5*R*)-*cis*-isomer was found to have a threshold of 46 μg/L in a red wine.

Table 2.4 Chiral GC-MS analysis on the composition of the samples synthesised by Wilkinson *et al*.

sample	(4*S*,5*S*)-*cis*-1a *	(4*R*,5*R*)-*cis*-1b	(4*S*,5*R*)-*trans*-2a*	(4*R*,5*S*)-*trans*-2b
(4*S*,5*S*)-*cis*-**1a** *	90	-	4	6
(4*R*,5*R*)-*cis*-**1b**	4	84	5	7
(4*S*,5*R*)-*trans*-**2a***	3	3	85	9
(4*R*,5*S*)-*trans*-**2b**	1	1	2	96

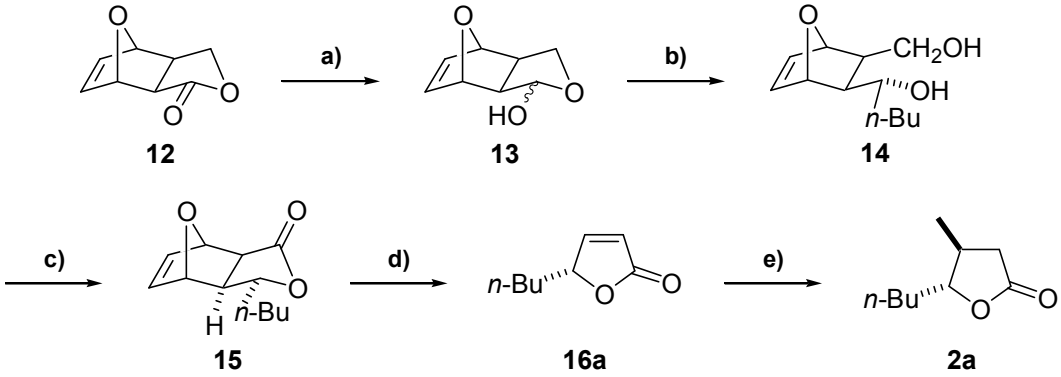
* naturally occurring oak lactone stereoisomers

Informal aroma descriptors were obtained for the *cis*-enriched solutions in white wine.[58] The (4*S*,5*S*)-stereoisomer was described as 'buttery', 'caramel', 'coconut', 'fruity', 'vanilla' and 'woody', while the (4*R*,5*R*)-stereoisomer was characterised as 'burnt apple', 'cinnamon', 'citrus', 'coconut', 'honey', 'lime' and 'vanilla'. In a separate sensory study, the *cis*-stereoisomer was associated with 'coconut' aroma in a Chardonnay wine and 'coconut', 'vanilla', 'berry', 'coffee' and 'dark chocolate' aromas in a Cabernet Sauvignon wine.[60]

Wilkinson *et al*. also conducted a duo-trio difference test to establish if there was a significant difference in aroma between the two *cis*-oak lactone isomers.[58] A young neutral white wine was spiked with either the (4*S*,5*S*)-isomer or the (4*R*,4*R*)-isomer at a concentration of 161.7 μg/L. From the panel of 25 judges, 20 people correctly identified the sample that was different from the reference, demonstrating that the two *cis*-isomers were significantly different at the 95% confidence level. This further brings into question the suitability of using racemic *cis*-oak lactone solutions for sensory studies.

The first diastereo- and enantio-selective synthesis of *trans*-oak lactone was reported by Bloch and Gilbert (Scheme 2.2).[61] This was used successfully to confirm the absolute configuration of quercus lactone a. Starting lactone **12**, available in greater than 98% enantiomeric excess from an enzymatic process[62] was reduced to the corresponding lactol **13** as a mixture (88:12) of diastereomers. Addition of *n*-butylmagnesium bromide in diethyl ether afforded diol **14** in 70% diastereomeric excess. The diol, oxidised with Jones reagent, led to pure lactone **15**, after chromatography and retrocycloaddition gave butenolide **16a**. Finally, conjugate addition of lithium dimethylcuprate with complete stereochemical control, the key

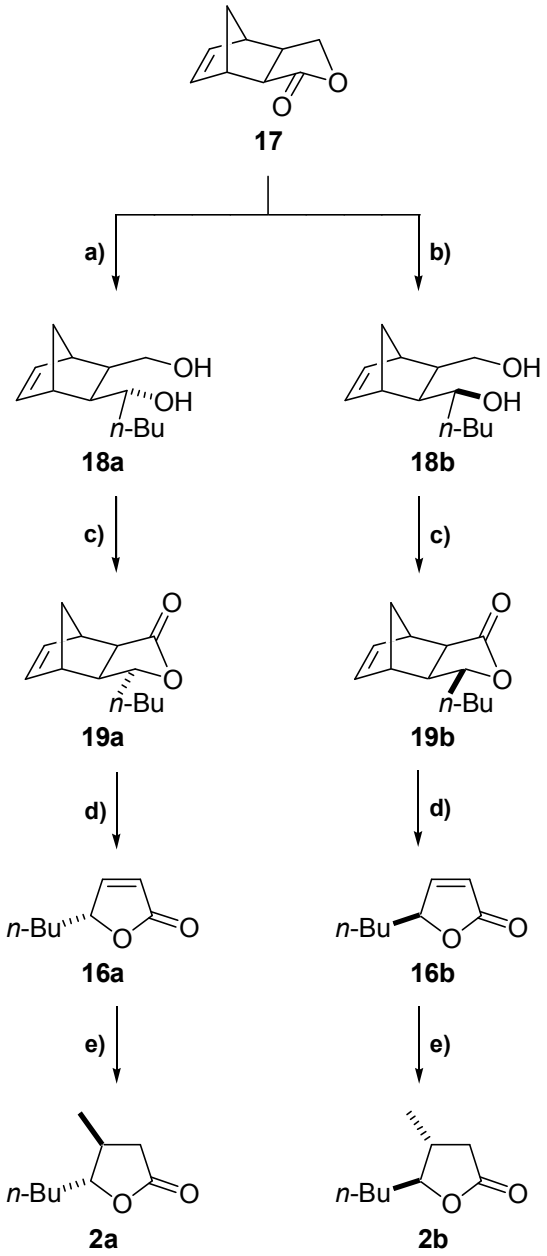
step in this pathway, gave **2a** in greater than 95% enantiomeric excess with a yield of 14% over the five steps. Use of tetrahydrofuran as the solvent in the Grignard addition produced the other isomer of **14** in 70-82% diastereomeric excess, which, although not reported, could be utilised to produce the other enantiomer of *trans*-oak lactone.



Reagents and conditions: a) DIBAL, PhMe, -78 °C, 3.5 hrs, 78%; **b)** *n*-BuMgBr, Et_2O, 0 °C-rt, o/n, 70%; **c)** i. Jones reagent, $(CH_3)_2CO$, 0 °C, 35%; ii. separation; **d)** PhMe, Δ, 3 hrs, 90%; **e)** Me_2CuLi, Et_2O, -60 °C-rt, 2 hrs, 80%.

Scheme 2.2

Recently, Suzuki *et al*.[63] reported a concise total synthesis of the enantiomers of *trans*-oak lactone, from chiral tricyclic lactone **17**, using the diastereoselective cuprate addition reaction (Scheme 2.3), as featured in the previous synthesis (Scheme 2.2).[61] Starting lactone **17** was reduced with di-*iso*-butylaluminium hydride in tetrahydrofuran or *n*-butyllithium and L-Selectride in toluene in one step, to diols **18a** and **18b** respectively, both in greater than 99% diastereomeric excess. Oxidation with tetra-*n*-propylammonium perruthenate (TPAP) (catalytic) in the presence of 4-methylmorpholine *N*-oxide (NMO) gave diastereomeric lactones **19a** and **19b**. Retrocycloaddition reaction from refluxing in *o*-dichlorobenzene (ODCB) gave the unsaturated enantiomeric lactones **16a** and **16b**, in 90% and 92% enantiomeric excess, respectively. The final reaction with dimethylcuprate in a 1,4-addition reaction produced the *trans*-stereoisomers of oak lactone (**2a** in 33% yield and **2b** in 24% yield over four steps).

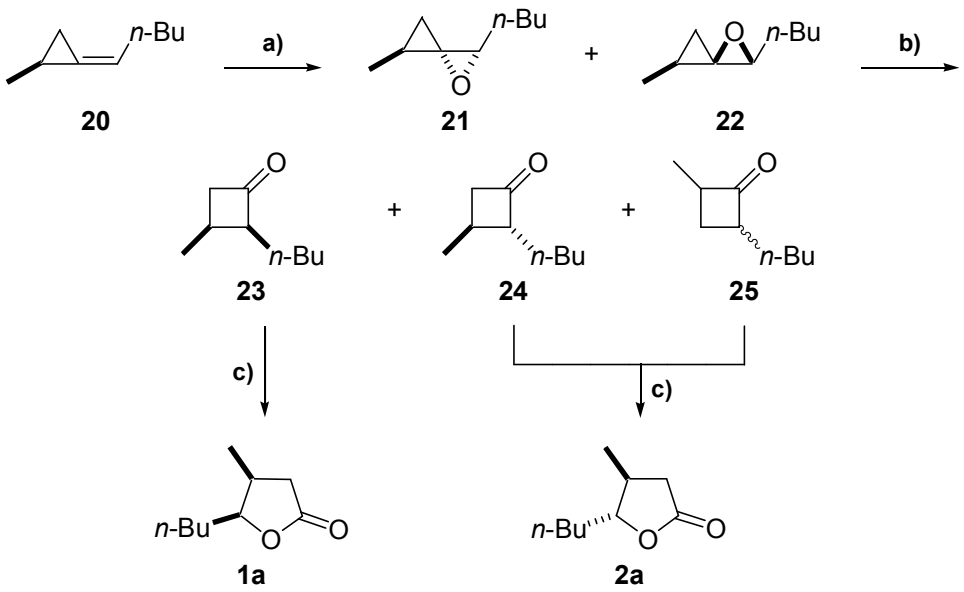


Reagents and conditions: a) i. DIBAL, THF, -78 °C, 1.5 hrs; ii. *n*-BuMgCl, THF, -78 °C, 5 hrs, 82%; **b)** i. *n*-BuLi, PhMe, -78 °C, 2 hrs; ii. L-Selectride, PhMe, -78 °C, 2.5 hrs, 78%; **c)** cat. TPAP, NMO, 4 Å molecular sieves, CH_2Cl_2, 0 °C-rt, 3 hrs, 66% for **19a**, 61% for **19b**; **d)** ODCB, Δ, 20 hrs, 85% for **16a**, 84% for **16b**; **e)** MeLi, CuI, Et_2O, -30 °C, 30 mins, -78 °C, 3.5 hrs, 72% for **2a**, 61% for **2b**.

Scheme 2.3

Chevtchoak *et al*. have developed a synthesis for both naturally occurring oak lactones (Scheme 2.4).[64] Epoxidation of **20** with *m*-chloroperbenzoic acid afforded a 70:30 mixture of inseparable diastereomeric oxaspiropentanes **21** and **22**. Treatment with a catalytic amount of lithium iodide resulted in ring expansion at the C_3-C_4

position to give a 55:17:28 mixture of (2*S*,3*S*)-**23**, (2*R*,3*S*)-**24** and the regioisomer-**25** in quantitative yield. *cis*-Cyclobutanone **23** was isolated by preparative gas chromatography (GC) and converted by Baeyer-Villiger oxidation using *m*-chloroperbenzoic acid to the *cis*-oak lactone isomer (**1a**), in greater than 89% enantiomeric excess with 84% overall yield. *trans*-Cyclobutanone **24** could not be separated from **25** and so was treated as a 72:28 mixture with *m*-chloroperbenzoic acid in a Baeyer Villiger reaction to yield *trans*-oak lactone isomer (**2a**), after column chromatography purification, in greater than 92% enantiomeric excess with 61% overall yield. The key step of this procedure involved the lithium iodide induced ring expansion of **21** and **22** followed by separation of the *cis*- and *trans*-cyclobutanone products.

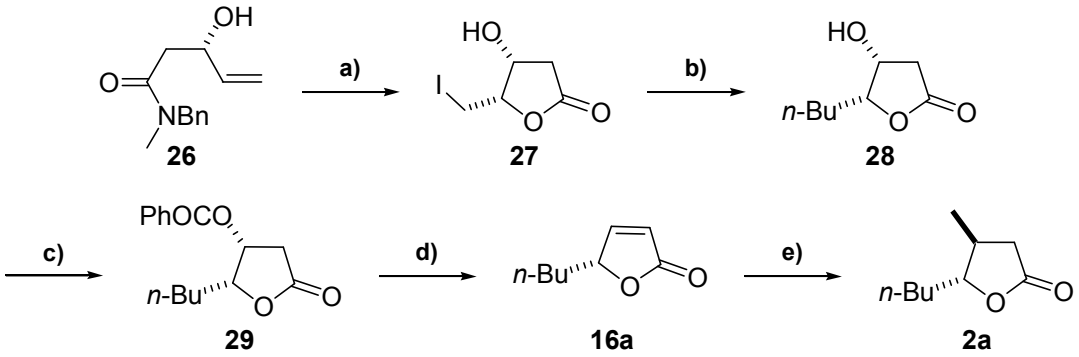


Reagents and conditions: a) *m*-CPBA-KF, CH_2Cl_2, 90%; **b)** cat. LiI, CH_2Cl_2, Δ, 5 hrs, 100%; **c)** *m*-CPBA, CH_2Cl_2, 0 °C, 93% for **1a**, 68% for **2a**.

Scheme 2.4

Takahata *et al*. synthesised the natural enantiomer of *trans*-oak lactone as depicted in Scheme 2.5.[65,66] The synthesis utilised the conjugate addition of lithium dimethylcuprate with complete diastereoselectivity (as was observed in the synthesis by Bloch and Gilbert).[61] Stereoselective iodolactonisation of *N*-benzyl-*N*-methyl-3-hydroxy-4-pentenamide (**26**) gave γ-lactone **27**, as an 8:1 *cis*:*trans* mixture. Cross coupling of *cis*-**27** with Grignard derived cuprates gave **28**, which was converted to butenolide **16a** by treatment with benzoyl chloride to give **29** and then an elimination

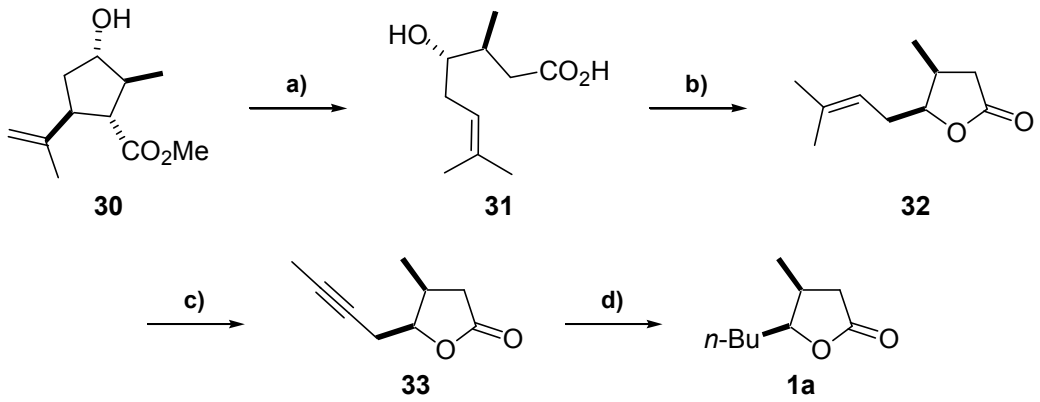
reaction, affording **16a** in 99% enantiomeric excess. Through conjugate addition with dimethyl cuprate, *trans*-oak lactone (**2a**) was synthesised with complete diastereoselectivity in 14% yield over a total of five steps. The stereocentre at C_5 is used to control the absolute stereochemistry at C_4, resulting from *anti*-attack of the cuprate with respect to the *n*-butyl group on the γ-lactone unit.



Reagents and conditions: a) I_2, DME-H_2O, 54%; **b)** *n*-C_3H_7MgBr-THF, CuBr-Me_2S, THF, -78 °C, 30 mins, rt, 6 hrs, 83%; **c)** PhCOCl, pyr, C_6H_6, rt, 12 hrs, 78%; **d)** NH_3/MeOH, rt, 10 mins, 63%; **e)** Me_2CuLi, Et_2O, -60 °C, 2 hrs, 63%.

Scheme 2.5

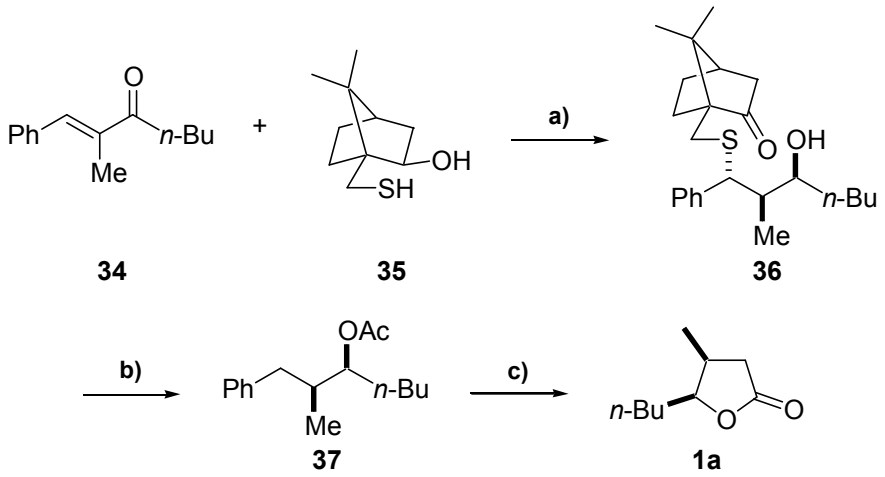
Suzuki *et al*. reported a novel and concise synthetic route to the natural enantiomer of *cis*-oak lactone (Scheme 2.6).[67] The key step of this synthesis utilised the γ,δ-unsaturated carbonyl system of **30** for the reductive carbon-carbon bond cleavage reaction. Reduction of **30** with a solution of sodium metal in hexamethyl phosphoramide (HMPA) produced **31**. Heating under reflux in benzene produced the γ-lactone backbone **32** with correct stereochemistry for the naturally occurring *cis*-oak lactone. Direct demethylation of the *iso*-propylidene group afforded the acetylenic compound **33**, which was hydrogenated to afford the *cis*-oak lactone (**1a**) in 9% overall yield for the four steps, with identical spectroscopic data to those reported in the literature.[59,68]



Reagents and conditions: a) i. Na, HMPA; ii. H_2O, Δ, 1 hr, 57%; **b)** C_6H_6, Δ, 100%; **c)** $NaNO_2$, AcOH-H_2O, 0-70 °C, 24%; **d)** H_2, Pd-C, EtOAc, 65%.

Scheme 2.6

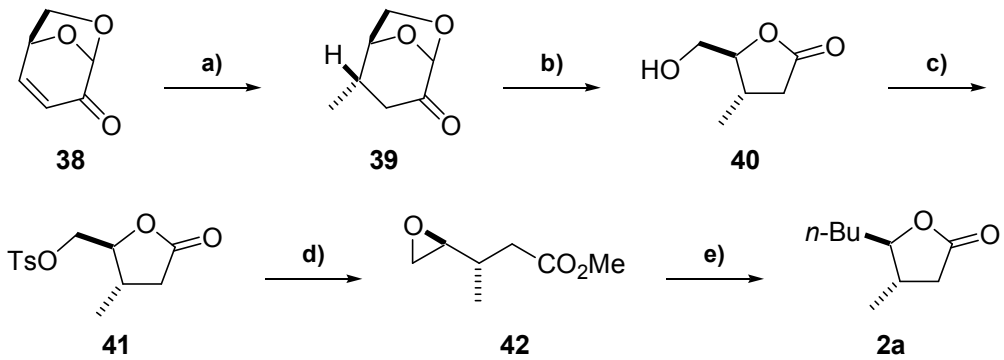
Ozeki *et al*. reported the preparation of the two stereocentres required in the final structure of *cis*-oak lactone in one step (Scheme 2.7).[69] Using the developed tandem Michael addition Meerwein-Ponndorf-Verley (MPV) reduction of α,β-unsaturated ketone **34** with (-)-10-mercapto-*iso*-borneol (**35**) gave optically active alcohol **36**.[70,71] With use of the starting alcohol **34** it was necessary to acetylate the alcohol and remove the chiral moiety with Raney nickel to produce **37** in 99% enantiomeric excess. Ruthenium catalysed oxidation of the phenyl group to the carboxylic acid, followed by hydrolysis of the acetate with sodium hydroxide led to *cis*-oak lactone (**1a**) upon lactonisation under acidic condition, with an overall yield for the six steps of 40%.



Reagents and conditions: a) Me_2AlCl, CH_2Cl_2, rt, 72%; **b)** i. Ac_2O, DMAP, pyr, rt; ii. Raney Ni (W2), EtOH, rt, 89%; **c)** i. $RuCl_3.xH_2O$, H_5IO_6, CCl_4/MeCN/H_2O, rt; ii. 1M aq NaOH, rt; iii. 10% aq HCl, rt, 62%.

Scheme 2.7

Ebata *et al*. have reported the synthesis of (4*S*,5*R*)-*trans*-oak lactone (Scheme 2.8).[68] The synthesis began with levoglucosenone (**38**), a highly functionalised chiral molecule, which has been shown to produce 4,5-disubstituted γ-lactones. Enone **38** was treated with lithium dimethylcuprate to give **39** with 100% diastereoselectivity. Oxidation to alcohol **40**, tosylation to **41** and treatment with potassium carbonate in methanol afforded epoxide **42**. A final reaction with lithium di-*n*-propylcuprate gave the nature identical *trans*-oak lactone (**2a**) in 36% overall yield (5 steps); enantiomeric purity not reported.



Reagents and conditions: a) Me_2CuLi, 84%; **b)** AcO_2H, 86%; **c)** TsCl; **d)** K_2CO_3, MeOH, 66% over two steps from **40**; **e)** *n*-Pr_2CuLi, 76%.

Scheme 2.8

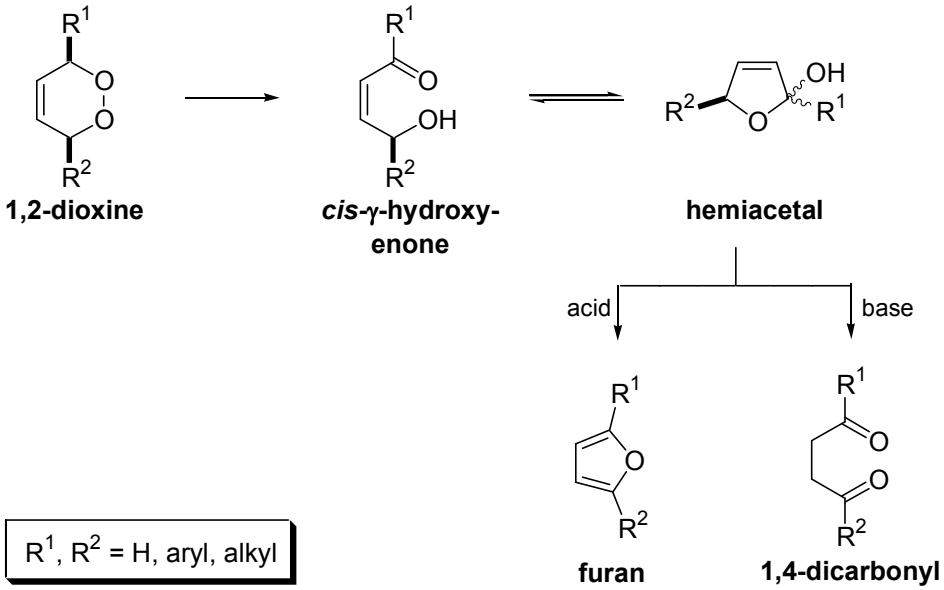
The reader is directed to additional references for further methods on the preparation of optically pure oak lactones.[72-82] Despite these numerous literature reports, there is no procedure for the preparation of all four stereoisomers, in optically pure form, from a common precursor. This void could potentially be filled using 1,2-dioxine chemistry.

2.3 Recent advances in 1,2-dioxine chemistry

2.3.1 Formation of *cis*-γ-hydroxyenones

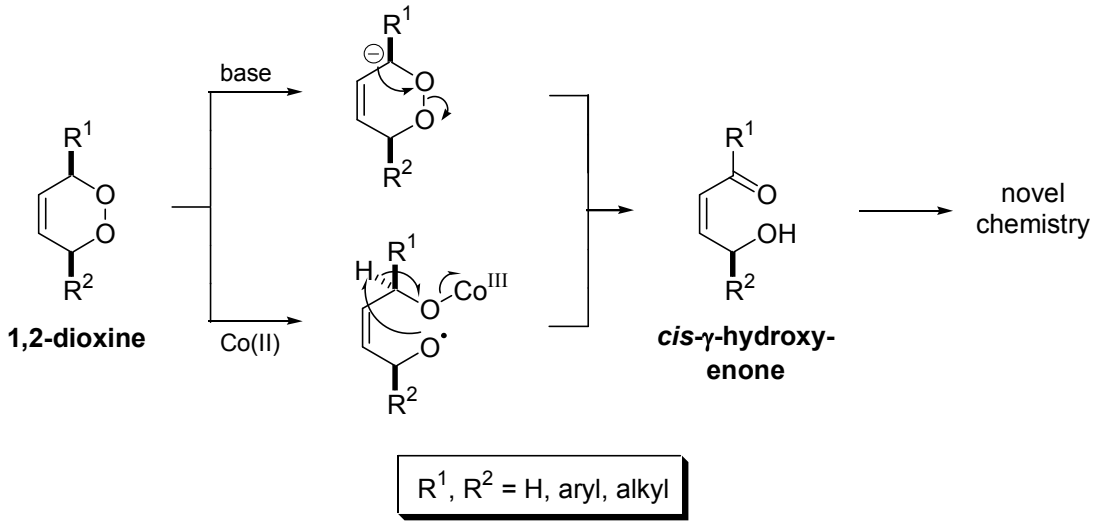
Until recently, monocyclic 1,2-dioxines (endoperoxides) have seen limited use in organic synthetic chemistry. This is largely due to the perceived instability of their oxygen-oxygen bond. Numerous examples in the literature now show this conception to be completely untrue; these compounds have become increasingly exploited as valuable precursors and intermediates in the synthesis of a wide range of substances.[83]

A key development in the use of 1,2-dioxines has been as masked *cis*-γ-hydroxyenones.[83] Previously, *cis*-γ-hydroxyenones and their isomeric hemiacetals had found little use due to their instability under acidic and basic conditions (Scheme 2.9). The formation of furans is observed when the reaction medium is acidic and dehydration occurs,[84] while 1,4-dicarbonyl compounds are detected in a basic environment under prolonged conditions from a Kornblum-De La Mare rearrangement of the hemiacetal.[85] This sensitivity had limited both the preparation and use of *cis*-γ-hydroxyenones.



Scheme 2.9

Two methods have been developed for the formation of *cis*-hydroxyenones from 1,2-dioxines (Scheme 2.10). Treatment with either mild base or metal will result in ring opening of the 1,2-dioxine and, provided the reaction conditions are monitored, the *cis*-γ-hydroxyenone will form without further undesirable products.[86-89] Addition of base removes the most acidic proton alpha to the oxygen-oxygen bond and forms the *cis*-enone. Continued exposure to base will see the Kornblum-De La Mare rearrangement product (i.e. the 1,4-dicarbonyl), which can be controlled by the addition of only a catalytic amount of base. Alternatively, transition metals, such as cobalt complexes, have been used to induce homolytic ring cleavage of the peroxide bond. As the reaction conditions for this latter procedure are free of acid and base reagents, quantitative isomerisation to the *cis*-enone is achievable.

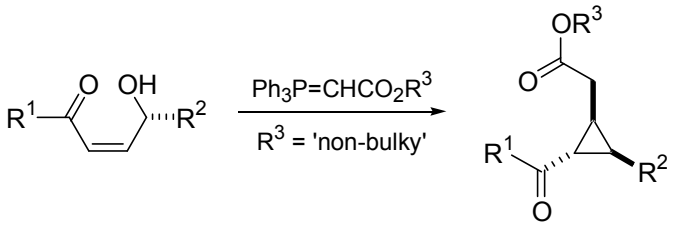


Scheme 2.10

2.3.2 Utilisation of 1,2-dioxines in organic chemistry

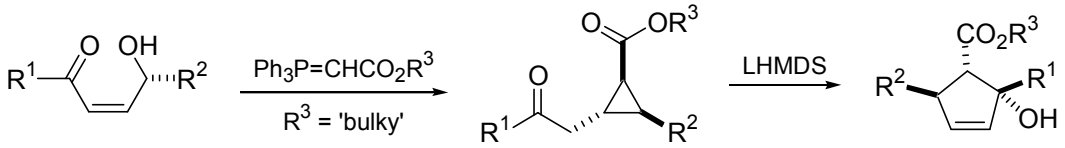
The chemistry of recently developed reactions of 1,2-dioxines and *cis*-γ-hydroxyenones is shown in Scheme 2.11. Initial investigations saw the conversion of *cis*-hydroxyenones, and their isomeric *trans*-counterparts, into cyclopropanes in a highly diastereoselective manner by reaction with stabilized phosphorus ylides (equation 1).[87,88,90,91] This was followed shortly by the discovery that reaction with a sterically bulky ylide alters the regiochemistry to give rise to structurally different cyclopropanes (equation 2).[86,92] These cyclopropanes can be further converted into cyclopentenols (equation 2).[93] 1,2-Dioxines can be converted *via* one pot syntheses into disubstituted thiophenes or pyrroles (equation 3),[94] and also into furans (equation 4).[95] The *cis*-γ-hydroxyenone equivalent can also be transformed into complex substituted furanones (equation 5),[96] tetrahydrofurans (equation 6),[97] and *trans*-epoxides (equation 7).[98] It was the formation of trisubstituted γ-lactones (furanones; equation 5), that was envisaged as a possible route to the oak lactones.

1)



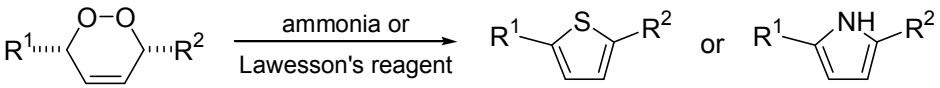
***cis*-γ-hydroxy-enone** **cyclopropane**

2)



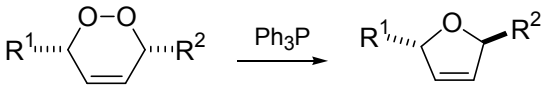
***cis*-γ-hydroxy-enone** **cyclopropane** **cyclopentenol**

3)



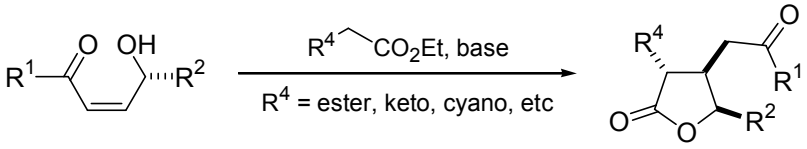
1,2-dioxine **thiophene** **pyrrole**

4)



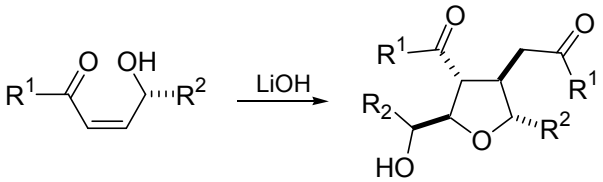
1,2-dioxine **dihydrofuran**

5)



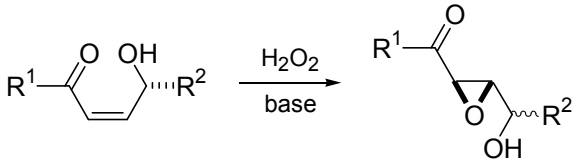
***cis*-γ-hydroxy-enone** **furanone**

6)



***cis*-γ-hydroxy-enone** **tetrahydrofuran**

7)



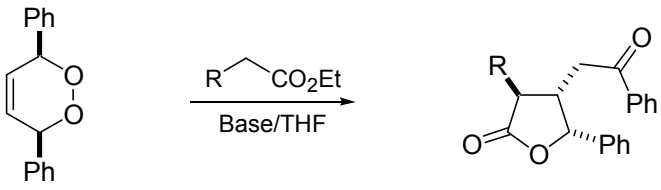
***cis*-γ-hydroxy-enone** ***trans*-epoxide**

Scheme 2.11

2.3.3 Synthesis of γ-lactones from 1,2-dioxines

The stereoselective synthesis of highly substituted γ-lactones features the addition of ester nucleophiles to 1,2-dioxines.[96] Initially, the 1,2-dioxine is converted to its *cis*-γ-hydroxyenone equivalent *via* heterolytic ring opening under basic conditions. Conjugate addition of the malonic ester enolate to the *cis*-enone, followed by protonation, affords the trisubstituted γ-lactone.

Preliminary investigations on this reaction focused on the use of 3,6-diphenyl-1,2-dioxine (**43**) as the model dioxine (Scheme 2.12).[96] Diethyl and cyano esters **44** and **45**, respectively, reacted with **43** to give excellent yields (93% each) of **48** and **49**, respectively, two equivalents of keto ester **46** were required in order to achieve a moderate yield of **50** (70%) and aromatic monoester **47** failed to react under a range of different conditions. This last ester saw the formation of the 1,4-dicarbonyl as depicted in Scheme 2.9. Products **48**, **49** and **50** were obtained with high diastereoselectivity, with only minor amounts (< 5%) of the all *cis*-isomer, as detected by ^{1}H NMR.



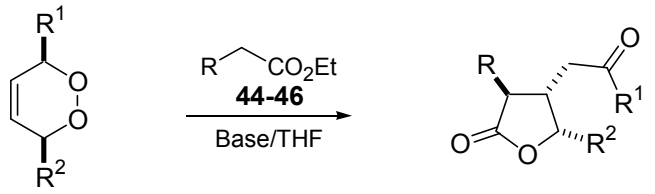
43

44; R = CO_2Et
45; R = CN
46; R = C(O)Me
47; R = Ph

48; R = CO_2Et
49; R = CN
50; R = C(O)Me

Scheme 2.12

The scope of this reaction was studied further with a range of 1,2-dioxines, **51**-**54**, and ester nucleophiles, **44**-**46**. A selection of these results, of relevance to the work described in the following chapter, is shown in Figure 2.3.[96] Each reaction was, again, found to be highly diastereoselective, with the products, **55**-**62**, obtained in moderate to excellent yields. The results represent the range of 1,2-dioxines and ester nucleophiles to which this reaction is applicable, and reveal the possibility of extending this procedure for the synthesis of many different trisubstituted γ-lactones.



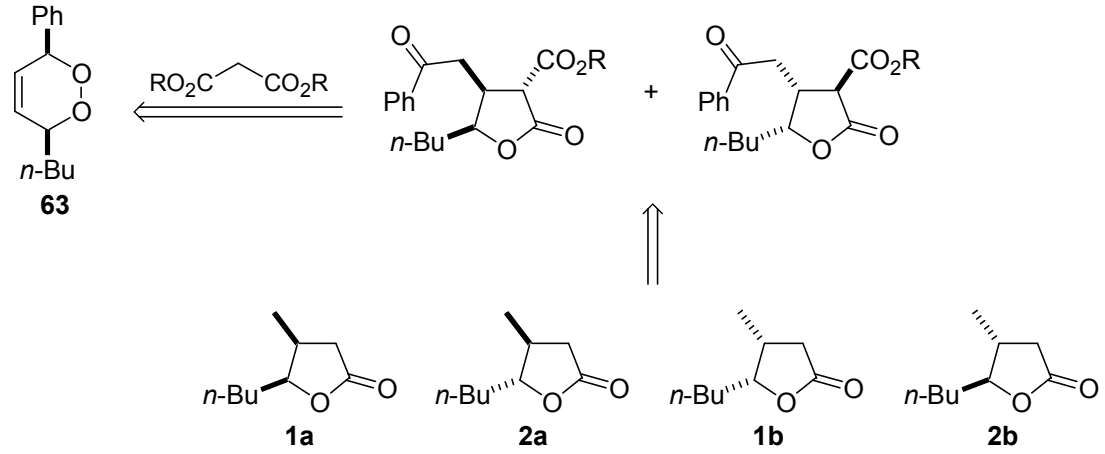
51-54 **55-62**

1,2-dioxine	R^1	R^2	malonate	R	product	yield (%)
51	Ph	Me	**44**	CO_2Et	**55**	97
51	Ph	Me	**45**	CN	**56**	78
51	Ph	Me	**46**	C(O)Me	**57**	85
52	Ph	H	**44**	CO_2Et	**58**	92
52	Ph	H	**45**	CN	**59**	65
52	Ph	H	**46**	C(O)Me	**60**	74
53	$-(CH_2)_2CH_3$	$-(CH_2)_2CH_3$	**44**	CO_2Et	**61**	56
54	Ph	$-(CH_2)_7CH_3$	**44**	CO_2Et	**62**	93

Figure 2.3 Reactions of ester nucleophiles with a range of 1,2-dioxines

2.4 Research aims

This project aimed to synthesise the four stereoisomers of oak lactone, in optically pure form, using 1,2-dioxine chemistry (Scheme 2.13). The strategy featured a common precursor, which could be manipulated to produce the naturally occurring oak lactone isomers, as well as their corresponding enantiomers. Suitably substituted 1,2-dioxine **63** would be converted to the corresponding *cis*-γ-hydroxyenone upon addition of base, and enable isolation of the trisubstituted γ-lactone through reaction with a malonate diester. Since the γ-lactone would be produced as a racemate, it would be necessary to invoke a resolving agent for the separation of the enantiomers through the formation of diastereomers. Standard chemical transformations would then be used to prepare the individual stereoisomers of oak lactone (**1a**, **1b**, **2a** and **2b**). With the final products envisaged to be of high enantiomeric purity, sensory studies would be undertaken to complete the odour profiles of the oak lactones through threshold testings in both white and red wines.


Ph
O
O
n-Bu
63
RO2C
CO2R
O
Ph
n-Bu
CO2R
O
O
+
O
Ph
n-Bu
CO2R
O
O
n-Bu
O
O
1a
n-Bu
O
O
2a
n-Bu
O
O
1b
n-Bu
O
O
2b

Scheme 2.13

3 Synthesis of enantiopure oak lactones

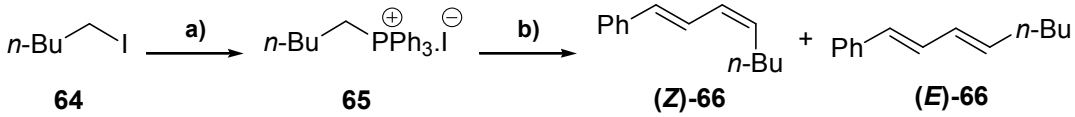
Previous work has seen the use of 1,2-dioxine chemistry for the preparation of trisubstituted γ-lactones. This chapter details the preliminary work in this area and extends the methodology for the stereoselective synthesis of the four stereoisomers of oak lactone.

3.1 Preliminary work towards the oak lactones *via* a 1,2-dioxine precursor

3.1.1 Synthesis of the 1,2-dioxine

Earlier experiments by the present author validated the use of a 1,2-dioxine as the key reactant for the synthesis of a trisubstituted γ-lactone.[99] In order to obtain the necessary alkyl chain at the C_5 position on the γ-lactone ring, 3-phenyl-6-*n*-butyl-1,2-dioxine (**63**) was required. 1,2-Dioxines can be prepared from the corresponding precursor diene and singlet oxygen in a photocycloaddition reaction;[100,101] the 1,2-dioxine required for this work was prepared from 1-phenylocta-1,3-diene.

1-Iodopentane (**64**) was reacted with triphenylphosphine in xylene to produce the corresponding phosphonium salt **65** in excellent yield (Scheme 3.1). The corresponding ylide was classified as reactive or non-stabilised[102] and was therefore generated *in situ* for the Wittig reaction with cinnamaldehyde. Diene **66** was prepared in excellent yield as a mixture of *trans*, *cis*- and *trans*, *trans*-geometric isomers, in the ratio of 4:1.



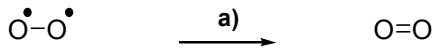
64 **65** **(*Z*)-66** **(*E*)-66**

Reagents and conditions: a) Ph_3P, xylene, Δ, 94%; **b)** i. $(CH_3)_3COK$, THF, 0 °C; ii. cinnamaldehyde, 0 °C-rt, 82%.

Scheme 3.1

The *trans*-configuration of the existing double bond, from cinnamaldehyde, was retained and the new double bond was found to have predominantly *cis*-geometry by ^{1}H NMR spectroscopy (J = 10.9 Hz). Approximately 20% of the minor isomer was detected (J = 15.4 Hz), with a coupling constant typical of *trans*-configured olefins. However, the geometry of the new double bond was not important (see below) and separation of the isomers was not attempted.

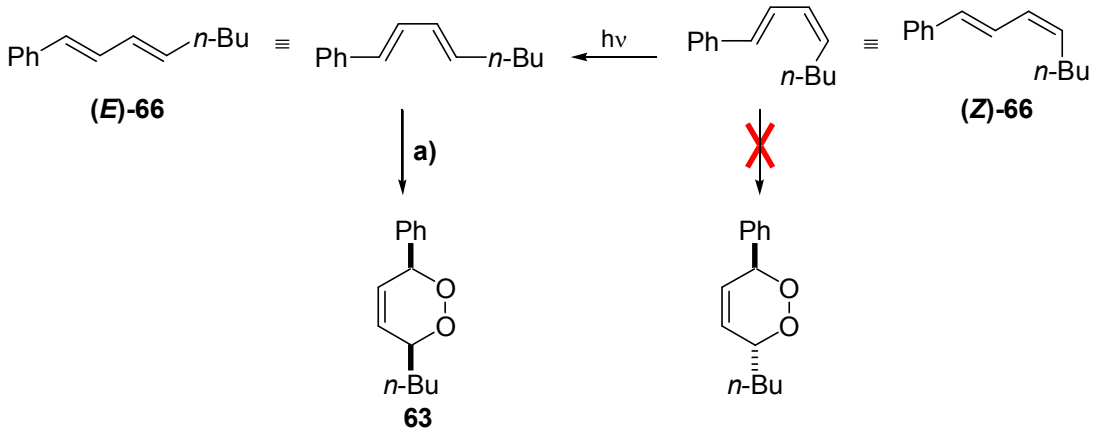
Following the Wittig reaction, the next step required was the photochemical addition of oxygen to the diene. The photo-oxidation featured the addition of oxygen as a dienophile across the diene system of **66** in a [4+2] cycloaddition reaction. The reaction required the presence of a photosensitiser to absorb light, such as rose bengal, a highly conjugated dye[103] (Scheme 3.2). This energy is then emitted and absorbed by oxygen to excite it from the triplet ground state to its singlet state, which is necessary before its addition as a dienophile to diene **66**.



Reagents and conditions: a) rose bengal, CH_2Cl_2, hυ.

Scheme 3.2

The minor product of the Wittig reaction, the *trans*, *trans*-configured diene, had the necessary s-*cis*-conformation required for the cycloaddition reaction. The major diene product, with *trans*, *cis*-configuration, cannot react directly due to steric interactions involving the *n*-butyl substituent. However, under the conditions of the photolysis reaction, the *trans*, *cis*-isomer of **66** was converted to the *trans*, *trans*-isomer of **66**. So, over time, the minor isomer was converted to the required s-*cis*-conformation, enabling formation of the *cis*-dioxine (Scheme 3.3). The cycloaddition reaction proceeded with high stereoselectivity; only *cis*-isomer **63** was obtained in the final (racemic) product.



Reagents and conditions: a) rose bengal, O_2, hυ, CH_2Cl_2, 0 °C, 10 hrs, 79%.

Scheme 3.3

The characteristic ^{1}H NMR signals indicative of dioxine formation include the olefinic protons and the protons adjacent to the oxygen-oxygen bond (Figure 3.1). The former of these protons, at C_4 and C_5, resonated at 6.19-6.02 ppm, as a two-proton multiplet, while the latter of these protons, at C_3 and C_6, resonated at 5.51 ppm and 4.58 ppm, both as multiplets.

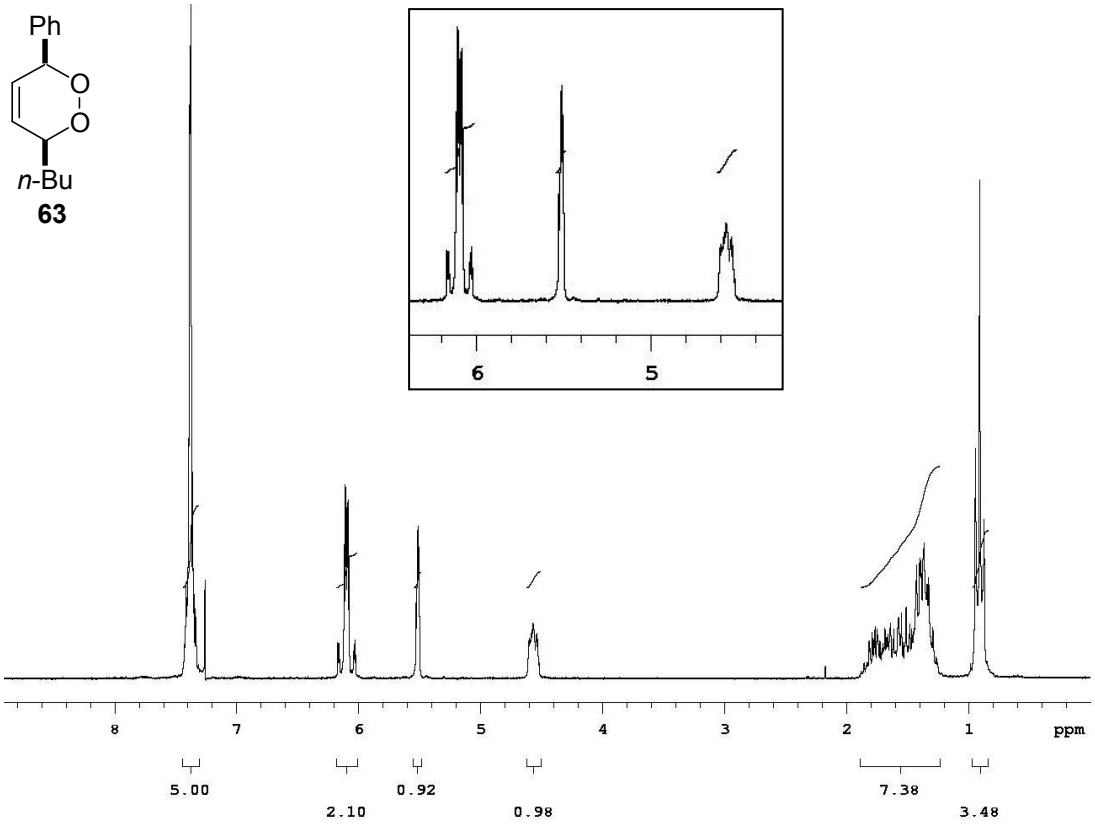
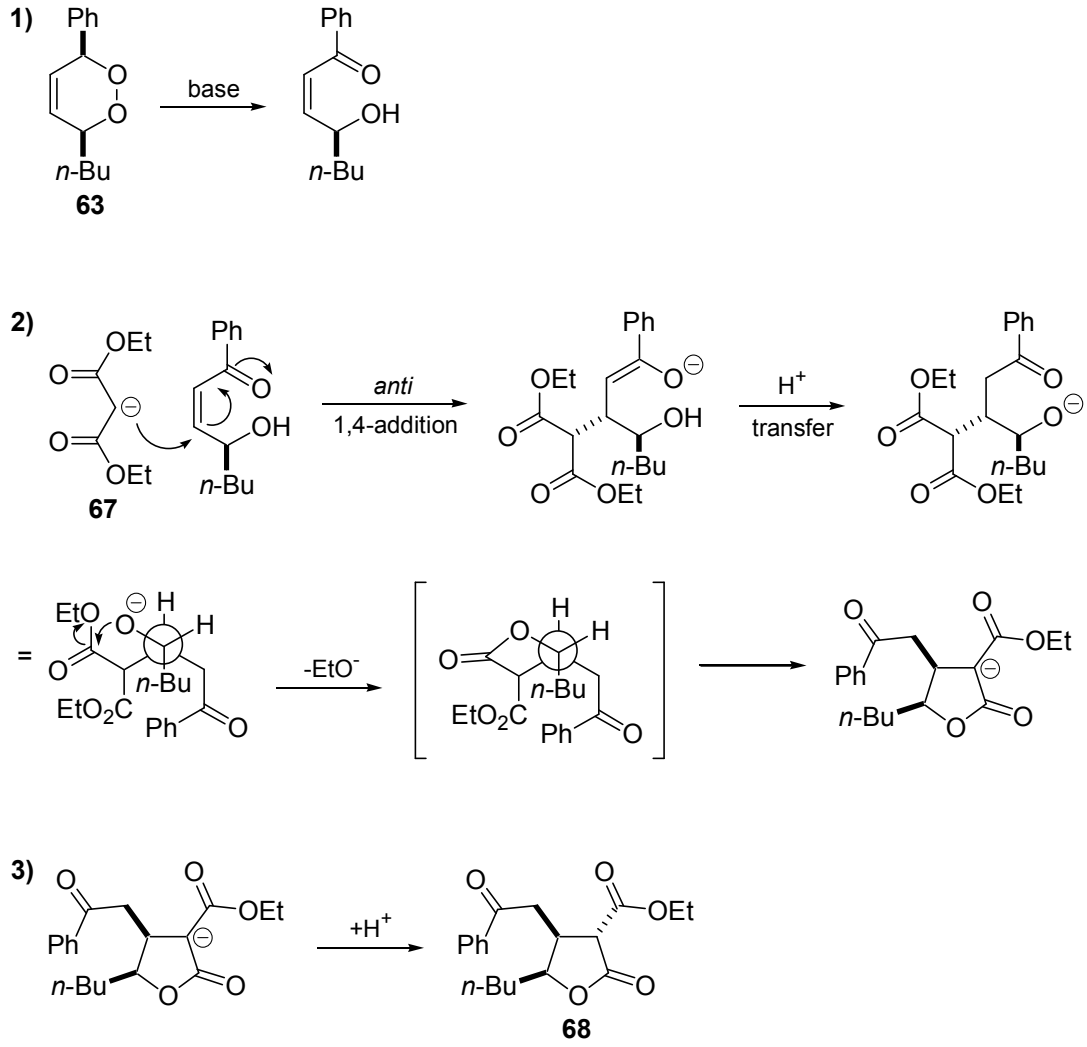


Figure 3.1 200 MHz ^{1}H NMR spectrum for 1,2-dioxine 63 in $CDCl_3$

3.1.2 Synthesis of the racemic trisubstituted γ-lactone

Dioxine **63** was converted to its *cis*-γ-hydroxyenone equivalent *via* heterolytic ring opening *in situ* with sodium hydride, prior to conjugate addition of the enolate of diethyl malonate (**67**) (Scheme 3.4). Trisubstituted γ-lactone **68** was formed upon protonation of the anion from the more hindered face of the molecule as shown by the formation of the thermodynamically stable *trans*, *cis*-product. There does, in theory, exist the possibility of protonation from either face of the carbanion; however, protonation from the less hindered face is seldom observed due to the increased energy in the all *cis*-arrangement around the lactone moiety.

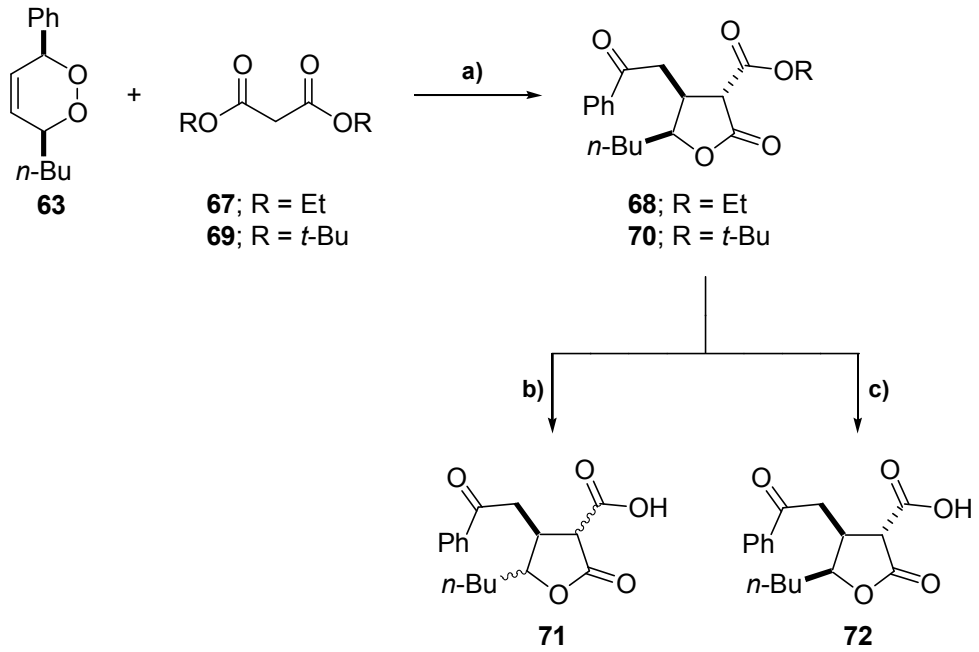


Scheme 3.4

Since the 1,2-dioxine used in the conjugate addition reaction was racemic, the trisubstituted γ-lactone product was also racemic. Thus, there was the need to

involve a resolution step in order to obtain enantiomerically pure oak lactone end products. It was anticipated that this would be achieved through cleavage of the ester moiety at C_3 and the addition of a chiral resolving agent. The resultant diastereomers should potentially be amenable to chromatographic separation.

Initial experiments involved the use of commercially available diethyl malonate (**67**) which resulted in the formation of ethyl ester **68** at the C_3 position on the lactone ring (Scheme 3.5). Although this reaction was successful, the ethyl ester product proved to be problematic, as hydrolysis under basic conditions was complicated by epimerisation of the C_3 and C_5 stereocentres. The use of di-*tert*-butyl malonate (**69**) formed *tert*-butyl ester **70** and enabled hydrolysis to be achieved under acidic conditions (Scheme 3.5). This overcame the epimerisation problems and trisubstituted 3-carboxy-γ-lactone **72** was isolated successfully.



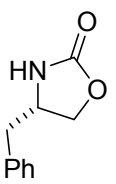
Reagents and conditions: a) NaH, THF, 0 °C-rt, 89% for **68**, quantitative for **70**; **b)** 50% aq EtOH, KOH, rt, o/n, 79%; **c)** TFA, CH_2Cl_2, rt, 3 hrs, 75%.

Scheme 3.5

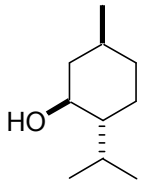
3.1.3 Attempted resolution

With trisubstituted 3-carboxy-γ-lactone **72** in hand, it required only conversion into diastereomers to enable separation before the oak lactone products could be obtained

by side chain manipulation. Two different chiral resolving agents were trialled; (*S*)-4-benzyl-2-oxazolidinone and (1*S*,2*R*,5*S*)-menthol (Figure 3.2). Preparation of diastereomeric amides with oxazolidinone was unsuccessful, while any attempts at separation of the menthol diastereomers by thin layer chromatography proved to be futile. Due to strict time constraints, this work could not be pursued further and the oak lactone stereoisomers were not synthesised by this route.



(*S*)-4-benzyl-2-oxazolidinone



(1*S*,2*R*,5*S*)-menthol

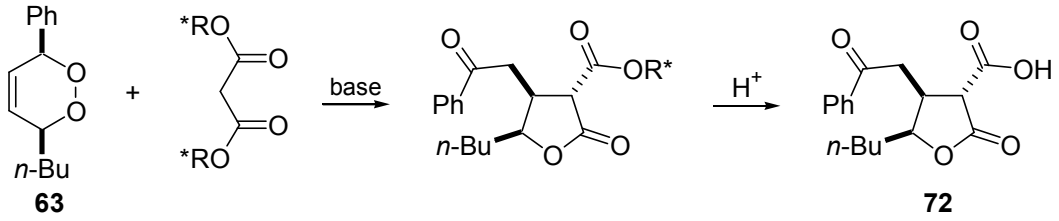
Figure 3.2 Structures of chiral resolving agents used for attempted chromatographic resolution

These preliminary experiments, although unsuccessful in terms of synthesis of the actual end products (the oak lactones), did, however, confirm the success of the 1,2-dioxine conjugate addition approach. Providing that the difficulty of separating the individual trisubstituted lactones could be overcome, then this route promised to provide access to all four desired oak lactones.

3.2 Synthesis the four stereoisomers of oak lactone

3.2.1 Utilisation of a chiral malonate for chromatographic resolution

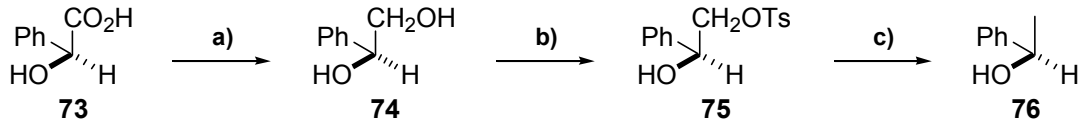
In order to achieve separation, and in view of the difficulties encountered in the earlier work while trying to add a resolving agent to the trisubstituted γ-lactone, it was envisaged that a chiral malonate could be used in lactone formation (Scheme 3.6). From the problems encountered when the lactone was treated with base,[99] it was considered necessary to use a chiral malonate that would form an ester in which hydrolysis could be achieved under acidic conditions.



Scheme 3.6

3.2.2 Synthesis of the diastereomeric trisubstituted γ-lactones

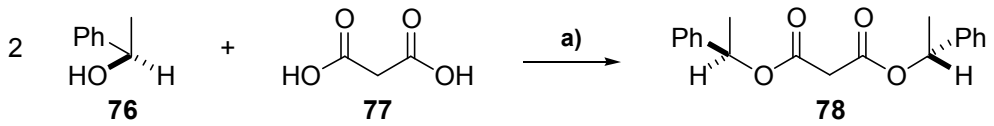
In keeping with the previously described necessity for an ester which could be cleaved under mild acidic conditions, a tertiary or benzyl ester was sought. It was decided to employ (*R*)-phenylethanol for this purpose. Although it is commercially available, it proved cheaper to synthesise the alcohol from the much less expensive (*S*)-mandelic acid (**73**) (Scheme 3.7).[104] Firstly, acid **73** was reduced with borane dimethylsufide to diol **74** in excellent yield, after purification by column chromatography. Diol **74** was selectively tosylated at the primary position, using *p*-toluenesulfonyl chloride, to **75**. Finally, tosylate **75** was reduced with lithium aluminium hydride to produce optically active alcohol **76**, which was used without further purification. Spectral data for the intermediate compounds[105-107] together with the final alcohol[108] were in good agreement with the literature.



Reagents and conditions: a) i. $BH_3.Me_2S$, THF, 0 °C-rt, 22.5 hrs; ii. 50% MeOH/THF, 0-50 °C, 4 hrs, 90%; **b)** *p*-TsCl, pyr, 0 °C-rt, 22 hrs, 91%; **c)** $LiAlH_4$, THF, 0 °C-rt, 4 hrs, 73%.

Scheme 3.7

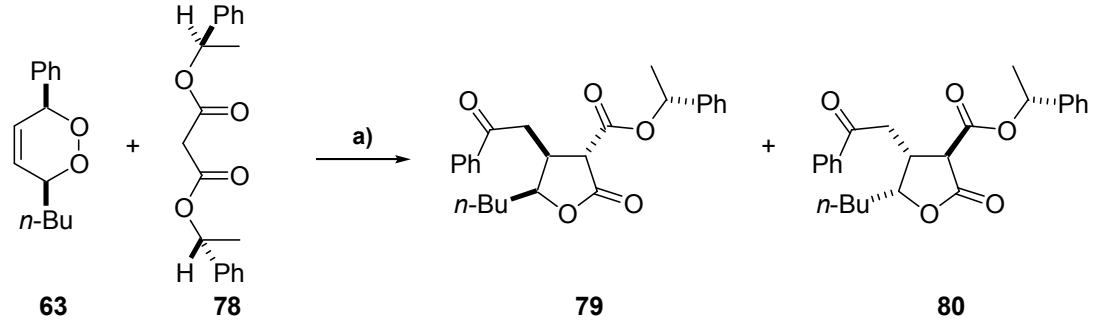
Initally, chiral malonate **78** was prepared by the reaction of alcohol **76** with malonyl dichloride in dichloromethane with pyridine and DMAP. Although the malonate was isolated after column chromatography, the procedure proved to be very low yielding (14%). Thus an alternative route was sought. Chiral malonate **78** was prepared from standard DCC mediated condensation of two equivalents of (*R*)-phenylethanol (**76**) with malonic acid (**77**) in excellent yield (84%) (Scheme 3.8).[109] The NMR data for the synthesised malonate matched those reported in the literature.[109]



Reagents and conditions: a) DCC, MeCN, rt, 24 hrs, 84%.

Scheme 3.8

Prior to the conjugate addition of chiral malonic ester **78**, dioxine **63** was first converted to its *cis*-γ-hydroxyenone equivalent. Treatment of the enolate derived from the chiral malonate diester **78** with **63** gave diastereomeric γ-lactones **79** and **80** in moderate yield (54%) after purification (Scheme 3.9).



Reagents and conditions: a) NaH, THF, 0 °C-rt, 24 hrs, 54%.

Scheme 3.9

The relative stereochemistry of trisubstituted γ-lactones **79** and **80** was confirmed by ^{1}H NMR spectroscopy (Figure 3.3), acquired in d-chloroform. The indicative proton at C_3, which appeared as a doublet at 3.40 ppm, featured a large coupling constant (J = 9.6 Hz) consistent with typical *trans*-coupling.[110] Coupling constants for the proton at C_4, 3.55 ppm, could not be calculated, as the signal appeared as a complex multiplet. The C_5 proton resonated as a quartet at 4.95 ppm with its coupling constant (J = 6.8 Hz) significantly smaller than that of the *trans*-proton coupling between C_3 and C_4. Previous studies on analogous products,[99] including X-ray crystal analysis,[96] confirm the relative stereochemistry obtained by this synthetic methodology.

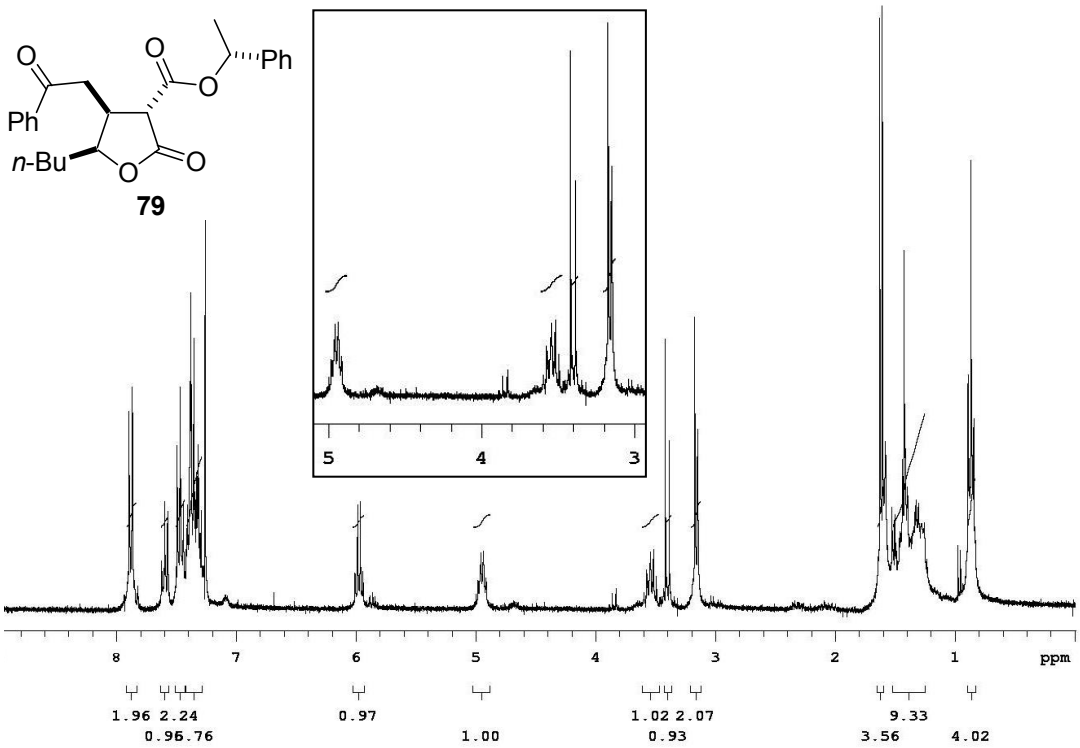


Figure 3.3 300 MHz ^{1}H NMR spectrum for diastereomer 79 in $CDCl_3$

Separation by column chromatography with silica gel provided pure samples of each diastereomer. ^{1}H NMR spectroscopy was used to determine the purity of the collected fractions (Table 3.1). Since at this stage it was not possible to determine which of the diastereomers, **79** or **80**, would lead to the nature-identical or the non-nature-identical isomers of oak lactone, the isomers were termed 'top' and 'bottom' in relation to their order of elution on thin layer chromatography. The 'top' isomer eluted first, ($\mathbf{R_f}$ = 0.26) while the 'bottom' isomer eluted second ($\mathbf{R_f}$ = 0.21). Since all four isomers of oak lactone were required products, both diastereomers were carried through to the end products.

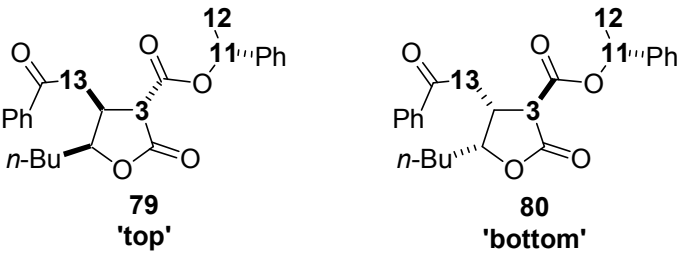
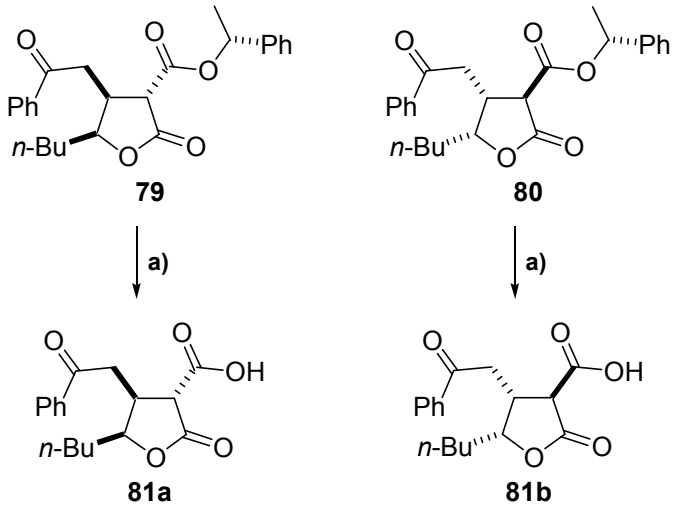


Table 3.1 Characteristic ^{1}H NMR data for 'top' and 'bottom' diastereomers

	'top'	**'bottom'**
H_{11}	5.98 (1H, q, J = 6.6)	5.96 (1H, q, J = 6.6)
H_3	3.40 (1H, d, J = 9.6)	3.43 (1H, d, J = 8.9)
H_{13}	3.16 (2H, d, J = 7.8)	3.18 (2H, d, J = 7.3)
H_{12}	1.62 (3H, d, J = 6.6)	1.60 (3H, d, J = 6.6)

Note: ^{1}H NMR data reported as chemical shift in ppm, relative integration, multiplicity (d = doublet; q = quartet) and coupling constant (J) in Hz; acquired in $CDCl_3$

Cleavage of the ester group at the C_3 position by treatment of **79** and **80**, separately, with trifluoroacetic acid in dichloromethane gave rise to enantiomers **81a** and **81b**, respectively, in excellent yields (Scheme 3.10). As anticipated, the use of a benzyl ester removed the problems of epimerisation observed in the preliminary experiments with base hydrolysis.



Reagents and conditions: a) TFA, CH_2Cl_2, rt, 2 hrs, 92% for **81a**, 93% for **81b**.

Scheme 3.10

The NMR spectrum for **81a** was initially obtained in d-chloroform. The C_4 proton was found to overlap with one of the C_{13} protons and so the spectrum was acquired

in d_6-benzene where complete resolution was obtained for each resonance (Figure 3.4). The distinctive C_5 proton resonated at 4.77 ppm as a quartet. The C_3 proton appeared as a doublet at 3.19 ppm with a large coupling constant (J = 10.2 Hz), consistent with *trans*-coupling to the C_4 proton.[110] The C_4 proton appeared at 3.38 ppm as a multiplet and the two protons for C_{13} at 2.99 ppm and 2.62 ppm, both as doublets of doublets.

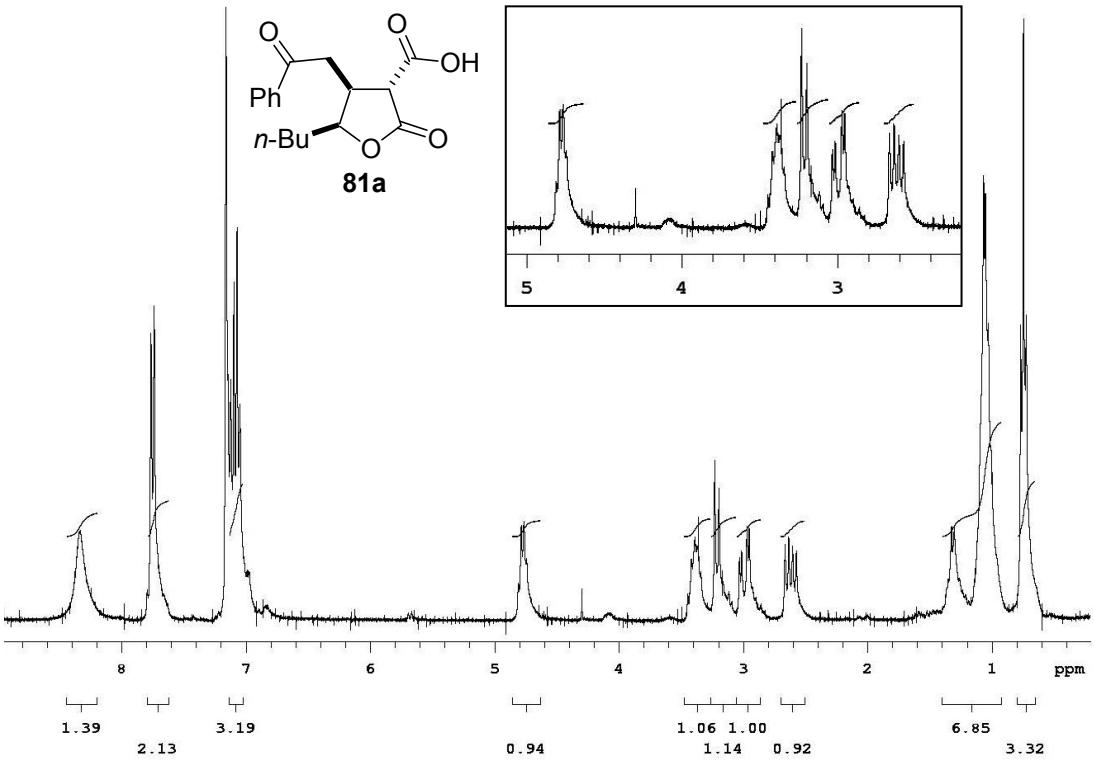


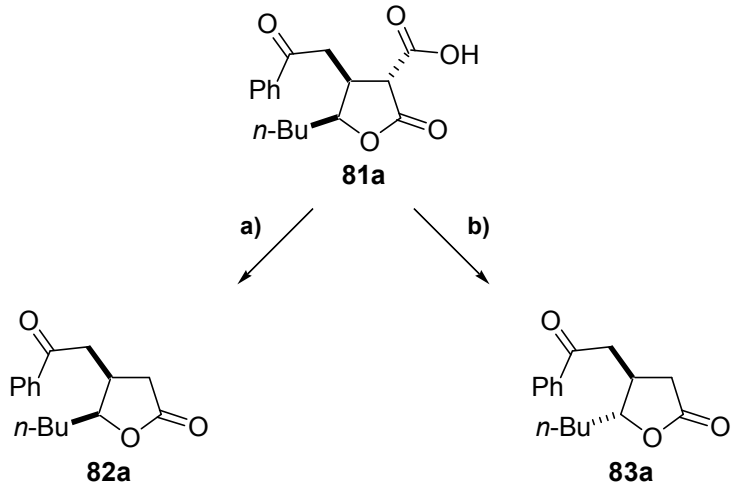
Figure 3.4 300 MHz ^{1}H NMR spectrum for acid 81a in C_6D_6

The remainder of this discussion is focused on the synthesis of the nature identical oak lactones from **81a**. In the same manner, the non-nature identical oak lactones were prepared from **81b** and will be outlined, albeit more briefly, at the end of this chapter.

3.2.3 Thermal decarboxylations

The key step in the conversion of the trisubstituted γ-lactones to the oak lactone stereoisomers was the loss of the carboxyl group at the C_3 position. This was directed to give either the *cis*- or the *trans*-decarboxylated product (Scheme 3.11).

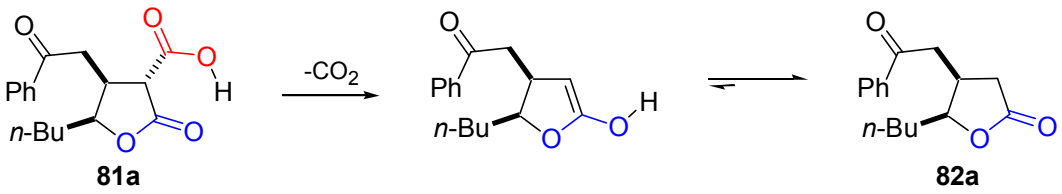
Preliminary experiments on racemic mixtures had suggested that the product of decarboxylation was highly solvent dependent. Reaction in toluene gave exclusively the *cis*-product, whereas 50% aqueous acetic acid gave predominantly (but not exclusively) the *trans*-product.[99]



Reagents and conditions: a) PhMe, Δ, 20 hrs, 76%, **b)** 50% aq AcOH, Δ, 20 hrs, 99%.

Scheme 3.11

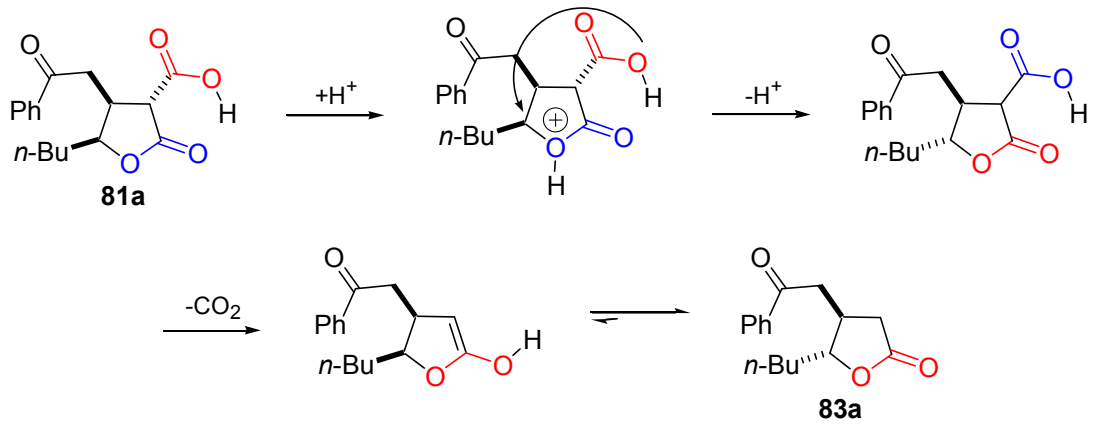
When the pure enantiomer **81a** was heated under reflux in toluene the decarboxylation proceeded cleanly to give **82a** in 76% yield. This product was completely free of contamination from epimer **83a**. Under these conditions, it was the external carboxylate group at C_3 that was lost directly (Scheme 3.12).



Scheme 3.12

Conversely, when **81a** was heated under reflux in 50% aqueous acetic acid, isomerisation was observed to give mainly (75%) the *trans*-decarboxylated product **83a** in 99% yield. The *trans*-product was actually a result of the loss of the internal carboxylate group (Scheme 3.13). Initially, the internal carboxyl group undergoes attack from the external carboxyl group, resulting in epimerisation at the C_5 centre,

presumably to relieve the steric buttressing observed in the original compound. This is then followed by loss of the original internal, but now external, carboxyl group.



Scheme 3.13

^{1}H NMR data were obtained in d_6-benzene for the decarboxylated products (Figure 3.5 and Figure 3.6). The selectivity for decarboxylation is clear in the ^{1}H spectra; the *cis*-isomer was obtained completely free of the *trans*-epimer, while the *trans*-isomer was obtained as a mixture (approximately a 3:1 ratio with the *cis*-epimer). The distinctive C_5 proton now appeared at 4.16 ppm and 3.71 ppm, for the *cis*-**82a** and *trans*-**83a** products, respectively. Another distinguishing feature between the two isomers was the resonance of the C_4 proton; for the *cis*-isomer it appeared as an approximate sextet at 2.26 ppm but for the *trans*-isomer it appeared as a multiplet at 2.28 ppm. For each isomer, the C_3 and C_{13} protons appeared as doublets of doublets (*cis*-isomer C_3 protons at 2.27 ppm and 1.89 ppm, C_{13} protons at 2.45 ppm and 2.26 ppm; *trans*-isomer C_3 protons at 2.52 ppm and 1.71 ppm, C_{13} protons at 2.41 ppm and 2.14 ppm). These protons were distinguishable from each other by heteronuclear multiple bond connectivity (HMBC) experiments, wherein the C_3 protons showed coupling to the lactone carbonyl, while the C_{13} protons showed coupling to the phenyl ketone.

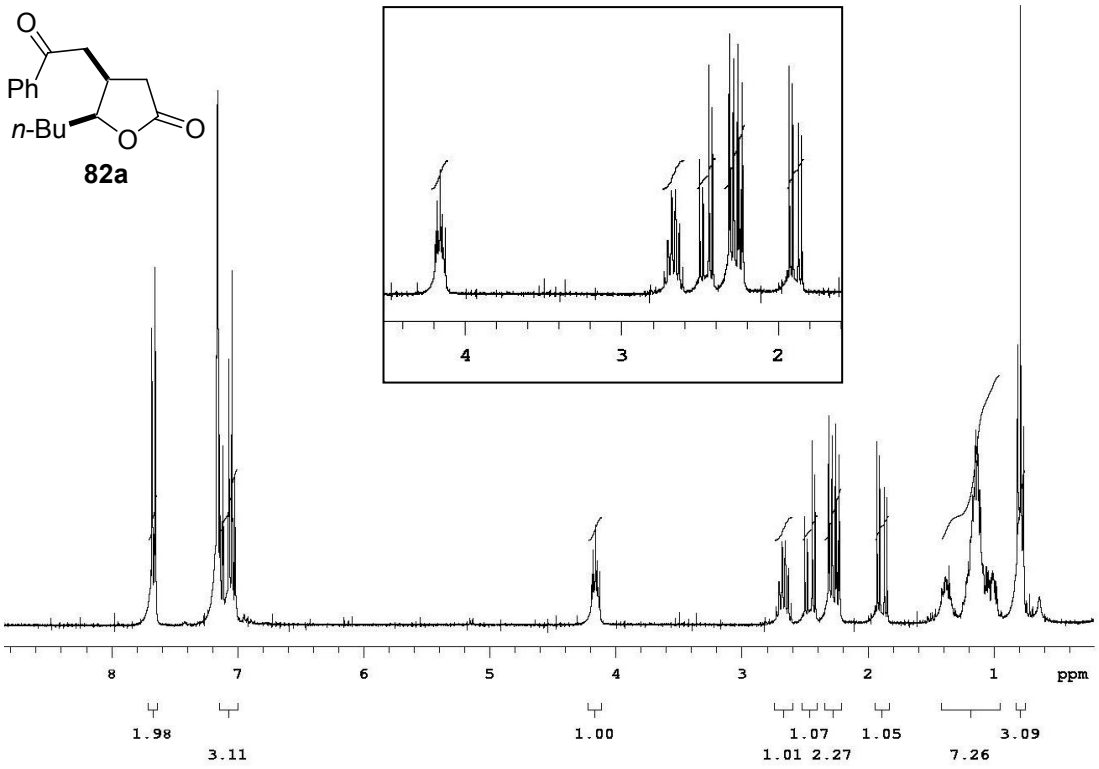


Figure 3.5 300 MHz ^{1}H NMR spectrum for *cis*-product 82a in C_6D_6

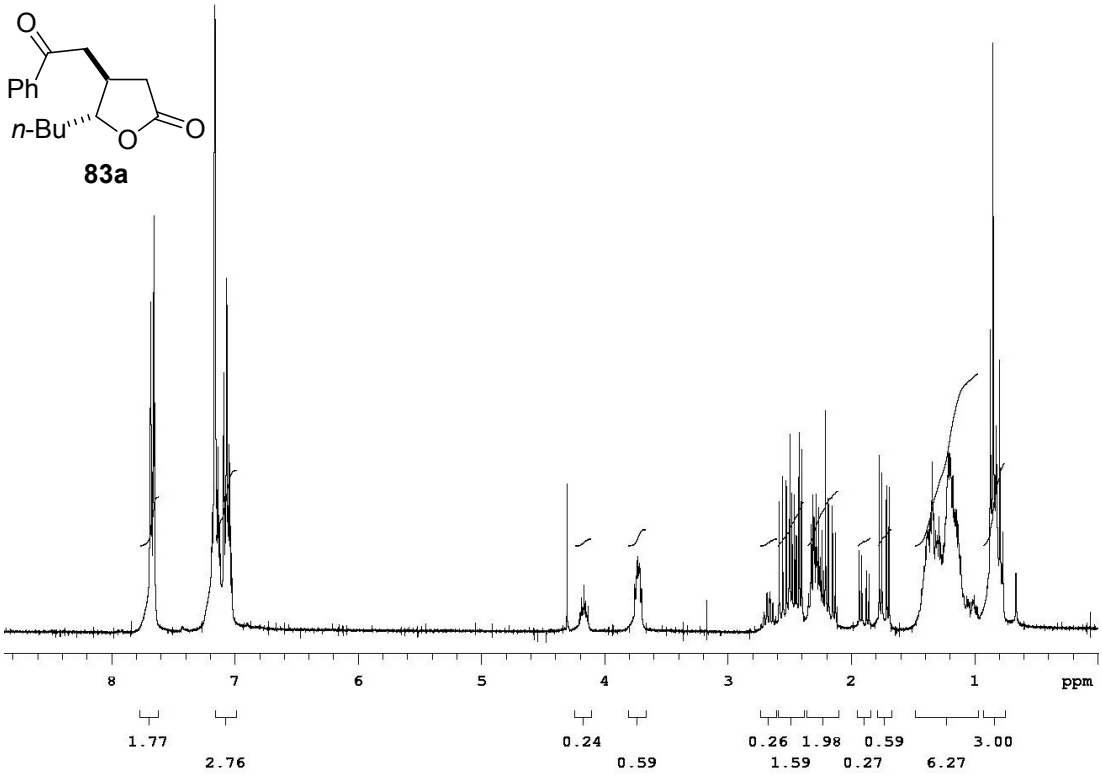


Figure 3.6 300MHz ^{1}H NMR spectrum for *trans*-product 83a in C_6D_6

Nuclear Overhauser effect (nOE) spectroscopy was used to differentiate between the *cis*- and *trans*-protons at C_3 for the two stereoisomers (Figure 3.7). For the *cis*-isomer, the C_4 proton showed strong correlation to the proton at 2.27 ppm but no correlation to the proton at 1.89 ppm. Thus the latter was assigned to be *trans*- and the former as *cis*-, relative to the stereocentre at C_4 with (*S*)-configuration. An nOe correlation between H_4 and H_5 confirmed the relative *cis*-stereochemistry that was obtained between these two protons for the isomer **82a**. For the trans-isomer, the proton at 2.52 ppm was assigned as *cis*- and the proton at 1.71 ppm as *trans*-relative to the (*S*)-stereocentre at C_4. The absence of an nOe correlation between H_4 and H_5 confirmed the relative *trans*-stereochemistry that was obtained between these two protons in the isomer **83a**.

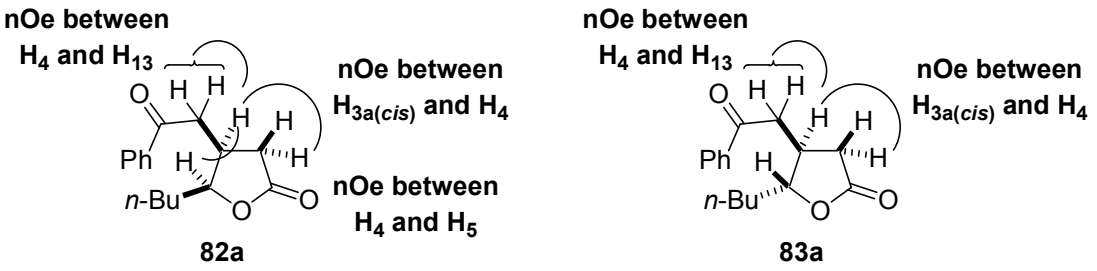


Figure 3.7 nOe correlations observed for the decarboxylated products *cis*-82a and *trans*-83a

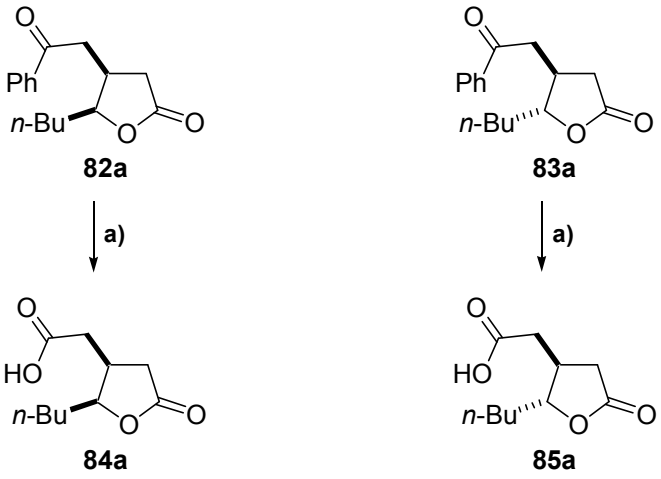
A small portion of *trans*-decarboxylated product **83a** was purified by recrystallisation from carbon tetrachloride and hexanes. This process was tedious but enabled spectroscopic data to be obtained on the individual stereoisomer. However, in the long run, it proved to be more convenient to purify the major isomer by column chromatography after the final step of the synthetic pathway.

3.2.4 Oxidation using ruthenium

The next step in the synthetic pathway was the oxidation of phenyl ketones **82a** and **83a** to carboxylic acids **84a** and **85a**, respectively (Scheme 3.14). Ruthenium tetroxide was first introduced as an organic oxidant in 1953.[111] It has seen widespread use for a large number of oxidative transformations, including alcohols, ethers, saturated and unsaturated hydrocarbons as well as nitrogen-containing compounds and aromatic rings.[112,113] Ruthenium is generally used in catalytic

quantities (1-5%), with periodate or hypochlorite-based oxidants in a four to five mole equivalent excess. Sharpless developed a ternary solvent system of acetonitrile, water and carbon tetrachloride for the optimised oxidation with ruthenium tetroxide.[114]

Initial attempts with the Sharpless method[114] were unsuccessful and large quantities of product corresponding to incomplete oxidation of the aromatic ring were observed. When the reaction was attempted as described elsewhere in the literature (1.1-1.7 molar equivalents of ruthenium trichloride),[115] the oxidation proceeded cleanly to give the desired lactone acids **84a** and **85a**. Contrary to literature reports suggesting extensive reaction time periods (18 days to two months),[115] the reaction proceeded in 20 hours in excellent yields.



Reagents and conditions: a) $RuCl_3$, $NaIO_4$, CCl_4/MeCN/H_2O, rt, 20 hrs, 83% for **84a**, 69% for **85a**.

Scheme 3.14

The diastereomeric purity of acids **84a** and **85a** was determined by ^1H NMR spectroscopy (Figure 3.8 and Figure 3.9) in d-chloroform. Prevention of any epimerisation of the C_5 stereocentre alleviated the need for tedious chromatography, especially for the *cis*-isomer **84a** which, thus far, was diastereomerically pure. Once again, the position of the C_5 proton was characteristic of the *cis*-**84a** or *trans*-**85a** isomers at either 4.59 ppm as a multiplet or at 3.62 ppm as a quartet, respectively. Under the conditions of the oxidation reaction, no epimerisation was observed.

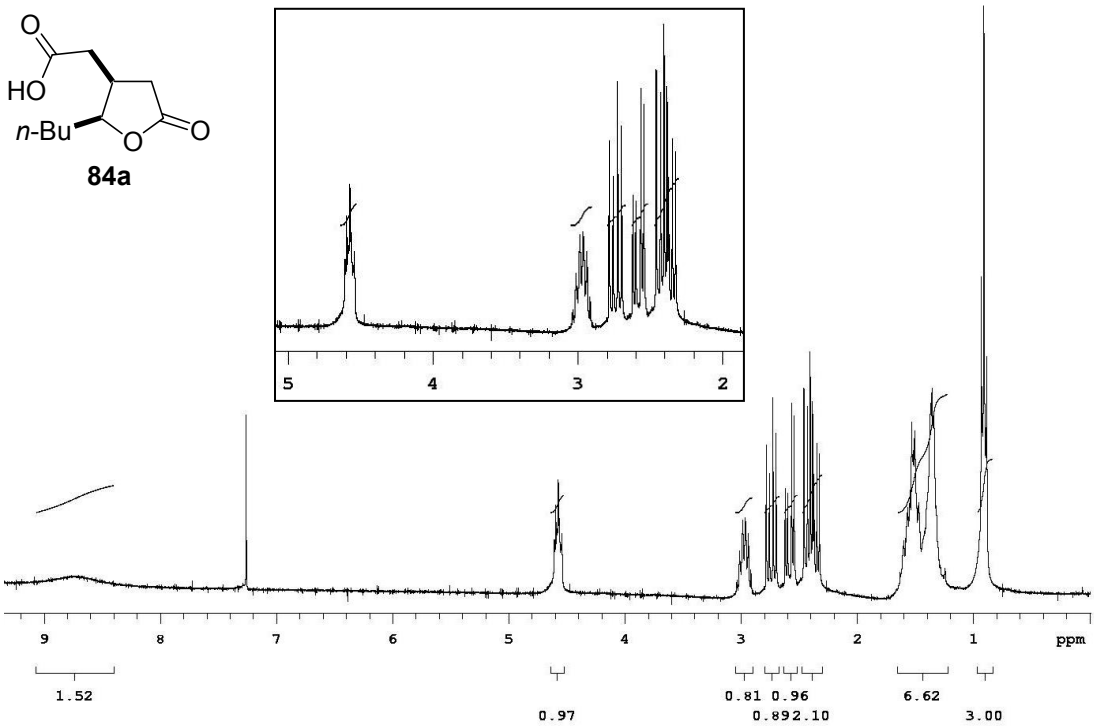


Figure 3.8 300 MHz ^{1}H NMR spectrum for *cis*-acid 84a in $CDCl_3$

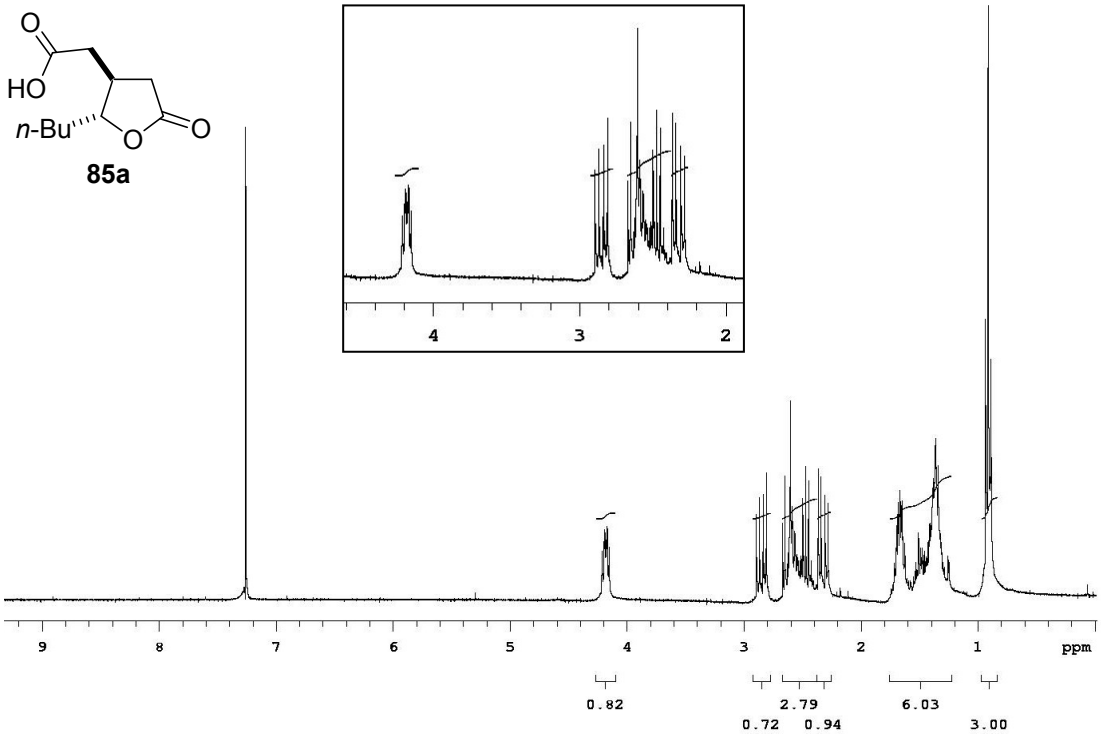


Figure 3.9 300 MHz ^{1}H NMR spectrum for *trans*-acid 85a in $CDCl_3$

For *cis*-isomer **84a**, it was possible to differentiate between the C_3 and C_{13} protons. Through the use of nuclear Overhauser spectroscopy, the *cis*- and *trans*-protons for

C_3 relative to the C_4 stereocentre, were assigned at 2.37 ppm and 2.74 ppm (J = 17.5 Hz), respectively (Figure 3.10). The protons at 2.58 ppm and 2.42 ppm (J = 16.7 Hz) were thus assigned as C_{13}. In the case of the *trans*-isomer **85a**, it was not possible to distinguish between the C_3 and C_{13} protons due to their proximity to the C_4 proton, even when analysed in d_6-benzene or on a high resolution spectrometer (600 MHz). For the *cis*-isomer **84a**, the presence of an nOe correlation between H_4 and H_5 confirmed the relative *cis*-stereochemistry, while the absence of an nOe correlation between these same protons in **85a** confirmed the relative *trans*-stereochemistry.

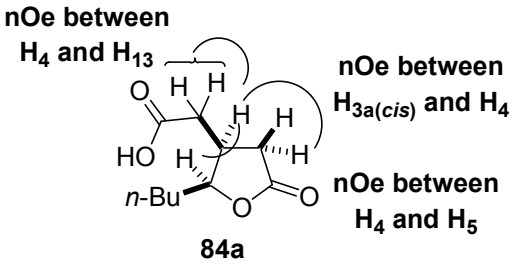
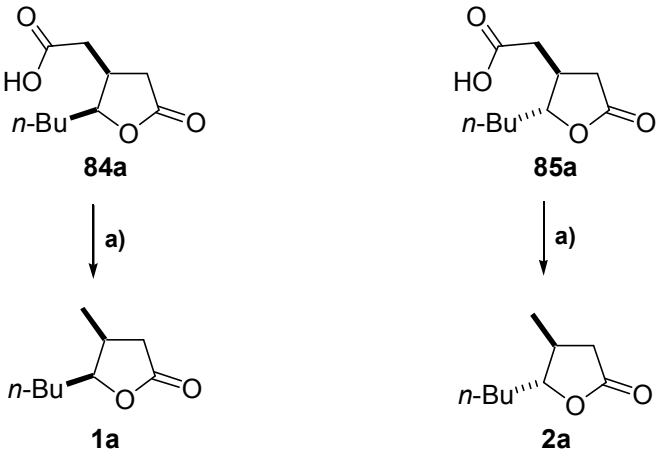


Figure 3.10 nOe correlations observed for oxidised product *cis*-84a

3.2.5 Barton ester formation and decarboxylation

The final step involved a Barton decarboxylation of acids **84a** and **85a** (Scheme 3.15).[116] The intermediate thiohydroxamic esters were formed from the reaction between carboxylic acids **84a** and **85a** and *N*-hydroxy-2-thiopyridone in DCC mediated coupling reactions.[117] The bright yellow, light sensitive ester thus formed undergoes a radical decarboxylative reduction, employing *tert*-butyl mercaptan as the hydrogen atom source.[118] Typically, this one pot procedure for decarboxylation affords excellent yields of the alkyl product;[119] the average yields obtained in this case could be attributed to the volatility of the oak lactone products.



Reagents and conditions: a) i. *N*-hydroxy-2-thiopyridone, DCC, DMAP, C_6H_6, rt, 2.5 hrs; ii. $(CH_3)_3CSH$, AIBN, C_6H_6, hυ, Δ, 4.5 hrs, 50% for **1a**, 49% for **2a**.

Scheme 3.15

The oak lactone products (**1a** and **2a**) were obtained after purification by column chromatography. Percentage yields for the reactions, from the formation of the decarboxylated product to the final oak lactone products, are shown in Table 3.2.

Table 3.2 Yields obtained for the synthesis of nature identical oak lactones

stereochemistry	decarboxylated product	oxidised lactone	oak lactone	overall[a]
(4*S*,5*S*)	76% (**82a**)	83% (**84a**)	50% (**1a**)	32%
(4*S*,5*R*)	99% (**83a**)	69% (**85a**)	49% (**2a**)	33%

[a] overall yield calculated from decarboxylated product (**82a** or **83a**) to oak lactone product (**1a** or **2a**)

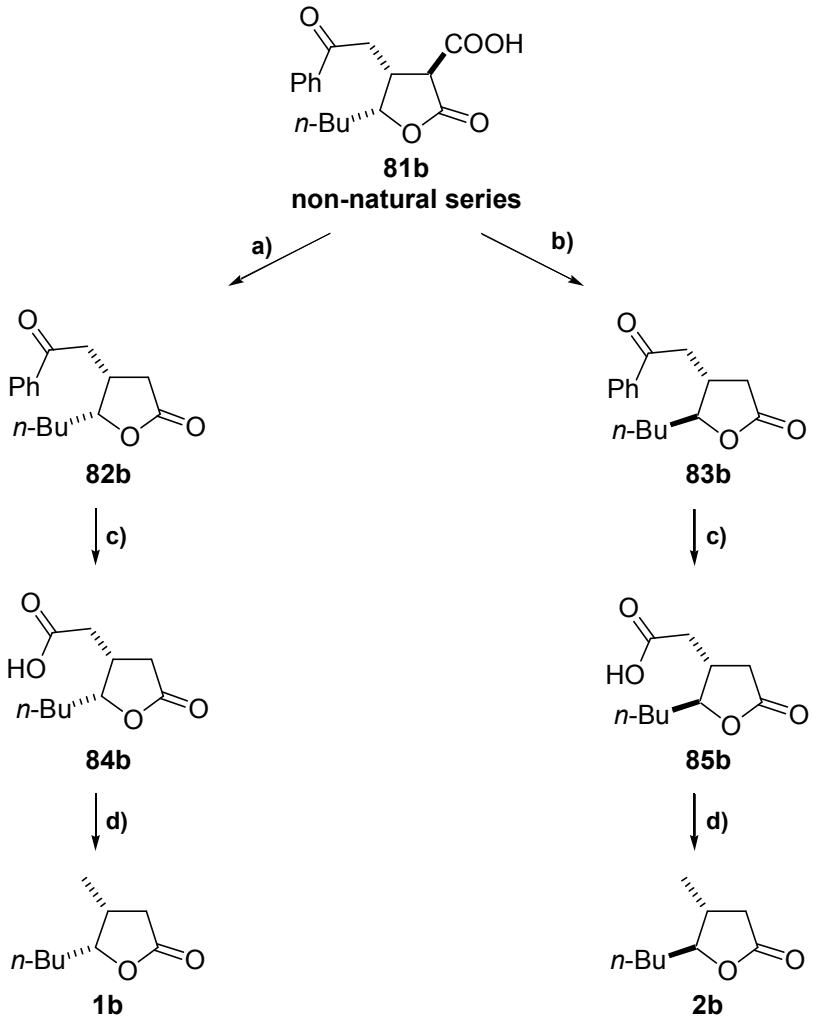
Table 3.3 lists literature ^{1}H NMR for the *cis*- and *trans*-oak lactones[59] compared with the data obtained for the synthetic samples. The NMR data are virtually identical to those previously reported, except where further splitting was observed for the C_5 proton.

Table 3.3 ^{1}H NMR data for literature and synthetic *cis*- and *trans*-oak lactones

	(4*S*,5*S*)-*cis*-**1a**		(4*S*,5*R*)-*trans*-**2a**	
	literature[59]	**synthetic**	**literature**[59]	**synthetic**
H_5	4.43 (m)	4.42 (ddd, *J* = 9.4, 5.6, 4.5)	4.01 (m)	4.00 (dt, *J* = 7.7, 4.0)
$H_{3a/b}$	2.70 (dd, *J* = 17, 8)	2.68 (dd, *J* = 16.9, 7.8)	2.67 (m)	2.66 (m)
H_4	2.58 (m)	2.57 (m)	2.21 (m)	2.29-2.12 (m)
$H_{3a/b}$	2.20 (dd, *J* = 17, 4)	2.19 (dd, *J* = 16.9, 3.8)		
$H_{6,7,8}$	1.65-1.36 (m)	1.74-1.26 (m)	1.74-1.25 (m)	1.74-1.27 (m)
H_{10}	1.02 (d, *J* = 7)	1.00 (d, *J* = 7.0)	1.14 (dd, *J* = 5, 2)	1.13 (dd, *J* = 6.5, 1.8)
H_9	0.93 (t, *J* = 7)	0.91 (t, *J* = 7.1)	0.92 (t, *J* = 7 Hz)	0.91 (t, *J* = 7.1)

Note: ^{1}H NMR data reported as chemical shift in ppm; splitting patterns quoted in parentheses as d = doublet, dd = doublet of doublets, ddd = doublet of doublet of doublets, dt = doublet of triplets, m = multiplet, t = triplet with coupling constant (*J*) in Hz; acquired in $CDCl_3$

In an identical manner, acid **81b** was taken through the synthetic scheme to obtain the non-nature identical oak lactones (**1b** and **2b**) (Scheme 3.16). Table 3.4 shows the product yields from decarboxylated isomers through to the final non-nature identical oak lactone stereoisomers. NMR data obtained matched that reported in the literature.[59] The assignment of absolute stereochemistry and enantiomeric purity for each of the four stereoisomers of oak lactone, based on chiral gas chromatography analysis, is described in the following chapter.



Reagents and conditions: a) PhMe, Δ, 20 hrs, 74%; **b)** 50% aq AcOH, Δ, 20 hrs, 87%; **c)** $RuCl_3$, $NaIO_4$, CCl_4/MeCN/H_2O, rt, 20 hrs, 77% for **84b** and **85b**; **d)** i. *N*-hydroxy-2-thiopyridone, DCC, DMAP, C_6H_6, rt, 2.5 hrs; ii. $(CH_3)_3CSH$, AIBN, C_6H_6, hυ, Δ, 4.5 hrs, 49% for **1b**, 27% for **2b**.

Scheme 3.16

Table 3.4 Yields obtained for synthesis of non-nature identical oak lactones

stereochemistry	decarboxylated product	oxidised lactone	oak lactone	overall[a]
(4*R*,5*R*)	74% (**82b**)	77% (**84b**)	49% (**1b**)	28%
(4*R*,5*S*)	87% (**83b**)	77% (**85b**)	27% (**2b**)	18%

[a] overall yield calculated from decarboxylated product (**82b** or **83b**) to oak lactone product (**1b** or **2b**)

3.3 Summary

All four possible oak lactone stereoisomers have been prepared and characterised. The initial reagent, a 1,2-dioxine, prepared from its corresponding diene in a photolysis reaction, was reacted with a chiral malonate to form diastereomeric trisubstituted γ-lactones. Separation of the diastereomers was achieved by column chromatography. Ester hydrolysis under acidic conditions produced the trisubstituted 3-carboxy-γ-lactone acid, which enabled selective decarboxylation to either the *cis*- or *trans*-4,5-disubstituted products. Through manipulation of the side chain *via* standard chemical transformations, the stereoisomers of oak lactone were obtained from a common intermediate.

4 Sensory studies on the oak lactones

Previous sensory work on the oak lactones has utilised mixtures of the four stereoisomers. This chapter describes the sensory studies undertaken on the synthetic enantiopure oak lactone samples. The odour detection thresholds are reported, along with the results from the duo-trio difference testings.

4.1 Assessment of optical purity and assignment of absolute stereochemistry for synthetic samples

Prior to using the synthetic oak lactone samples in any sensory studies, it was necessary to evaluate their diastereomeric and enantiomeric purity, so that the exact composition was known. The importance of optical purity was two-fold. Firstly, numerous sensory studies conducted in various media have shown that racemates of the *cis*-isomers **1a** and **1b** were substantially more potent than racemates of their *trans*-counterparts **2a** and **2b**.[47,53,54] To determine successfully the individual odour detection thresholds for the four stereoisomers of oak lactone (**1a**, **1b**, **2a** and **2b**), it was vital that the samples were of high diastereomeric purity. Secondly, there is limited literature on the individual thresholds for the four oak lactone stereoisomers; with one exception,[58] sensory studies have been conducted on racemic mixtures. In order to fill this void, it was crucial that the samples were of high enantiomeric purity. From the ^{1}H NMR spectra, it was evident that each synthetic sample was of high chemical purity and also of high diastereomeric purity. This was clear by the position of the proton at H_5: in *cis*-isomers **1a** and **1b** it resonated at 4.42 ppm, while in *trans*-isomers **2a** and **2b** it resonated at 4.00 ppm. There was also a visible difference in the ^{13}C NMR spectrum, with the position of the carbon at C_5 which resonated at 83.7 ppm for *cis*-isomers **1a** and **1b** but at 87.4 ppm for the *trans*-isomers **2a** and **2b**. The four stereoisomers of oak lactone are shown in Figure 4.1.

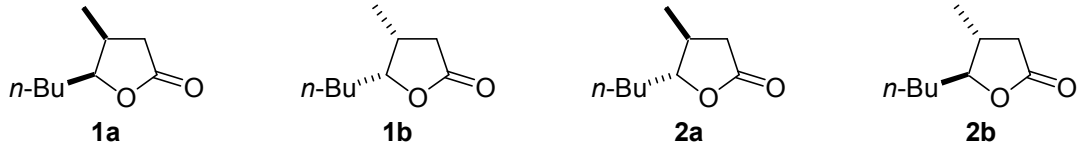


Figure 4.1 The four possible stereoisomers of oak lactone

Chiral gas chromatography (chiral GC) was then used to determine the absolute stereochemistry as well as to establish the enantiomeric purity of each synthetic sample. A commercially available mixture of the four oak lactone stereoisomers was resolved by chiral GC and each peak assigned their correct absolute stereochemistry by comparison with a natural oak extract and with samples of lower enantiomeric purity synthesised by Wilkinson *et al*.[58] (Table 4.1, Figure 4.2). Each of the synthetic samples was measured to have an optical purity of 99%, i.e. enantiomeric excess of 98%. This high level of purity corresponds to an impurity of only 1% from the opposite enantiomer. Thus, the synthesised oak lactone samples were deemed to be highly suitable for sensory work. The chromatograms for the individual stereoisomers are shown in Figure 4.3.

Table 4.1 Oak lactone stereoisomers on a chiral gas chromatography column and purity of the synthetic samples

peak	retention time	stereoisomer	synthetic samples	optical purity	enantio purity
1	27.390	(4*S*,5*R*)-**2a***	'top'	99%	98%
2	27.832	(4*R*,5*S*)-**2b**	'bottom'	99%	98%
3	29.019	(4*S*,5*S*)-**1a***	'top'	99%	98%
4	29.258	(4*R*,5*R*)-**1b**	'bottom'	99%	98%

*naturally occurring oak lactone stereoisomers, confirmed by comparison with an oak wood extract

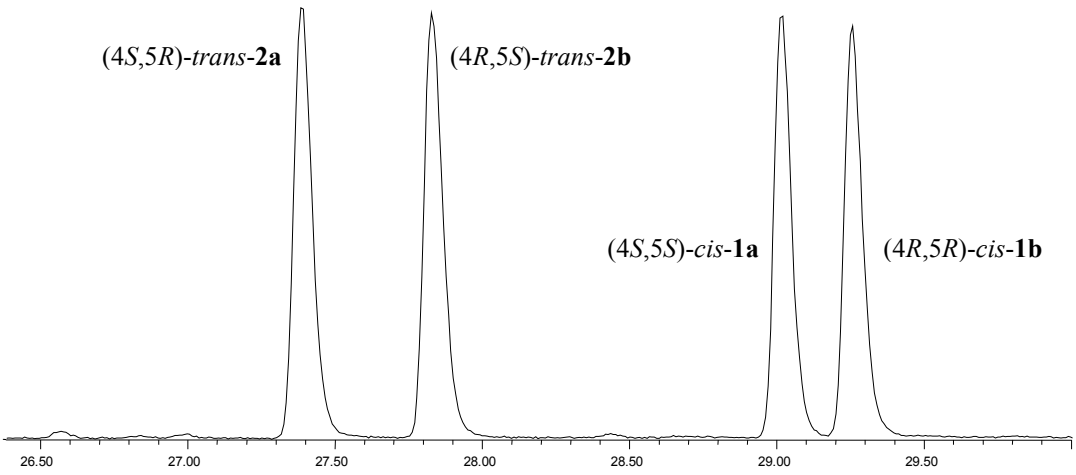


Figure 4.2 Chromatogram for the commercial sample showing the four stereoisomers of oak lactone on a chiral column

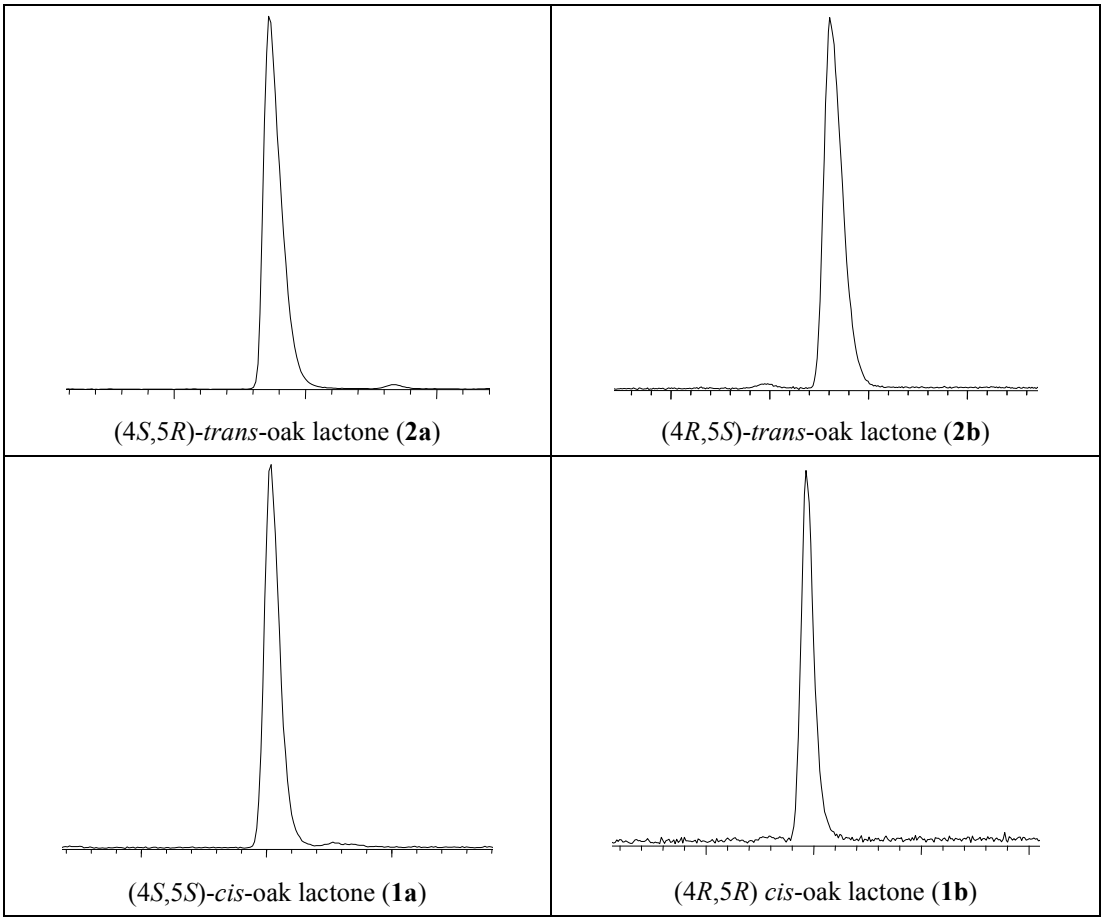


Figure 4.3 Chromatograms for synthetic oak lactone samples on a chiral column

Specific rotations were not recorded for any of the oak lactone stereoisomers. It was observed in a previous study[58] that the measured specific rotations were very close to those reported.[59] However, chiral GC analysis revealed the samples to be mixtures

containing small amounts of the other stereoisomers, with the 'correct' specific rotation being a composite. It is strongly felt that chiral GC analysis provides a superior, more reliable measure of both diastereomeric and enantiomeric purity.

4.2 Odour threshold definitions

An odour threshold is the limit of sensory ability. There are four different classifications for an odour threshold: the *absolute* or *detection threshold* is the lowest concentration required from a particular aroma that will produce a sensation; the *recognition threshold* is the level at which a certain aroma can be identified; the *difference threshold* is the required change in aroma compound concentration necessary to note a difference; the *terminal threshold* is the point at which there is no difference in aroma intensity above this level.[120]

This work focused on the determination of the odour detection thresholds for the oak lactones. The detection threshold of an aroma compound is generally considered to be the point at which 50% of the sample population can no longer identify a difference between a spiked sample and a control. One commonly used method for determining odour detection thresholds is that outlined by the American Society for Testing and Materials (ASTM).[120] The method E679 was chosen for threshold determination. This rapid method of threshold determination allows for the use of a large panel, unlike the method E1432 where individuals are retested up to five times at each concentration. Although the latter intermediate method ensures that the results are bias free, this can be avoided in the former method by retesting panel members who fall into the upper or lower limits of the range of concentrations under examination.

The alternative forced choice test was used, where six sets of three samples are presented to each panellist (a group of at least 24 judges) in ascending order of concentration, spaced by a factor of three. Of the three samples in each set, two are control samples and one is a spiked sample. This is also known as the 'triangle test'. From the results of the test, each panellist was assigned a best estimate threshold (BET), this being the geometric mean of the highest concentration missed and the

next higher concentration tested. The individual BETs were used to determine the odour detection threshold of the group for that particular compound in the designated medium. Odour detection thresholds, however determined, are dependent on the panel and medium amongst other factors.

4.3 Determination of odour detection thresholds in white and red wine

Odour thresholds were determined in a white and a red wine for each stereoisomer of oak lactone using the ASTM method E679. There were 28 judges who participated in every threshold test. This group is referred to as the 'common 28'. The total number of judges who were involved ranged from 33 to 43 people across all tests. Odour thresholds were determined for the common 28 and also for the total number of judges in each test (Table 4.2). The difference in odour thresholds between the common 28 and the total number of judges for each stereoisomer was minor. Full details of the odour threshold data are listed in Chapter 11 (Appendices).

Table 4.2 Group odour detection threshold values in white and red wine (μg/L)

judges	(4*S*,5*S*)-*cis*-1a*	(4*R*,5*R*)-*cis*-1b	(4*S*,5*R*)-*trans*-2a*	(4*R*,5*S*)-*trans*-2b
		white		
'common 28'	20	132	140	330
all	24 (N = 37)	132 (N = 43)	172 (N = 37)	305 (N = 36)
		red		
'common 28'	54	170	370	305
all	57 (N = 33)	175 (N = 33)	380 (N = 34)	285 (N = 34)

*naturally occurring oak lactone stereoisomers; number in parentheses indicates total number of judges

The distributions of the individual BET values for each stereoisomer of oak lactone are collected over Figure 4.4 to Figure 4.7. The histograms for the common 28 and for all judges are presented and show broadly a typical bell curve, or skewed bell curve shape. All data are presented for comparison.

The histograms for (4*S*,5*S*)-*cis*-oak lactone (**1a**) are presented in Figure 4.4. The

odour detection threshold in white wine was calculated to be 20 μg/L for the common 28 and 24 μg/L for all the judges (N = 37). These results are in excellent agreement with the threshold value determined earlier of 23 μg/L using a sample of lower isomeric purity (90%),[58] despite a different panel and different wines being used in the two studies. In red wine, the odour detection thresholds were 54 μg/L and 57 μg/L, for the common 28 and for all the judges (N = 33), respectively. The results in red wine are not only in good agreement with each other, but also with the earlier determined value of 46 μg/L.[58] The distribution of individual BETs, however, differed somewhat between the two studies. In the earlier case, a greater proportion of individual BETs was at the lower or higher ends of the concentration range and this indicates the variation that can occur between panels and between individuals in panels.

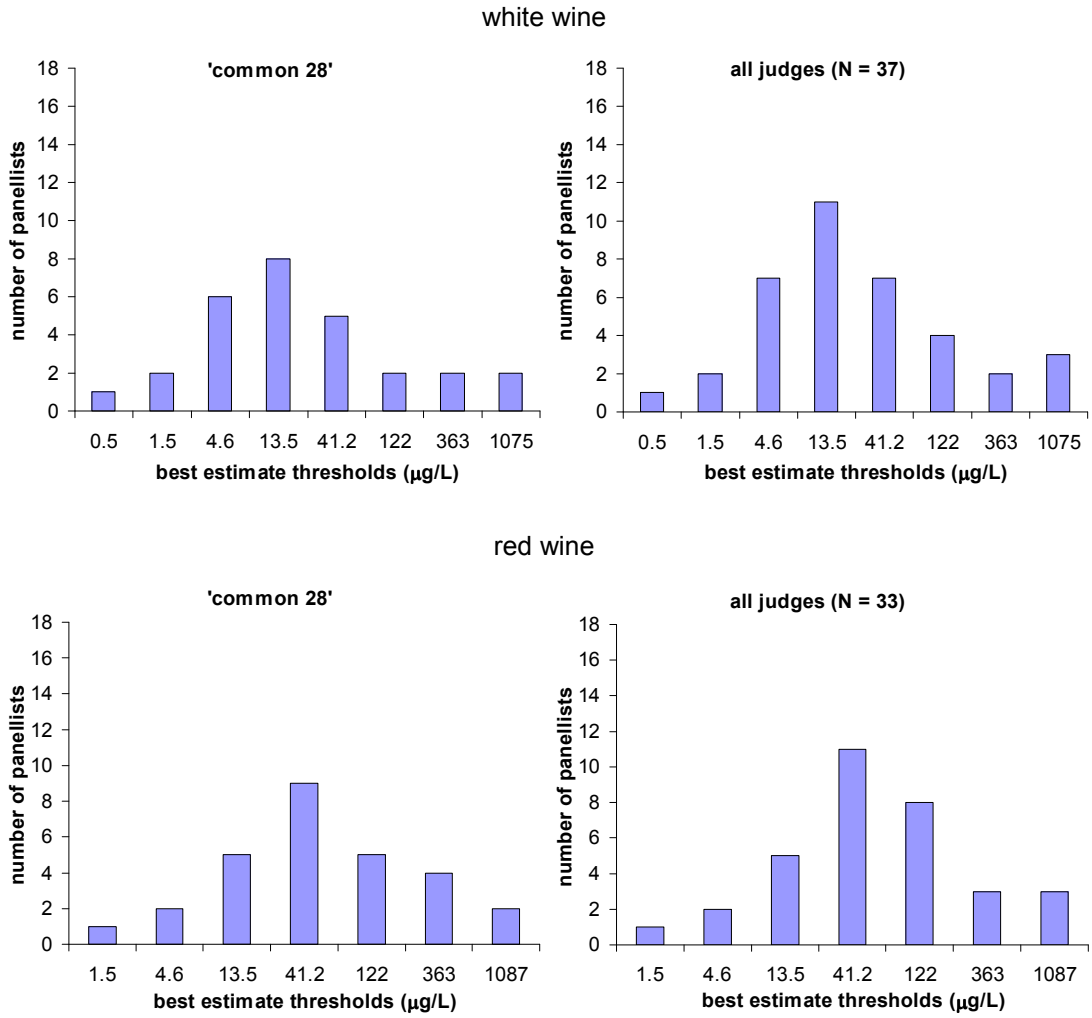


Figure 4.4 Histograms showing the distribution of best estimate thresholds for (4*S*,5*S*)-*cis*-oak lactone (1a) in white and red wine

The histograms for (4*R*,5*R*)-*cis*-oak lactone (**1b**) are presented in Figure 4.5. The odour detection threshold in white wine was calculated to be 132 μg/L by both the common 28 and by all the judges (N = 43). The reported threshold was 82 μg/L for a sample is of low isomeric purity (84%).[58] It is possible that the contamination, namely from the 4% of the more potent (4*S*,5*S*)-stereoisomer, lowered the odour threshold. The histograms follow the typical bell curve distribution. The odour detection threshold in red wine was calculated to be 170 μg/L for the common 28 and 175 μg/L for all the judges (N = 33). The distribution of individual BETs is slightly skewed, with the spread at the higher end of the concentration range.

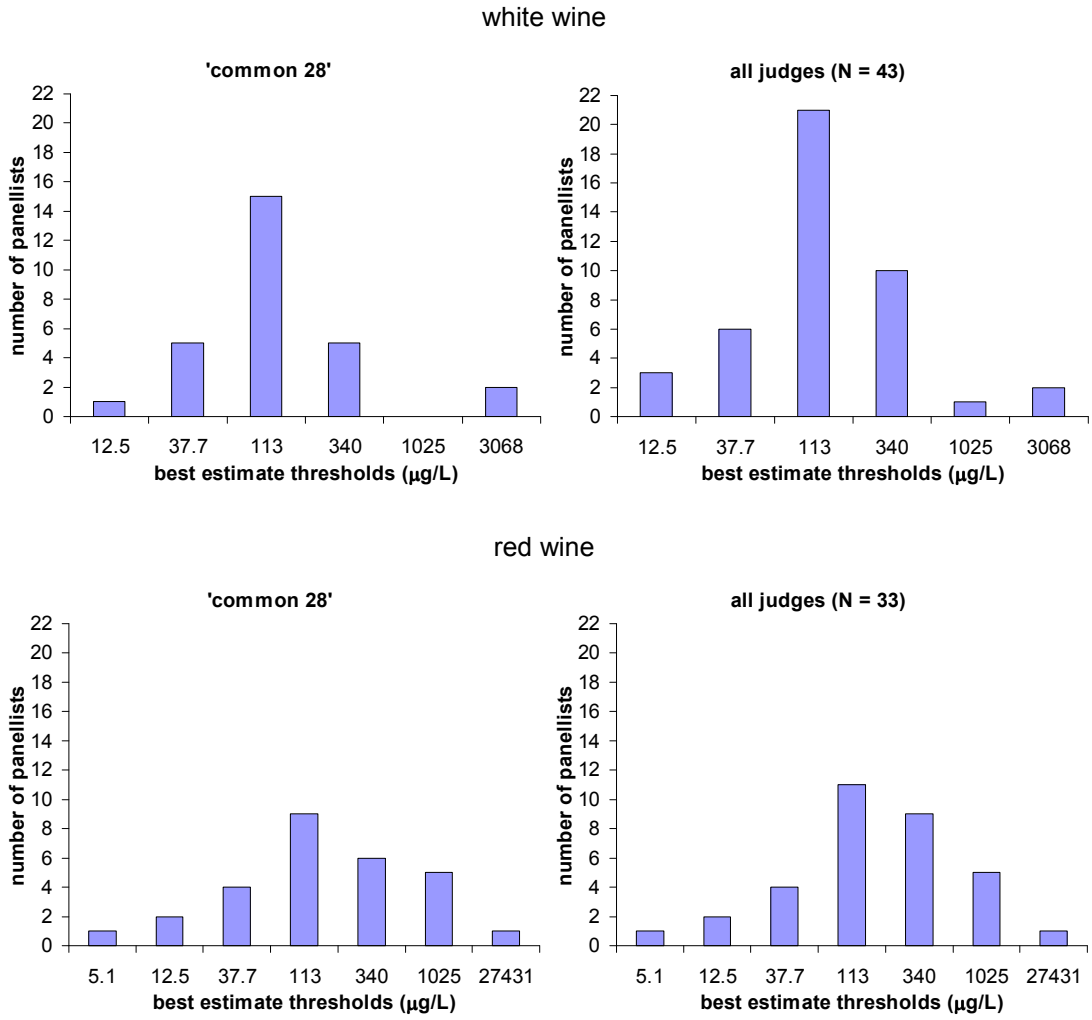


Figure 4.5 Histograms showing the distribution of best estimate thresholds for (4*R*,5*R*)-*cis*-oak lactone (1b) in white and red wine

This sensory study reports, for the first time, the odour thresholds for (4*S*,5*R*)- and (4*R*,5*S*)-*trans*-oak lactone (**2a** and **2b**) in white and red wine. (4*S*,5*R*)-*trans*-Oak

lactone (**2a**) was found to have a threshold of 140 μg/L and 172 μg/L in white wine for the common 28 and all the judges (N = 37), respectively. The histograms, in this case, reveal a wide variation in sensitivity to this isomer in white wine. This does not appear to be the case in red wine, with the data displaying a typically bell curve shape and threshold values of 370 μg/L and 380 μg/L for the common 28 and all the judges (N = 34), respectively (Figure 4.6).

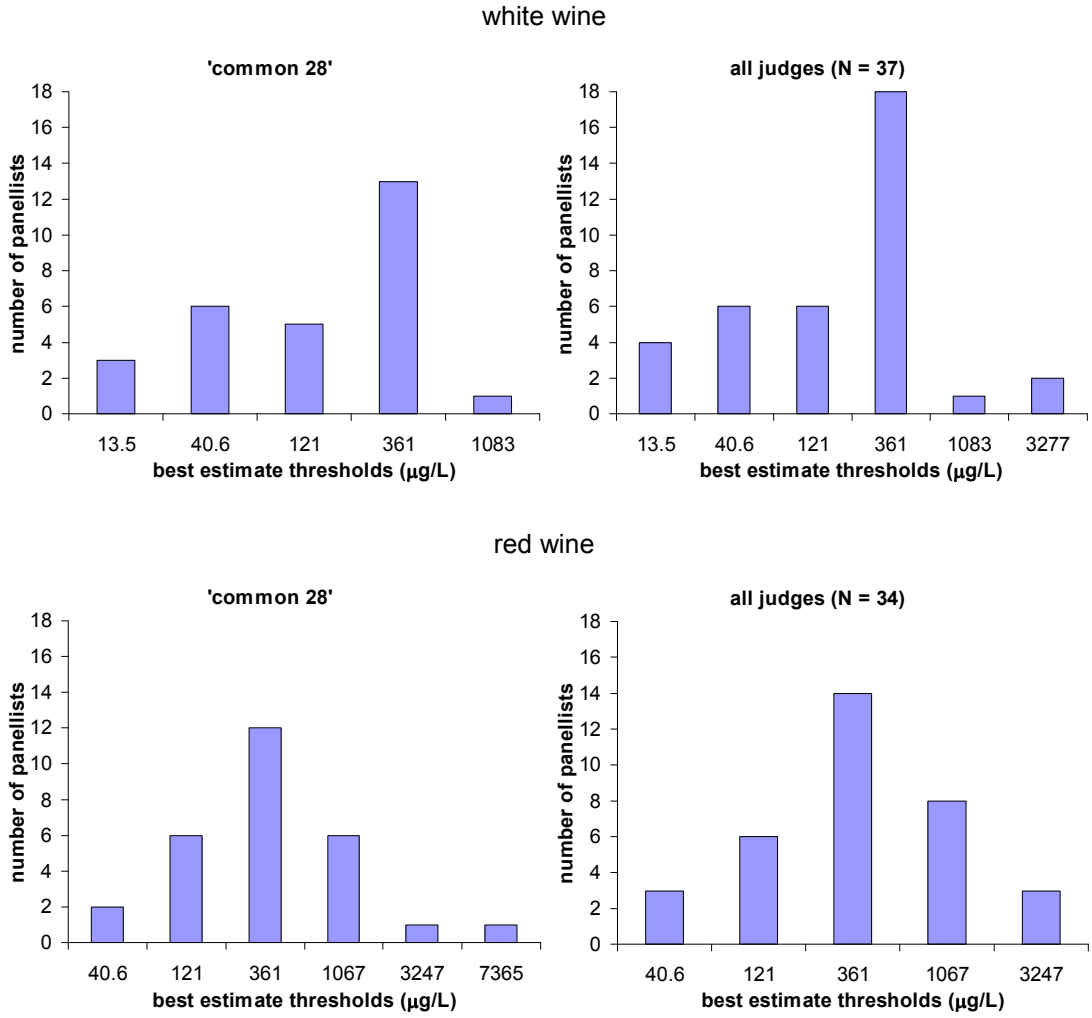


Figure 4.6 Histograms showing the distribution of best estimate thresholds for (4*S*,5*R*)-*trans*-oak lactone (2a) in white and red wine

For (4*R*,5*S*)-*trans*-oak lactone (**2b**), the odour threshold in white wine was 330 μg/L (common 28), or 305 μg/L (36 judges). The histograms show roughly a bell curve distribution (Figure 4.7). In red wine, the threshold was 305 μg/L (common 28), or 285 μg/L (34 judges). This is the only stereoisomer to have a slightly lower odour threshold in red wine than in white. In this medium, the histograms do not display a

bell curve, but rather two apparent sub-populations (Figure 4.7).

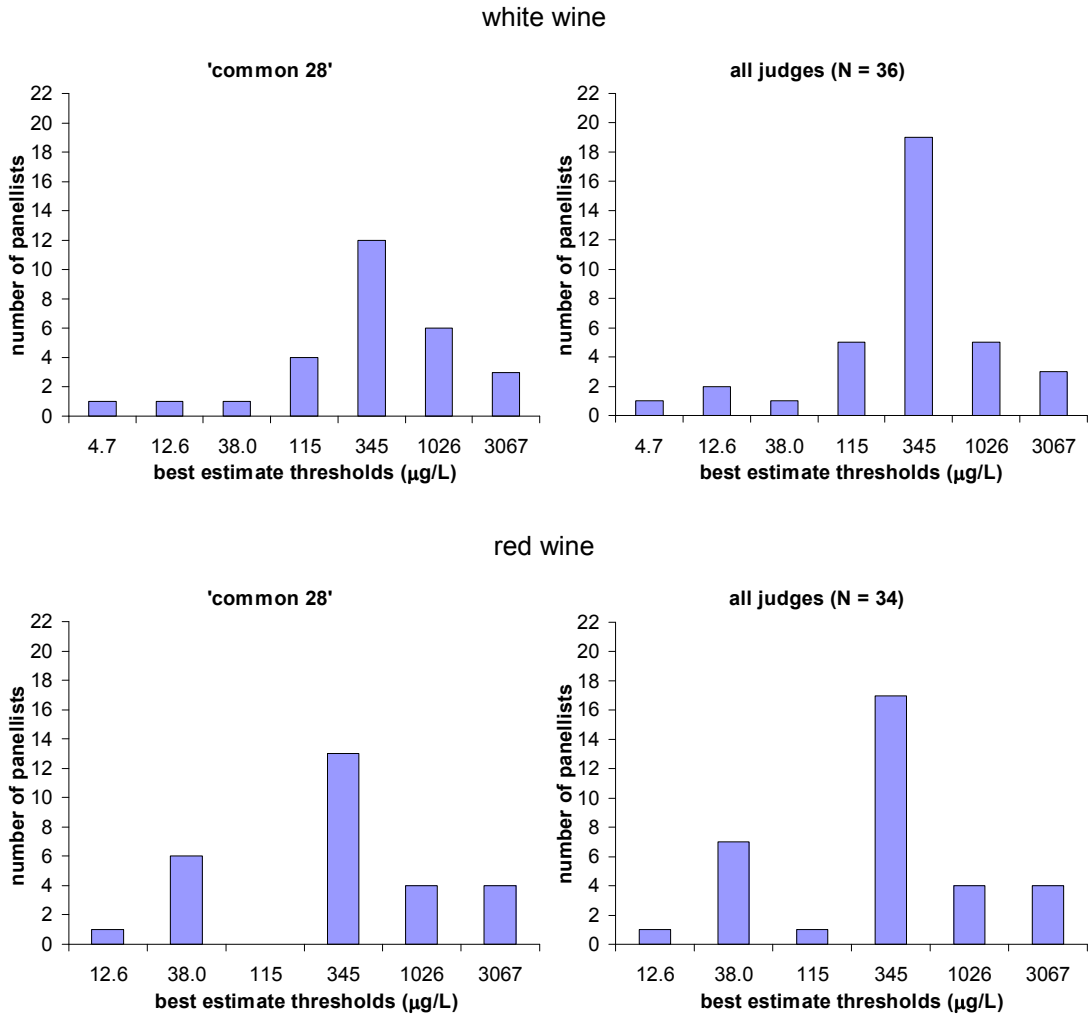


Figure 4.7 Histograms showing the distribution of best estimate thresholds for (4*R*,5*S*)-*trans*-oak lactone (2b) in white and red wine

4.4 Duo-trio difference tests

Compounds with similar odour detection thresholds can be distinguished from each other if their odour qualities are different. In order to determine if there is a perceivable difference between two compounds, it is necessary to assess any differences separately from previous odour detection threshold studies. A duo-trio difference test can be used to measure this difference.[121] A set of three samples is presented to each panellist, with one marked as the reference and the other two containing the two compounds of interest, one of which matches the reference. The

panellist must identify the sample that corresponds to the reference.

4.4.1 The pairs of enantiomers of oak lactone

A duo-trio difference test was conducted, in order to determine whether or not there is a significant difference between the enantiomers when compared directly against each other. This was undertaken in a white and a red wine, at a different concentration for each test, depending on the medium and the isomers tested. Each panel consisted of the same 36 judges. The results are presented in Table 4.3.

Table 4.3 Duo-trio difference test results for the pairs of enantiomers of oak lactone in white and red wine (N = 36)

stereoisomers tested	medium	correct responses	significance[a]
(4*S*,5*S*)-**1a** vs. (4*R*,5*R*)-**1b**	white[b]	28	p = 0.001
	red[c]	28	p = 0.001
(4*S*,5*R*)-**2a** vs. (4*R*,5*S*)-**2b**	white[d]	22	p = 0.121
	red[e]	23	p = 0.066

[a] $p > 0.05$ not significant; [b] concentration of 150 μg/L; [c] concentration of 300 μg/L; [d] concentration of 250 μg/L; [e] concentration of 500 μg/L

For the *cis*-oak lactones (**1a** and **1b**), there was a significant difference (at the 99% confidence level; $p < 0.01$) between the two isomers in both white and red wine with 28 correct responses. It is possible that this is a direct reflection of the difference in odour threshold data. In contrast, for the *trans*-oak lactones (**2a** and **2b**) there was no significant difference ($p > 0.05$) between the two isomers in white or red wine. This could be due to the smaller differences in odour threshold values. This does not indicate that there is no difference between the *trans*-enantiomers but, rather, that there is no more than a minority of panellists who can identify a difference.

It is important, in the case of the *trans*-oak lactone difference tests, to understand the use of statistics for the determination of a significantly different result. Generally, the convention used is when the probability of the result having been obtained by chance is less than, or equal to, one in twenty, i.e. where $p < 0.05$ the result is considered to be significant. In the *trans*-difference test in red wine, it would have required only one of the 13 judges who answered incorrectly to have identified the

correct sample for the probability to drop from 7% to less than 5% ($p = 0.03$).

4.4.2 Oak lactone comparison between American and French oak

The final duo-trio test undertaken involved an equal mixture of *cis*-oak lactone (**1a**) and *trans*-oak lactone (**2a**) compared with only the *cis*-stereoisomer **1a**. This was to investigate whether the presence of a significant amount of *trans*-oak lactone (**2a**) in wine could alter the sensory impact of the *cis*-isomer **1a**. French oak barrels impart an approximately equal ratio of *cis*-**1a** and *trans*-**2a** isomers into wine, while American oak barrels impart a much higher ratio of *cis*-oak lactone (**1a**) into the wine, with generally only 10-15% of the *trans*-isomer **2a**. This difference testing utilised only the nature identical oak lactone enantiomers of each oak lactone diastereomer. The results are presented in Table 4.4.

In white and red wine, the reference sample was *cis*-oak lactone (**1a**) at concentrations of 150 μg/L and 300 μg/L, respectively. This was tested against an identical sample with the same amount of the natural *cis*-isomer **1a** plus an equal amount of the natural *trans*-oak lactone (**2a**) (total oak lactone concentration was 300 μg/L in the white and 600 μg/L in the red). In neither medium was the difference found to be statistically significant ($p > 0.05$). There were, however, some panellists who were able to identify a difference. These results lie in a similar situation to those of the *trans*-enantiomeric difference testings, where only one more correct answer, in white wine, would have changed the probability of the answer to a significant result ($p < 0.05$).

Table 4.4 Duo-trio difference test results for mixtures of (4*S*,5*S*)-*cis*-1a and (4*S*,5*R*)-*trans*-2a stereoisomers in white and red wine (N = 36)

stereoisomers tested	medium	correct responses	significance[a]
(4*S*,5*S*)-**1a** vs. (4*S*,5*S*)-**1a** + (4*S*,5*R*)-**2a**	white[b]	23	p = 0.066
	red[c]	22	p = 0.121

[a] $p > 0.05$ not significant; [b] *cis*-isomer **1a** at 150 μg/L, each isomer **1a** and **2a** at 150 μg/L in the mixture; [c] *cis*-isomer **1a** at 300 μg/L, each isomer **1a** and **2a** at 300 μg/L in the mixture

4.5 Conclusions

The nature identical forms of *cis*- and *trans*-oak lactone (**1a** and **2a**) have been prepared, along with their enantiomeric counterparts **1b** and **2b** (Chapter 3). The synthetic methodology featured 1,2-dioxine chemistry, with the addition of a chiral malonate to enable separation of the furanone diastereomers by column chromatography. Selective decarboxylation of the ester cleaved 3-carboxy-γ-lactones could be directed to give the *cis*- or *trans*-products. The final oak lactone stereoisomers were obtained in high enantiomeric purity (99%), suitable for use as standards in sensory studies. This work presents the first reported procedure the for preparation of the individual stereoisomers of oak lactone from a common precursor.

The sensory data obtained for the oak lactones confirm the conclusions from earlier studies that *cis*-isomer **1a** is likely to be more important to wine odour than *trans*-isomer **2a**. The odour detection threshold for *cis*-isomer **1a** was approximately one-seventh that of its *trans*-counterpart **2a**, in both white and red wine. The odour detection threshold for the nature-identical *cis*-oak lactone (**1a**) is much lower than that which is stated in literature reports for racemic *cis*-oak lactone in white wine, which reveals the sensory impact of this stereoisomer to be even greater than previously thought. The results of the difference test between *cis*-enantiomers **1a** and **1b** at the 99% confidence level, combined with its potent threshold, bring into question the validity of using racemic *cis*-oak lactone for sensory studies. There was a trend towards a difference between the two *trans*-isomers, but this was not statistically significant according to the commonly used criteria ($p < 0.05$).

The difference tests with the mixtures of *cis*-oak lactone (**1a**) and *trans*-oak lactone (**2a**) showed that adding the latter to the former had little apparent impact on the aroma, although, as discussed above, there might have been a minority of judges who could detect a difference. Nevertheless, the sensory differences observed between American and French oak-aged wines are more likely to be related to the influence of the absolute concentration of *cis*-isomer **1a** or to that of other oak wood volatiles, rather than the relative amount of the *trans*-isomer **2a**.

5 Experimental – Part A

5.1 General procedures

Solvents and reagents

Chemicals were purchased from Sigma-Aldrich and either used as supplied, or dried and distilled using standard procedures.[122] Anhydrous Et_2O and THF were distilled over sodium and benzophenone under N_2 and used immediately. Anhydrous CH_2Cl_2 was distilled over calcium hydride under N_2 and used immediately. Anhydrous C_6H_6 was distilled over phosphorus pentoxide under N_2 and stored over molecular sieves (4 Å). All other solvents used for reactions, extractions or purifications were distilled prior to use. Reactions employing moisture sensitive reagents were handled under N_2 and performed in flame dried glassware.

Chromatography

Analytical thin layer chromatography (TLC) was performed with Merck silica gel 60 F_{254} (20 x 20 cm) aluminium sheets and visualised under a 254 nm UV lamp where applicable or treated with either anisaldehyde dip (*p*-anisaldehyde, 9.2 mL; H_2SO_4, 12.5 mL; AcOH, 3.75 mL; EtOH, 338 mL) or potassium permanganate dip ($KMnO_4$, 3 g, K_2CO_3, 20 g, 5% NaOH, 5 mL; H_2O, 300 mL) and developed upon heating with a heat gun. The retention factor ($\mathbf{R_f}$) reported is rounded to the nearest 0.01. Column chromatography was performed with Merck silica gel 60 (particle size: 0.040-0.063 mm). When purifying compounds of an acid sensitive nature, buffered silica gel was used, and prepared by spinning silica gel (100 g) with pH 7 phosphate buffer (10 mL) on a rotary evaporator at atmospheric pressure overnight.

Nuclear Magnetic Resonance spectroscopy

Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Varian Gemini 200 or 300 spectrometer operating at frequencies of 200 MHz or 300 MHz for proton and 50 MHz or 75.5 MHz for carbon nuclei, respectively. Spectra were acquired in $CDCl_3$ (^{1}H NMR referenced at 7.26 ppm; ^{13}C NMR referenced at 77.0 ppm) or C_6D_6 (^{1}H NMR referenced at 7.16 ppm; ^{13}C NMR referenced at 128.0 ppm) at ambient temperature, as indicated. ^{1}H NMR data are recorded as follows: chemical shift (δ)

in ppm, relative integration, multiplicity (s = singlet; d = doublet; t = triplet; q = quartet; qn = quintet; sx = sextet; sp = septet; m = multiplet; b = broad; app. = apparent), coupling constant(s) (*J*) in Hz and interpretation of signal. In the case of ^{13}C NMR spectra with observed splitting, the data are recorded as follows: chemical shift (δ) in ppm, multiplicity, coupling constant(s) (*J*) in Hz and interpretation of signal. Where further resolution was required for NMR signals, ^{1}H and ^{13}C spectra were recorded on a Varian Unity Inova 600 spectrometer operating at frequencies of 600 MHz and 150 MHz, respectively, as indicated. The assignments of the signals observed in the ^{1}H and ^{13}C NMR spectra were assisted by conducting homonuclear (^{1}H-^{1}H) correlation spectroscopy (COSY), nuclear Overhauser effect (nOe) spectroscopy (NOESY), heteronuclear (^{1}H-^{13}C) correlation spectroscopy (HETCOR or HMQC) and long range heteronuclear (^{1}H-^{13}C) correlation spectroscopy (HMBC) experiments.

Melting points

Melting points were recorded on a Reichert hot-stage apparatus and are uncorrected.

Optical rotations

All specific rotations were measured with a PolAAr 21 polarimeter and referenced to the D sodium line (589 nm) at 20 °C in a cell with a 1 dm path length. The concentration (*c*) is specified in g/100 mL and the solvent used is reported.

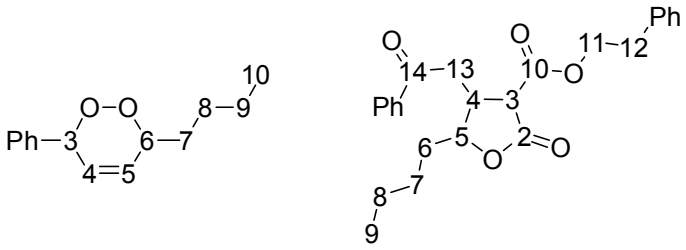
Mass spectrometry

Low resolution gas chromatography-mass spectrometry (GC-MS) was performed on an Agilent 6890A gas chromatogram fitted to an Agilent 5973N mass spectrometer. Mass spectral data are presented as mass to charge ratio (*m/z*) and intensity of peak relative to the base peak. High resolution mass spectrometry was performed on a Bruker BioApex 47e FTMS fitted with an Analytica electrospray source (ESI). Mass spectral data are presented as molecular formula, molecular ion ($[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$ or $[M+K]^+$), calculated mass and accurate mass.

Numbering system

For use in ^{1}H and ^{13}C NMR data, compounds are reported following their systematic numbering. The numbering for the 1,2-dioxine and the trisubstituted γ-lactone are

shown below.

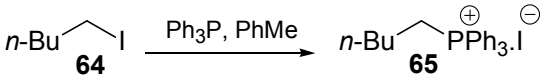


Note

Room temperature (rt) varied between 20-25 °C.

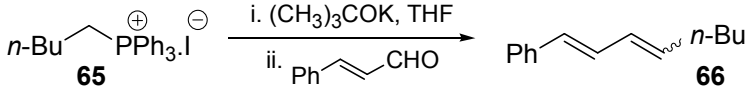
5.2 Experimental procedures for Chapter 3

n-Pentyltriphenylphosphonium iodide (65)



1-Iodopentane (**64**) (49.83 g, 246.57 mmol) was added slowly to a stirred solution of Ph_3P (58.37 g, 220.03 mmol) in xylene (400 mL) and the mixture was heated under reflux for 24 hrs. The cooled salt product was collected by vacuum filtration. Recrystallisation from *iso*-propanol followed by vacuum filtration afforded salt **65** (94.75 g, 94%) as fine white crystals. **mpt** 172-173 °C (lit.[123] **mpt** 170 °C); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.90-7.65 (15H, m, H_{Ar}), 3.78-3.58 (2H, m, H_1), 1.67-1.56 (4H, m, $H_{2,3}$), 1.31 (2H, sx, *J* = 7.2, H_4), 0.82 (3H, t, *J* = 7.2, H_5); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 135.1 (d, *J* = 3.4, C_{Ar}), 133.7 (d, *J* = 9.7, C_{Ar}), 130.5 (d, *J* = 12.6, C_{Ar}), 118.2 (d, *J* = 85.9, C_{Ar}), 32.4 (d, *J* = 15.5, $C_{2,3}$), 23.0 (d, *J* = 50.4, C_1), 22.3 (d, *J* = 4.6, $C_{2,3}$), 22.2 (C_4), 13.6 (C_5).

trans, *cis*- and *trans*, *trans*-1-Phenyl-1,3-octadiene (66)



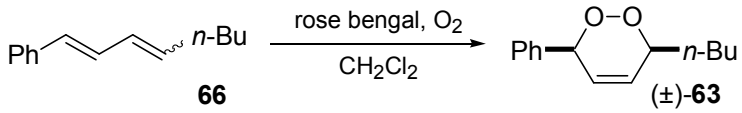
To a stirred suspension of salt **65** (90.04 g, 195.59 mmol) in anhydrous THF (1 L)

under an atmosphere of N_2 at 0 °C was added $(CH_3)_3COK$ (24.14 g, 204.35 mmol) slowly and the mixture stirred for 30 mins. Cinnamaldehyde (25.15 g, 184.59 mmol) was added dropwise, at 0 °C, to the stirred orange solution and the resulting cream solution was stirred for 40 hrs at rt. The filtrate was collected by vacuum filtration and concentrated under reduced pressure. Trituration with hexanes (150 mL x 4) followed by rotary evaporation gave purified diene **66** (33.57 g, 82%) as a pale yellow oil upon distillation (95 °C / 50 mm Hg), as a 4:1 mixture of *trans*, *cis*- and *trans*, *trans*-isomers determined by ^{1}H NMR.

Major *trans*, *cis*-**66**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.61-7.01 (5H, m, H_{Ar}), 7.07 (1H, ddd, *J* = 15.5, 10.9, 1.1, H_2), 6.53 (1H, d, *J* = 15.5, H_1), 6.16 (1H, t, *J* = 10.9, H_3), 5.54 (1H, dt, *J* = 7.8, 10.9, H_4), 2.30 (2H, app. q, *J* = 7.3, H_5), 1.49-1.30 (4H, m, $H_{6,7}$), 0.97-0.87 (3H, m, H_8); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 137.6 (C_{Ar}), 133.2 (H_4), 131.9 (C_1), 128.7 (C_3), 128.5, 127.2, 126.3 (C_{Ar}), 124.4 (C_2), 31.8 ($C_{6,7}$), 27.7 (C_5), 22.3 ($C_{6,7}$), 13.9 (C_8).

Minor *trans*, *trans*-**66**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.66-7.02 (5H, m, H_{Ar}), 6.76 (1H, dd, *J* = 10.4, 15.4, H_2), 6.44 (1H, d, *J* = 15.4, H_1), 6.21 (1H, dd, *J* = 10.4, 15.4, H_3), 5.84 (1H, dt, *J* = 7.3, 15.4, H_4), 2.16 (2H, app. q, *J* = 6.9, H_5), 1.51-1.25 (4H, m, $H_{6,7}$), 0.99-0.87 (3H, m, H_8); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 137.6 (C_{Ar}), 135.8 (C_4), 130.5 (C_3), 129.9 (C_1), 129.4 (C_2), 128.5, 127.0, 126.1 (C_{Ar}), 32.5 (C_5), 31.4, 22.2 ($C_{6,7}$), 13.9 (C_8).

(±)-3-Phenyl-6-*n*-butyl-3,6-dihydro-1,2-dioxine (63)

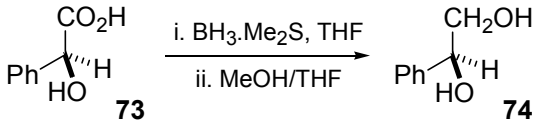


66 (±)-**63**

1-Phenyl-1,3-octadiene (**66**) (5.55 g, 29.79 mmol) and rose bengal (0.11 g) were dissolved in CH_2Cl_2 (100 mL). The mixture was cooled to 0 °C and a stream of O_2 was passed through the solution. The mixture was irradiated with two tungsten halogen lamps (500 W), approximately 5-10 cm from the reactor vessel, for 10 hrs. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexanes; 50% (v/v) CH_2Cl_2/hexanes, $\mathbf{R_f}$ = 0.24) to yield dioxine **63** (4.60 g, 79%) as a pale yellow oil. **HRMS** calculated for $C_{14}H_{18}O_2H^+$

$[M+H]^+$: 219.1385; found: 219.1377; calculated for $C_{14}H_{18}O_2NH_4^+$ $[M+NH_4]^+$: 236.1651; found: 236.1634, calculated for $C_{14}H_{18}O_2Na^+$ $[M+Na]^+$: 241.1205; found: 241.1199; **^{1}H NMR** (200 MHz, $CDCl_3$) δ = 7.44-7.32 (5H, m, H_{Ar}), 6.18-6.02 (2H, m, $H_{4,5}$), 5.52 (1H, m, H_3), 4.56 (1H, m, H_6), 1.90-1.24 (6H, m, $H_{7,8,9}$) 0.91 (3H, t, *J* = 7.0, H_{10}); **^{13}C NMR** (50 MHz, $CDCl_3$) δ = 137.6, 128.8 (C_{Ar}), 128.7 ($C_{4,5}$), 128.5, 128.4 (C_{Ar}), 126.2 ($C_{4,5}$), 80.1 (C_3), 78.4 (C_6), 32.7, 27.6, 22.5 ($C_{7,8,9}$), 13.9 (C_{10}).

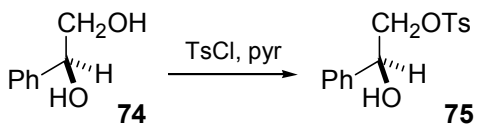
(*S*)-1-Phenyl-1,2-ethanediol (74)



73 **74**

$BH_3.Me_2S$ (12 mL, 126.50 mmol) was added dropwise to a solution of (*S*)-mandelic acid (**73**) (15.03 g, 97.79 mmol) in anhydrous THF (125 mL) under an atmosphere of N_2 at 0 °C and the mixture was stirred for 1 hr. $BH_3.Me_2S$ (3 mL, 31.59 mmol) was added dropwise at rt and stirring continued for 1.5 hrs followed by a further quantity of $BH_3.Me_2S$ (6 mL, 63.31 mmol) with additional stirring for 20 hrs. MeOH (25 mL) in anhydrous THF (25 mL) was added slowly to the stirred solution at 0 °C, followed by an additional amount of MeOH (175 mL) and the mixture was stirred at 45 °C for 4 hrs. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (40% (v/v) hexanes/EtOAc, $\mathbf{R_f}$ = 0.17) to yield diol **74** (12.17 g, 90%) as a white solid. **mpt** 62-64 °C (lit.[105] **mpt** 66-67 °C); $\mathbf{[\alpha]_D}$ = +70.1 (*c* = 0.99, $CHCl_3$) (lit.[106] $\mathbf{[\alpha]_D^{22}}$ = +40.8 (*c* = 4, EtOH)); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.43-7.27 (5H, m, H_{Ar}), 4.81 (1H, dd, *J* = 3.6, 8.1, H_1), 3.78 (1H, dd, *J* = 3.5, 11.4, H_2), 3.67 (1H, dd, *J* = 8.1, 11.4, H_2), 2.57 (2H, bs, H_{OH}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 140.5, 128.5, 128.0, 126.0 (C_{Ar}), 74.7 (C_1), 68.0 (C_2).

(*S*)-1-Phenyl-1,2-ethanediol 2-tosylate (75)

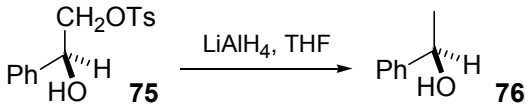


74 **75**

p-Toluene sulfonyl chloride (55.83 g, 286.98 mmol) was added portionwise to a stirred solution of diol **74** (38.26 g, 276.91 mmol) in pyr (350 mL) at 0 °C. The

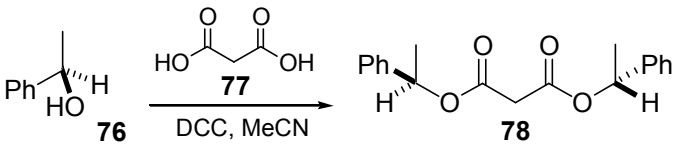
mixture was stirred at rt for 20 hrs. Further *p*-toluene sulfonyl chloride (5.13 g, 26.93 mmol) was added and the mixture stirred for 2 hrs. Ice was added and stirring continued for 1 hr. Et_2O (200 mL) was added, the pyr layer removed and the organic layer washed with saturated $CuSO_4$ (100 mL x 10), H_2O (75 mL x 2) and brine (75 mL x 2), then dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to yield tosylate **75** (73.91 g, 91%) as a pale yellow crystalline solid. **mpt** 67-69 °C (lit.[107] **mpt** 75 °C); $[\alpha]_D$ = +49.6 (*c* = 1.17, $CHCl_3$) (lit.[105] $[\alpha]_D$ = +33.2 (*c* = 2, EtOH)); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.82-7.73 (2H, m, H_{Ar}), 7.46-7.27 (8H, m, H_{Ar}), 4.98 (1H, dd, *J* = 3.3, 8.6, H_1), 4.15 (1H, dd, *J* = 3.3, 10.4, H_2), 4.04 (1H, dd, *J* = 8.6, 10.4, H_2), 2.45 (3H, s, H_{Me}), 2.16 (1H, bs, H_{OH}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 145.1, 138.2, 132.6, 129.9, 129.6, 128.7, 128.5, 127.9, 126.2 (C_{Ar}), 74.3 (C_2), 71.9 (C_1), 21.6 (C_{Me}).

(*R*)-1-Phenylethanol (76)



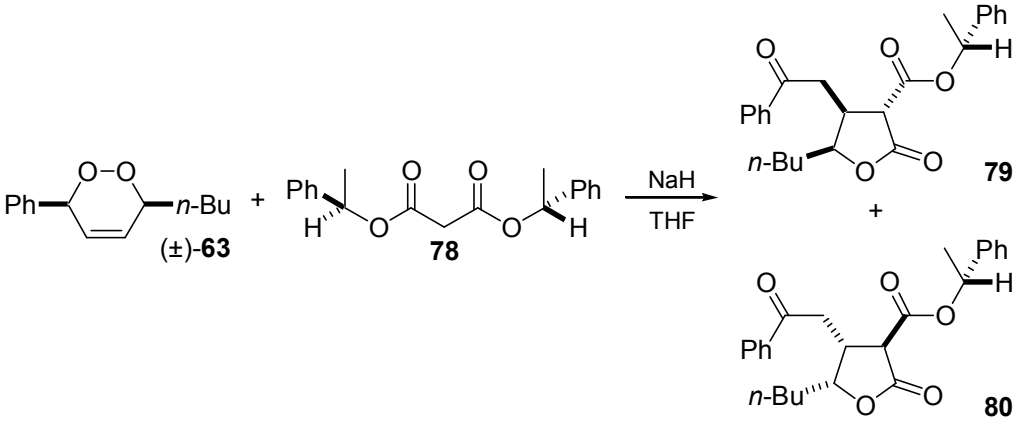
To a solution of $LiAlH_4$ (11.20 g, 280.37 mmol) in anhydrous THF (250 mL) under N_2 at 0 °C was added tosylate **75** (73.86 g, 252.63 mmol) in anhydrous THF (250 mL) slowly over 0.5 hr and the mixture stirred at rt for 4 hrs. The mixture was cooled, acetone (5 mL) was added dropwise followed by saturated Na_2SO_4 solution (100 mL). The mixture was filtered, dried (Na_2SO_4), filtered again and the solvent was removed under reduced pressure. The residue was triturated with hexanes (50 mL x 6), filtered and concentrated under reduced pressure to yield alcohol **76** (22.68 g, 73%) as a colourless oil, which was used without further purification. $[\alpha]_D$ = +38.7 (*c* = 1.18, EtOH) (lit.[108] $[\alpha]_D$ = +44.16 (neat)); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.49-7.21 (5H, m, H_{Ar}), 4.90 (1H, q, *J* = 6.5, H_1), 2.00 (1H, bs, H_{OH}), 1.50 (3H, d, *J* = 6.5, H_2); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 145.7, 128.2, 127.1, 125.3 (C_{Ar}), 69.9 (C_1), 24.9 (C_2).

***bis*-[(*R*)-(+)-1-Phenylethyl]malonate (78)**



A solution of malonic acid (**77**) (9.31 g, 88.57 mmol) in MeCN (400 mL) was treated with (*R*)-1-phenylethanol (**76**) (21.61 g, 176.88 mmol) and DCC (37.50 g, 179.93 mmol) and the mixture was stirred at rt for 5 hrs. An additional quantity of DCC (5.00 g, 23.99 mmol) was added and the mixture stirred at rt for 24 hrs. The mixture was filtered, the solvent was removed by rotary evaporation and the residue taken up in EtOAc (250 mL). This was washed with H_2O (100 mL x 2) and brine (100 mL x 2), then dried (Na_2SO_4), filtered and the solvent removed under reduced pressure. Purification by column chromatography (30% (v/v) EtOAc/hexanes, $\mathbf{R_f}$ = 0.27) afforded malonate **78** (23.16 g, 84%) as a colourless oil. $\mathbf{[\alpha]_D}$ = +98.6 (*c* = 1.46, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.36-7.26 (5H, m, H_{Ar}), 5.94 (2H, q, *J* = 6.6, H_1), 3.41 (2H, s, H_3), 1.55 (6H, d, *J* = 6.6, H_{Me}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 165.7(C_2), 140.9, 128.5, 128.0, 126.1 (C_{Ar}), 73.6 (C_1), 42.1 (C_3), 21.9 (C_{Me}).

(3*R*,4*S*,5*S*)- and (3*S*,4*R*,5*R*)-(*R*)-1-Phenylethyl 2-oxo-4-(2-oxo-2-phenylethyl)-5-*n*-butyltetrahydrofuran-3-carboxylate (79 and 80)



Malonate **78** (21.44 g, 68.64 mmol) was added to a suspension of NaH (2.99 g, 74.75 mmol) in anhydrous THF (250 mL) at 0 °C, under N_2, and the mixture was stirred at rt for 4 hrs. Dioxine **63** (15.01 g, 68.76 mmol) was added and the solution stirred for 24 hrs. The reaction was neutralised with saturated NH_4Cl and extracted with CH_2Cl_2 (200 mL x 3). The organic layers were combined, dried (Na_2SO_4), filtered

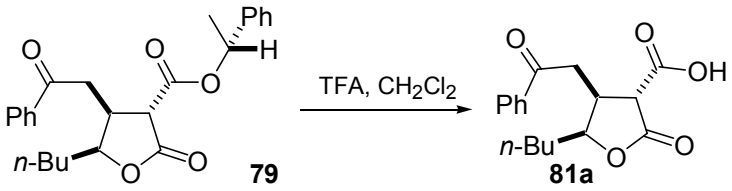
and the solvent removed *in vacuo*. Purification by column chromatography (CH_2Cl_2, $\mathbf{R_f}$ = 0.20) afforded diastereomers **79** and **80** (12.68 g, 54%) as a pale yellow oil. **HRMS** calculated for $C_{25}H_{28}O_5H^+$ $[M+H]^+$: 409.2016; found: 409.2000; calculated for $C_{25}H_{28}O_5NH_4^+$ $[M+NH_4]^+$: 426.2281; found: 426.2269; calculated for $C_{25}H_{28}O_5Na^+$ $[M+Na]^+$: 431.1835; found: 431.1822.

Further purification by column chromatography (30% (v/v) Et_2O/hexanes) separated the diastereomers, both as pale yellow oils.

79: $\mathbf{R_f}$ = 0.26; $\mathbf{[\alpha]_D}$ = -50.3 (*c* = 1.00, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.96-7.81 (2H, m, H_{Ar}), 7.64-7.56 (1H, m, H_{Ar}), 7.52-7.24 (7H, m, H_{Ar}), 5.98 (1H, q, *J* = 6.6, H_{11}), 4.95 (1H, q, *J* = 6.8, H_5), 3.55 (1H, m, H_4), 3.40 (1H, d, *J* = 9.6, H_3), 3.16 (2H, d, *J* = 7.8, H_{13}), 1.62 (3H, d, *J* = 6.6, H_{12}), 1.54-1.21 (6H, m, $H_{6,7,8}$), 0.87 (3H, t, *J* = 7.2, H_9); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 196.9 (C_{14}), 170.8, 166.7 ($C_{2,10}$), 140.8, 135.9, 133.7, 128.7, 128.5, 128.1, 127.9, 126.1 (C_{Ar}), 81.4 (C_5), 74.5 (C_{11}), 51.6 (C_3), 38.5 (C_4), 37.3 (C_{13}), 29.9, 27.8, 22.2, 22.0 ($C_{6,7,8,12}$), 13.8 (C_9).

80: $\mathbf{R_f}$ = 0.21; $\mathbf{[\alpha]_D}$ = -124.5 (*c* = 1.02, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.98-7.89 (2H, m, H_{Ar}), 7.70-7.22 (8H, m, H_{Ar}), 5.96 (1H, q, *J* = 6.6, H_{11}), 4.91 (1H, q, *J* = 6.7, H_5), 3.56 (1H, m, H_4), 3.43 (1H, d, *J* = 8.9, H_3), 3.18 (2H, d, *J* = 7.3, H_{13}), 1.60 (3H, d, *J* = 6.6, H_{12}), 1.57-1.22 (6H, m, $H_{6,7,8}$), 0.87 (3H, t, *J* = 7.2, H_9); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 197.0 (C_{14}), 170.7, 166.4 ($C_{2,10}$), 140.8, 135.9, 133.7, 128.8, 128.55, 128.49, 127.9, 125.8 (C_{Ar}), 81.4 (C_5), 74.4 (C_{11}), 52.0 (C_3), 38.4 (C_4), 37.1 (C_{13}), 29.9, 27.8, 22.3, 22.2 ($C_{6,7,8,12}$), 13.8 (C_9).

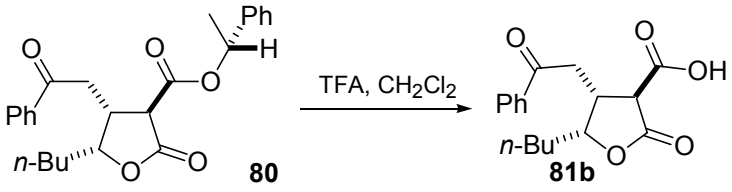
(3*R*,4*S*,5*S*)-2-Oxo-4-(2-oxo-2-phenylethyl)-5-*n*-butyltetrahydrofuran-3-carboxylic acid (81a)



TFA (3.5 mL) was added slowly to a solution of **79** (663 mg, 1.62 mmol) in CH_2Cl_2 (10 mL) and the mixture was stirred at rt for 2 hrs. The reaction was concentrated

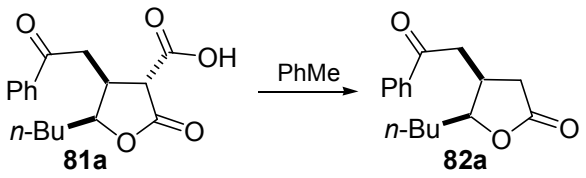
under reduced pressure, the residue taken up in Et_2O (50 mL) and washed with saturated $NaHCO_3$ solution (20 mL x 3). The aqueous layers were combined, acidified with 10% HCl (pH 2) and extracted with CH_2Cl_2 (25 mL x 3). The organic layers were combined, dried (Na_2SO_4), filtered and the solvent removed *in vacuo* to yield acid **81a** (452 mg, 92%) as a colourless oil. $[\alpha]_D$ = -142.0 (*c* = 1.23, $CHCl_3$); **HRMS** calculated for $C_{17}H_{20}O_5H^+$ $[M+H]^+$: 305.1389; found: 305.1384; calculated for $C_{17}H_{20}O_5NH_4^+$ $[M+NH_4]^+$: 322.1654; found: 322.1647; calculated for $C_{17}H_{20}O_5Na^+$ $[M+Na]^+$: 327.1208; found: 327.1200; **^{1}H NMR** (300 MHz, C_6D_6) δ = 8.33 (1H, bs, OH), 7.81-7.62 (2H, m, H_{Ar}), 7.26-6.92 (3H, m, H_{Ar}), 4.77 (1H, q, *J* = 7.1, H_5), 3.38 (1H, m, H_4), 3.19 (1H, d, *J* = 10.2, H_3), 2.99 (1H, dd, *J* = 5.2, 18.2, $H_{13a/b}$), 2.62 (1H, dd, *J* = 8.9, 18.2, $H_{13a/b}$), 1.40-0.90 (6H, m, $H_{6,7,8}$), 0.75 (3H, t, *J* = 6.8, H_9); **^{13}C NMR** (75.5 MHz, C_6D_6) δ = 197.3 (C_{14}), 172.2, 170.8 ($C_{2,10}$), 136.4, 133.4, 128.8 (2C, C_{Ar}), 82.0 (C_5), 50.9 (C_3), 38.4 (C_4), 37.7 (C_{13}), 29.8, 28.1, 22.4 ($C_{6,7,8}$), 13.9 (C_9).

(3*S*,4*R*,5*R*)-2-Oxo-4-(2-oxo-2-phenylethyl)-5-*n*-butyltetrahydrofuran-3-carboxylic acid (81b)



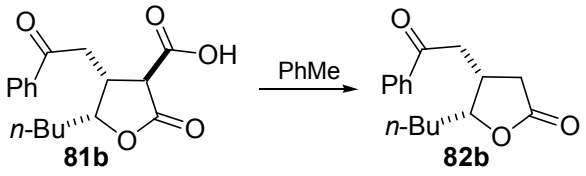
Acid **81b** was synthesised, as per **81a**, from **80** (632 mg, 1.55 mmol) with TFA (3.2 mL) in CH_2Cl_2 (9 mL) to yield the title compound **81b** (441 mg, 93%) as a colourless oil. $[\alpha]_D$ = +141.0 (*c* = 1.17, $CHCl_3$); **HRMS** calculated for $C_{17}H_{20}O_5H^+$ $[M+H]^+$: 305.1389; found: 305.1384; calculated for $C_{17}H_{20}O_5NH_4^+$ $[M+NH_4]^+$: 322.1654; found: 322.1650; calculated for $C_{17}H_{20}O_5Na^+$ $[M+Na]^+$: 327.1208; found: 327.1206; **^{1}H NMR** (300 MHz, C_6D_6) δ = 7.74-7.66 (2H, m, H_{Ar}), 7.22-6.98 (3H, m, H_{Ar}), 4.71 (1H, ddd, *J* = 3.1, 7.5, 10.7, H_5), 3.12 (1H, m, H_4), 3.05 (1H, dd, *J* = 5.3, 18.0, $H_{13a/b}$), 2.79 (1H, d, *J* = 10.1, H_3), 2.45 (1H, dd, *J* = 9.3, 18.0, $H_{13a/b}$), 1.38-0.82 (6H, m, $H_{6,7,8}$), 0.73 (3H, t, *J* = 7.0, H_9); **^{13}C NMR** (75.5 MHz, C_6D_6) δ =197.5 (C_{14}), 172.2, 171.3 ($C_{2,10}$), 136.5, 133.5, 128.8, 128.4 (C_{Ar}), 82.2 (C_5), 51.3 (C_3), 38.7 (C_4), 37.8 (C_{13}), 30.0, 28.3, 22.6 ($C_{6,7,8}$), 14.1 (C_9).

(4*S*,5*S*)-*cis*-5-*n*-Butyl-4-(benzoylmethyl)-4,5-dihydro-2(3H)-furanone (82a)



Acid **81a** (298 mg, 0.98 mmol) was heated under reflux in PhMe (15 mL) for 20 hrs. The mixture was cooled, the solvent removed under reduced pressure and the residue purified by column chromatography (30% (v/v) EtOAc/hexanes, $\mathbf{R_f}$ = 0.22) to yield *cis*-decarboxylated **82a** (195 mg, 76%) as a white crystalline solid. $\mathbf{[\alpha]_D}$ = -34.6 (*c* = 1.07, $CHCl_3$); HRMS calculated for $C_{16}H_{20}O_3H^+$ $[M+H]^+$: 261.1490; found: 261.1483; calculated for $C_{16}H_{20}O_3NH_4^+$ $[M+NH_4]^+$: 278.1756; found: 278.1748; calculated for $C_{16}H_{20}O_3Na^+$ $[M+Na]^+$: 283.1310; found: 283.1299; **^{1}H NMR** (600 MHz, C_6D_6) δ = 7.68-7.65 (2H, m, H_{Ar}), 7.15-7.12 (1H, m, H_{Ar}), 7.06-7.02 (2H, m, H_{Ar}), 4.16 (1H, ddd, *J* = 10.0, 6.5, 3.5, H_5), 2.66 (1H, app. sx, *J* = 7.0, H_4), 2.45 (1H, dd, *J* = 6.8, 17.8, $H_{13a/b}$), 2.27 (1H, dd, *J* = 8.2, 17.2, $H_{3a(cis)}$), 2.26 (1H, dd, *J* = 7.6, 17.8, $H_{13a/b}$), 1.89 (1H, dd, *J* = 6.2, 17.2, $H_{3b(trans)}$), 1.38 (1H, m, $H_{6,7,8}$), 1.23-1.08 (4H, m, $H_{6,7,8}$), 1.02 (1H, m, $H_{6,7,8}$), 0.79 (3H, t, *J* = 7.2, H_9); **^{13}C NMR** (150 MHz, C_6D_6) δ = 196.9 (C_{14}), 174.9 (C_2), 136.9, 133.2, 128.7 (2C, C_{Ar}), 81.7 (C_5), 37.3 (C_{13}), 35.2 (C_3), 34.2 (C_4), 30.1, 28.5, 22.6 ($C_{6,7,8}$), 14.0 (C_9).

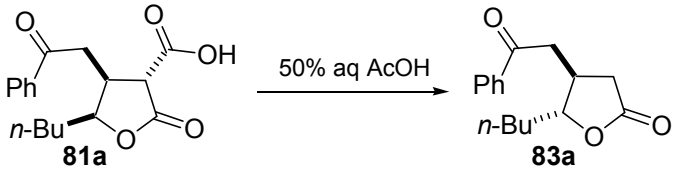
(4*R*,5*R*)-*cis*-5-*n*-Butyl-4-(benzoylmethyl)-4,5-dihydro-2(3H)-furanone (82b)



cis-Decarboxylated **82b** was synthesised, as per **82a**, from **81b** (146 mg, 0.48 mmol) and PhMe (10 mL) to yield the title compound **82b** (93 mg, 74%) as a white crystalline solid. $\mathbf{[\alpha]_D}$ = +36.2 (*c* = 1.05, $CHCl_3$); **HRMS** calculated for $C_{16}H_{20}O_3H^+$ $[M+H]^+$: 261.1490; found: 261.1484; calculated for $C_{16}H_{20}O_3NH_4^+$ $[M+NH_4]^+$: 278.1756; found: 278.1750; calculated for $C_{16}H_{20}O_3Na^+$ $[M+Na]^+$: 283.1310; found: 283.1304; **^{1}H NMR** (300 MHz, C_6D_6) δ = 7.72-7.63 (2H, m, H_{Ar}), 7.15-7.00 (3H, m, H_{Ar}), 4.17 (1H, ddd, *J* = 3.5, 6.3, 9.7, H_5), 2.66 (1H, app. sx, *J* = 7.0, H_4), 2.45 (1H, dd, *J* = 6.7, 17.9, H_{13}), 2.27 (1H, dd, *J* = 8.1, 17.3, $H_{3a(cis)}$) 2.25 (1H, dd, *J* = 7.6, 17.9,

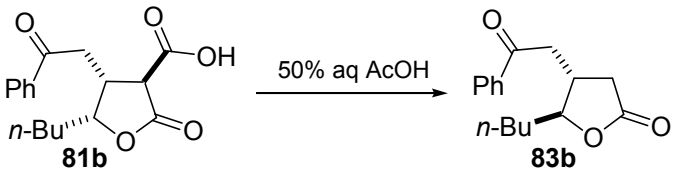
H_{13}) 1.88 (1H, dd, *J* = 6.3, 17.3, $H_{3b(trans)}$), 1.48-0.96 (6H, m, $H_{6,7,8}$), 0.79 (3H, t, *J* = 6.9, H_9); **^{13}C NMR** (75.5 MHz, C_6D_6) δ = 197.1 (C_{14}), 175.2 (C_2), 136.9, 133.2, 128.7, 128.2 (C_{Ar}), 82.0 (C_5), 37.4 (C_{13}), 35.1 (C_3), 34.2 (C_4), 30.1, 28.5, 22.6 ($C_{6,7,8}$), 14.0 (C_9).

(4*S*,5*R*)-*trans*-5-*n*-Butyl-4-(benzoylmethyl)-4,5-dihydro-2(3H)-furanone (83a)



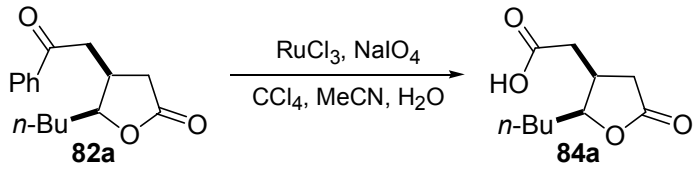
Acid **81a** (317 mg, 1.04 mmol) was heated under reflux in 50% aqueous AcOH (15 mL) for 20 hrs. The mixture was cooled, neutralised to pH 7 (10% NaOH) and extracted with CH_2Cl_2 (20 mL x 3). The organic layers were combined, dried (Na_2SO_4), filtered and the solvent removed *in vacuo*. Purification by column chromatography (30% EtOAc/hexanes, $\mathbf{R_f}$ = 0.20) afforded the decarboxylated product **83a** (267 mg, 99%), a pale yellow solid, as a 3:1 mixture of *trans*- and *cis*-isomers determined by ^{1}H NMR. **HRMS** calculated for $C_{16}H_{20}O_3H^+$ $[M+H]^+$: 261.1490; found: 261.1487; calculated for $C_{16}H_{20}O_3NH_4^+$ $[M+NH_4]^+$: 278.1756; found: 278.1750; calculated for $C_{16}H_{20}O_3Na^+$ $[M+Na]^+$: 283.1310; found: 283.1304. Recrystallisation from CCl_4/hexanes afforded a small portion of *trans*-decarboxylated **83a** (15 mg, 6%) as fine white crystals. $\mathbf{[\alpha]_D}$ = +48.0 (*c* = 0.75, $CHCl_3$); **^{1}H NMR** (600 MHz, C_6D_6) δ = 7.66-7.64 (2H, m, H_{Ar}), 7.14 (1H, m, H_{Ar}), 7.07-7.03 (2H, m, H_{Ar}), 3.71 (1H, ddd, *J* = 8.1, 5.3, 4.0, H_5), 2.52 (1H, dd, *J* = 8.8, 17.7, $H_{3a(cis)}$), 2.41 (1H, dd, *J* = 6.6, 17.6, $H_{13a/b}$), 2.28 (1H, m, H_4), 2.14 (1H, dd, *J* = 7.2, 17.6, $H_{13a/b}$), 1.71 (1H, dd, *J* = 6.5, 17.7, $H_{3b(trans)}$), 1.43-1.06 (6H, m, $H_{6,7,8}$), 0.84 (3H, t, *J* = 7.2, H_9); **^{13}C NMR** (150 MHz, C_6D_6) δ = 196.9 (C_{14}), 174.9 (C_2), 136.9, 133.2, 128.6, 128.3 (C_{Ar}), 84.7 (C_5), 41.9 (C_{13}), 35.8 (C_4), 34.7 (C_3), 34.6, 28.0, 22.8, ($C_{6,7,8}$), 14.0 (C_9).

(4*R*,5*S*)-*trans*-5-*n*-Butyl-4-(benzoylmethyl)-4,5-dihydro-2(3H)-furanone (83b)



trans-Decarboxylated **83b** was synthesised, as per **83a**, from **81b** (288 mg, 0.95 mmol) and 50% aqueous AcOH (10 mL) to afford the title compound **83b** (215 mg, 87%), a white crystalline solid, as a 3:1 mixture of *trans*- and *cis*-isomers determined by ^{1}H NMR. **HRMS** calculated for $C_{16}H_{20}O_3H^+$ $[M+H]^+$: 261.1490; found: 261.1490; calculated for $C_{16}H_{20}O_3NH_4^+$ $[M+NH_4]^+$: 278.1756; found: 278.1760; calculated for $C_{16}H_{20}O_3Na^+$ $[M+Na]^+$: 283.1310; found: 283.1306. **83b**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 8.02-7.89 (2H, m, H_{Ar}), 7.64-7.43 (3H, m, H_{Ar}), 4.23 (1H, ddd, *J* = 4.4, 5.8, 8.1, H_5), 3.26 (1H, dd, *J* = 5.9, 17.6, $H_{3a(cis)}$), 3.08 (1H, dd, *J* = 7.7, 17.7, $H_{13a/b}$), 2.93 (1H, dd, *J* = 8.8, 17.6, $H_{13a/b}$), 2.23 (1H, dd, *J* = 6.7, 17.6, $H_{3b(trans)}$), 1.83-1.28 (6H, m, $H_{6,7,8}$), 0.91 (3H, t, *J* = 7.2, H_9); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 197.3 (C_{14}), 176.0 (C_2), 136.1, 133.3, 128.5, 127.7 (C_{Ar}), 85.1 (C_5), 41.5 (C_{13}), 35.9 (C_4), 34.7 (C_3), 33.9, 27.4, 22.1 ($C_{6,7,8}$), 13.6 (C_9).

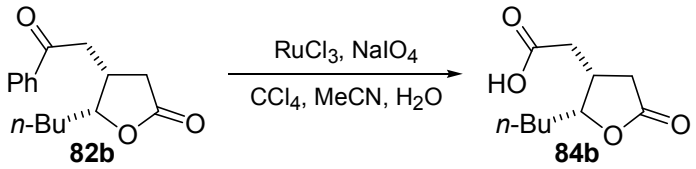
(4*S*,5*S*)-*cis*-5-*n*-Butyl-4-(carboxymethyl)-4,5-dihydro-2(3H)-furanone (84a)



cis-Decarboxylated **82a** (118 mg, 0.45 mmol) was stirred in a mixture of CCl_4 (2.2 mL), MeCN (2.2 mL) and pH 7 buffer solution (3.3 mL) with $NaIO_4$ (1.47 g, 6.74 mmol) and $RuCl_3$ (121 mg, 0.58 mmol) at rt for 20 hrs. The mixture was diluted with H_2O (20 mL) and extracted with CH_2Cl_2 (15 mL x 3). The organic layers were combined and extracted with saturated $NaHCO_3$ solution (20 mL x 3). The combined aqueous layers were acidified to pH 1 (10% HCl) and extracted with CH_2Cl_2 (25 mL x 3). The organic layers were combined, dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to yield acid **84a** (65 mg, 83%) as a colourless oil. $[\alpha]_D$ = -42.6 (*c* = 1.48, $CHCl_3$); **HRMS** calculated for $C_{10}H_{16}O_4H^+$ $[M+H]^+$: 201.1127; found: 201.1120; calculated for $C_{10}H_{16}O_4NH_4^+$ $[M+NH_4]^+$:

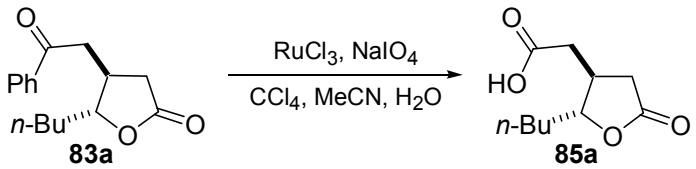
218.1392; found: 218.1388; calculated for $C_{10}H_{16}O_4Na^+$ $[M+Na]^+$: 223.0946; found: 223.0940; **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 8.51 (1H, bs, H_{OH}), 4.59 (1H, m, H_5), 2.98 (1H, m, H_4), 2.74 (1H, dd, *J* = 8.1, 17.5, $H_{3b(trans)}$), 2.58 (1H, dd, *J* = 6.1, 16.7, $H_{13a/b}$), 2.42 (1H, dd, *J* = 9.4, 16.7, $H_{13a/b}$), 2.37 (1H, dd, *J* = 6.4, 17.5, $H_{3a(cis)}$), 1.70-1.22 (6H, m, $H_{6,7,8}$), 0.91 (3H, t, *J* = 7.2, H_9); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 176.8, 176.0 ($C_{2,10}$), 82.3 (C_5), 34.79 (C_4), 34.76 (C_3), 33.1 (C_{13}), 29.7, 27.9, 22.3 ($C_{6,7,8}$), 13.8 (C_9).

(4*R*,5*R*)-*cis*-5-*n*-Butyl-4-(carboxymethyl)-4,5-dihydro-2(3H)-furanone (84b)



Acid **84b** was synthesised, as per **84a**, from **82b** (93 mg, 0.36 mmol) in a mixture of CCl_4 (1.8 mL), MeCN (1.8 mL) and pH 7 buffer solution (2.8 mL) with $NaIO_4$ (1.32 g, 6.05 mmol) and $RuCl_3$ (127 mg, 0.61 mmol) to yield the title compound **84b** (55 mg, 77%) as a colourless oil. **$[\alpha]_D$** = +39.5 (*c* = 1.60, $CHCl_3$); **HRMS** calculated for $C_{10}H_{16}O_4H^+$ $[M+H]^+$: 201.1127; found: 201.1116; calculated for $C_{10}H_{16}O_4NH_4^+$ $[M+NH_4]^+$: 218.1392; found: 218.1381; calculated for $C_{10}H_{16}O_4Na^+$ $[M+Na]^+$: 223.0946; found: 223.0939; **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 10.28 (1H, bs, OH), 4.56 (1H, ddd, *J* = 3.5, 6.5, 9.9, H_5), 2.96 (1H, m, H_4), 2.72 (1H, dd, *J* = 8.1, 17.4, $H_{3b(trans)}$), 2.56 (1H, dd, *J* = 6.2, 16.8, $H_{13a/b}$), 2.40 (1H, dd, *J* = 9.1, 16.8, $H_{13a/b}$), 2.35 (1H, dd, *J* = 6.5, 17.4, $H_{3a(cis)}$), 1.66-1.24 (6H, m, $H_{6,7,8}$), 0.89 (3H, t, *J* = 7.1, H_9); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 176.8, 176.2 ($C_{2,10}$), 82.4 (C_5), 34.70 (C_4), 34.67 (C_3), 33.1 (C_{13}), 29.6, 27.8, 22.3 ($C_{6,7,8}$), 13.7 (C_9).

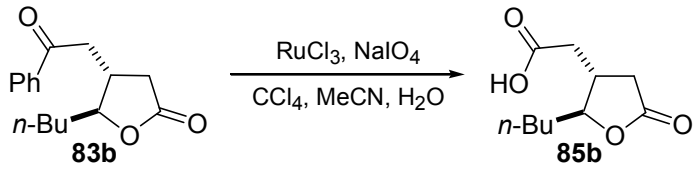
(4*S*,5*R*)-*trans*-5-*n*-Butyl-4-(carboxymethyl)-4,5-dihydro-2(3H)-furanone (85a)



Acid **85a** was synthesised, as per **84a**, from **83a** (14.3 mg, 0.054 mmol) in a mixture of CCl_4 (0.3 mL), MeCN (0.3 mL) and pH 7 buffer solution (2.5 mL) with $NaIO_4$

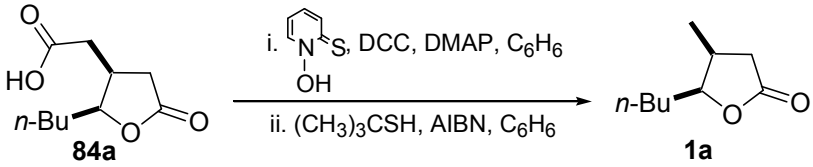
(207.2 mg, 0.950 mmol) and $RuCl_3$ (21.6 mg, 0.104 mmol) to yield the title compound **85a** (7.4 mg, 69%) as a colourless oil. $[\alpha]_D$ = +62.7 (*c* = 0.34, $CHCl_3$); **HRMS** calculated for $C_{10}H_{16}O_4H^+$ $[M+H]^+$: 201.1127; found: 201.1124; calculated for $C_{10}H_{16}O_4NH_4^+$ $[M+NH_4]^+$: 218.1392; found: 218.1386; calculated for $C_{10}H_{16}O_4Na^+$ $[M+Na]^+$: 223.0946; found: 223.0937; **^{1}H NMR** (600 MHz, C_6D_6) δ = 3.53 (1H, q, *J* = 6.1, H_5), 2.36 (1H, dd, *J* = 8.5, 17.6, $H_{3,13}$), 1.89 (1H, m, H_4), 1.87 (1H, dd, *J* = 6.2, 17.3, $H_{3,13}$), 1.74 (1H, dd, *J* = 7.9, 17.6, $H_{3,13}$), 1.66 (1H, dd, *J* = 7.9, 17.3, $H_{3,13}$), 1.20-1.07 (6H, m, $H_{6,7,8}$), 0.81 (3H, t, *J* = 7.2, H_9); **^{13}C NMR** (150 MHz, C_6D_6) δ = 176.4, 174.8 ($C_{2,10}$), 84.0 (C_5), 36.7, 36.5 ($C_{3,4,13}$), 34.6 ($C_{3,13}$), 34.1, 27.8, 22.6 ($C_{6,7,8}$), 14.0 (C_9).

(4*R*,5*S*)-*trans*-5-*n*-Butyl-4-(carboxymethyl)-4,5-dihydro-2(3H)-furanone (85b)



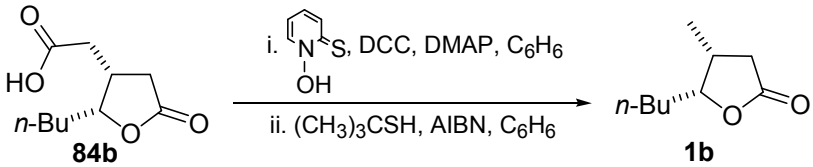
Acid **85b** was synthesised, as per **84a**, from **83b** (207 mg, 0.80 mmol; as a 3:1 mixture with **82b**) in a mixture of CCl_4 (4.1 mL), MeCN (4.1 mL) and pH 7 buffer solution (6.2 mL) with $NaIO_4$ (2.90 g, 13.29 mmol) and $RuCl_3$ (255 mg, 1.23 mmol) to yield the title compound **85b** (123 mg, 77%), a colourless oil, as a 3:1 mixture of *trans*- and *cis*-isomers as determined by ^{1}H NMR. **HRMS** calculated for $C_{10}H_{16}O_4H^+$ $[M+H]^+$: 201.1127; found: 201.1116; calculated for $C_{10}H_{16}O_4NH_4^+$ $[M+NH_4]^+$: 218.1392; found: 218.1382; calculated for $C_{10}H_{16}O_4Na^+$ $[M+Na]^+$: 223.0946; found: 223.0939. **85b**: **^{1}H NMR** (300 MHz, C_6D_6) δ = 11.15 (1H, bs, OH), 3.62 (1H, q, *J* = 5.8, H_5), 2.47 (1H, dd, *J* = 7.8 17.1, $H_{3,13}$), 2.02 (1H, m, H_4), 1.99 (1H, dd, *J* = 5.4, 18.0, $H_{3,13}$), 1.87 (1H, dd, *J* = 7.7, 17.1, $H_{3,13}$), 1.80 (1H dd, *J* = 9.9, 18.0, $H_{3,13}$), 1.39-1.02 (6H, m, $H_{6,7,8}$), 0.83 (3H, t *J* = 6.9, H_9); **^{13}C NMR** (75.5 MHz, C_6D_6) δ = 176.4, 176.3 ($C_{2,10}$), 84.8 (C_5), 36.9, 36.7 ($C_{3,4,13}$), 34.9 ($C_{3,13}$), 34.1, 27.9, 22.7 ($C_{6,7,8}$), 14.0 (C_9).

(4*S*,5*S*)-*cis*-5-*n*-Butyl-4-methyl-4,5-dihydro-2(3H)-furanone (1a)



To a solution of acid **84a** (64.7 mg, 0.32 mmol), *N*-hydroxy-2-thiopyridone (89.1 mg, 0.70 mmol) and DMAP (54.1 mg, 0.44 mmol) in anhydrous C_6H_6 (2.5 mL) was added DCC (108.1 mg, 0.52 mmol) in anhydrous C_6H_6 (1.5 mL). The mixture was stirred in the dark under N_2 at rt for 2.5 hrs, then added to a boiling solution of $(CH_3)_3CSH$ (150 μL, 1.80 mmol) and AIBN (catalytic) in anhydrous C_6H_6 (4 mL), under N_2, whilst being irradiated with a 300 W tungsten lamp for 4.5 hrs. The reaction was cooled and concentrated under reduced pressure. Purification by column chromatography (40% (v/v) Et_2O/hexanes, $\mathbf{R_f}$ = 0.12) afforded *cis*-oak lactone (**1a**) (25.1 mg, 50%) as a colourless oil. **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 4.42 (1H, ddd, *J* = 4.5, 5.6, 9.4, H_5), 2.68 (1H, dd, *J* = 7.8, 16.9, $H_{3a(cis)}$), 2.57 (1H, m, H_4), 2.19 (1H, dd, *J* = 3.8, 16.9, $H_{3b(trans)}$), 1.74-1.26 (6H, m, $H_{6,7,8}$), 1.00 (3H, d, *J* = 7.0, H_{10}), 0.91 (3H, t, *J* = 7.1, H_9); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 176.9 (C_2), 83.7 (C_5), 37.5 (C_3), 33.0 (C_4), 29.6, 28.0, 22.5 ($C_{6,7,8}$), 13.9 (C_9), 13.8 (C_{14}).

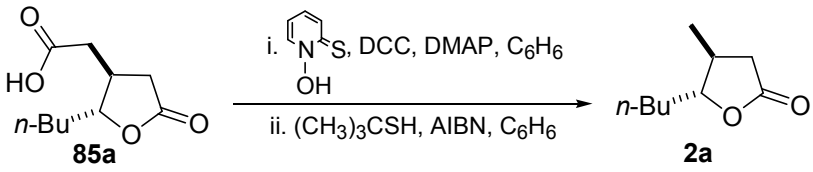
(4*R*,5*R*)-*cis*-5-*n*-Butyl-4-methyl-4,5-dihydro-2(3H)-furanone (1b)



cis-Oak lactone (**1b**) was synthesised, as per **1a**, from **84b** (56.2 mg, 0.28 mmol) using *N*-hydroxy-2-thiopyridone (74.0 mg, 0.58 mmol) and DMAP (56.4 mg, 0.46 mmol) in anhydrous C_6H_6 (3 mL) with DCC (87.3 mg, 0.42 mmol) in anhydrous C_6H_6 (1.5 mL) and $(CH_3)_3CSH$ (150 μL, 1.80 mmol) and AIBN (catalytic) in anhydrous C_6H_6 (4 mL) to yield the title compound **1b** (21.7 mg, 49%) as a colourless oil. **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 4.42 (1H, ddd, *J* = 4.5, 5.6, 9.4, H_5), 2.68 (1H, dd, *J* = 7.7, 16.8, $H_{3a(cis)}$), 2.56 (1H, m, H_4), 2.18 (1H, dd, *J* = 3.8, 16.8, $H_{3b(trans)}$), 1.74-1.26 (6H, m, $H_{6,7,8}$), 1.00 (3H, d, *J* = 7.0, H_{10}), 0.91 (3H, t, *J* = 7.1, H_9); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 176.9 (C_2), 83.6 (C_5), 37.5 (C_3), 33.0 (C_4),

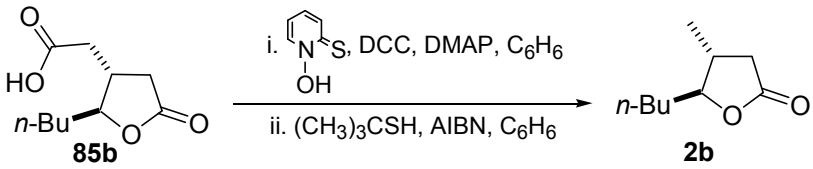
29.5, 28.0, 22.5 ($C_{6,7,8}$), 13.9 (C_9), 13.8 (C_{14}).

(4*S*,5*R*)-*trans*-5-*n*-Butyl-4-methyl-4,5-dihydro-2(3H)-furanone (2a)



trans-Oak lactone (**2a**) was synthesised, as per **1a**, from **85a** (48.3 mg, 0.24 mmol) using *N*-hydroxy-2-thiopyridone (64.8 mg, 0.51 mmol) and DMAP (49.4 mg, 0.40 mmol) in anhydrous C_6H_6 (2.5 mL) with DCC (75.5 mg, 0.37 mmol) in anhydrous C_6H_6 (1.5 mL) and $(CH_3)_3CSH$ (150 μL, 1.80 mmol) and AIBN (catalytic) in anhydrous C_6H_6 (4 mL) to yield the title compound **2a** (18.2 mg, 49%) as a colourless oil. **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 4.00 (1H, dt, *J* = 4.0, 7.7, H_5), 2.66 (1H, m, $H_{3a/b}$), 2.29-2.12 (2H, m, $H_{3a/b,4}$), 1.74-1.27 (6H, m, $H_{6,7,8}$), 1.13 (3H, dd, *J* = 1.8, 6.5, H_{10}), 0.91 (3H, t, *J* = 7.1, H_9); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 176.5 (C_2), 87.4 (C_5), 37.1 (C_3), 36.0 (C_4), 33.7, 27.8, 22.4 ($C_{6,7,8}$), 17.5 (C_9), 13.8 (C_{14}).

(4*R*,5*S*)-*trans*-5-*n*-Butyl-4-methyl-4,5-dihydro-2(3H)-furanone (2b)



trans-Oak lactone (**2b**) was synthesised, as per **1a**, from **85b** (98.0 mg, 0.49 mmol), using *N*-hydroxy-2-thiopyridone (129.0 mg, 1.01 mmol) and DMAP (91.0 mg, 0.74 mmol) in anhydrous C_6H_6 (3 mL) with DCC (162.0 mg, 0.79 mmol) in anhydrous C_6H_6 (2 mL) and $(CH_3)_3CSH$ (200 μL, 2.40 mmol) and AIBN (catalytic) in anhydrous C_6H_6 (4 mL) to yield the title compound **2b** (21.0 mg, 27%) as a colourless oil. **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 4.00 (1H, dt, *J* = 4.1, 7.7, H_5), 2.67 (1H, m, $H_{3a/b}$), 2.30-2.10 (2H, m, $H_{3a/b,4}$), 1.75-1.27 (6H, m, $H_{6,7,8}$), 1.12 (3H, dd, *J* = 1.8, 6.5, H_{10}), 0.90 (3H, t, *J* = 7.1, H_9); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 176.6 (C_2), 87.4 (C_5), 37.1 (C_3), 36.0 (C_4), 33.6, 27.8, 22.4 ($C_{6,7,8}$), 17.4 (C_9), 13.8 (C_{14}).

5.3 Experimental procedures for Chapter 4

Chiral GC-MS analysis

The prepared oak lactone samples were analysed by chiral GC-MS to determine the diastereomeric and enantiomeric purity, as described by Wilkinson *et al*.[58]

Solvent and wine

EtOH was purchased from Sigma-Aldrich and purified prior to use. The white wine used was a young (< 12 months old), unwooded cask 'bag in a box' wine (pH 3.22, 9.0% EtOH, SO_2 levels 188 mg/L total and 26 mg/L free). The red wine used was a 2005 vintage McLaren Vale Shiraz (pH 3.65, 14.8% EtOH, SO_2 levels 44 mg/L total and 4 mg/L free), which had not received any oak treatment and showed no oak lactone on analysis.[124,125]

Odour detection thresholds

The odour detection thresholds were determined according to the American Standards for Testings and Materials (ASTM) method E 679-79[120] in white and red wine. The sensory testings took place over approximately one month. Most panellists had previous experience with difference testing and with sensory evaluation of wine. The judges were Australian Wine Research Institute (AWRI) staff and students of various ethnic origins, aged between 20 and 55, with similar numbers of males and females. There were 28 panellists who participated in every threshold test, and this group is referred to as 'the common 28'. For purposes of comparison, thresholds were determined for both the common 28 and for the full panel, which ranged from 33 to 43 panellists.

Solutions of the various oak lactone isomers were prepared from stock solutions and each test concentration was quantified by GC-MS analysis using d_4-oak lactone as an internal standard.[124,125] The spiked samples were prepared by adding an EtOH solution (< 0.5 mL) of the isomer of interest to 1L of wine. An equal volume of EtOH was added to the corresponding blank samples. The samples in each individual triangle test were presented in random order and identified only by three-digit random numbers. The sample that was different from the other two was always the spiked sample. Panellists smelled, but did not taste the samples.

Wines were presented as 20 mL portions in randomly coded glasses, covered with a watch glass (as part of a triangle test) in ascending order of oak lactone concentration. Those panellists who were successful at detecting all concentrations were retested at lower concentrations, while panellists who were unsuccessful at detecting the highest concentration were retested at higher concentrations. Each individual panellist was assigned a best estimate threshold (BET) value, being the geometric mean of the highest concentration tested that was identified correctly and the next lowest concentration. The geometric mean of all the individual BETs was then calculated to give the final threshold value.

The concentrations employed were as follows:

- (4*S*,5*S*)-*cis*-oak lactone (**1a**): 2.7, 7.7, 23.7, 71.5, 209 and 632 μg/L, with retest concentrations for the low end of 0.3 and 0.9 μg/L and for the high end of 1,869 and 5,498 μg/L.
- (4*R*,5*R*)-*cis*-oak lactone (**1b**): 7.2, 21.7, 65.5, 195, 593 and 1,772 μg/L, with retest concentrations for the low end of 0.8 and 2.4 μg/L and for the high end of 5,231 and 15,693 μg/L.
- (4*S*,5*R*)-*trans*-oak lactone (**2a**): 7.8, 23.4, 70.3, 208, 625 and 1,875 μg/L, with retest concentrations for the low end of 0.9 and 2.6 μg/L and for the high end of 5,729 and 16,926 μg/L.
- (4*R*,5*S*)-*trans*-oak lactone (**2b**): 7.3, 21.9, 66.0, 200, 595 and 1,771 μg/L, with retest concentrations for the low end of 0.8 and 2.4 μg/L and for the high end of 5,413 and 16,039 μg/L.

Duo-trio difference tests

The duo-trio difference tests were conducted according to standard procedures[121] using 36 panellists. The same group of panellists participated in every test. In general, three samples were presented to each panellist, two of which were spiked with identical quantities of one isomer, with one of these solutions being marked as the reference sample. The third sample contained the corresponding enantiomer, or a mixture of *cis*- and *trans*-isomers, depending on the test. Panellists smelled, but did not taste the samples. Panellists were asked to pick which of the two test samples was identical to the reference sample.

The concentrations employed were as follows:

- (4*S*,5*S*)-*cis*-oak lactone (**1a**) vs. (4*R*,5*R*)-*cis*-oak lactone (**1b**): 150 μg/L in white wine, 300 μg/L in red wine
- (4*S*,5*R*)-*trans*-oak lactone (**2a**) vs. (4*R*,5*S*)-*trans*-oak lactone (**2b**): 250 μg/L in white wine, 500 μg/L in red wine
- (4*S*,5*S*)-*cis*-oak lactone (**1a**) vs. (4*S*,5*S*)-*cis*-oak lactone (**1a**) and (4*S*,5*R*)-*trans*-oak lactone (**2a**): 150 μg/L in white wine (300 μg/L total concentration in the mixture), 300 μg/L in red wine (600 μg/L total concentration in the mixture).

6 Introduction to grape-derived lactones

Lactones are heterocyclic compounds found throughout nature in a wide variety of foods and beverages. This chapter discusses the sensory properties of some γ- and δ-lactones, with the focus on a series of γ-lactones and their occurrence, biosynthesis and quantification in wine.

6.1 Lactones in food and beverages

The structural class of lactones, characterised by a cyclic ester group, features widely in the literature as important aroma or flavour compounds.[126] They can contain rings of various sizes but the most common are five- or six-membered rings. They can also possess varying degrees of saturation as well as having different side chains. Figure 6.1 depicts the simplest γ-lactone, γ-butyrolactone, the longest five-substituted alkyl chain γ-lactone considered for this study, γ-dodecalactone, and an example of a δ-lactone, δ-octalactone.

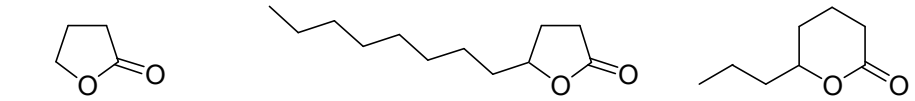


Figure 6.1 Examples of some lactones

Lactones exist as naturally occurring odorants in all major food groups including fruits and vegetables, bread and nut products, dairy and meat products and miscellaneous foods such as honey and popcorn. Lactones are also present in many different beverages including tea, coffee and wine.[126] Table 6.1 lists some of these food and beverage products.

Table 6.1 Food and beverage products in which lactones have been identified

apricot	cocoa	milk	popcorn
animal fat	coconut	nectarine	pork
beef	coffee	nuts	raspberry
bread products	cream	orange	strawberry
butter	dried mushroom	passionfruit	tea
celery	honey	peach	tomato
cheese	lamb	pineapple	vegetable oils
chicken	mango	plum	wine

6.2 Sensory properties of some γ- and δ-lactones

Lactones are generally pleasant, potent odorants that contribute a variety of aromas, including 'coconut' and 'sweet fruit'. Sensory descriptors for the series of γ- and δ-octalactone, nonalactone, decalactone and dodecalactone are listed in Table 6.2.[126,127]

Table 6.2 Odour descriptors for lactones

compound	**descriptors**
γ-octalactone	coconut, strong fruity, walnut
δ-octalactone	coconut
γ-nonalactone	strong coconut
δ-nonalactone	milky/oily
γ-decalactone	peach-like, pleasant fruity
δ-decalactone	oily, peachy
γ-dodecalactone	buttery/fatty, peachy
δ-dodecalactone	oily, strong fresh fruit

Aroma and tasted detection threshold studies have been completed on numerous lactones in various media including taste thresholds in an artificial peach beverage,[128] and odour thresholds in water,[129] model wine[130] and white wine.[131,132] Table 6.3 presents the numerous threshold values for the γ- and δ-lactones, as listed above. A general inspection of the threshold values reveals the γ-lactones to be more potent than the corresponding δ-lactones. A direct comparison is possible between γ- and δ-octalactone in water[129] (γ-octalactone over fifty times more potent), γ- and δ-

nonalactone in a sweet white wine[132] (γ-nonalactone over five times more potent) and γ- and δ-decalactone in water[129] (γ-decalactone nine times more potent).

Table 6.3 Odour threshold values (μg/L) for lactones in water or wine medium

compound	threshold	medium	reference
γ-octalactone	7-8	H_2O	Buttery *et al*. 1971,[133] Buttery and Ling 1998,[134] Buttery *et al*. 1999[135]
	7	H_2O	Engel *et al*. 1988[129]
	14	H_2O	Boonbumrung *et al*. 2001[136]
δ-octalactone	2,400	sweet white wine	Etievant *et al*. 1983[132]
	400	H_2O	Engel *et al*. 1988[129]
γ-nonalactone	460	sweet white wine	Etievant *et al*. 1983[132]
	30	simple white wine	Nakamura *et al*. 1988[131]
δ-nonalactone	2,600	sweet white wine	Etievant *et al*. 1983[132]
γ-decalactone	11	H_2O	Engel *et al*. 1988[129]
	1-10	H_2O	Larsen and Poll 1992[137]
	10	model wine[a]	Moyano *et al*. 2002[130]
δ-decalactone	4,200	sweet white wine	Etievant *et al*. 1983[132]
	100	H_2O	Engel *et al*. 1988[129]
γ-dodecalactone	7	H_2O	Engel *et al*. 1988[129]
δ-dodecalactone			odour threshold unknown

[a] 14% EtOH, tartaric acid to pH 3.5

This study focuses on γ-octalactone (**86a** and **86b**), γ-nonalactone (**87a** and **87b**), γ-decalactone (**88a** and **88b**) and γ-dodecalactone (**89a** and **89b**) (Figure 6.2) in white and red wine.

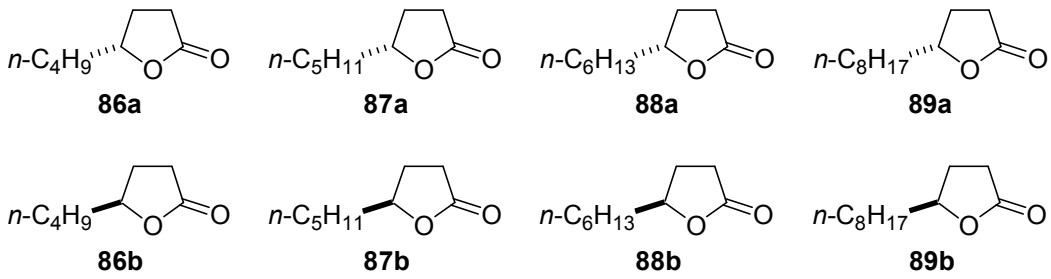


Figure 6.2 Structures of γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone

6.3 Chirality of γ-lactones

6.3.1 Enantiomeric distribution of γ-lactones in some fruits

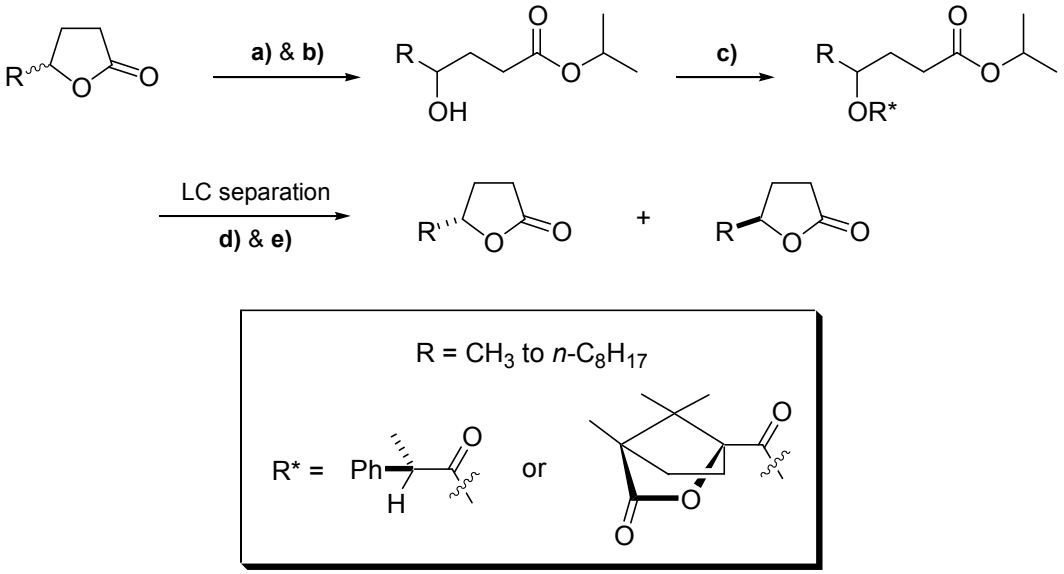
All five-alkyl substituted γ-lactones exist as a pair of enantiomers (as shown above, Figure 6.2). The abundance of the individual enantiomers in nature varies greatly, although there is a general trend towards the dominance of the (*R*)-stereoisomers (Table 6.4).

Table 6.4 Enantiomeric ratios (*R*/*S*) in some fruit

fruit	86 (C_8)	87 (C_9)	88 (C_{10})	89 (C_{12})	reference
apricot	89/11	84/16	94/6	100/0	Bernreuther *et al*. 1989[138]
	86/14-93/07	64/36-91/09	91/09-98/02	93/07-> 99/01	Guichard *et al*. 1990[139]
	85/15		100/0	100/0	Greger and Schieberle 2007[140]
lulo del chocó	23/77		20/80		Morales and Duque 2000[141]
mango	53/47	73/27	66/34	100/0	Bernreuther *et al*. 1989[138]
nectarine			87/13		Engel *et al*. 1988[129]
passionfruit	59/41	93/7	91/9	98/2	Bernreuther *et al*. 1989[138]
	72/28	66/34	93/7	100/0	Nitz *et al*. 1991[142]
	55/45-78/22	50/50-56/44	87/13-93/7	99/1-100/0	Nitz *et al*. 1991[142]
			93/7	99/1	Werkhoff *et al*. 1998[143]
peach			89/11		Feuerbach *et al*. 1988[144]
	87/13	85/15	87/13	96/4	Bernreuther *et al*. 1989[138]
pineapple	67/33-75/25	61/39-77/23	63/37-91/9	82/18-100/0	Nitz *et al*. 1991[142]
plum	86/14	70/30	91/9	100/0	Nitz *et al*. 1991[142]
raspberry	40/60				Bernreuther *et al*. 1989[138]
	44/56	28/72	49/51	50/50	Nitz *et al*. 1991[142]
strawberry			100/0	100/0	Tressl and Albrecht 1986[145]
	66/34	64/36	98/2	98/2	Bernreuther *et al*. 1989[138]
			100/0	100/0	Mosandl *et al*. 1989[146]
	85/15	88/12	99/1	100/0	Nitz *et al*. 1991[142]

6.3.2 Sensory analysis on the enantiomers of γ-lactones

A series of optically pure five-alkyl substituted γ-lactones, from pentalactone through to dodecalactone, was prepared in 1989 by Mosandl and Günther (Scheme 6.1).[147] They were prepared from the commercially available racemic γ-lactones *via* ring opening under basic conditions, with separation of the enantiomers effected by the use of a chiral auxiliary. Liquid chromatography was used to separate the diastereomers, which featured either (*R*)-2-phenylpropionic acid or (1*S*,4*R*)-camphanoic acid as the resolving agent. Base hydroylsis and re-lactonisation gave the individual enantiomers of this particular series of γ-lactones with high optical purity (enantiomeric excess ≥ 99.8%).



Reagents and conditions: a) KOH, MeOH, rt, o/n; **b)** $(CH_3)_2CHBr$, DMF, rt, 24 hrs; **c)** for (*R*)-phenylpropionic acid: i. $(COCl)_2$, 20 °C, 15 mins; ii. DMAP, CCl_4, 20 °C, 3 hrs; for (1*S*,4*R*)-camphanoic acid: i. $SOCl_2$, 82 °C, 2 hrs; ii. DMAP, CCl_4, 20 °C, 2 hrs; **d)** KOH, MeOH, rt, 24 hrs; **e)** HCl, 50 °C, 24 hrs.

Scheme 6.1

A descriptive sensory study was completed on the enantiomers; the results of relevance to work completed in this thesis are listed in Table 6.5.[147] A 1% solution of each compound in propylene glycol was prepared and tested by smelling on strips. It was observed that an increase in alkyl chain length led to a decrease in coconut aroma and an increase in fruity-sweet notes. In this study, the (*R*)-enantiomers appeared to have greater odour strength than the corresponding (*S*)-isomers.

Table 6.5 Odour descriptors for the individual enantiomers of five-alkyl substituted γ-lactones

	odour descriptors
(*R*)-γ-octalactone (**86a**)	coconut tones with almond notes, spicy-green
(*S*)-γ-octalactone (**86b**)	coconut notes, fatty
(*R*)-γ-nonalactone (**87a**)	soft coconut with fatty-milky aspects, strong, sweet
(*S*)-γ-nonalactone (**87b**)	fatty, mouldy, weak coconut notes
(*R*)-γ-decalactone (**88a**)	caramel, fatty-sweet fruity note, soft coconut, strong
(*S*)-γ-decalactone (**88b**)	soft, sweet coconut note with fruity-fatty aspects
(*R*)-γ-dodecalactone (**89a**)	bloomy notes with aldehyde and woody aspects, strong, fruity-sweet
(*S*)-γ-dodecalactone (**89b**)	fatty-fruity, milky notes

6.4 Biosynthesis of γ-lactones

Long chain fatty acids have been shown to be precursor compounds in the biosynthesis of γ-lactones. Most of the natural fatty acids exist as even-numbered carbon chains, due to the involvement of acetyl-CoA, a coenzyme that carries a two carbon atom group, in their formation. The production of even-numbered γ-lactones, in particular γ-decalactone and γ-dodecalactone, has been investigated in fruits and microorganisms, using isotopically labelled compounds.[148,149] The fundamental first step was the introduction of oxygen into the carbon chain. Cultures of the yeast *Sporobolomyces odorus* have been shown to metabolise oleic acid to (*R*)-γ-decalactone (**88a**) and both enantiomers of γ-dodecalactone (**89a** and **89b**) (Figure 6.3).[148] It was proposed that the initial step involved enantioselective (*R*)-12-hydroxylation of oleic acid, catalysed by hydroxylase, followed by a series of β-oxidation cycles of the resultant ricinoleic acid to (*R*)-γ-decalactone. Oleic acid has also been shown to be a precursor compound for γ-dodecalactone, possibly catalysed by 10-hydratase or 9,10-epoxygenase, although the metabolic degradation is unclear.[148]

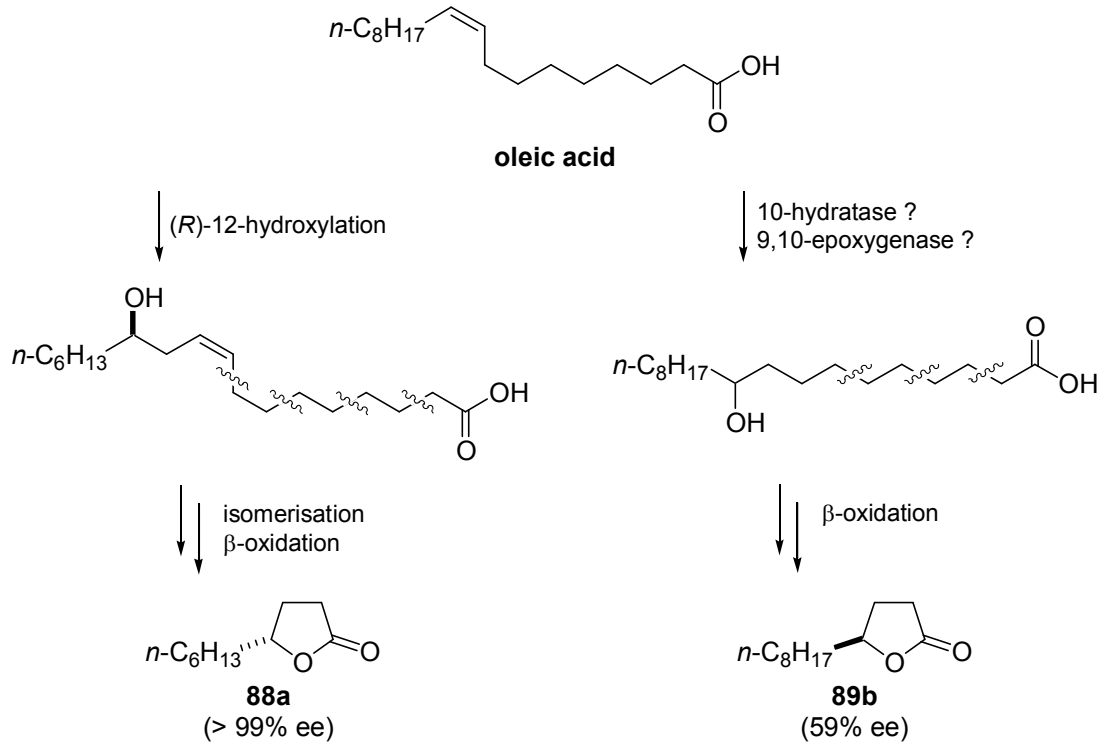


Figure 6.3 Proposed formation of (*R*)-γ-decalactone and (*R*)- and (*S*)-γ-dodecalctone from oleic acid

Sporobolomyces odorus has been shown to produce epoxygenated analogues of palmitoleic and oleic acids (Figure 6.4).[149] A second proposed biosynthetic pathway involves the action of an epoxide hydrolase to produce 5,6- or 9,10-dihydroxy fatty acids from palmitoleic and oleic acids, respectively.[150] Subsequent degradation of the dihydroxy fatty acid intermediates leads to hydroxyl γ-, δ- and ε-lactones[151] and finally to aroma active γ-lactones through β-oxidations to generate the linear precursor of correct chain length.[150] Cyclisation followed by elimination of water and reduction of the alkenyl intermediate leads to the formation of the natural products, γ-decalactone (**88a**) and γ-dodecalactone (**89a**) from palmitoleic and oleic acids, respectively, with (*R*)-stereochemistry.

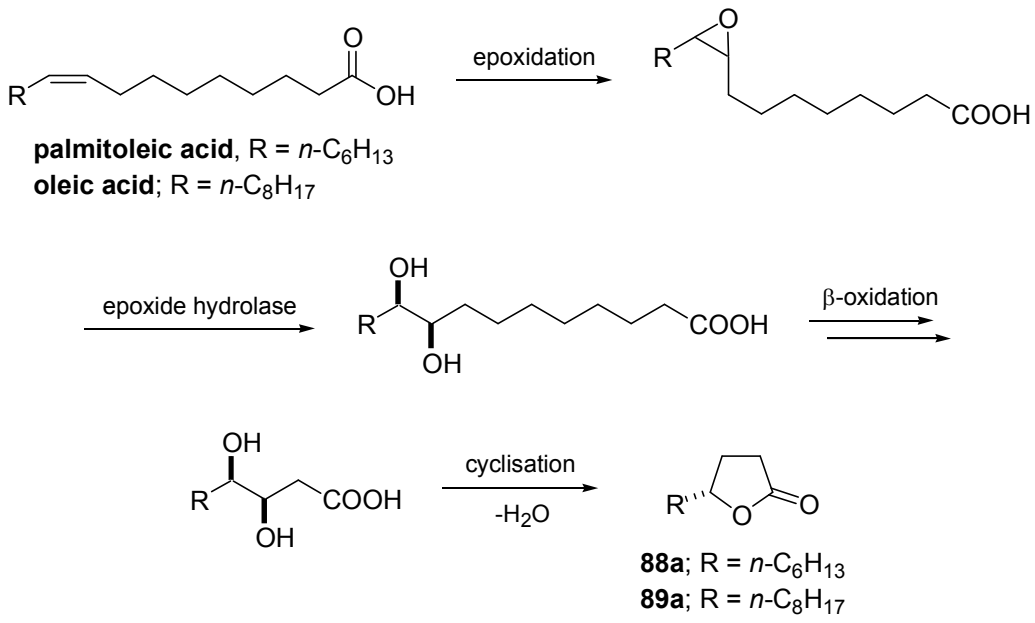


Figure 6.4 Proposed biosynthesis of (*R*)-γ-decalactone and (*R*)-γ-dodecalactone from palmitoleic and oleic acids, respectively

Degradation of oxygenated fatty acids by β-oxidation yields lactones with an even number of carbon atoms and cannot explain the formation of lactones with an odd number of carbon atoms. The yeast *Saccharomyces cerevisiae* has been shown, using deuterium labelling, to produce γ-nonalactone from linoleic acid by two biosynthetic pathways.[152,153] The first features 13-lipoxygenation of linoleic acid to (*S*)-13-hydroxyoctadecadienoic acid, followed by four β-oxidation cycles and finally α-oxidation to (*S*)-γ-nonalactone (**87b**) (50-70% ee). The second features 9-lipoxygenation of linoleic acid to (*R*)-9-hydroxyoctadecadienoic acid then Baeyer-Villiger type oxidation to (3*Z*)-nonen-1-ol, which is further metabolised to the (*R*)-enantiomer (**87a**) (approximately 50% ee).

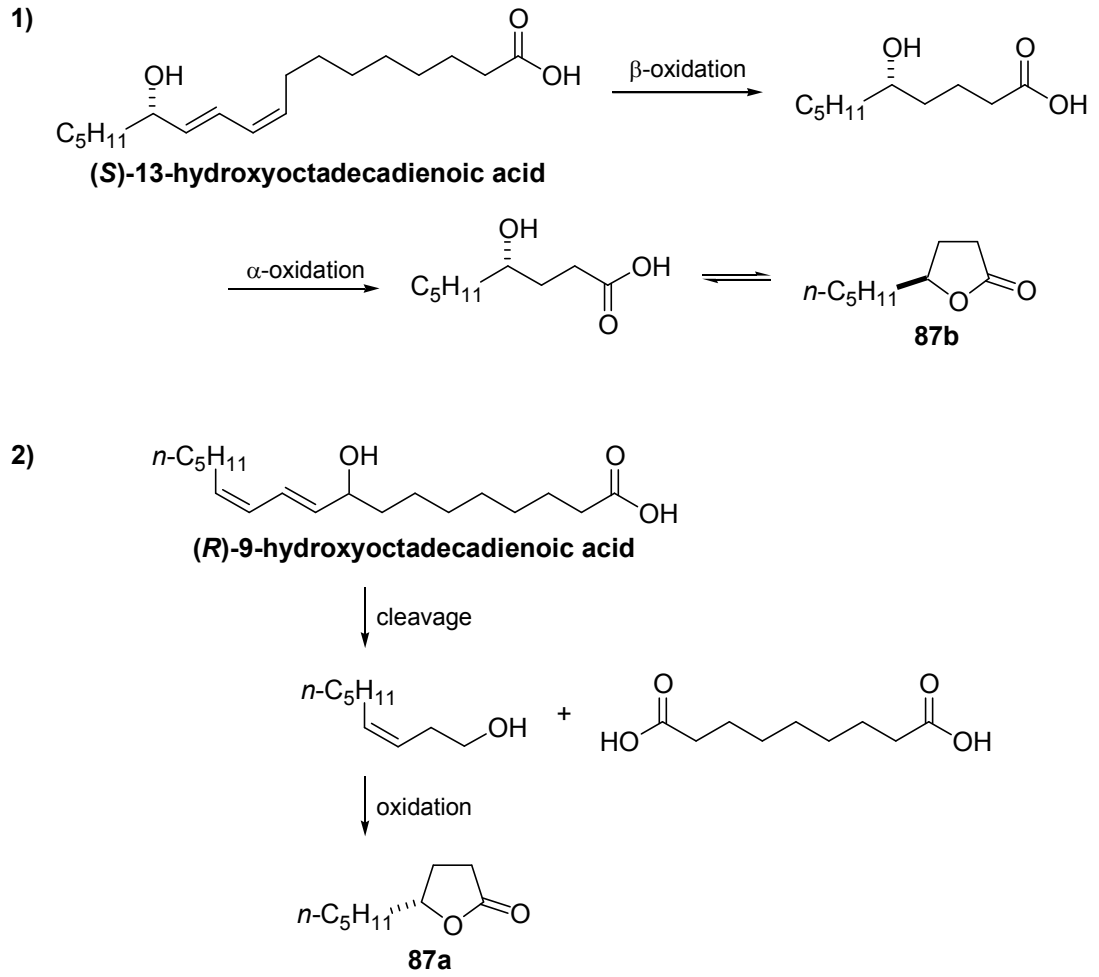


Figure 6.5 Proposed formation of (*S*)- and (*R*)-γ-nonalactone from linoleic acid

6.5 γ-Lactones in wine

6.5.1 Identification of γ-lactones

γ-Nonalactone was first identified in distilled beverages by Kahn *et al.* in 1969[152] and then later in wine by Schreier *et al.* in 1974,[153] who also identified γ-decalactone in wine in 1976.[154] γ-Octalactone was first identified in sherry by Fagan *et al.* in 1982[155] with the earliest report in wine following a decade later.[156] γ-Lactones continue to be discovered in wine, with the identification of γ-dodecalactone reported by Barbe *et al.* in 1998.[157]

6.5.2 Quantification of γ-lactones

Various methods are described in the literature for the quantification of γ-lactones in wine. Two decades ago, γ-nonalactone was quantified in six white wines and 32 red wines, using a continuous extraction procedure with freon 11.[131] *Iso*-butylbenzoate was used as the internal standard, where γ-nonalactone was found to range from not detected to 43 μg/L. More recently, a large study of 52 red wines was completed and included the quantification of γ-nonalactone and γ-decalactone, with an average of 16.2 μg/L and 1 μg/L, respectively.[158] In a similar sized study of 57 Spanish red wines, γ-nonalactone was quantified using solid-phase extraction as the method of sample clean-up.[159] Other studies with smaller sample sets have been published, namely on the identification and quantification of a large number of odorants in different wine varieties. Included in the extensive list of aroma compounds were γ-nonalactone and γ-decalactone. Table 6.6 lists a number of these results.

Table 6.6 Concentrations measured (μg/L) for γ-nonalactone and γ-decalactone in wines

	concentration	reference
	white	
γ-nonalactone (**87**)	0-16, average of 7 (N = 6)	Nakamura *et al*. 1988[131]
	23 (N = 1)	Escudero *et al*. 2004[160]
	18.7 (N = 9)	Gómez-Míguez *et al*. 2007[161]
γ-decalactone (**88**)	0.5 (N = 1)	Escudero *et al*. 2004[160]
	0.9 (N = 9)	Gómez-Míguez *et al*. 2007[161]
	red	
γ-nonalactone (**87**)	12-43, average of 23 (N = 32)	Nakamura *et al*. 1988[131]
	3.3-40.8, average of 16.2 (N = 52)	Ferreira *et al*. 2000[158]
	70.6 (N = 1)	Ferreira *et al*. 2002[162]
	1.7-23, average of 10 (N = 57)	López *et al*. 2002[159]
	10.3-18.0, average of 13.5 (N = 6)	Fang and Qian 2006[163]
	12.8-20, average of 15.7 (N = 6)	Carrillo *et al*. 2006[164]
	3.1-15, average of 8.8 (N = 5)	Escudero *et al*. 2007[165]
γ-decalactone (**88**)	0.67-2.9, average of 1 (N = 52)	Ferreira *et al*. 2000[158]
	0.4 (N = 1)	Ferreira *et al*. 2002[162]
	2.2-73, average of 26 (N = 5)	Escudero *et al*. 2007[165]

Note: N denotes number of wines analysed

Recently, a study on C_8 to C_{12} aliphatic lactones was published by Ferreira *et al.* and included the oak lactones, γ-octalactone, γ-nonalactone, γ-decalactone, δ-decalactone, γ-undecalactone and γ-dodecalactone.[166] The method utilised solid-phase extraction for sample preparation and GC-MS for analysis with 2-octanol as the internal standard. This work represents the first reported data for the quantification of γ-octalactone and γ-dodecalactone in white and red wines. A selection of the results, for the γ-lactones of interest to work completed in this thesis, are shown in Table 6.7.

Table 6.7 Concentrations measured (μg/L) for γ-lactones in wines

	aged red (N = 5)	**young red (N = 4)**	**white (N = 4)**
γ-octalactone (**86**)	1.4-4.6 (2.3)	1.3-1.6 (1.4)	0.0
γ-nonalactone (**87**)	3.7-27.0 (13.4)	6.1-16.4 (10.2)	2.2-9.6 (5.9)
γ-decalactone (**88**)	0.0-1.5 (0.5)	0.1- 0.3 (0.2)	0.0-0.4 (0.1)
γ-dodecalactone (**89**)	0.7-17.7 (4.6)	0.4-2.5 (1.6)	0.0

Note: N denotes number of wines analysed; number in parentheses indicates average concentration measured

It is noteworthy that in the above listed quantification studies, none of the research addressed the distribution of the individual enantiomers in wine.

6.6 Stable isotope dilution assay (SIDA)

In order to quantify trace aroma compounds accurately, a sensitive and reproducible analytical method is required. The characteristic odorants generally account for only a small fraction of the total sample and therefore have to be enriched, often by multiple concentration steps, prior to analysis. These difficulties can be overcome through the use of an internal standard, where analyte losses through extraction, and analysis, can be corrected. However, this method only provides accurate data when the analyte and the internal standard have similar properties. Thus, the best choice for an internal standard is a compound that is as close as possible (in terms of structural and physical properties) to the actual analyte.

The most accurate method for the quantification of volatile flavour compounds in food and beverages, particularly in wine, involves the use of a stable isotope dilution assay (SIDA). The procedure employs isotopically labelled analogues of analytes, typically deuterated compounds, as internal standards. A precise quantity of the labelled analogue is added to the sample, prior to extraction. The labelled standard is practically identical in its physical and chemical properties to that of the analyte and thus will behave identically during sample preparation; any loss of analyte due to mechanical or extraction problems will be mirrored exactly by the internal standard.

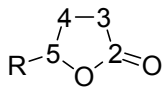
It follows then that one of the distinct advantages of using labelled analogues over other internal standards is that complete extraction of the volatiles is no longer necessary.[50] Throughout sample preparation and analysis, the ratio of isotopically labelled standard to the analyte remains constant. As a consequence, the accuracy of the quantification results is not affected by incomplete isolation, nor by degradation of the analyte. Thus, methods can be developed for the rapid extraction of a sample.

In most cases using SIDA, the labelled analogues require synthesis, which is usually costly and time consuming. However, the efficiency and accuracy of this technique have seen its widespread use for the quantification of potent odorants in food and beverages. The first reported use of SIDA for quantitative analysis was over 40 years ago.[167] Deuterated glucose was used as the internal standard. Through the use of GC-MS, the ratio of protons to deuteriums was determined. Today, SIDA has been widely applied to the quantification of trace aroma compounds in food and beverages including bread crusts,[168] β-damascenone in foods including roasted coffee, black tea, honey and beer,[169] the (*R*)- and (*S*)-enantiomers of linalool in beer[170] and odour-active compounds in fresh pineapple.[171] Examples of its use in wine include the quantification of (*E*) 1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB),[172] the oak lactones in barrel-aged wines,[124] methoxypyrazines[173] including 2-methoxy-3,5-dimethylpyrazine (fungal must) in corks soaked in wine,[174] β-damascenone,[175] β-ionone,[176] and most recently vitispirane.[177] SIDA methods have also been developed for the quantification of multiple compounds in a single run including four related cinnamic acid esters,[178] four monoterpene alcohols[179] and 31 volatile wine fermentation products which included ethyl esters, acetates, acids and alcohols.[180]

6.7 Research aims

The aims behind this work were threefold. Firstly, this project aimed to develop a SIDA method using GC-MS for the quantification of γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone in a single run employing synthesised labelled standards. The method would then be applied to a large survey of white and red wine varieties in order to examine the abundance of the γ-lactones in Australian wines. Secondly, this project aimed to investigate the distribution of the individual enantiomers in wines by developing a chiral SIDA method utilising optically pure standards of the γ-lactones as reference materials. Finally, odour detection threshold values of the individual enantiomers for each γ-lactone would be determined, where considered appropriate, in order to assess their likely impact on wine aroma.

Note: any compound with a five-membered cyclic ester is a γ-lactone. Each member of this class is a unique compound, with its own common name. Throughout this thesis, the following numbering system is used, in keeping with the compounds' belonging to the furanone family.



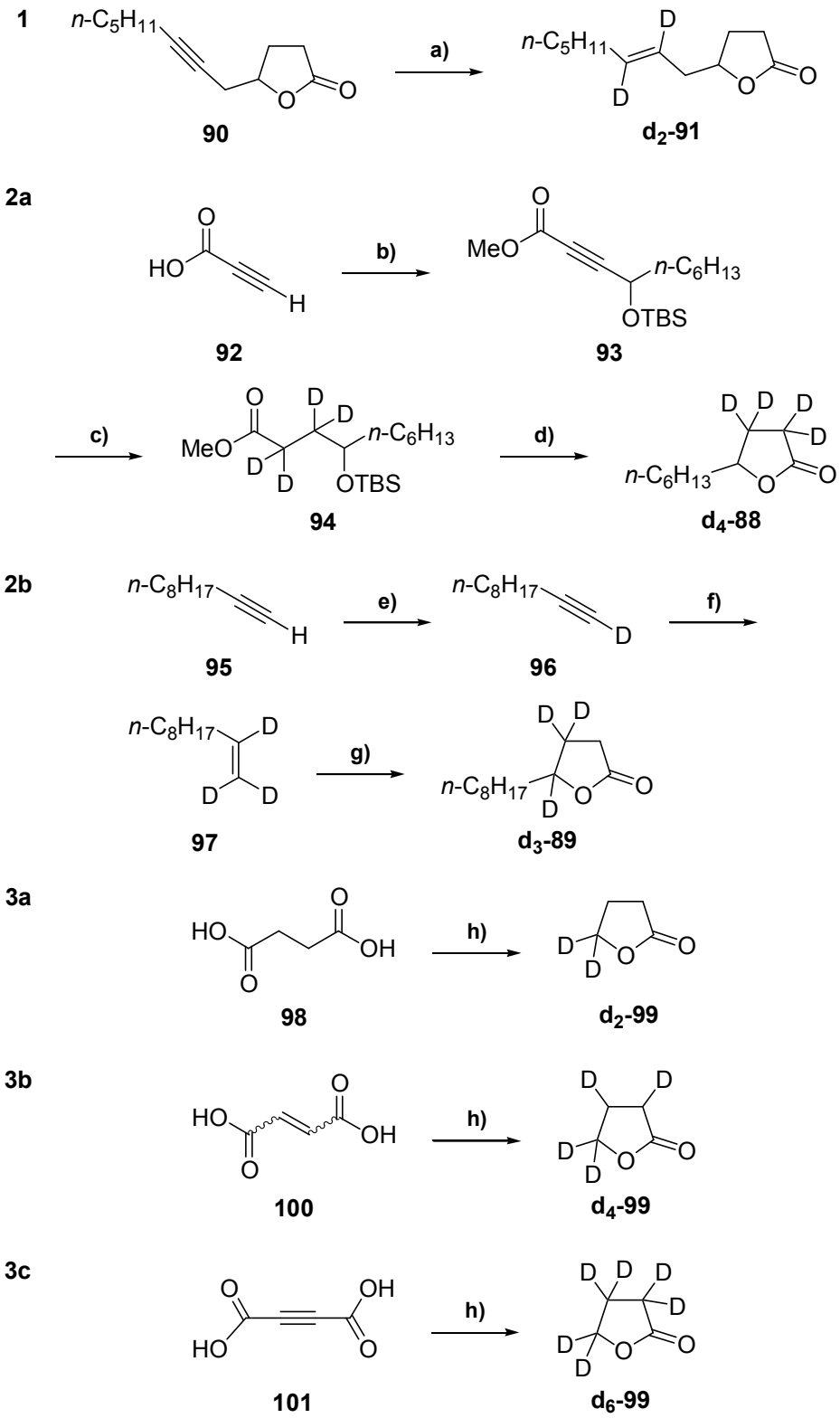
furanone numbering

7 Synthesis of deuterated standards and development of stable isotope dilution analysis (SIDA) methods in wine

Stable isotope dilution analysis (SIDA) is the most accurate method for the quantification of aroma compounds in food and beverages. This chapter explores the synthesis of deuterated analogues and details the development of a SIDA method for the measurement of γ-lactones in wine.

7.1 Previous syntheses of deuterated γ-lactones

There are several articles published in the literature detailing the synthesis of γ-lactones. Few, however, allow for the incorporation of deuterium atoms into the γ-lactone unit (Scheme 7.1). There are procedures described for the preparation of d_2-standards of 6-dodeceno-γ-lactone with the deuterium atoms substituted around the double bond at C_7 and C_8.[181,182] One such example involved a modified approach for the synthesis of 6-dodeceno-γ-lactone,[181] where the incorporation of deuterium atoms was achieved by reduction of (*Z*)-6-dodecyne-γ-lactone with sodium borodeuteride in d_1-methanol (equation 1).[182] Two further synthetic methods were recently published for deuterated γ-lactones, with differing degrees of deuteration.[183] The d_4-analogues of γ-octalactone, γ-decalactone and γ-dodecalactones were prepared by reduction of the doubly protected hydroxypropiolic acid with deuterium gas, with the deuterium atoms at C_3 and C_4 (e.g. equation 2a). The products were obtained with greater than 89% deuterium incorporation. d_3-γ-Octalactone and d_3-γ-dodecalactone were also prepared by free radical addition of 2-iodoacetamide to the corresponding deuterated olefins (e.g. equation 2b). The deuterium atoms were positioned at C_4 and C_5 with greater than 92% deuterium incorporation. A one-step procedure was published earlier this year for the preparation of γ-butyrolactones with two, four or six deuterium atoms, from saturated succinic acid, or unsaturated maleic, fumaric or acetylenedicarboxylic acids in the presence of a ruthenium catalyst with deuterium gas (equations 3a, 3b and 3c).[184]

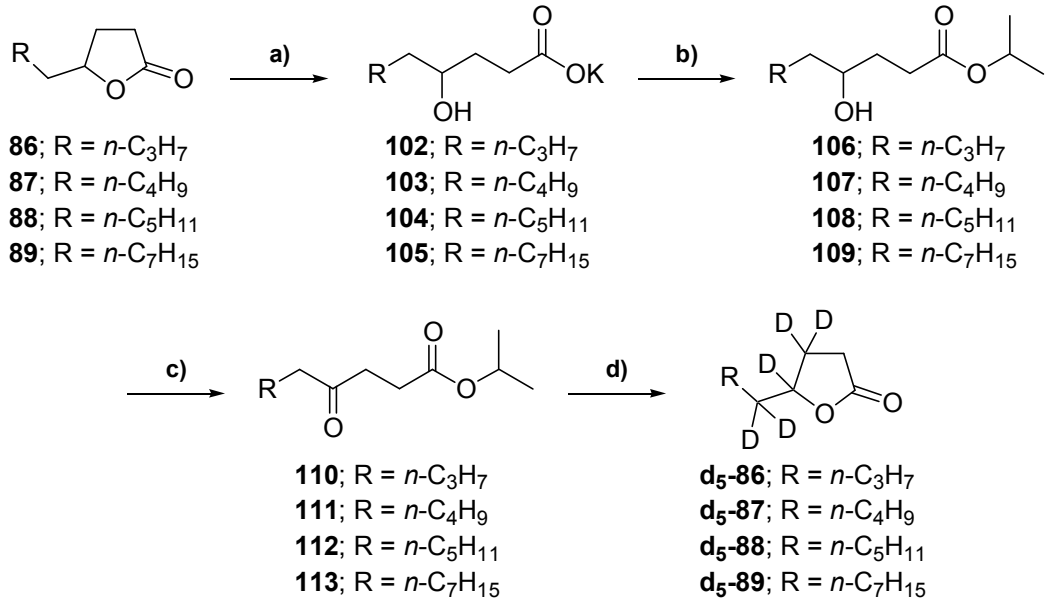


Reagents and conditions: a) $NaBD_4$, MeOD, no yield reported; **b)** i. LDA, -78 °C, THF, then heptanal, TMEDA, 44%; ii. CH_2N_2, Et_2O, 65%; iii. *tert*-$BuMe_2SiCl$, imidazole, DMF, 66%; **c)** D_2, $RhCl(PPh_3)_3$, C_6H_6, 97%; **d)** HCl (cat.), THF, 92%; **e)** i. *n*-BuLi, -78 °C, THF; ii. D_2O , 97%; **f)** D_2, Lindlar's cat., quinoline, pentane, 61%; **g)** ICH_2CONH_2, ACCN, H_2O, C_6H_6, 23%; **h)** D_2, THF, 180 bar, 180 °C, $Ru_4H_4(CO)_8(PBu_3)_4$, 100%.

Scheme 7.1

7.2 Synthesis of penta-deuterated γ-lactones

Racemic γ-octalactone (**86**), γ-nonalactone (**87**), γ-decalactone (**88**) and γ-dodecalactone (**89**) were obtained commercially and employed as the starting reagents in the synthesis of the deuterated standards. Isotoptically labelled analogues of γ-octalactone (**d_5-86**), γ-nonalactone (**d_5-87**), γ-decalactone (**d_5-88**) and γ-dodecalactone (**d_5-89**) were prepared as shown in Scheme 7.2.



Reagents and conditions: a) KOH, MeOH, rt, 3 days; **b)** $(CH_3)_2CHBr$, DMSO, rt, 20 hrs; **c)** Swern [O]; **d)** i. NaH, D_2O, rt, 24 hrs; ii. $NaBD_4$, D_2O, rt, 24 hrs; iii. HCl, rt, 24 hrs.

Scheme 7.2

Racemic γ-lactones **86**, **87**, **88** and **89** were ring-opened under basic conditions and isolated as potassium carboxylate salts **102**, **103**, **104** and **105**, respectively (Scheme 7.2). Oxidation of the C_4 hydroxyl group into the corresponding keto functionality was then attempted. This key reaction served two purposes: firstly, re-lactonisation was not possible with the ketone and, secondly, the presence of the ketone activated the flanking α-hydrogens towards deuterium exchange. Two common reagents for oxidation were investigated. Aqueous potassium permanganate gave only a (blackened) mixture of products, with the desired ketone constituting only a small fraction of the mixture. Chromium trioxide in anhydrous pyridine was then trialled, as reported for the synthesis of the ring-opened ketone analogues of the oak

lactones.[124] As with the previous attempt, the crude reaction mixture proved to be difficult to purify and resulted in very low yields (approximately 10%). With both oxidants proving to be unsatisfactory, an alternative route to the ring opened ketones was explored.

The potassium salts of the γ-lactones **102**, **103**, **104** and **105** were converted into the corresponding *iso*-propyl esters **106**, **107**, **108** and **109**, respectively, with *iso*-propyl bromide in dimethyl sulfoxide (Scheme 7.2). Purification by column chromatography on silica afforded the esters as colourless oils, in yields of 80% or greater (Table 7.1). The esters were found to be sufficiently resistant to lactonisation to allow for purification. However, exposure to acid (e.g. standing in deuterated chloroform) over time led to re-lactonisation.

Oxidation of the C_4 hydroxyl group was successfully achieved under Swern conditions (dimethyl sulfoxide, oxalyl chloride and triethylamine in dichloromethane) (Scheme 7.2).[185] Each of the keto esters **110**, **111**, **112** and **113** was obtained in quantitative yield and, due to the high purity of the crude product as detected by nuclear magnetic resonance (NMR) spectroscopy, was used without further purification (Table 7.1). Despite the additional effort involved in the preparation and isolation of the *iso*-propyl esters, this second route proved both cleaner and higher yielding than the original endeavour.

The final step in this sequence involved the deuterium exchange of the α-hydrogens under basic conditions (Scheme 7.2). Following a procedure described in the literature which was used in the preparation of deuterated analogues of the oak lactones,[124] the ring-opened keto esters **110**, **111**, **112** and **113** were treated with sodium hydride in deuterium oxide. Under these conditions, all four α-hydrogens were exchanged within 24 hours at room temperature. Due to cleavage of the *iso*-propyl esters and thus formation of the carboxylate anions under basic conditions, exchange of the C_2 protons was not observed. Sodium borodeuteride effected the reduction of the keto functionality, and the desired d_5-lactones were obtained after acidification of the mixture. Each γ-lactone was deuterated *via* the same pathway (Scheme 7.2), with similar yields obtained for each intermediate (Table 7.1). The overall yields achieved for the labelled analogues of γ-octalactone (**d_5-86**), γ-

nonalactone (**d_5-87**), γ-decalactone (**d_5-88**) and γ-dodecalactone (**d_5-89**) were 76%, 73%, 77% and 70%, respectively.

Table 7.1 Reaction yields obtained for the synthesis of the d_5-γ-lactones

	γ-octalactone	**γ-nonalactone**	**γ-decalactone**	**γ-dodecalactone**
iso-propyl ester	85% (**106**)	86% (**107**)	89% (**108**)	80% (**109**)
keto ester	quantitative (**110**)	quantitative (**111**)	quantitative (**112**)	quantitative (**113**)
d_5-product	89% (**d_5-86**)	85% (**d_5-87**)	86% (**d_5-88**)	88% (**d_5-89**)
overall	76%	73%	77%	70%

Note: number for chemical structure denoted in parentheses

The presence of the deuterium atoms in each of the γ-lactones, three on the lactone ring (C_4 and C_5) and two on the alkyl side chain (C_6), was confirmed by both NMR and gas chromatography-mass spectrometry (GC-MS) analysis. Table 7.2 and Table 7.3 list the ^{1}H NMR and ^{13}C NMR parameters, respectively, for the starting (unlabelled) lactones, and their corresponding d_5-analogues.

Table 7.2 ^{1}H NMR data for analytes and d_5-labelled analogues of the γ-lactones

	H_5	**H_3**	**$H_{4a/b}$**	**$H_{4a/b,n\text{-alkyl}}$[a]**	**H_{methyl}[b]**
γ-octalactone (**86**)	4.48 (qn)	2.52 (dd)	2.31 (sx)	1.91-1.26 (m)	0.91 (t)
d_5-γ-octalactone (**d_5-86**)	-	2.51 (s)	-	1.48-1.27 (m)[c]	0.91 (t)
γ-nonalactone (**87**)	4.48 (qn)	2.52 (dd)	2.31 (sx)	1.91-1.24 (m)	0.89 (t)
d_5-γ-nonalactone (**d_5-87**)	-	2.34 (s)	-	1.36-1.10 (m)[c]	0.75 (t)
γ-decalactone (**88**)	4.48 (qn)	2.53 (dd)	2.31 (sx)	1.91-1.22 (m)	0.88 (t)
d_5-γ-decalactone (**d_5-88**)	-	2.33 (s)	-	1.32-1.02 (m)[c]	0.71 (t)
γ-dodecalactone (**89**)	4.48 (qn)	2.53 (dd)	2.31 (sx)	1.91-1.22 (m)	0.88 (t)
d_5-γ-dodecalactone (**d_5-89**)	-	2.48 (s)	-	1.44-1.30 (m)[c]	0.84 (t)

Note: ^{1}H NMR data reported as chemical shift in ppm; splitting patterns quoted in parentheses as dd = doublet of doublets, m = multiplet, qn = quintet, s = singlet, sx = sextet, t = triplet; acquired in $CDCl_3$; [a] refers to *n*-alkyl chain at C_5 position on γ-lactone moiety, data do not include terminal methyl centre; [b] refers to terminal methyl group in *n*-alkyl chain; [c] multiplet for labelled analogues integrates for two less protons than the analyte

Table 7.3 ^{13}C NMR data for analytes and d_5-labelled analogues of the γ-lactones

	C_2	C_5	C_4[a]	C_3	$C_{n\text{-alkyl}}$[b,c]	C_{methyl}[d]
γ-octalactone (**86**)	177.2	80.9	27.8	28.7	35.1, 27.2, 22.3	13.7
d_5-γ-octalactone (**d_5-86**)	176.6	79.6 (t)	33.6 (qn), 26.7 (qn)	27.9	26.4, 21.7	13.2
γ-nonalactone (**87**)	177.1	80.8	27.7	28.6	35.3, 31.2, 24.6, 22.2	13.7
d_5-γ-nonalactone (**d_5-87**)	177.0	80.0 (t)	34.2 (qn), 26.7 (qn)	28.8	31.1, 24.3, 22.1	13.5
γ-decalactone (**88**)	177.2	80.9	27.8		35.4, 31.5, 28.8,[e] 28.7,[e] 25.0, 22.4	13.9
d_5-γ-decalactone (**d_5-88**)	176.8	79.9 (t)	34.1 (qn), 26.7 (qn)	28.1	31.2, 28.5, 24.5, 22.0	13.5
γ-dodecalactone (**89**)	177.2	80.9	27.8	28.7	35.4, 31.7, 29.3, 29.2, 29.0, 25.1, 22.5	13.9
d_5-γ-dodecalactone (**d_5-89**)	177.2	80.4 (t)	34.9 (qn), 27.1 (qn)	31.7	31.7, 29.3, 29.2, 29.1, 25.0, 22.5	14.0

Note: ^{1}H NMR data reported as chemical shift in ppm; splitting patterns quoted in parentheses as qn = quintet, t = triplet; acquired in $CDCl_3$; [a] in the case of the deuterated analogue the peak for C_4 was indistinguishable from C_6; [b] refers to *n*-alkyl chain at C_5 position on γ-lactone moiety, data do not include terminal methyl centre; [c] in the case of the deuterated analogue data do not include C_6; [d] refers to terminal methyl group in *n*-alkyl chain; [e] peak for C_3 indistinguishable from *n*-alkyl chain

Figure 7.1 shows mass spectra for the labelled and unlabelled γ-lactones. In all cases, the base peaks were *m/z* 85 (unlabelled) and 88 (labelled); this fragment corresponded to the lactone ring after loss of the alkyl side chain, and confirmed that three labels were incorporated within the ring, with the two remaining labels located on the side chain. GC-MS analysis also confirmed the purity of each labelled standard as greater than 98%.

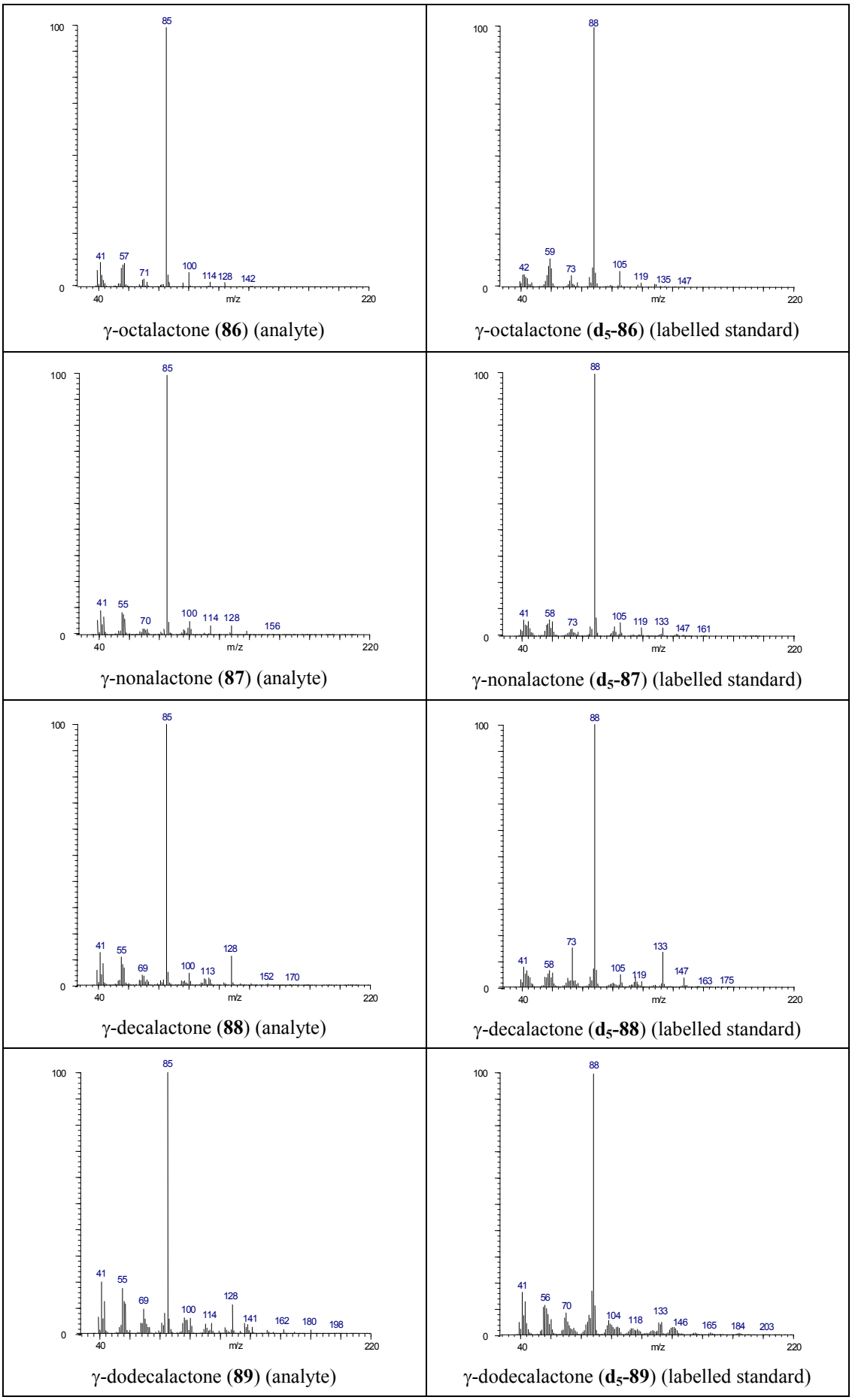


Figure 7.1 Mass spectra of unlabelled (left) and d_5-labelled (right) γ-lactones

7.3 Development of head space solid-phase microextraction (HS SPME) method

7.3.1 Target and qualifier ions for d_5-analogues

Solutions of the γ-lactones and the deuterated analogues were analysed by GC-MS in scan mode to enable selection of suitable target and qualifier ions (Figure 7.1). With the easy fragmentation of the alkyl side chain, the spectra were dominated by a large base peak (*m/z* 85 for unlabelled; *m/z* 88 for labelled) with only small peaks for the other fragment ions. With the molecular ion of low abundance, there was little choice to be made; the ions selected were *m/z* 85, 86, 100 and 128 for the analytes and *m/z* 88, 89, 105 and 133 for the labelled standards (Table 7.4). In the case of γ-octalactone, the ions of *m/z* 128 (unlabelled) and 133 (labelled) were not observed. The base peaks were used for quantification by peak area and the remaining ions used as qualifiers to ensure the identity of the analytes. For the quantification of the γ-lactones, the mass spectra were recorded in selected ion monitoring (SIM) mode.

Table 7.4 Target and qualifier ions monitored in SIM mode

	retention time	**target *m/z***	**qualifiers *m/z***
γ-octalactone (**86**)	8.984	85	86, 100
d_5 γ-octalactone (**d_5-86**)	8.978	88	89, 105
γ-nonalactone (**87**)	9.888	85	86, 100, 128
d_5 γ-nonalactone (**d_5-87**)	9.876	88	89, 105, 133
γ-decalactone (**88**)	10.948	85	86, 100, 128
d_5 γ-decalactone (**d_5-88**)	10.942	88	89, 105, 133
γ-dodecalactone (**89**)	13.459	85	86, 100, 128
d_5 γ-dodecalactone (**d_5-89**)	13.441	88	89, 105, 133

7.3.2 Optimisation of solid-phase microextraction (SPME) procedure

The use of head space solid-phase microextraction (HS SPME) was investigated. White wine was selected as the medium of choice for the development of the SIDA method, as it was assumed to be an easier matrix to work with compared with red wine. A range of different parameters was explored to lower the limit of detection

and hence increase the sensitivity of the method.

Five different fibres were tested in duplicate for their affinity towards the target lactones: the polydimethylsiloxane (PDMS) fibre; the polydimethylsiloxane/divinyl benzene (PDMS/DVB) fibre; the white polyacrylate fibre; the carbowax/divinyl benzene (CW/DVB) fibre; and the divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre. By comparing the relative areas of the base peak for each γ-lactone, the PDMS/DVB and the DVB/CAR/PDMS fibres were discovered to have the highest affinity across the range of the four γ-lactones. These two fibres were investigated further at lower concentrations, with the latter fibre ultimately being selected for the development of the SPME method.

Briefly, the factors which were optimised for good signal intensity and low background noise were:

- nature of the SPME fibre ((DVB/CAR/PDMS) fibre found to be optimal);
- presence or absence of sodium chloride (presence found to be optimal);
- extraction conditions (agitation and incubation at 50 °C, 40 minute adsorption time found to be optimal);
- ethanol content (50% dilution with water found to be optimal).

7.3.3 Calibration functions, reproducibility and precision of method for solid-phase microextraction (SPME)

Calibration functions were obtained through the analysis of a series of spiked solutions in a white wine (‘bag in a box’ fresh dry white). Head space analysis was performed using SPME, in duplicate. The calibration functions obtained were linear across the concentration range (0-100 μg/L) for each γ-lactone, and are shown in both graphical form (Figure 7.2) and in tabular form (Table 7.5).

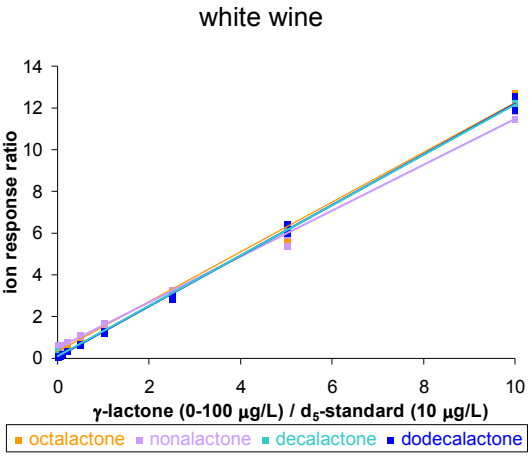


Figure 7.2 Calibration functions for the γ-lactones in a white wine

Table 7.5 Correlation coefficients (r^2) and equation of the line for the calibration functions in a white wine

	r^2	equation of the line
γ-octalactone (**86**)	0.997[a]	y = 1.19x + 0.33
γ-nonalactone (**87**)	0.997[a]	y = 1.10x + 0.50
γ-decalactone (**88**)	1.000[a]	y = 1.21x + 0.11
γ-dodecalactone (**89**)	0.999[a]	y = 1.22x + 0.53

[a] range 0-100 µg/L; N = 9 x 2 (number of data points, with each being measured in duplicate)

The HS SPME method was verified for reproducibility and precision by the analysis of seven replicates at two different concentrations (5 µg/L and 25 µg/L) and found to be within the acceptable range for accuracy and precision (less than 10%) (Table 7.6).

Table 7.6 Comparison of seven replicate determinations for accuracy and precision in a white wine

	spike (μg/L)	average (μg/L)	standard deviation[a]	difference (%)[b]
γ-octalactone (**86**)	5	5.54	0.14	10.80
	25	23.79	1.67	-4.84
γ-nonalactone (**87**)	5	5.29	0.13	5.80
	25	25.35	0.72	1.40
γ-decalactone (**88**)	5	5.14	0.18	2.80
	25	25.25	0.34	1.00
γ-dodecalactone (**89**)	5	5.49	0.12	9.80
	25	26.60	0.62	6.40

[a] standard deviation from the average measured level of γ-lactone, [b] percentage difference between the spiked level and the averaged measured level of γ-lactone

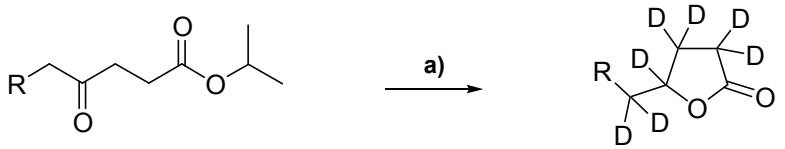
7.3.4 Application of solid-phase microextraction (SPME) method

With the SPME method developed, a survey of Australian white wines was undertaken. A selection of 34 white wines was analysed for the four γ-lactones. Unfortunately, in several of the bottled white wine samples, the method suffered from the interference of co-eluting compounds that gave several of the selected ions. Although the method was satisfactory for some of the wines analysed it was not sufficiently robust to enable the determination of the four γ-lactones in all the 34 wines. An alternative method was therefore developed for sample preparation with a particular focus on sample clean-up.

7.4 Synthesis of septua-deuterated standards

Prior to the improvement of the quantification method, it was envisaged that the incorporation of further deuterium atoms into the γ-lactone structure might remove some problems associated with the SPME method. As previously discussed, it was necessary for the additional deuterium atoms to be included in the γ-lactone ring. This was achieved through the synthesis of d_7-labelled analogues of γ-octalactone (**d_7-86**), γ-nonalactone (**d_7-87**), γ-decalactone (**d_7-88**) and γ-dodecalactone (**d_7-89**).

As utilised for the deuterium exchange under basic conditions, ring opened keto esters **110**, **111**, **112** and **113** were employed for deuterium exchange under strong acidic conditions (deuterium chloride in deuterium oxide, heated under reflux for 1–4 weeks) (Scheme 7.3). The acidic environment ensured protonation of the carboxylate anions and thus enabled exchange of the C_2 protons, previously not possible under basic conditions. The incorporation of the deuterium atoms into the ring opened oxidised γ-lactones was observed by NMR spectroscopy. The signals for the protons at C_3 and C_5 and lastly the protons at C_2, were monitored until no longer detectable. Sodium borodeuteride was again used to reduce the ketone back to the alcohol, which simultaneously incorporated the seventh deuterium atom at the C_4 position, followed by acidification to the final deuterated γ-lactones *via* ring closure.



110; R = *n*-C_3H_7
111; R = *n*-C_4H_9
112; R = *n*-C_5H_{11}
113; R = *n*-C_7H_{15}

d$_7$-86; R = *n*-C_3H_7
d$_7$-87; R = *n*-C_4H_9
d$_7$-88; R = *n*-C_5H_{11}
d$_7$-89; R = *n*-C_7H_{15}

Reagents and conditions: a) i. DCl, D_2O, Δ, 1-4 wks; ii. $NaBD_4$, D_2O, rt, 24 hrs; iii. DCl/D_2O, rt, 24 hrs.

Scheme 7.3

Each γ-lactone was synthesised separately *via* the same pathway (Scheme 7.3). Due to the harsh nature of the reaction conditions necessary to force deuterium exchange, a large amount of degraded product was observed in the γ-nonalactone and γ-dodecalactone products, with lower yields of the purified product collected, 13 and 17%, respectively (Table 7.7). γ-Octalactone and γ-decalactone were prepared in more satisfactory yields of 75% and 40%, respectively (Table 7.7).

Table 7.7 Reactions yields obtained for the synthesis of the d$_7$-γ-lactones

	γ-octalactone	**γ-nonalactone**	**γ-decalactone**	**γ-dodecalactone**
d_7-product	75% (**d$_7$-86**)	13% (**d$_7$-87**)	40% (**d$_7$-88**)	17% (**d$_7$-89**)
overall yield	64%	11%	36%	14%

Note: number for chemical structure denoted in parentheses

As before, the presence of the deuterium atoms in the synthesised d_7-standards was confirmed by NMR and GC-MS analysis. Table 7.8 and Table 7.9 list the ^1H and ^{13}C NMR parameters, respectively, for the starting (unlabelled) lactones, and their corresponding d_7-analogues. Figure 7.3 shows the mass spectra for the labelled and unlabelled γ-lactones. Similar to the case of the d_5-analogues, the base peaks were *m/z* 85 (unlabelled) and 90 (labelled); this fragment corresponded to the lactone ring after loss of the alkyl side chain, and confirmed that five labels were incorporated within the ring, with the two remaining labels located on the side chain. GC-MS analysis also confirmed the purity of each labelled standard as greater than 98%.

Table 7.8 ^1H NMR data for analytes and d_7-labelled analogues of the γ-lactones

	H_5	H_3	$H_{4a/b}$	$H_{4a/b,n\text{-alkyl}}$[a]	H_{methyl}[b]
γ-octalactone (**86**)	4.48 (qn)	2.52 (dd)	2.31 (sx)	1.91-1.26 (m)	0.91 (t)
d_7 γ-octalactone (**d_7-86**)	-	-	-	1.48-1.26 (m)[c]	0.91 (t)
γ-nonalactone (**87**)	4.48 (qn)	2.51 (dd)	2.31 (sx)	1.91-1.24 (m)	0.89 (t)
d_7 γ-nonalactone (**d_7-87**)	-	-	-	1.51-1.21 (m)[c]	0.89 (t)
γ-decalactone (**88**)	4.48 (qn)	2.53 (dd)	2.31 (sx)	1.91-1.22 (m)	0.88 (t)
d_7 γ-decalactone (**d_7-88**)	-	-	-	1.52-1.20 (m)[c]	0.88 (t)
γ-dodecalactone (**89**)	4.48 (qn)	2.53 (dd)	2.31 (sx)	1.91-1.22 (m)	0.88 (t)
d_7 γ-dodecalactone (**d_7-89**)	-	-	-	1.50-1.19 (m)[c]	0.88 (t)

Note: ^1H NMR data reported as chemical shift in ppm; splitting patterns quoted in parentheses as dd = doublet of doublets, m = multiplet, qn = quintet, s = singlet, sx = sextet, t = triplet; acquired in $CDCl_3$; [a] refers to *n*-alkyl chain at the C_5 position on γ-lactone moiety, data do not include terminal methyl centre; [b] refers to terminal carbon centre in the *n*-alkyl chain; [c] multiplet for labelled analogues integrates for two less protons than the analyte

Table 7.9 ^{13}C NMR data for analytes and d_7-labelled analogues of the γ-lactones

	C_2	C_5	C_4[a]	C_3	$C_{n\text{-alkyl}}$[b,c]	C_{methyl}[d]
γ-octalactone (**86**)	177.2	80.9	27.8	28.7	35.1, 27.2, 22.3	13.7
d_7 γ-octalactone (**d_7-86**)	176.9	79.9 (t)	33.8 (qn), 26.4 (qn)	27.5 (qn)	26.6, 21.8	13.4
γ-nonalactone (**87**)	177.1	80.8	27.7	28.6	35.3, 31.2, 24.6, 22.2	13.7
d_7 γ-nonalactone (**d_7-87**)	177.3	80.4 (t)	34.6 (qn), 27.2 (qn)	28.0 (qn)	31.4, 24.6, 22.4	13.9
γ-decalactone (**88**)	177.2	80.9	27.8		35.4, 31.5, 28.8,[e] 28.7,[e] 25.0, 22.4	13.9
d_7 γ-decalactone (**d_7-88**)	177.1	80.2 (t)	34.4 (qn), 26.7 (qn)	27.8 (qn)	31.4, 28.7, 24.7, 22.3	13.8
γ-dodecalactone (**89**)	177.2	80.9	27.8	28.7	35.4, 31.7, 29.3, 29.2, 29.0, 25.1, 22.5	13.9
d_7 γ-dodecalactone (**d_7-89**)	177.3	80.4 (t)	34.6 (qn), 28.0 (qn)	28.6 (qn)	31.8, 29.4, 29.2, 29.1, 24.9, 22.6	14.0

Note: ^{1}H NMR data reported as chemical shift in ppm; splitting patterns quoted in parentheses as qn = quintet, t = triplet; acquired in $CDCl_3$; [a] in the case of the deuterated analogue the peak for C_4 was indistinguishable from C_6; [b] refers to *n*-alkyl chain at C_5 position on γ-lactone moiety, data do not include terminal methyl centre; [c] in the case of the deuterated analogue data do not include C_6; [d] refers to terminal methyl group in *n*-alkyl chain; [e] peak for C_3 indistinguishable from *n*-alkyl chain

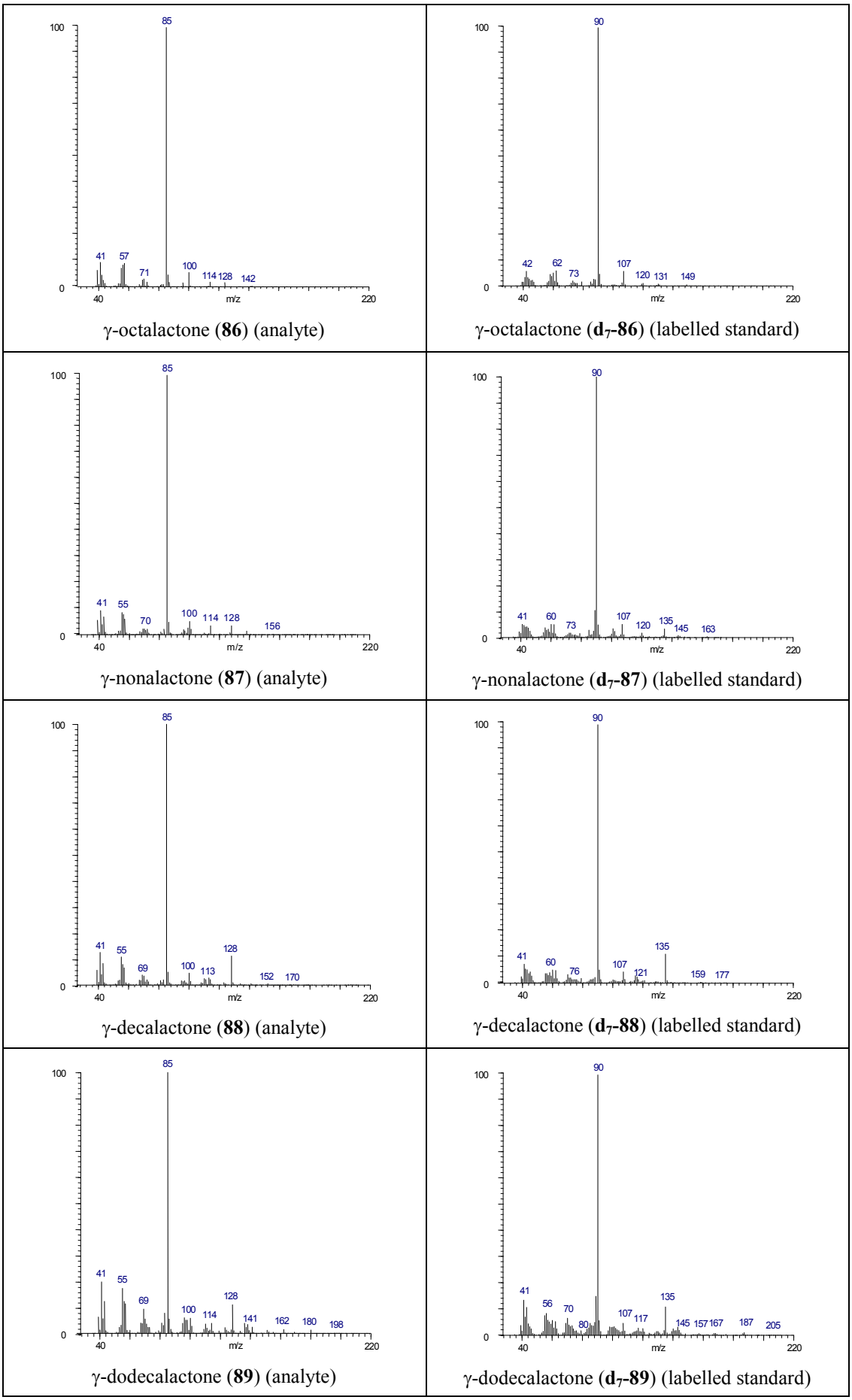


Figure 7.3 Mass spectra of unlabelled (left) and d_7-labelled (right) γ-lactones

7.5 Development of the solid-phase extraction (SPE) method

7.5.1 Target and qualifier ions for d_7-analogues

Solid-phase extraction (SPE) is an analytical technique used widely for either sample extraction or sample clean-up. A recently published method for the determination of aliphatic lactones in wine featuring SPE[166] has been extended here to incorporate d_7-analogues of the γ-lactones as internal standards.

The same target and qualifier ions were selected as for the SPME method, the major difference being that the ions for the internal standards were now two mass units heavier compared with the d_5-standards (Table 7.10). For the quantification of the γ-lactones, the mass spectra were recorded in SIM mode.

Table 7.10 Target and qualifier ions monitored in SIM mode

	retention time	**target *m/z***	**qualifiers *m/z***
γ-octalactone (**86**)	8.901	85	86, 100
d_7 γ-octalactone (**d_7-86**)	8.880	90	91, 107
γ-nonalactone (**87**)	9.792	85	86, 100, 128
d_7 γ-nonalactone (**d_7-87**)	9.765	90	91, 107, 135
γ-decalactone (**88**)	10.856	85	86, 100, 128
d_7 γ-decalactone (**d_7-88**)	10.829	90	91, 107, 135
γ-dodecalactone (**89**)	13.378	85	86, 100, 128
d_7 γ-dodecalactone (**d_7-89**)	13.336	90	91, 107, 135

7.5.2 Calibration functions, reproducibility and precision of method using solid-phase extraction (SPE)

The calibration functions obtained for each γ-lactone in both white ('bag in a box' fresh dry white) and red wine ('bag in a box' dry red) were linear throughout the concentration range (0-100 μg/L), with excellent correlation coefficients. These are shown both in graphical form (Figure 7.2) and in tabular form (Table 7.11).

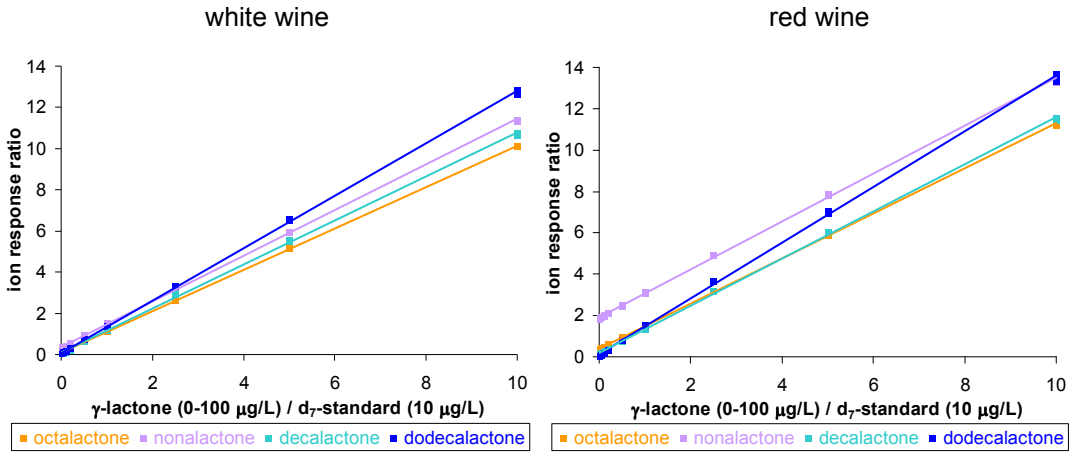


Figure 7.4 Calibration functions for the γ-lactones in a white and a red wine

Table 7.11 Correlation coefficients (r^2) and equation of the line for the calibration functions in a white and a red wine

	r^2	equation of the line
white wine		
γ-octalactone (**86**)	1.000[a]	y = 1.00x + 0.12
γ-nonalactone (**87**)	1.000[a]	y = 1.11x + 0.36
γ-decalactone (**88**)	0.999[a]	y = 1.07x + 0.09
γ-dodecalactone (**89**)	1.000[a]	y = 1.27x + 0.08
red wine		
γ-octalactone (**86**)	1.000[a]	y = 1.09x + 0.39
γ-nonalactone (**87**)	0.999[a]	y = 1.16x + 1.92
γ-decalactone (**88**)	1.000[a]	y = 1.14x + 0.19
γ-dodecalactone (**89**)	0.999[a]	y = 1.35x + 0.12

[a] range 0-100 µg/L; N = 9 x 2 (number of data points, with each being measured in duplicate)

The SIDA method was verified for reproducibility and precision through the analysis of seven replicates at two different concentrations (5 µg/L and 25 µg/L) (Table 7.12). The difference between the spike level and the average measured level was within the acceptable range of error (less than 10%).

Table 7.12 Comparison of seven replicate determinations for accuracy and precision in a white and a red wine

	spike level (μg/L)	average (μg/L)	standard deviation	difference (%)
white wine				
γ-octalactone (**86**)	5	5.11	0.02	2.20
	25	25.37	0.18	1.48
γ-nonalactone (**87**)	5	5.12	0.04	2.40
	25	26.52	0.18	6.08
γ-decalactone (**88**)	5	4.99	0.04	-0.20
	25	26.09	0.15	4.36
γ-dodecalactone (**89**)	5	5.04	0.05	0.80
	25	25.49	0.21	1.96
red wine				
γ-octalactone (**86**)	5	5.03	0.05	0.60
	25	25.21	0.08	-0.84
γ-nonalactone (**87**)	5	4.91	0.04	-1.80
	25	25.41	0.14	-1.64
γ-decalactone (**88**)	5	5.00	0.04	0.00
	25	25.41	0.17	-1.64
γ-dodecalactone (**89**)	5	4.99	0.04	-0.20
	25	25.37	0.25	-1.48

7.5.3 Application of solid-phase extraction (SPE) method

The SIDA method developed using SPE was found to be accurate and reproducible. The limit of detection was determined to be 0.10 μg/L. An initial screening of 18 wines was completed to verify the effectiveness of the SPE sample clean-up and to check the target and qualifier ions for co-eluters in the SIDA method (Table 7.13). Based on results from the literature,[166] red wine was the focus of the preliminary analysis. A selection of 16 red wines of different varieties and regions was divided into two groups of older (1996-1999) and younger (2002-2005) wines. Only two white wines were included in the first selection for quantification. No difficulties were encountered with co-eluting peaks in any of these wine samples.

Notwithstanding the small number of white wines that were analysed, the results were in agreement with those reported in the literature and showed that these particular γ-lactones are found in lower concentrations in white wines than in red wines (Table 7.13) (reported average concentrations in four white wines: γ-octalactone at 0.0 μg/L; γ-nonalactone at 5.9 μg/L; γ-decalactone at 0.1 μg/L; γ-dodecalactone at 0.0 μg/L).[166] The initial screening of red wines showed the younger wines to contain a higher average concentration of γ-nonalactone (7.66 μg/L) than the older wines (4.44 μg/L). In the case of γ-octalactone and γ-decalactone, the highest average concentrations were measured in the older wines (0.96 μg/L and 0.60 μg/L, respectively) rather than in the younger wines (0.50 μg/L and 0.18 μg/L, respectively) (Table 7.14). The results in red wine were in general agreement with published data by Ferreira *et al*. for five aged wines (γ-octalactone at 2.3 μg/L; γ-nonalactone at 13.4 μg/L; γ-decalactone at 0.5 μg/L; γ-dodecalactone at 4.6 μg/L) and four young red wines (reported average concentrations: γ-octalactone at 1.4 μg/L; γ-nonalactone at 10.2 μg/L; γ-decalactone at 0.2 μg/L; γ-dodecalactone at 1.6 μg/L), with the exception of γ-dodecalactone which was not detected in any of the 16 red wines. [166]

Table 7.13 Concentrations measured for the γ-lactones in white and red wines (μg/L)

variety	year	region	C_8	C_9	C_{10}	C_{12}
		white				
Sauvignon Blanc	2005	Adelaide Hills	<0.10	<0.10	<0.10	<0.10
Semillon	2005	Barossa Valley	0.33	0.69	0.45	<0.10
		red (1996-1999)				
Cabernet Sauvignon	1998	Limestone Coast	<0.10	0.21	<0.10	<0.10
Cabernet Sauvignon Merlot	1997	Coonawarra	<0.10	<0.10	<0.10	<0.10
Cabernet Sauvignon Petit Verdot Shiraz	1998	McLaren Vale	0.61	<0.10	<0.10	<0.10
Merlot	1997	Coonawarra	1.12	<0.10	0.82	<0.10
Merlot	1998	Barossa Valley	0.75	7.16	0.77	<0.10
Shiraz	1996	Heathcote	0.44	<0.10	<0.10	<0.10
Shiraz	1997	Barossa Valley	0.75	1.54	0.47	<0.10
Shiraz	1999	Grampians	2.09	8.83	0.33	<0.10
		red (2002-2005)				
Cabernet Sauvignon	2004	Eden Valley	<0.10	7.28	<0.10	<0.10
Cabernet Sauvignon	2004	Clare Valley	0.47	16.98	0.22	<0.10
Cabernet Sauvignon Merlot Cab. Franc	2003	Coonawarra	<0.10	<0.10	<0.10	<0.10
Shiraz	2003	Eden Valley	0.23	9.71	<0.10	<0.10
Shiraz	2004	Clare Valley	0.37	9.61	0.14	<0.10
Shiraz Cabernet Franc Viognier	2004	Yarra Valley	0.91	4.64	<0.10	<0.10
Shiraz Grenache	2002	McLaren Vale	0.50	2.45	<0.10	<0.10
Shiraz Grenache	2005	Barossa Valley	<0.10	2.95	<0.10	<0.10

Table 7.14 Minimum, maximum and average concentrations for the γ-lactones in red wines (μg/L)

	minimum	maximum	average[a]
older (1996-1999, N = 8)			
γ-octalactone (**86**)	<0.10	2.09	0.96 (6)[b]
γ-nonalactone (**87**)	<0.10	8.83	4.44 (4)[b]
γ-decalactone (**88**)	<0.10	0.82	0.60 (4)[b]
γ-dodecalactone (**89**)	<0.10	<0.10	<0.10
younger (2002-2005, N = 8)			
γ-octalactone (**86**)	<0.10	0.91	0.50 (5)[b]
γ-nonalactone (**87**)	<0.10	16.98	7.66 (7)[b]
γ-decalactone (**88**)	<0.10	0.22	0.18 (2)[b]
γ-dodecalactone (**89**)	<0.10	<0.10	<0.10

[a] only wines exceeding LOD (0.10 μg/L) included in average; [b] number in parentheses indicates total wines used in average

7.6 Wine survey

A large wine survey of 164 wines (44 white wines and 120 red wines) was conducted to determine the presence of the four target γ-lactones (Table 7.15 and Table 7.16). The wines ranged in price, vintage, region and variety. Full details of the quantification results are listed in Chapter 11 (Appendices).

7.6.1 White wine analysis for γ-lactone content

For the white wines, the varieties chosen were Chardonnay (12 wines), Riesling (12 wines), Sauvignon Blanc (10 wines) and Semillon (10 wines) (Table 7.15). The wines represented a wide cross-section of quality, including wine from both the top end and the bottom end of the market. The wines were chosen from the different wine-making regions within Australia. The ages of the wines ranged from the most recent vintage (2006) back to 1994.

The concentration of the γ-lactones ranged from below the limit of detection (0.10 μg/L) to 3.48 μg/L for γ-octalactone in a Semillon (Table 7.15). The most abundant

γ-lactone in Chardonnay, Riesling and Sauvignon Blanc was found to be γ-nonalactone at average concentration levels of 0.62 μg/L, 1.59 μg/L and 0.21 μg/L, respectively, while γ-octalactone was recorded to be the most prevalent in Semillon, with an average concentration level of 0.78 μg/L. The average concentration for γ-octalactone and γ-nonalactone across the whole range of white wines was calculated to be essentially the same at 0.69 μg/L and 0.71 μg/L, respectively. In this work, the concentration of γ-octalactone was found to be higher than that recorded in the literature (0.0 μg/L),[166] while the concentration of γ-nonalactone was significantly lower than that in the literature (average concentrations from various studies include 6.8 μg/L,[131] 23 μg/L[160], 18.7 μg/L,[161] and 5.9 μg/L[166]). Neither γ-decalactone nor γ-dodecalactone was detected in any of the white wines. These results were in close approximation to those in the literature (average concentrations from various studies include 0.5 μg/L[160], 0.9 μg/L,[161] and 0.1 μg/L for γ-decalactone;[166] 0.0 μg/L for γ-dodecalactone[166]).

Table 7.15 Minimum, maximum and average concentrations for the γ-lactones in white wines (μg/L)

	minimum	maximum	average[a]
Chardonnay (N = 12)			
γ-octalactone (**86**)	<0.10	1.19	0.31 (5)[b]
γ-nonalactone (**87**)	<0.10	0.82	0.62 (3)[b]
γ-decalactone (**88**)	<0.10	<0.10	<0.10
γ-dodecalactone (**89**)	<0.10	<0.10	<0.10
Riesling (N = 12)			
γ-octalactone (**86**)	0.28	1.68	0.98 (12)[b]
γ-nonalactone (**87**)	<0.10	3.34	1.59 (3)[b]
γ-decalactone (**88**)	<0.10	<0.10	<0.10
γ-dodecalactone (**89**)	<0.10	<0.10	<0.10
Sauvignon Blanc (N = 10)			
γ-octalactone (**86**)	<0.10	<0.10	<0.10
γ-nonalactone (**87**)	<0.10	0.21	0.21 (1)[b]
γ-decalactone (**88**)	<0.10	<0.10	<0.10
γ-dodecalactone (**89**)	<0.10	<0.10	<0.10
Semillon (N = 10)			
γ-octalactone (**86**)	<0.10	3.48	0.78 (7)[b]
γ-nonalactone (**87**)	<0.10	0.45	0.41 (2)[b]
γ-decalactone (**88**)	<0.10	<0.10	<0.10
γ-dodecalactone (**89**)	<0.10	<0.10	<0.10

[a] only wines exceeding LOD (0.10 μg/L) included in average; [b] number in parentheses indicates total number of wines used in average

7.6.2 Red wine analysis for γ-lactone content

As with the white wines, the red wines were chosen from across the different Australian wine-making regions, and also covered a wide range of quality. They comprised Cabernet Sauvignon (30 wines), Durif (6 wines), Merlot (25 wines), Pinot Noir (17 wines) and Shiraz (42 wines) (Table 7.16). The ages of the wines ranged from the most recent vintage (2006) back to 1991.

Compared with the white wines, the red wines which were analysed were found to contain significantly higher levels of the γ-lactones, ranging from below the limit of

detection (0.10 μg/L) to 39.75 μg/L for γ-nonalactone in a Durif (Table 7.16). The highest level of γ-octalactone was also found in a Durif at 4.18 μg/L and also its highest average concentration at 0.87 μg/L. γ-Nonalactone was the most abundant γ-lactone in all red wine varieties, with the highest average in the Cabernet Sauvignon wines at 7.27 μg/L. The highest level of γ-decalactone was found in a Cabernet Sauvignon at 4.00 μg/L, with the highest average concentration for this compound in the same wine variety at 1.01 μg/L. γ-Dodecalactone was not measured above the limit of detection in any of the red wines. The average concentrations across all red wine varieties were calculated to be 0.50 μg/L, 4.95 μg/L and 0.74 μg/L for γ-octalactone, γ-nonalactone and γ-decalactone, respectively. The average concentrations measured for γ-octalactone and γ-nonalactone were lower than those given in the literature compared with work by Ferreira *et al*. (γ-octalactone at 2.3 μg/L and 1.4 μg/L and γ-nonalactone at 13.4 μg/L and 10.2 μg/L for aged and young reds, respectively), for γ-decalactone the results were in close agreement with the literature (0.5 μg/L and 0.2 μg/L for aged and young reds, respectively) but the results were considerably different for γ-dodecalactone, which was not detected in any of the Australian red wines analysed (4.6 and 1.6 μg/L for aged and young reds, respectively).[166]

Table 7.16 Minimum, maximum and average concentrations for the γ-lactones in red wines (μg/L)

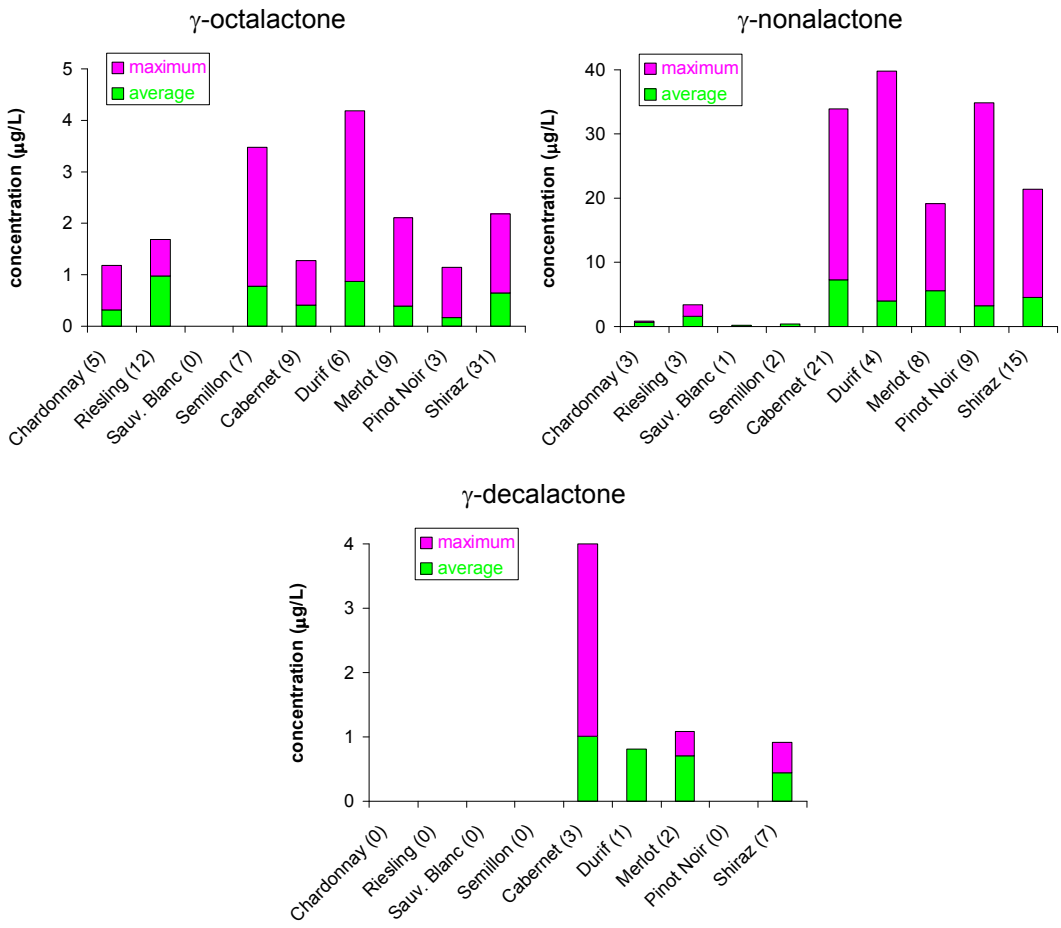
	minimum	maximum	average[a]
Cabernet Sauvignon (N = 30)			
γ-octalactone (**86**)	<0.10	1.27	0.41 (9)[b]
γ-nonalactone (**87**)	<0.10	33.94	7.27 (21)[b]
γ-decalactone (**88**)	<0.10	4.00	1.01 (3)[b]
γ-dodecalactone (**89**)	<0.10	<0.10	<0.10
Durif (N = 6)			
γ-octalactone (**86**)	0.25	4.18	0.87 (6)[b]
γ-nonalactone (**87**)	<0.10	39.75	4.04 (4)[b]
γ-decalactone (**88**)	<0.10	0.81	0.81 (1)[b]
γ-dodecalactone (**89**)	<0.10	<0.10	<0.10
Merlot (N = 25)			
γ-octalactone (**86**)	<0.10	2.11	0.39 (9)[b]
γ-nonalactone (**87**)	<0.10	19.12	5.57 (8)[b]
γ-decalactone (**88**)	<0.10	1.08	0.71 (2)[b]
γ-dodecalactone (**89**)	<0.10	<0.10	<0.10
Pinot Noir (N = 17)			
γ-octalactone (**86**)	<0.10	1.14	0.17 (3)[b]
γ-nonalactone (**87**)	<0.10	34.84	3.28 (9)[b]
γ-decalactone (**88**)	<0.10	<0.10	<0.10
γ-dodecalactone (**89**)	<0.10	<0.10	<0.10
Shiraz (N = 42)			
γ-octalactone (**86**)	<0.10	2.19	0.65 (31)[b]
γ-nonalactone (**87**)	<0.10	21.37	4.57 (15)[b]
γ-decalactone (**88**)	<0.10	0.92	0.44 (7)[b]
γ-dodecalactone (**89**)	<0.10	<0.10	<0.10

[a] only wines exceeding LOD (0.10 μg/L) included in average; [b] number in parentheses indicates total number of wines used in average

7.6.3 Graphical representation of concentration results for γ-lactones in white and red wines

The average and maximum concentrations measured in the white and red wines for γ-octalactone, γ-nonalactone and γ-decalactone are presented graphically in Figure

7.5. The significantly higher levels of γ-nonalactone compared with γ-octalactone and γ-decalactone are perhaps more obvious in this representation.



Note: number in parentheses indicates total number of wines used in average

Figure 7.5 Average and maximum concentrations for γ-octalactone, γ-nonalactone and γ-decalactone in white and red wines

γ-Octalactone was found to be present in 22 white and 56 red wines (Figure 7.6). For the wines that contained γ-octalactone, the concentration in white wine ranged from 0.20-3.48 µg/L, while in red wine it ranged from 0.11-4.18 µg/L. Most of the white and red wines were quantified in the range of 0-2 µg/L (20 for white wine, 53 for red wine).

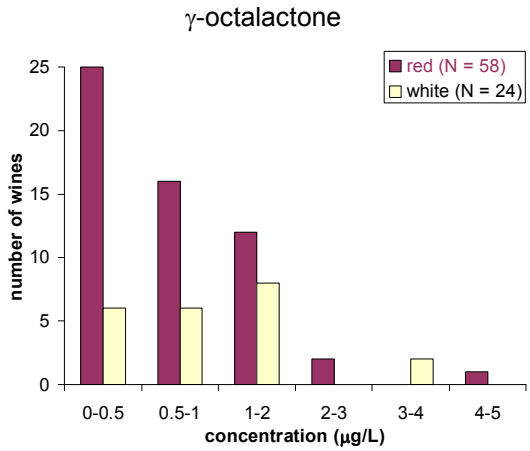


Figure 7.6 Wines grouped by concentration for γ-octalactone in the white and red wines

γ-Nonalactone was detected in eight white and 57 red wines in the concentration ranges of 0.21-3.34 µg/L and 0.13-39.75 µg/L, respectively (Figure 7.7). Of the total number of white wines analysed, only a small portion contained γ-nonalactone and more than half of these were within 0-1 µg/L. The concentrations measured in the red wines were distributed over a significantly larger range and nearly two-thirds of the results were greater than the maximum measured in white wine (4-40 µg/L).

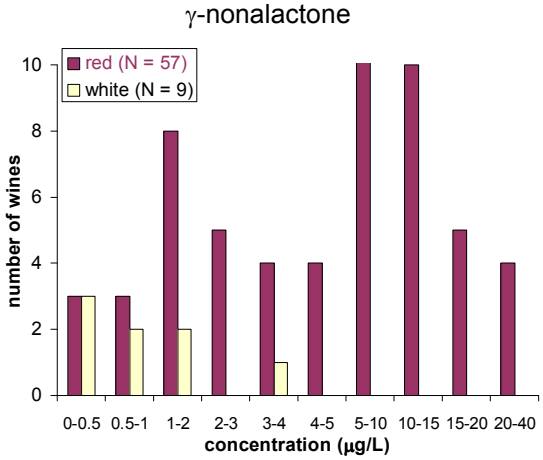


Figure 7.7 Wines grouped by concentration for γ-nonalactone in the white and red wines

γ-Decalactone was not measured in any of the 44 white wines and in only 13 of the 120 red wines (Figure 7.8). The concentrations in red wines ranged from 0.17-4.00 µg/L. Over 75% of the wines quantified with γ-decalactone were within 0-1 µg/L.

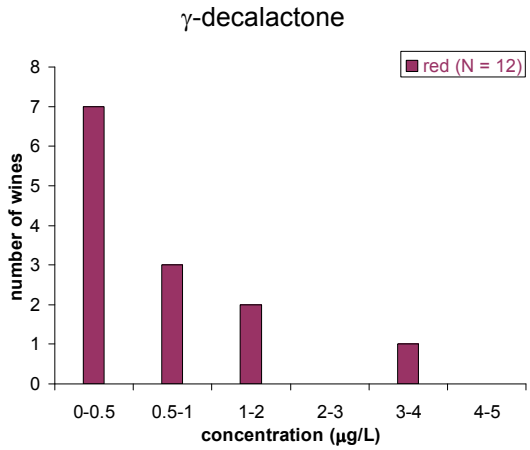
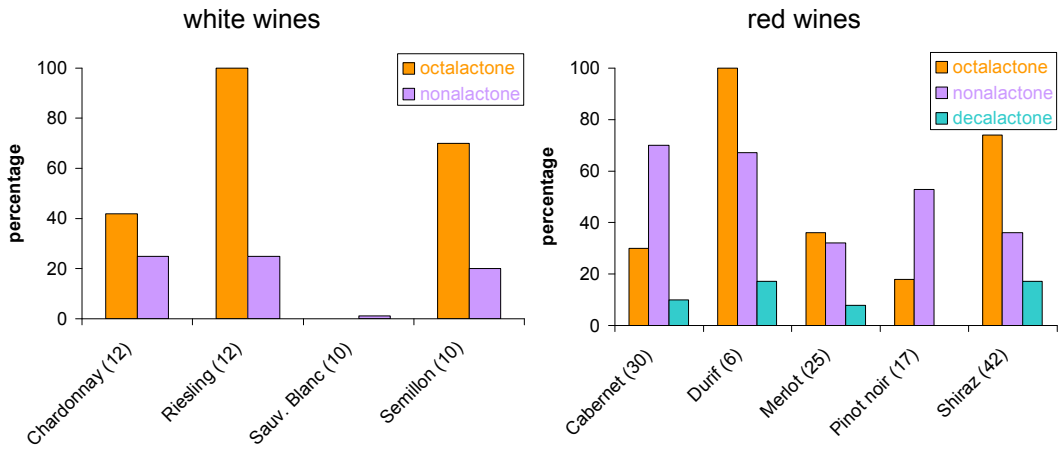


Figure 7.8 Wines grouped by concentration for γ-decalactone in the red wines

7.6.4 Concentrations measured for the γ-lactones grouped by variety and vintage

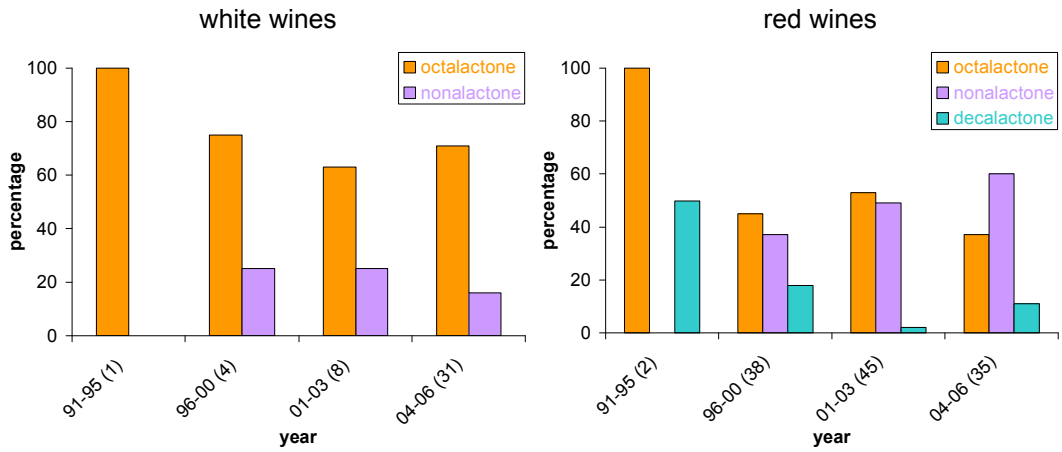
Figure 7.9 shows the percentage of white and red wines that contained γ-octalactone, γ-nonalactone and γ-decalactone grouped by variety. For the white wines, γ-octalactone, the most widespread γ-lactone, was detected in all the Riesling wines, a majority of the Semillon wines and just under half of the Chardonnay wines, while γ-nonalactone was not detected in more than a quarter of any of the white wine varieties. For the red wines, γ-octalactone, γ-nonalactone and γ-decalactone were detected across the range of varieties, with the exception of γ-decalactone which was not measured in any of the Pinot Noir wines. γ-Decalactone was detected in only a small percentage of the different red wine varieties (less than a quarter), while γ-octalactone and γ-nonalactone were both present, on average, in just over half of the red wines analysed.



Note: number in parentheses indicates total number of wines analysed for that variety

Figure 7.9 Percentage of white and red wines that contained γ-octalactone, γ-nonalactone and γ-decalactone grouped by variety (above LOD)

Figure 7.10 shows the percentage of white and red wines that contained γ-octalactone, γ-nonalactone and γ-decalactone grouped by vintage. For the white wines, both γ-octalactone and γ-nonalactone were found across the range of different vintages, with the exception of γ-nonalactone which was not detected in the earliest year bracket (1991-1995) although there was only one wine analysed in this group. For the red wines, γ-octalactone, γ-nonalactone and γ-decalactone were detected throughout the range of vintages, again with the exception of γ-nonalactone which was not found in the earliest age bracket (1991-1995) although there were only two wines analysed in this group.



Note: number in parentheses indicates total number of wines analysed for that year period

Figure 7.10 Results grouped by age as a percentage of white and red wines analysed for that age bracket (above LOD)

7.7 Summary

The initial quantification methodology of γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone with HS SPME and synthetic d_5-analogues of the γ-lactones as internal standards was successful in the production of calibration functions over the concentration range of 0-100 µg/L. The SIDA method was confirmed for reproducibility and precision with the wine used for method development. During the analysis of multiple commercial wine samples, co-eluters sometimes interfered with the measurement of the target and qualifier ions of both the analytes and deuterated analogues. An existing method which employed SPE was therefore modified to incorporate synthetic d_7-analogues of the γ-lactones as internal standards. The reproducibility and precision of the method were confirmed to be excellent (less than 10%) with a low LOD (0.10 µg/L).

A large wine survey was completed and the levels of γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone were quantified in 44 white and 120 red wines. γ-Octalactone was the most common γ-lactone across the range of white wines (average of 0.69 µg/L and 0.50 µg/L in white and red wines, respectively), γ-nonalactone was found to be the most abundant lactone in both white and red wines (average of 0.71 µg/L and 4.95 µg/L in white and red wines, respectively), γ-

decalactone was measured only in the red wines (average of 0.74 μg/L) and γ-dodecalactone was not measured above the limit of detection (0.10 μg/L).

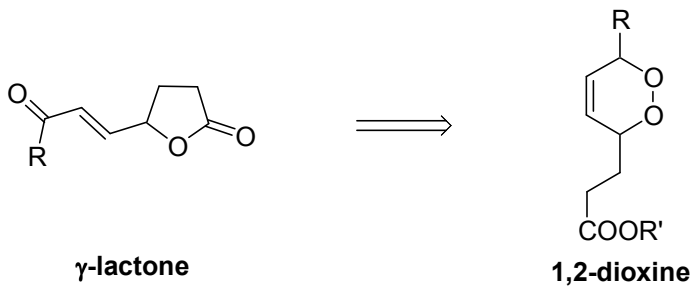
8 Synthesis of optically pure γ-lactones and development of chiral quantification method

Due to the presence of a stereocentre in the γ-lactone structure, the compounds exist as pairs of enantiomers. This chapter discusses the work completed in order to investigate the distribution of the individual enantiomers in wine.

8.1 Attempted synthesis using 1,2-dioxine chemistry

8.1.1 Proposed synthetic methodology

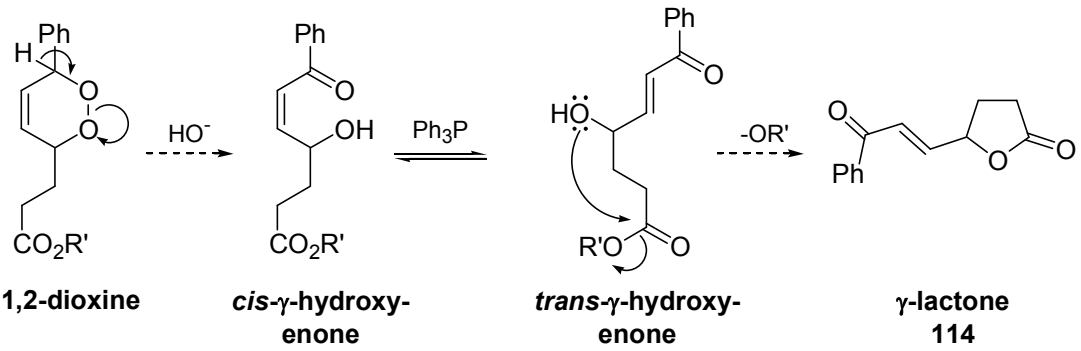
Following the successful utilisation of 1,2-dioxine chemistry in the synthesis of the oak lactones (see Chapter 3), it was anticipated that a suitably substituted 1,2-dioxine could be manipulated to prepare the (*R*)- and (*S*)-enantiomers of γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone. The 1,2-dioxine would again feature as the key intermediate, prepared from a series of standard chemical transformations, in the fundamental reaction step to form the γ-lactone backbone. Rather than employing the 1,2-dioxine in a 1,4-nucleophilic addition reaction, as described in Chapter 3 for the synthesis of the oak lactones, the objective for this reaction step was to form the γ-lactone backbone from a lactonisation step (Scheme 8.1).



γ-lactone **1,2-dioxine**

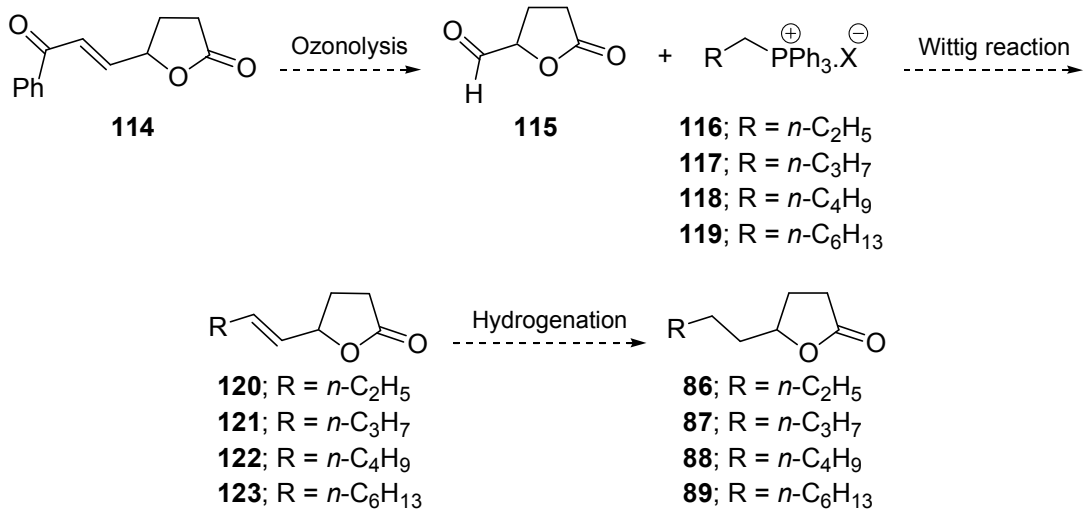
Scheme 8.1

cis-γ-Hydroxyenones are known to form from 1,2-dioxines by base catalysis or by use of a transition metal (see Chapter 2).[83] When an amine base is employed, *cis*-γ-hydroxyenones can undergo rearrangement to form their isomeric 1,4-diketones. This rearrangement can be avoided with the use of a hydroxyl base. The intermediate enone is known to be in equilibrium with its hemiacetal equivalent. The functionality of the γ-hydroxyenone can be exploited by isomerisation to produce the *trans*-isomer, where formation of the corresponding hemiacetal is not possible. It was anticipated that the *trans*-enone intermediate, prepared from a suitably substituted 1,2-dioxine, would undergo intramolecular transesterification (i.e. lactonisation) to give the γ-lactone moiety (Scheme 8.2).



Scheme 8.2

It was proposed that the final γ-lactone products could be prepared by ozonolysis followed by a series of Wittig reactions for the addition of the various alkyl chains. The final products would be obtained after a series of hydrogenation reactions (Scheme 8.3).



Scheme 8.3

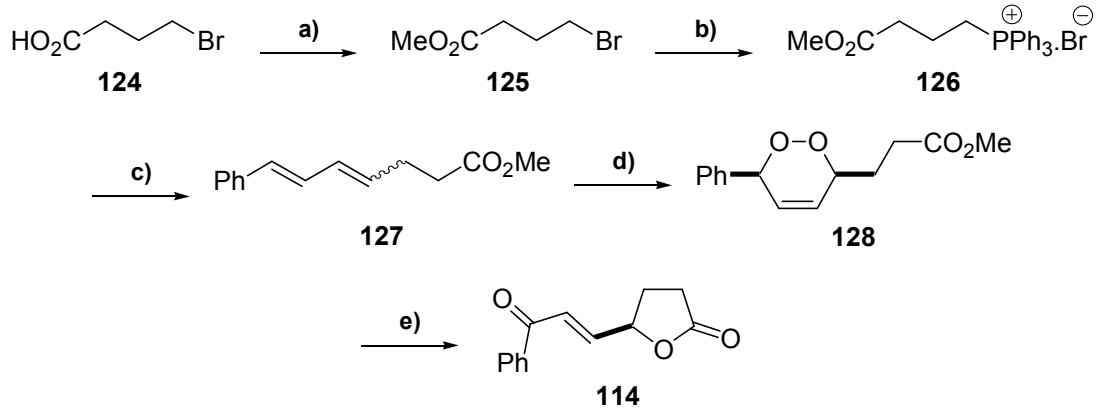
8.1.2 Validation of the methodology

There were two novel points in the methodology that required verification for the synthesis of the optically pure γ-lactones. Firstly, the synthesis of the γ-lactone unit from a 1,2-dioxine required verification. This reaction would form the lactone moiety, from which the final products would be synthesised by appropriate chemical transformations. Secondly, a resolution step was required to separate the stereoisomeric 1,2-dioxines. The success of the diastereomeric separation was crucial for the preparation of optically pure products, necessary for sensory threshold studies. Thus, it was envisaged that both aspects would be addressed separately and amalgamated once optimised for the overall synthesis of the γ-lactones.

A preliminary study was completed for the synthesis of racemic 1,2-dioxine **128** from the ester **125** to validate the photolysis reaction with an alkyl extended methyl ester functionality at the C_6 position in the 1,2-dioxine (Scheme 8.4). 4-Bromobutyric acid (**124**) was esterified in methanol using Dowex ion exchange resin as a mild source of acid. Ester **125** was converted into its corresponding phosphorane salt **126** by treatment with triphenyl phosphine in acetonitrile. A standard Wittig reaction using cinnamaldehyde then gave the desired diene **127** in excellent yield (86%). Spectral analysis by ^{1}H NMR showed the product to be a mixture comprising approximately 90% of the (*E*,*Z*) isomer. A cycloaddition reaction between this diene and singlet oxygen then gave the desired dioxine **128** in

reasonable yield (60%) after column chromatography.

With 1,2-dioxine **128** in hand, the lactonisation reaction was explored. The first reaction featured 40% triphenyl phosphine with an equi-molar amount of lithium hydroxide in tetrahydrofuran. After a period of five days, a small amount of product **114** was isolated by column chromatography (15%, or 28% based on recovered starting material). The reaction showed promise and further attempts were made with a) 50% triphenyl phosphine and an equi-molar amount of lithium hydroxide, and b) an equi-molar amount of triphenyl phosphine and a five-fold excess of lithium hydroxide. Neither of these attempts improved on the yield obtained in the original reaction.



Reagents and conditions: a) Dowex, MeOH, Δ, 24 hrs, 59%; **b)** Ph_3P, MeCN, Δ, 48 hrs, 79%; **c)** i. NaH, 0 °C, DMF, THF; ii. cinnamaldehyde, 0 °C-rt, THF, 16 hrs, 86%; **d)** O_2, rose bengal, 0 °C, CH_2Cl_2, 6.5 hrs, 60%; **e)** LiOH, Ph_3P, THF, rt, 5 days, 28%.

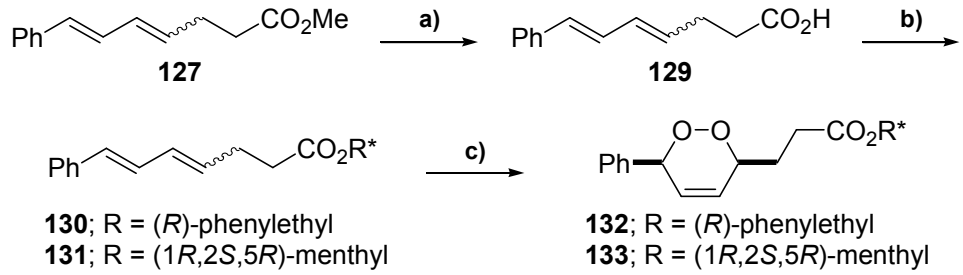
Scheme 8.4

Concurrently, separation of the diastereomeric dioxine was investigated. It was felt necessary to use a chiral resolving group to enable separation of the diastereomers produced in the photolysis reaction. Subsequent reaction of each with lithium hydroxide and triphenyl phosphine should produce enantiomerically pure samples.

Diene **127** was hydrolysed with sodium hydroxide in 10% aqueous methanol to give dienoic acid **129** in excellent yield (95%). Two different chiral alcohols were investigated for their potential to serve as a resolving agent: (*R*)-1-phenylethanol and (1*R*,2*S*,5*R*)-menthol were each coupled with diene acid **129** and successfully used to

produce the corresponding diastereomeric 1,2-dioxines, **132** and **133**, in moderate yields (60% and 55%, respectively).

Following an initial purification step for the removal of the rose bengal, 1,2-dioxines **132** and **133** were subjected to a wide range of different solvent systems using thin layer chromatography. Despite the extensive search for a suitable solvent system, neither mixture could be separated into its constituent isomers. It was decided to abandon this particular route to the lactone aldehyde after an alternative synthetic route was found that did not require resolution.



127 **129**

130; R = (*R*)-phenylethyl
131; R = (1*R*,2*S*,5*R*)-menthyl

132; R = (*R*)-phenylethyl
133; R = (1*R*,2*S*,5*R*)-menthyl

Reagents and conditions: a) NaOH, 10% H_2O/MeOH, rt, 23 hrs, 95%; **b)** R*-OH, DMAP, DCC, CH_2Cl_2, rt, 23 hrs, 63% for **130**; R*-OH, $(COCl)_2$, CH_2Cl_2, rt, 16 hrs, 12% for **131**; **c)** O_2, rose bengal, 0 °C, CH_2Cl_2, 9-10 hrs, 60% for **132**, 55% for **133**.

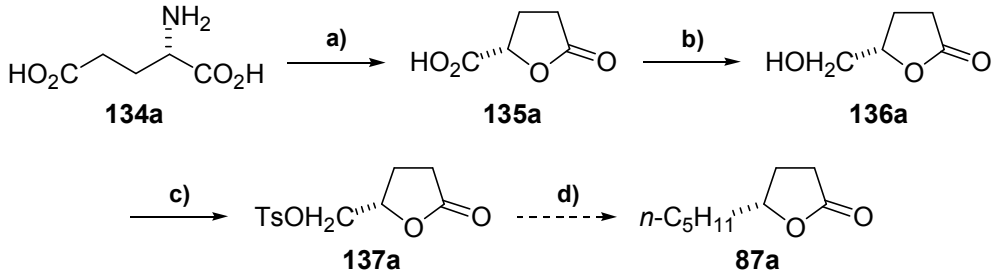
Scheme 8.5

8.2 Synthesis of γ-lactones from glutamic acid

8.2.1 Cuprate addition to tosylate

A general synthesis of optically active five-alkyl or five-alkenyl γ-lactones has been reported from the commercially available enantiomers of glutamic acid.[186,187] These were for the synthesis of both the (*R*)- and (*S*)-enantiomers of γ-caprolactone, γ-nonalactone and γ-6-dodecen-5-olide. The key step was a selective tosylate displacement reaction, rather than ring opening of the lactone, through addition of lithium dialkylcuprate or dialkenylcuprate (see below).[188] This reported procedure was chosen as a suitable methodology for the synthesis of the enantiomers of γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone.

L-Glutamic acid (**134a**) was treated with nitrous acid (generated *in situ* from hydrochloric acid and sodium nitrite) to produce acid **135a**. The reaction is known to proceed with complete retention of configuration.[189] Acid **135a** was obtained in low yield (37%), consistent with the findings reported in the literature.[187] The solubility of the acid was a hindrance during purification when trying to remove the sodium chloride by-product. The subsequent step involved reduction of acid **135a** to alcohol **136a**. The reaction proceeded cleanly with borane dimethyl sulfide as the reducing agent to give alcohol **136a** in excellent yield following purification by column chromatography. Tosylation of alcohol **136a** with tosyl chloride in anhydrous pyridine was the final step prior to cuprate addition. Tosylate **137a** was recrystallised and collected as a fine white material. Spectral data for both the alcohol and tosylate were in good agreement with the literature.[187] With the key intermediate at hand, the tosylate displacement reaction was attempted. Lithium di-*n*-butylcuprate was generated *in situ* by the addition of *n*-butyllithium to a suspension of copper (I) cyanide and to this was added tosylate **137a**. Despite literature precedents for the preparation of γ-lactones from cuprate additions to tosylated γ-lactones, the reaction was unsuccessful. The formation of γ-nonalactone (**87a**) was not detected in any of the attempts made (Scheme 8.6).



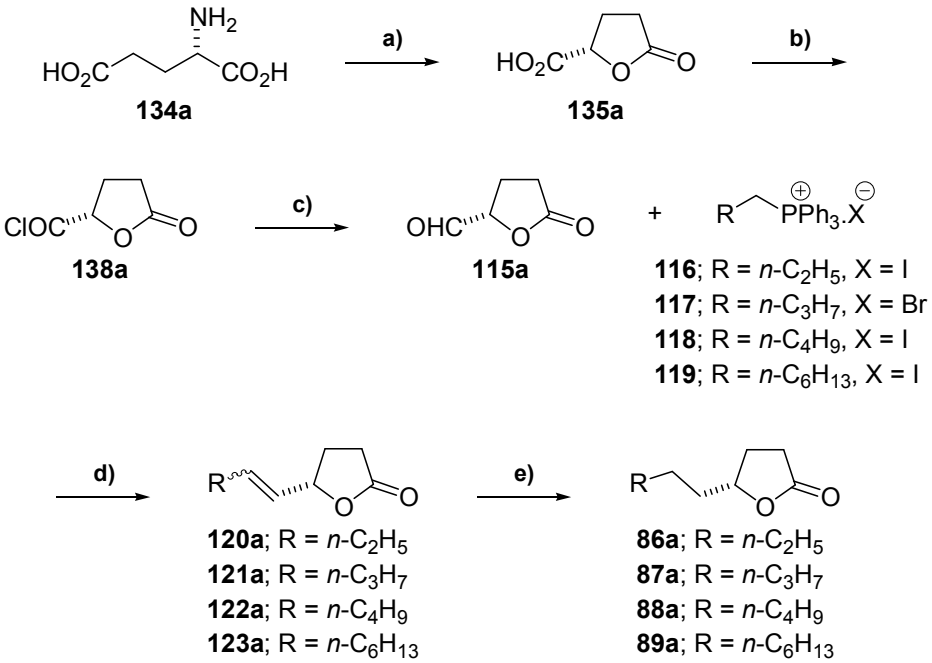
Reagents and conditions: a) HCl, $NaNO_2$, 0 °C-rt, H_2O, 16 hrs, 37%; **b)** i. $BH_3.Me_2S$, THF, rt, 2.5 hrs; ii. 50% H_2O/THF, 84%; **c)** TsCl, 0 °C-rt, pyr, 16 hrs, 45%; **d)** *n*-BuLi, Cu(I)CN, -78 °C-rt, THF, 3.5 hrs.

Scheme 8.6

8.2.2 Wittig reaction for alkyl chain addition

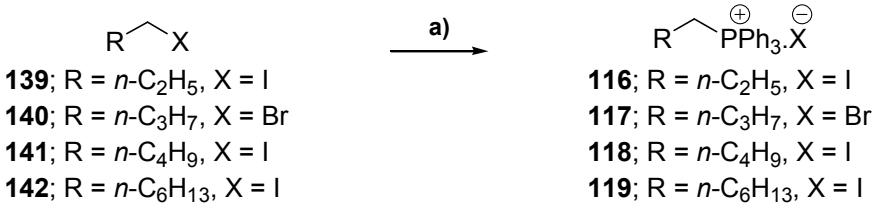
Following a procedure for the synthesis of the sex pheromone of the Japanese beetle, (*R*,*Z*)-5-1-decenyldihydro-2(3H)-furanone, the individual enantiomers of γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone were prepared.[190]

This sequence began with L- or D-glutamic acids and utilised the stereospecificity of the deamination reaction, as outlined above, shown in Scheme 8.7. Acid **135a** was converted to acid chloride **138a** and used immediately to prepare aldehyde **115a**. Rosenmund reduction of acid chloride **138a** to aldehyde **115a** was undertaken at 60-70 °C using hydrogen gas in toluene with palladium on barium sulfate as the catalyst. Attempts to purify this aldehyde were unsuccessful and it was therefore used immediately upon preparation.[190] The key step in this pathway was the Wittig reaction that enabled the addition of the alkyl side chain. By altering the Wittig salt (*n*-propyl **116**, *n*-butyl **117**, *n*-pentyl **118** and *n*-heptyl **119**) (Scheme 8.8), the length of the side chain was easily manipulated to produce the desired γ-lactone. Due to the use of acid chloride **138a** and aldehyde **115a** *in situ*, the yields of alkenyl γ-lactones **120a**, **121a**, **122a** and **123a** were calculated over the three step sequence beginning with acid **135a** (Table 8.1). The alkenyl γ-lactones were obtained over a range of yields (13-64%) with the average yield, for the three steps, being quite modest (43%).



Reagents and conditions: a) HCl, $NaNO_2$, 0 °C-rt, H_2O, 16 hrs, 37%; **b)** $(COCl)_2$, Δ, CH_2Cl_2, 2.75 hrs; **c)** H_2, Pd-$BaSO_4$, PhMe, 3 hrs; **d)** $(CH_3)_3COK$, 0 °C-rt, THF, 1.5 hrs; **e)** H_2, Pd-$BaSO_4$, rt, EtOAc, 6-72 hrs.

Scheme 8.7



139; R = *n*-C_2H_5, X = I
140; R = *n*-C_3H_7, X = Br
141; R = *n*-C_4H_9, X = I
142; R = *n*-C_6H_{13}, X = I

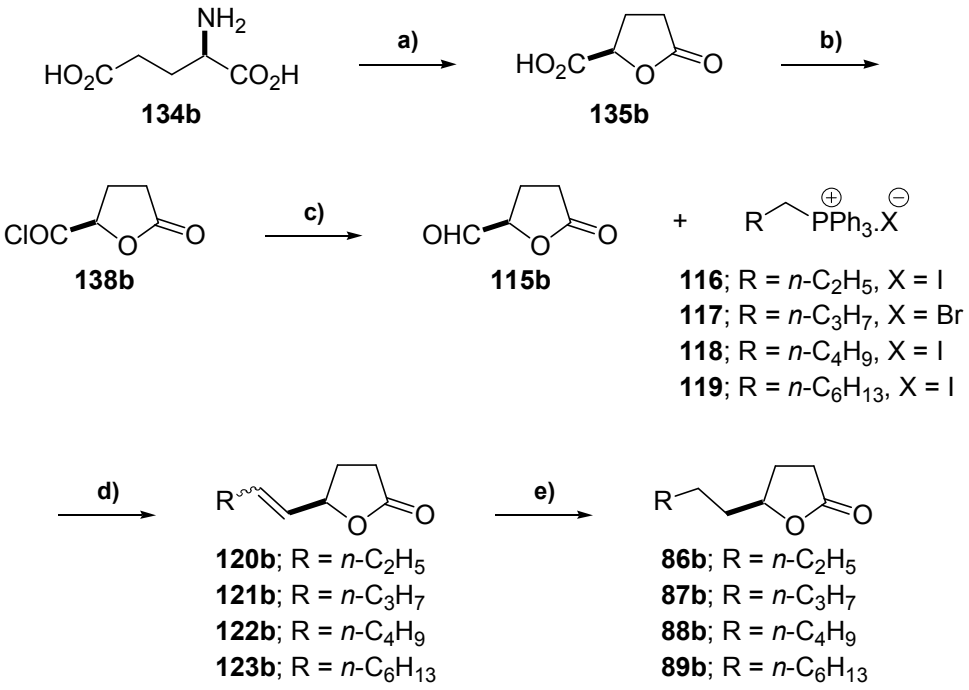
116; R = *n*-C_2H_5, X = I
117; R = *n*-C_3H_7, X = Br
118; R = *n*-C_4H_9, X = I
119; R = *n*-C_6H_{13}, X = I

Reagents and conditions: a) PPh_3, Δ, PhMe, 93% for **116**, 70% for **117**, 96% for **118**; PPh_3, Δ, MeCN, 99% for **119**.

Scheme 8.8

The final γ-lactone products were obtained after hydrogenation of the corresponding alkenyl precursors. A range of catalysts was trialled including palladium on carbon, palladium on barium sulfate, palladium hydroxide, palladium on calcium carbonate and, despite literature reports on the use of rhodium on carbon,[190] were all unsuccessful in the satisfactory hydrogenation of the alkenyl γ-lactones. As reported in the literature, extensive ring opening and the production of large quantities of the alkyl carboxylic acids were detected.[190] The most effective means of hydrogenation was through the use of palladium on barium sulfate, although the presence of the ring opened acid was detected in all hydrogenation reactions. Purification by column chromatography with aluminium oxide plug followed by silica gel proved to be the best method for sample clean up, with final products **86a**, **87a**, **88a** and **89a** with (*R*)-configuration obtained in low to excellent yields (32-79%) (Table 8.1).

In an identical manner, D-glutamic acid (**134b**) was used to prepare the series of (*S*)-configured γ-lactones, **86b**, **87b**, **88b** and **89b** (Scheme 8.9), with the yields also contained in Table 8.1.



Reagents and conditions: a) HCl, $NaNO_2$, 0 °C-rt, H_2O, 16 hrs, 37%; **b)** $(COCl)_2$, Δ, CH_2Cl_2, 2.75 hrs; **c)** H_2, Pd-$BaSO_4$, PhMe, 3 hrs; **d)** $(CH_3)_3COK$, 0 °C-rt, THF, 1.5 hrs; **e)** H_2, Pd-$BaSO_4$, rt, EtOAc, 6-72 hrs.

Scheme 8.9

Table 8.1 Experimental yields for alkenyl and final γ-lactone products

	isomer	**alkene**[a]	**γ-lactone**[b]	**$[\alpha]_D$ γ-lactone**	**ee**
γ-octalactone	*R*	52% (**120a**)	32% (**86a**)	+52.9 (**86a**)	> 98%
	S	45% (**120b**)	41% (**86b**)	-52.2 (**86b**)	> 98%
γ-nonalactone	*R*	43% (**121a**)	79% (**87a**)	+47.9 (**87a**)	> 98%
	S	64% (**121b**)	63% (**87b**)	-49.8 (**87b**)	> 98%
γ-decalactone	*R*	13% (**122a**)	63% (**88a**)	+48.4 (**88a**)	> 98%
	S	47% (**122b**)	57% (**88b**)	-44.3 (**88b**)	> 98%
γ-dodecalactone	*R*	35% (**123a**)	53% (**89a**)	+41.9 (**89a**)	> 98%
	S	43% (**123b**)	61% (**89b**)	-41.0 (**89b**)	> 98%

Note: number for chemical structure denoted in parentheses; [a] percentage yield from acid lactone **135a** or **135b**; [b] percentage yield from the respective alkenyl lactone **120a**, **120b**, **121a**, **121b**, **122a**, **122b**, **123a**, or **123b**

8.3 Development of chiral quantification method

8.3.1 Chiral analysis of synthetic γ-lactone samples

The optically pure γ-lactone compounds synthesised were analysed by NMR spectroscopy to determine their overall purity. From both ^{1}H and ^{13}C NMR spectrum, it was evident that each synthetic sample was of extremely high purity. Chiral GC-MS analysis (Figure 8.1 for racemic γ-lactones) showed each sample to have an enantiomeric excess of > 98% (Figure 8.2). This was of utmost importance for the sensory studies as discussed later (Chapter 9). From the elution times of the prepared compounds, it was observed that the (*R)*-enantiomers eluted first in all cases. The same target and qualifier ions were used for the chiral quantification method as chosen for the solid-phase extraction method (Table 8.2).

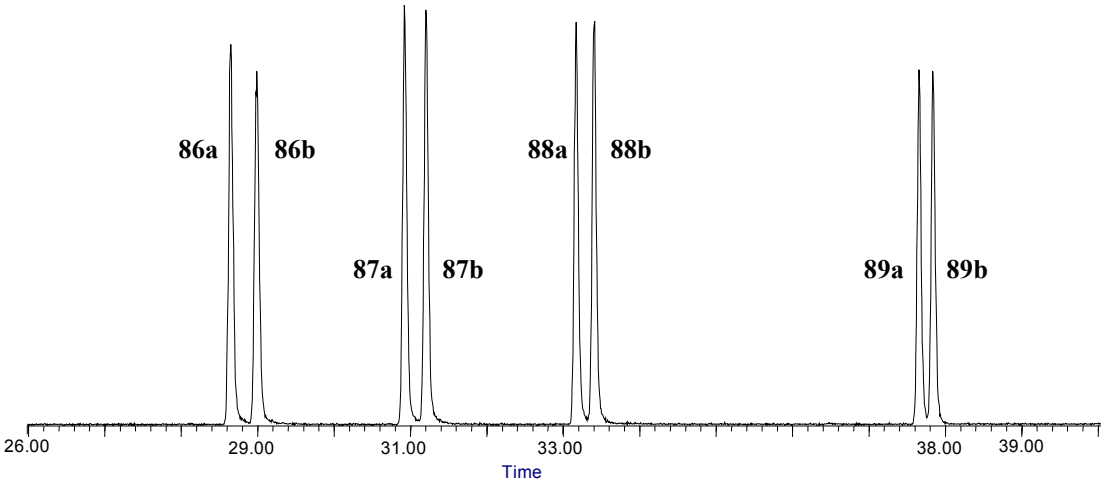


Figure 8.1 Chromatogram for racemic γ-lactones on a chiral column

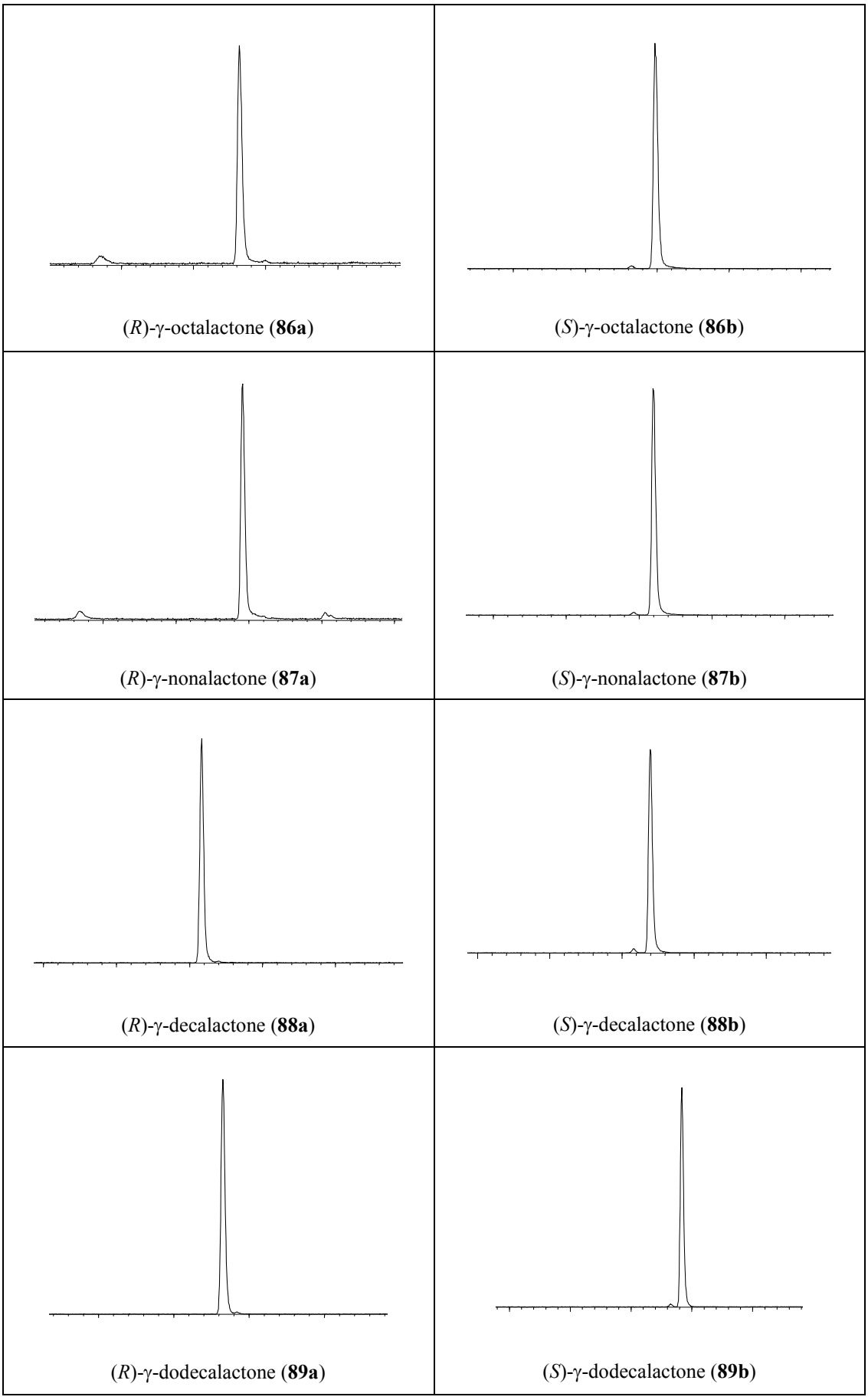


Figure 8.2 Chromatograms for synthetic γ-lactones on a chiral column

Table 8.2 Target and qualifier ions monitored in SIM mode

	retention time	target *m/z*	qualifiers *m/z*
(*R*)-γ-octalactone (**86a**)	28.418	85	86, 100
d_7-(*R*)-γ-octalactone (**d_7-86a**)	28.453	90	91, 107
(*S*)-γ-octalactone (**86b**)	28.764	85	86, 100
d_7-(*S*)-γ-octalactone (**d_7-86b**)	28.798	90	91, 107
(*R*)-γ-nonalactone (**87a**)	30.712	85	86, 100, 128
d_7-(*R*)-γ-nonalactone (**d_7-87a**)	30.719	90	91, 107, 135
(*S*)-γ-nonalactone (**87b**)	30.988	85	86, 100, 128
d_7-(*S*)-γ-nonalactone (**d_7-87b**)	30.995	90	91, 107, 135
(*R*)-γ-decalactone (**88a**)	32.957	85	86, 100, 128
d_7-(*R*)-γ-decalactone (**d_7-88a**)	32.944	90	91, 107, 135
(*S*)-γ-decalactone (**88b**)	33.192	85	86, 100, 128
d_7-(*S*)-γ-decalactone (**d_7-88b**)	33.179	90	91, 107, 135
(*R*)-γ-dodecalactone (**89a**)	37.462	85	86, 100, 128
d_7-(*R*)-γ-dodecalactone (**d_7-89a**)	37.455	90	91, 107, 135
(*S*)-γ-dodecalactone (**89b**)	37.649	85	86, 100, 128
d_7-(*S*)-γ-dodecalactone (**d_7-89b**)	37.642	90	91, 107, 135

8.3.2 Calibration functions, reproducibility and precision of method for chiral analysis

The analytical method described previously (see Chapter 7) was extended for use with a chiral gas chromatography column, using the d_7-analogues as internal standards. Since the isotopically labelled standards were prepared from commercially available γ-lactones, i.e. racemic mixtures, the d_7-analogues existed as equal mixtures of (*R*)- and (*S*)-enantiomers. The calibration functions were developed from the prepared wine extracts purified through the SPE procedure in white and red wine (Figure 8.3). The calibration functions are shown both graphically (Figure 8.3) and in tabular form (Table 8.3). Precision and accuracy of the method were verified as before, at two different concentrations, 2.5 μg/L and 12.5 μg/L, with the difference between the spiked level and the average measured level falling within the acceptable range of error. The limit of detection was determined to be 2.00 μg/L.

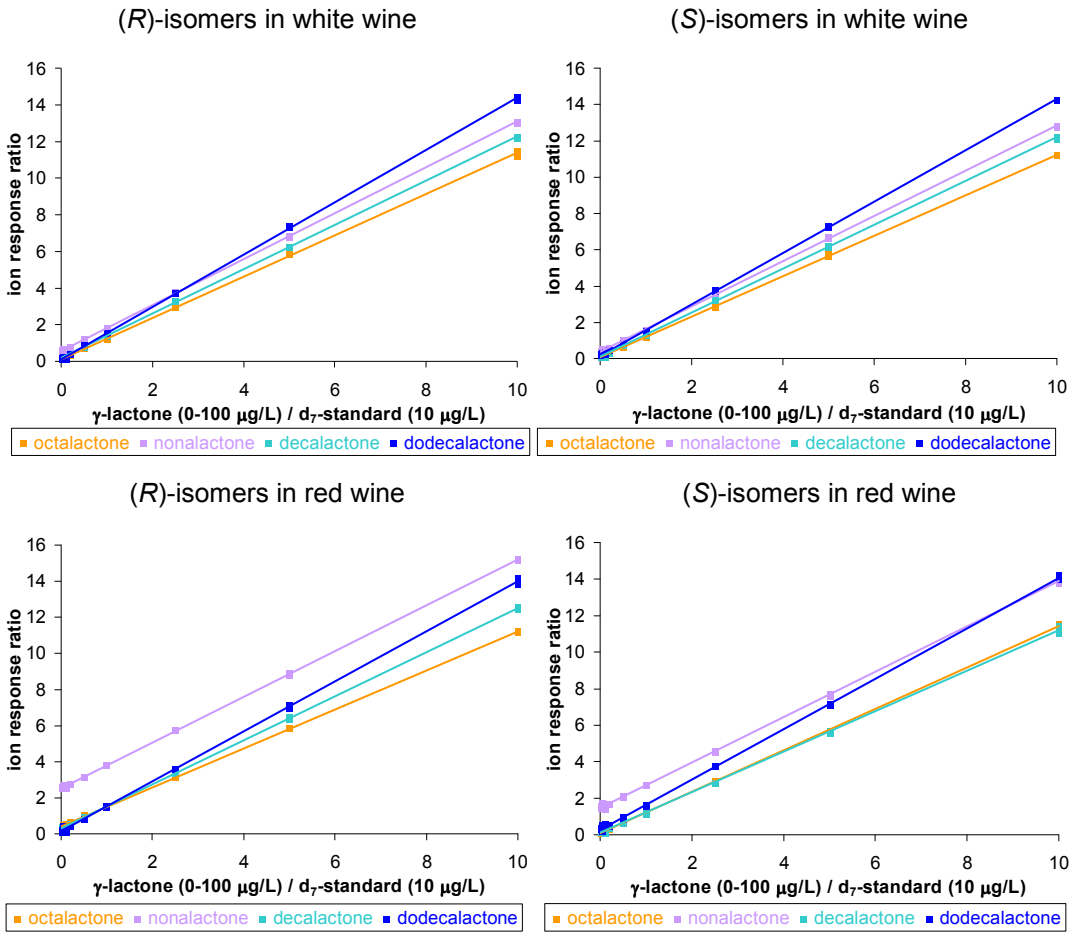


Figure 8.3 Calibration functions for (*R*)- and (*S*)-γ-lactones in a white and a red wine

Table 8.3 Correlation coefficients (r^2) and equation of the line for the calibration functions in a white and a red wine

	enantiomer	r^2	equation of line
		white wine	
γ-octalactone	*R* (**86a**)	1.000[a]	y = 1.13x + 0.12
	S (**86b**)	1.000[a]	y = 1.12x + 0.07
γ-nonalactone	*R* (**87a**)	1.000[a]	y = 1.25x + 0.57
	S (**87b**)	1.000[a]	y = 1.25x + 0.37
γ-decalactone	*R* (**88a**)	1.000[a]	y = 1.21x + 0.17
	S (**88b**)	1.000[a]	y = 1.21x + 0.10
γ-dodecalactone	*R* (**89a**)	1.000[a]	y = 1.43x + 0.11
	S (**89b**)	1.000[a]	y = 1.41x + 0.17
		red wine	
γ-octalactone	*R* (**86a**)	1.000[a]	y = 1.08x + 0.41
	S (**86b**)	1.000[a]	y = 1.13x + 0.07
γ-nonalactone	*R* (**87a**)	1.000[a]	y = 1.27x + 2.53
	S (**87b**)	1.000[a]	y = 1.25x + 1.45
γ-decalactone	*R* (**88a**)	1.000[a]	y = 1.22x + 0.30
	S (**88b**)	1.000[a]	y = 1.11x + 0.10
γ-dodecalactone	*R* (**89a**)	1.000[a]	y = 1.39x + 0.14
	S (**89b**)	0.999[a]	y = 1.38x + 0.27

[a] range 0-50 μg/L; N = 9 x 2 (number of data points, with each being measured in duplicate)

Table 8.4 Comparison of seven replicate determinations for accuracy and precision in a white and a red wine

	isomer	spike (μg/L)	average (μg/L)	standard deviation[a]	difference (%)[b]
white wine					
γ-octalactone	*R* (**86a**)	2.5	2.48	0.03	-0.80
		12.5	12.48	0.04	-0.16
	S (**86b**)	2.5	2.51	0.03	0.40
		12.5	12.52	0.13	0.16
γ-nonalactone	*R* (**87a**)	2.5	2.55	0.06	2.00
		12.5	12.88	0.07	3.04
	S (**87b**)	2.5	2.55	0.08	2.00
		12.5	12.84	0.15	2.72
γ-decalactone	*R* (**88a**)	2.5	2.59	0.05	3.60
		12.5	12.90	0.05	3.20
	S (**88b**)	2.5	2.54	0.02	1.60
		12.5	12.92	0.06	3.36
γ-dodecalactone	*R* (**89a**)	2.5	2.52	0.01	0.80
		12.5	12.55	0.07	0.40
	S (**89b**)	2.5	2.56	0.08	2.40
		12.5	12.47	0.11	-0.24
red wine					
γ-octalactone	*R* (**86a**)	2.5	2.56	0.06	2.40
		12.5	12.45	0.14	-0.40
	S (**86b**)	2.5	2.49	0.06	-0.40
		12.5	12.42	0.13	-0.64
γ-nonalactone	*R* (**87a**)	2.5	2.42	0.05	-3.20
		12.5	12.55	0.17	0.40
	S (**87b**)	2.5	2.56	0.07	2.40
		12.5	12.47	0.18	-0.24
γ-decalactone	*R* (**88a**)	2.5	2.54	0.12	1.60
		12.5	12.51	0.08	0.08
	S (**88b**)	2.5	2.45	0.05	-2.00
		12.5	12.27	0.19	-1.84
γ-dodecalactone	*R* (**89a**)	2.5	2.46	0.03	-1.60
		12.5	12.49	0.17	-0.08
	S (**89b**)	2.5	2.55	0.27	2.00
		12.5	12.30	0.15	-1.60

[a] standard deviation from the average measured level of γ-lactone, [b] percentage difference between the spiked level and the averaged measured level of γ-lactone

8.4 Wine survey

From the initial set of wines analysed using the quantification method (see Chapter 7), the wines with γ-nonalactone ((*R*)- and (*S*)-enantiomers) greater than 4 μg/L were analysed with the chiral analytical method (Table 8.5). On average, the red wines contained slightly higher amounts of the (*R*)-enantiomer (58% for these five wines), with the exception of one wine measured as a racemate of γ-nonalactone.

Table 8.5 Distribution of (*R*)- and (*S*)-enantiomers of γ-nonalactone in red wines

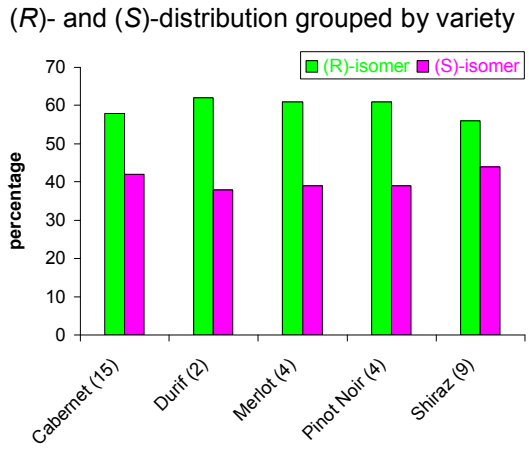
variety	year	concentration (μg/L)	% *R*	% *S*
Cabernet Sauvignon	2004	16.98	61	39
Cabernet Sauvignon	2004	7.28	52	48
Merlot	1998	7.16	58	42
Shiraz	1999	8.83	58	42
Shiraz	2003	9.71	62	38
Shiraz	2004	9.61	50	50

From the large group of wines analysed using the achiral method (see Chapter 7), the wines which contained γ-nonalactone ((*R*)- and (*S*)-enantiomers) greater than 4 μg/L) were analysed using the chiral method (Table 8.6). No other γ-lactones were present in high enough concentrations to warrant chiral analysis. However, should these be detected in other varieties at higher concentrations, as reported by Ferreira *et al*.,[166] then the method for the other γ-lactones can be applied. In the wines analysed using the chiral method, the (*R*)-enantiomer for γ-nonalactone was present, on average, in greater amounts than the (*S*)-enantiomer, across the five different red wine varieties (Figure 8.4). There was however, no overwhelming predominance of any one isomer, with the highest average of 62% for the (*R*)-enantiomer in Durif followed closely by 61% in Pinot Noir (Table 8.6). The average for the (*R*)-enantiomer across all red wines analysed was calculated to be 59%, or an 18% enantiomeric excess in favour of the (*R*)-isomer. Full details of the enantiomeric distribution for γ-nonalactone are listed in Chapter 11 (Appendices).

Table 8.6 Distribution of (*R*)- and (*S*)-enantiomers of γ-nonalactone in red wines

	minimum		maximum		average	
	R	***S***	***R***	***S***	***R***	***S***
Cabernet (15)	46	37	63	54	58	42
Durif (2)	61	38	62	39	62	38
Merlot (4)	48	32	68	52	59	41
Pinot Noir (4)	60	37	63	40	61	39
Shiraz (9)	48	39	61	52	56	44

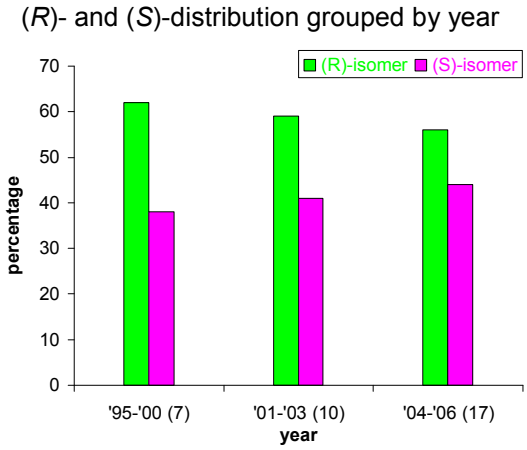
Note: number in parentheses denotes number of wines analysed for that variety



Note: number in parentheses indicates number of wines analysed for that variety

Figure 8.4 Percentage distribution of (*R*)- and (*S*)-enantiomers of γ-nonalactone grouped by variety

Figure 8.5 shows the percentage results for the distribution of the enantiomers of γ-nonalactone grouped by year. The earliest vintage bracket (1995-2000) contained 62% of the (*R*)-stereoisomer, followed by 59% in the next age bracket (2001-2003) and finally 56% in the youngest wines (2004-2006).



Note: number in parentheses indicates number of wines analysed for that year period

Figure 8.5 Percentage distribution of (*R*)- and (*S*)-enantiomers of γ-nonalactone grouped by year

8.5 Summary

The SPE method was successfully extended for use with a chiral gas chromatography column. This enabled the enantiomeric distribution of γ-nonalactone to be investigated. The (*R*)-enantiomer was found to be dominant across the red wines analysed, at an average of 59%, although there were wines analysed with the (*S*)-enantiomer in greater amounts. With further investigations into different wine varieties, it is anticipated that all γ-lactones will be able to be analysed by chiral GC-MS and their enantiomeric ratio identified.

9 Sensory studies on the γ-lactones

To the best of our knowledge, there are no reported odour detection threshold values for the individual stereoisomers of γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone in any medium. Presented in this chapter are the results of the sensory studies completed on the (*R*)- and (*S*)-enantiomers in red wine.

9.1 Determination of odour detection thresholds in red wine

Following the development of the stable isotope dilution assay (SIDA) and its subsequent use for quantification of γ-octalactone, γ-nonalactone, γ-decalactone and γ-decalactone, at varying levels in red and white wine, odour detection thresholds for the enantiomers of these compounds were determined. Although there are limited threshold data for γ-lactones in wine, there are no reported threshold data to date for the individual stereoisomers in any medium. This information was required to determine whether or not these γ-lactones are likely to contribute to the overall aroma of wine at the concentrations measured and to evaluate any sensory differences between the enantiomers. The structures of the γ-lactones are shown in Figure 9.1.

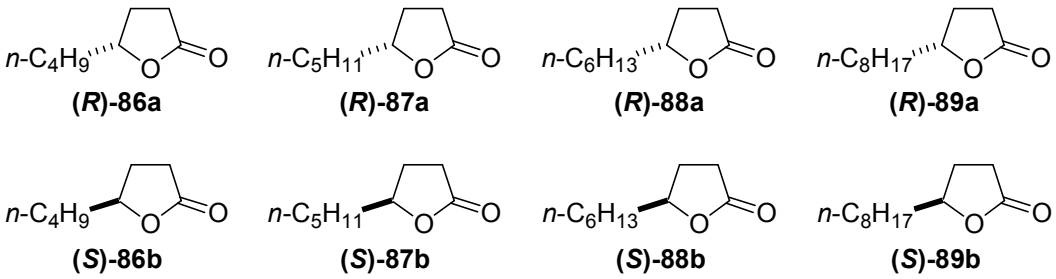


Figure 9.1 The γ-lactones under investigation in this work

From literature sensory reports, racemic γ-nonalactone has a threshold of 30 μg/L in a simple white wine[131] and 460 μg/L in a sweet white wine,[132] while racemic γ-

decalactone has a threshold of 10 μg/L in a model wine solution.[130] Model wine threshold values are not always good indicators of threshold values in wine.[24] It was therefore decided to determine odour threshold values for the individual enantiomers (ee > 98%, see Chapter 8) in real wine.

Red wine was chosen as the medium for this sensory threshold study. With the γ-lactones consistently detected at significantly higher levels in red wines, in agreement with literature reports,[166] it has been proposed that they are more likely to be important to the aroma of red wine than to that of white wine.

The odour thresholds were determined in a red wine for each stereoisomer of γ-octalactone (**86a** and **86b**), γ-nonalactone (**87a** and **87b**), γ-decalactone (**88a** and **88b**) and γ-dodecalactone (**89a** and **89b**) using the American Standards for Testings and Materials (ASTM) method E679[120] (Table 9.1) (see Chapter 4 for details of method used). There were 25 judges who participated in each threshold test. For each judge, an individual best estimate threshold (BET) was calculated and the group threshold was determined as the geometric mean of the individual BETs. Full details of the odour threshold data are listed in Chapter 11 (Appendices).

Table 9.1 Group odour detection threshold values in red wine (μg/L)

isomer	γ-octalactone	γ-nonalactone	γ-decalactone	γ-dodecalactone
R	238 (**86a**)	285 (**87a**)	34 (**88a**)	8 (**89a**)
S	135 (**86b**)	91 (**87b**)	47 (**88b**)	39 (**89b**)

Note: compound number identified in parentheses

The distributions of the individual BET values for each pair of enantiomers for the γ-lactones are presented in Figure 9.2 to Figure 9.5. It is interesting to note the wide range in sensitivity of the panellists to each compound.

In this study, (*R*)-γ-octalactone (**86a**) had an odour threshold of 238 μg/L and (*S*)-γ-octalactone (**86b**) of 135 μg/L. The distributions for both (*R*)-γ-octalactone (**86a**) and (*S*)-γ-octalactone (**86b**) broadly fit a typical bell curve (Figure 9.2).

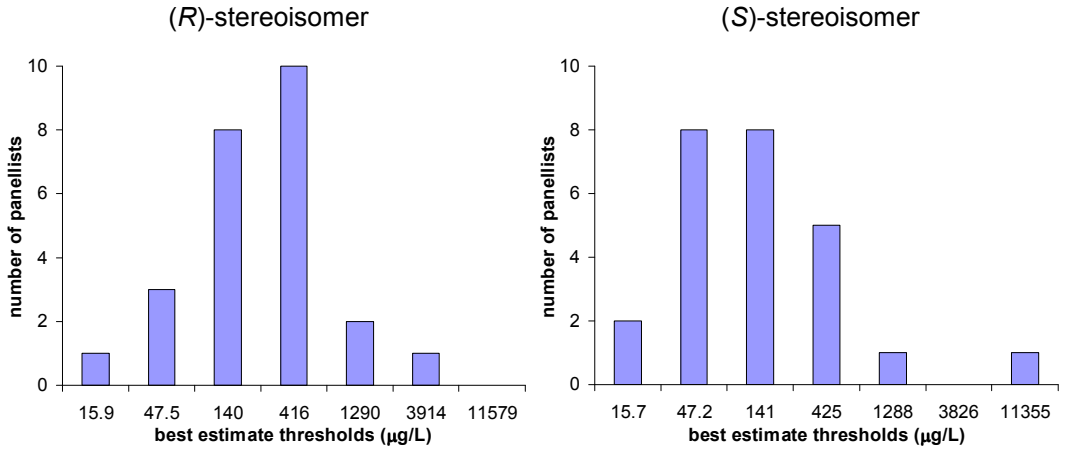


Figure 9.2 Histograms showing the distribution of best estimate thresholds for the enantiomers of γ-octalactone (86a and 86b)

(*R*)-γ-Nonalactone (**87a**) was determined to have the highest odour threshold value of all the compounds tested, at 285 µg/L, while its enantiomeric counterpart, (*S*)-γ-nonalactone (**87b**), had a threshold approximately one third that value, at 91 µg/L. The distribution for (*R*)-nonalactone (**87a**) showed a relatively even spread of panellists across the concentration range, while (*S*)-nonalactone (**87b**) appeared to feature two sub-populations of judges displayed with the two skewed bell curves (Figure 9.3).

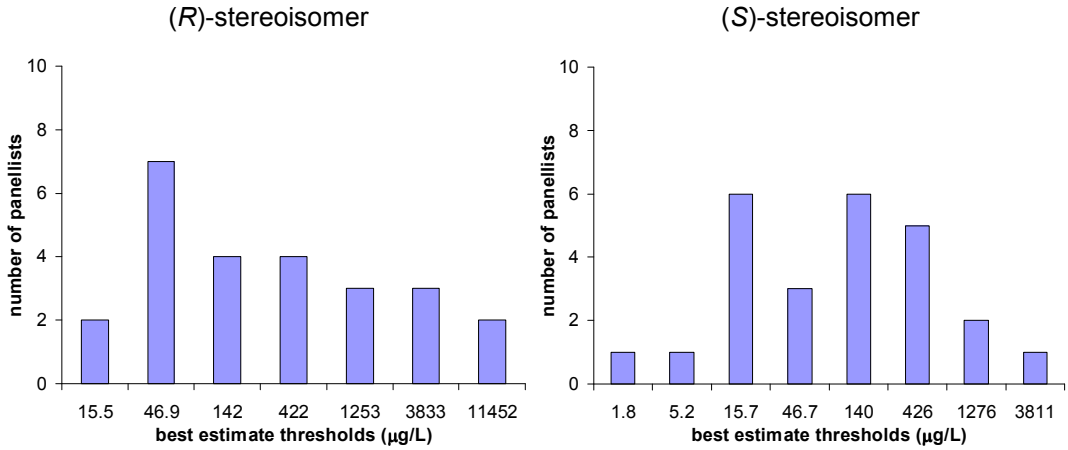


Figure 9.3 Histograms showing the distribution of best estimate thresholds for the enantiomers of γ-nonalactone (87a and 87b)

(*R*)-γ-Decalactone (**88a**) and (*S*)-γ-decalactone (**88b**) were the only pair of enantiomers with similar thresholds, 34 µg/L and 47 µg/L, respectively. The best

estimate thresholds for (*R*)-decalactone (**88a**) fit a skewed bell curve, while the distribution for (*S*)-decalactone (**88b**) is concentrated largely around 16 μg/L (Figure 9.4).

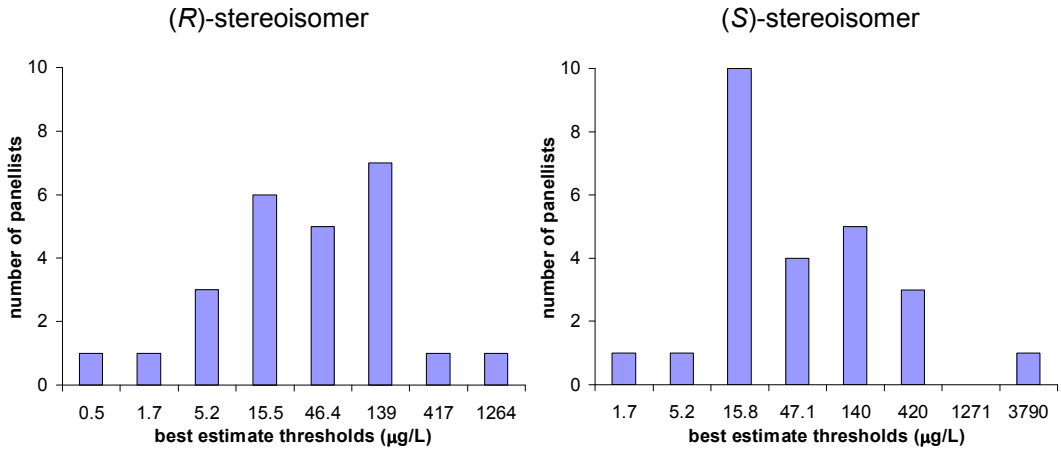


Figure 9.4 Histograms showing the distribution of best estimate thresholds for the enantiomers of γ-decalactone (88a and 88b)

(*R*)-γ-Dodecalactone (**89a**) was found to be the most potent compound in this study, with an odour detection threshold value of 8 μg/L. Its enantiomer, (*S*)-γ-dodecalactone (**89b**), had an odour threshold value of 39 μg/L, almost five times the value of (*R*)-isomer **89a** – the largest difference found amongst the pairs of enantiomers for this series of γ-lactones. Although there were 25 judges who participated in the threshold test for (*S*)-γ-dodecalactone (**89b**), there was one panel member who was not assigned a threshold value as she was anosmic to this compound and was therefore excluded from the calculations. The distribution for both γ-dodecalactones (**89a** and **89b**) featured two apparent sub-populations. When a significant outlier at the high end of the concentration range for the (*R*)-enantiomer was omitted, the threshold decreased to 6 μg/L.

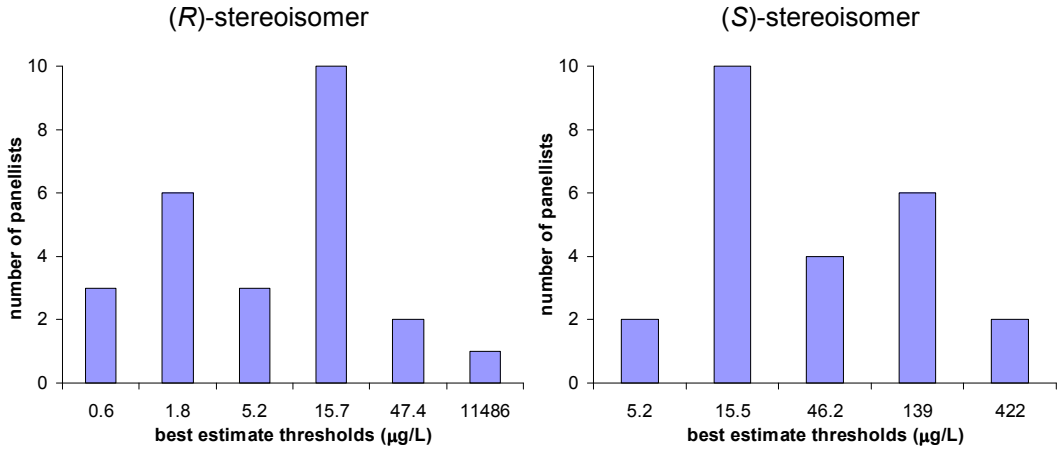


Figure 9.5 Histograms showing the distribution of best estimate thresholds for the enantiomers of γ-dodecalactone (89a and 89b)

9.2 Odour threshold value trends across the series of (*R*)- and (*S*)-enantiomers for the γ-lactones

A general trend for a decrease in odour threshold value was observed with an increase in alkyl chain length (Figure 9.6). This trend was substantially stronger for the (*R*)-series (with the exception of γ-nonalactone (**87a**)) than with the (*S*)-series (Table 9.1). There was a decrease in threshold value for the (*R*)-enantiomers from γ-octalactone (**86a**) (238 µg/L) to γ-dodecalactone (**89a**) (8 µg/L) of nearly thirty-fold, but the corresponding decrease in threshold value for the (*S*)-enantiomers from γ-octalactone (**86b**) (135 µg/L) to γ-dodecalactone (**89b**) (39 µg/L) was only by a factor of three and a half. As a result, the threshold value for the (*R*)-stereoisomer of γ-octalactone (**86a**) (238 µg/L) was higher than its enantiomeric counterpart **86b** (135 µg/L), while the threshold value for the (*S*)-stereoisomer of γ-dodecalactone (**89b**) (39 µg/L) was significantly higher than its enantiomeric counterpart **89b** (8 µg/L) (Table 9.1).

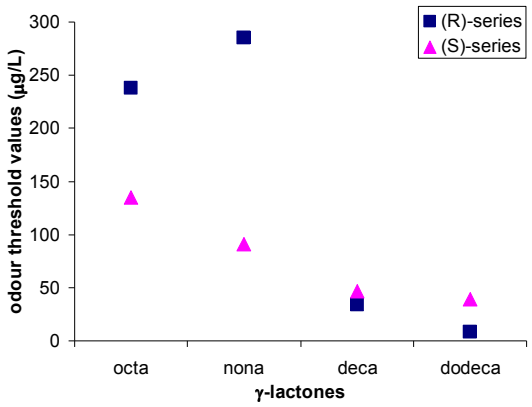


Figure 9.6 Group odour threshold values for the γ-lactones as a function of alkyl chain length

9.3 Conclusions

Odour threshold values have been determine for each enantiomer of γ-octalactone (**86a** and **86b**), γ-nonalactone (**87a** and **87b**), γ-decalactone (**88a** and **88b**) and γ-dodecalactone (**89a** and **89b**) in a red wine. (*R*)-γ-dodecalactone (**89a**) was found to be the most potent compound in this series, with an odour threshold of 8 µg/L, while (*R*)-γ-nonalactone (**87a**) was found to be the least potent compound, at 285 µg/L. When the threshold values are related to the concentration of γ-lactones quantified in the red wines, it is evident that the γ-lactones were not measured above their individual odour detection thresholds in any of the Australian wines investigated.

It might be tempting to conclude that this series of γ-lactones do not have any impact on red wine aroma. However, these odour threshold values illustrate merely the concentration at which 50% of the sampled population could detect a difference between a spiked sample, of *one single compound*, compared with the control.

Odour threshold studies by Ferriera *et al*. have been conducted on a mixture of lactones, γ-octalactone, γ-nonalactone, γ- and δ-decalactone, γ-undecalactone and γ-dodecalactone, based on the average concentration at which they were measured in wine.[191] The results indicated these lactones to be significant contributors to wine aroma as a cluster, rather than as individual odorants. The work of Ferriera *et al*.

highlights the synergistic or additive effect a group of aroma compounds could have on the perceived odour, and hence the overall sensory threshold value.[191]

When considering the odour properties of a group of aroma compounds, it is necessary to consider the compounds as a whole and as individual odorants. With a larger number of compounds, there is a greater probability that members on a sensory panel could detect at least one of the odorants under investigation even when each compound is below its odour detection threshold concentration for a group of panellists. This is a direct result of panel members having different sensitivities to the individual compounds. For an understanding of the possible additive or synergetic effects for mixtures of odorants, odour threshold values would need to be determined for each panellist on the individual compounds as well as different combinations of the compounds – a complex and time consuming task that is beyond the scope of this thesis.

This work has developed SIDA methodologies for the quantification of γ-octalactone (**86**), γ-nonalactone (**87**), γ-decalactone (**88**) and γ-dodecalactone (**89**), and their constituent stereoisomers (**86a** and **86b**; **87a** and **87b**; **88a** and **88b**; **89a** and **89b**), in wine. Deuterated analogues were prepared from commercially available racemic γ-lactones through deuterium exchange to the corresponding oxidised ring-opened products. Optically pure standards were synthesised from the enantiomers of glutamic acid, necessary to determine the order of elution of the stereoisomers in the chiral gas chromatogram and also for use as standards in the sensory threshold study in red wine. Further odour detection threshold values need to be determined, in white wine and in water, the latter of which would enable application of the data to the wider food and beverage industry. With the development of a quantification method using SIDA and the production of enantiomerically pure sample for each γ-lactone, there is now the opportunity to pursue this area of research in other fields.

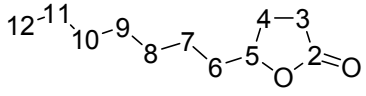
10 Experimental – Part B

10.1 General procedures

Refer to Chapter 5, Experimental – Part A; 5.1 General procedures.

Numbering system

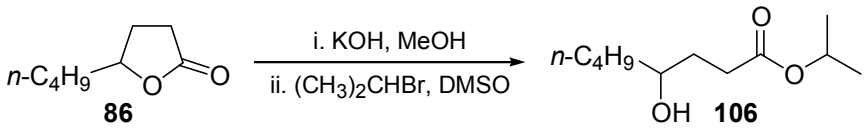
The γ-lactones are numbered as furanone compounds as shown below.



10.2 Experimental procedures for Chapter 7

10.2.1 Synthesis of d_5-analogues of γ-lactones

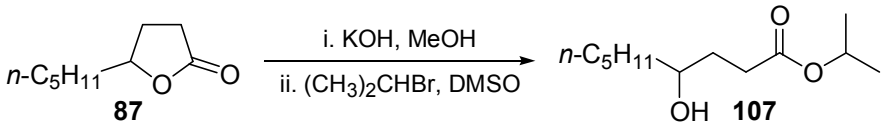
(±) *Iso*-propyl 4-hydroxyoctanoate (106)



To a stirred solution of (±)-γ-octalactone (**86**) (4.99 g, 34.04 mmol) in MeOH (65 mL) was added KOH (2.23 g, 33.78 mmol) and the mixture was stirred at rt for 3 days. The solvent was removed under reduced pressure to afford the potassium carboxylate as a white solid (6.24 g). This salt (5.21 g, 26.27 mmol) was dissolved in DMSO (100 mL) with heating and to this cooled solution was added $(CH_3)_2CHBr$ (12.2 mL, 123.43 mmol) and the mixture was stirred at rt for 20 hrs. The solution was diluted with H_2O (100 mL) and extracted with Et_2O (75 mL x 3). The combined organic extracts were washed with H_2O (100 mL x 3), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The resulting oil was purified by column chromatography (CH_2Cl_2; 5% (v/v) Et_2O/CH_2Cl_2, $\mathbf{R_f}$ = 0.14) to afford alcohol **106** as a colourless oil (4.24 g, 85%). **HRMS** calculated for $C_{11}H_{22}O_3H^+$ [M+H$^+$]:

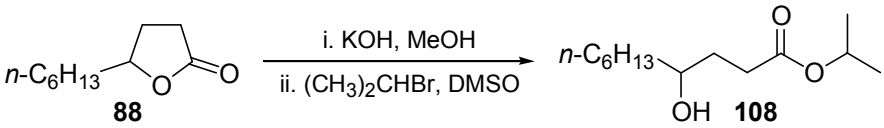
203.1647; found: 203.1649; calculated for $C_{11}H_{22}O_3Na^+$ [M+Na^+]: 225.1467; found: 225.1463; calculated for $C_{11}H_{22}O_3K^+$ [M+K^+]: 241.1206; found: 241.1201; **^{1}H NMR** (300 MHz, C_6D_6) δ = 5.03 (1H, sp, *J* = 6.1, H_9), 3.36 (1H, m, H_4), 2.41-2.25 (2H, m, H_2), 1.75-1.52 (2H, m, H_3), 1.36-1.10 (7H, m, $H_{5,6,7,OH}$), 1.05 (6H, d, *J* = 6.1, H_{10}), 0.85 (3H, t, *J* = 6.9, H_8); **^{13}C NMR** (75.5 MHz, C_6D_6) δ = 173.5 (C_1), 70.9 (C_4), 67.4 (C_9), 37.6 ($C_{5,6,7}$), 32.8 (C_3), 31.3 (C_2), 28.1, 23.0 ($C_{5,6,7}$), 21.8 (C_{10}), 14.2 (C_8).

(±) *Iso*-propyl 4-hydroxynonanoate (107)



Alcohol **107** was synthesised as per **106**. (±) γ-Nonalactone (**87**) (4.97 g, 30.86 mmol) and KOH (2.24 g, 33.93 mmol) in MeOH (50 mL) generated the potassium carboxylate as a white solid (6.79 g). This salt (5.28 g, 24.87 mmol) in DMSO (130 mL) with $(CH_3)_2CHBr$ (12.0 mL, 121.43 mmol) afforded alcohol **107** (4.62 g, 86%) as a colourless oil upon purification by column chromatography (5% (v/v) Et_2O/CH_2Cl_2, $\mathbf{R_f}$ = 0.26). **HRMS** calculated for $C_{12}H_{24}O_3H^+$ [M+H^+]: 217.1804; found: 217.1800; calculated for $C_{12}H_{24}O_3Na^+$ [M+Na^+]: 239.1623; found: 239.1616; calculated for $C_{12}H_{24}O_3K^+$ [M+K^+]: 255.1363; found: 255.1351; **^{1}H NMR** (300 MHz, C_6D_6) δ = 5.03 (1H, sp, *J* = 6.3, H_{10}), 3.38 (1H, m, H_4), 2.42-2.26 (2H, m, H_2), 1.76-1.53 (2H, m, H_3), 1.38-1.10 (9H, m, $H_{5,6,7,8,OH}$), 1.05 (6H, d, *J* = 6.3, H_{11}), 0.88 (3H, t, *J* = 6.9, H_9); **^{13}C NMR** (75.5 MHz, C_6D_6) δ = 173.5 (C_1), 70.9 (C_4), 67.5 (C_{10}), 37.9 ($C_{5,6,7,8}$), 32.8 (C_3), 32.2 ($C_{5,6,7,8}$), 31.3 (C_2), 25.7, 23.0 ($C_{5,6,7,8}$), 21.8 (C_{11}), 14.2 (C_9).

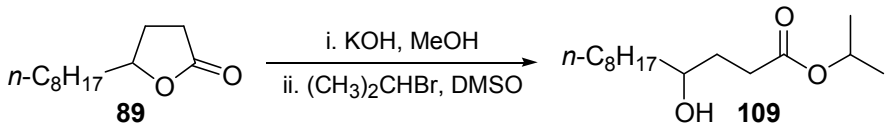
(±) *Iso*-propyl 4-hydroxydecanoate (108)



Alcohol **108** was synthesised as per **106**. (±) γ-Decalactone (**88**) (5.00 g, 29.08 mmol) and KOH (2.13 g, 32.27 mmol) in MeOH (50 mL) generated the potassium carboxylate as a white solid (6.77 g). This salt (5.68 g, 25.09 mmol) in DMSO (150 mL) with $(CH_3)_2CHBr$ (12.0 mL, 121.43 mmol) afforded alcohol **108** (5.17 g, 89%)

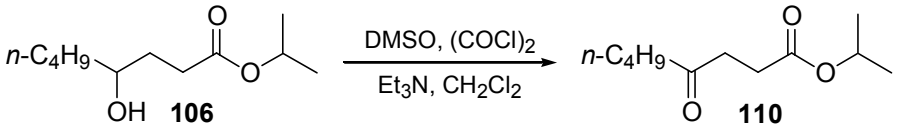
as a colourless oil upon purification by column chromatography (50% (v/v) Et_2O/CH_2Cl_2, $\mathbf{R_f}$ = 0.27). **HRMS** calculated for $C_{13}H_{26}O_3H^+$ [M+H$^+$]: 231.1960; found: 231.1965; calculated for $C_{13}H_{26}O_3Na^+$ [M+Na$^+$]: 253.1780; found: 253.1770; calculated for $C_{13}H_{26}O_3K^+$ [M+K$^+$]: 269.1519; found: 269.1514; **^{1}H NMR** (300 MHz, C_6D_6) δ = 5.03 (1H, sp, *J* = 6.3, H_{11}), 3.39 (1H, m, H_4), 2.43-2.26 (2H, m, H_2), 1.77-1.54 (2H, m, H_3), 1.38-1.14 (11H, m, $H_{5,6,7,8,9,OH}$), 1.05 (6H, d, *J* = 6.3, H_{12}), 0.90 (3H, t, *J* = 6.8, H_{10}); **^{13}C NMR** (75.5 MHz, C_6D_6) δ = 173.4 (C_1), 71.0 (C_4), 67.4 (C_{11}), 38.0 ($C_{5,6,7,8,9}$), 32.8 (C_3), 32.2 ($C_{5,6,7,8,9}$), 31.3 (C_2), 29.7, 26.0, 23.0 ($C_{5,6,7,8,9}$), 21.8 (C_{12}), 14.3 (C_{10}).

(±) *Iso*-propyl 4-hydroxydodecanoate (109)



Alcohol **109** was synthesised as per **106**. (±) γ-Dodecalactone (**89**) (6.04 g, 29.55 mmol) and KOH (2.26 g, 34.24 mmol) in MeOH (60 mL) generated the potassium carboxylate as a white solid (7.90 g). This salt (6.41 g, 25.20 mmol) in DMSO (150 mL) with $(CH_3)_2CHBr$ (12.0 mL, 121.43 mmol) afforded alcohol **109** (5.18 g, 80%) as a colourless oil upon purification by column chromatography (50% (v/v) Et_2O/hexanes, $\mathbf{R_f}$ = 0.26). **HRMS** calculated for $C_{15}H_{30}O_3H^+$ [M+H$^+$]: 259.2273; found: 259.2277; calculated for $C_{15}H_{30}O_3Na^+$ [M+Na$^+$]: 281.2093; found: 281.2087; calculated for $C_{15}H_{30}O_3K^+$ [M+K$^+$]: 297.1832; found: 297.1832; **^{1}H NMR** (300 MHz, C_6D_6) δ = 5.03 (1H, sp, *J* = 6.3, H_{13}), 3.41 (1H, m, H_4), 2.43-2.27 (2H, m, H_2), 1.78-1.54 (2H, m, H_3), 1.38-1.18 (15H, m, $H_{5,6,7,8,9,10,11,OH}$), 1.05 (6H, d, *J* = 6.3, H_{14}), 0.92 (3H, t, *J* = 6.7, H_{12}); **^{13}C NMR** (75.5 MHz, C_6D_6) δ = 173.4 (C_1), 71.0 (C_4), 67.4 (C_{13}), 38.0 ($C_{5,6,7,8,9,10,11}$), 32.8 (C_3), 32.3 ($C_{5,6,7,8,9,10,11}$), 31.3 (C_2), 30.1, 30.0, 29.7, 26.1, 23.1 ($C_{5,6,7,8,9,10,11}$), 21.8 (C_{14}), 14.3 (C_{12}).

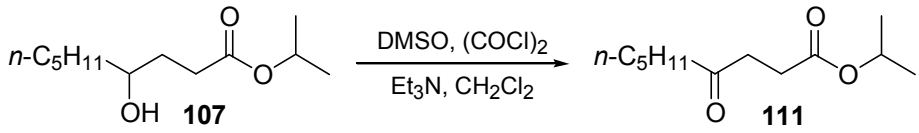
Iso-propyl 4-oxooctanoate (110)



To a stirred solution of DMSO (4.6 mL, 64.11 mmol) in anhydrous CH_2Cl_2 (90 mL)

at -78 °C under N_2 was added oxalyl chloride (16.2 mL of a 2 M solution in CH_2Cl_2, 32.38 mmol) over 2 mins. After the mixture was stirred at -78 °C for 30 mins, a solution of alcohol **106** (4.37 g, 21.60 mmol) in anhydrous CH_2Cl_2 (15 mL) was added and the resulting mixture was stirred at -78 °C for 45 mins. Et_3N (20 mL, 142.77 mmol) was added slowly and the white solution was stirred at -78 °C for 30 mins, at 0 °C for 30 mins and finally at rt for 30 mins. The reaction was poured into a rapidly stirring 1 M solution of $NaHSO_4$ (125 mL), the layers were separated and the aqueous layer extracted with Et_2O (75 mL x 3). The combined organic extracts were concentrated under reduced pressure, the residue taken up in Et_2O (100 mL) and then washed with 1 M solution of $NaHSO_4$ (40 mL x 3), H_2O (40 mL), saturated $NaHCO_3$ (40 mL) and brine (40 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated under reduced pressure to yield keto ester **110** as a yellow oil (4.37 g, quantitative), which was used without further purification. **HRMS** calculated for $C_{11}H_{20}O_3H^+$ [M+H$^+$]: 201.1491; found: 201.1488; calculated for $C_{11}H_{20}O_3Na^+$ [M+Na$^+$]: 223.1310; found: 223.1303; calculated for $C_{11}H_{20}O_3K^+$ [M+K$^+$]: 239.1050; found: 239.1045; **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 4.98 (1H, sp, J = 6.3, H_9), 2.70 (2H, t, J = 6.6, $H_{2,3}$), 2.53 (2H, t, J = 6.6, $H_{2,3}$), 2.44 (2H, t, J = 7.5, H_5), 1.57 (2H, qn, J = 7.5, H_6), 1.31 (2H, sx, J = 7.5, H_7), 1.21 (6H, d, J = 6.3, H_{10}), 0.89 (3H, t, J = 7.5, H_8); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 209.1 (C_4), 172.2 (C_1), 67.8 (C_9), 42.4 (C_5), 37.0, 28.3 ($C_{2,3}$), 25.8 (C_6), 22.2 (C_7), 21.7 (C_{10}), 13.7 (C_8).

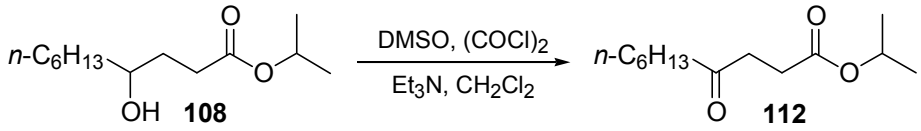
Iso-propyl 4-ketononanoate (111)



Keto ester **111** was synthesised, as per **110**, from alcohol **107** (4.17 g, 19.28 mmol) in anhydrous CH_2Cl_2 (15 mL) under Swern conditions of DMSO (4.3 mL, 59.93 mmol) in anhydrous CH_2Cl_2 (85 mL) with oxalyl chloride (15 mL of a 2 M solution in CH_2Cl_2, 30.00 mmol) and Et_3N (17 mL, 121.34 mmol). Keto ester **111** was obtained as a yellow oil (4.14 g, quantitative) and used without further purification. **HRMS** calculated for $C_{12}H_{22}O_3H^+$ [M+H$^+$]: 215.1647; found: 215.1643; **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 4.98 (1H, sp, J = 6.3, H_{10}), 2.70 (2H, t, J = 6.6, $H_{2,3}$), 2.53 (2H, t, J = 6.6, $H_{2,3}$), 2.43 (2H, t, J = 7.5, H_5), 1.58 (2H, qn, J = 7.5, H_6), 1.38-1.23

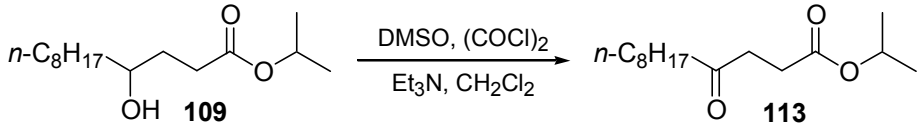
(4H, m, $H_{7,8}$), 1.22 (6H, d, J = 6.3, H_{11}), 0.88 (3H, t, J = 7.0, H_9); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 209.0 (C_4), 172.2 (C_1), 67.8 (C_{10}), 42.6 (C_5), 36.9 ($C_{2,3}$), 31.3 ($C_{7,8}$), 28.2 ($C_{2,3}$), 23.4 (C_6), 22.3 ($C_{7,8}$), 21.6 (C_{11}), 13.8 (C_9).

Iso-propyl 4-ketodecanoate (112)



Keto ester **112** was synthesised, as per **110**, from alcohol **108** (4.53 g, 19.67 mmol) in anhydrous CH_2Cl_2 (15 mL) under Swern conditions of DMSO (4.4 mL, 61.33 mmol) in anhydrous CH_2Cl_2 (85 mL) with oxalyl chloride (15 mL of a 2 M solution in CH_2Cl_2, 30.00 mmol) and Et_3N (17 mL, 121.34 mmol). Keto ester **112** was obtained as a yellow oil (4.61 g, quantitative) and used without further purification. **HRMS** calculated for $C_{13}H_{24}O_3H^+$ [M+H$^+$]: 229.1804; found: 229.1804; calculated for $C_{13}H_{24}O_3Na^+$ [M+Na$^+$]: 251.1623; found: 251.1616; calculated for $C_{13}H_{24}O_3K^+$ [M+K$^+$]: 267.1363; found: 267.1363; **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 4.98 (1H, sp, J = 6.3, H_{11}), 2.69 (2H, t, J = 6.6, $H_{2,3}$), 2.53 (2H, t, J = 6.6, $H_{2,3}$), 2.43 (2H, t, J = 7.4, H_5), 1.57 (2H, qn, J = 7.4, H_6), 1.36-1.23 (6H, m, $H_{7,8,9}$), 1.22 (6H, t, J = 6.3, H_{12}), 0.87 (3H, t, J = 6.7, H_{10}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 209.2 (C_4), 172.3 (C_1), 67.9 (C_{11}), 42.8 (C_5), 37.0 ($C_{2,3}$), 31.5, 28.9 ($C_{7,8,9}$), 28.3 ($C_{2,3}$), 23.8 (C_6), 22.4 ($C_{7,8,9}$), 21.8 (C_{12}), 14.0 (C_{10}).

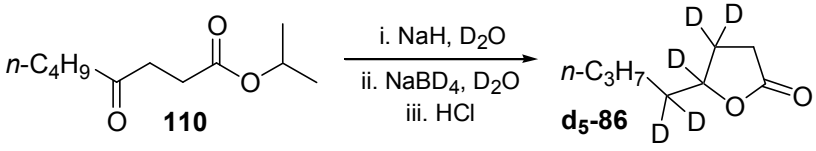
Iso-propyl 4-ketododecanoate (113)



Keto ester **113** was synthesised, as per **110**, from alcohol **109** (4.76 g, 18.42 mmol) in anhydrous CH_2Cl_2 (15 mL) under Swern conditions of DMSO (4.3 mL, 59.93 mmol) in anhydrous CH_2Cl_2 (85 mL) with oxalyl chloride (15 mL of a 2 M solution in CH_2Cl_2, 30.00 mmol) and Et_3N (17 mL, 121.34 mmol). Keto ester **113** was obtained as a yellow oil (4.87 g, quantitative) and used without further purification. **HRMS** calculated for $C_{15}H_{28}O_3H^+$ [M+H$^+$]: 257.2117; found: 257.2115; calculated for $C_{15}H_{28}O_3Na^+$ [M+Na$^+$]: 279.1936; found: 279.1927; **^{1}H NMR** (300 MHz, $CDCl_3$)

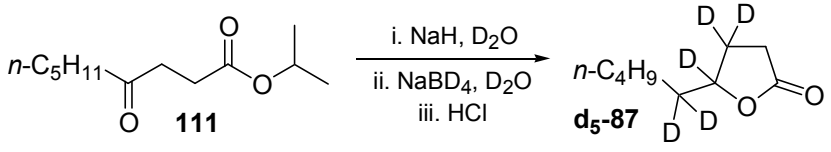
δ = 4.98 (1H, sp, *J* = 6.3, H_{13}), 2.70 (2H, t, *J* = 6.6, $H_{2,3}$), 2.53 (2H, t, *J* = 6.6, $H_{2,3}$), 2.43 (2H, t, *J* = 7.4, H_5), 1.57 (2H, qn, *J* = 7.4, H_6), 1.33-1.21 (10H, m, $H_{7,8,9,10,11}$), 1.22 (6H, d, *J* = 6.3, H_{14}), 0.87 (3H, t, *J* = 6.8, H_{12}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 209.1 (C_4), 172.2 (C_1), 67.8 (C_{13}), 42.7 (C_5), 37.0 ($C_{2,3}$), 31.7, 29.3, 29.1, 29.0, ($C_{7,8,9,10,11}$), 28.3 ($C_{2,3}$), 23.7 (C_6), 22.5 ($C_{7,8,9,10,11}$), 21.7 (C_{14}), 14.0 (C_{12}).

(±) γ-Octalactone (d_5-86)



To a stirred solution of NaH (0.59 g, 14.75 mmol) in D_2O (10 mL) was added a suspension of keto ester **110** (0.95 g, 4.74 mmol) in D_2O (5 mL) and the mixture was stirred under N_2 at rt for 24 hrs. $NaBD_4$ (1.01 g, 23.65 mmol) and D_2O (5 mL) were added and the mixture was stirred under N_2 at rt for 24 hrs. The reaction was quenched by the careful addition of concentrated HCl (pH 2) and stirred under N_2 at rt for 24 hrs. The product was extracted with Et_2O (20 mL x 3), the organic layers combined, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by column chromatography (50% (v/v) Et_2O/hexanes, $\mathbf{R_f}$ = 0.18) to afford γ-lactone **d_5-86** as a colourless oil (0.62 g, 89%). **MS** *m/z* (%) 147 (M^+, 1), 119 (1), 105 (6), 89 (9), 88 (100), 73 (7), 59 (11), 43 (5); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 2.51 (2H, s, H_3), 1.48-1.27 (4H, m, $H_{7,8}$), 0.91 (3H, t, *J* = 6.9, H_9); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 176.6 (C_2), 79.6 (t, *J* = 22.9, C_5), 33.6 (qn, *J* = 19.2, $C_{4,6}$), 27.9 (C_3), 26.7 (qn, *J* = 20.3, $C_{4,6}$), 26.4, 21.7 ($C_{7,8}$), 13.2 (C_9).

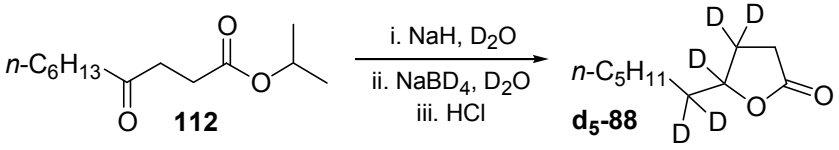
(±) γ-Nonalactone (d_5-87)



Labelled γ-nonalactone (**d_5-87**) was synthesised, as per **d_5-86**, from keto ester **111** (1.11 g, 5.18 mmol) in D_2O (5 mL) *via* a three step sequence of NaH (0.64 g, 16.00 mmol) in D_2O (10 mL), $NaBD_4$ (0.98 g, 22.94 mmol) in D_2O (5 mL) and concentrated HCl (pH 2). Purification by column chromatography (50% (v/v)

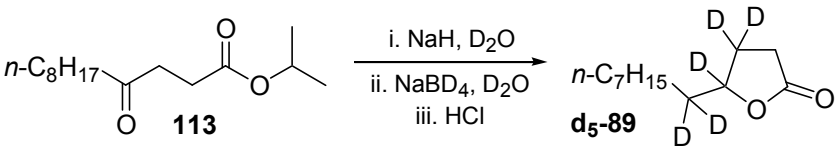
Et_2O/hexanes, $\mathbf{R_f}$ = 0.23) afforded γ-lactone **d_5-87** as a colourless oil (0.71 g, 85%). **MS** *m/z* (%) 161 (M^+, <1), 133 (3), 119 (3), 105 (5), 89 (7), 88 (100), 73 (2), 57 (5), 43 (4); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 2.34 (2H, s, H_3), 1.36-1.10 (6H, m, $H_{7,8,9}$), 0.75 (3H, t, *J* = 6.5, H_{10}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.0 (C_2), 80.0 (t, *J* = 22.9, C_5), 34.2 (qn, *J* = 19.1, $C_{4,6}$), 31.1 ($C_{7,8,9}$), 28.8 (C_3), 26.7 (qn, *J* = 20.3, $C_{4,6}$), 24.3, 22.1 ($C_{7,8,9}$), 13.5 (C_{10}).

(±) γ-Decalactone (d_5-88)



Labelled γ-decalactone (**d_5-88**) was synthesised, as per **d_5-86**, from keto ester **112** (1.18 g, 5.17 mmol) in D_2O (5 mL) *via* a three step sequence of NaH (0.66 g, 16.50 mmol) in D_2O (10 mL), $NaBD_4$ (1.02 g, 23.88 mmol) in D_2O (5 mL) and concentrated HCl (pH 2). Purification by column chromatography (50% (v/v) Et_2O/hexanes, $\mathbf{R_f}$ = 0.20) afforded γ-lactone **d_5-88** as a colourless oil (0.78 g, 86%). **MS** *m/z* (%) 175 (M^+, <1), 133 (13), 119 (2), 105 (4), 89 (6), 88 (100), 87 (7), 57 (5), 43 (6); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 2.33 (2H, s, H_3), 1.32-1.02 (8H, m, $H_{7,8,9,10}$), 0.71 (3H, t, *J* = 6.5, H_{11}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 176.8 (C_2), 79.9 (t, *J* = 22.9, C_5), 34.1 (qn, *J* = 19.2, $C_{4,6}$), 31.2, 28.5 ($C_{7,8,9,10}$), 28.1 (C_3), 26.7 (qn, *J* = 20.5, $C_{4,6}$), 24.5, 22.0 ($C_{7,8,9,10}$), 13.5 (C_{11}).

(±) γ-Dodecalactone (d_5-89)



Labelled γ-dodecalactone (**d_5-89**) was synthesised, as per **d_5-86**, from keto ester **113** (1.30 g, 5.07 mmol) in D_2O (5 mL) *via* a three step sequence of NaH (0.67 g, 16.75 mmol) in D_2O (10 mL), $NaBD_4$ (0.99 g, 23.18 mmol) in D_2O (5 mL) and concentrated HCl (pH 2). Purification by column chromatography (50% (v/v) Et_2O/hexanes, $\mathbf{R_f}$ = 0.23) afforded γ-lactone **d_5-89** as a colourless oil (0.91 g, 88%). **MS** *m/z* (%) 203 (M^+, <1), 133 (5), 119 (1), 115 (2), 105 (3), 89 (11), 88 (100), 57

(10), 43 (13); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 2.48 (2H, s, H_3), 1.44-1.30 (12H, m, $H_{7,8,9,10,11,12}$), 0.84 (3H, t, *J* = 6.7, H_{13}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.2 (C_2), 80.4 (t, *J* = 22.9, C_5), 34.9 (qn, *J* = 19.2, $C_{4,6}$), 31.7, 29.3, 29.2, 29.1 ($C_{7,8,9,10,11,12}$), 28.5 (C_3), 27.1 (qn, *J* = 22.6, $C_{4,6}$), 25.0, 22.5 ($C_{7,8,9,10,11,12}$), 14.0 (C_{13}).

10.2.2 Method development for head space solid-phase microextraction (HS SPME)

Sample preparation

In a glass vial (15 mL), wine (10 mL) was spiked with the d_5-standards (25 μL of a 4,000 μg/L solution; spike at 10 μg/L for each γ-lactone) and a sample (5 mL) was diluted with H_2O (5 mL) in an SPME vial (22 mL) with NaCl (2 g) added. For the calibration functions, increasing concentrations of the analytes (0-100 μg/L) were added to the wine (10 mL) prior to dilution.

Calibration functions and validation of method

The method was validated by a series of duplicate standard additions of the analytes (0 μg/L, 0.5 μg/L, 1 μg/L, 2 μg/L, 5 μg/L, 10 μg/L, 25 μg/L, 50 μg/L and 100 μg/L) to a white wine ('bag in a box' fresh dry white wine, pH 3.23, 9.2% EtOH, SO_2 levels 155 mg/L total and 16 mg/L free) containing the d_5-standards (25 μL of a 4,000 μg/L solution; spike at 10 μg/L for each γ-lactone). The blank wine was analysed and shown to contain no γ-lactones. The reproducibility of the analyses was determined at two concentrations, 5 μg/L and 25 μg/L, by spiking seven samples of the same wine with the analytes.

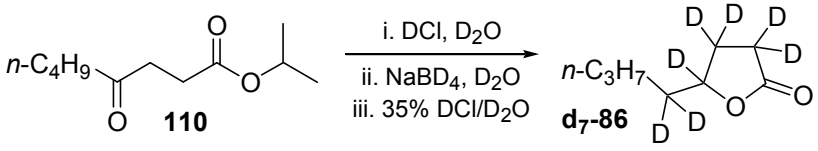
Instrumental analysis

An Agilent 6890A gas chromatogram fitted to an Agilent 5973N mass spectrometer was used with an Innowax capillary column, 60 m x 0.25 mm I.D. and 0.25 μm film thickness. The carrier gas was He with a flow rate of 1.8 mL/min. The initial column temperature was 50 °C, held for 1 min, then increased to 190 °C at 40 °C/min and finally to 250 °C at 5 °C/min and held for 20 mins with the transfer line at 250 °C. The extraction was carried out with a Gerstel MPS2 auto sampler using a grey divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre in splitless mode. During splitless mode, a pressure pulse of 45.0 psi was applied. The

fibre was incubated at 50 °C and agitated at 250 rpm with an extraction time of 40 mins. The fibre was desorbed for 15 mins. The mass spectrometer detector was initially used in scan mode and recorded in the range of *m/z* 35-350. For method development and validation, the mass spectrometer detector was used in selected ion monitoring (SIM) mode with a solvent delay of 6 mins. The ions monitored for the analytes were *m/z* 85, 86, 100, 128 and the ions monitored for the d_5-standards were *m/z* 88, 89, 105, 133. The underlined ions were the target ions used for quantification and the remaining ions were the qualifying ions used for compound verification.

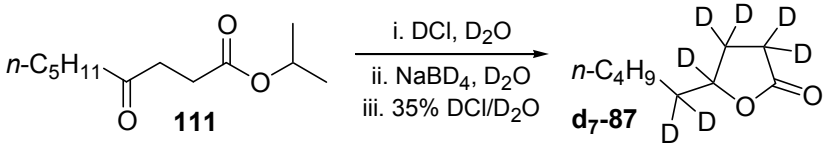
10.2.3 Synthesis of d_7-analogues of γ-lactones

(±) γ-Octalactone (d_7-86)



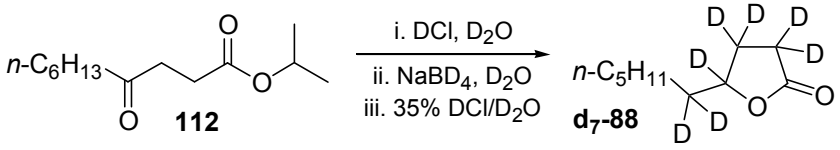
Keto ester **110** (0.50 g, 2.50 mmol) was heated under reflux in 35% (w/v) DCl/D_2O solution (1.5 mL) and D_2O (8.5 mL) under N_2 for 6 days. Any loss in volume of the DCl/D_2O solution was replaced as required. The reaction was allowed to cool and was then extracted with Et_2O (10 mL x 3). The organic extracts were combined, dried (Na_2SO_4), filtered and concentrated under reduced pressure. To the residue was added $NaBD_4$ (0.48 g, 11.26 mmol) and D_2O (10 mL) and the mixture was stirred under N_2 at rt for 24 hrs. The reaction was quenched by the careful addition of 35% (w/v) DCl/D_2O solution (pH 2) and stirred at rt under N_2 for 24 hrs. The product was extracted with Et_2O (25 mL x 3), the organic layers were combined, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by column chromatography (50% (v/v) Et_2O/hexanes, $\mathbf{R_f}$ = 0.18) to afford γ-lactone **d_7-86** as a colourless oil (0.28 g, 75%). **MS** *m/z* (%) 149 (M^+, 1), 107 (6), 91 (5), 90 (100), 73 (2), 59 (4), 43 (3); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 1.48-1.26 (4H, m, $H_{7,8}$), 0.91 (3H, t, J = 7.1, H_9); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 176.9 (C_2), 79.9 (t, J = 22.9, C_5), 33.8 (qn, J = 19.2, $C_{4,6}$), 27.5 (qn, J = 20.2, C_3), 26.6 ($C_{7,8}$), 26.4 (qn, J = 20.6, $C_{4,6}$), 21.8 ($C_{7,8}$), 13.4 (C_9).

(±) γ-Nonalactone (d_7-87)



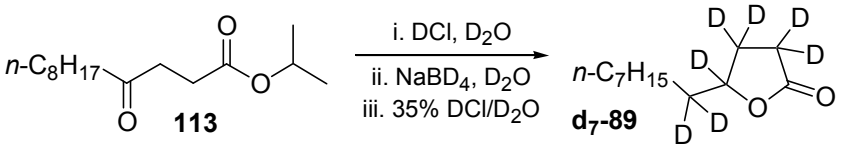
Labelled γ-nonalactone (**d_7-87**) was synthesised, as per **d_7-86**, from keto ester **111** (0.49 g, 2.30 mmol) in D_2O (8 mL) *via* a three step sequence of 35% (w/v) DCl/D_2O solution (2 mL) for 15 days, $NaBD_4$ (0.21 g, 4.89 mmol) in D_2O (10 mL) and 35% (w/v) DCl/D_2O solution (pH 1). Purification by column chromatography (50% (v/v) Et_2O/hexanes, $\mathbf{R_f}$ = 0.23) afforded γ-lactone **d_7-87** as a colourless oil (49.2 mg, 13%). **MS** *m/z* (%) 163 (M^+, <1), 135 (3), 121 (1), 107 (5), 91 (5), 90 (100), 73 (2), 57 (3), 43 (4); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 1.51-1.21 (6H, m, $H_{7,8,9}$), 0.89 (3H, t, *J* = 6.8, H_{10}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.3 (C_2), 80.4 (t, *J* = 22.6, C_5), 34.6 (qn, *J* = 18.9, $C_{4,6}$), 31.4 ($C_{7,8,9}$), 28.0 (qn, *J* = 21.9, C_3), 27.2 (qn, *J* = 21.9, $C_{4,6}$), 24.6, 22.4 ($C_{7,8,9}$), 13.9 (C_{10}).

(±) γ-Decalactone (d_7-88)



Labelled γ-decalactone (**d_7-88**) was synthesised, as per **d_7-86**, from keto ester **112** (0.51 g, 2.25 mmol) in D_2O (8.5 mL) *via* a three step sequence of 35% (w/v) DCl/D_2O solution (1.5 mL) for 29 days, $NaBD_4$ (0.50 g, 11.65 mmol) in D_2O (10 mL) and 35% (w/v) DCl/D_2O solution (pH 2). Purification by column chromatography (50% (v/v) Et_2O/hexanes, $\mathbf{R_f}$ = 0.20) afforded γ-lactone **d_7-88** as a colourless oil (0.16 g, 40%). **MS** *m/z* (%) 177 (M^+, <1), 135 (12), 121 (1), 107 (5), 91 (5), 90 (100), 87 (2), 57 (4), 43 (6); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 1.52-1.20 (8H, m, $H_{7,8,9,10}$), 0.88 (3H, t, *J* = 6.6, H_{11}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.1 (C_2), 80.2 (t, *J* = 23.2, C_5), 34.4 (qn, *J* = 19.2, $C_{4,6}$), 31.4, 28.7 ($C_{7,8,9,10}$), 27.8 (qn, *J* = 20.2, C_3), 26.7 (qn, *J* = 20.3, $C_{4,6}$), 24.7, 22.3 ($C_{7,8,9,10}$), 13.8 (C_{11}).

(±) γ-Dodecalactone (d_7-89)



Labelled γ-dodecalactone (**d_7-89**) was synthesised, as per **d_7-86**, from keto ester **113** (0.51 g, 1.98 mmol) in D_2O (8 mL) *via* a three step sequence of 35% (w/v) DCl/D_2O solution (2 mL) for 17 days, $NaBD_4$ (0.24 g, 5.60 mmol) in D_2O (10 mL) and 35% (w/v) DCl/D_2O solution (pH 1). Purification by column chromatography (50% (v/v) Et_2O/hexanes, $\mathbf{R_f}$ = 0.23) afforded γ-lactone **d_7-89** as a colourless oil (69.2 mg, 17%). **MS** *m/z* (%) 205 (M^+, <1), 135 (10), 121 (1), 115 (2), 107 (5), 91 (6), 90 (100), 101 (3), 57 (6), 43 (11); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 1.50-1.19 (12H, m, $H_{7,8,9,10,11,12}$), 0.88 (3H, t, *J* = 6.8, H_{13}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.3 (C_2), 80.4 (t, *J* = 22.4, C_5), 34.6 (qn, *J* = 18.9, $C_{4,6}$), 31.8, 29.4, 29.2, 29.1 ($C_{7,8,9,10,11,12}$), 28.6 (qn, *J* = 22.9, C_3), 28.0 (qn, t, *J* = 20.0, $C_{4,6}$), 24.9, 22.6 ($C_{7,8,9,10,11,12}$), 14.0 (C_{13}).

10.2.4 Method development for solid-phase extraction (SPE)

Sample preparation

Varian Bond Elut-ENV 200 mg prepacked 3 mL cartridges were placed in an extraction manifold system and conditioned by rinsing with MeOH (2 mL) and H_2O (4 mL). Wine (50 mL) spiked with the d_7-standards (50 μL of a 10,000 μg/L solution; spike at 10 μg/L for each γ-lactone) was passed through and the cartridges were rinsed with H_2O (5 mL) and 40% (v/v) MeOH/H_2O with 1% (w/v) $NaHCO_3$ (20 mL). The cartridges were allowed to dry by passing air through for 30 mins. The analytes were extracted with CH_2Cl_2 (2 mL), dried through a glass pipette with $MgSO_4$ and concentrated under a gentle stream of N_2 at rt to fit into a vial insert (100 μL) for analysis by GC-MS. Liquid extracts were stored at -18 °C for chiral GC-MS analysis (Chapter 8).

Calibration functions and validation of method

The method was validated by a series of duplicate standard additions of the analytes (0 μg/L, 0.5 μg/L, 1 μg/L, 2 μg/L, 5 μg/L, 10 μg/L, 25 μg/L, 50 μg/L and 100 μg/L)

to a red wine ('bag in a box' dry red wine, pH 3.50, 12.8% EtOH, SO_2 levels 117 mg/L total and 21 mg/L free) or a white wine ('bag in a box' fresh dry white wine, pH 3.23, 9.2% EtOH, SO_2 levels 155 mg/L total and 16 mg/L free) containing the d_7-standards (50 μL of a 10,000 μg/L solution; spike at 10 μg/L for each γ-lactone). The blank wines were analysed and showed to contain no γ-lactones. The reproducibility of the analyses was determined at two concentrations, 5 μg/L and 25 μg/L, by spiking seven samples of the same wine with the analytes.

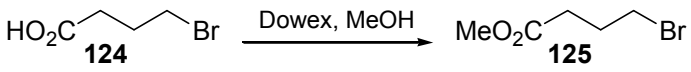
Instrumental analysis

An Agilent 6890A gas chromatogram fitted to an Agilent 5973N mass spectrometer was used with an Innowax capillary column, 60 m x 0.25 mm I.D. and 0.25 μm film thickness. The carrier gas was He at 1.5 mL/min. The initial column temperature was 50 °C, held for 1 min, then increased to 190 °C at 40 °C/min and finally to 250 °C at 5 °C/min and held for 20 mins with the transfer line at 250 °C. The injection was carried out with a Gerstel MPS2 auto sampler using a 10 μL syringe in splitless mode. During splitless mode, a pressure pulse of 45.0 psi was applied. The injected volume was 2 μL. The mass spectrometer detector was initially used in scan mode and recorded in the range of *m/z* 35-350. For method development and validation, the mass spectrometer detector was used in SIM mode with a solvent delay of 5 mins. The ions monitored for the analytes were *m/z* <u>85</u>, 86, 100, 128 and the ions monitored for the d_7-standards were *m/z* <u>90</u>, 91, 107, 135. The underlined ions were the target ions used for quantification and the remaining ions were the qualifying ions used for compound verification.

10.3 Experimental procedures for Chapter 8

10.3.1 Synthesis of optically pure γ-lactones

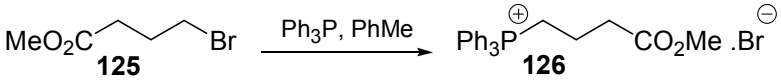
Methyl 4-bromobutanoate (125)



Acid **124** (1.92 g, 11.27 mmol) in MeOH (40 mL) was heated under reflux with pre-

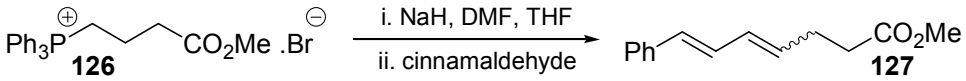
treated Dowex for 24 hrs. The reaction was cooled and the solvent removed under reduced pressure. The residue was purified by column chromatography (20% (v/v) Et_2O/hexanes, $\mathbf{R_f}$ = 0.26) to afford ester **125** (1.21 g, 59%) as a colourless oil. **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 3.69 (3H, s, H_{Me}), 3.46 (2H, t, *J* = 6.7, H_4), 2.51 (2H, t, *J* = 7.1, H_2), 2.17 (2H, dqn, *J* = 0.6, 6.7, H_3); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 172.7 (C_1), 51.5 (C_{Me}), 32.5 (C_4), 32.0 (C_2), 27.6 (C_3).

(4-Methoxy-4-oxobutyl)triphenylphosphonium bromide (126)



Methyl 4-bromobutanoate (**125**) (19.42 g, 107.28 mmol) was added to a stirred solution of Ph_3P (28.78 g, 108.63 mmol) in PhMe (250 mL) and heated under reflux for 48 hrs. The cooled crude white crystalline product **126** (37.57 g, 79%) was collected by vacuum filtration. **mpt** 165-168 °C (lit.[192] **mpt** 166-168 °C); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.98-7.64 (15H, m, H_{Ar}), 4.08-3.98 (2H, m, H_4), 3.64 (3H, s, H_{Me}), 2.90 (2H, t, *J* = 6.4, H_2), 2.20-1.84 (2H, m, H_3); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 173.1 (C_1), 134.8 (d, *J* = 2.9, C_{Ar}), 133.4 (d, *J* = 10.3, C_{Ar}), 130.3 (d, *J* = 12.6, C_{Ar}), 117.8 (d, *J* = 85.9, C_{Ar}), 51.5 (C_{Me}), 32.8 (d, *J* = 18.3, C_2), 21.5 (d, *J* = 51.5, C_4), 17.8 (C_3).

***trans*, *cis*- and *trans*, *trans*-Methyl 7-phenyl-4,6-heptadienoate (127)**

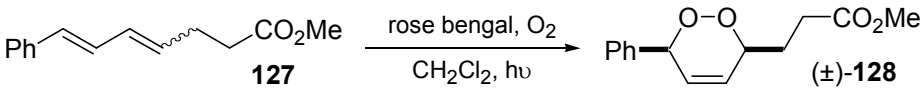


To a stirred suspension of salt **126** (1.02 g, 2.31 mmol) in anhydrous THF (25 mL) with DMF (0.8 mL) under an atmosphere of N_2 at 0 °C was added NaH (0.10 g, 2.50 mmol) and the mixture was stirred for 30 mins. Cinnamaldehyde (0.31 g, 2.35 mmol) was added dropwise, at 0 °C, to the stirred yellow solution and the resulting cream mixture was stirred under N_2 at rt for 16 hrs. The filtrate was collected by vacuum filtration through celite and the solvent removed under reduced pressure. The residue was purified by column chromatography (15% (v/v) Et_2O/hexanes, $\mathbf{R_f}$ = 0.25) to afford the product diene **127** (283.3 mg, 86%) with greater than 90% *trans*, *cis*-configuration as determined by ^{1}H NMR.

Major *trans*, *cis*-**127**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.50-7.15 (5H, m, H_{Ar}), 7.07 (1H, ddd, *J* = 15.5, 11.0, 1.1, H_6), 6.55 (1H, d, *J* = 15.5, H_7), 6.20 (1H, t, *J* = 11.0, H_5), 5.48 (1H, dt, *J* = 7.8, 11.0, H_4), 3.69 (3H, s, H_{Me}), 2.63 (2H, q, *J* = 7.3, H_3), 2.45 (2H, t, *J* = 7.3, H_2); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 173.4 (C_1), 137.4 (C_{Ar}), 133.0 (C_7), 130.04, 130.00 ($C_{4,5}$), 128.6, 127.5, 126.4 (C_{Ar}), 123.8 (C_6), 51.6 (C_{Me}), 34.0 (C_2), 23.5 (C_3).

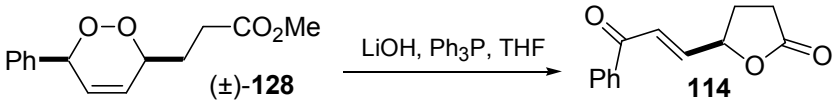
Minor *trans*, *trans*-**127**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.50-7.15 (5H, m, H_{Ar}), 6.74 (1H, dd, *J* = 10.3, 15.8, H_6), 6.47 (1H, d, *J* = 15.8, H_7), 6.25 (1H, dd, *J* = 10.6, 14.9, H_5), 5.81 (1H, dt, *J* = 6.9, 15.2, H_4), 3.69 (3H, s, H_{Me}), 2.63 (2H, q, *J* = 7.3, H_3), 2.45 (2H, t, *J* = 7.3, H_2); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 173.4 (C_1), 137.4 (C_{Ar}), 132.8 (C_4), 131.6 (C_5), 131.0 (C_7), 128.9 (C_6), 127.3, 126.2 (C_{Ar}), 51.6 (C_{Me}), 33.7 (C_2), 28.0 (C_3). One C_{Ar} peak overlaps with *trans*, *cis*-**127** C_{Ar} peaks.

(±)-3-Phenyl-6-ethylcarboxymethyl-3,6-dihydro-1,2-dioxine (128)



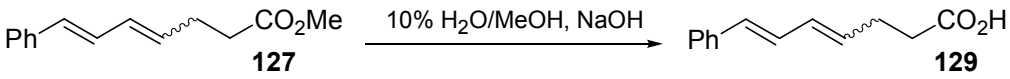
Diene **127** (106.5 mg, 0.49 mmol) and rose bengal (35.6 mg) were dissolved in CH_2Cl_2 (100 mL). The pink solution was cooled to 0 °C and a stream of O_2 was passed through the solution, while being irradiated with two tungsten halogen lamps (500 W), approximately 5-10 cm from the reactor vessel, for 6.5 hrs. The solvent was removed under reduced pressure and the residue purified by column chromatography (30% (v/v) hexanes/CH_2Cl_2, $\mathbf{R_f}$ = 0.30) to yield dioxine **128** (56.6 mg, 60%) as a pale yellow oil. **HRMS** calculated for $C_{14}H_{16}O_4NH_4^+$ [M+NH_4^+]: 266.1393; found: 266.1384; calculated for $C_{14}H_{16}O_4Na^+$ [M+Na^+]: 271.0947; found: 271.0943; **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.41-7.34 (5H, m, H_{Ar}), 6.16-6.06 (2H, m, $H_{4,5}$), 5.59 (1H, m, H_3), 4.59 (1H, m, H_6), 3.67 (3H, s, H_{Me}), 2.52 (2H, dt, *J* = 2.6, 7.4, H_8), 2.19-1.97 (2H, m, H_7); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 173.6 (C_{ester}), 136.9, 128.9, 128.59, 128.57 (C_{Ar}), 127.9, 127.3 ($C_{4,5}$), 80.3 (C_3), 77.3 (C_6), 51.6 (C_{Me}), 29.8 (C_8), 28.2 (C_7).

(±)-*trans*-Dihydro-5-(1-propenyl-3-oxo-3-phenyl)-2(3H)-furanone (114)



LiOH (1.1 mg, 0.046 mmol) was added to a stirred solution of dioxine **128** (11.0 mg, 0.044 mmol) and Ph_3P (5.1 mg, 0.019 mmol) in anhydrous THF (0.5 mL). The mixture was stirred under N_2 at rt for 5 days. The solvent was removed under reduced pressure and the residue purified by column chromatography (CH_2Cl_2, $\mathbf{R_f}$ = 0.41) to yield *trans*-alkene γ-lactone **114** (1.4 mg, 28%) as a colourless oil. **^{1}H NMR** (300 MHz, C_6D_6) δ = 7.10-7.00 (6H, m, $H_{Ar,7}$), 6.70 (1H, dd, *J* = 4.1, 15.4, H_6), 4.19 (1H, m, H_5), 1.85-1.55 (2H, m, H_3), 1.21 (1H, m, $H_{4a/b}$), 0.92 (1H, m, $H_{4a/b}$).

***trans*, *cis*- and *trans*, *trans*-7-Phenyl-4,6-heptadienoic acid (129)**



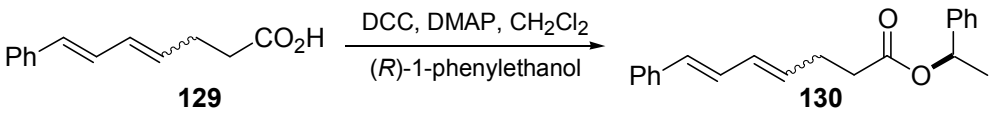
To a solution of diene **127** in 10% aqueous MeOH (100 mL) was added NaOH (1.35 g, 33.75 mmol) and the solution was stirred at rt for 23 hrs. The mixture was concentrated under reduced pressure, the residue taken up in H_2O (100 mL), washed with EtOAc (50 mL x 2) and acidified with 10% HCl (pH 1). The aqueous layer was extracted with CH_2Cl_2 (75 mL x 3), dried (Na_2SO_4), filtered and concentrated under reduced pressure to yield the title compound **129** (2.91 g, 95%) as a bright yellow viscous oil with greater than 90% *trans*, *cis*-configuration as determined by ^{1}H NMR, which was used without further purification. **HRMS** calculated for $C_{13}H_{14}O_2H^+$ [M+H$^+$]: 203.1072; found: 203.1072; calculated for $C_{13}H_{14}O_2Na^+$ [M+Na$^+$]: 225.0892; found: 225.0893.

Major *trans*, *cis*-**129**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.47-7.18 (5H, m, H_{Ar}), 7.06 (1H, ddd, *J* = 15.5, 11.0, 1.1, H_6), 6.56 (1H, d, *J* = 15.5, H_7), 6.21 (1H, t, *J* = 11.0, H_5), 5.49 (1H, m, H_4), 2.63 (2H, q, *J* = 7.2, H_3), 2.49 (2H, t, *J* = 7.2, H_2); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 179.3 (C_1), 137.3 (C_{Ar}), 133.2 (C_7), 130.2 (C_5), 129.6 (C_4), 128.6, 127.6, 126.4 (C_{Ar}), 123.7 (C_6), 34.0 (C_2), 23.1 (C_3).

Minor *trans*, *trans*-**129**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.47-7.18 (5H, m, H_{Ar}),

6.74 (1H, dd, J = 10.6, 15.7, H_6), 6.48 (1H, d, J = 16.1, H_7), 6.30 (1H, m, H_5), 5.80 (1H, m, H_4), 2.63 (2H, q, J = 7.2, H_3), 2.49 (2H, t, J = 7.2, H_2); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 179.3 (C_1), 137.3 (C_{Ar}), 132.3 (C_4), 131.8 (C_5), 131.2 (C_7), 128.8 (C_6), 127.3, 126.2 (C_{Ar}), 33.7 (C_2), 27.7 (C_3). One C_{Ar} peak overlaps with *trans*, *cis*-**129** C_{Ar} peaks.

trans, *cis*- and *trans*, *trans*-(*R*)-1-Phenylethyl 7-phenyl-4,6-heptadienoate (130)



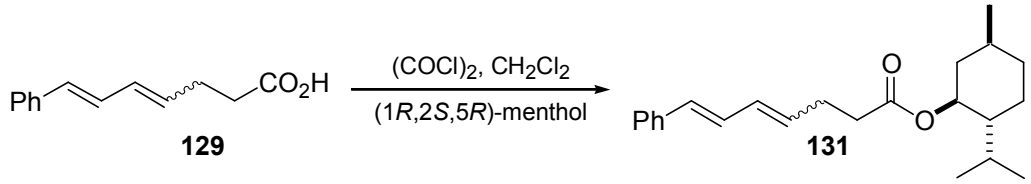
129 **130**

A solution of diene **129** (155.3 mg, 0.77 mmol) and DMAP (99.6 mg, 0.82 mmol) in CH_2Cl_2 (2 mL) was treated with (*R*)-1-phenylethanol (91.8 mg, 0.75 mmol) and DCC (154.7 mg, 0.75 mmol). The resulting mixture was stirred at rt for 23 hrs, filtered and concentrated under reduced pressure. Purification of the residue by column chromatography (15% (v/v) Et_2O/hexanes, $\mathbf{R_f}$ = 0.37) afforded the title compound **130** (145.4 mg, 63%) as a pale yellow oil with greater than 90% *trans*, *cis*-configuration as determined by ^{1}H NMR. **HRMS** calculated for $C_{21}H_{22}O_2Na^+$ [M+Na^+]: 329.1518; found: 329.1513.

Major *trans*, *cis*-**130**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.46-7.19 (12H, m, H_{Ar}), 7.06 (1H, dd, J = 11.0, 15.6, H_6), 6.54 (1H, d, J = 15.6, H_7), 6.19 (1H, t, J = 11.0, H_5), 5.91 (1H, q, J = 6.6, $H_{CH\text{-}O}$), 5.47 (1H, dt, J = 7.6, 11.0, H_4), 2.63 (2H, q, J = 7.5, H_3), 2.46 (2H, m, H_2), 1.54 (3H, d, J = 6.7, H_{Me}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 172.1 (C_1), 141.6, 137.3 (C_{Ar}), 132.9 (C_7), 130.0 (C_5), 129.9 (C_4), 128.5, 128.4, 127.8, 127.5, 126.4, 126.0 (C_{Ar}), 123.8 (C_6), 72.3 ($C_{CH\text{-}O}$), 34.4 (C_2), 23.4 (C_3), 22.1 (C_{Me}).

Minor *trans*, *trans*-**130**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.46-7.19 (12H, m, H_{Ar}), 6.71 (1H, dd, J = 10.5, 15.6, H_6), 6.43 (1H, d, J = 15.6, H_7), 6.26 (1H, m, H_5), 5.91 (1H, q, J = 6.6, $H_{CH\text{-}O}$), 5.78 (1H, m, H_4), 2.63 (2H, q, J = 7.5, H_3), 2.46 (2H, m, H_2), 1.54 (3H, d, J = 6.7, H_{Me}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 172.1 (C_1), 141.6, 137.3 (C_{Ar}), 132.7 (C_4), 131.6 (C_5), 130.9 (C_7), 128.8 (C_6), 127.2, 126.1 (C_{Ar}), 72.2 ($C_{CH\text{-}O}$), 34.1 (C_2), 28.0 (C_3), 22.1 (C_{Me}). One C_{Ar} peak overlaps with *trans*, *cis*-**130** C_{Ar} peaks.

***trans*, *cis*- and *trans*, *trans*-(1*R*,2*S*,5*R*)-Menthyl 7-phenyl-4,6-heptadienoate (131)**



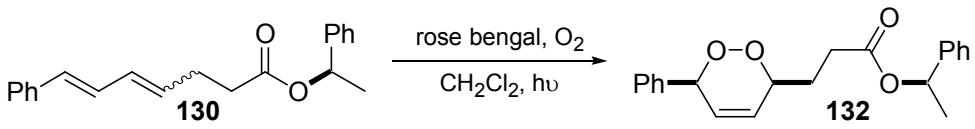
129 **131**

To a solution of diene **129** (106.4 mg, 0.53 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C was added oxalyl chloride (65 μL, 0.75 mmol) and the mixture was stirred at 0 °C for 5 mins and at rt for 30 mins. (1*R*,2*S*,5*R*)-Menthol (118.1 mg, 0.76 mmol) was added and the mixture was stirred at rt for 16 hrs. The solvent was removed under reduced pressure and the residue purified by column chromatography (5% (v/v) Et_2O/hexanes, $\mathbf{R_f}$ = 0.32) to yield the title compound **131** (20.9 mg, 12%) as a pale yellow oil with greater than 90% *trans*, *cis*-configuration as determined by ^{1}H NMR. **HRMS** calculated for $C_{23}H_{32}O_2Na^+$ [M+Na^+]: 363.2300; found: 363.2300.

Major *trans*, *cis*-**131**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.42-7.20 (5H, m, H_{Ar}), 7.07 (1H, dd, *J* = 10.6, 15.6, H_6), 6.54 (1H, d, *J* =15.6, H_7), 6.19 (1H, t, *J* = 10.6, H_5), 5.48 (1H, dt, *J* = 7.4, 10.6, H_4), 4.70 (1H, dt, *J* = 4.6, 10.9, $H_{CH\text{-}O}$), 2.62 (2H, q, *J* = 7.4, H_3), 2.42 (2H, t, *J* = 7.4, H_2), 2.05-1.28 (5H, m, $H_{menthol}$), 1.13-0.75 (11H, m, $H_{menthol}$); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 172.5 (C_1), 137.4 (C_{Ar}), 132.9 (C_7), 130.3 (C_4), 129.9 (C_5), 128.6, 127.5, 126.2 (C_{Ar}), 124.0 (C_6), 74.3 ($C_{CH\text{-}O}$), 47.0, 40.9 ($C_{menthol}$), 34.6 (C_2), 34.2, 31.3, 26.3 ($C_{menthol}$), 23.7 (C_3), 23.4, 21.9, 20.7, 16.3 ($C_{menthol}$).

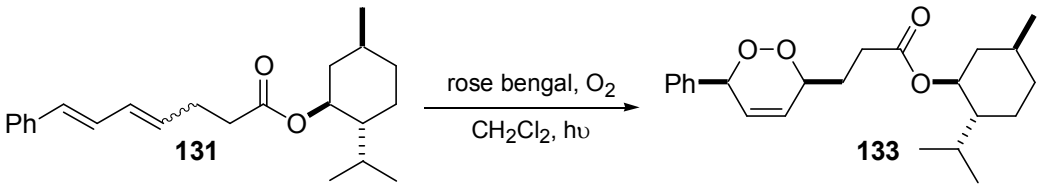
Minor *trans*, *trans*-**131**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.42-7.20 (5H, m, H_{Ar}), 6.73 (1H, dd, *J* = 10.6, 15.6, H_6), 6.45 (1H, d, *J* =15.6, H_7), 6.26 (1H, m, H_5), 5.80 (1H, dt, *J* = 7.6, 15.6, H_4), 4.70 (1H, dt, *J* = 4.6, 10.9, $H_{CH\text{-}O}$), 2.62 (2H, q, *J* = 7.4, H_3), 2.42 (2H, t, *J* = 7.4, H_2), 2.05-1.28 (5H, m, $H_{menthol}$), 1.13-0.75 (11H, m, $H_{menthol}$); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 172.4 (C_1), 137.4 (C_{Ar}), 132.8 (C_4), 131.5 (C_5), 130.8 (C_7), 128.8 (C_6), 127.2, 126.1 (C_{Ar}), 74.1 ($C_{CH\text{-}O}$), 46.9, 41.0 ($C_{menthol}$), 34.9 (C_2), 34.2, 31.3 ($C_{menthol}$), 28.2 (C_3), 25.4, 24.6, 22.0, 20.7, 16.2 ($C_{menthol}$). One C_{Ar} peak overlaps with *trans*, *cis*-**131** C_{Ar}.

(3*S*,6*S*)- and (3*R*,6*R*)-3-Phenyl-6-(ethylcarboxyl-(*R*)-1-phenylethyl)-3,6-dihydro-1,2-dioxine (132)



Dioxine **132** was synthesised, as per **128**, from diene **130** (139.9 mg, 0.46 mmol) with rose bengal (90.7 mg) in CH_2Cl_2 (100 mL) for 9 hrs and purified by column chromatography (30% (v/v) hexanes/CH_2Cl_2, $\mathbf{R_f}$ = 0.27) to yield the title compound **132** (93.8 mg, 60%) as a pale yellow oil. **HRMS** calculated for $C_{21}H_{22}O_4NH_4^+$ [M+NH_4^+]: 356.1862; found: 356.1853; calculated for $C_{21}H_{22}O_4Na^+$ [M+Na^+]: 361.1416; found: 361.1415; **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.50-7.18 (10H, m, H_{Ar}), 6.13-6.04 (2H, m, $H_{4,5}$), 5.89 (1H, q, *J* = 6.6, $H_{CH\text{-}O}$), 5.57 (1H, m, H_3), 4.56 (1H, m, H_6), 2.66-2.44 (2H, m, H_8), 2.20-1.94 (2H, m, H_7), 1.53 (3H, t, *J* = 6.6, H_{Me}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 172.43, 172.41 (C_9), 141.6, 136.9, 128.9, 128.6, 128.54, 128.45, 128.4, 128.0 (C_{Ar}), 127.8, 127.2 ($C_{4,5}$), 126.03, 125.96 (C_{Ar}), 80.3 (C_3), 77.3 (C_6), 72.4 ($C_{CH\text{-}O}$), 30.29, 30.25 (C_8), 28.15, 28.09 (C_7), 22.23, 22.17 (C_{Me}).

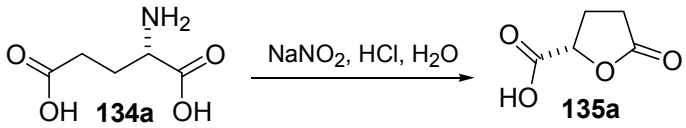
(3*S*,6*S*)- and (3*R*,6*R*)-3-Phenyl-6-(ethylcarboxyl-(1*R*,2*S*,5*R*)-Menthyl)-3,6-dihydro-1,2-dioxine (133)



Dioxine **133** was synthesised, as per **128**, from diene **131** (130.3 mg, 0.38 mmol) with rose bengal (75.4 mg) in CH_2Cl_2 (100 mL) for 10 hrs and purified by column chromatography (50% (v/v) CH_2Cl_2/hexanes, $\mathbf{R_f}$ = 0.36) to yield the title compound **133** (78.0 mg, 55%) as a clear oil. **HRMS** calculated for $C_{23}H_{32}O_4NH_4^+$ [M+NH_4^+]: 390.2645; found: 390.2637; calculated for $C_{23}H_{32}O_4Na^+$ [M+Na^+]: 395.2199; found: 395.2192. **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.40-7.34 (5H, m, H_{Ar}), 6.15-6.07 (2H, m, $H_{4,5}$), 5.57 (1H, m, H_3), 4.68 (1H, dt, *J* = 4.4, H_{10}), 4.58 (1H, m, H_6), 2.55-2.40 (2H, m, H_8), 2.19-1.91 (2H, m, H_7), 1.84 (1H, dq, *J* = 2.7, 7.0, $H_{menthol}$), 1.74-1.30 (5H, m, $H_{menthol}$), 1.12-0.81 (9H, m, $H_{menthol}$), 0.75 (3H, t, *J* = 6.9, $H_{menthol}$); **^{13}C NMR**

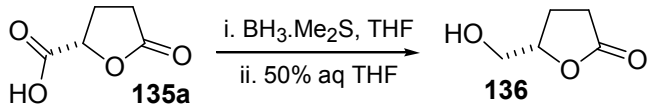
(75.5 MHz, $CDCl_3$) δ = 172.8 (C_9), 137.0, 128.9, 128.60, 128.56 (C_{Ar}), 128.1, 128.0, 127.1 ($C_{4,5}$), 80.3 (C_3), 77.4 (C_6), 74.2 (C_{10}), 46.9, 40.9, 34.2, 31.3 ($C_{menthol}$), 30.4, 30.3 (C_8), 28.3, 28.2 (C_7), 26.24, 26.19, 23.41, 23.36, 22.0, 20.7, 16.30, 16.26 ($C_{menthol}$).

(*S*)-5-Oxo-2-tetrahydrofurancarboxylic acid (135a)



To a stirred suspension of L-glutamic acid (**134a**) (25.08 g, 168.75 mmol) in H_2O (75 mL) at 0 °C was added simultaneously a solution of $NaNO_2$ (17.72 g, 256.81 mmol) in H_2O (100 mL) and a solution of concentrated HCl (25 mL of a 37% solution, 255.00 mmol) in H_2O (75 mL) over 2 hrs. The mixture was stirred at rt for 16 hrs. The water was removed under reduced pressure, the residue extracted into hot EtOAc and filtered through celite. The solvent was removed under reduced pressure with PhMe to ensure that all traces of water had been removed. Recrystallisation from $CHCl_3$ afforded acid **135a** (8.20 g, 37%) as a white crystalline solid. **mpt** 68-71 °C (lit.[189] **mpt** 71 – 72 °C); $[\alpha]_D$ = +16.9 (*c* = 1.01, EtOH) (lit.[187] $[\alpha]_D$ = +15.6 (*c* = 2.0, EtOH)); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 8.71 (1H, bs, H_{OH}), 5.00 (1H, dd, *J* = 4.3, 8.3, H_5), 2.75-2.52 (1H, m, $H_{3,4}$), 2.51-2.31 (1H, m, H_4); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 176.3, 174.6 ($C_{2,6}$), 75.2 (C_5), 26.7 (C_3), 25.8 (C_4).

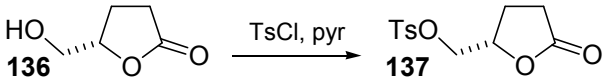
(*S*)-Dihydro-5-(hydroxymethyl)-2(3H)-furanone (136)



To a solution of acid **135a** (203.2 mg, 1.56 mmol) in anhydrous THF (0.5 mL) was added $BH_3.Me_2S$ (1 mL of a 2 M solution in THF, 2.00 mmol) slowly and the mixture was stirred at rt for 2.5 hrs. Decomposition of the resulting mixture was effected by the careful addition of 50% aqueous THF (1 mL). The reaction was concentrated under reduced pressure, the residue taken up in EtOAc (20 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc, R_f = 0.17) to yield alcohol **136** (151.4

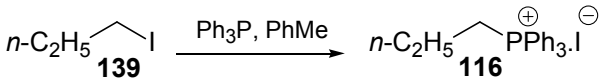
mg, 84%) as a colourless oil. **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 4.63 (1H, m, H_5), 4.00 (1H, dd, *J* = 2.9, 12.6, H_6), 3.64 (1H, dd, *J* = 4.7, 12.6, H_6), 2.68-2.53 (2H, m, H_3), 2.50 (1H, bs, H_{OH}), 2.32-2.08 (2H, m, H_4); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 178.1 (C_2), 80.9 (C_5), 63.7 (C_6), 28.5, 23.0 ($C_{3,4}$).

(*S*)-Dihydro-5-[[[(4-methylphenyl)sulfonyl]oxy]methyl]-2(3H)-furanone (137)



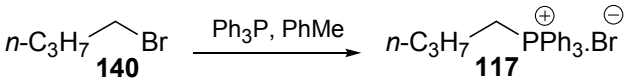
To a solution of alcohol **136** (78.7 mg, 0.68 mmol) in anhydrous pyr (1.5 mL) was added *p*-toluenesulfonyl chloride (202.0 mg, 1.06 mmol) at 0 °C under an atmosphere of N_2 and the mixture was stirred at rt for 16 hrs. After this time, H_2O (10 mL) and EtOAc (20 mL) were added. The organic layer was washed with saturated $CuSO_4$ (10 mL x 4), H_2O (10 mL x 2) and brine (10 mL), then dried (Na_2SO_4), filtered and concentrated under reduced pressure. The product was recrystallised from C_6H_6/hexanes to yield tosylate **137** (83.3 mg, 45%) as fine white crystals. **mpt** 85-87 °C (lit.[193] **mpt** 85-87 °C); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.79 (2H, d, *J* = 8.2, H_{Ar}), 7.36 (2H, d, *J* = 8.5, H_{Ar}), 4.68 (1H, m, H_5), 4.21-4.11 (2H, m, H_6), 2.68-2.49 (2H, m, H_3), 2.46 (3H, s, H_{Me}), 2.35 (1H, m, H_4), 2.13 (1H, m, H_4); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 176.0 (C_2), 145.4, 132.2, 130.1, 128.0 (C_{Ar}), 76.4, 69.9 ($C_{5,6}$), 27.9, 23.5, 21.7 ($C_{3,4,Me}$).

***n*-Propyltriphenylphosphonium iodide (116)**



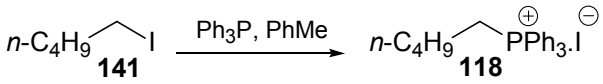
1-Iodopropane (**139**) (19.99 g, 116.4 mmol) was added slowly to a stirred solution of Ph_3P (31.07 g, 117.3 mmol) in PhMe (125 mL) and heated under reflux for 24 hrs. The cooled salt **116** (46.68 g, 93%) was collected by vacuum filtration as a white crystalline product. **mpt** 208-210 °C (lit.[193] **mpt** 203-204 °C); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.89-7.62 (15H, m, H_{Ar}), 3.76-3.58 (2H, m, H_1), 1.80-1.62 (2H, m, H_2), 1.25 (3H, dt, *J* = 1.5, 7.3, H_3); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 135.0 (d, *J* = 2.9, C_{Ar}), 133.5 (d, *J* = 10.3, C_{Ar}), 130.5 (d, *J* = 12.6, C_{Ar}), 117.9 (d, *J* = 85.9, C_{Ar}), 24.7 (d, *J* = 49.8, C_1), 16.4 (d, *J* = 4.6, C_3), 15.3 (d, *J* = 17.2, C_2).

n-Butyltriphenylphosphonium bromide (117)



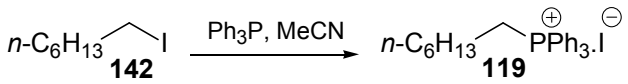
1-Bromobutane (**140**) (15.09 g, 110.1 mmol) was added slowly to a stirred solution of Ph_3P (28.96 g, 109.3 mmol) in PhMe (75 mL) and heated under reflux for 24 hrs. The cooled salt **117** (30.43 g, 70%) was collected by vacuum filtration as a white crystalline product. **mpt** 241-244 °C (lit.[194] **mpt** 242-243 °C); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.89-7.63 (15H, m, H_{Ar}), 3.84-3.64 (2H, m, H_1), 1.76-1.49 (4H, m, $H_{2,3}$), 0.88 (3H, t, *J* = 6.8, H_4); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 134.9 (d, *J* = 2.9, C_{Ar}), 133.4 (d, *J* = 10.3, C_{Ar}), 130.4 (d, *J* = 12.0, C_{Ar}), 118.1 (d, *J* = 85.9, C_{Ar}), 24.4 (d, *J* = 4.6, $C_{2,3}$), 23.6 (d, *J* = 16.0, $C_{2,3}$), 22.4 (d, *J* = 49.8, C_1), 13.6 (C_4).

n-Pentyltriphenylphosphonium iodide (118)



1-Iodopentane (**141**) (20.13 g, 99.6 mmol) was added slowly to a stirred solution of Ph_3P (26.32 g, 99.3 mmol) in PhMe (100 mL) and heated under reflux for 24 hrs. The cooled salt **118** (43.98 g, 96%) was collected by vacuum filtration as a white crystalline product. **mpt** 171-173 °C (lit.[195] **mpt** 165-170 °C); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.87-7.65 (15H, m, H_{Ar}), 3.69-3.49 (2H, m, H_1), 1.72-1.51 (4H, m, $H_{2,3}$), 1.39-1.20 (2H, m, H_4), 0.79 (3H, t, *J* = 7.4, H_5); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 134.9 (d, *J* = 2.9, C_{Ar}), 133.3 (d, *J* = 10.3, C_{Ar}), 130.4 (d, *J* = 12.6, C_{Ar}), 117.8 (d, *J* = 85.9, C_{Ar}), 32.1 (d, *J* = 15.5, C_2), 22.8 (d, *J* = 49.8, C_1), 21.95 (d, *J* = 4.6, C_3), 21.87 (C_4), 13.4 (C_5).

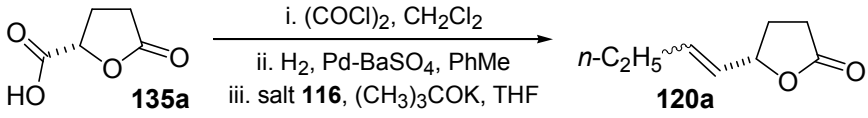
n-Heptyltriphenylphosphonium iodide (119)



1-Iodoheptane (**142**) (10.04 g, 43.5 mmol) was added slowly to a stirred solution of Ph_3P (11.60 g, 43.8 mmol) in MeCN (100 mL) and the mixture was heated under reflux for 24 hrs. The solvent was removed under reduced pressure and the resulting viscous oil was refluxed in Et_2O (150 mL x 2). The cooled salt **119** (21.06 g, 99%)

was collected by vacuum filtration as a white crystalline solid. **mpt** 89-91 °C (lit.[196] **mpt** 128-131 °C); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.87-7.64 (15H, m, H_{Ar}), 3.71-3.50 (2H, m, H_1), 1.71-1.52 (4H, m, $H_{2,3}$), 1.33-1.09 (6H, m, $H_{4,5,6}$), 0.80 (3H, t, *J* = 6.7, H_7); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 135.0 (d, *J* = 2.9, C_{Ar}), 133.4 (d, *J* = 9.7, C_{Ar}), 130.4 (d, *J* = 12.6, C_{Ar}), 117.9 (d, *J* = 85.9, C_{Ar}), 31.1 ($C_{4,5,6}$), 30.2 (d, *J* = 15.5, $C_{2,3}$), 28.6 ($C_{4,5,6}$), 22.9 (d, *J* = 50.4, C_1), 22.4 (d, *J* = 4.6, $C_{2,3}$), 22.3 ($C_{4,5,6}$), 13.8 (C_7).

cis- and *trans*-(*S*)-5-(1-Butenyl)dihydro-2(3H)-furanone (120a)



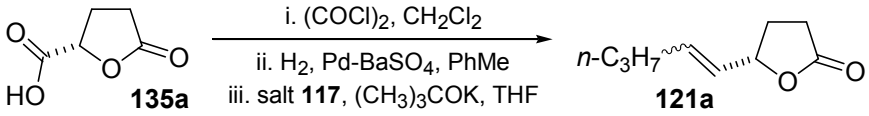
Acid **135a** (1.48 g, 11.38 mmol) was refluxed in oxalyl chloride (2.6 mL, 30.42 mmol) and anhydrous CH_2Cl_2 (2.6 mL) under N_2 for 2.75 hrs. The solvent and excess oxalyl chloride were removed under reduced pressure to yield acid chloride **138a** as a pale yellow oil. To a suspension of Pd-$BaSO_4$ (0.51 g) in anhydrous PhMe (45 mL) was bubbled through H_2 for 30 mins, prior to the addition of acid chloride **138a** in anhydrous PhMe (5 mL). H_2 was bubbled through the mixture which was stirred at 60-70 °C for 3 hrs. The mixture was filtered through celite, rinsed with anhydrous CH_2Cl_2 and concentrated under reduced pressure to yield aldehyde **115a** as a colourless oil. $(CH_3)_3COK$ (1.59 g, 13.46 mmol) was added to a suspension of salt **116** (6.23 g, 14.41 mmol) in anhydrous THF (35 mL) and the mixture was stirred under N_2 at 0 °C for 1.5 hrs. A bright orange solution resulted, indicative of ylide formation. Aldehyde **115a** was added in anhydrous THF (5 mL) at 0 °C and stirring continued under N_2 at rt for 16 hrs. The reaction was filtered through celite and concentrated under reduced pressure. The residue was purified by column chromatography (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.15) to afford alkene **120a** (0.82 g, 52%), a colourless oil, as a 3:1 mixture of *cis*- and *trans*-isomers determined by ^{1}H NMR.

Major *cis*-**120a**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 5.67 (1H, dt, *J* = 10.8, 7.6, H_7), 5.45 (1H, m, H_6), 5.25 (1H, q, *J* = 7.8, H_5), 2.59-2.50 (2H, m, H_3), 2.38 (1H, m, H_4), 2.22-2.04 (2H, m, H_8), 1.94 (1H, m, H_4), 1.01 (3H, t, *J* = 7.5, H_9); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.1 (C_2), 137.2 (C_7), 126.6 (C_6), 76.3 (C_5), 29.2 (C_4), 28.9 (C_3),

21.1 (C_8), 14.1 (C_9).

Minor *trans*-**120a**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 5.86 (1H, dt, *J* = 15.3, 6.3, H_7), 5.45 (1H, m, H_6), 5.25 4.89 (1H, q, *J* = 7.1, H_5), 2.59-2.50 (2H, m, H_3), 2.38 (1H, m, H_4), 2.22-2.04 (2H, m, H_8), 1.94 (1H, m, H_4), 1.01 (3H, t, *J* = 7.5, H_9); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.0 (C_2), 137.0 (C_7), 126.4 (C_6), 81.1 (C_5), 28.75, 28.66 ($C_{3,4}$), 25.0 (C_8), 12.9 (C_9).

cis- and *trans*-(*S*)-5-(1-Pentenyl)dihydro-2(3H)-furanone (121a)

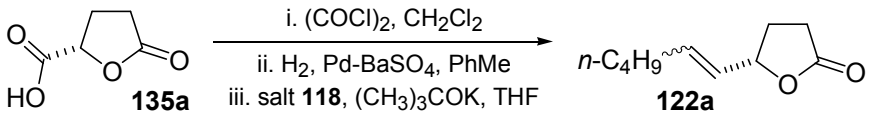


Alkene **121a** was synthesised, as per **120a**, *via* a three step sequence from acid **135a** (1.57 g, 12.07 mmol). Acid chloride **138a** was prepared with oxalyl chloride (2.8 mL, 32.76 mmol) in anhydrous CH_2Cl_2 (2.8 mL), aldehyde **115a** from a Rosenmund reduction with Pd-$BaSO_4$ (0.51 g) in anhydrous PhMe (45 mL) and alkene **121a** by Wittig olefination with $(CH_3)_3COK$ (1.72 g, 14.56 mmol) and salt **117** (6.71 g, 16.80 mmol) in anhydrous THF (35 mL). Purification by column chromatography (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.20) gave alkene **121a** (0.79 g, 43%), a colourless oil, as a 9:1 mixture of *cis*- and *trans*-isomers determined by ^{1}H NMR.

Major *cis*-**121a**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 5.66 (1H, dt, *J* = 10.9, 7.6, H_7), 5.48 (1H, m, H_6), 5.25 (1H, q, *J* = 7.8, H_5), 2.58-2.53 (2H, m, H_3), 2.38 (1H, m, H_4), 2.17-2.02 (2H, m, H_8), 1.94 (1H, m, H_4), 1.41 (2H, dsx, *J* = 7.4, 2.1, H_9), 0.91 (2.7H, t, *J* = 7.4, H_{10}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.0 (C_2), 135.3 (C_7), 127.3 (C_6), 76.2 (C_5), 29.5 (C_8), 29.1 (C_4), 28.8 (C_3), 22.3 (C_9), 13.4 (C_{10}).

Minor *trans*-**121a**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 5.80 (1H, dt, *J* = 14.7, 7.2, H_7), 5.48 (1H, m, H_6), 4.89 (1H, q, *J* = 7.1, H_5), 2.58-2.53 (2H, m, H_3), 2.38 (1H, m, H_4), 2.17-2.02 (2H, m, H_8), 1.94 (1H, m, H_4), 1.41 (2H, dsx, *J* = 7.4, 2.1, H_9), 0.90 (1H, t, *J* = 7.4, H_{10}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.0 (C_2), 135.1 (C_7), 127.5 (C_6), 80.9 (C_5), 33.9 (C_4), 28.6, 28.5 ($C_{3,8}$), 21.7 (C_9), 13.4 (C_{10}).

***cis*- and *trans*-(*S*)-5-(1-Hexenyl)dihydro-2(3H)-furanone (122a)**

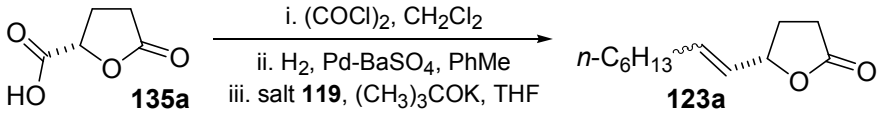


Alkene **122a** was synthesised, as per **120a**, *via* a three step sequence from acid **135a** (1.56 g, 11.99 mmol). Acid chloride **138a** was prepared with oxalyl chloride (2.8 mL, 32.76 mmol) in anhydrous CH_2Cl_2 (2.8 mL), aldehyde **115a** from a Rosenmund reduction with Pd-$BaSO_4$ (0.51 g) in anhydrous PhMe (45 mL) and alkene **122a** by Wittig olefination with $(CH_3)_3COK$ (1.70 g, 14.39 mmol) and salt **118** (6.98 g, 15.16 mmol) in anhydrous THF (35 mL). Purification by column chromatography (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.24) gave alkene **122a** (0.26 g, 13%), a yellow oil, as a 19:1 mixture of *cis*- and *trans*-isomers determined by ^{1}H NMR.

Major *cis*-**122a**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 5.67 (0.95H, dt, *J* = 10.8, 7.7, H_7), 5.47 (1H, m, H_6), 5.25 (1H, q, *J* = 7.8, H_5), 2.59-2.53 (2H, m, H_3), 2.37 (1H, m, H_4), 2.17-2.05 (2H, m, H_8), 1.94 (1H, m, H_4), 1.47-1.19 (4H, m, $H_{9,10}$), 0.92-0.88 (3H, m, H_{11}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 176.9 (C_2), 135.4 (C_7), 127.1 (C_6), 76.1 (C_5), 31.3 ($C_{9,10}$), 29.0 (C_4), 28.7 (C_3), 27.2 (C_8), 21.9 ($C_{9,10s}$), 13.6 (C_{11}).

Minor *trans*-**122a**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 5.80 (1H, dt, *J* = 14.8, 7.1, H_7), 5.47 (1H, m, H_6), 4.89 (1H, q, *J* = 7.2, H_5), 2.59-2.53 (2H, m, H_3), 2.37 (1H, m, H_4), 2.17-2.05 (2H, m, H_8), 1.94 (1H, m, H_4), 1.47-1.19 (4H, m, $H_{9,10}$), 0.92-0.88 (3H, m, H_{11}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 176.9 (C_2), 135.3 (C_7), 127.2 (C_6), 80.9 (C_5), 31.5, 30.6, 28.6, 28.4, 21.8 ($C_{3,4,8,9,10}$), 13.6 (C_{11}).

***cis*- and *trans*-(*S*)-5-(1-Octenyl)dihydro-2(3H)-furanone (123a)**



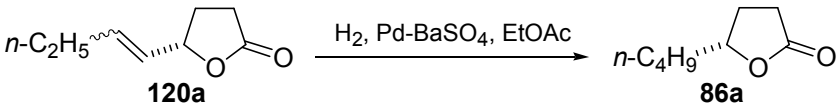
Alkene **123a** was synthesised, as per **120a**, *via* a three step sequence from acid **135a** (1.64, 12.61 mmol). Acid chloride **138a** was prepared with oxalyl chloride (3 mL, 35.10 mmol) in anhydrous CH_2Cl_2 (3 mL), aldehyde **115a** from a Rosenmund reduction with Pd-$BaSO_4$ (0.50 g) in anhydrous PhMe (45 mL) and alkene **123a** by

Wittig olefination with $(CH_3)_3COK$ (2.04 g, 18.19 mmol) and salt **119** (8.84 g, 18.09 mmol) in anhydrous THF (35 mL). Purification by column chromatography (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.24) gave alkene **123a** (0.87 g, 35%), a yellow oil, as a 9:1 mixture of *cis*- and *trans*-isomers determined by ^{1}H NMR.

Major *cis*-**123a**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 5.67 (1H, dt, *J* = 10.9, 7.6, H_7), 5.46 (1H, m, H_6), 5.24 (1H, q, *J* = 7.8, H_5), 2.58-2.53 (2H, m, H_3), 2.37 (1H, m, H_4), 2.18-2.04 (2H, m, H_8), 1.93 (1H, m, H_4), 1.44-1.21 (8H, m, $H_{9,10,11,12}$) 0.88 (3H, t, *J* = 6.7, H_{13}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.1 (C_2), 135.7 (C_7), 127.2 (C_6), 76.3 (C_5), 31.5 ($C_{9,10,11,12}$), 29.3, 29.2 ($C_{4,9,10,11,12}$), 28.9 (C_3), 28.7 ($C_{9,10,11,12}$), 27.7 (C_8), 22.5 ($C_{9,10,11,12}$), 13.9 (C_{13}).

Minor *trans*-**123a**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 5.80 (1H, dt, *J* = 14.7, 7.2 Hz, H_7), 5.46 (1H, m, H_6), 4.89 (1H, q, *J* = 7.1, H_5), 2.58-2.53 (2H, m, H_3), 2.37 (1H, m, H_4), 2.18-2.04 (2H, m, H_8), 1.93 (1H, m, H_4), 1.44-1.21 (8H, m, $H_{9,10,11,12}$) 0.88 (3H, t, *J* = 6.7, H_{13}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.1 (C_2), 135.6 (C_7), 127.3 (C_6), 81.1 (C_5), 32.0, 28.66, 28.63 ($C_{3,4,8,9,10,11,12}$), 13.9 (C_{13}); four peaks overlap with *cis*-**128a** for ($C_{3,4,8,9,10,11,12}$).

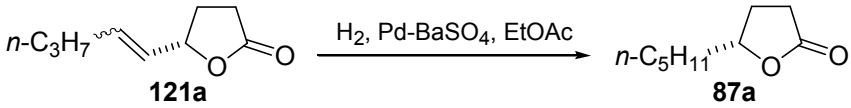
(*R*)-Octalactone (86a)



120a **86a**

Alkene **120a** (215.9 mg, 1.54 mmol) was stirred in a suspension of Pd-$BaSO_4$ (47.5 mg) with EtOAc (5mL) under an atmosphere of H_2 for 16 hrs. The reaction was filtered through celite and concentrated under reduced pressure. The residue was passed through an Al_2O_3 plug (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.39) and purified by column chromatography (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.18) to afford γ-lactone **86a** (69.9 mg, 32%) as a colourless oil. $\mathbf{[\alpha]_D}$ = +52.9 (*c* = 1.21, MeOH) (lit.[147] $\mathbf{[\alpha]_D}$ = +56.2 (*c* = 1.5-2.5, MeOH)); **MS** *m/z* (%) 142 (M^+, <1), 114 (2), 100 (6), 86 (5), 85 (100), 70 (3), 57 (9), 43 (3); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 4.48 (1H, qn, *J* = 6.7, H_5), 2.52 (2H, dd, *J* = 6.7, 9.9, H_3), 2.31 (1H, sx, *J* = 6.4, $H_{4a/b}$), 1.91-1.26 (7H, m, $H_{4a/b,6,7,8}$), 0.91 (3H, t, *J* = 6.7, H_9); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.2 (C_2), 80.9 (C_5), 35.1 ($C_{6,7,8}$), 28.7 (C_3), 27.8 (C_4), 27.2, 22.3 ($C_{6,7,8}$), 13.7 (C_9).

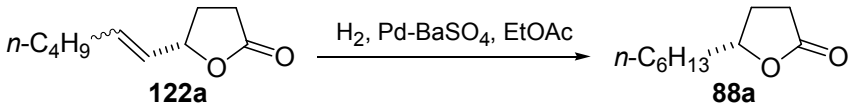
(*R*)-Nonalactone (87a)



121a **87a**

γ-Nonalactone (**87a**) was prepared, as per **86a**, from alkene **121a** (117.7 mg, 0.76 mmol) and Pd-$BaSO_4$ (5.2 mg) in EtOAc (4mL) for 6 hrs. The residue was passed through an Al_2O_3 plug (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.38) and purified by column chromatography (50% (v/v) Et_2O/hexanes, $\mathbf{R_f}$ = 0.21) to afford γ-lactone **87a** (94.3 mg, 79%) as a colourless oil. $\mathbf{[\alpha]_D}$ = +47.9 (*c* = 0.84, MeOH) (lit.[147] $\mathbf{[\alpha]_D}$ = +51.8 (*c* = 1.5-2.5, MeOH)); **MS** *m/z* (%) 156 (M^+, <1), 128 (3), 114 (3), 100 (5), 86 (5), 85 (100), 71 (2), 57 (6), 43 (7); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 4.48 (1H, qn, *J* = 6.8, H_5), 2.52 (2H, dd, *J* = 6.8, 9.4, H_3), 2.31 (1H, sx, *J* = 6.8, $H_{4a/b}$), 1.91-1.24 (9H, m, $H_{4a/b,6,7,8,9}$), 0.89 (3H, t, *J* = 6.9, H_{10}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.1 (C_2), 80.8 (C_5), 35.3, 31.2 ($C_{6,7,8,9}$), 28.6 (C_3), 27.7 (C_4), 24.6, 22.2 ($C_{6,7,8,9}$), 13.7 (C_{10}).

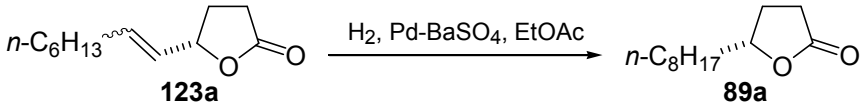
(*R*)-Decalactone (88a)



122a **88a**

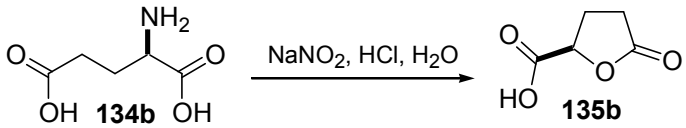
γ-Decalactone (**88a**) was prepared, as per **86a**, from alkene **122a** (172.9 mg, 1.03 mmol) and Pd-$BaSO_4$ (16.4 mg) in EtOAc (4mL) for 24 hrs, followed by a further portion of Pd-$BaSO_4$ (18.1 mg) for another 24 hrs. The residue was passed through an Al_2O_3 plug (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.41) and purified by column chromatography (50% (v/v) Et_2O/hexanes, $\mathbf{R_f}$ = 0.21) to afford γ-lactone **88a** (109.8 mg, 63%) as a colourless oil. $\mathbf{[\alpha]_D}$ = +48.4 (*c* = 1.08, MeOH), (lit.[147] $\mathbf{[\alpha]_D}$ = +48.5 (*c* = 1.5-2.5, MeOH)); **MS** *m/z* (%) 170 (M^+, <1), 128 (11), 114 (2), 100 (5), 86 (5), 85 (100), 57 (7), 43 (8); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 4.48 (1H, qn, *J* = 6.7, H_5), 2.53 (2H, dd, *J* = 6.7, 9.7, H_3), 2.31 (1H, sx, *J* = 6.7, $H_{4a/b}$), 1.91-1.22 (11H, m, $H_{4a/b,6,7,8,9,10}$), 0.88 (3H, t, *J* = 6.7, H_{11}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.2 (C_2), 80.9 (C_5), 35.4, 31.5 ($C_{6,7,8,9,10}$), 28.8, 28.7 ($C_{3,6,7,8,9,10}$), 27.8 (C_4), 25.0, 22.4 ($C_{6,7,8,9,10}$), 13.9 (C_{11}).

(*R*)-Dodecalactone (89a)



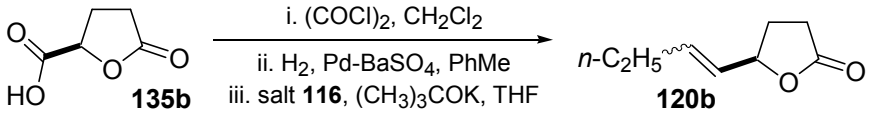
γ-Dodecalactone (**89a**) was prepared, as per **86a**, from alkene **123a** (406.0 mg, 2.07 mmol) and Pd-$BaSO_4$ (43.3 mg) in EtOAc (4mL) for 48 hrs, followed by a further portion of Pd-$BaSO_4$ (39.1 mg) for another 24 hrs. The residue was passed through an Al_2O_3 plug (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.40) and purified by column chromatography (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.22) to afford γ-lactone **89a** (217.2 mg, 53%) as a colourless oil. $\mathbf{[\alpha]_D}$ = +41.9 (*c* = 1.17, MeOH) (lit.[147] $\mathbf{[\alpha]_D}$ = +42.2 (*c* = 1.5-2.5, MeOH)); **MS** *m/z* (%) 198 (<1), 128 (11), 114 (4), 113 (1), 100 (6), 86 (6), 85 (100), 57 (11), 43 (12); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 4.48 (1H, qn, *J* = 6.7, H_5), 2.53 (2H, dd, *J* = 6.7, 9.8, H_3), 2.31 (1H, sx, *J* = 6.7, $H_{4a/b}$), 1.91-1.22 (15H, m, $H_{4a/b,6,7,8,9,10,11,12}$), 0.88 (3H, t, *J* = 6.6, H_{13}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.2 (C_2), 80.9 (C_5), 35.4, 31.7, 29.3, 29.2, 29.0 ($C_{6,7,8,9,10,11,12}$), 28.7 (C_3), 27.8 (C_4), 25.1, 22.5 ($C_{6,7,8,9,10,11,12}$), 13.9 (C_{13}).

(*R*)-5-Oxo-2-tetrahydrofurancarboxylic acid (135b)



Acid **135b** was prepared, as per **135a**, from D-glutamic acid (**134b**) (15.00 g, 100.90 mmol) in H_2O (50 mL) with sodium nitrite (10.63 g, 154.06 mmol) in H_2O (50 mL) and concentrated HCl (15 mL of a 37% solution, 153.00 mmol) in H_2O (45 mL). Recrystallisation from $CHCl_3$ afforded acid **135b** (4.84 g, 37%) as a white crystalline solid. **mpt** 67-70 °C (lit.[197] **mpt** 73.5-74.0 °C); $\mathbf{[\alpha]_D}$ = -14.9 (*c* = 1.14, EtOH) (lit.[187] $\mathbf{[\alpha]_D}$ = -15.7 (*c* = 2.4, EtOH)); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 9.14 (1H, bs, H_{OH}), 5.00 (1H, dd, *J* = 4.4, 8.6, H_5), 2.74-2.53 (3H, m, $H_{3,4}$), 2.52-2.32 (1H, m, H_4); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 176.2, 174.5 ($C_{2,6}$), 75.2 (C_5), 26.7 (C_3), 25.8 (C_4).

***cis*- and *trans*-(*R*)-5-(1-Butenyl)dihydro-2(3H)-furanone (120b)**

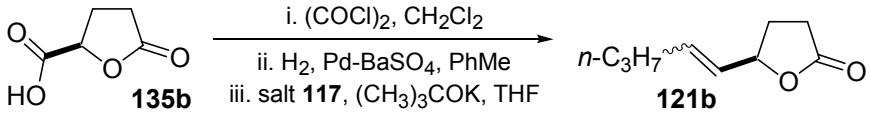


Alkene **120b** was synthesised, as per **120a**, *via* a three step sequence from acid **135b** (1.55 g, 11.91 mmol). Acid chloride **138b** was prepared with oxalyl chloride (2.8 mL, 32.76 mmol) in anhydrous CH_2Cl_2 (2.8 mL), aldehyde **115b** from a Rosenmund reduction with Pd-$BaSO_4$ (0.50 g) in anhydrous PhMe (45 mL) and alkene **120b** by Wittig olefination with $(CH_3)_3COK$ (1.64g, 13.88 mmol) and salt **116** (6.15 g, 14.23 mmol) in anhydrous THF (35 mL). Purification by column chromatography (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.15) gave alkene **120b** (0.75 g, 45%), a colourless oil, as a 9:1 mixture of *cis*- and *trans*-isomers determined by ^{1}H NMR.

Major *cis*-**120b**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 5.67 (1H, dt, *J* = 10.8, 7.6, H_7), 5.42 (1 H, t, *J* = 9.7, H_6), 5.25 (1H, q, *J* = 7.8, H_5), 2.59-2.50 (2H, m, H_3), 2.38 (1H, m, H_4), 2.22-2.04 (2H, m, H_8), 1.94 (1H, m, H_4), 1.01 (3H, t, *J* = 7.5, H_9); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 176.8 (C_2), 136.7 (C_7), 126.4 (C_6), 75.9 (C_5), 28.9 (C_4), 28.5 (C_3), 20.7 (C_8), 13.7 (C_9).

Minor *trans*-**120b**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 5.85 (1H, dt, *J* = 15.4, 6.3, H_7), 5.49 (1H, t, *J* = 7.8, H_6), 4.89 (1H, q, *J* = 7.2, H_5), 2.59-2.50 (2H, m, H_3), 2.38 (1H, m, H_4), 2.22-2.04 (2H, m, H_8), 1.94 (1H, m, H_4), 1.01 (3H, t, *J* = 7.5, H_9); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 176.8 (C_2), 136.5 (C_7), 126.2 (C_6), 80.8 (C_5), 28.4, 28.3 ($C_{3,4}$), 24.7 (C_8), 12.6 (C_9).

***cis*- and *trans*-(*R*)-5-(1-Pentenyl)dihydro-2(3H)-furanone (121b)**



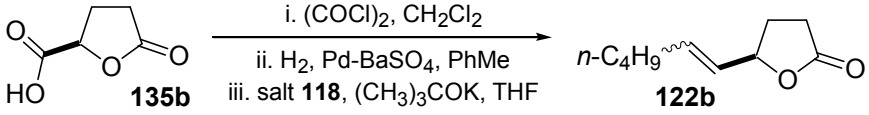
Alkene **121b** was synthesised, as per **120a**, *via* a three step sequence from acid **135b** (1.52 g, 11.64 mmol). Acid chloride **138b** was prepared with oxalyl chloride (2.8 mL, 32.76 mmol) in anhydrous CH_2Cl_2 (2.8 mL), aldehyde **115b** from a Rosenmund reduction with Pd-$BaSO_4$ (0.51 g) in anhydrous PhMe (45 mL) and alkene **121b** by

Wittig olefination with $(CH_3)_3COK$ (1.66 g, 14.18 mmol) and salt **117** (6.14 g, 15.39 mmol) in anhydrous THF (35 mL). Purification by column chromatography (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.20) gave alkene **121b** (1.14 g, 64%), a colourless oil, as a 4:1 mixture of *cis*- and *trans*-isomers determined by ^{1}H NMR.

Major *cis*-**121b**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 5.66 (1H, dt, *J* = 10.9, 7.7, H_7), 5.47 (1H, m, H_6), 5.25 (1H, q, *J* = 7.9, H_5), 2.58-2.50 (2H, m, H_3), 2.37 (1H, m, H_4), 2.19-2.02 (2H, m, H_8), 1.94 (1H, m, H_4), 1.41 (2H, dsx, *J* = 7.4, 2.1, H_9), 0.91 (3H, t, *J* = 7.4, H_{10}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.0 (C_2), 135.2 (C_7), 127.3 (C_6), 76.2 (C_5), 29.5 (C_8), 29.1 (C_4), 28.8 (C_3), 22.3 (C_9), 13.40 (C_{10}).

Minor *trans*-**121b**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 5.80 (1H, dt, *J* = 14.8, 7.3, H_7), 5.47 (1H, m, H_6), 4.89 (1H, q, *J* = 7.2, H_5), 2.58-2.50 (2H, m, H_3), 2.37 (1H, m, H_4), 2.19-2.02 (2H, m, H_8), 1.94 (1H, m, H_4), 1.41 (2H, dsxt, *J* = 7.4, 2.1, H_9), 0.90 (0.45H, t, *J* = 7.2, H_{10}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.0 (C_2), 135.1 (C_7), 127.4 (C_6), 80.9 (C_5), 33.9 (C_4), 28.6, 28.5 ($C_{3,8}$), 21.7 (C_9), 13.36 (C_{10}).

***cis*- and *trans*-(*R*)-5-(1-Hexenyl)dihydro-2(3H)-furanone (122b)**



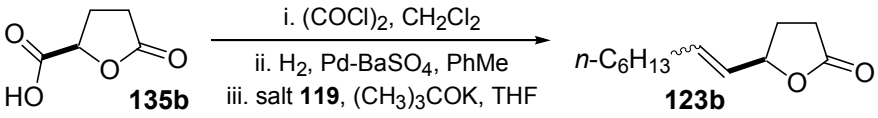
Alkene **122b** was synthesised, as per **120a**, *via* a three step sequence from acid **135b** (1.49 g, 11.47 mmol). Acid chloride **138b** was prepared with oxalyl chloride (2.8 mL, 32.76 mmol) in anhydrous CH_2Cl_2 (2.8 mL), aldehyde **115b** from a Rosenmund reduction with Pd-$BaSO_4$ (0.51 g) in anhydrous PhMe (45 mL) and alkene **122b** by Wittig olefination with $(CH_3)_3COK$ (1.69 g, 14.35 mmol) and salt **118** (6.86 g, 14.35 mmol) in anhydrous THF (35 mL). Purification by column chromatography (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.24) gave alkene **122b** (0.91 g, 47%), a pale yellow oil, as a 4:1 mixture of *cis*- and *trans*-isomers determined by ^{1}H NMR.

Major *cis*-**122b**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 5.67 (1H, dt, *J* = 10.8, 7.6, H_7), 5.47 (1H, m, H_6), 5.25 (1H, q, *J* = 7.8, H_5), 2.59-2.53 (2H, m, H_3), 2.38 (1H, m, H_4), 2.22-2.04 (2H, m, H_8), 1.94 (1H, m, H_4), 1.43-1.24 (4H, m, $H_{9,10}$), 0.90 (3H, t, *J* = 7.0, H_{11}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 176.9 (C_2), 135.4 (C_7), 127.1 (C_6), 76.2

(C_5), 31.3 ($C_{9,10}$), 29.0 (C_4), 28.7 (C_3), 27.2 (C_8), 21.9 ($C_{9,10}$), 13.6 (C_{11}).

Minor *trans*-**122b**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 5.80 (1H, dt, *J* = 14.8, 7.3, H_7), 5.47 (1H, m, H_6), 4.89 (1H, q, *J* = 7.1, H_5), 2.59-2.53 (2H, m, H_3), 2.38 (1H, m, H_4), 2.22-2.04 (2H, m, H_8), 1.94 (1H, m, H_4), 1.43-1.24 (4H, m, $H_{9,10}$), 0.90 (3H, t, *J* = 7.0, H_{11}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 176.8 (C_2), 135.3 (C_7), 127.2 (C_6), 80.9 (C_5), 31.5 ($C_{3,4,8,9,10}$), 30.7 ($C_{3,4,8,9,10}$), 28.6, 28.5 ($C_{3,4,8,9,10}$), 21.8 ($C_{3,4,8,9,10}$), 13.6 (C_{11}).

cis- and *trans*-(*R*)-5-(1-Octenyl)dihydro-2(3H)-furanone (123b)



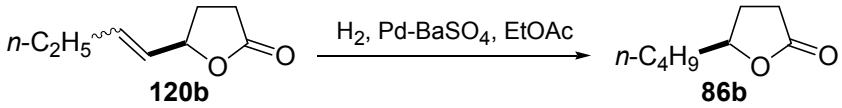
Alkene **123b** was synthesised, as per **120a**, *via* a three step sequence from acid **135b** (1.57, 12.08 mmol). Acid chloride **138b** was prepared with oxalyl chloride (2.8 mL, 32.76 mmol) in anhydrous CH_2Cl_2 (2.8 mL), aldehyde **115b** from a Rosenmund reduction with Pd-$BaSO_4$ (0.52 g) in anhydrous PhMe (45 mL) and alkene **123b** by Wittig olefination with $(CH_3)_3COK$ (1.76 g, 14.92 mmol) and salt **119** (8.06 g, 16.50 mmol) in anhydrous THF (35 mL). Purification by column chromatography (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.24) gave alkene **123b** (1.02 g, 43%), a pale yellow oil, as a 4:1 mixture of *cis*- and *trans*-isomers determined by ^{1}H NMR.

Major *cis*-**123b**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 5.67 (1H, dt, *J* = 10.8, 7.7, H_7), 5.47 (1H, m, H_6), 5.25 (1H, q, *J* = 7.8, H_5), 2.59-2.50 (2H, m, H_3), 2.38 (1H, m, $H_{4a/b}$), 2.18-2.02 (2H, m, H_8), 1.94 (1H, m, $H_{4a/b}$), 1.42-1.21 (8H, m, $H_{9,10,11,12}$) 0.88 (3H, t, *J* = 6.6, H_{13}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.0 (C_2), 135.6 (C_7), 127.1 (C_6), 76.2 (C_5), 31.4 ($C_{9,10,11,12}$), 29.2, 29.1 ($C_{4,9,10,11,12}$), 28.8 (C_3), 28.8 ($C_{9,10,11,12}$), 27.6 (C_8), 22.4 ($C_{9,10,11,12}$), 13.9 (C_{13}).

Minor *trans*-**123b**: **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 5.80 (1H, dt, *J* = 14.6, 7.2 Hz, H_7), 5.47 (1H, m, H_6), 4.89 (1H, q, *J* = 7.1, H_5), 2.59-2.50 (2H, m, H_3), 2.38 (1H, m, $H_{4a/b}$), 2.18-2.02 (2H, m, H_8), 1.94 (1H, m, $H_{4a/b}$), 1.42-1.21 (8H, m, $H_{9,10,11,12}$) 0.88 (3H, t, *J* = 6.6, H_{13}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 176.9 (C_2), 135.4 (C_7), 127.3 (C_6), 81.0 (C_5), 31.9, 28.64, 28.56 ($C_{3,4,8,9,10,11,12}$), 13.9 (C_{13}); four peaks overlap with

cis-**123b** for ($C_{3,4,8,9,10,11,12}$).

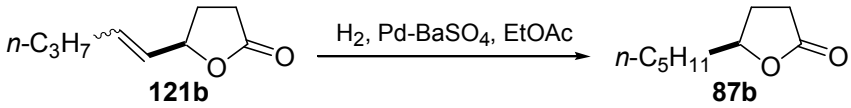
(*S*)-Octalactone (86b)



120b **86b**

γ-Octalactone (**86b**) was prepared, as per **86a**, from alkene **120b** (395.3 mg, 2.82 mmol) and Pd-$BaSO_4$ (41.0 mg) in EtOAc (10 mL) for 48 hrs, followed by a further portion of Pd-$BaSO_4$ (44.8 mg) for another 24 hrs. The residue was passed through an Al_2O_3 plug (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.39) and purified by column chromatography (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.18) to afford γ-lactone **86b** (164.6 mg, 41%) as a colourless oil. $\mathbf{[\alpha]_D}$ = -52.2 (c=1.23, MeOH) (lit.[147] $\mathbf{[\alpha]_D}$ = -56.6 (*c* = 1.5-2.5, MeOH)); **MS** *m/z* (%) 142 (M^+, <1), 114 (2), 100 (6), 86 (5), 85 (100), 70 (3), 57 (9), 43 (3); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 4.48 (1H, qn, *J* = 6.8, H_5), 2.52 (2H, dd, *J* = 6.8, 9.9, H_3), 2.31 (1H, sx, *J* = 6.8, $H_{4a/b}$), 1.91-1.28 (7H, m, $H_{4a/b,6,7,8}$), 0.91 (3H, t, *J* = 6.7, H_9); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.0 (C_2), 80.8 (C_5), 35.0 ($C_{6,7,8}$), 28.6 (C_3), 27.7 (C_4), 27.1, 22.1 ($C_{6,7,8}$), 13.6 (C_9).

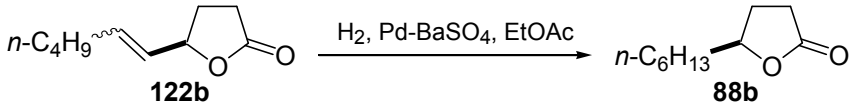
(*S*)-Nonalactone (87b)



121b **87b**

γ-Nonalactone (**87b**) was prepared, as per **86a**, from alkene **121b** (428.2 mg, 2.78 mmol) and Pd-$BaSO_4$ (54.2 mg) in EtOAc (10 mL) for 48 hrs. The residue was passed through an Al_2O_3 plug (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.38) and purified by column chromatography (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.21) to afford γ-lactone **87b** (274.5 mg, 63%) as a colourless oil. $\mathbf{[\alpha]_D}$ = -49.8 (*c* = 1.05, MeOH) (lit.[147] $\mathbf{[\alpha]_D}$ = -51.6 (*c* = 1.5-2.5, MeOH)); **MS** *m/z* (%) 156 (M^+, <1), 128 (3), 114 (3), 100 (5), 86 (5), 85 (100), 71 (2), 57 (6), 43 (7); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 4.43 (1H, qn, *J* = 6.7, H_5), 2.47 (2H, dd, *J* = 6.7, 9.3, H_3), 2.27 (1H, sx, *J* = 6.7, $H_{4a/b}$), 1.86-1.19 (9H, m, $H_{4a/b,6,7,8,9}$), 0.83 (3H, t, *J* = 7.1, H_{10}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.2 (C_2), 80.9 (C_5), 35.4, 31.3 ($C_{6,7,8,9}$), 28.7 (C_3), 27.8 (C_4), 24.7, 22.3 ($C_{6,7,8,9}$), 13.8 (C_{10}).

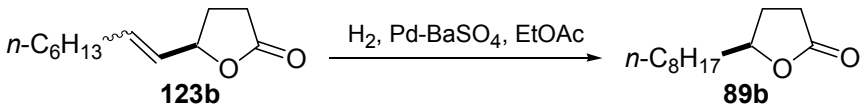
(*S*)-Decalactone (88b)



122b **88b**

γ-Decalactone (**88b**) was prepared, as per **86a**, from alkene **122b** (403.9 mg, 2.40 mmol) and Pd-$BaSO_4$ (49.4 mg) in EtOAc (10 mL) for 3 days. The residue was passed through an Al_2O_3 plug (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.41) and purified by column chromatography (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.21) to afford γ-lactone **88b** (231.6 mg, 57%) as a colourless oil. $\mathbf{[\alpha]_D}$ = -44.3 (*c* = 1.15, MeOH) (lit.[147] $\mathbf{[\alpha]_D}$ = -48.1 (*c* = 1.5-2.5, MeOH)); **MS** *m/z* (%) 170 (M^+, <1), 128 (11), 114 (2), 100 (5), 86 (5), 85 (100), 57 (7), 43 (8); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 4.48 (1H, qn, *J* = 6.7, H_5), 2.52 (2H, dd, *J* = 6.7, 9.8, H_3), 2.31 (1H, sx, *J* = 6.7, $H_{4a/b}$), 1.91-1.22 (11H, m, $H_{4a/b,6,7,8,9,10}$), 0.88 (3H, t, *J* = 6.7, H_{11}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.2 (C_2), 81.0 (C_5), 35.5, 31.5 ($C_{6,7,8,9,10}$), 28.9, 28.8 ($C_{3,6,7,8,9,10}$), 27.9 (C_4), 25.1, 22.4 ($C_{6,7,8,9,10}$), 13.9 (C_{11}).

(*S*)-Dodecalactone (89b)



123b **89b**

γ-Dodecalactone (**89b**) was prepared, as per **86a**, from alkene **123b** (377.3 mg, 1.92 mmol) and Pd-$BaSO_4$ (42.6 mg) in EtOAc (10 mL) for 6 hrs, followed by a further portion of Pd-$BaSO_4$ (56.3 mg) for 24 hrs. The residue was passed through an Al_2O_3 plug (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.40) and purified by column chromatography (50% (v/v) ether/hexanes, $\mathbf{R_f}$ = 0.22) to afford γ-lactone **89b** (230.8 mg, 61%) as a colourless oil. $\mathbf{[\alpha]_D}$ = -41.0 (*c* = 1.22, MeOH) (lit.[147] $\mathbf{[\alpha]_D}$ = -42.6 (*c* = 1.5-2.5, MeOH)); **MS** *m/z* (%) 198 (<1), 128 (11), 114 (4), 113 (1), 100 (6), 86 (6), 85 (100), 57 (11), 43 (12); **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 4.48 (1H, qn, *J* = 6.8, H_5), 2.53 (2H, dd, *J* = 6.8, 9.4, H_3), 2.31 (1H, sx, *J* = 6.8, $H_{4a/b}$), 1.91-1.18 (15H, m, $H_{4a/b,6,7,8,9,10,11,12}$), 0.88 (3H, t, *J* = 6.7, H_{13}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ = 177.1 (C_2), 80.9 (C_5), 35.4, 31.6, 29.2, 29.1, 29.0 ($C_{6,7,8,9,10,11,12}$), 28.7 (C_3), 27.8 (C_4), 25.0, 22.4 ($C_{6,7,8,9,10,11,12}$), 13.9 (C_{13}).

10.3.2 Method development for chiral analysis

Sample preparation

The stored liquid extracts (details described in 10.2.4 Method development for solid-phase extraction (SPE) analysis) were allowed to warm to room temperature prior to chiral GC-MS analysis.

Instrumental analysis

An Agilent 6890A gas chromatogram fitted to an Agilent 5973N mass spectrometer was used with an Rt-βDEXcst capillary column, 30 m x 0.25 mm I.D. and 0.25 μm film thickness. The carrier gas was He at a flow rate of 1.1 mL/min. The initial column temperature was 50 °C, held for 1 min and then increased to 220 °C at 4 °C/min and held for 11 mins with the transfer line at 220 °C. The injection was carried out with a Gerstel MPS2 auto sampler using a 10 μL syringe in splitless mode. During splitless mode, a pressure pulse of 25.0 psi was applied. The injected volume was 1 μL. The mass spectrometer detector was used in SIM mode with a solvent delay of 5 mins. The ions monitored for the analytes were *m/z* 85, 86, 100, 128 and the ions monitored for the d_7-standards were *m/z* 90, 91, 107, 135. The underlined ions were the target ions used for quantification and the remaining ions were the qualifying ions used for compound verification. The optically pure γ-lactone samples were analysed to determine the enantiomeric purity prior to their use as standards.

10.4 Experimental procedures for Chapter 9

γ-Lactone standards

The prepared γ-lactone samples (details described in 10.3.1 Synthesis of optically pure γ-lactones) were used to determine the odour thresholds in a red wine. A selection of spiked wines, from each γ-lactone threshold test, was quantified using the stable isotope dilution analysis (SIDA) SPE chiral GC-MS method with the d_7-standards.

Solvent and wine

EtOH was purchased from Sigma-Aldrich and purified prior to use. The red wine used was a 'bag in a box' dry red wine (pH 3.50, 12.8% EtOH, SO_2 levels 117 mg/L total and 21 mg/L free) and was shown to contain no γ-lactones.

Odour detection thresholds

The odour detection thresholds in red wine were determined according to the American Standards for Testings and Materials (ASTM) method E 679-79[120] (details described in 5.3 Experimental procedures for Chapter 4). There were 25 panellists who participated in the threshold tests. From the results of the triangle tests, each individual panellist was assigned a best estimate threshold (BET) value. The group BET was then calculated to determine the final threshold value.

The concentrations employed were as follows:

- (*R*)-γ-Octalactone (**86a**): 3.1, 9.2, 27.5, 82.1, 239 and 726 μg/L, with retest concentrations for the high end of 2,292 and 6,685 μg/L
- (*S*)-γ-Octalactone (**86b**): 9.1, 27.4, 81.3, 244, 739 and 2,244 μg/L, with retest concentrations for the high end of 6,600 and 19,536 μg/L
- (*R*)-γ-Nonalactone (**87a**): 9.0, 26.7, 82.5, 246, 727 and 2,160 μg/L, with retest concentrations for the low end of 1.0 and 3.0 μg/L and for the high end of 6,678 and 19,640 μg/L
- (*S*)-γ-Nonalactone (**87b**): 9.0, 27.2, 80.0, 245, 740 and 2,200 μg/L, with retest concentrations for the low end of 1.0 and 3.0 μg/L and for the high end of 6,600 and 20,000 μg/L
- (*R*)-γ-Decalactone (**88a**): 9.0, 26.8, 80.3, 239, 727 and 2,199 μg/L, with retest concentrations for the low end of 1.0 and 3.0 μg/L and for the high end of 6,501 and 19,120 μg/L
- (*S*)-γ-Decalactone (**88b**): 9.1, 27.5, 80.8, 242, 727 and 2,222 μg/L, with retest concentrations for the low end of 1.0 and 3.0 μg/L and for the high end of 6,464 and 20,200 μg/L
- (*R*)-γ-Dodecalactone (**89a**): 9.0, 27.4, 82.1, 242, 736 and 2,185 μg/L, with retest concentrations for the low end of 0.3, 1.0 and 3.0 μg/L and for the high end of 6,670 and 19,780 μg/L

- (*S*)-γ-Dodecalactone (**89b**): 3.0, 8.9, 26.9, 79.2, 243 and 733 μg/L, with retest concentrations for the low end of 0.4 and 1.0 μg/L and for the high end of 2,178 and 6,534 μg/L.

11 Appendices

11.1 Odour threshold data for the oak lactones in white and red wine

Table 11.1 Odour threshold data for each judge for (4*S*,5*S*)-*cis*-oak lactone in white wine

	concentration (μg/L)										
judge	**0.3**	**0.9**	**2.7**	**7.7**	**23.7**	**71.5**	**209**	**632**	**1,869**	**5,498**	**BET**
1	0	1	0	1	1	1	1	1			4.6
2			0	0	1	1	1	1			13.5
3			1	0	1	1	1	1			13.5
4			0	0	1	1	1	1			13.5
5			1	0	0	1	1	1			41.2
6			1	0	0	1	1	1			41.2
7			1	0	0	0	1	1			122
8			0	1	1	0	1	1			122
9			0	0	0	0	0	0	1	1	1,075
10			1	0	0	1	0	0	1	1	1,075
11			0	0	1	0	0	1	1	1	363
12	1	0	1	1	1	1	1	1			1.5
13			0	1	0	0	0	1	1	1	363
14	1	0	1	1	1	1	1	1			1.5
15	0	1	1	1	1	1	1	1			0.5
16			0	0	0	1	1	1			41.2
17			1	0	0	1	1	1			41.2
18			0	1	1	1	1	1			4.6
19			0	0	1	1	1	1			13.5
20			0	1	1	1	1	1			4.6
21			0	1	1	1	1	1			4.6
22			0	0	1	1	1	1			13.5
23			0	0	1	1	1	1			13.5
24			1	0	1	1	1	1			13.5
25			0	0	1	1	1	1			13.5
26			0	1	1	1	1	1			4.6
27			0	1	1	1	1	1			4.6
28			0	0	0	1	1	1			41.2
29	0	0	0	1	1	1	1	1			4.6
30			0	1	0	1	1	1			41.2
31			0	1	0	0	1	1			122
32			1	0	0	0	0	1			1,075
33			0	0	0	1	1	1			41.2
34			0	0	1	1	1	1			13.5
35			0	0	1	1	1	1			13.5
36			0	1	1	0	1	1			122
37			0	0	1	1	1	1			13.5

Table 11.2 Odour threshold data for each judge for (4*S*,5*S*)-*cis*-oak lactone in red wine

	concentration (μg/L)										
judge	**0.3**	**0.9**	**2.7**	**7.7**	**23.7**	**71.5**	**209**	**632**	**1,869**	**5,635**	**BET**
1			0	0	0	1	1	1			41.2
2			0	0	0	0	1	1			122
3			0	0	0	1	1	1			41.2
4			0	0	0	1	1	1			41.2
5			0	0	1	1	1	1			13.5
6			0	0	1	1	1	1			13.5
7			0	0	1	0	0	0	0	1	1,087
8			0	1	0	1	0	1	1	1	363
9			0	0	0	1	0	0	1	1	1,087
10			0	1	0	0	1	1			122
11			0	1	0	0	0	1	1	1	363
12			0	1	0	1	1	1			41.2
13			1	0	0	1	1	1	1	1	363
14			0	0	0	1	1	1			41.2
15			0	0	0	1	1	1			41.2
16			1	0	0	1	1	1			122
17			1	1	0	0	1	1			122
18			0	0	1	1	1	1			13.5
19			0	1	1	1	1	1			4.6
20			0	1	0	1	1	1			41.2
21			0	0	1	1	1	1			13.5
22			0	1	0	1	1	1			41.2
23			1	0	0	0	1	1			122
24			1	0	1	1	1	1			13.5
25			0	0	0	1	1	1			41.2
26	1	0	1	1	1	1	1	1			1.5
27			0	1	1	1	1	1			122
28			0	0	0	0	0	1	1	1	363
33			1	0	1	0	1	1			122
38			1	1	0	0	1	1			122
39			0	0	0	1	1	1			41.2
40			1	1	0	1	1	1			122
41			0	0	0	0	1	1			41.2

Table 11.3 Odour threshold data for each judge for (4*R*,5*R*)-*cis*-oak lactone in white wine

	concentration (μg/L)								
judge	**7.2**	**21.7**	**65.5**	**195**	**593**	**1,772**	**5,231**	**15,693**	**BET**
1	0	1	1	1	1	1			12.5
2	0	0	1	1	1	1			37.7
3	1	0	0	1	1	1			113
4	0	0	0	1	1	1			113
5	0	0	1	1	1	1			37.7
6	1	0	0	1	1	1			113
7	1	0	1	0	1	1			340
8	0	0	1	1	1	1			113
9	0	0	1	1	1	1			37.7
10	0	0	0	0	0	0	1	1	3,068
11	1	0	0	1	1	1			113
12	0	0	0	1	1	1			113
13	0	1	1	1	0	0	1	1	3,068
14	0	0	0	1	1	1			113
15	0	0	1	1	1	1			37.7
16	0	0	0	0	1	1			340
17	0	1	0	1	1	1			113
18	1	0	0	1	1	1			113
19	1	0	0	1	1	1			113
20	0	0	0	1	1	1			113
21	0	1	0	0	1	1			340
22	0	0	0	1	1	1			113
23	0	0	0	1	1	1			113
24	0	1	0	0	1	1			340
25	0	0	0	0	1	1			340
26	0	0	0	1	1	1			113
27	0	0	0	1	1	1			113
28	0	0	1	1	1	1			37.7
30	1	0	0	1	1	1			113
31	0	0	0	0	1	1			340
33	0	1	0	0	1	1			340
34	0	0	0	1	1	1			113
35	1	1	0	1	1	1			113
36	0	1	1	0	1	1			340
37	1	0	0	1	1	1			113
38	1	0	0	0	1	1			340
39	1	0	0	1	1	1			113
40	0	0	1	1	1	1			37.7
42	0	1	1	1	1	1			12.5
43	0	1	0	1	1	1			113
44	0	0	1	1	0	1	1	1	1,025
45	0	1	1	1	1	1			12.5
47	0	0	0	0	1	1			340

Table 11.4 Odour threshold data for each judge for (4*R*,5*R*)-*cis*-oak lactone in red wine

	concentration (μg/L)											
judge	**0.8**	**2.4**	**7.2**	**21.7**	**65.5**	**195**	**593**	**1,772**	**5,231**	**15,693**	**47,950**	**BET**
1			1	0	1	1	1	1				37.7
2			0	0	0	1	1	1				113
3			0	0	0	1	1	1				113
4			0	0	0	1	1	1				113
5			0	0	0	1	1	1				340
6			0	1	1	1	1	1				12.5
7	1	0	0	1	1	1	1	1				12.5
9			0	0	0	1	1	1				113
10			0	1	0	0	0	1	1	1		1,025
11			0	1	1	1	0	0	1	1		3,020
12			0	0	1	1	1	1				37.7
13			1	0	1	1	0	0	0	0	1	27,431
14			0	0	1	1	1	1				37.7
15			1	1	0	1	1	1				113
16			0	1	0	0	1	1				340
17			0	1	0	1	1	1				113
18			0	1	1	1	1	1				12.5
19			1	1	1	0	1	1				340
20			0	1	0	0	0	1	1	1		1,025
21			0	0	1	0	1	1				340
22			0	1	0	0	1	1				340
23			1	1	1	0	0	1	1	1		1,025
24			0	1	0	1	1	1				113
25			1	1	0	0	1	1				340
26			1	1	0	1	1	1				113
27			0	0	0	1	1	1				113
28			0	0	1	1	1	1				37.7
30			0	0	0	1	1	1				113
36	0	0	1	1	1	1	1	1				5.1
37			0	0	1	0	1	1				340
39			0	0	0	1	1	1				113
41			0	0	0	0	1	1				340
47			0	0	0	0	1	1				340

Table 11.5 Odour threshold data for each judge for (4*S*,5*R*)-*trans*-oak lactone in white wine

	concentration (μg/L)										
judge	**0.9**	**2.6**	**7.8**	**23.4**	**70.3**	**208**	**625**	**1,875**	**5,729**	**16,926**	**BET**
1			0	1	1	1	1	1			13.5
2			0	0	0	0	1	1			361
3	0	0	0	1	1	1	1	1			13.5
4			0	0	1	1	1	1			40.6
5			0	0	1	0	1	1			361
6			1	0	1	1	1	1			40.6
7			0	0	1	1	1	1			40.6
8			1	0	1	0	0	0	1	1	3,277
9			1	0	1	0	1	1			361
10			0	0	1	0	1	1			361
11			0	1	0	0	1	1			361
12	1	1	0	1	1	1	1	1			13.5
13			0	1	0	0	0	1	1	1	361
14			0	0	1	1	1	1			40.6
15			0	0	0	1	1	1			121
16			0	0	0	1	1	1			121
17			0	0	0	0	1	1			361
18			0	0	0	1	1	1			121
19			1	0	1	1	1	1			40.6
20			0	0	0	0	1	1			361
21			0	0	0	1	1	1			121
22			1	1	0	1	1	1			121
23			0	0	0	0	1	1			361
24			0	1	1	0	1	1			361
25			0	0	0	0	1	1			361
26			1	1	0	0	1	1			361
27			0	1	0	0	1	1			361
28			1	0	1	1	1	1			40.6
29			0	0	0	0	1	1			361
30			0	0	1	0	1	1			361
31			1	0	0	0	1	0	1	1	3,277
32			0	1	1	1	1	1			13.5
33			0	0	0	0	1	1			361
34			1	0	0	0	1	1			361
35			1	1	0	0	0	1	1	1	1,083
36			0	1	1	0	0	1	1	1	361
37			1	1	0	1	1	1			121

Table 11.6 Odour threshold data for each judge for (4*S*,5*R*)-*trans*-oak lactone in red wine

	concentration (μg/L)								
judge	**7.8**	**23.4**	**70.3**	**208**	**625**	**1,875**	**5,208**	**10,416**	**BET**
1	1	0	0	0	1	1			361
2	1	0	0	0	1	1			361
3	1	0	0	0	1	1			361
4	1	0	0	0	1	1			361
5	0	1	1	0	1	1			361
6	1	0	1	1	1	1			40.6
7	0	0	0	1	1	1			121
8	1	0	0	1	0	1	1	1	1,067
9	0	0	0	1	1	1			121
10	0	0	1	1	1	1			40.6
11	0	0	1	1	0	1	1	1	1,067
12	0	1	1	0	0	1	1	1	1,067
13	0	0	1	0	0	0	0	1	7,365
14	0	0	0	0	0	0	1	1	3,247
15	1	0	0	0	1	1			361
16	0	0	0	0	0	1	1	1	1,067
17	1	0	0	1	1	1			121
18	0	0	0	0	0	1	1	1	1,067
19	0	0	1	0	1	1			361
20	0	1	0	1	1	1			121
21	0	0	0	0	1	1			361
22	0	0	0	0	1	1			361
23	0	0	0	1	0	1	1	1	1,067
24	1	0	1	0	1	1			361
25	0	0	0	0	1	1			361
26	0	1	0	1	1	1			121
27	0	1	1	0	1	1			361
28	1	1	0	1	1	1			121
33	0	0	0	0	0	1			1,067
38	0	0	1	0	1	1			361
39	1	0	0	1	0	1	1	1	1,067
40	1	0	1	0	1	1			361
47	1	0	0	0	0	1	1	1	1,067
48	0	0	1	1	1	1			40.6

Table 11.7 Odour threshold data for each judge for (4*R*,5*S*)-*trans*-oak lactone in white wine

	concentration (μg/L)										
judge	**0.8**	**2.4**	**7.3**	**21.9**	**66.0**	**201**	**595**	**1,771**	**5,413**	**16,039**	**BET**
1			1	1	0	0	1	1			345
2			1	0	0	1	1	1			115
3			0	0	0	0	1	1			345
4			0	1	0	1	1	1			115
5			0	0	0	1	1	1			115
6			0	0	0	0	1	1			345
7			0	1	0	1	0	1	1	1	345
8			0	0	1	1	1	1			38.0
9			0	0	0	0	1	1			345
10			0	0	0	0	1	1	1	1	345
11			0	0	0	1	1	0	1	1	3,067
12			0	0	0	1	0	1	1	1	345
13			0	0	0	0	0	0	1	1	3,067
14			0	0	0	0	1	1			345
15			1	0	0	0	1	1			345
16			0	0	1	0	0	1	1	1	1,026
17			0	0	0	0	1	1			345
18			1	0	1	1	0	0	1	1	3,067
19			0	0	0	1	1	1			115
20			0	1	1	1	1	1			12.6
21	1	0	1	1	1	1	1	1			4.7
22			1	1	1	1	0	1	1	1	1,026
23			1	0	0	1	0	1	1	1	1,026
24			0	1	1	0	1	1			345
25			1	0	0	0	0	1	1	1	1,026
26			0	1	0	0	0	1	1	1	1,026
27			0	1	1	0	1	1			345
28			0	0	0	0	1	1			345
30			0	0	0	1	1	1			115
37			1	1	1	0	1	1			345
40			0	0	1	0	1	1			345
45			0	1	1	1	1	1			12.6
46			0	1	0	0	1	1			345
47			1	0	0	0	1	1			345
48			1	1	0	0	1	1			345
49			0	0	0	0	1	1			345

Table 11.8 Odour threshold data for each judge for (4*R*,5*S*)-*trans*-oak lactone in red wine

	concentration (μg/L)								
judge	**7.3**	**21.9**	**66.0**	**201**	**595**	**1,771**	**5,413**	**16,039**	**BET**
1	1	0	1	1	1	1			38.0
2	0	1	1	0	1	1			345
3	0	0	1	1	1	1			38.0
4	1	0	1	1	1	1			38.0
5	0	1	0	0	1	1			345
6	0	1	1	1	1	1			12.6
7	0	1	0	1	0	1	1	1	345
8	1	1	1	0	1	1			345
9	0	1	0	0	0	1	1	1	1,026
10	1	0	1	0	1	1			345
11	0	1	0	1	0	0	1	1	3,067
12	1	1	0	1	0	1	1	1	1,026
13	0	1	0	0	1	1	1	1	345
14	1	1	0	1	0	1	1	1	1,026
15	1	1	1	0	1	1			345
16	1	0	1	0	1	1			345
17	0	0	1	0	1	1			345
18	0	0	1	1	1	1			38.0
19	1	0	1	1	1	1			38.0
20	1	0	1	0	1	1			345
21	1	1	0	0	0	1	1	1	345
22	0	0	0	0	0	0	1	1	3,067
23	1	1	0	0	1	1			345
24	0	0	1	0	0	1	1	1	345
25	1	0	1	1	0	0	1	1	3,067
26	0	0	1	0	0	1	1	1	1,026
27	0	1	0	0	0	0	1	1	3,067
28	0	0	1	1	1	1			38.0
30	0	0	1	1	1	1			38.0
31	0	1	0	0	1	1			345
34	1	1	0	0	1	1			345
37	0	0	0	0	1	1			345
40	1	1	1	0	1	1			345
48	0	1	0	1	1	1			115

11.2 Quantification results for γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone in white and red wines

Table 11.9 Concentrations measured for γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone in various white wines (μg/L)

wine	winery	year	origin	C_8	C_9	C_{10}	C_{12}
			Chardonnay				
CH 1	Cape Mentelle	2005	WA	<0.10	<0.10	<0.10	<0.10
CH 2	Curly Flat	2003	VIC	1.19	<0.10	<0.10	<0.10
CH 3	Curly Flat	2004	VIC	<0.10	<0.10	<0.10	<0.10
CH 4	d'Arenberg	2006	SA	<0.10	<0.10	<0.10	<0.10
CH 5	De Bortoli	2003	VIC	<0.10	<0.10	<0.10	<0.10
CH 6	De Bortoli	2004	VIC	0.31	<0.10	<0.10	<0.10
CH 7	McWilliam's Hanwood	2005	SA	<0.10	<0.10	<0.10	<0.10
CH 8	Mountadam	2004	SA	0.78	0.62	<0.10	<0.10
CH 9	Petaluma	2001	SA	<0.10	<0.10	<0.10	<0.10
CH 10	Petaluma	2003	SA	<0.10	0.82	<0.10	<0.10
CH 11	Stonier	2005	VIC	<0.10	<0.10	<0.10	<0.10
CH12	Yering Station	2005	VIC	<0.10	<0.10	<0.10	<0.10
			Riesling				
RI 1	d'Arenberg	2002	SA	1.01	<0.10	<0.10	<0.10
RI 2	Frankland Estate	2004	WA	1.65	1.59	<0.10	<0.10
RI 3	Frankland Estate	2006	WA	1.14	<0.10	<0.10	<0.10
RI 4	Grosset	2003	SA	0.44	<0.10	<0.10	<0.10
RI 5	Grosset	2004	SA	1.17	<0.10	<0.10	<0.10
RI 6	Grosset	2005	SA	0.75	<0.10	<0.10	<0.10
RI 7	Grosset	2006	SA	0.28	<0.10	<0.10	<0.10
RI 8	Petaluma	2004	SA	1.48	<0.10	<0.10	<0.10
RI 9	Petaluma	2006	SA	0.67	3.34	<0.10	<0.10
RI 10	Sevenhill	2006	SA	0.91	1.53	<0.10	<0.10
RI 11	Stefano Lubiana	2005	TAS	0.95	<0.10	<0.10	<0.10
RI 12	Temple Bruer	2002	SA	1.68	<0.10	<0.10	<0.10
			Sauvignon Blanc				
SA 1	Bridgewater Mill	2005	SA	<0.10	<0.10	<0.10	<0.10
SA 2	Bridgewater Mill	2006	SA	<0.10	0.21	<0.10	<0.10
SA 3	Brown Brothers	2005	VIC	<0.10	<0.10	<0.10	<0.10
SA 4	Henschke	2006	SA	<0.10	<0.10	<0.10	<0.10
SA 5	Katnook Estate	2005	SA	<0.10	<0.10	<0.10	<0.10
SA 6	Mitchelton Wines	2006	VIC	<0.10	<0.10	<0.10	<0.10
SA 7	Nepenthe	2006	SA	<0.10	<0.10	<0.10	<0.10
SA 8	O'Leary Walker	2006	SA	<0.10	<0.10	<0.10	<0.10
SA 9	PHI	2006	VIC	<0.10	<0.10	<0.10	<0.10
SA 10	Stefano Lubiana	2006	TAS	<0.10	<0.10	<0.10	<0.10

Continued: Table 11.9 Concentrations measured for γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone in various white wines (μg/L)

wine	winery	year	origin	C_8	C_9	C_{10}	C_{12}
			Semillon				
SE 1	Kaesler	2005	SA	0.48	<0.10	<0.10	<0.10
SE 2	Kaesler	2006	SA	<0.10	<0.10	<0.10	<0.10
SE 3	Knappstein Lenswood	2000	SA	<0.10	0.37	<0.10	<0.10
SE 4	Moss Wood	2004	WA	0.20	<0.10	<0.10	<0.10
SE 5	Mount Horrocks	2006	SA	<0.10	<0.10	<0.10	<0.10
SE 6	Rockfords	2003	SA	0.25	0.45	<0.10	<0.10
SE 7	Tyrell's Wines	1994	NSW	1.08	<0.10	<0.10	<0.10
SE 8	Tyrell's Wines	1999	NSW	3.48	<0.10	<0.10	<0.10
SE 9	Tyrell's Wines	2000	NSW	3.09	<0.10	<0.10	<0.10
SE 10	Will Taylor	2000	NSW	0.78	<0.10	<0.10	<0.10

Table 11.10 Concentrations measured for γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone in various red wines (μg/L)

wine	winery	year	origin	C_8	C_9	C_{10}	C_{12}
			Cabernet Sauvignon				
CA 1	Dalwhinnie	2000	VIC	<0.10	<0.10	<0.10	<0.10
CA 2	Dalwhinnie	2001	VIC	<0.10	0.14	<0.10	<0.10
CA 3	Dalwhinnie	2004	VIC	<0.10	<0.10	<0.10	<0.10
CA 4	d'Arenberg	1997	SA	0.21	1.88	<0.10	<0.10
CA 5	d'Arenberg	2001	SA	0.28	12.54	<0.10	<0.10
CA 6	d'Arenberg	2003	SA	<0.10	7.27	<0.10	<0.10
CA 7	d'Arenberg	2004	SA	0.37	16.48	<0.10	<0.10
CA 8	Hardy's Tintara	2000	SA	<0.10	4.14	<0.10	<0.10
CA 9	Heathvale	2004	SA	<0.10	7.84	<0.10	<0.10
CA 10	Lake Breeze	2000	SA	<0.10	<0.10	<0.10	<0.10
CA 11	Lengs & Cooter	2002	SA	0.41	10.18	<0.10	<0.10
CA 12	McWilliam's	1999	NSW	<0.10	<0.10	<0.10	<0.10
CA 13	McWilliam's	2000	NSW	<0.10	<0.10	<0.10	<0.10
CA 14	McWilliam's	2001	NSW	<0.10	<0.10	<0.10	<0.10
CA 15	Mitchelton Wines	2005	VIC	<0.10	<0.10	<0.10	<0.10
CA 16	Mosswood	2004	WA	<0.10	0.13	<0.10	<0.10
CA 17	O'Leary Walker	2004	SA	1.27	17.47	0.17	<0.10
CA 18	Paracombe	2001	SA	<0.10	10.52	<0.10	<0.10
CA 19	Rosemount Estate	1997	SA	<0.10	<0.10	<0.10	<0.10
CA 20	Rouge Homme	1996	SA	<0.10	0.81	1.01	<0.10
CA 21	Serafino	2004	SA	<0.10	12.99	<0.10	<0.10
CA 22	Stone Haven	2002	SA	<0.10	16.41	<0.10	<0.10
CA 23	Taylors	2002	SA	<0.10	6.98	<0.10	<0.10
CA 24	Taylors	2004	SA	<0.10	6.10	<0.10	<0.10
CA 25	Turkey Flat	1998	SA	0.58	1.55	<0.10	<0.10
CA 26	Turkey Flat	2004	SA	0.94	33.94	<0.10	<0.10
CA 27	Whale Coast	2001	SA	0.91	11.53	<0.10	<0.10
CA 28	Woodstock	2002	SA	<0.10	2.63	<0.10	<0.10
CA 29	Woodstock	2004	SA	<0.10	4.19	4.00	<0.10
CA 30	Yalumba	2003	SA	<0.10	<0.10	<0.10	<0.10
			Durif				
DU 1	Brown Brothers	2003	VIC	0.68	<0.10	<0.10	<0.10
DU 2	Casella Wines	2005	VIC	0.97	5.59	<0.10	<0.10
DU 3	Kingston Estate	2003	SA	1.03	2.49	<0.10	<0.10
DU 4	Massena	2004	SA	4.18	39.75	0.81	<0.10
DU 5	Stanton & Killeen	2001	VIC	0.76	0.62	<0.10	<0.10
DU 6	Stanton & Killeen	2002	VIC	0.25	<0.10	<0.10	<0.10

Continued: Table 11.10 Concentrations measured for γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone in various red wines (μg/L)

wine	winery	year	origin	C_8	C_9	C_{10}	C_{12}
			Merlot				
ME 1	Barossa Ridge	1998	SA	0.99	7.23	0.33	<0.10
ME 2	Barossa Ridge	2000	SA	2.11	10.05	1.08	<0.10
ME 3	Beresford	2003	SA	<0.10	<0.10	<0.10	<0.10
ME 4	Ceravolo	2002	SA	0.21	3.25	<0.10	<0.10
ME 5	Elderton	1997	SA	0.37	<0.10	<0.10	<0.10
ME 6	Fox Creek	1997	SA	1.50	<0.10	<0.10	<0.10
ME 7	Fox Creek	1999	SA	0.39	<0.10	<0.10	<0.10
ME 8	Geoff Merrill	2002	SA	0.18	<0.10	<0.10	<0.10
ME 9	Grant Burge	2004	SA	0.74	19.12	<0.10	<0.10
ME 10	Kay Brothers Amery	2000	SA	<0.10	<0.10	<0.10	<0.10
ME 11	Kay Brothers Amery	2003	SA	<0.10	<0.10	<0.10	<0.10
ME 12	Mike Press	2003	SA	<0.10	<0.10	<0.10	<0.10
ME 13	Padthaway Estate	2000	SA	<0.10	<0.10	<0.10	<0.10
ME 14	Pepper Tree Wines	1996	SA	<0.10	<0.10	<0.10	<0.10
ME 15	Pirramimma	1998	SA	<0.10	<0.10	<0.10	<0.10
ME 16	Preece	2005	VIC	<0.10	<0.10	<0.10	<0.10
ME 17	Smith & Hooper	2000	SA	<0.10	<0.10	<0.10	<0.10
ME 18	Smith & Hooper	2003	SA	<0.10	<0.10	<0.10	<0.10
ME 19	Tatachilla	2001	SA	<0.10	0.41	<0.10	<0.10
ME 20	Three Hills	1999	WA	<0.10	<0.10	<0.10	<0.10
ME 21	Tyrell's Wines	1999	SA	<0.10	0.77	<0.10	<0.10
ME 22	Wild Geese Wines	2003	SA	<0.10	3.90	<0.10	<0.10
ME 23	Xanadu	2002	WA	<0.10	<0.10	<0.10	<0.10
ME 24	Yangarra Park	2001	SA	0.35	10.12	<0.10	<0.10
ME 25	Yarra Ridge	1997	VIC	<0.10	<0.10	<0.10	<0.10
			Pinot Noir				
PI 1	Beechtree Wines	2003	SA, VIC	0.11	<0.10	<0.10	<0.10
PI 2	Curly Flat	2003	VIC	0.17	3.28	<0.10	<0.10
PI 3	Curly Flat	2004	VIC	<0.10	<0.10	<0.10	<0.10
PI 4	De Bortoli	2005	VIC	<0.10	<0.10	<0.10	<0.10
PI 5	Diamond Valley	2005	VIC	<0.10	<0.10	<0.10	<0.10
PI 6	Geoff Weaver	1999	SA	<0.10	1.53	<0.10	<0.10
PI 7	Geoff Weaver	2004	SA	<0.10	13.30	<0.10	<0.10
PI 8	Grosset	2000	SA	1.14	34.84	<0.10	<0.10
PI 9	Grosset	2001	SA	<0.10	18.25	<0.10	<0.10
PI 10	Grosset	2004	SA	<0.10	9.56	<0.10	<0.10
PI 11	Kooyong	2003	VIC	<0.10	<0.10	<0.10	<0.10
PI 12	Kooyong Meres	2004	VIC	<0.10	1.92	<0.10	<0.10
PI 13	Kooyong Estate	2004	VIC	<0.10	1.72	<0.10	<0.10
PI 14	Moss Wood	2001	WA	<0.10	1.78	<0.10	<0.10
PI 15	Stefano Lubiana	2006	TAS	<0.10	<0.10	<0.10	<0.10
PI 16	Stonier	2005	VIC	<0.10	<0.10	<0.10	<0.10
PI 17	Wignalls	2004	WA	<0.10	<0.10	<0.10	<0.10

Continued: Table 11.10 Concentrations measured for γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone in various red wines (μg/L)

wine	winery	year	origin	C_8	C_9	C_{10}	C_{12}
			Shiraz				
SH 1	Barossa Valley Estate	2002	SA	1.14	<0.10	<0.10	<0.10
SH 2	Brokenwood	1997	SA	0.15	1.48	0.25	<0.10
SH 3	Brown Brothers	1997	VIC	0.89	21.37	<0.10	<0.10
SH 4	Brown Brothers	2002	VIC	0.89	<0.10	<0.10	<0.10
SH 5	Chalk Hill	2001	SA	0.35	2.80	<0.10	<0.10
SH 6	Cigale	2005	SA	0.46	11.81	<0.10	<0.10
SH 7	d'Arenberg	2004	SA	1.52	13.20	0.39	<0.10
SH 8	De Bortoli	2001	VIC	<0.10	<0.10	<0.10	<0.10
SH 9	De Bortoli	2004	VIC	0.54	<0.10	<0.10	<0.10
SH 10	Grant Burge	2004	SA	0.38	5.09	<0.10	<0.10
SH 11	Heartland	2005	SA	<0.10	<0.10	<0.10	<0.10
SH 12	Leeuwin Estate	2001	WA	0.38	<0.10	<0.10	<0.10
SH 13	Leeuwin Estate	2003	WA	0.36	<0.10	<0.10	<0.10
SH 14	Majella	2003	SA	<0.10	<0.10	<0.10	<0.10
SH 15	Mike Press Wines	2005	SA	<0.10	<0.10	<0.10	<0.10
SH 16	Mitchell	2004	SA	0.65	9.78	<0.10	<0.10
SH 17	Mitchelton	1991	VIC	<0.10	<0.10	<0.10	<0.10
SH 18	Mitchelton	1996	VIC	<0.10	<0.10	<0.10	<0.10
SH 19	Mitchelton	2000	VIC	<0.10	<0.10	<0.10	<0.10
SH 20	Mitchelton	2002	VIC	0.89	<0.10	<0.10	<0.10
SH 21	Mount Pleasant	2000	VIC	<0.10	<0.10	<0.10	<0.10
SH 22	Mt Langi Ghiran	1995	VIC	0.99	<0.10	0.18	<0.10
SH 23	Mt Langi Ghiran	1999	VIC	2.19	8.80	0.44	<0.10
SH 24	Mt Langi Ghiran	2000	VIC	1.33	<0.10	<0.10	<0.10
SH 25	Mt Langi Ghiran	2003	VIC	1.16	<0.10	<0.10	<0.10
SH 26	Orlando Wines	1997	SA	<0.10	<0.10	<0.10	<0.10
SH 27	Orlando Wines	1999	SA	0.25	<0.10	0.92	<0.10
SH 28	Orlando Wines	2000	SA	1.46	9.42	<0.10	<0.10
SH 29	Pikes	2004	SA	1.13	4.35	<0.10	<0.10
SH 30	Pirramimma	2002	SA	0.50	<0.10	<0.10	<0.10
SH 31	Seppelt	1999	VIC	1.63	1.67	<0.10	<0.10
SH 32	Seppelt	2003	VIC	0.69	4.57	<0.10	<0.10
SH 33	Seppelt	2003	VIC	0.81	3.26	<0.10	<0.10
SH 34	Shingleback	2003	SA	0.37	<0.10	<0.10	<0.10
SH 35	St John's Road	2005	SA	<0.10	<0.10	<0.10	<0.10
SH 36	Tatachilla	1995	SA	1.20	<0.10	<0.10	<0.10
SH 37	Tatachilla	1997	SA	0.30	<0.10	<0.10	<0.10
SH 38	Tatachilla	1999	SA	<0.10	<0.10	0.54	<0.10
SH 39	Tatachilla	2001	SA	0.31	2.70	0.49	<0.10
SH 40	Woodstock	2004	SA	0.29	2.55	<0.10	<0.10
SH 41	Wynns Estate	2000	SA	0.46	<0.10	<0.10	<0.10
SH 42	Yellowtail	2003	SA	<0.10	<0.10	<0.10	<0.10

11.3 Enantiomeric distribution of γ-nonalactone in red wines

Table 11.11 Distribution of (*R*)- and (*S*)-isomers of γ-nonalactone in red wines

wine	C_9 (µg/L)[a]	*R* (µg/L)[b]	*S* (µg/L)[b]	total (µg/L)[c]	diff. (%)[d]	*R* (%)	*S* (%)
Cabernet Sauvignon							
CA 5	12.54	7.32	4.82	12.14	-3.19	60	40
CA 6	7.27	3.86	3.22	7.08	-2.61	55	45
CA 7	16.48	9.49	6.74	16.23	-1.52	58	42
CA 8	4.14	2.34	1.77	4.11	-0.72	57	43
CA 9	7.84	4.18	3.62	7.80	-0.51	54	46
CA 11	10.18	6.06	4.25	10.31	1.28	59	41
CA 17	17.47	10.72	7.07	17.79	1.83	60	40
CA 18	10.52	6.90	4.06	10.96	4.18	63	37
CA 21	12.99	7.71	5.33	13.04	0.39	59	41
CA 22	16.41	9.85	6.44	16.29	-0.73	60	40
CA 23	6.98	4.21	2.67	6.88	-1.43	61	39
CA 24	6.10	3.37	2.77	6.14	0.66	55	45
CA 26	33.94	20.57	13.97	34.54	1.77	60	40
CA 27	11.53	6.63	5.23	11.86	2.86	56	44
CA 29	4.19	1.99	2.30	4.29	2.39	46	54
Durif							
DU 2	5.59	3.45	2.14	5.59	0.00	62	38
DU 4	39.75	25.18	15.97	41.15	3.52	61	39
Merlot							
ME 1	7.23	4.39	2.73	7.12	-1.52	62	38
ME 2	10.05	6.76	3.25	10.01	-0.40	68	32
ME 9	19.12	11.30	8.08	19.38	1.36	58	42
ME 24	10.12	5.87	4.40	10.27	1.48	57	43
Pinot Noir							
PI 7	13.30	8.16	5.13	13.29	-0.08	61	39
PI 8	34.84	22.04	13.02	35.06	0.63	63	37
PI 9	18.25	11.13	7.19	18.32	0.38	61	39
PI 10	9.56	5.70	3.73	9.43	-1.36	60	40
Shiraz							
SH 3	21.37	12.71	8.11	20.82	-2.57	61	39
SH 6	11.81	6.87	4.98	11.85	0.34	58	42
SH 7	13.20	7.79	5.16	12.95	-1.89	60	40
SH 10	5.09	2.84	2.69	5.53	8.64	51	49
SH 16	9.78	4.90	4.92	9.82	0.41	50	50
SH 23	8.80	5.42	3.71	9.13	3.75	59	41
SH 28	9.42	5.90	3.77	9.67	2.65	61	39
SH 29	4.35	2.26	2.05	4.31	-0.92	48	52
SH 32	4.57	2.62	2.05	4.67	2.19	56	44

[a] refers to level measured using solid-phase extraction (SPE) stable isotope dilution analysis (SIDA); [b] refers to level measured using chiral SPE SIDA method; [c] total refers to (*R*) plus (*S*), as measured using chiral SIDA method; [d] difference between measured level from SPE SIDA method and chiral SIDA method

11.4 Odour threshold data for γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone in red wine

Table 11.12 Odour threshold data for each judge for (*R*)-γ-octalactone

	concentration (μg/L)								
judge	**3.1**	**9.2**	**27.5**	**82.1**	**239**	**726**	**2,292**	**6,685**	**BET**
2	0	0	1	0	1	1			140
6	0	0	0	0	0	1	1	1	416
9	0	0	1	0	0	0	1	1	1,290
13	0	0	0	0	0	1	1	1	416
17	1	0	0	1	0	1	1	1	416
23	1	1	1	0	0	1	1	1	416
28	0	1	0	0	1	1			140
34	0	0	1	0	1	0	0	1	3,914
36	1	0	0	0	1	1			140
40	1	1	0	1	1	1			47.5
66	0	1	0	1	0	1	1	1	416
89	1	0	0	1	1	1			47.5
216	0	0	0	0	1	1			140
259	1	0	1	0	1	1			140
265	0	0	1	0	1	1			140
284	0	1	1	0	0	1	1	1	416
305	1	0	1	1	1	1			15.9
310	1	1	0	0	1	1			140
312	0	0	0	0	0	1	1	1	416
313	0	1	0	1	1	1			47.5
317	0	0	1	0	0	1	1	1	416
318	0	1	0	0	1	1			140
319	1	1	0	0	0	0	1	1	1,290
320	1	1	1	0	0	1	1	1	416
321	0	1	1	0	0	1	1	1	416

Table 11.13 Odour threshold data for each judge for (*S*)-γ-octalactone

	concentration (µg/L)								
judge	**9.1**	**27.4**	**81.3**	**244**	**739**	**2,244**	**6,600**	**19,536**	**BET**
2	0	0	1	1	1	1			47.2
6	1	0	1	1	1	1			47.2
9	0	0	0	1	1	1			141
13	1	0	1	1	1	1			47.2
17	0	1	1	1	1	1			15.7
23	0	0	0	1	1	1			141
28	1	0	1	1	1	1			141
34	0	1	0	1	0	1	0	1	11,355
36	0	0	0	1	1	1			141
40	0	0	0	0	1	1			425
46	0	1	0	1	1	1			141
66	0	0	1	1	1	1			47.2
89	1	0	1	1	1	1			47.2
216	1	0	1	0	1	1			425
259	1	0	1	1	1	1			47.2
265	0	1	0	0	1	1			425
267	0	1	1	0	1	1			425
284	0	1	0	1	1	1			141
305	1	0	1	1	1	1			47.2
310	1	0	1	1	1	1			47.2
313	0	1	1	0	1	1			425
317	1	0	0	1	0	1	1	1	1,288
318	1	1	0	1	1	1			141
320	0	1	1	1	1	1			15.7
321	0	0	0	1	1	1			141

Table 11.14 Odour threshold data for each judge for (*R*)-γ-nonalactone

	concentration (μg/L)										
judge	**1.0**	**3.0**	**9.0**	**26.7**	**82.5**	**246**	**727**	**2,160**	**6,678**	**19,840**	**BET**
2			0	1	1	0	1	1			422
6			0	0	1	1	1	1			46.9
13			1	0	1	1	1	1			46.9
17			0	1	0	1	1	1			142
23			0	0	0	0	1	1			422
28			0	1	0	1	0	1	1	1	1,253
34			1	0	1	1	1	0	1	1	3,833
36			1	1	0	1	1	1			142
40			0	0	1	0	0	1	1	1	1,253
64			0	0	0	1	1	1			142
66			1	0	0	0	1	1			422
89	0	0	0	1	1	1	1	1			15.5
216			0	0	1	0	0	0	0	1	11,452
259			0	0	1	0	0	0	1	1	3,833
264			0	0	1	1	1	1			46.9
265			0	0	0	0	0	1	0	1	11,452
284			1	0	1	1	0	1	1	1	1,253
305			1	0	1	1	1	1			46.9
310			1	0	0	1	1	1			142
312			0	0	1	1	1	1			46.9
313	1	0	0	1	1	1	1	1			15.5
317			0	0	0	0	0	0	1	1	3,833
318			0	1	1	0	1	1			422
319			0	0	1	1	1	1			46.9
321			0	0	1	1	1	1			46.9

Table 11.15 Odour threshold data for each judge for (*S*)-γ-nonalactone

	concentration (μg/L)										
judge	**1.0**	**3.0**	**9.0**	**27.2**	**80.0**	**246**	**740**	**2,200**	**6,600**	**20,000**	**BET**
2			0	0	0	0	1	1			426
6			0	0	1	1	1	1			46.7
9			0	0	0	1	1	1			140
13			0	0	0	0	1	1			426
17			0	1	0	1	1	1			140
23			0	0	0	0	1	1			426
28			0	1	1	1	1	1			15.7
34			1	0	0	0	0	1	1	1	1,276
36			0	0	0	1	1	1			140
40			0	0	0	0	1	1			426
66			1	1	0	0	1	1			426
89	0	1	1	1	1	1	1	1			1.8
216			1	0	1	0	0	0	1	1	3,811
259			0	1	1	1	1	1			15.7
264			0	1	1	1	1	1			15.7
265			0	0	0	1	1	1			140
284			0	0	0	1	1	1			140
290			0	1	1	1	1	1			15.7
305			0	1	1	1	1	1			15.7
310			0	0	1	1	1	1			46.7
313	1	1	0	1	1	1	1	1			15.7
317			1	0	1	0	0	1	1	1	1,276
318			0	0	0	1	1	1			140
321	1	0	1	1	1	1	1	1			5.2

Table 11.16 Odour threshold data for each judge for (*R*)-γ-decalactone

	concentration (μg/L)										
judge	**1.0**	**3.0**	**9.0**	**26.8**	**80.3**	**239**	**727**	**2,199**	**6,501**	**19,120**	**BET**
2			0	0	0	1	1	1			139
6			0	0	1	1	1	1			46.4
9			1	1	0	0	1	1			417
13			0	0	0	1	1	1			139
17			0	1	1	1	1	1			15.5
23			1	0	1	1	1	1			46.4
28	0	1	1	1	1	1	1	1			1.7
34			0	1	0	1	1	1			139
36			0	0	0	1	1	1			139
40			1	0	1	1	1	1			46.4
46	1	0	1	1	1	1	1	1			5.2
66			1	1	0	1	1	1			139
89	1	1	1	1	1	1	1	1			0.5
216			1	0	1	1	1	1			46.4
265			0	0	0	0	0	1	1	1	1,264
267			0	1	1	1	1	1			15.5
284			0	1	1	1	1	1			15.5
305			0	0	1	1	1	1			46.4
310	1	0	1	1	1	1	1	1			5.2
313			0	1	1	1	1	1			15.5
316	0	0	1	1	1	1	1	1			5.2
317			0	0	0	1	1	1			139
318			0	0	0	1	1	1			139
320			0	1	1	1	1	1			15.5
321			0	1	1	1	1	1			15.5

Table 11.17 Odour threshold data for each judge for (*S*)-γ-decalactone

	concentration (μg/L)										
judge	**1.0**	**3.0**	**9.1**	**27.5**	**80.8**	**242**	**727**	**2,222**	**6,464**	**20,200**	**BET**
2			1	0	0	0	1	1			420
6			0	1	1	1	1	1			15.8
9			1	0	1	1	1	1			47.1
13			0	0	1	0	1	1			420
17			0	0	1	1	1	1			47.1
28			0	0	0	1	1	1			140
34			0	1	1	1	1	1			15.8
36			1	0	0	1	1	1			140
40			1	0	1	1	1	1			47.1
46	0	1	0	1	1	1	1	1			15.8
66			1	1	1	0	1	1			420
89	1	0	1	1	1	1	1	1			5.2
216			1	1	0	1	1	1			140
264			0	1	1	1	1	1			15.8
265			0	0	1	1	1	0	1	1	3,790
267			1	0	1	1	1	1			47.1
284			0	1	1	1	1	1			15.8
290			0	1	1	1	1	1	1	1	15.8
305			0	0	0	1	1	1			140
310			0	1	1	1	1	1			15.8
313			0	1	1	1	1	1			15.8
316	0	1	1	1	1	1	1	1			1.7
317			1	0	0	1	1	1			140
320			0	1	1	1	1	1			15.8
321	0	0	0	1	1	1	1	1			15.8

Table 11.18 Odour threshold data for each judge for (*R*)-γ-dodecalactone

	concentration (μg/L)											
judge	**0.3**	**1.0**	**3.0**	**9.0**	**27.4**	**82.1**	**242**	**736**	**2,185**	**6,670**	**19,780**	**BET**
2				0	0	1	1	1	1			47.4
6	0	0	1	1	1	1	1	1	1			1.8
9	0	0	0	1	1	1	1	1	1			15.7
13				0	1	1	1	1	1			15.7
17	1	0	1	1	1	1	1	1	1			1.8
23	0	1	1	1	1	1	1	1	1			0.6
34				0	1	1	1	1	1			15.7
36	0	0	1	1	1	1	1	1	1			1.8
46	0	0	1	1	1	1	1	1	1			1.8
66				1	0	1	1	1	1			47.4
89	0	1	1	1	1	1	1	1	1			0.6
216	0	0	1	0	1	1	1	1	1			15.7
264				0	1	1	1	1	1			15.7
265				0	1	0	0	0	1	0	1	11,486
267	1	0	1	1	1	1	1	1	1			1.8
284				0	1	1	1	1	1			15.7
290	0	0	1	1	1	1	1	1	1			1.8
305				0	1	1	1	1	1			15.7
310	0	1	1	1	1	1	1	1	1			0.6
313	0	1	0	1	1	1	1	1	1			5.2
316	0	1	0	1	1	1	1	1	1			5.2
317	0	0	0	0	1	1	1	1	1			15.7
320	1	1	0	1	1	1	1	1	1			5.2
321	1	1	1	0	1	1	1	1	1			15.7

Table 11.19 Odour threshold data for each judge for (*S*)-γ-dodecalactone

	concentration (μg/L)										
judge	**0.4**	**1.0**	**3.0**	**8.9**	**26.9**	**79.2**	**243**	**733**	**2,178**	**6,534**	**BET**
2	1	1	0	1	1	1	1	1			5.2
6			1	0	1	0	1	1			139
9			0	1	0	0	0	1	1	1	422
13			0	1	0	0	1	1			139
17			1	0	1	1	1	1			15.5
23			0	0	1	1	1	1			15.5
28			0	0	1	1	1	1			15.5
34			1	0	0	0	1	1			139
36			0	0	1	1	1	1			15.5
40			0	0	1	1	1	1			15.5
46			1	0	1	1	1	1			15.5
66			1	1	1	1	0	1	1	1	422
89			1	0	0	1	1	1			46.2
216			1	0	1	0	1	1			139
265			0	0	1	0	0	1	0	0	-
267			1	0	1	1	1	1			15.5
284			1	0	1	1	1	1			15.5
305			0	0	1	1	1	1			15.5
310			0	0	0	0	1	1			139
313			0	1	0	1	1	1			46.2
316			0	1	1	1	1	1			5.2
317			0	0	0	1	1	1			46.2
318			0	0	1	1	1	1			15.5
320			0	1	0	1	1	1			46.2
321			0	0	0	0	1	1			139

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