



**MONASH University**

**A Stereoselective Isoprenoid Synthetic Methodology  
and the Preparation of a Mycobacterial Antigen**

*A thesis submitted in fulfilment of the requirements for the degree of*

**DOCTOR OF PHILOSOPHY**

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## ERRATA

- Page vi, line 21: octadiene
- Page 2, line 5: , respectively (add a comma before respectively)
- Page 5, 3<sup>rd</sup> last line: **with the enolate** of 1.18
- Pages 6 and 7: Baker's, use upper case B
- Page 8, line 4: **and** homoallylic
- Page 9, line 3 up: **Menthol**
- Page 11, line 4: delete reference 28
- Page 12, line 6: **the** menthol manufacturing
- Page 18, last line: **an** alternative
- Page 25, line 4: add “(the preparation of 1.69 see section 1.3)” after 1.69
- Page 27, line 1: delete reference 26
- Page 29, footnotes in scheme 1.23: “5 h” for “5h”
- Page 31, line 5: add “, confirmed from its specific rotation” after “93 % ee”; line 9: delete “amine”; line 14: add “, confirmed from its specific rotation” after “93 % ee”; line 16: delete “amine”
- Page 34, line 10: **an** alternative attempt
- Page 35, scheme 1.28: “5 M HCl” for “5M HCl”; line 5: as **a** colourless; scheme 1.31: structures 1.160 and 1.161 – OH groups should be properly positioned; line 11: in **the** <sup>1</sup>H NMR; line 13: **of the** methyl group
- Page 36, line 5: “a dt” for “peaks”; line 7: in **the** <sup>31</sup>P NMR; line 8: Itaconic acid
- Page 37, footnotes in scheme 1.34: (1.1 atm.), - one comma only
- Page 40, line 13: add “we found” after “Fortunately,”
- Page 41, line 5: “the ketone” for “ketone’s”; scheme 1.38: 77 % overall yield; scheme 1.38 title: “2-methylalkanols” for “2-methyl alkanols”
- Page 43, line 10: Because **the** hydroxyl group
- Page 44, 3<sup>rd</sup> paragraph: Conversion **of** both
- Page 45, line 15: add “which was proved by <sup>1</sup>H NMR spectroscopy” after “75 %”
- Page 50, 4<sup>th</sup> last line: CaH<sub>2</sub>
- Page 51, line 13: add “The title mixture (86 mg, 74 %) was obtained as a volatile colourless liquid” before “The analytical data”
- Page 52, line 11: gave **1.91** (delete “the”) (56 mg, 35 %), and 1.92 with **a** small;
- Page 53, line 6 and 17: To **a** solution of...
- Page 54, line 6: 1.50 g, ... ; line 15: **Reduction** of keto-alcohol; line 17: 0.50 g, ...
- Page 55, line 5: 0.20 g, ....
- Page 74, structure of 1.142 should be the one drawn in scheme 1.34 on page 37
- Page 78, line 7: in **the** dark **in the** laboratory
- Page 82, line 21: “Negishi, E-i.” for “Negishi , E.”
- Page 82, line 20: “*Chem. Rev.*” for “*Chemical Reviews*”
- Page 83, line 8: “*Justus Liebigs Ann. Chem.*” for “*Justus Liebigs Annalen der Chemie*”
- Page 86, line 1: “*Bioorg*” for “*Bioorga*”

Page 88, line 1 in 3<sup>rd</sup> paragraph: attracting **the** opposite sex; line 3 in 3<sup>rd</sup> paragraph: Superior to **the** traditional insecticides

Page 89, line 2: extensive studies **of** thousands

Page 92, Scheme 2.2: the configuration of the Br bearing carbon in structure 2.13 should be inverted

Page 93, 3rd last line: “for the  $CH_2CO$  protons” for “for the protons in the ketone  $\alpha$ -methylenes”

Page 95, line 9: comma before respectively

Page 97, line 3 up: died **from** it

Page 101, in table 2.1: delete “extracting”

Page 105, line 12: from **the** fragrance

Page 106, line 9: **nutty** and fatty

Page 109, line 8: “was not” for ““wasn’t””; line 15, “in 90 %” for “in 90 % yield””; line 16: on **such** solution

Page 110, line 3: phenylpentanenitrile – all one word

Page 113, line 13: cinnamyl bromide – two words

Page 115, last line: delete “in”

Page 117, first two lines in paragraph 1: Coronary heart disease is clinically characterised as **the** blockage of coronary arteries, and the major cause of **such** blockage is high concentration of cholesterol in the blood.

Page 117, line 12: cholesterol-lowering

Page 121, line 4: **The compound** 2.91; “olefin” for “olefine”; line 8: delete “olefination”; 3rd last line: of **the** base

Page 130, last line: in humans and animals

Page 134, line 4: carboalumination

Page 136, line 11: **the** Appel reaction

Page 137, line 4: enantiomerically pure

Page 139, line 2: Ring cleavage of five, six, and seven member lactones **using** alkyllithiums

Page 140, line 2, chain extended alcohol

Page 161, line 2: “*Br. Med. Bull.*” for “*British Medical Bulletin*”; line 7: “*H.-S. Z. Physiol. Chem.*” for “*Hoppe-Seyler's Zeitschrift für physiologische Chemie*”; line 28: “*J. Chem. Ecol.*” for “*J. Chemical Ecol.*”

Page 162, line 8: “*J. Chem. Soc., Perkin Trans. I*” for “*J. Chem. Soc. Perk. TI*”

Page 164, line 4: “*J. Chem. Soc., Perkin Trans. I*” for “*J. Chem. Soc. Perk. TI*”; line 29: “*Helv. Chim. Acta.*” for “*Helvetica Chimica Acta*”

Page 165, line 1: “**D**’Abrosca”; line 5: “*J. Mol. Med.*” for “*Journal of Molecular Medicine*”; line 7: “*Bioorg. Med. Chem.*” for “*Bioorgan. Med. Chem.*”

Page 166, line 8: **the** glycosidic acetal bond

Page 168, line 14: influence **on**

Page 169, 4<sup>th</sup> last line: “one missing proton” for “one proton shorter”; 3<sup>rd</sup> last line: “full NMR characterisation was not given” for ““only partial of NMR data were given””

Page 171, line 6 up: as **the** intermediate; line 7 up: started **from**

Page 172, scheme 3.3 title: using chloride **3.40** as

Page 173, last line: “confirmed from its specific rotation” for “confirmed by measuring specific rotation”

Page 174, line 4 and 5: in **the** <sup>1</sup>H NMR ... for **the**  $\alpha$ -anomer

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# ***Declaration***

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

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# ***Abstract***

## ***Chapter 1***

In Chapter 1, the need for exploring new methodology for the synthesis of naturally occurring chiral isoprenoids was outlined, and a lactone ring cleavage method was developed. Reaction optimisation studies were carried out on the commercially available lactone,  $\gamma$ -valerolactone. In addition, methods were found to be feasible for the preparation of (*S*)- and (*R*)-3-methylbutyrolactone in high yields and enantiomeric purity.

## ***Chapter 2***

The methodology developed in Chapter 1 was applied to the synthesis of several selected natural products containing one or more chiral isoprenoid moieties, including a sex pheromone, fragrances, the side chain of zaragozic acid C, an intermediate of tuberculostearic acid, and the side chain of vitamin E. A purported natural product, with antibacterial and antifungal activities, (*R*)-2-methylheptyl isonicotinate, was also prepared. Our results indicated that the structure assigned to the natural product was incorrect. The application of such lactone ring cleavage reactions was extended to unbranched lactones, making it an efficient method for chain elongation in general.

## ***Chapter 3***

In this Chapter, a mycobacterial antigen was synthesised for investigation of the underlying mechanisms involved in the CD1-antigen-T cell interaction. Initial attempts at coupling an acetyl-protected galactosyl iodide to acetonide-protected glycerol followed by installing the required acyl groups after hydrolysis of the acetonide group, were unsuccessful. To prepare the antigen and enable the biological studies to begin as soon as possible, the method developed by Gervay-Hague and co-workers was successfully adopted. This involved coupling a TMS-protected galactosyl iodide with an appropriate diacylglycerol to give the required product. Biological studies showed that this synthetic galactosyl diacylglycerol could activate NKT cells to licence DCs, and resulted in CCL17 expression in splenic DCs.

# Abbreviations

$\delta$	chemical shift
$^{\circ}\text{C}$	degrees Celsius
$[\alpha]_{\text{D}}$	specific rotation
Ac	acetyl
AcOH	acetic acid
AcOOH	acetic peroxide
Ac <sub>2</sub> O	acetic anhydride
Ar	argon
b	broad
BINAP	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
Bn	benzyl
Bu	butyl
BzCl	benzoyl chloride
<i>c</i>	concentration (g/100 mL)
cat.	catalytic
Calcd.	calculated
CCL	chemokine (C-C motif) ligand
CD1	cluster of differentiation
cm	centimetres
COD	1,5-cyclo-octadiene
DBU	1,8-diazabicycloundec-7-ene
DCC	1,3-dicyclohexylcarbodiimide
DCs	dendritic cells
DHP	dihydropyran
DIBALH	diisobutylaluminium hydride
DMAP	4-( <i>N,N</i> -dimethylaminopyridine)
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethylsulfoxide
DIPEA	( <i>N,N</i> -diisopropylethylamine)
ee	enantiomeric excess
eq.	equivalents
Et	ethyl
Et <sub>2</sub> O	diethyl ether

EtOAc	ethyl acetate
g	grams
h	hour(s)
HCOOH	performic acid
HMG-CoA	3-hydroxy-3-methylglutaryl CoA
HRMS	high resolution mass spectrometry
<i>i</i> -	iso-
IR	infrared
<i>J</i>	coupling constant
KHMDS	potassium bis(trimethylsilyl)amide
lit.	literature
LDA	lithium diisopropylamide
LiH <sub>2</sub> NBH <sub>3</sub>	lithium amidotrihydroborate
m	multiplet (NMR); medium (IR)
M	molar
M <sup>+</sup>	molecular ion
<i>m</i> CPBA	<i>meta</i> -chloroperbenzoic acid
Me	methyl
MeCN	acetonitrile
MeOH	methanol
MHz	megahertz
min	minutes
mL	millilitres
mmol	millimole
m.p.	melting point
MPM	β-mannosyl phosphomycoketides
MOM	methoxymethyl
MsCl	methanesulfonyl chloride
MS	mass spectrometry
m/z	mass to charge ratio
<i>n</i>	normal
NaHMDS	sodium bis(trimethylsilyl)amide
NBS	<i>N</i> -bromosuccinimide
<i>n</i> -BuLi	normal butyllithium
NKT cells	natural killer T cells
NMR	nuclear magnetic resonance

NaN(TMS) <sub>2</sub>	sodium hexamethyldisilazide
PLE	pig liver esterase
PCL	pseudomonas cepacia lipase
Ph	phenyl
ppm	parts per million
Pd/C	palladium on carbon
<i>i</i> -Pr	<i>iso</i> -propyl
R	alkyl or aryl group
Rh	rhodium
r.t.	room temperature
Ru	ruthenium
s	singlet (NMR); strong (IR)
<i>tert</i>	tertiary
<i>t</i> -BuLi	tertiary butyl lithium
TBS	<i>t</i> -butyldimethylsilyl
THF	tetrahydrofuran
THP	tetrahydropyranyl
TMEDA	tetramethylethylenediamine
TsCl	<i>para</i> -toluenesulfonyl chloride
TsOH	<i>para</i> -toluenesulfonic acid
TBSCl	<i>tert</i> -butyldimethylsilyl chloride
™	trademark
TMS	trimethylsilyl
TBAI	tetrabutylammonium iodide
μg	micrograms
μL	microlitres
$\nu_{\max}$	infra-red absorption maxima
w	weak
Zn	zinc
Zr	zirconium

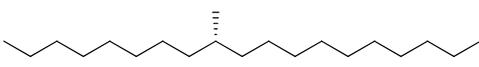
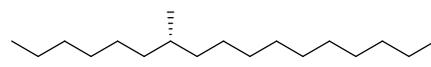
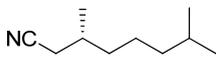
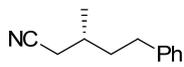
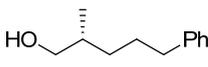
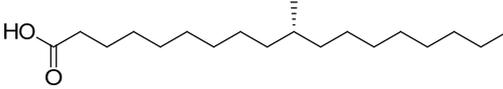
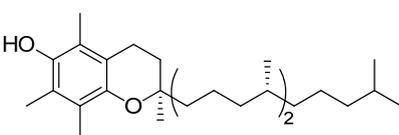
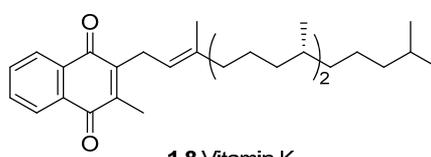
# Chapter 1. Methodology Development for the Synthesis of Chiral Isoprenoid Moieties

*In this Chapter, a new methodology was developed for the synthesis of chiral isoprenoid moieties. The reaction conditions were explored and optimised. The preparation of the key enantiomerically enriched lactones was investigated, and the most practical methods were identified and adopted for this work. The new methodology is potentially useful for the stereoselective synthesis of a variety of naturally-occurring isoprenoids.*

## 1.1. Background on natural products with chiral isoprenoid moieties

Chiral isoprenoid units are important structural fragments in natural products, such as pheromones (**1.1-1.2**),<sup>1-3</sup> fragrances (**1.3-1.5**),<sup>4</sup> and vitamins (**1.7-1.8**)<sup>5</sup> (Table 1.1).

**Table 1.1:** Examples of natural products with chiral isoprenoid moieties

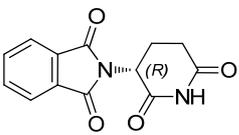
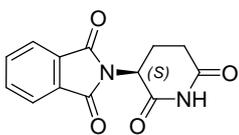
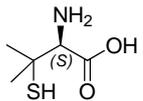
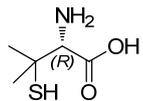
 <p><b>1.1</b> Sex pheromone of cotton leafworm</p>	 <p><b>1.2</b> Sex pheromone of hemlock looper moth</p>
 <p><b>1.3</b> (R)-3,7-Dimethyloctanenitrile</p>	 <p><b>1.4</b> (R)-Citrinal Nitrile</p>
 <p><b>1.5</b> (R)-Rosaphen</p>	 <p><b>1.6</b> Tuberculostearic acid</p>
 <p><b>1.7</b> Vitamin E</p>	 <p><b>1.8</b> Vitamin K</p>

(*S*)-9-Methylnonadecane **1.1** and (*S*)-7-methylheptadecane **1.2** are two sex pheromones of the female moth, cotton leafworm (*Alabama argillacea*),<sup>6</sup> and looper moth (*Lambdina athasaria* and *L. pellucidaria*)<sup>7</sup> respectively. Although the fragrances (**1.3-1.5**) are commercially available as racemates, each enantiomer presents different olfactive notes and odorant. Tuberculostearic acid **1.6** is a methyl-branched, saturated fatty acid isolated from *Mycobacterium tuberculosis*. Vitamins E **1.7** and K **1.8** are two bioactive natural compounds with repeating chiral isoprenoid moieties.

### 1.1.1. The need for the synthesis of enantiomerically enriched natural products

Absolute stereochemistry is an important property for the bioactivity of natural products. In medicinal chemistry, the lack of understanding of the bioactivity of enantiomers may result in serious consequences (**Table 1.2**). Probably the most notorious and noteworthy example is thalidomide. Racemic thalidomide was used by pregnant woman as a sedative drug to treat morning sickness and aid sleep in the 1950s.<sup>8</sup> However, whilst the *R*-enantiomer **1.9** provided beneficial tranquilizer effects, the *S*-enantiomer **1.10** proved to be an extreme teratogen. As a consequence, this resulted in more than ten thousand children in 46 countries being born with deformities from the 1950s to early 1960s.<sup>8</sup> This tragedy led to more consideration of side effects of enantiomers and stricter testing for medicine licensing afterwards. Another example is penicillamine, used to treat rheumatoid arthritis. The *S*-enantiomer **1.11** is an effective chelating agent for the removal of Pb, Au and Hg, while the *R*-enantiomer **1.12** has been found to cause optic atrophy or blindness.<sup>9</sup>

**Table 1.2:** Examples of stereoisomers' side effects in medicinal chemistry

Name	Effective isomer	Isomer with side effects
Thalidomide	 <p style="text-align: center;"><b>1.9</b></p> <p style="text-align: center;">Sedative, hypnotic</p>	 <p style="text-align: center;"><b>1.10</b></p> <p style="text-align: center;">Teratogenic</p>
Penicillamine	 <p style="text-align: center;"><b>1.11</b></p> <p style="text-align: center;">Effective chelator for Pb, Au, Hg</p>	 <p style="text-align: center;"><b>1.12</b></p> <p style="text-align: center;">Can cause optic atrophy</p>

Stereochemistry also plays an important role in pheromone and fragrance properties. It is important to determine the absolute stereochemistry of natural products before understanding their bioactivity. The most straightforward method of determining absolute stereochemistry is X-ray diffraction. However, many natural products are liquids or amorphous solids and can not be analysed by X-ray crystallography. In addition, due to their extremely low concentrations in organisms, many natural products are usually only isolated in small amounts. With such limited availability, it is often difficult to examine their structures by degradation reactions to give known compounds with absolute configurations. Authentic samples of optically active compounds provide valuable data in order to establish the absolute and relative stereochemistry of natural compounds. Thus, reliable and unambiguous methodologies for the total synthesis, in the context of the work outlined in this thesis, of enantiomerically enriched methyl-branched compounds is of critical importance.

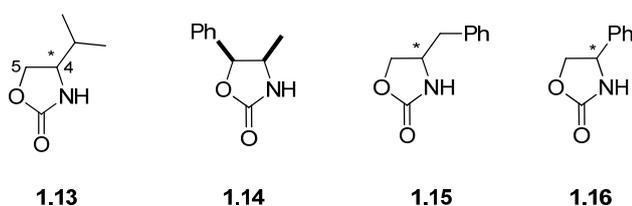
Furthermore, effective total synthesis of these bioactive compounds may also provide opportunities for commercial manufacture. Paclitaxel was firstly isolated from the bark of the pacific yew tree, and was found to display potent antileukemic and tumor inhibitory properties, ultimately leading to its use in cancer chemotherapy.<sup>10</sup> The isolation of paclitaxel involves tedious work and great amount of natural sources. For example, isolation of 1 kg of paclitaxel needs around 7 tons of *Taxus brevifolia* bark, which means several thousand trees would be cut for its production.<sup>11</sup> *T. brevifolia* nowadays is on the threatened species list. The scarcity of paclitaxel in Nature and its increasing demand from the clinic treatment of cancer prompted the total synthesis of paclitaxel. Many groups have reported total syntheses of paclitaxel. For example, Sorensen and co-workers disclosed a total synthesis in 1994 giving paclitaxel in 40 steps.<sup>12</sup> None of these is commercially viable.

## 1.1.2. Previous synthesis of enantiomerically enriched methyl-branched moieties

### 1.1.2.1. Chiral auxiliary approaches to chiral, methyl-branched moieties

Chiral auxiliaries are enantiomerically pure compounds specifically modified to assist in the installation of new chiral centres in substrates. Generally, after being temporarily attached to the substrate, chiral auxiliaries can control the relative stereochemistry of newly-created stereocentres during their formation. Subsequently, the auxiliary can be removed to give the optically active product.<sup>13</sup>

Many chiral auxiliaries have been reported. Oxazolidinones are a group of widely used chiral auxiliaries developed by Evans and co-workers for, inter alia, asymmetric aldol and alkylation reactions.<sup>14</sup> There are several commercially available oxazolidinones, for example, **1.13-1.16** in **Figure 1.1**, all with chiral bulky substituents such as isopropyl, phenyl, or benzyl groups at C4 or C5.

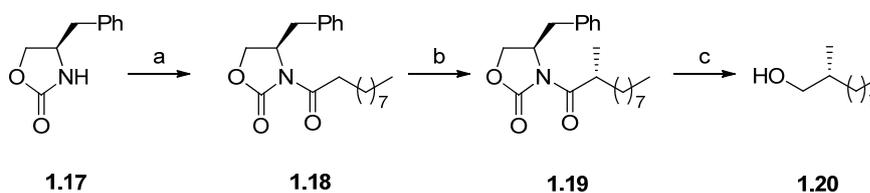


**Figure 1.1:** Examples of commercially available oxazolidinones

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Benjamin Cao and co-workers from Melbourne University reported the synthesis of chiral alcohol **1.20** from oxazolidinones **1.17** (**Scheme 1.1**).<sup>15</sup> The key step of introducing the methyl group stereoselectively was achieved by reacting MeI with the imide **1.18**, which was generated from acid chloride and oxazolidinone **1.17**. Due to the steric hindrance of the benzyl group in imide **1.18**, the methyl group in imide **1.19** was introduced stereoselectively. After removal of the auxiliary group, chiral 2-methyl

branched alcohol **1.20** was obtained in 76 % yield.



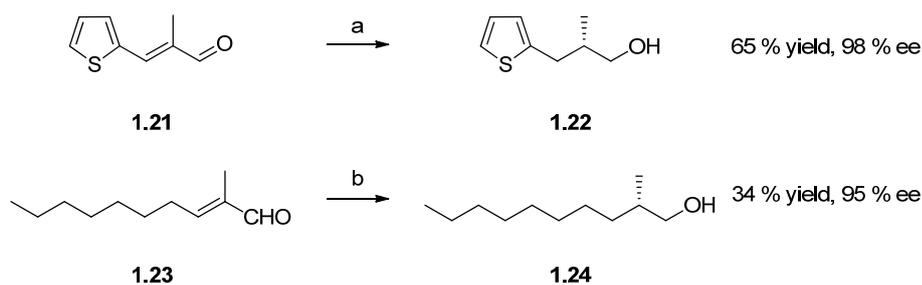
Reagents and conditions: a) *n*-BuLi, C<sub>9</sub>H<sub>19</sub>COCl, THF, -78 °C to r.t.; b) NaHMDS, THF, -78 °C, 1 h then MeI, -78 °C, 2 h; c) NaBH<sub>4</sub>, THF/H<sub>2</sub>O, 0 °C, overnight.

**Scheme 1.1:** Benjamin Cao's synthesis of chiral methyl moiety with auxiliary **1.17**<sup>15</sup>

Asymmetric synthesis mediated by chiral auxiliaries is still attractive for the high selectivity and reliability usually achieved. However, the use of chiral auxiliaries has important limitations, including the requirement of additional steps for the installation and removal of the auxiliary, as well as the often substantial cost of the auxiliaries themselves.

### 1.1.2.2. Asymmetric synthesis of chiral, methyl-branched moieties based on biocatalysts

*Saccharomyces cerevisiae*, commonly known as Baker's yeast, has been used as a biocatalyst for the reduction of both carbon-oxygen and carbon-carbon double bonds to give optically active compounds.<sup>16</sup> Högberg and co-workers reported the asymmetric formation of chiral methyl-branched thiophene derivatives with baker's yeast. The reduction of thiophene substituted aldehyde **1.21** gave the chiral alcohol **1.22** in 75 % yield and 94 % ee.<sup>17</sup> However, only the *S*-configured enantiomer was accessible via this method. For the synthesis of linear alcohols, the reduction of aldehyde **1.23**, which was prepared from octanol and propanal via aldol reaction in 25 % yield, gave chiral alcohol **1.24** in 34 % yield and 95 % ee (**Scheme 1.2**).<sup>17</sup>



Reagents and conditions: a) Baker's yeast, water, glucose, O<sub>2</sub>, 72 h fermentation; b) Baker's yeast, water, glucose, O<sub>2</sub>, 75 h fermentation.

**Scheme 1.2:** Asymmetric reductions with baker's yeast<sup>17</sup>

### 1.1.2.3. Asymmetric hydrogenation for preparing chiral, methyl-branched moieties via transition metal-ligand catalysts

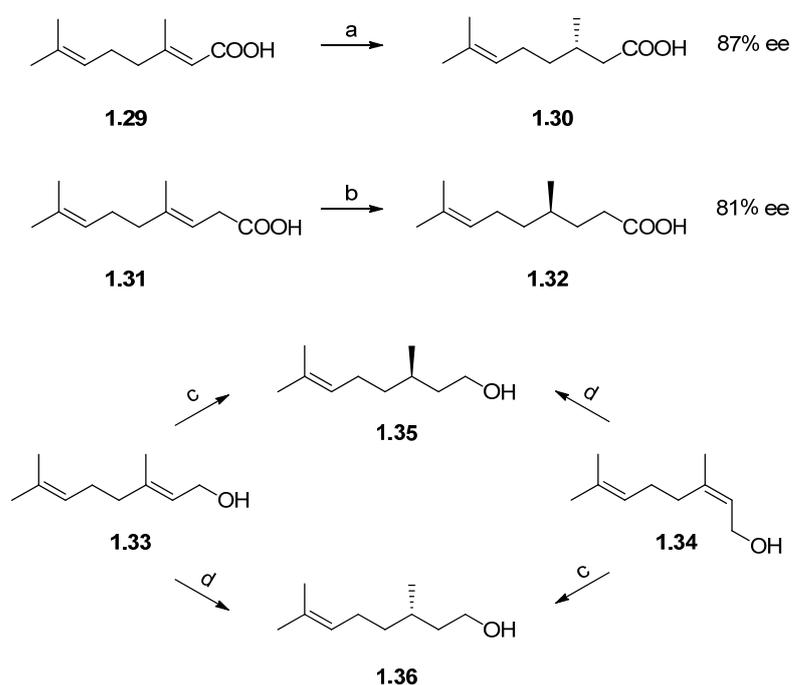
An enantiomerically pure ligand coordinates to a metal centre by chelation to form asymmetric catalysts, which direct the stereochemistry of reactions and allow the preparation of enantiomerically enriched compounds. This field was pioneered by Knowles,<sup>18</sup> Noyori,<sup>19</sup> Sharpless,<sup>20</sup> and numerous ligands and transition metals have since then been applied in asymmetric synthesis.

BINAP ligands, **1.25** and **1.26**, have axial chirality because of the restricted rotation of the naphthyl groups, and exert strong steric influences on transition-metal complexes. They can determine the chirality at the coordination sites in asymmetric synthesis (**Figure 1.2**).<sup>21</sup>



**Figure 1.2:** Structure of BINAP ligands<sup>21</sup>

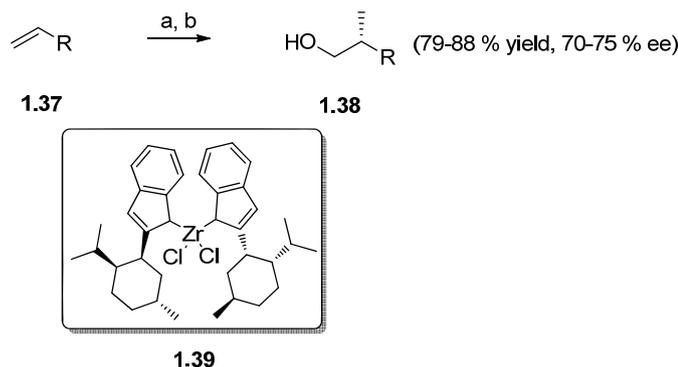
BINAP phosphine ligands chelating with Ru(II) are outstanding catalysts for asymmetric hydrogenation of various functionalised olefins with polar functional groups, such as  $\alpha,\beta$ - and  $\beta,\gamma$ -unsaturated carboxylic acids (such as **1.29** and **1.31**),<sup>19</sup> homoallylic alcohols (such as **1.33** and **1.34**) (**Scheme 1.3**).<sup>22</sup> The stereoselectivity of these asymmetric reductions is highly dependent on the catalysts involved, substituents present in the starting materials, as well as reaction conditions.<sup>19,22,23</sup>



Reagents and conditions: a) 0.36 mol% (*R*)-Ru(II)-BINAP, 101 atm H<sub>2</sub>; b) 0.67 mol% (*S*)-Ru(II)-BINAP, 50 atm H<sub>2</sub>; c) 0.2 mol% (*S*)-Ru(II)-BINAP, > 30 atm H<sub>2</sub>; d) 0.2 mol% (*R*)-Ru(II)-BINAP, > 30 atm H<sub>2</sub>.

**Scheme 1.3:** Examples of asymmetric hydrogenation with Ru(II)-BINAP<sup>19,22,23</sup>

Introducing chiral methyl groups by Zr-catalysed enantioselective methylalumination of terminal alkenes **1.37** was reported by Negishi and co-workers (**Scheme 1.4**).<sup>24</sup> With (3-neomenthyl-1-indenyl)<sub>2</sub>ZrCl<sub>2</sub> **1.39** as catalyst, unactivated terminal alkenes **1.37** could be converted to corresponding alcohols **1.38**. This reaction gave optically active alcohols **1.38** from terminal alkenes in 79-88 % yield and 70-75 % ee.<sup>24</sup>

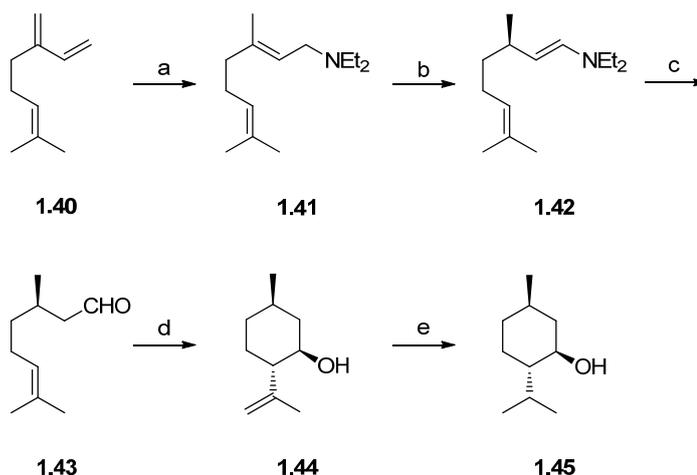


Reagents and conditions: a)  $\text{Me}_3\text{Al}$ , 2 mol% **1.39**; b)  $\text{O}_2$ .

**Scheme 1.4:** (3-Neomenthyl-1-indenyl) $_2\text{ZrCl}_2$  **1.39** catalysed alkylations<sup>24</sup>

The stereo-specific alkylation methodology catalysed by (3-3-neomenthyl-1-indenyl) $_2\text{ZrCl}_2$  **1.39** stands out in the synthesis of chiral methyl-branched compounds for its high efficiency, requiring no protection-deprotection steps. It has been applied to the synthesis of natural compounds with repeating methyl branches, such as the side chains of vitamins E and K.<sup>25</sup> On the other hand, currently the enantiomeric purity of the generated chiral alcohol is limited to 75 % ee, and more investigation is required to improve the enantioselectivity.

The commercial production of menthol is a well known example of successful application of asymmetric hydrogenation in industry with transition metal-ligand as catalyst. Menthol is used in many consumer products, such as toothpaste, food additives, and pharmaceutical products for relieving minor throat irritation. Before its availability from asymmetric synthesis, it was obtained from cornmint, peppermint or other mint oils. It is manufactured in thousands of tons annually by Takasago International Cooperation (**Scheme 1.5**).<sup>26</sup> The key step is the asymmetric isomerisation of amine **1.41** catalysed by < 1 mol% Rh(I)-(S)-BINAP complex to give chiral intermediate **1.42**. (-)-Methanol **1.45** is produced in with 96 % ee via this procedure. Natural citronellal occurs in 80 % ee, while the commercial process provides citronellal **1.43** in greater than 95 % ee.<sup>27</sup>



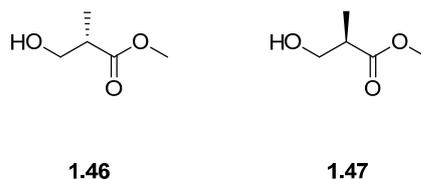
Reagents and conditions: a) Li,  $(\text{C}_2\text{H}_5)_2\text{NH}$ ; b)  $< 1 \text{ mol}\% \text{ Rh(I)-(S)-BINAP}$ ,  $\text{H}_2$ ; c)  $\text{H}^+$ ; d)  $\text{ZnBr}_2$ ; e)  $\text{H}_2$ , cat. Ni.

**Scheme 1.5:** Asymmetric synthesis of menthol with Rh(I)-(S)-BINAP complex<sup>26</sup>

There are many other commercially available chiral compounds being synthesised through asymmetric hydrogenation commercially at relatively low cost.<sup>21</sup> The increasing range of chiral compounds available from large-scale asymmetric synthesis provides a substantially larger pool of materials for use as starting materials for the synthesis of other chiral products. Overall, transition metal catalysed asymmetric synthesis plays an important role for preparing chiral methyl-branched compounds.

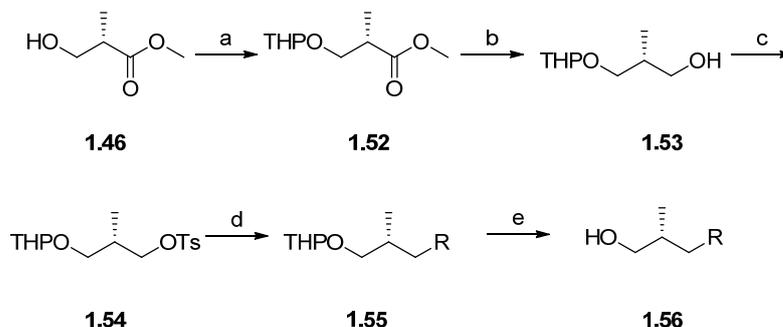
#### 1.1.2.4. Total synthesis of chiral, methyl-branched moieties from the chiral pool

The preparation of optically enriched compounds from optically active starting materials is a well-established strategy in asymmetric synthesis. The chirality is built in the starting materials and usually retained in the consequent reactions to generate the desired products with the same chirality. Citronellol **1.43**, menthol **1.45**, Roche esters **1.46** and **1.47** are common and widely used chiral pool compounds for the synthesis of chiral methyl units (**Figure 1.3**).<sup>13</sup> They are all commercially available in high enantiomeric purity.



**Figure 1.3:** Structures of Roche esters

To incorporate chiral Roche esters into chiral isoprenoid compounds, they are converted to other compounds suitable for coupling reactions. Seeberger and co-workers reported preparing 2-methyl alcohols **1.56** from Roche ester **1.46** (Scheme 1.6).<sup>28,29</sup> The conversion started with protection of the hydroxyl group in **1.46** to give ether **1.52**, followed by reduction of the methyl ester group with LiAlH<sub>4</sub> to give alcohol **1.53**, which was converted to tosylate **1.54** by reacting with TsCl. **1.54** was then coupled with a Grignard reagent in the presence of Li<sub>2</sub>CuCl<sub>4</sub> to give ether **1.55**, followed by removal of the THP group to give the alcohol **1.56**. After five steps, the chiral Roche ester **1.46** was converted to chiral alcohol **1.56**, which was later used for the synthesis of tuberculostearic acid.<sup>28</sup>

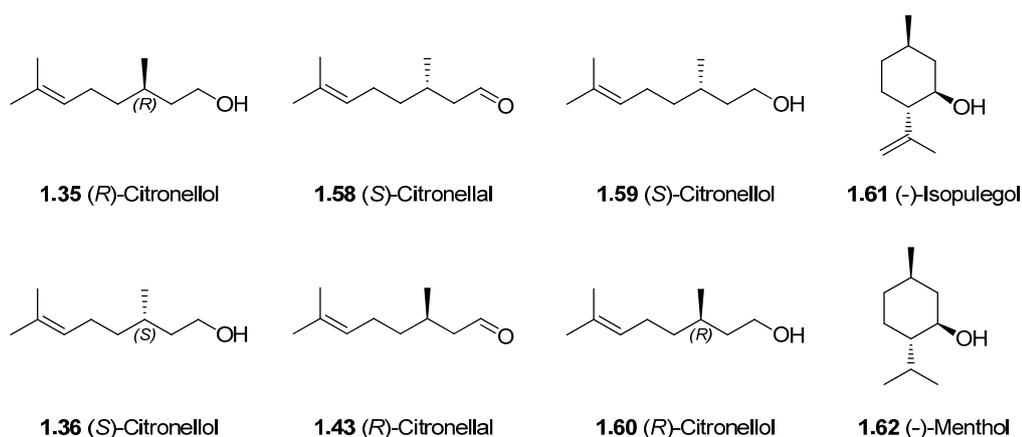


Reagents and conditions: a) DHP, TsOH; b) LiAlH<sub>4</sub>; c) TsCl; d) RMgBr, Li<sub>2</sub>CuCl<sub>4</sub>; e) TsOH.

**Scheme 1.6:** Conversion of Roche esters to chiral alkyl building blocks<sup>28,29</sup>

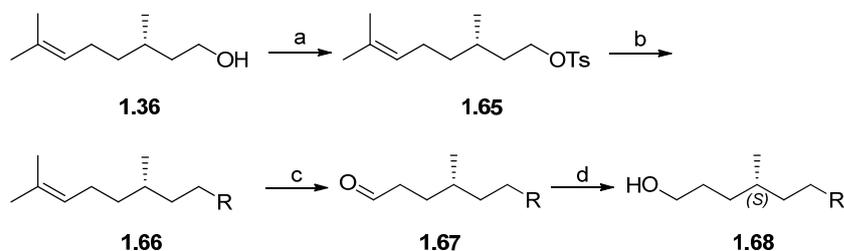
Although Roche esters are useful chiral building blocks, and both enantiomers are commercially available, the synthesis requires protection, reduction and deprotection steps, making it an inefficient procedure.

The availability of chiral pool compounds is a main limitation of chiral pool-based asymmetric synthesis. For many naturally-derived chiral pool compounds, only one enantiomer is available, the other being either unavailable or prohibitively expensive. Modern development of asymmetric synthesis has greatly enriched the availability of chiral compounds, often providing both enantiomers at a reasonable price, such as chiral compounds listed in **Figure 1.4** available from menthol manufacturing industry.



**Figure 1.4:** Chiral compounds from menthol manufacturing industry<sup>26</sup>

In 2006, Larsen and co-workers reported an effective synthesis of chiral methyl-branched alcohols from (*S*)-citronellol over four steps (**Scheme 1.7**). After converting citronellol **1.36** to tosylate **1.65**, it was coupled with a Grignard reagent to give an alkene **1.66**. Cleavage **1.66** with O<sub>3</sub> followed by reduction gave methyl-branched alcohol **1.68**, which again was used for the synthesis tuberculostearic acid.<sup>30</sup>

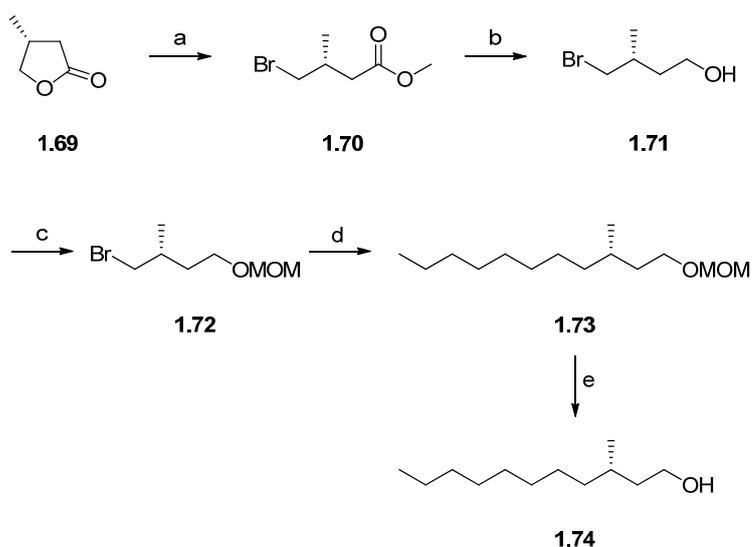


Reagents and conditions: a) TsCl, pyridine; b) RMgBr, Li<sub>2</sub>CuCl<sub>4</sub>, THF; c) i - O<sub>3</sub>; ii - Dimethyl sulfide, MeOH; d) LiAlH<sub>4</sub>.

**Scheme 1.7:** Synthesis of chiral methyl moieties from (*S*)-citronellol<sup>30</sup>

Although citronellol is an economical starting material, the conversion includes oxidation and reduction steps, making the procedure less efficient. In addition, only chiral 4-methyl branched alcohols are obtainable, and it is not useful for synthesis of compounds with repeating isoprenoid moieties.

Tian and co-workers reported a method of synthesising chiral methyl-branched alcohols from (*R*)-3-methylbutyrolactone **1.69** (Scheme 1.8).<sup>31</sup> Lactone **1.69** was treated with HBr in MeOH to give the bromoester **1.70**. After reduction of **1.70** to alcohol **1.71**, the hydroxyl group was protected to give bromide **1.72**. This was coupled with a Grignard reagent to give ether **1.73**, which upon hydrolysis yielded alcohol **1.74**.



Reagents and conditions: a) HBr, MeOH; b) LiAlH<sub>4</sub>, Et<sub>2</sub>O; c) MOMCl, DIPEA; d) Li<sub>2</sub>CuCl<sub>4</sub>, C<sub>7</sub>H<sub>15</sub>Br; e) HCl, MeOH.

**Scheme 1.8:** Tian's synthesis of chiral methyl-branched alcohol from chiral lactone<sup>31</sup>

This method involves reduction, protection, and deprotection reactions, and takes five steps from the lactone to the chiral methyl-branched alcohol. In our view, a more efficient methodology would be desirable.

## **1.2. Methodology development towards chiral isoprenoid units**

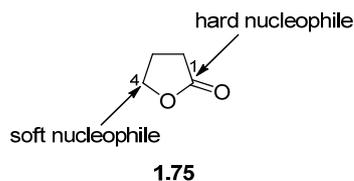
### **1.2.1. Synthetic objectives and challenges**

Generally speaking, there is no simple answer for the best asymmetric synthetic approach, but an acceptable methodology should adhere to some fundamental criteria, such as (a) providing short synthetic pathways with high yields and good enantiomeric selectivity, (b) being environmentally benign, (c) avoiding expensive starting materials, and (d) leading to easily purified products.

There have been some significant reports on the synthesis of chiral, saturated isoprenoid compounds.<sup>15,17,21,24,26,28,29</sup> However, considering the numerous natural products with such moieties and the disadvantages of existing methods, there is still room to explore more efficient approaches for general methyl-branched compounds with short synthetic routes, cheap starting materials, and feasible scale up procedures. Even more importantly there is still a need to develop reliable methods for target molecules with repeating isoprenoid units, such as the side chains of vitamins E and K.

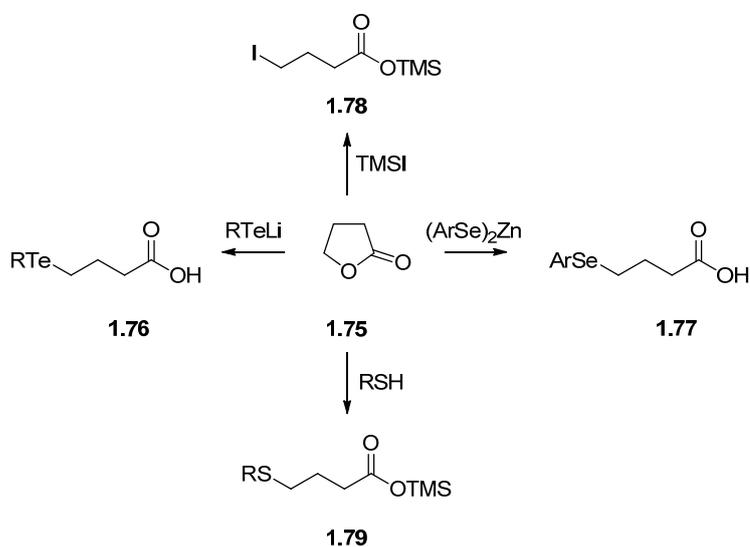
### **1.2.2. Lactone reactivity - general considerations**

Lactones can be hydrolysed under either acidic or basic conditions. Nucleophilic attack can also cause lactone ring cleavage. There are two sites in the lactone which can be potentially attacked by nucleophiles. According to Markiewicz, “soft” nucleophiles are preferred to attack the alcohol carbon at C4 position, while “hard” nucleophiles tend to attack at the carbonyl group (**Figure 1.5**).<sup>32</sup>



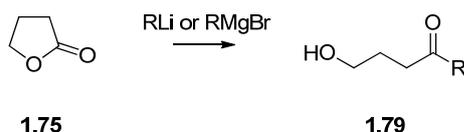
**Figure 1.5:** Markiewicz's report about nucleophilic attack of lactone

It has been reported that thiols,<sup>33</sup> TMSI,<sup>34,35</sup> Zn selenolates,<sup>36</sup> and organotellurolates<sup>37</sup> could attack C4 position, giving acids as products (**Scheme 1.9**).



**Scheme 1.9:** Ring cleavage of lactone by “soft” nucleophiles<sup>33-37</sup>

When “hard” nucleophiles, such as alkyllithiums or Grignard reagents are used, the nucleophilic attack occurs at the carbonyl group, and results in keto-alcohols **1.79** after aqueous work up (**Scheme 1.10**).<sup>38-40</sup>

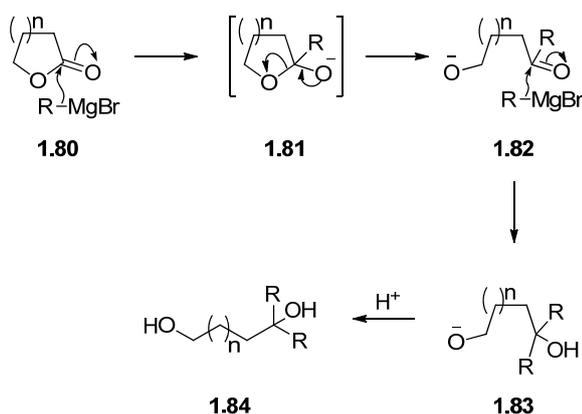


**Scheme 1.10:** Cleavage of lactone by alkyllithiums or Grignard reagents<sup>38,39</sup>

Ring cleavage of lactones with strong nucleophiles, such as alkyllithiums or Grignard reagents, appears to be an under-utilised method for carbon chain elongation and forms the basis of the methodology reported in this section of the thesis.<sup>40</sup>

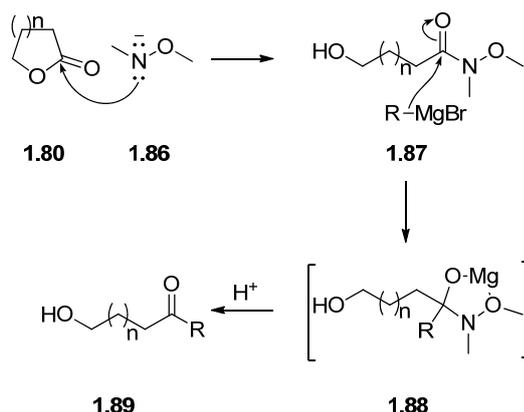
### 1.2.3. Ring cleavage with Grignard reagents via Weinreb amides

Grignard reagents tend to form tertiary alcohols on reaction with lactones **1.80** (**Figure 1.6**), because the ketones **1.82** generated from the first addition of Grignard reagent are more reactive than lactones **1.80**, which will enable the second addition of Grignard reagents to give the tertiary alcohols **1.84**.<sup>38,39</sup>



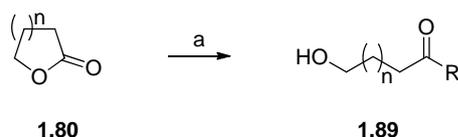
**Figure 1.6:** Reaction of lactones with Grignard reagents

However, Weinreb amides **1.87**, derived from lactones **1.80** and *N,O*-dimethylhydroxylamine·HCl, form stable metal-chelated intermediates **1.88** with Grignard reagents, thus inhibit further addition and give ketones **1.89** as the major, often exclusive, products (**Figure 1.7**).<sup>41</sup>



**Figure 1.7:** Mechanism of Weinreb ketone synthesis<sup>41</sup>

Xu and co-workers reported the synthesis of keto-alcohols **1.89** in good yields from the reaction of lactones **1.80**, *N,O*-dimethylhydroxylamine·HCl salt and Grignard reagents in one pot (**Scheme 1.11**).<sup>42</sup> This efficient and convenient approach provides a feasible method for the preparation of keto-alcohols **1.89** from lactones **1.80**. However, it requires 8 eq. of the Grignard reagent to ensure good yields, including 1 eq. to neutralise the *N,O*-dimethylhydroxylamine·HCl salt and a further 1 eq. to deprotonate the hydroxylamine.<sup>42</sup>

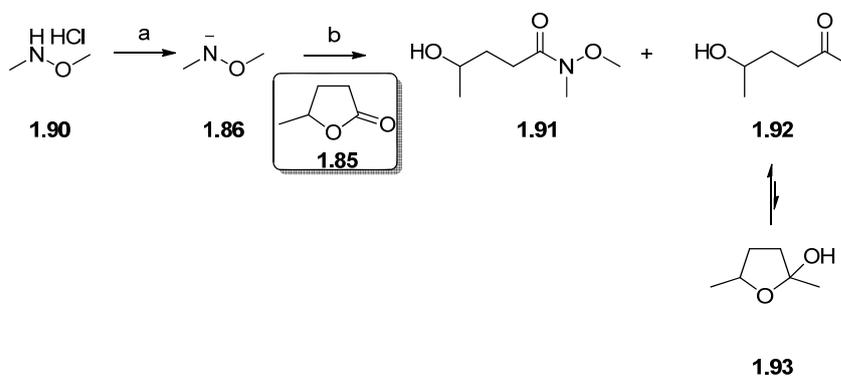


Reagents and conditions: a) MeNHOMe·HCl (1.2 eq.), NaOMe (0.25 eq.), RMgBr (8 eq.), H<sup>+</sup> work up.

**Scheme 1.11:** Synthesis of keto-alcohols in one pot with Weinreb amide<sup>42</sup>

Where Grignard reagents are not commercially available or are prohibitively expensive, reducing the number of equivalents of Grignard reagents is essential. 2 eq. Grignard reagents, which were used to neutralise or deprotonate the amine, could be replaced by cheaper, commercially available organometallics such as *n*-BuLi. Thus the following procedure was attempted. 2.2 eq. *n*-BuLi was added to the solution of MeNHOMe·HCl salt **1.90** in THF at -78 °C. After stirring for 10 min,  $\gamma$ -valerolactone

**1.85** and 1 eq. of MeMgBr were added sequentially. The reaction was warmed to  $-20\text{ }^{\circ}\text{C}$  and allowed to react for 2 h, and then stirred at r.t. for 12 h. The reaction was monitored by  $^1\text{H}$  NMR spectroscopy. The signal arising from methoxy group in amide **1.91** in  $^1\text{H}$  NMR spectrum was a singlet at 3.70 ppm, and the signal for  $\text{CH}_3\text{C}=\text{O}$  in **1.92** was at 2.16 ppm. **1.92** was in equilibrium with hemiketal **1.93**. The isolated yield of keto-alcohol **1.92** and hemiketal **1.93** was 40 % after purification, and the isolated yield of amide **1.91** was 35 %. The yield of **1.92** and **1.93** was improved to 56 % by further heating the reaction at  $50\text{ }^{\circ}\text{C}$  for 5 h, together with amide **1.91** in 15 % yield (Scheme 1.12).



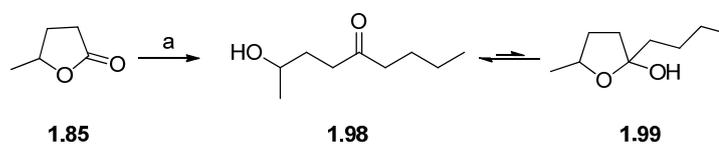
Reagents and conditions: a) *n*-BuLi (2.2 eq.),  $-78\text{ }^{\circ}\text{C}$ ; b) MeMgBr (1 eq.),  $-20\text{ }^{\circ}\text{C}$ , 2 h, then r.t. 12 h.

**Scheme 1.12:** Attempts to reduce the usage of Grignard reagent

To optimise the reaction yield, adding more *n*-BuLi was attempted, but the excess *n*-BuLi simply ring opened the γ-valerolactone. Alternatively, more Grignard reagent was added. The best result was achieved using 2.2 eq. of *n*-BuLi, together with 2.5 eq. of MeMgBr, which gave **1.92** and **1.93** in 73 % yield. Although the procedure gave a relatively good yield, the usage of expensive Weinreb salts and 2.5 eq. Grignard reagent suggested that development of alternative procedure was still required.



**1.98**, and the molar ratio of **1.98:1.99** was 20:1 (NMR spectroscopy).



Reagents and conditions: a) *n*-BuLi, THF/Et<sub>2</sub>O as solvents, -78 °C.

**Scheme 1.13:** Model reaction of *n*-BuLi reaction with γ-valerolactone

The yields of the model reactions are summarised in **Table 1.3**. It was found that using Et<sub>2</sub>O as solvent gave a better yield of keto-alcohol **1.98** and hemiketal **1.99** than using THF, which was consistent with the result reported by Umani-Ronchi.<sup>39</sup> It was found that using 1.1 eq. of the lactone, the yield was improved from 63 % to 68 % (entry 3 and 4), and less tertiary alcohol was produced. Varying the reaction time from 3 h to 5 h (Entry 3, 4, and 5) made no significant difference on the yield. The best yield of the keto-alcohol was obtained by treating *n*-BuLi with 1.1 eq. γ-valerolactone **1.85** in Et<sub>2</sub>O at -78 °C for 3 h.

**Table 1.3:** Experiments at -78 °C with various conditions

	Solvent	Molar ratio ( <b>1.85</b> : <i>n</i> -BuLi)	Time (h)	Yield of <b>1.98</b> and <b>1.99</b> (%)
1	THF	1 : 1	2	47
2	Et <sub>2</sub> O	1 : 1	2	53
3	Et <sub>2</sub> O	1 : 1	3	63
4	Et <sub>2</sub> O	1.1 : 1	3	68
5	Et <sub>2</sub> O	1.1 : 1	4	67
6	Et <sub>2</sub> O	1.1 : 1	5	67

Cooke and Rubottom proposed that quenching with TMSCl would consumed any remaining alkyllithium and alkoxyllithium immediately, leaving no alkyllithium to

react with the keto-alcohol **1.102** (Figure 1.9).<sup>38,43</sup> In order to further optimise the yield of the desired keto-alcohol product, the reaction was quenched with TMSCl. 1.1 eq. of  $\gamma$ -Valerolactone **1.85** was reacted with *n*-BuLi in Et<sub>2</sub>O at -78 °C for 3 h, and then the reaction was treated with 3 eq. of TMSCl at -78 °C and stirred for further 5 min. It was then warmed to 0 °C and quenched with 1 M aqueous HCl. The isolated yield of the keto-alcohol **1.98** and hemiketal **1.99** was improved to 71 %.

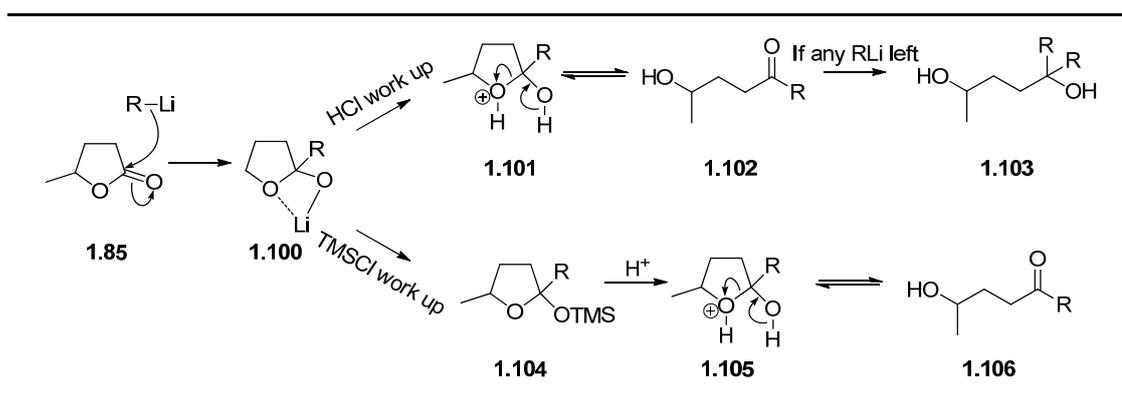


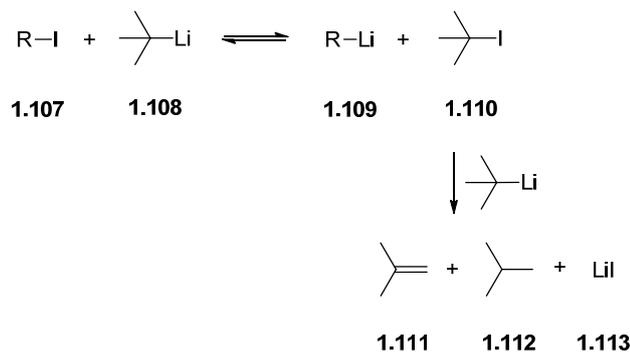
Figure 1.9: Workup conditions comparison

### 1.2.5. Preparation of alkyllithiums from halides

Alkyl halides are readily converted into alkyllithiums.<sup>44-49</sup> On an industrial scale, alkyllithiums are prepared by the reaction of alkyl halides with Li metal.<sup>50</sup> The use of alkyl iodides often suffers from significant side reactions such as homo-Wurtz-coupling.<sup>49</sup> This can be avoided by using less reactive halides, such as chlorides and bromides, but requiring high reaction temperatures and longer refluxing periods.<sup>49</sup> The unreliable yield of this reaction also makes it necessary to titrate the generated alkyllithiums each time.

Preparation of alkyllithium reagents by halogen-lithium exchange was initially proposed by Gilman.<sup>51</sup> Negishi and co-workers reported using 2 eq. of *t*-BuLi to prepare alkyllithiums from primary alkyl iodides in quantitative yield.<sup>45,52</sup> The first equivalent of *t*-BuLi form the equilibrium between RI **1.107** and *t*-BuI **1.110**, and a

second equivalent of *t*-BuLi **1.108** rapidly consumes the generated *t*-BuI **1.110** to give *i*-butylene **1.111**, *i*-butane **1.112**, and LiI **1.113** (Scheme 1.14).<sup>53</sup> The principal advantage of using *t*-BuLi is because the use of 2 eq. of *t*-BuLi pushes the exchange equilibrium between alkyl iodide and alkyllithium irreversibly in the direction of alkyllithium formation.



**Scheme 1.14:** Preparation of alkyllithiums **1.109** from *t*-BuLi and alkyl iodides<sup>53</sup>

The preparation of alkyllithiums from iodides with *t*-BuLi was carried out in our lab many times.<sup>50</sup> The optimal conditions involved adding 2.1-2.2 eq. of *t*-BuLi to a solution of alkyl halide in Et<sub>2</sub>O at -78 °C for 1 h, followed by warming to r.t. for 30 min, during which Et<sub>2</sub>O served as a scavenger to consume any excess *t*-BuLi, generating the corresponding alkyllithium in > 95 % yield.

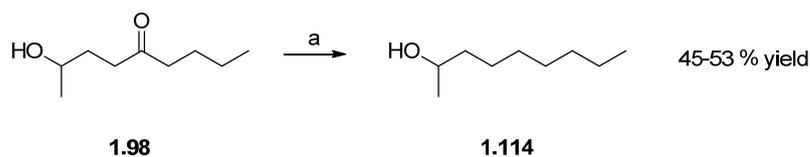
## 1.2.6. Deoxygenation of ketones

### 1.2.6.1. Wolff-Kishner reduction

The direct reduction of ketones to methylenes has been developed over several decades. Clemmensen reduction<sup>54</sup> and Wolff-Kishner reduction<sup>55</sup> are two of the most well known methods for this reduction. Instead of heating the ketone with hydrazine and NaOEt in a sealed vessel, the Huang-Minlon modified Wolff-Kishner reduction was carried out by heating the hydrazine with base (such as KOH) in high boiling point solvents like diethylene glycol or triethylene glycol, at about 180-200 °C.<sup>56-58</sup>

### 1.2.6.2. Initial attempts to reduce keto-alcohol via Wolff-Kishner reduction

Keto-alcohol **1.98**, produced from *n*-BuLi and  $\gamma$ -valerolactone **1.85**, was used to investigate reduction methods. Initially the reduction of keto-alcohol **1.98** via the Huang-Minlon modified Wolff-Kishner reduction was investigated (**Scheme 1.15**). After heating the ketone **1.98** with 3 eq. KOH and 2.5 eq.  $\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}$  in triethylene glycol at 190 °C for 5 h, the reaction mixture was cooled, diluted with water, and extracted with  $\text{Et}_2\text{O}$ . The alcohol **1.114** was isolated in 51 % yield. The harsh reaction conditions, the tedious work to extract the product from triethylene glycol, and the unsatisfying yields made alternatives desired.



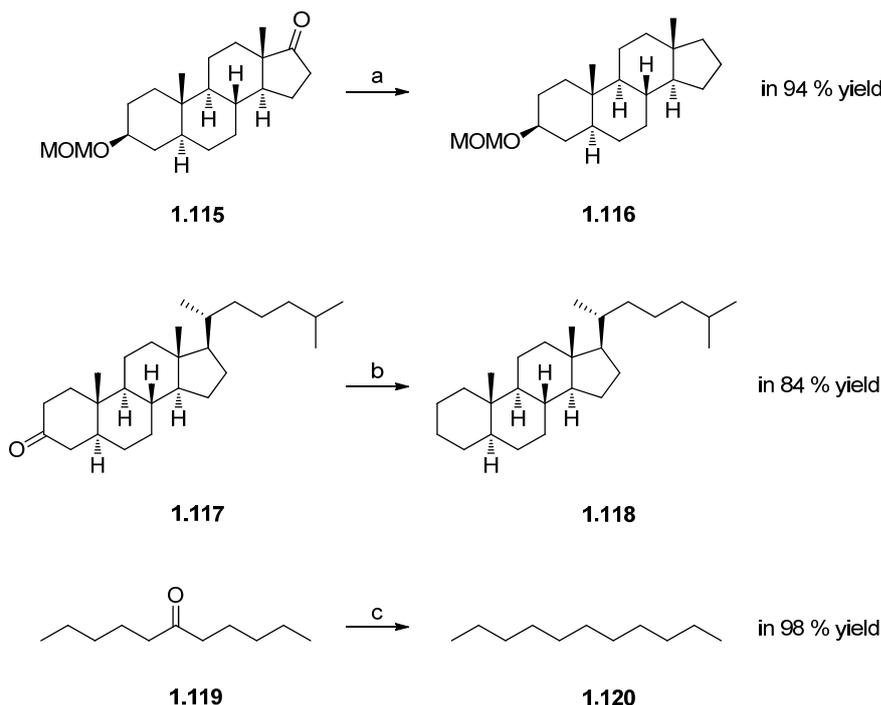
Reagents and conditions: a) KOH (3 eq.),  $\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}$  (2.5 eq.), triethylene glycol, 190 °C, 5 h.

**Scheme 1.15:** Model reaction of Wolff-Kishner reduction

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### 1.2.6.3. Clemmensen reduction

Clemmensen reduction is a general method by which aldehydes or ketones are converted to the corresponding hydrocarbons with amalgamated Zn and HCl.<sup>59-61</sup> The primary problem with the original method was the highly toxic amalgam. The modified Clemmensen reduction developed by Yamamura and co-workers used activated Zn, saturated HCl in  $\text{Et}_2\text{O}$  at 0 °C, and gave the reduction products in around 75 % yield.<sup>59-61</sup> This method was further modified by Arimoto and co-workers, who used 100 eq. each of  $\text{TMSCl}$  and Zn in a mixture solvent of  $\text{CH}_2\text{Cl}_2$  and MeOH or  $\text{CH}_2\text{Cl}_2$  and EtOH, giving excellent yields of the reduction products (**Scheme 1.16**).<sup>62</sup>

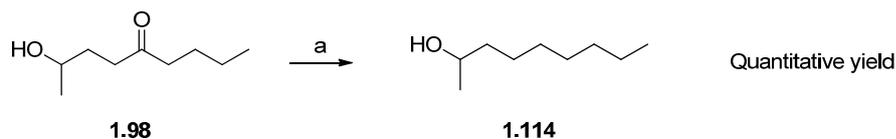


Reagents and conditions: a) TMSCl (100 eq.), Zn dust (100 eq.), MeOH/CH<sub>2</sub>Cl<sub>2</sub> (3:1), 0 °C, 1 h; b) TMSCl (100 eq.), Zn dust (100 eq.), EtOH/CH<sub>2</sub>Cl<sub>2</sub> (3:1), 0 °C, 5 h; c) TMSCl (100 eq.), Zn dust (100 eq.), MeOH/CH<sub>2</sub>Cl<sub>2</sub> (3:1), 0 °C, 30 min.

**Scheme 1.16:** Selected reductions using modified Clemmensen reduction<sup>62</sup>

#### 1.2.6.4. Successful reduction of keto-alcohol via Clemmensen reduction

The modified Clemmensen reduction developed by Arimoto was attractive for its mild reaction conditions and excellent yields.<sup>62</sup> However, the requirement for 100 eq. TMSCl and Zn as reported literature represents significant waste. It was found that the 100 eq. TMSCl and Zn was not necessary for less hindered linear ketones. With the use of only 10 eq. each of TMSCl and Zn in MeOH/Et<sub>2</sub>O (3:1) at 0 °C, the reduction of **1.98** gave alcohol **1.114** after 1 h in 97 % yield after purification by flash chromatography (Scheme 1.17).

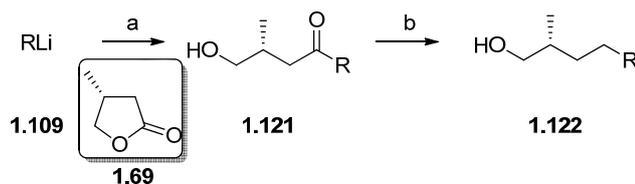


Reagents and conditions: a) TMSCl (10 eq.), Zn (10 eq.), MeOH/Et<sub>2</sub>O (3:1), 0 °C, 1 h.

**Scheme 1.17:** Model reaction of modified Clemmensen reduction

### 1.2.7. Proposed synthetic scheme for chiral methyl-branched moieties

After all the above model reactions, the chiral 3-methylbutyrolactone **1.69** was chosen as a key starting material for the synthesis of chiral isoprenoid moieties. A synthetic scheme for chiral methyl-branched moieties was proposed (**Scheme 1.18**). (*R*)-3-Methylbutyrolactone **1.69** could be opened by nucleophilic attack of an alkyllithium to give keto-alcohol **1.121**. Reduction of the ketone was then expected to give corresponding alcohol **1.122** as useful building moieties for a variety of natural products with chiral methyl branches. The enantiomer of **1.122** can be also prepared by employing the (*S*)-enantiomer as the starting material.



Reagents and conditions: a) **1.69** (1.1 eq.), Et<sub>2</sub>O, -78 °C; b) TMSCl (10 eq.), Zn (10 eq.), MeOH/Et<sub>2</sub>O (3:1), 0 °C.

**Scheme 1.18:** Proposed synthetic scheme for compounds with one isoprenoid moiety

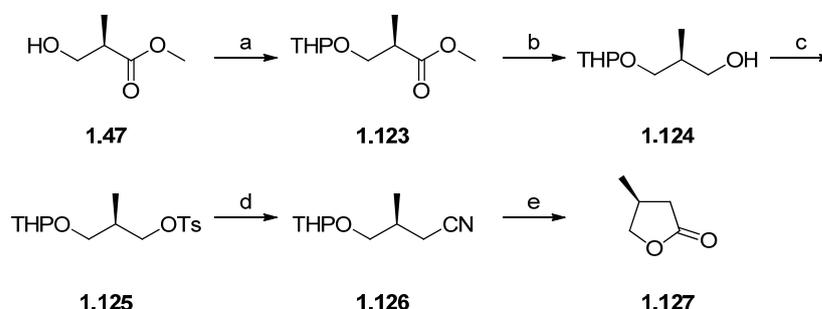
## 1.3. Preparation of enantiomerically enriched 3-methylbutyrolactones

### 1.3.1. Previous synthesis

Both enantiomers of chiral 3-methylbutyrolactones have been regarded as important building blocks for asymmetric synthesis.<sup>63</sup> Various synthetic methods have been developed for their preparation.

#### 1.3.1.1. From commercially available Roche esters

Mori reported synthesis of (*S*)-3-methylbutyrolactone **1.127** from commercially available (*R*)-Roche ester **1.47** (Scheme 1.19).<sup>64,65</sup> The hydroxyl group in **1.47** was protected to give ester **1.123**, followed by the reduction to the corresponding alcohol **1.124** up on treatment with LiAlH<sub>4</sub>. Esterification of **1.124** gave tosylate **1.125**. After converting tosylate **1.125** to cyanide **1.126**, alkaline hydrolysis, followed by ring closing under acidic conditions, gave the chiral lactone **1.127**. Although both (*R*)-Roche ester **1.47** and its enantiomer are commercially available, allowing access to both enantiomers of the product, it took five steps from **1.47** to lactone **1.127** in an overall yield of 19 %.

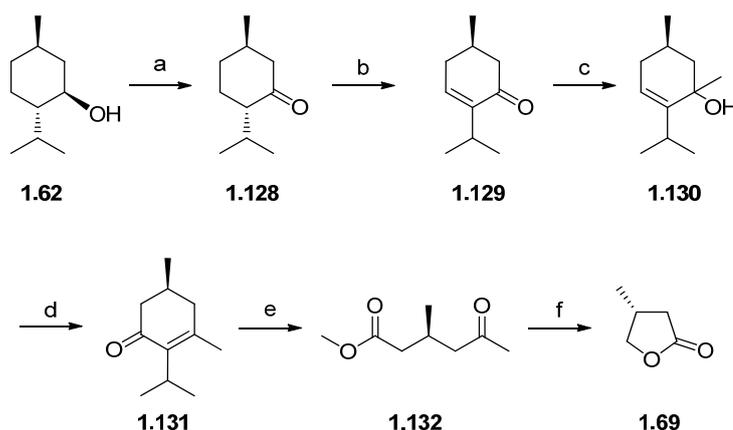


Reagents and conditions: a) Dihydropyran, TsOH, Et<sub>2</sub>O, r.t.; b) LiAlH<sub>4</sub>, Et<sub>2</sub>O, 0-5 °C, 4 h; c) TsCl, pyridine, 0-5 °C, overnight; d) NaCN, DMSO; e) KOH, ethylene glycol, reflux, 6 h, then H<sup>+</sup>.

**Scheme 1.19:** Synthesis of (*S*)-3-methylbutyrolactone **1.27** from Roche esters<sup>65</sup>

### 1.3.1.2. Synthesis from L-menthol

Tolstikov reported a synthesis of (*R*)-3-methylbutyrolactone **1.69** from L-menthol **1.62** over 10 steps in 21 % yield and 98 % ee (**Scheme 1.20**).<sup>66,67</sup> The whole synthetic approach, including oxidation and hydrolysis, was laborious.

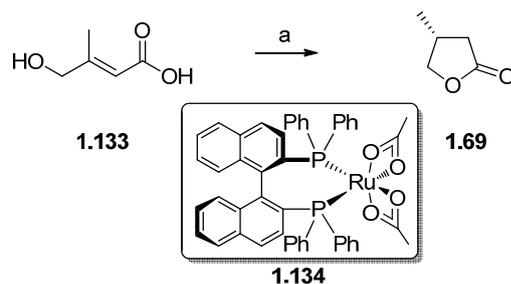


Reagents and conditions: a) H<sub>2</sub>O<sub>2</sub>; b) i - NBS; ii - Quinoline; c) MeLi, -78 °C; d) PCC, CH<sub>2</sub>Cl<sub>2</sub>; e) i - O<sub>3</sub>, MeOH and CH<sub>2</sub>Cl<sub>2</sub>; ii - TsOH, MeOH; f) i - *m*CPBA; ii - KOH, MeOH; iii - HCl.

**Scheme 1.20:** Synthesis of (*R*)-3-methylbutyrolactone from L-menthol<sup>67</sup>

### 1.3.1.3. Asymmetric hydrogenation with Ru(II)-BINAP complex

In 1987, Noyori reported that with Ru(II)-BINAP catalyst **1.134**, (*E*)-4-hydroxy-3-methylbut-2-enoic acid **1.133** could be asymmetrically reduced to lactone **1.69** with good enantiomeric purity. The reaction employed 0.69 mol% catalyst **1.134** in MeOH at r.t. under 86 atm H<sub>2</sub> for 16 h. The resultant lactone **1.69** was obtained in 100 % conversion and 93 % ee (**Scheme 1.21**).<sup>19</sup>

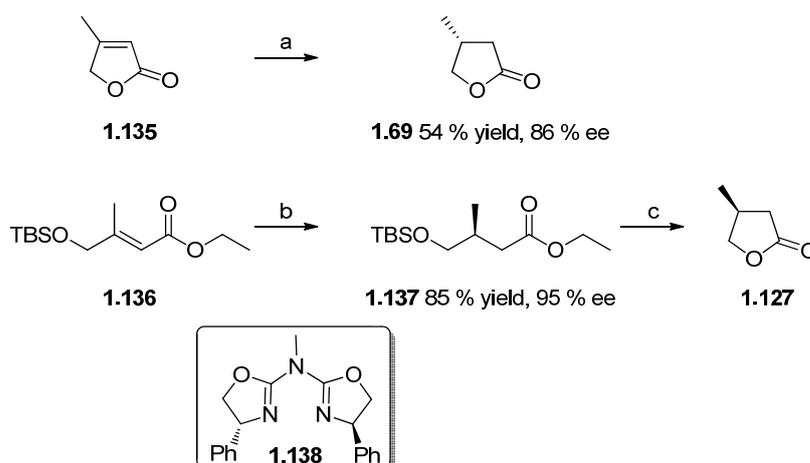


Reagents and conditions: a) **1.134** (0.69 mol%), 86 atm H<sub>2</sub>, MeOH, 15-30 °C, 16 h.

**Scheme 1.21:** Synthesis of chiral lactone with Ru(II)-BINAP catalyst<sup>19</sup>

### 1.3.1.4. Asymmetric conjugate reduction of $\alpha,\beta$ -unsaturated ester with Co(II)-azabis(oxazoline)

Reiser and co-workers reported an asymmetric conjugate reduction of  $\alpha,\beta$ -unsaturated esters using a Co(II)-azabis(oxazoline) as catalyst (**Scheme 1.22**).<sup>63</sup> With 3 mol% CoCl<sub>2</sub> and 2.5 mol% ligand **1.138**, the asymmetric reduction of unsaturated lactone **1.135** gave (*R*)-3-methylbutyrolactone **1.69** in 54 % yield and 86% ee. Better enantiomeric purity was achieved by asymmetric conjugate reduction of unsaturated ethyl ester **1.136**, and the chiral ethyl ester **1.137** was obtained in 85 % yield and 95 % ee, which could be easily cyclised to (*S*)-lactone **1.127** in quantitative yield.<sup>63</sup>



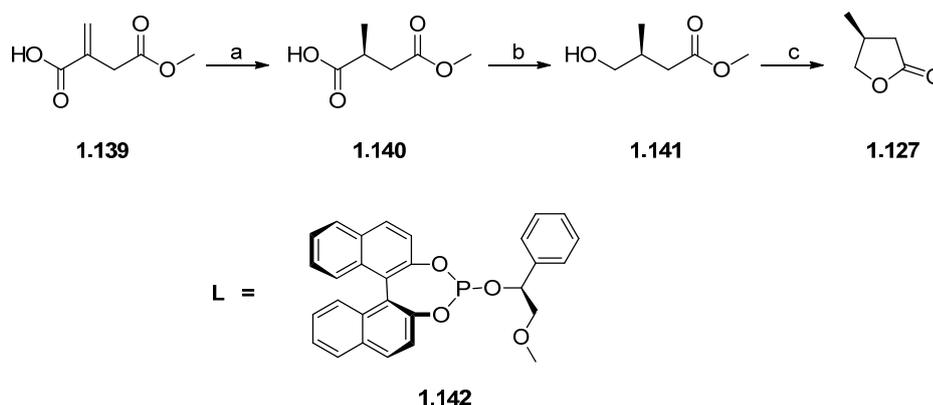
Reagents and conditions: a) CoCl<sub>2</sub> (3 mol%), **1.138** (2.5 mol%), NaBH<sub>4</sub>, EtOH, 4 h, r.t.; b) CoCl<sub>2</sub> (3 mol%), **1.138** (2.5 mol%), NaBH<sub>4</sub>, EtOH/diglyme (1:1), 24 h, r.t.; c) TsOH, MeOH, r.t., 2 h.

**Scheme 1.22:** Synthesis of chiral 3-methylbutyrolactone by conjugate reduction<sup>63</sup>

### 1.3.1.5. Asymmetric synthesis with Rh(I)-phosphite ligand **1.142** as catalyst

Helmchen reported the Rh(I)-phosphite ligand catalysed asymmetric hydrogenation of 4-methoxy-2-methylene-4-oxobutanoic acid **1.139**, and both **1.127** and its enantiomer could be prepared from the cheap starting material itaconic acid (Scheme 1.23).<sup>68</sup>

The asymmetric hydrogenation step used 0.1 mol%  $[\text{Rh}(\text{COD})_2(\text{ligand } \mathbf{1.142})_2]\text{BF}_4$  as catalyst under 1.1 atm  $\text{H}_2$  to give the chiral acid **1.140** in 92 % yield and > 98 % ee. After the reduction of the acid **1.140** to alcohol **1.141**, followed by acid-catalysed lactonisation, the chiral lactone **1.127** was obtained in 98 % yield and 99 % ee.<sup>68</sup>

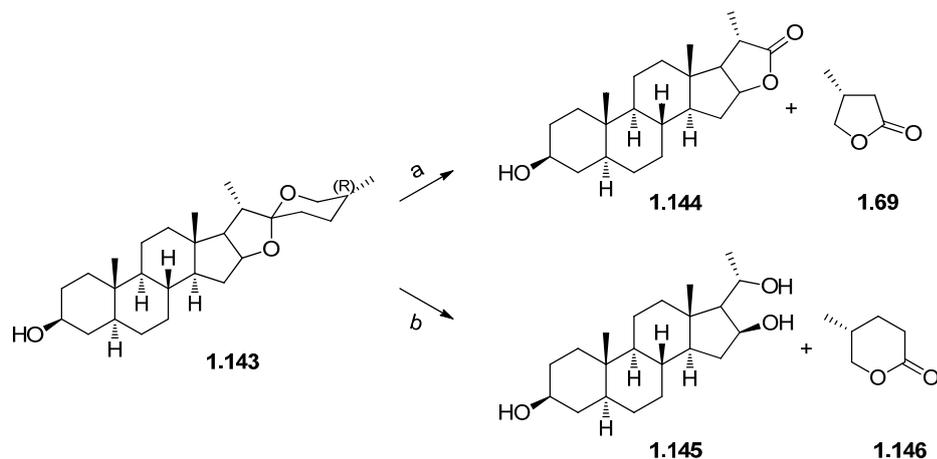


Reagents and conditions: a)  $\text{H}_2$  (1.1 atm),  $[\text{Rh}(\text{COD})_2(\text{ligand } \mathbf{1.142})_2]\text{BF}_4$  (0.1 mol %),  $(\text{CH}_2\text{Cl})_2$ , r.t., 24 h; b)  $\text{BH}_3 \cdot \text{SMe}_2$ , THF, 0 °C, 5h; c) cat. TsOH,  $\text{CH}_2\text{Cl}_2$ .

**Scheme 1.23:** Synthesis of lactone **1.127** with  $[\text{Rh}(\text{COD})_2(\text{ligand } \mathbf{1.142})_2]\text{BF}_4$ <sup>68</sup>

### 1.3.1.6. Baeyer-Villiger oxidation from tigogenin

Tian and co-workers reported preparing (*R*)-3-methylbutyrolactone **1.69** and (*R*)-4-methyl- $\delta$ -valerolactone **1.146** via Baeyer-Villiger oxidation from tigogenin **1.143** (Scheme 1.24).<sup>69</sup> Usually, lactone **1.144** and diol **1.145** are used for the synthesis of steroidal medicines,<sup>70</sup> while lactones **1.69** and **1.146** are regarded as by-products.



Reagents and conditions: a) H<sub>2</sub>O<sub>2</sub>, H<sub>2</sub>SO<sub>4</sub>, I<sub>2</sub>, AcOOH; b) H<sub>2</sub>O<sub>2</sub>, H<sub>2</sub>SO<sub>4</sub>, HCOOH.

**Scheme 1.24:** Degradation of tigogenin **1.143** for lactones<sup>69</sup>

The degradation of tigogenin **1.143** uses AcOH or HCOOH, and H<sub>2</sub>O<sub>2</sub> for the generation of peroxide *in situ*. When I<sub>2</sub> was used as catalyst,  $\gamma$ -lactone **1.69** was generated, and without I<sub>2</sub>,  $\delta$ -lactone **1.146** was obtained.

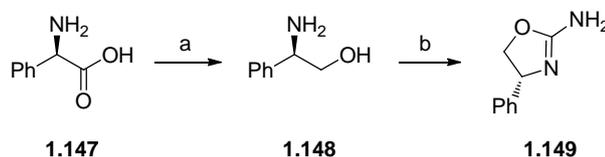
### 1.3.2. Evaluation of the reported methods for the synthesis of enantiomerically enriched 3-methylbutyrolactone

The asymmetric hydrogenation of unsaturated acids reported by Noyori with Ru(II)-BINAP dicarboxylate complex is a powerful procedure for the synthesis (*R*)-3-methylbutyrolactone.<sup>19</sup> Unfortunately, without access to a high pressure reaction vessel (86 atm), alternative methodologies were desired for exploring the most efficient asymmetric synthesis of chiral 3-methylbutyrolactone.

#### 1.3.2.1. Asymmetric hydrogenation by Reiser's method

Our attention was attracted by the asymmetric hydrogenation reported by Reiser and co-workers.<sup>63</sup> With Co(II)-azabis(oxazoline) ligand **1.138** as catalyst, the reaction was carried out at r.t. under ambient conditions, giving high enantiomeric selectivity for the chiral 3-methylbutyrolactone in moderate yield.

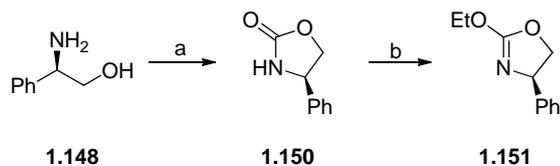
The preparation of the azabis(oxazoline) ligand **1.138** was carried out from phenylglycine **1.147** as reported (Scheme 1.25).<sup>71</sup> Thus phenylglycine **1.147** was reduced to 2-phenylglycinol **1.148** using 2.5 eq. NaBH<sub>4</sub> and 1.2 eq. H<sub>2</sub>SO<sub>4</sub> in THF at r.t. for 12 h.<sup>72</sup> Purification of **1.148** was carried out by recrystallisation from hexane/EtOAc giving **1.148** in 81 % yield and 93 % ee. The identity of the product **1.148** was confirmed by the appearance of peaks at 3.75 ppm in the <sup>1</sup>H NMR spectrum. Aminooxazoline **1.149** was prepared by reacting alcohol **1.148** with Br<sub>2</sub> and NaCN in MeOH at r.t. for 2 h in 88 % yield. The reaction was monitored by <sup>1</sup>H NMR spectroscopy and a new peak at 5.09 ppm corresponding to the amine CH in **1.149**.



Reagents and conditions: a) NaBH<sub>4</sub> (2.5 eq.), H<sub>2</sub>SO<sub>4</sub> (1.2 eq.), r.t., 12 h; b) Br<sub>2</sub> (1.1 eq.), NaCN (1.1 eq.), r.t., 1.5 h.

Scheme 1.25: Synthesis of azabis(oxazoline) ligand<sup>71</sup>

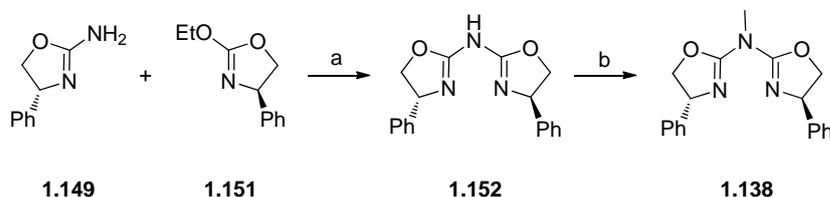
The oxazolidinone **1.150** was prepared from 2-phenylglycinol **1.148** and diethyl carbonate using NaOEt, which was generated *in situ* from Na metal and EtOH (Scheme 1.26).<sup>71</sup> The reaction was refluxed in EtOH for 12 h, and the remaining starting material was removed by washing with cold Et<sub>2</sub>O, giving **1.150** in 89 % yield and 93 % ee. The identity of **1.150** was confirmed by the appearance of peaks at 4.74 ppm in its <sup>1</sup>H NMR spectrum, which accounted for the methylene protons, as well as a multiplet at 4.95 ppm corresponding to the amine CH proton. **1.150** was then treated with triethyloxonium tetrafluoroborate to give ethoxyoxazoline **1.151** in 61 % yield after stirring in CH<sub>2</sub>Cl<sub>2</sub> at r.t. for 24 h, which was used directly in the next step.<sup>71</sup>



Reagents and conditions: a) Diethyl carbonate (1.1 eq.), EtOH, Na (1.1 eq.), reflux 12 h; b) Triethyloxonium tetrafluoroborate (1.3 eq.), CH<sub>2</sub>Cl<sub>2</sub>, r.t., 24 h.

**Scheme 1.26:** Synthesis of azabis(oxazoline) ligand<sup>71</sup>

Refluxing aminooxazoline **1.149** and ethoxyoxazoline **1.151** with cat. TsOH in toluene for 36 h gave azabis(oxazoline) **1.152**, which was first purified by flash chromatography followed by recrystallisation from acetone to give **1.152** as white crystals in 39 % yield.<sup>71</sup> Deprotonation with *n*-BuLi followed by treatment with MeI in THF gave methyl-substituted azabis(oxazoline) **1.138** as a yellow oil in 93 % yield and > 95 % ee (**Scheme 1.27**).<sup>63</sup>

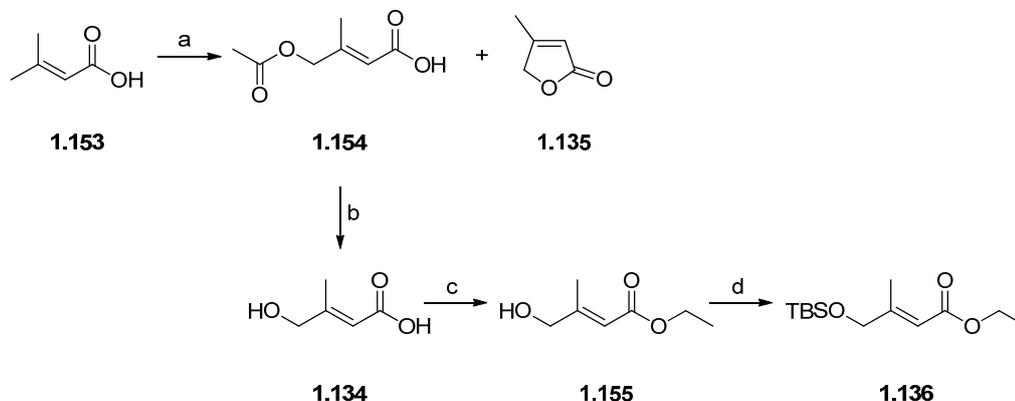


Reagents and conditions: a) TsOH (0.1 eq.), toluene, reflux, 36 h; b) MeI (5 eq.), *n*-BuLi (1.1 eq.), r.t., 20 h.

**Scheme 1.27:** Synthesis of azabis(oxazoline) ligand **1.138**<sup>63,71</sup>

TBS-protected  $\alpha,\beta$ -unsaturated ester **1.136** could be prepared as shown in **Scheme 1.28**.<sup>73</sup> 3-Methyl-2-butenic acid **1.153** was oxidized with SeO<sub>2</sub> to give the ester **1.154** as a colourless oil in 38 % yield, together with the lactone **1.135** in 16 % yield. **1.154** was hydrolysed in aqueous NaOH and then treated with 5 M HCl to give acid **1.134** as a pale yellow solid in 84 % yield. The acid **1.134** was esterified to give **1.155** as a volatile colourless liquid in 50 % yield after careful distillation. **1.155** was TBS-protected after treatment with TBSCl and imidazole in DMF at r.t. 16 h, giving **1.136**

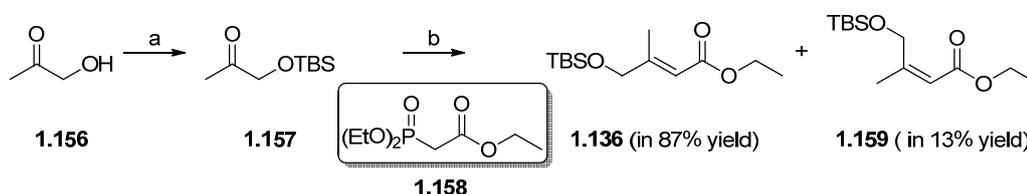
in 92 % yield. The whole procedure gave the TBS protected **1.136** in 15 % over four steps from commercially available acid **1.153**.



Reagents and conditions: a)  $\text{SeO}_2$  (0.5 eq.), AcOH, reflux 9 h; b) NaOH (2.5 M)/MeOH (vol/vol 2:1), reflux 4 h and then 5M HCl; c)  $\text{SOCl}_2$  (1.6 eq.), EtOH, r.t., 14 h; d) TBSCl (1.1 eq.), imidazole (2.5 eq.), DMF, r.t., 16 h.

**Scheme 1.28:** Synthesis of TBS protected ester **1.136**

A more efficient synthesis of TBS protected **1.136** was attempted (**Scheme 1.29**). TBS-protected ketone **1.157** was prepared from acetol **1.156** with TBSCl and imidazole in DMF in 98 % yield after stirring at r.t. for 20 h. Stereoselective synthesis of *E*-isomer **1.136** was achieved via Horner-Wadsworth-Emmons reaction with ethyl phosphoroacetate **1.158** and NaH, giving **1.136** and **1.159** in a ratio of 87:13.<sup>73</sup>

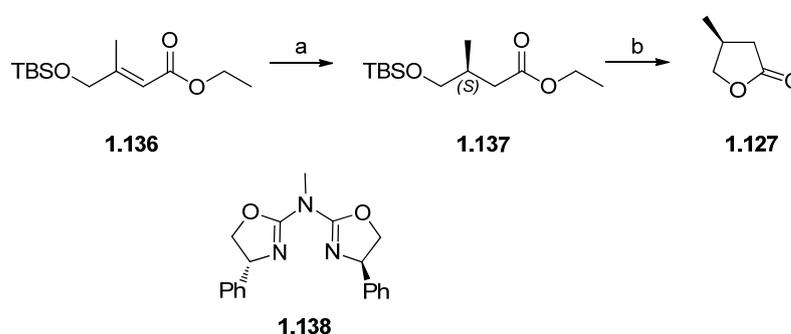


Reagents and conditions: a) TBSCl (1.1 eq.), imidazole (2 eq.), DMF, r.t., 20 h; b) NaH (1.3 eq.), THF, r.t., 1 h.

**Scheme 1.29:** Stereoselective synthesis of **1.136**<sup>73</sup>

### 1.3.2.2. Asymmetric conjugate reduction of unsaturated ester with Co(II)-azabis(oxazoline) catalysts

The reduction of **1.136** was carried out with 2.5 mol % CoCl<sub>2</sub>-ligand **1.138** and 2.5 eq. NaBH<sub>4</sub> in EtOH/diglyme (1:1) at r.t. for 24 h, giving **1.137** in 85 % yield as a colourless oil. **1.137** was easily cyclised by stirring with cat. TsOH to give lactone **1.127**. The whole procedure took four steps, giving lactone **1.127** in 69 % yield and 71 % ee (**Scheme 1.30**).



Reagents and conditions: a) CoCl<sub>2</sub> (3 mol%), **1.138** (2.5 mol%), NaBH<sub>4</sub> (2.5 eq.), EtOH/diglyme (1:1), 24 h, r.t.; b) TsOH (0.1 eq.), MeOH, r.t., 2 h.

**Scheme 1.30:** Synthesis of (*R*)-3-methylbutyrolactone by conjugate reduction

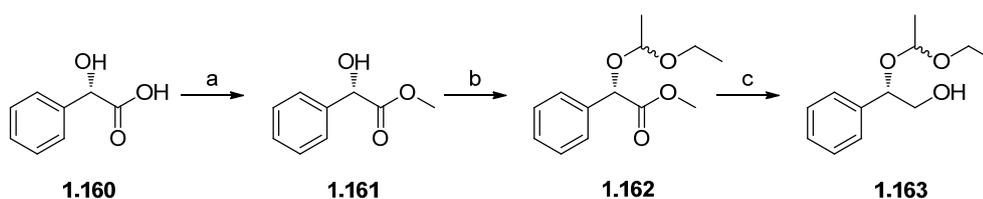
Disappointingly, the asymmetric conjugate reduction of ester **1.136** failed to give the chiral lactone **1.127** in expected 86 % ee as reported by Reiser, which prompted alternative attempt for synthesis of chiral  $\gamma$ -lactone.<sup>63</sup>

### 1.3.2.3. Asymmetric synthesis by Helmchen's method

Preparation of chiral 3-methylbutyrolactone via [Rh(COD)<sub>2</sub>(ligand**1.142**)<sub>2</sub>]BF<sub>4</sub> catalysed hydrogenation of 4-methoxy-2-methylene-4-oxobutanoic acid, in turn prepared from readily available itaconic acid, was reported by Helmchen.<sup>68</sup> The experiments were reproduced in our lab according to the reported procedure.<sup>68</sup>

Alcohol **1.163** was synthesised from mandelic acid **1.160** (**Scheme 1.31**).<sup>74</sup> Firstly,

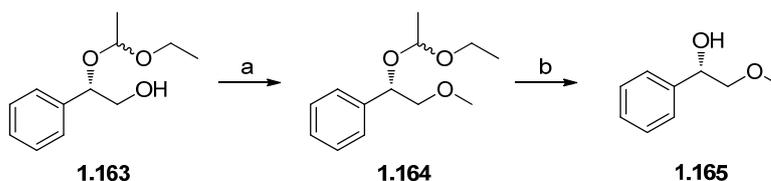
mandelic acid **1.160** was esterified in MeOH with cat. TsOH. Then the alcohol **1.161** was protected as 1-ethoxy-1-ethyl ether by reacting with ethyl vinyl ether in CH<sub>2</sub>Cl<sub>2</sub> with the presence of cat. pyridinium *p*-toluenesulfonate. After stirring at r.t. for 40 min, **1.162** was obtained in quantitative yield as a 1:1 mixture of diastereomers, followed by reduction with LiAlH<sub>4</sub> in THF at 0 °C for 30 min to give the alcohol **1.163** as colourless oil in 99 % yield.



Reagents and conditions: a) TsOH (0.1 eq.), MeOH, reflux, 3 h; b) Ethyl vinyl ether (2.5 eq.), pyridinium *p*-toluenesulfonate (0.1 eq.), CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 40 min; c) LiAlH<sub>4</sub> (1 eq.), THF, 0 °C, 30 min.

**Scheme 1.31:** Synthesis of alcohol **1.163** from mandelic acid<sup>74</sup>

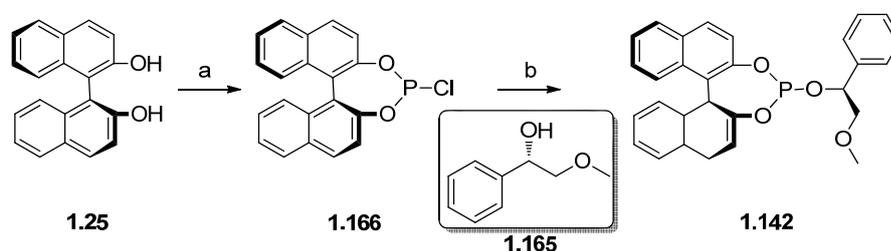
The methylation of alcohol **1.163** with MeI and NaH in THF at r.t. for 24 h gave **1.164** in quantitative yield (**Scheme 1.32**). Then the protecting group was removed by stirring **1.164** in MeOH with cat. TsOH at r.t. for 24 h to give alcohol **1.165** as a colourless liquid in quantitative yield. The identity of **1.165** was confirmed by the appearance of peaks at 4.82-4.97 ppm region in <sup>1</sup>H NMR spectrum, which accounted for the CH proton, the peaks at 3.40-3.58 ppm region accounted for the methylene protons, and the peak at 3.44 ppm corresponding to the protons in methyl group. The enantiomeric purity of **1.165** was confirmed by measuring its specific rotation, and was found to be of 98 % ee.<sup>74</sup>



Reagents and conditions: a) MeI (2 eq.), NaH (1.5 eq.), THF, r.t., 24 h; b) TsOH (0.1 eq.), MeOH.

**Scheme 1.32:** Synthesis of (*S*)-2-methoxy-1-phenylethanol **1.165**<sup>74</sup>

Phosphite ligand **1.142** was prepared from alcohol **1.165** and (*S*)-1,1-binaphthol **1.25** (Scheme 1.33).<sup>68</sup> (*S*)-1,1-Binaphthol **1.25** was refluxed in neat  $\text{PCl}_3$  for 5 h. Excess  $\text{PCl}_3$  was removed *in vacuo* to give the phosphite **1.166** as a white solid, followed by treatment with **1.165** and  $\text{Et}_3\text{N}$  to give **1.142** as a white solid in 87 % yield. The identity of the phosphite ligand **1.142** was confirmed by the appearance of peaks at 5.46 ppm in  $^1\text{H}$  NMR spectrum, which accounted for the phosphite *CH* proton, and a shift at 151.52 ppm in  $^{31}\text{P}$  NMR.



Reagents and conditions: a)  $\text{PCl}_3$  (10 eq.), reflux, 5 h; b)  $\text{Et}_3\text{N}$  (1 eq.), toluene, r.t., 14 h.

Scheme 1.33: Synthesis of ligand **1.142**<sup>68</sup>

Itaconid acid **1.167** can be selectively esterified at the non-conjugated carboxylic acid. Thus treatment of itaconic acid with Amberlyst 15, gave monomethylated **1.139** in 94 % yield (Scheme 1.34). For asymmetric hydrogenation, a balloon charged with  $\text{H}_2$  and 0.1 mol % catalyst was used to give chiral ester **1.140** in 92 % yield and 98 % ee. The disappearance of the peaks at 5.80-6.50 ppm, which accounted for the terminal alkene  $\text{CH}_2$  protons, meant the consumption of **1.139**. The identity of the **1.140** was confirmed by the appearance of a peak at 2.96 ppm in the  $^1\text{H}$  NMR spectrum, which accounted for the *CH* proton, and a shift at 2.74 ppm of the peaks corresponding to the methylene protons.



#### 1.3.2.4. Baeyer-Villiger oxidation from tigogenin

*Agave sisalana*, commonly known as sisal (**Figure 1.10**), is an agave that produces a stiff fibre, used for making rope, twine, paper, cloth, wall coverings, carpets, and so on.<sup>75</sup> Global sisal fibre production was up to hundreds of thousand tons in 2008.<sup>76</sup> However, only less than 5 % of the sisal leaves is used to make fibre, leaving the rest of the plant as waste, which causes serious environmental problems.<sup>76</sup>

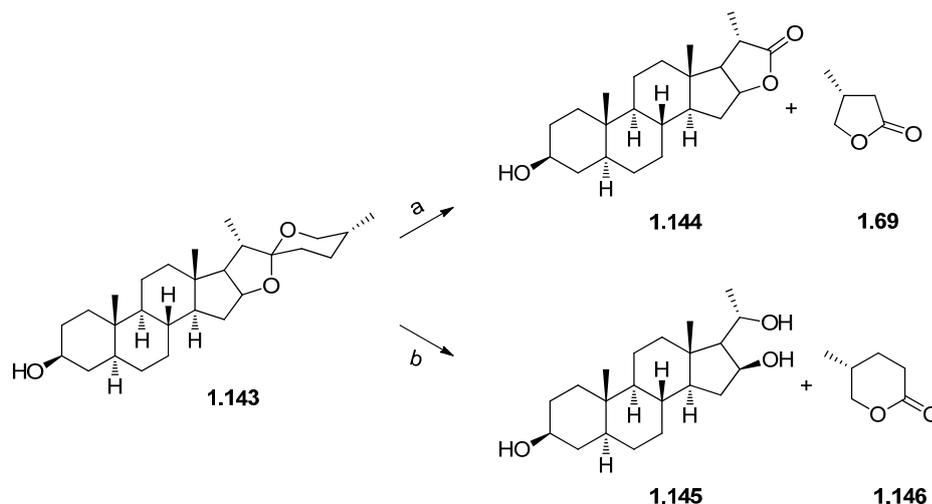


**Figure 1.10:** The sisal plant

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Considerable research has been conducted towards the utilisation of sisal waste. Tigogenin is a steroid contained in sisal leaves and flowering stalks.<sup>77</sup> The extraction of tigogenin from sisal has been well studied,<sup>78</sup> and in China, tigogenin has been produced from sisal industry waste,<sup>69,79,80</sup> making it an economical material for use in the synthesis of steroidal derived compounds.

(*R*)-3-Methylbutyrolactone and (*R*)-4-methyl- $\delta$ -valerolactone are degradation products of tigogenin (**Scheme 1.36**).<sup>69</sup> Usually, the steroidal lactone **1.144** and diol **1.145** are used for total synthesis of steroidal medicines, while lactones **1.69** and **1.146** are regarded as by-products, and isolated from sisal industrial waste.<sup>70</sup>



Reagents and conditions: a) H<sub>2</sub>O<sub>2</sub> (11 eq.), H<sub>2</sub>SO<sub>4</sub> (0.9 eq.), I<sub>2</sub> (0.1 eq.), AcOH, r.t., 12 h; b) H<sub>2</sub>O<sub>2</sub> (8 eq.), HCOOH, 50 °C, 6 h.

**Scheme 1.36:** Degradation of tigogenin **1.143** for lactones **1.69** and **1.146**<sup>69</sup>

When I<sub>2</sub> is used as catalyst, (*R*)-3-methylbutyrolactone **1.69**, is the degradation product. However, without I<sub>2</sub>, the degradation reaction gives (*R*)-4-methyl-δ-valerolactone **1.146**.

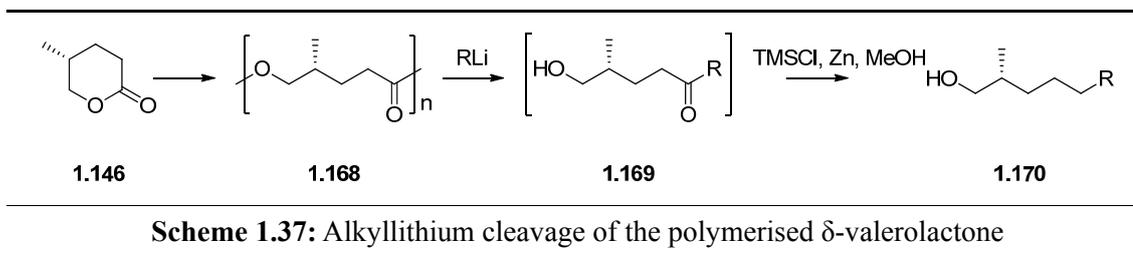
To prepare (*R*)-3-methylbutyrolactone **1.69** AcOOH served as peroxide, and was prepared *in situ* from AcOH and H<sub>2</sub>O<sub>2</sub> (w/w 30 %) in the presence of H<sub>2</sub>SO<sub>4</sub> as catalyst. To a mixture of tigogenin **1.143** in AcOH were added H<sub>2</sub>SO<sub>4</sub> and I<sub>2</sub>, followed by the prepared AcOOH. The mixture was stirred in dark for 12 h. After quenching the reaction with NaHSO<sub>3</sub>, solvents were removed *in vacuo*, and the mixture of the crude product was basified, converting the lactone **1.69** to the sodium salt, which was soluble in water. The undesired product **1.144** was removed by extracting out with organic solvents. After that, the basic aqueous layer was acidified with HCl to pH 2, and the lactone **1.69** was extracted with organic solvents. The resulting red oil was purified by flash chromatography to give **1.69** as a colourless liquid in 37 % yield and > 97 % ee.

To prepare (*R*)-4-methyl-δ-valerolactone **1.146**, H<sub>2</sub>O<sub>2</sub> (w/w 30 %) was added to the mixture of tigogenin **1.143** in HCOOH. The mixture was stirred at 50 °C for 6 h. After

quenching the reaction with  $\text{NaHSO}_3$ , the solvents were removed *in vacuo*, and the mixture of the crude product was basified by refluxing in aqueous  $\text{NaOH}$  solution, and the diol **1.145** was removed by extracting out with organic solvents. After acidifying with  $\text{HCl}$ , the lactone **1.146** was extracted with organic solvents. The resulting yellow oil was purified by flash chromatography to give **1.146** as a colourless liquid in 33 % yield and 99 % ee.

Thus both lactones **1.69** and **1.146** were synthesised with high enantiomeric purity making them excellent starting materials for our chiral isoprenoid synthesis.

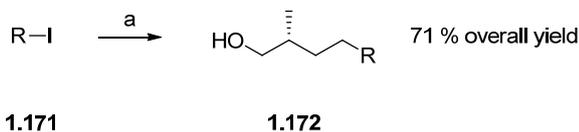
It is notable that (*R*)-3-methylbutyrolactone **1.69** is stable at r.t. for at least 3 months, while (*R*)-4-methyl- $\delta$ -valerolactone **1.146** polymerises after one month at r.t.. The stability of  $\gamma$ -butyrolactone and the propensity of  $\delta$ -valerolactone to polymerise are consistent with a quantum mechanics study carried out by Houk.<sup>81</sup> Fortunately, reaction of alkyllithiums with the polymerised lactone gave relatively clean products. This might be due to the fact that the polymerisation product, ester **1.168**, could also react with alkyllithiums to give the keto-alcohol **1.169** (Scheme 1.37).



## 1.4. The “one pot” procedure

In an attempt to improve reaction yields and shorten the synthetic approach further, we explored a “one pot” procedure. It was noticed that after the lactone ring cleavage reaction, the generated keto-alcohol existed in equilibrium with the corresponding hemiketal. The hemiketal could be reduced with the same reduction conditions as ketone's. In addition, the TMSCl used in the improved Clemmensen reduction could also serve as quenching reagent for the lactone ring cleavage reaction. Thus, in principle, the reduction step could be carried out without the need for isolation and purification of the keto-alcohol. Therefore, the sequence of ring cleavage followed by modified Clemmensen reduction in “one pot” was next investigated.

The general procedure of the “one pot” approach could start from iodides or alkyllithiums. For those alkyllithiums that are not commercially available, iodide **1.171** was converted to alkyllithium by reacting with *t*-BuLi at -78 °C for 1 h in Et<sub>2</sub>O. The generated alkyllithium was added to 1.1 eq. of lactone **1.69** in Et<sub>2</sub>O and reacted at -78 °C for 3 h, followed by quenching with 10 eq. TMSCl. After stirring for 5 min at -78 °C, the reaction mixture was gradually warmed to 0 °C. Zn dust and MeOH were added and the reaction was stirred at 0 °C for 1 h. After work up with 1 M HCl and purification by flash chromatography, alcohol **1.172** was obtained in around 77 % yield (Scheme 1.38).



Reagents and conditions: a) i - *t*-BuLi (2.2 eq.), Et<sub>2</sub>O, -78 °C, 1 h; ii - lactone **1.69** (1.1 eq.), Et<sub>2</sub>O, -78 °C, 3 h; iii - TMSCl (10 eq.), Zn (10 eq.), MeOH/Et<sub>2</sub>O (3:1), 0 °C, 1 h.

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**Scheme 1.38:** The “one pot” procedure for prepare chiral 2-methyl alkanol **1.172**

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The optimised yield for lactone ring cleavage step and the reduction step for the model reactions was 71 % (including keto-alcohol and hemiketal) and 96 % respectively, giving an overall yield over two steps 68 %, while the “one pot” procedure gave the chiral alcohol in 77 % yield. The combined “one pot” procedure not only saved a purification step in between, but also improved the yield, making the methodology of preparing chiral 2-methyl alcohols even more effective.

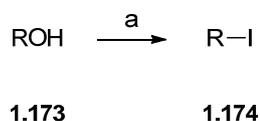
## 1.5. Extension of the methodology to the synthesis of oligo-isoprenoids

Several natural products contain repeating isoprenoid segments, such as vitamins E and K. Our procedure, in principle, could be applied to their preparation. In order to achieve this, the primary alcohol from the first iteration of our methodology needs to be converted effectively into a new alkyllithium ready for the next cycle. In this section current methods are reviewed and an improved procedure is described.

### 1.5.1. Preparation of alkyllithiums from alcohols via halides

The alcohols generated from first ring cleavage need to be converted to alkyllithiums for a second cycle ring cleavage. Because hydroxyl group is a poor leaving group, it is necessary to convert it to other functional groups before alkyllithiums could be prepared, and halides are good intermediates here. It is feasible to convert alcohols to alkyllithiums via halides.

Many methods exist for the conversion of alcohols to halides. Alcohols **1.173** could be halogenated to iodides **1.174** through Appel reactions (Scheme 1.39).<sup>82,83</sup>



Reagents and conditions: a) PPh<sub>3</sub> (1.1 eq.), I<sub>2</sub> (1.1 eq.), CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 1 h.

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**Scheme 1.39:** Appel reaction<sup>85,86</sup>

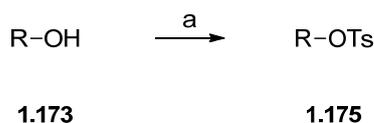
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## 1.5.2. Preparation of alkyllithiums from alcohols via tosylates and halides

### 1.5.2.1. Preparation of tosylates from alcohols

Alcohols could also be converted to halides via their tosylates. As good leaving groups, tosylates are easily displaced by halides through substitution reactions, making tosylates good intermediates for this reaction.

Tosylates **1.175** were generated in good yields from alcohols **1.173** and TsCl in the presence of pyridine (3 eq.) and DMAP (0.1 eq.) in CH<sub>2</sub>Cl<sub>2</sub> or CHCl<sub>3</sub> for 12-48 h at r.t. (Scheme 1.40).<sup>84</sup>

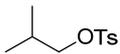
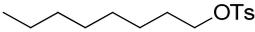
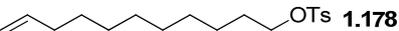


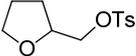
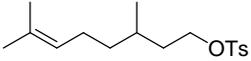
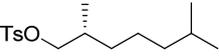
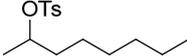
Reagents and conditions: a) TsCl (1.5 eq.), DMAP (0.1 eq.), pyridine (3 eq.), CH<sub>2</sub>Cl<sub>2</sub>, r.t., 24 h.

**Scheme 1.40:** Conversion of alcohols to tosylates

Conversion both primary and secondary alcohols to tosylates **1.176-1.182** were performed according to the above general procedure in > 90 % yields (Table 1.4).

**Table 1.4:** Conversion of alcohols to tosylates

Products	Reaction time (h)	Yield (%)
 <b>1.176</b>	24	98
 <b>1.177</b>	12	95
 <b>1.178</b>	12	95

 <b>1.179</b>	24	93
 <b>1.180</b>	24	93
 <b>1.181</b>	24	95
 <b>1.182</b>	48	90

### 1.5.2.2. An improved method for the preparation of iodides from tosylates

Tosylates can be converted to iodides **1.174** employing a modified Finkelstein reaction, by refluxing tosylates **1.175** and NaI in acetone for several hours (Scheme 1.41).<sup>85</sup>

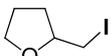
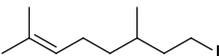
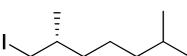
To reduce the reported reaction times, we explored the use of microwave irradiation. Gratifyingly, we found that primary tosylates were quantitatively converted to iodides after irradiation for 1 to 2 h under solvent-free conditions. The typical procedure involved heating the tosylate with 3 eq. NaI at 100-150 W in a sealed 10 mL microwave vial using a CEM Discover<sup>TM</sup> microwave reactor irradiating for 0.5-2 h, during which time the temperature was maintained at 100-140 °C. Several alcohols were used as examples, and reactions were carried out to optimise the conversion yields for each specific alcohol, and the results were summarised in Table 1.5.

It was found all the primary tosylates gave iodides (**1.183-1.188**) in > 94 % yields, while for conversion of secondary tosylate to corresponding iodides (**1.189**), competing elimination reduced the yield to 75 %.



**Scheme 1.41:** Conversion of tosylates to iodides

**Table 1.5:** Conversion of tosylates to iodides under solvent-free microwave irradiation (with 3 eq. NaI)

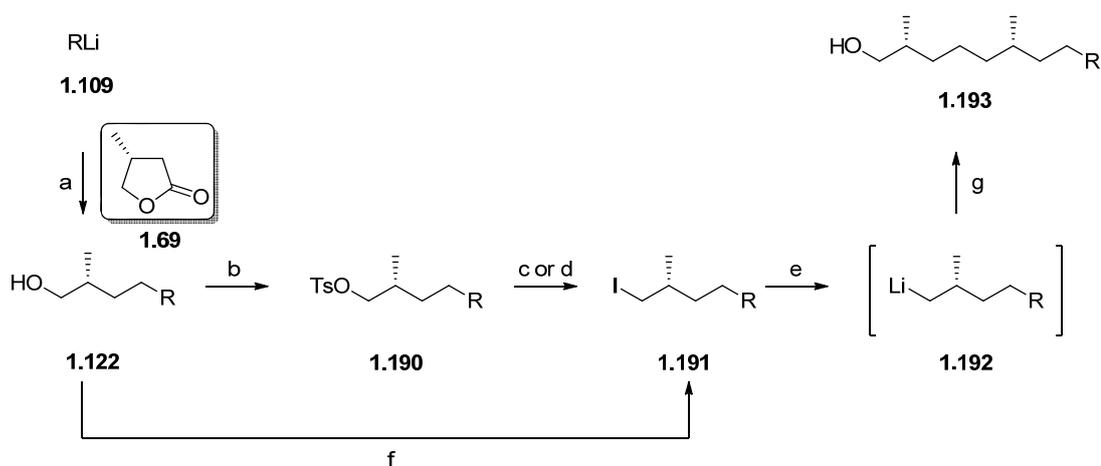
Products	Temperature (°C)	Power (W)	Time (min)	Yield (%)	Scale (mmol)
 <b>1.183</b>	120	100	30	>99	0.4
 <b>1.184</b>	120	120	45	95	0.3
 <b>1.185</b>	140	150	30	95	0.2
 <b>1.186</b>	115	100	90	94	0.2
 <b>1.187</b>	120	120	45	98	0.3
 <b>1.188</b>	120	110	45	94	0.2
 <b>1.189</b>	115	100	60	75	0.3

Thus the conversion of primary tosylates to iodides under solvent-free, microwave irradiation condition gives neat iodides in high yield with relatively short reaction time. This procedure is especially useful for the *in situ* generation of volatile iodides.

### 1.5.3. Proposed synthetic scheme for the synthesis of oligo-isoprenoids

In summary, a new methodology for generating highly enantiomerically enriched isoprenoid moieties has been developed (**Scheme 1.42**). Firstly, lactone **1.69** is ring opened with alkylolithiums, followed by modified Clemmensen reduction *in situ*. This

“one pot” procedure gives alcohols **1.122**, which can be used for the synthesis of several natural products. For natural products with repeating saturated isoprenoid moieties, the generated alcohols **1.122** from the first ring cleavage can be halogenated to iodides **1.191**, which, after lithiation with *t*-BuLi *in situ*, are then inserted into a new iteration of our process described above.



Reagents and conditions: a) i - **1.69** (1.1 eq.), Et<sub>2</sub>O, -78 °C, 3 h; ii - TMSCl (10 eq.), Zn (10 eq.), MeOH/Et<sub>2</sub>O (3:1), 0 °C, 1 h; b) TsCl (1.5 eq.), DMAP (0.1 eq.), pyridine (3 eq.), r.t., 24 h; c) NaI (3 eq.), 120 °C, 120 W, 45 min; d) NaI (3 eq.), acetone, reflux, 12 h; e) PPh<sub>3</sub> (1.1 eq.), imidazole (1.1 eq.), I<sub>2</sub> (1.1 eq.), CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 2 h; f) *t*-BuLi (2.2 eq.), Et<sub>2</sub>O, -78 °C, 1 h; g) i - **1.69** (1.1 eq.), Et<sub>2</sub>O, -78 °C, 3 h; ii - TMSCl (10 eq.), Zn (10 eq.), MeOH/Et<sub>2</sub>O (3:1), 0 °C, 1 h.

**Scheme 1.42:** Proposed synthetic scheme for repeating isoprenoid units

Many isoprenoid targets could be prepared similarly via our new methodology. Generally speaking, each ring cleavage takes two stages. The first stage is the “one pot” procedure giving alcohols with one chiral methyl centre. The second stage converts the alcohols to iodides, which after lithiation can be used in the next cycle.

An optimised preparation of the key starting material, the (*R*)-3-methylbutyrolactone was also investigated. Several reported synthetic methods were evaluated. It is found the oxidation from tigogenin is the most straightforward method, from which either (*R*)-3-methylbutyrolactone **1.69** or (*R*)-4-methyl- $\delta$ -valerolactone **1.146** can be

obtained as in one step. On the other hand, asymmetric reduction with Rh(I)-phosphite ligand **1.142** as catalyst is an effective process for the preparation of the both (*R*)-3-methylbutyrolactone **1.69** and its enantiomer **1.127** in large scale, from relatively cheap starting materials and with easy work up procedures.

## 1.6. Experimental

### 1.6.1. General procedures

Melting points were measured on Reichert hot stage melting point apparatus (Reichert, Austria).

Optical Rotations were measured on a PolAAR 2001 automatic polarimeter (Optical Activity Ltd., Huntingdon, Cambridgeshire, UK) with 1 dm cell in CHCl<sub>3</sub>, at a wavelength of 589 nm (sodium D line) at r.t., and were assigned as  $[\alpha]_D$ , temperature, concentration  $c$  (g/100 mL) and solvent.

<sup>1</sup>H NMR spectra were determined at 300 MHz with a Bruker Avance DPX300 spectrometer or at 400 MHz with a Bruker Avance DRX400 spectrometer (Bruker BioSpin Corp., Billerica, Massachusetts, USA). The chemical shifts ( $\delta$ , ppm) were referenced to the residual CHCl<sub>3</sub> signal ( $\delta$  7.26 ppm). Each resonance was quoted with chemical shifts measured in parts per million (ppm), multiplicities, coupling constants ( $J$  Hz), number of protons, and structural assignments. Multiplicities were denoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or b (broad).

<sup>13</sup>C NMR spectra were recorded on 75 MHz with a Bruker Avance DPX300 spectrometer or at 100 MHz with a Bruker Avance DRX400 spectrometer (Bruker BioSpin Corp., Billerica, Massachusetts, USA) in CDCl<sub>3</sub>, referenced with the residual CHCl<sub>3</sub> signal ( $\delta$  77.16 ppm). Each resonance was quoted with chemical shifts measured in parts per million (ppm) and structural assignment.

Low resolution electrospray ionisation mass spectrometry was measured on a Micromass Platform spectrometer (Micromass Ltd., Wythenshawe, Manchester, UK). Accurate mass determinations were made on an Agilent G1969A LC-TOF system (Agilent Technologies, Santa Clara, California, USA) with reference and mass

correction at 4000 V capillary voltage for electrospray ionisation.

Infra red spectra were obtained using a Bruker Equinox IFS55 FTIR, fitted with a mercury-cadmium-telluride detector and a Specac Golden Gate single reflection diamond ATR element. The operation condition was 50 scans at a resolution of  $4\text{ cm}^{-1}$ . The intensity of absorption bands for all samples have been specified as either s (strong), m (medium), w (weak) or b (broad).

Kugelrohr distillations were carried on a Büchi GKR-50 apparatus *in vacuo*.

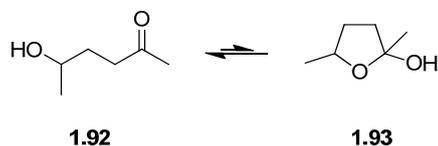
Analytical TLC was performed on Merck Silica Gel 60 F<sub>254</sub>, and visualised under a 254 nm UV lamp and/or stained using an alkaline KMnO<sub>4</sub> solution. Flash chromatography was performed using Silica Gel 60 (230-400 mesh, no. 9385) from Merck or SiliaFlash<sup>®</sup> P60 (230-400 mesh) from Silicycle.

### 1.6.2. Materials

Commercially available reagents and chemicals were purchased from Sigma-Aldrich Company. (Milwaukee, Wisconsin, USA), AK Scientific Inc. (Union City, California, USA), Merck (Darmstadt, Hesse, Germany), Ajax Finechem Pty. Ltd. (Sydney, New South Wales, Australia), or Lancaster Synthesis Ltd. (Morecambe, Lancashire, UK), and are reagent grade unless specified. Anhydrous solvents were freshly distilled under N<sub>2</sub> prior to use as the following procedure: Toluene and Et<sub>3</sub>N were distilled from CaH; CH<sub>2</sub>Cl<sub>2</sub> was distilled from P<sub>2</sub>O<sub>5</sub>; THF and Et<sub>2</sub>O were distilled from Na using benzophenone as indicator; (CH<sub>2</sub>Cl)<sub>2</sub> was dried over activated 4 Å molecular sieves. The solvents used in alkyllithiums or Grignard reagents involved reactions were degassed by sparging with Ar for 45 min after distillation from Na.

### 1.6.3. Ring cleavage with Grignard reagents via Weinreb amides

#### 1.6.3.1. Employing 8 eq. Grignard reagent

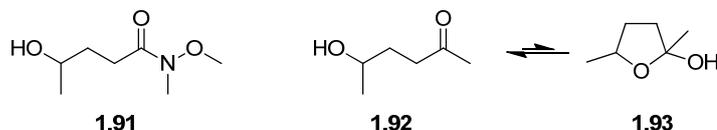


To a solution of  $\gamma$ -valerolactone (0.10 g, 1.0 mmol), *N,O*-dimethylhydroxylamine·HCl (0.12 g, 1.2 mmol) and NaOMe (14 mg, 0.25 mmol) in THF (10 mL) at  $-20\text{ }^{\circ}\text{C}$ , MeMgBr (1.4 M in THF, 6.9 mL, 9.6 mmol) was added dropwisely. The reaction was stirred at  $-20\text{ }^{\circ}\text{C}$  for 2 h and then warmed gradually to r.t. for 12 h. After that, saturated  $\text{NH}_4\text{Cl}$  solution (10 mL) was added, and then stirred at r.t. for 2 h. THF was removed *in vacuo*, and  $\text{CH}_2\text{Cl}_2$  (30 mL) was added, washed with water (30 mL), dried over  $\text{Na}_2\text{SO}_4$ , filtered, and the solvent removed *in vacuo* to give a colourless liquid. Purification by flash chromatography (hexane/ $\text{Et}_2\text{O}$  1:1) gave a mixture of keto-alcohol **1.92** and hemiketal **1.93** in a ratio of 30:1, as calculated by the integration of the peaks at 3.72-3.84 ppm ( $\text{CHCH}_3$  in **1.92**) and 4.10-4.24 ppm ( $\text{CHCH}_3$  in **1.93**) in  $^1\text{H}$  NMR spectrum. The analytical data were in agreement with literature values.<sup>86</sup>

#### 5-Hydroxyhexan-2-one (**1.92**)

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  3.84-3.72 (br s, 1 H, CH), 2.58 (t,  $J = 7.1$  Hz, 2 H,  $\text{CH}_2\text{C}=\text{O}$ ), 2.16 (s, 3 H,  $\text{C}=\text{OCH}_3$ ), 1.81-1.72 (m, 2 H,  $\text{CHCH}_2$ ), 1.19 (d,  $J = 6.2$  Hz, 3 H,  $\text{CHCH}_3$ ).

### 1.6.3.2. Employing 1 eq. Grignard reagent and 2.5 eq. *n*-BuLi

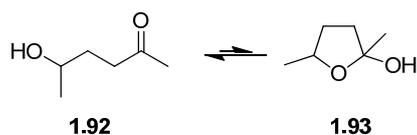


To a solution of *N,O*-dimethylhydroxylamine·HCl salt (0.10 g, 1.0 mmol) in THF (10 mL) under at  $-78\text{ }^{\circ}\text{C}$ , *n*-BuLi (1.8 ml, 1.5 M, 2.6 mmol) was added dropwisely. After stirring for 10 min, the reaction mixture was gradually warmed to  $-20\text{ }^{\circ}\text{C}$ ,  $\gamma$ -valerolactone (0.10 g, 1.0 mmol) in THF (10 mL) was added, and followed by MeMgBr (1.4 M in THF, 0.7 mL, 1.0 mmol). The reaction was stirred at  $-20\text{ }^{\circ}\text{C}$  for 2 h and then warmed to r.t., and stirred at r.t. for 12 h. Saturated  $\text{NH}_4\text{Cl}$  (10 mL) was added, and the mixture was continued stirred for 1 h. THF was removed *in vacuo*, and  $\text{CH}_2\text{Cl}_2$  (30 mL) was added, washed with water, dried over  $\text{Na}_2\text{SO}_4$ , filtered, and the solvent removed *in vacuo* to give a colourless liquid. Purification by flash chromatography (hexane/ $\text{Et}_2\text{O}$  1:1) gave the **1.91** (56 mg, 35%), and **1.92** with small amount **1.93** (46 mg, 40 % altogether) as a volatile colourless liquid. The analytical data of **1.91** were in agreement with literature values.<sup>86</sup> The  $^1\text{H}$  NMR of **1.92** was in consistent with analytical data in section 1.6.3.1.

#### 4-Hydroxy-*N*-methoxy-*N*-methylpentanamide (1.91)

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  3.91-3.77 (m, 1 H, CH), 3.70 (s, 3 H,  $\text{OCH}_3$ ), 3.19 (s, 3 H,  $\text{NCH}_3$ ), 2.73-2.46 (m, 2 H,  $\text{CHCH}_2$ ), 1.92-1.66 (m, 2 H,  $\text{CHCH}_2$ ), 1.22 (d,  $J = 6.2\text{ Hz}$ , 3 H,  $\text{CHCH}_3$ ).

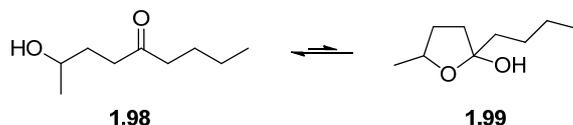
### 1.6.3.3. Employing 2.5 eq. Grignard reagent



To a solution of *N,O*-dimethylhydroxylamine·HCl (0.10 g, 1.0 mmol) in THF (10 mL)

under at  $-78\text{ }^{\circ}\text{C}$ , *n*-BuLi (1.8 ml, 1.5 M, 2.6 mmol) was added dropwisely. After stirring for 10 min, the reaction mixture was gradually warmed to  $-20\text{ }^{\circ}\text{C}$ ,  $\gamma$ -valerolactone (0.10 g, 1.0 mmol) in THF (10 mL) was added, and followed by MeMgBr (1.4 M in THF, 1.79 mL, 2.5 mmol). The reaction was stirred at  $-20\text{ }^{\circ}\text{C}$  for 2 h and then warmed to r.t., and stirred at r.t. for 12 h. Saturated aqueous  $\text{NH}_4\text{Cl}$  (10 mL) was added, and the mixture was continued stirred for 1 h. THF was removed *in vacuo*, and  $\text{CH}_2\text{Cl}_2$  (30 mL) was added, washed with water, dried over  $\text{Na}_2\text{SO}_4$ , filtered, and the solvent removed *in vacuo* to give a colourless liquid. Purification by flash chromatography (hexane/ $\text{Et}_2\text{O}$  1:1) gave keto-alcohol **1.92** and hemiketal **1.93** (84 mg, 73 % altogether) as a volatile colourless liquid. The  $^1\text{H}$  NMR of **1.92** was in consistent with analytical data in section 1.6.3.1.

#### 1.6.4. Ring cleavage with alkyllithiums



##### 1.6.4.1. Quenching with HCl solution

To the solution of  $\gamma$ -valerolactone (1.5 g, 15.0 mmol) in  $\text{Et}_2\text{O}$  (100 mL) at  $-78\text{ }^{\circ}\text{C}$  was added *n*-BuLi (1.6 M in pentane, 8.5 mL, 13.6 mmol) dropwise. After stirring at  $-78\text{ }^{\circ}\text{C}$  for 3 h, the reaction was quenched by adding HCl (5M, 10 mL), and gradually warmed to r.t.. The mixture was washed with 1 M HCl (100 mL), and the aqueous phase extracted with  $\text{Et}_2\text{O}$  ( $2 \times 100\text{ mL}$ ). The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , filtered and the solvent removed *in vacuo*. Purification by flash chromatography (hexane/ $\text{Et}_2\text{O}$  2:1) gave a mixture of keto-alcohol **1.98** and hemiketal **1.99** (1.3 g, 68 %) as a colourless liquid. The molar ratio of **1.98**:**1.99** was 20:1, as calculated by integration of the peaks at 3.66-3.78 ppm ( $\text{CHCH}_3$  in **1.98**) and 4.21-4.35 ppm ( $\text{CHCH}_3$  in **1.99**) in the  $^1\text{H}$  NMR spectrum. The analytical data were in agreement with literature values.<sup>39</sup>

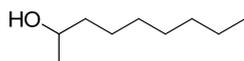
## 2-Hydroxynonan-5-one (1.98)

$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  3.78 - 3.66 (m, 1 H,  $\text{CHOH}$ ), 2.56 (t,  $J = 7.2$  Hz, 2 H,  $\text{CH}_2\text{C}=\text{O}$ ), 2.43 (t,  $J = 7.2$  Hz, 2 H,  $\text{CH}_2\text{C}=\text{O}$ ), 1.61 - 1.31 (m, 6 H,  $3 \times \text{CH}_2$ ), 1.20 (d,  $J = 6.2$  Hz, 3 H,  $\text{CHCH}_3$ ), 0.90 (t,  $J = 7.3$  Hz, 3 H,  $\text{CH}_2\text{CH}_3$ ).

### 1.6.4.2. Quenching with TMSCl

To the solution of  $\gamma$ -valerolactone (1.5 g, 15.0 mmol) in  $\text{Et}_2\text{O}$  (100 mL) at  $-78$  °C was added  $n$ -BuLi (1.6 M in pentane, 8.5 mL, 13.6 mmol) dropwise. After stirring at  $-78$  °C for 3 h, the reaction was quenched by adding TMSCl (5.2 mL, 41.0 mmol). The mixture was warmed to r.t. over 10 min, and then washed with 1 M HCl (100 mL). The aqueous phase was extracted with  $\text{Et}_2\text{O}$  ( $2 \times 100$  mL). The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , filtered and the solvent removed *in vacuo*. Purification by flash chromatography (hexane/ $\text{Et}_2\text{O}$  2:1) gave keto-alcohol **1.98** and hemiketal **1.99** (1.7 g, 71 % altogether) as a colourless liquid. The analytical data were in consistence with analytical data in section **1.6.4.1**.<sup>39</sup>

## 1.6.5. Reductions of keto-alcohol



1.114

### 1.6.5.1. Wolff-Kishner reduction

To the solution of keto-alcohol **1.98** (0.5 g, 3.2 mmol) in triethylene glycol (8 mL) was added KOH (0.53 g, 9.5 mmol) and  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$  (0.39 g, 7.9 mmol). The mixture was stirred at  $190$  °C for 5 h, and then cooled, diluted with water (50 mL), and extracted with  $\text{Et}_2\text{O}$  ( $2 \times 50$  mL). The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , filtered and the solvent removed *in vacuo*. The product was purified by flash chromatography (hexane/ $\text{Et}_2\text{O}$  2:1) to give the *title compound* (0.23 g, 51 %) as a clear liquid. The analytical data were in agreement with literature values.<sup>87</sup>

### Nonan-2-ol (1.114)

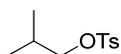
$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  3.84 - 3.70 (m, 1H), 1.64 (s, 1 H, OH), 1.56 - 1.09 (m, 12 H,  $\text{CH}_2$ ), 1.17 (d,  $J = 6.2$  Hz, 3 H,  $\text{CHCH}_3$ ), 0.87 (t,  $J = 6.9$  Hz, 3 H,  $\text{CH}_2\text{CH}_3$ ).

#### 1.6.5.2. Modified Clemmensen reduction

To a solution of keto-alcohol **1.98** (0.2 g, 1.26 mmol) in MeOH (12 mL) and  $\text{CH}_2\text{Cl}_2$  (4 mL) at 0 °C, was added Zn dust (0.83 g, 12.6 mmol) and TMSCl (1.8 mL, 12.6 mmol). The reaction was stirred at 0 °C for 1 h, and quenched by adding  $\text{NaHCO}_3$  (1.27 g, 15.2 mmol). After stirring for 5 min at r.t., the suspension was filtered through celite, and the solvent was removed *in vacuo*. HCl (1 M, 30 mL) was added and the mixture was extracted with  $\text{Et}_2\text{O}$  (3  $\times$  30 mL). The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , filtered, and the solvent removed *in vacuo*. The crude product was purified by flash chromatography (Hexane/ $\text{Et}_2\text{O}$  2:1) to give the *title compound* (0.18 g, quantitative yield) as a colourless liquid. The analytical data were in consistence with analytical data in section 1.6.5.1.<sup>87</sup>

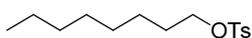
#### 1.6.6. Conversion of alcohols to tosylates

General experiment procedure: To a solution of alcohol in  $\text{CH}_2\text{Cl}_2$  (0.1 M) was added TsCl (1.5 eq.), pyridine (3 eq.) and DMAP (0.1 eq.). The reaction was stirred at r.t. for 24 h. The reaction mixture was washed by HCl solution (1 M) and aqueous  $\text{NaHCO}_3$ , dried over  $\text{Na}_2\text{SO}_4$ , filtered, and the solvent removed *in vacuo*. The products for primary alcohols were pure enough for the next step without further purification. The tosylate product for secondary alcohol was purified by flash chromatography (**Table 1.4**).

**1.6.6.1. *i*-Butyl *p*-toluenesulfonate (1.176)****1.176**

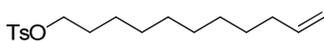
Tosylate **1.176** was prepared according to the above general procedure after stirring at r.t. for 24 h in 98 % yield. The analytical data were in agreement with literature values.<sup>87</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.78 (d, *J* = 8.3 Hz, 2 H, ArH), 7.34 (d, *J* = 7.9 Hz, 2 H, ArH), 3.79 (d, *J* = 6.5 Hz, 2 H, CH<sub>2</sub>), 2.44 (s, 3 H, CH<sub>3</sub> for Ts), 1.94 (dp, *J* = 13.3, 6.7 Hz, 1 H, CH), 0.89 (d, *J* = 6.8 Hz, 6 H, 2 × CH<sub>3</sub>).

**1.6.6.2. Octyl *p*-toluenesulfonate (1.177)****1.177**

Tosylate **1.177** was prepared according to the above general procedure after stirring at r.t. for 12 h in 95 % yield. The analytical data were in agreement with literature values.<sup>88</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.79 (d, *J* = 8.3 Hz, 2 H, ArH), 7.34 (d, *J* = 8.0 Hz, 2 H, ArH), 4.02 (t, *J* = 6.5 Hz, 2 H, CH<sub>2</sub>OTs), 2.45 (s, 3 H, CH<sub>3</sub> for Ts), 1.69-1.56 (m, 2 H, CH<sub>2</sub>), 1.37-1.13 (m, 10 H, 5 × CH<sub>2</sub>), 0.87 (t, *J* = 7.0 Hz, 3 H, CH<sub>3</sub>).

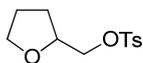
**1.6.6.3. Undec-10-en-1-yl *p*-toluenesulfonate (1.178)****1.178**

Tosylate **1.178** was prepared according to the above general procedure after stirring at r.t. for 12 h in 95 % yield. The analytical data were in agreement with literature values.<sup>89</sup>

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.79 (d,  $J = 8.3$  Hz, 2 H, ArH), 7.39-7.29 (m, 2 H, ArH), 5.86-5.74 (m, 1 H,  $\text{CH}=\text{CH}_2$ ), 5.04-4.87 (m, 2 H,  $\text{CH}=\text{CH}_2$ ), 4.02 (t,  $J = 6.5$  Hz, 2 H,  $\text{CH}_2\text{OTs}$ ), 2.44 (s, 3 H,  $\text{CH}_3$  of Ts), 2.09-1.96 (m, 2 H,  $\text{CH}-\text{CH}_2$ ), 1.70-1.56 (m, 2 H,  $\text{CH}_2$ ), 1.41-1.15 (m, 12 H,  $6 \times \text{CH}_2$ ).

**$^{13}\text{C}$  NMR** (100 MHz,  $\text{CDCl}_3$ )  $\delta$  144.73(ArC), 139.27( $\text{CH}=\text{CH}_2$ ), 133.45(ArC), 129.92(ArC), 128.01(ArC), 114.28( $\text{CH}=\text{CH}_2$ ), 70.81( $\text{CH}_2\text{OTs}$ ), 33.89( $\text{CH}-\text{CH}_2$ ); 29.43, 29.16, 29.02, 28.94 and 25.44( $\text{CH}_2$ ), 21.74 ( $\text{CH}_3$  of Ts).

#### 1.6.6.4. Tetrahydrofuran-2-ylmethyl *p*-toluenesulfonate (1.179)

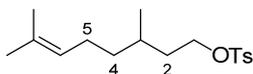


**1.179**

Tosylate **1.179** was prepared according to the above general procedure after stirring at r.t. for 24 h in 93 % yield. The analytical data were in agreement with literature values.<sup>90</sup>

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.80 (d,  $J = 8.3$  Hz, 2 H, ArH), 7.34 (d,  $J = 8.0$  Hz, 2 H, ArH), 4.13-4.04 (m, 1 H,  $\text{CH}_2\text{OTs}$ ), 4.04-3.94 (m, 2 H,  $\text{CH}_2\text{OTs}$  and CH), 3.83-3.68 (m, 2 H,  $\text{CH}_2\text{O}$ ), 2.45 (s, 3 H,  $\text{CH}_3$ ), 2.03-1.92 (m, 1 H,  $\text{CH}_2\text{CH}$ ), 1.91-1.82 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{CH}_2$ ), 1.72-1.61 (m, 1 H,  $\text{CH}_2\text{CH}$ ).

#### 1.6.6.5. 3,7-Dimethyloct-6-en-1-yl *p*-toluenesulfonate (Citronellyl tosylate) (1.180)



**1.180**

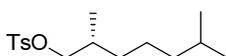
Tosylate **1.180** was prepared according to the above general procedure after stirring at r.t. for 24 h in 93 % yield. The analytical data were in agreement with literature values.<sup>91</sup>

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.79 (d,  $J = 8.3$  Hz, 2 H, ArH), 7.34 (d,  $J = 8.0$  Hz, 2 H, ArH), 5.06-4.99 (m, 1 H,  $\text{C}=\text{CH}$ ), 4.13-4.00 (m, 2 H,  $\text{CH}_2\text{OTs}$ ), 2.45 (s, 3 H,

$\text{CH}_3$  for Ts), 2.04-1.04 (m, 7 H,  $\text{CH}$  and  $3 \times \text{CH}_2$ ), 1.67 and 1.57 (2 s, 6 H,  $2 \times \text{CCH}_3$ ), 0.82 (d,  $J = 6.5$  Hz, 3 H,  $\text{CHCH}_3$ ).

$^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  144.76 (ArC), 133.44 (ArC), 131.61 ( $\text{CCH}_3$ ), 129.94 (ArC), 128.02 (ArC), 124.46 ( $\text{C}=\text{CH}$ ), 69.19 ( $\text{CH}_2\text{OTs}$ ), 36.85 (C2), 35.81 (C4), 29.03 (CH), 25.82 ( $\text{CCH}_3$ ), 25.40 (C5), 21.76 ( $\text{CH}_3$  of Ts), 19.19 ( $\text{CHCH}_3$ ), 17.76 ( $\text{CCH}_3$ ).

#### 1.6.6.6. (*R*)-2,6-Dimethylheptyl 4-methylbenzenesulfonate (1.181)

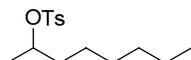


1.181

Tosylate **1.181** was prepared according to the above general procedure after stirring at r.t. for 24 h in 95 % yield.

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.79 (d,  $J = 8.3$  Hz, 2 H, ArH), 7.34 (d,  $J = 8.0$  Hz, 2 H, ArH), 3.88 (dd,  $J = 9.3, 5.7$  Hz, 1 H,  $\text{CH}_2\text{OTs}$ ), 3.81 (dd,  $J = 9.3, 6.5$  Hz, 1 H,  $\text{CH}_2\text{OTs}$ ), 2.45 (s, 3 H,  $\text{CH}_3$  for Ts), 1.83-1.70 (m, 1 H, CH), 1.53-1.41 (m, 1 H,  $\text{CH}(\text{CH}_3)_2$ ), 1.34-1.01 (m, 6 H,  $3 \times \text{CH}_2$ ), 0.88 (d,  $J = 6.7$  Hz, 3 H,  $\text{CHCH}_3$ ), 0.83 (d,  $J = 6.6$  Hz, 6 H,  $\text{CH}(\text{CH}_3)_2$ ).

#### 1.6.6.7. Octan-2-yl 4-methylbenzenesulfonate (1.182)



1.182

Tosylate **1.182** was prepared according to the above general procedure after stirring at r.t. for 48 h in 90 % yield after flash chromatography purification (hexane/ $\text{Et}_2\text{O}$  4:1).

The analytical data were in agreement with literature values.<sup>92</sup>

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.79 (d,  $J = 8.3$  Hz, 2 H, ArH), 7.33 (d,  $J = 8.0$  Hz, 2 H, ArH), 4.60 (dq,  $J = 12.5, 6.3$  Hz, 1 H, CH), 2.44 (s, 3 H,  $\text{CH}_3$  of Ts), 1.65-1.54 (m, 1 H,  $\text{CH}_2$ ), 1.52-1.41 (m, 1 H,  $\text{CH}_2$ ), 1.26 (d,  $J = 6.3$  Hz, 3 H,  $\text{CHCH}_3$ ), 1.24-1.10 (m, 8 H,  $4 \times \text{CH}_2$ ), 0.85 (t,  $J = 7.0$  Hz, 3 H,  $\text{CH}_3$ ).

### 1.6.7. Conversion of tosylates to iodides under microwave irradiation

General procedure: NaI (3 eq.) was added to neat primary tosylate in a 10 mL microwave vial, which was sealed and heated under microwave irradiation in a CEM Discover<sup>TM</sup> microwave reactor, and subject to 100-150 W irradiations for 0.5-2 h (Table 1.5).

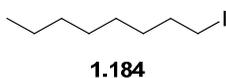
#### 1.6.7.1. *i*-BuI (1.183)



<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.14 (d, *J* = 5.9 Hz, 2 H, CH<sub>2</sub>I), 1.78-1.67 (m, 1 H, CH), 1.00 (d, *J* = 6.6 Hz, 6 H, 2 × CH<sub>3</sub>).

The analytical data were in agreement with literature values.<sup>87</sup>

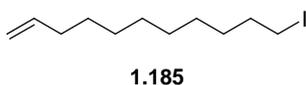
#### 1.6.7.2. *n*-C<sub>8</sub>H<sub>17</sub>I (1.184)



<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.19 (t, *J* = 7.1 Hz, 2 H, CH<sub>2</sub>I), 1.82 (dt, *J* = 14.5, 7.1 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>I), 1.45-1.23 (m, 10 H, 5 × CH<sub>2</sub>), 0.88 (t, *J* = 6.9 Hz, 3 H, CH<sub>3</sub>).

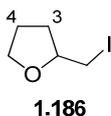
The analytical data were in agreement with literature values.<sup>93</sup>

#### 1.6.7.3. Undec-10-en-1-yl iodide (1.185)



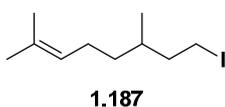
<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.81 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1 H, CH=CH<sub>2</sub>), 5.03-4.90 (m, 2 H, CH=CH<sub>2</sub>), 3.19 (t, *J* = 7.1 Hz, 2 H, CH<sub>2</sub>I), 2.10-1.96 (m, 2 H, CH-CH<sub>2</sub>), 1.82 (dt, *J* = 14.5, 7.1 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>I), 1.46-1.19 (m, 12 H, 6 × CH<sub>2</sub>).

The analytical data were in agreement with literature values.<sup>94</sup>

**1.6.7.4. Tetrahydrofuran-2-ylmethyl iodide (1.186)**

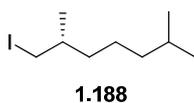
$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  4.06-3.89 (m, 2 H,  $\text{CH}_2\text{O}$ ), 3.88-3.76 (m, 1 H,  $\text{CH}$ ), 3.23 (qd,  $J = 9.9, 5.9$  Hz, 2 H,  $\text{CH}_2\text{I}$ ), 2.18-2.06 (m, 1 H, H3a), 2.04-1.83 (m, 2 H, H4), 1.76-1.59 (m, 1 H, H3b).

The analytical data were in agreement with literature values.<sup>95</sup>

**1.6.7.5. Citronellyl iodide (1.187)**

$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.13-5.05 (m, 1 H,  $\text{C}=\text{CH}$ ), 3.32-3.08 (m, 2 H,  $\text{CH}_2\text{I}$ ), 2.10-1.79 (m, 3 H, H2a and H5), 1.76-1.47 (m, 2 H, H2b and H3), 1.68, 1.61 (2 s, 6 H,  $2 \times \text{CCH}_3$ ), 1.41-1.08 (m, 2 H, H4), 0.90 (t,  $J = 7.1$  Hz, 3 H,  $\text{CHCH}_3$ ).

The analytical data were in agreement with literature values.<sup>96</sup>

**1.6.7.6. (R)-1-Iodo-2,6-dimethylheptane (1.188)**

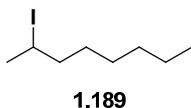
$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  3.23 (dd,  $J = 9.6, 4.7$  Hz, 1 H,  $\text{CH}_2\text{I}$ ), 3.15 (dd,  $J = 9.6, 6.1$  Hz, 1 H,  $\text{CH}_2\text{I}$ ), 1.61-1.11 (m, 8 H,  $2 \times \text{CH}$  and  $3 \times \text{CH}_2$ ), 0.98 (d,  $J = 6.5$  Hz, 3 H,  $\text{CH}_3$ ), 0.91-0.83 (m, 6 H,  $2 \times \text{CH}_3$ ).

**IR**  $\nu_{\text{max}}$  2958(s), 2928(s), 2870(m), 1463(m), 1381(m), 1263(m), 1196(m), 1099(m), 1020(m), 911(m), 806(m), 737(m).

**EI-MS:** Calcd. for  $[\text{C}_9\text{H}_{19}\text{I}]$ : 254.1, found: 254.1.

The analytical data was in agreement with the literature values.<sup>25</sup>

### 1.6.7.7. 2-Iodoctane (1.189)



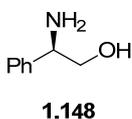
$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  4.25-4.04 (m, 1 H, CH), 1.92 (d,  $J = 6.8$  Hz, 3 H,  $\text{CH}_3\text{CH}$ ), 1.91-1.16 (m, 10 H,  $5 \times \text{CH}_2$ ), 0.87-0.90 (m, 3 H,  $\text{CH}_3$ ).

The analytical data were in agreement with literature values.<sup>97</sup>

### 1.6.8. Preparation of (*S*)-3-methylbutyrolactone by Reiser's method

The preparation of (*S*)-3-methylbutyrolactone from asymmetric hydrogenation with Co(II)-azabis(oxazoline) complex as catalyst was carried out according to the method reported by Reiser and co-workers.<sup>63,71,98</sup>

#### 1.6.8.1. (*R*)-2-Amino-2-phenylethanol (1.148)



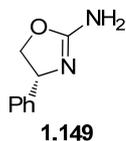
To a mixture of  $\text{NaBH}_4$  (2 g, 53 mmol) in THF (20 mL) at 0 °C was added (*R*)-phenylglycine (3.1 g, 20.5 mmol).  $\text{H}_2\text{SO}_4$  (1.2 mL, 24.2 mmol) in  $\text{Et}_2\text{O}$  (5 mL) was then added dropwise, and the resulting mixture was stirred at r.t. for 12 h. The solvent was removed *in vacuo*. Aqueous NaOH (5 M, 20 mL) was added, and the mixture was refluxed for 2 h, and then filtered through celite. The solids were washed with water (30 mL). The combined filtrations were extracted with  $\text{CH}_2\text{Cl}_2$ , and the solvent of the combined organic layer was removed *in vacuo* to give white solids. Purification by recrystallisation (hexane/ $\text{EtOAc}$ ) gave the *title compound* (2.3 g, 81 %) as white crystals, m.p. 75-76 °C (lit.<sup>85</sup> m.p. 76-78 °C). The analytical data were in agreement with literature values.<sup>98-100</sup>

$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.38-7.26 (m, 5 H, ArH), 4.05 (dd,  $J = 8.2, 4.5$  Hz, 1 H,

CH), 3.75 (dd,  $J = 10.7, 4.5$  Hz, 1 H, CH<sub>2</sub>), 3.55 (dd,  $J = 10.7, 8.2$  Hz, 1 H, CH<sub>2</sub>).

**Specific Rotation**  $[\alpha]_{\text{D}}^{22} -31.1^{\circ}$  ( $c$  0.8, MeOH) (Lit.<sup>100</sup>:  $[\alpha]_{\text{D}}^{24} -33.5^{\circ}$  ( $c$  1, MeOH))

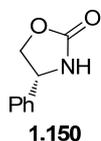
#### 1.6.8.2. (R)-4-Phenyl-4,5-dihydrooxazol-2-amine (1.149)



To a solution of Br<sub>2</sub> (1.8 g, 0.58 mL, 11.26 mmol) in MeOH (15 mL) at 0 °C was added NaCN (0.54 g, 11.02 mmol), and then the alcohol **1.148** (1.37 g, 10 mmol) in MeOH (3 mL) was added. The reaction mixture was stirred at r.t. for 1.5 h, and then ammonia solution (25 %, 5 mL, 10 mmol) was added. The mixture was further stirred for 15 min. MeOH was removed *in vacuo*, and then NaOH (3M, 15 mL) was added, and the aqueous solution was extracted with EtOAc (4 × 20 mL). The combined organic layers were washed with brine (80 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent removed *in vacuo* to give the *title compound* as a light yellow solid (1.4 g, 88 %), m.p. 118-120 °C. The analytical data were in agreement with literature values.<sup>101</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.37-7.23 (m, 5 H, ArH), 5.09 (dd,  $J = 9.2, 7.3$  Hz, 1 H, CH), 4.63 (dd,  $J = 9.2, 8.0$  Hz, 1 H, CH<sub>2</sub>), 4.08 (dd,  $J = 8.0, 7.3$  Hz, 1 H, CH<sub>2</sub>).

#### 1.6.8.3. (R)-4-Phenyloxazolidin-2-one (1.150)



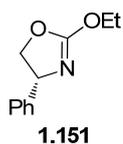
To the mixture of Na metal (0.3 g, 13 mmol) in EtOH (20 mL) was added a solution of alcohol **1.148** (1.6 g, 11.7 mmol) in EtOH (18 mL). Diethyl carbonate (1.5 mL, 12.4 mmol) was then added dropwise. The reaction was refluxed for 12 h, and then the solvent was removed *in vacuo*. CH<sub>2</sub>Cl<sub>2</sub> (30 mL) was added, and then washed by saturated aqueous NH<sub>4</sub>Cl. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the

solvent removed *in vacuo* to give a yellow solid. Cold Et<sub>2</sub>O (5 mL) was added to the crude product, and the precipitates were collected by filtering to give the *title compound* as a pale yellow solid (1.7 g, 89 %), m.p. 132-133 °C (Lit.<sup>102</sup> m.p. 130-133 °C). The analytical data were in agreement with literature values.<sup>101</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.47-7.29 (m, 5 H, ArH), 5.42 (br s, 1 H, NH), 4.95 (t, *J* = 7.2 Hz, 1 H, CH), 4.74 (t, *J* = 8.7 Hz, 1 H, CH<sub>2</sub>), 4.20 (dd, *J* = 8.6, 6.9 Hz, 1 H, CH<sub>2</sub>).

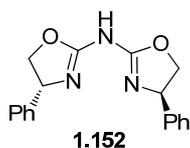
**Specific Rotation**  $[\alpha]_{\text{D}}^{22}$  -51.1 ° (*c* 1.0, CHCl<sub>3</sub>) (Lit.:  $[\alpha]_{\text{D}}^{26}$  -54.9° (*c* 1.0, CHCl<sub>3</sub>)).<sup>101</sup>

#### 1.6.8.4. (R)-2-Ethoxy-4-phenyloxazolidine (1.151)



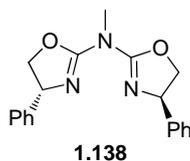
To a solution of oxazolidinone **1.150** (1.75 g, 10.7 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (25 mL) at 0 °C, triethyloxonium tetrafluoroborate (1 M in CH<sub>2</sub>Cl<sub>2</sub>, 14.3 mL, 14.3 mmol) was added dropwise. The reaction mixture was stirred at r.t. for 24 h. Then the solids were removed by filtering through celite, and the filtration was poured over cold saturated Na<sub>2</sub>CO<sub>3</sub> solution. The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 40 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and the solvent removed *in vacuo*. Et<sub>2</sub>O (20 mL) was added to the brown mixture, and the remaining solid was removed by filtering. The solvent in the filtration was removed *in vacuo* to give a yellow oil (1.1 g, 61 %), and was used in the next step without further purification. The analytical data were in agreement with literature values.<sup>101</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.42-7.21 (m, 5 H, ArH), 5.15 (dd, *J* = 9.5, 7.6 Hz, 1 H, CH), 4.73 (dd, *J* = 9.5, 8.1 Hz, 1 H, CHCH<sub>2</sub>O), 4.44-4.30 (m, 2 H, OCH<sub>2</sub>CH<sub>3</sub>), 4.19 (dd, *J* = 8.1, 7.6 Hz, 1 H, CHCH<sub>2</sub>O), 1.40 (t, *J* = 7.1 Hz, 3 H, CH<sub>3</sub>).

**1.6.8.5. (R)-Bis((R)-4-phenyl-4,5-dihydrooxazol-2-yl)amine (1.152)**

To a solution of oxazolamine **1.149** (0.68 g, 3.6 mmol) and ether **1.151** (0.59 g, 3.7 mmol) in toluene (40 mL) was added TsOH·H<sub>2</sub>O (0.1 g, 0.55 mmol). The mixture was refluxed for 36 h. The solvent was removed *in vacuo*, and the resulting brown solid was purified by flash chromatography (hexane/EtOAc 1:1 with 2% Et<sub>3</sub>N, silica was flushed with solvent system before loading) to give a white solid, which was further recrystallised from acetone to afford the *title compound* (0.43 g, 39 %) as white crystals. The analytical data were in agreement with literature values.<sup>71</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.42-7.27 (m, 10 H, ArH), 5.14 (dd, *J* = 9.2, 7.4 Hz, 2 H, CH<sub>2</sub>), 4.72 (dd, *J* = 9.2, 8.7 Hz, 2 H, CH<sub>2</sub>), 4.19 (dd, *J* = 8.6, 7.4 Hz, 2 H, 2 × CH).

**1.6.8.6. (R)-N-Methyl-4-phenyl-N-((R)-4-phenyl-4,5-dihydrooxazol-2-yl)-4,5-dihydrooxazol-2-amine (1.138)**

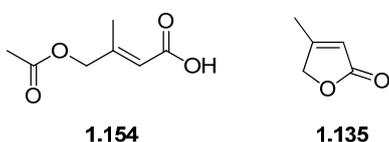
To a solution of amine **1.152** (92 mg, 0.3 mmol) in THF (5 mL) at -78 °C, was added *n*-BuLi (205 μL, 1.5 M, 0.31 mmol) dropwise. The mixture was stirred at -78 °C for 30 min, and then MeI (92 mL, 1.5 mmol) was added. After stirring the reaction mixture at r.t. for 20 h, the solvent was removed *in vacuo*. CH<sub>2</sub>Cl<sub>2</sub> (30 mL) was added to the resulting brown oil, and then the organic layer was washed by saturated NaHCO<sub>3</sub> (20 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent removed *in vacuo*. The resulting yellow oil was purified by flash chromatography (hexane/EtOAc 2:1 with 2% Et<sub>3</sub>N, silica was flushed with solvent system before loading) to give the *title compound* (89 mg, 93 %) as a clear oil. The analytical data were in agreement with

literature values.<sup>101</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.57-7.05 (m, 10 H, ArH), 5.23 (dd, *J* = 9.4, 7.9 Hz, 2 H, CH<sub>2</sub>), 4.81 (dd, *J* = 9.5, 8.3 Hz, 2 H, CH<sub>2</sub>), 4.27 (t, *J* = 8.0 Hz, 2 H, CH), 3.55 (s, 3 H, CH<sub>3</sub>).

**Specific Rotation**  $[\alpha]_{\text{D}}^{22} +76.7^{\circ}$  (*c* 3.3, CH<sub>2</sub>Cl<sub>2</sub>). (Lit.:  $[\alpha]_{\text{D}}^{20} +61.4^{\circ}$  (*c* 1.0, CH<sub>2</sub>Cl<sub>2</sub>))<sup>101,103</sup>

#### 1.6.8.7. Oxidation of 3-methyl-2-butenic acid with SeO<sub>2</sub>



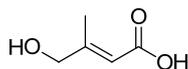
To a solution of 3-methyl-2-butenic acid (0.25 g, 2.5 mmol) in AcOH (15 mL) was added SeO<sub>2</sub> (0.14 g, 1.25 mmol). The mixture was refluxed for 9 h, and the solvent removed *in vacuo*. To the resultant was added EtOAc (30 mL) and water (30 mL). The organic layer was washed with saturated NaHCO<sub>3</sub> (30 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent removed *in vacuo*. The resulting brown oil was purified by flash chromatography (hexane/Et<sub>2</sub>O 2:1) to give the *title compound* **1.154** (0.15 g, 38 %) as a colourless oil, together with the starting material 3-methyl-2-butenic acid (0.11 g, 44 %) and the ring closed product **1.135** 4-methylfuran-2(5*H*)-one (40 mg, 16 %). The analytical data were in agreement with literature values.<sup>104</sup>

#### (*E*)-4-Acetoxy-3-methylbut-2-enoic acid (**1.154**)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.90 (dd, *J* = 3.0, 1.5 Hz, 1 H, CH=C), 4.61 (d, *J* = 1.0 Hz, 2 H, CH<sub>2</sub>O), 2.16-2.15 (2 s, 6 H, 2 × CH<sub>3</sub>).

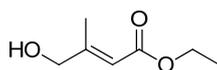
#### 4-Methylfuran-2(5*H*)-one (**1.135**)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.92-5.78 (m, 1 H, CH=C), 4.78-4.62 (m, *J* = 1.6, 0.8 Hz, 2 H, CH<sub>2</sub>), 2.16-2.09 (m, *J* = 1.6, 0.8 Hz, 3 H, CH<sub>3</sub>).

**1.6.8.8. (E)-4-Hydroxy-3-methylbut-2-enoic acid (1.134)****1.134**

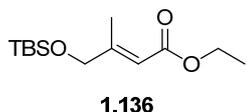
To a solution of the ester **1.154** (0.47 g, 3 mmol) in MeOH (20 mL), aqueous NaOH (2.5 M, 10 mL) was added. The mixture was refluxed for 4 h. Then the solvent was removed *in vacuo*, and the resultant solid was dissolved in water (30 mL), and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with NH<sub>4</sub>Cl (100 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent removed *in vacuo*. The resulting yellow solid was purified by flash chromatography (hexane/EtOAc 1:2) to give the *title compound* (0.29 g, 84 %) as a pale yellow solid, m.p. 162-165 °C (Lit.<sup>104</sup> m.p. 160-165 °C). The analytical data were in agreement with literature values.<sup>104</sup>

<sup>1</sup>H NMR (400 MHz, MeOD) δ 6.01-5.91 (m, 1 H, CH=C), 4.05 (dd, *J* = 1.7, 0.6 Hz, 2 H, CH<sub>2</sub>), 2.06-2.04 (m, 3 H, CH<sub>3</sub>).

**1.6.8.9. (E)-Ethyl 4-hydroxy-3-methylbut-2-enoate (1.155)****1.155**

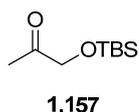
To a solution of acid **1.134** (0.97 g, 8.4 mmol) in EtOH (20 mL) at 0 °C, SOCl<sub>2</sub> (1 mL, 13.8 mmol) was added drop by drop. The reaction was stirred at r.t. for 14 h. Then the solvent was removed *in vacuo*, and the resultant liquid was dissolved in EtOAc (40 mL), and washed with saturated NaHCO<sub>3</sub> (40 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent removed *in vacuo*. The resulting liquid was purified by flash chromatography (hexane/Et<sub>2</sub>O 2:1) to give the *title compound* (0.6 g, 50 %) as a colourless liquid. The analytical data were in agreement with literature values.<sup>105</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.00-5.96 (m, 1 H, CH=C), 4.16 (q, *J* = 7.1 Hz, 4 H, CH<sub>2</sub>), 4.13 (s, 1 H, OH), 2.08 (br s, 3 H, CCH<sub>3</sub>), 1.27 (t, *J* = 7.1 Hz, 3 H, OCH<sub>2</sub>CH<sub>3</sub>).

**1.6.8.10. (E)-4-((*t*-Butyldimethylsilyl)oxy)-3-methylbut-2-enoic ethylester (1.136)**

To a solution of alcohol **1.155** (0.11 g, 0.95 mmol) in DMF (5 mL) was added imidazole (0.16 g, 2.4 mmol) and TBSCl (0.16 g, 1 mmol). The mixture was stirred at r.t. for 16 h. And then to the mixture was added water (50 mL) and Et<sub>2</sub>O (50 mL). The organic layer was separated and washed with water (50 mL), saturated NH<sub>4</sub>Cl (50 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent removed *in vacuo*. The resulting colourless liquid was purified by flash chromatography (hexane/Et<sub>2</sub>O 5:1) to give the *title compound* (0.2 g, 92 %) as a colourless liquid. The analytical data were in agreement with literature values.<sup>73</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.97 (d, *J* = 1.4 Hz, 1 H, C=CH), 4.16 (q, *J* = 7.1 Hz, 2 H, OCH<sub>2</sub>CH<sub>3</sub>), 4.11-4.08 (m, 2 H, CH<sub>2</sub>OSi), 2.05-2.03 (m, 3 H, CH=CCH<sub>3</sub>), 1.27 (t, *J* = 7.1 Hz, 3 H, OCH<sub>2</sub>CH<sub>3</sub>), 0.92 (s, 9 H, C(CH<sub>3</sub>)<sub>3</sub>), 0.08 (s, 6 H, Si(CH<sub>3</sub>)<sub>2</sub>).

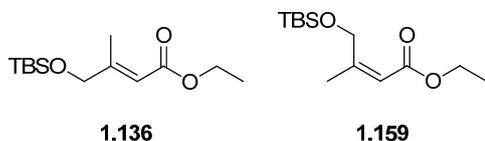
**1.6.8.11. 1-(*tert*-Butyldimethylsilyloxy)-2-propanone (1.157)**

To a solution of acetol (1 g, 13.5 mmol) and imidazole (1.84 g, 27.0 mmol) in DMF (25 mL) at 0 °C was added TBSCl (2.34 g, 15.5 mmol). The reaction mixture was stirred at r.t. for 20 h, and then was diluted with water (200 mL). The mixture was extracted with Et<sub>2</sub>O (200 mL), and the organic layer was washed by saturated brine (200 mL) and saturated NH<sub>4</sub>Cl solution (200 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent removed to give the *title compound* (2.5 g, 98 %) as a colourless liquid which was pure enough for the next step. The analytical data were in agreement with literature values.<sup>106</sup>

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 4.14 (s, 2 H, CH<sub>2</sub>), 2.17 (s, 3 H, C=OCH<sub>3</sub>), 0.93 (s,

9 H, C(CH<sub>3</sub>)<sub>3</sub>), 0.09 (s, 6 H, Si(CH<sub>3</sub>)<sub>2</sub>).

#### 1.6.8.12. (*E*)-4-(*tert*-Butyldimethylsilyloxy)-3-methyl-but-2-enoic ethylester (**1.136**)



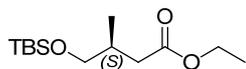
To the mixture of NaH (60 % in mineral oil, 0.166 g, 4.1 mmol) in THF (16 mL) at 0 °C was added triethyl phosphonoacetate (0.79 g, 3.5 mmol). The reaction mixture was slowly warmed to r.t. and stirred at r.t. for 1 h. Then the mixture was cooled to 0 °C, and **1.157** (0.6 g, 3.2 mmol) in THF (5 mL) was added dropwisely. The reaction was stirred at r.t. for 2 h. Then NH<sub>4</sub>Cl solution (10 mL) was added, followed by water (10 mL) and Et<sub>2</sub>O (30 mL). The aqueous layer was collected and further extracted with Et<sub>2</sub>O (30 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent removed to give a colourless liquid, which was a mixture of *E*- and *Z*-configuration (*E*:*Z* 83:17). The *title compound* (0.70 g, 2.7 mmol) was isolated by flash chromatography (hexane/Et<sub>2</sub>O 20:1 to 10:1) as a colourless oil. The *Z*-configured isomer (0.10 g, 0.4 mmol) was also isolated. The analytical data were in agreement with literature values.<sup>73</sup>

#### For *E*-configured (**1.136**):

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.97 (d, *J* = 1.4 Hz, 1 H, C=CH), 4.16 (q, *J* = 7.1 Hz, 2 H, OCH<sub>2</sub>CH<sub>3</sub>), 4.11-4.08 (m, 2 H, CH<sub>2</sub>OSi), 2.05-2.03 (m, 3 H, CH=CCH<sub>3</sub>), 1.27 (t, *J* = 7.1 Hz, 3 H, OCH<sub>2</sub>CH<sub>3</sub>), 0.92 (s, 9 H, C(CH<sub>3</sub>)<sub>3</sub>), 0.08 (s, 6 H, Si(CH<sub>3</sub>)<sub>2</sub>).

#### For the minor *Z*-configured (**1.159**):

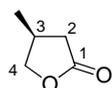
<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.69-5.65 (m, 1 H, C=CH), 4.79 (dd, *J* = 1.8, 0.8 Hz, 2 H, CH<sub>2</sub>OSi), 4.13 (q, *J* = 7.1 Hz, 2 H, OCH<sub>2</sub>CH<sub>3</sub>), 1.96 (dt, *J* = 1.5, 0.8 Hz, 3 H, CH=CCH<sub>3</sub>), 1.27 (t, *J* = 7.1 Hz, 3 H, OCH<sub>2</sub>CH<sub>3</sub>), 0.91 (s, 9 H, C(CH<sub>3</sub>)<sub>3</sub>), 0.08 (s, 6 H, Si(CH<sub>3</sub>)<sub>2</sub>).

**1.6.8.13. (S)-4-(tert-Butyldimethylsilyloxy)-3-methylbutyric ethyl ester (1.137)****1.137**

To the mixture of amine ligand **1.138** (4.8 mg, 0.015 mmol) in EtOH (0.5 mL) was added  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  (4.2 mg, 0.013 mmol, 2.5 mol %). The mixture was stirred for 10 min at r.t. and then (*E*)-ester **1.136** (0.139 g, 0.54 mmol) in EtOH (0.5 mL) was added. After cooling to 0 °C,  $\text{NaBH}_4$  (51 mg, 1.35 mmol) was added slowly. The reaction mixture was stirred at r.t. for 24 h. Water (10 mL) was added and the mixture was extracted with  $\text{CH}_2\text{Cl}_2$  (3 × 15 mL). The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , filtered, and the solvent removed *in vacuo*. The resulting light yellow liquid was purified by flash chromatography (hexane/ $\text{Et}_2\text{O}$  20:1) to give the *title compound* (0.12 g, 85 %) as a colourless liquid. The analytical data were in agreement with literature values.<sup>63</sup>

<sup>1</sup>H NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  4.14 (q,  $J = 7.1$  Hz, 2 H,  $\text{OCH}_2\text{CH}_3$ ), 3.51 (dd,  $J = 9.8, 5.4$  Hz, 1 H,  $\text{CH}_2\text{OSi}$ ), 3.42 (dd,  $J = 9.8, 6.3$  Hz, 1 H,  $\text{CH}_2\text{OSi}$ ), 2.52-2.04 (m, 3 H,  $\text{CHCH}_2\text{C}=\text{OO}$  and  $\text{CHCH}_3$ ), 1.26 (t,  $J = 7.1$  Hz, 3 H,  $\text{OCH}_2\text{CH}_3$ ), 0.94 (d,  $J = 6.6$  Hz, 3 H,  $\text{CHCH}_3$ ), 0.90 (s, 9 H,  $\text{C}(\text{CH}_3)_3$ ), 0.05 (s, 6 H,  $\text{Si}(\text{CH}_3)_2$ ).

**Specific Rotation**  $[\alpha]_{\text{D}}^{22} -3.4^\circ$  ( $c$  2.7,  $\text{CHCl}_3$ ) (Lit.:  $[\alpha]_{\text{D}}^{22} -5.2^\circ$  ( $c$  1,  $\text{CHCl}_3$ ))<sup>63</sup>

**1.6.8.14. (S)-3-Methylbutyrolactone (1.127)****1.127**

To a solution of ester **1.137** (0.143 g, 0.55 mmol) in  $\text{CH}_2\text{Cl}_2$  (5 mL) was added  $\text{TsOH} \cdot \text{H}_2\text{O}$  (10 mg). The reaction mixture was stirred at r.t. for 24 h.  $\text{CH}_2\text{Cl}_2$  (15 mL) was added, and the mixture was washed with saturated  $\text{NaHCO}_3$  (15 mL), dried over  $\text{Na}_2\text{SO}_4$ , filtered, and the solvent removed *in vacuo* to give a colourless liquid, which

was purified by flash chromatography (hexane/Et<sub>2</sub>O 3:1) to give the *title compound* (53 mg, 97 %) as a colourless liquid. The analytical data were in agreement with literature values.<sup>63</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 4.40 (dd, *J* = 8.8, 7.3 Hz, 1 H, H4a), 3.86 (dd, *J* = 8.8, 6.4 Hz, 1 H, H4b), 2.72-2.58 (m, 2 H, H3 and H2a), 2.21-2.07 (m, 1 H, H2b), 1.16 (d, *J* = 6.6 Hz, 3 H, CH<sub>3</sub>).

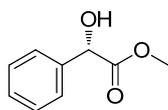
<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 177.37(C=O), 74.82(C4), 36.27(C2), 30.53(C3), 18.07(CH<sub>3</sub>).

**Specific Rotation**  $[\alpha]_{\text{D}}^{22}$  -15.8° (*c* 1.3, CHCl<sub>3</sub>). (Lit.:  $[\alpha]_{\text{D}}^{20}$  -22.2° (*c* 0.6, CHCl<sub>3</sub>))<sup>63</sup>

### 1.6.9. Preparation of (*S*)-3-methylbutyrolactone by Helmchen's method

The preparation of (*S*)-3-methylbutyrolactone via Rh catalysed asymmetric hydrogenation was carried out according to the procedure reported by Helmchen and co-workers.<sup>68</sup>

#### 1.6.9.1. (*S*)-Methyl 2-hydroxy-2-phenylacetate (1.161)



1.161

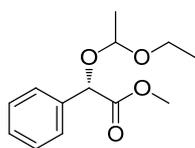
To a solution of (*S*)-mandelic acid (1.52 g, 10 mmol) in MeOH (30 mL), TsOH·H<sub>2</sub>O (0.19 g, 1 mmol) was added. The reaction was refluxed for 3 h, and then solvent was removed *in vacuo*. The resultant was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (50 mL), washed with saturated NaHCO<sub>3</sub> (50 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and solvent was removed *in vacuo* to give the crude product as a white solid, which was purified by recrystallisation (hexane and EtOAc) to give the *title compound* (1.6 g, 98 %) as white

crystals, m.p. 55-57 °C (lit. m.p. 56-57 °C)<sup>107</sup>. The analytical data were in agreement with literature values.<sup>68,107,108</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.47-7.28 (m, 5 H, ArH), 5.18 (d, *J* = 5.7 Hz, 1 H, CH), 3.77 (s, 3 H, CH<sub>3</sub>), 3.42 (d, *J* = 5.7 Hz, 1 H, OH).

**Specific Rotation**  $[\alpha]_{\text{D}}^{22} +178^{\circ}$  (*c* 1.0, CHCl<sub>3</sub>) (Lit.<sup>107</sup>:  $[\alpha]_{\text{D}}^{22} +177^{\circ}$  (*c* 1.5, CHCl<sub>3</sub>))

#### 1.6.9.2. (2S)-Methyl 2-(1-ethoxyethoxy)-2-phenylacetate (1.162)

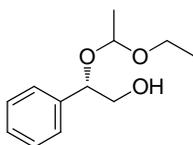


**1.162**

To a solution of ester **1.161** (2.65 g, 15.9 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (40 mL), pyridinium *p*-toluenesulfonate (40 mg, 0.16 mmol) and ethyl vinyl ether (3.8 ml, 2.86 g, 39.7 mmol) was added at 0 °C. The reaction was stirred at r.t. for 40 min. The reaction mixture was washed with water (40 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and solvent was removed *in vacuo* to give the *title compound* (3.79 g, 15.9 mmol) as a colorless oil (1:1 mixture of two diastereoisomers by <sup>1</sup>H NMR), which was used for next step without further purification.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.46 (dt, *J* = 7.5, 1.5 Hz, 4 H, ArH), 7.40-7.29 (m, 6 H, ArH), 5.25 (s, 1 H, PhCHC=O), 5.20 (s, 1 H, PhCHC=O), 4.94 (q, *J* = 5.4 Hz, 1 H, OCHCH<sub>3</sub>), 4.78 (q, *J* = 5.4 Hz, 1 H, OCHCH<sub>3</sub>), 3.71 (s, 6 H, two diastereoisomers' OCH<sub>3</sub>), 3.68-3.39 (m, 4 H, two diastereoisomers' OCH<sub>2</sub>CH<sub>3</sub>), 1.36 (dd, *J* = 5.4, 1.1 Hz, 6 H, two diastereoisomers' CHCH<sub>3</sub>), 1.14 (td, *J* = 7.1, 2.9 Hz, 6 H, two diastereoisomers' OCH<sub>2</sub>CH<sub>3</sub>).

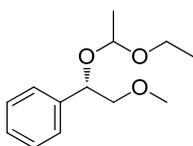
<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 128.75 (ArC), 128.70 (ArC), 128.58 (ArC), 127.40 (ArC), 127.27 (ArC), 99.38 (OCHCH<sub>3</sub>), 98.90 (OCHCH<sub>3</sub>), 75.60 (PhCHC=O), 75.26 (PhCHC=O), 60.84 (OCH<sub>2</sub>CH<sub>3</sub>), 60.70 (OCH<sub>2</sub>CH<sub>3</sub>), 52.42 (OCH<sub>3</sub>), 52.37 (OCH<sub>3</sub>), 20.15 (CHCH<sub>3</sub>), 19.89 (CHCH<sub>3</sub>), 15.29 (OCH<sub>2</sub>CH<sub>3</sub>), 15.27 (OCH<sub>2</sub>CH<sub>3</sub>).

**1.6.9.3. (S)-2-(1-Ethoxyethoxy)-2-phenylethanol (1.163)****1.163**

To a suspension of  $\text{LiAlH}_4$  (0.38 g, 10 mmol) in THF (40 ml) at 0 °C, a solution of **1.162** (2.4 g, 10 mmol) in THF (40 ml) was added dropwise. The mixture was stirred at 0 °C for 30 min.  $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$  (1 g, 6 mmol) was added in 3 portions, and the mixture was stirred at 0 °C for further 15 min. The reaction mixture was filtered through celite, and the solvent removed *in vacuo*.  $\text{Et}_2\text{O}$  (100 mL) was added, washed with water (100 mL), saturated brine (100 mL), dried over  $\text{Na}_2\text{SO}_4$ , filtered, and the solvent removed *in vacuo* to give the *title compound* (2.1 g, 99 %) as colourless oil (1:1 mixture of two diastereoisomers calculated by integrations of corresponding peaks in  $^1\text{H}$  NMR spectrum), which was pure enough for the next step. The analytical data were in agreement with literature values.<sup>109</sup>

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.42-7.27 (m, 10 H,  $2 \times \text{ArH}$ ), 4.82-4.72 (m, 2 H,  $2 \times \text{CH}$ ), 4.63 (dt,  $J = 8.2, 4.6$  Hz, 2 H,  $2 \times \text{CH}$ ), 3.77-3.50 (m, 7 H,  $\text{CH}_2$ ), 3.26 (dq,  $J = 9.3, 7.1$  Hz, 1 H,  $\text{CH}_2$ ), 2.67 (d,  $J = 6.8$  Hz, 1 H, OH), 2.32 (dd,  $J = 9.3, 4.0$  Hz, 1 H, OH), 1.33 (dd,  $J = 5.3, 3.5$  Hz, 6 H,  $2 \times \text{CHCH}_3$ ), 1.21 (t,  $J = 7.0$  Hz, 3 H,  $\text{OCH}_2\text{CH}_3$ ), 1.02 (t,  $J = 7.1$  Hz, 3 H,  $\text{OCH}_2\text{CH}_3$ ).

**Specific Rotation**  $[\alpha]_{\text{D}}^{22} +143$  ( $c$  5.2,  $\text{CHCl}_3$ ) (Lit.<sup>109</sup>:  $[\alpha]_{\text{D}}^{20} +140$  ( $c$  4.9,  $\text{CHCl}_3$ ))

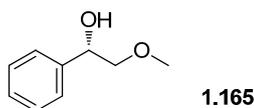
**1.6.9.4. ((S)-1-(1-Ethoxyethoxy)-2-methoxyethyl)benzene (1.164)****1.164**

To a suspension of NaH (0.36 g, 15 mmol) in THF (40 mL) at 0 °C was added alcohol **1.163** (2.1 g, 10 mmol) in THF (10 mL) dropwise. MeI (1.9 mL, 4.3 g, 30.5 mmol)

was then added. The reaction was stirred at r.t. for 24 h. MeOH (5 mL) was added dropwise at 0 °C and the solvent was removed *in vacuo*. To the resulting colourless liquid, Et<sub>2</sub>O (50 mL) was added, and washed with saturated NH<sub>4</sub>Cl (50 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent removed *in vacuo* to give the *title compound* (2.3 g, 100 %) as a colourless liquid.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.47-7.27 (m, 10 H, ArH), 4.95-4.77 (m, 2 H, 2 × PhCH), 4.72 (dd, *J* = 7.7, 4.1 Hz, 1 H, CHCH<sub>3</sub>), 4.60 (q, *J* = 5.4 Hz, 1 H, CHCH<sub>3</sub>), 3.68-3.52 (m, 5 H, 2 × CH<sub>2</sub>), 3.45 (ddd, *J* = 10.4, 4.2, 2.7 Hz, 2 H, CH<sub>2</sub>), 3.37 (d, *J* = 2.2 Hz, 6 H, 2 × OCH<sub>3</sub>), 3.26 (dq, *J* = 9.3, 7.1 Hz, 1 H, CH<sub>2</sub>), 1.28 (d, *J* = 5.3 Hz, 6 H, 2 × CHCH<sub>3</sub>), 1.17 (t, *J* = 7.1 Hz, 3 H, OCH<sub>2</sub>CH<sub>3</sub>), 1.01 (t, *J* = 7.1 Hz, 3 H, OCH<sub>2</sub>CH<sub>3</sub>).

#### 1.6.9.5. (S)-2-Methoxy-1-phenylethanol (1.165)

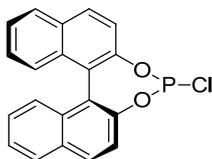


To a solution of ether **1.164** (2 g, 8.92 mmol) in MeOH (50 mL) was added TsOH·H<sub>2</sub>O (0.04 g, 0.2 mmol). The reaction mixture was stirred at r.t. for 24 h. The solvent was removed *in vacuo*. CH<sub>2</sub>Cl<sub>2</sub> (50 mL) was then added to the resulting colourless liquid, and the mixture was washed by saturated NaHCO<sub>3</sub> (50 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent removed *in vacuo* to give the *title compound* (1.36 g, 100 %) as a colourless liquid. The analytical data were in agreement with literature values.<sup>74</sup>

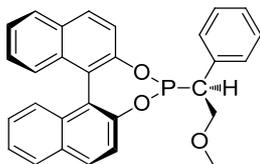
<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.48-7.28 (m, 5 H, ArH), 4.97-4.82 (m, 1 H, CH), 3.58-3.40 (m, 2 H, CH<sub>2</sub>), 3.44 (s, 3 H, CH<sub>3</sub>), 2.72 (d, *J* = 2.3 Hz, 1 H, OH).

IR  $\nu_{\max}$  3416(s), 3062(w), 2982(s), 2888(s), 2823(s), 1493(w), 1451(s), 1323(m), 1193(s), 1115(s), 1062(s), 1027(s), 967(m), 903(m), 755(s), 698(s), 628(m) cm<sup>-1</sup>.

**Specific Rotation**  $[\alpha]_{\text{D}}^{22} +51.1$  (*c* 1.5, CH<sub>2</sub>Cl<sub>2</sub>) (Lit.<sup>74</sup>:  $[\alpha]_{\text{D}}^{22} +51.1$  (*c* 1.4, CH<sub>2</sub>Cl<sub>2</sub>))

**1.6.9.6. (11b*S*)-4-Chlorodinaphtho[2,1-*d*:1',2'-*f*][1,3,2]dioxaphosphepine (1.166)****1.166**

To (*S*)-(-)-1,1'-bi(2-naphthol) (0.5 g, 11.0 mmol, 1.75 mmol) in a schlenk flask (50 mL) was added  $\text{PCl}_3$  (freshly distilled at 80 °C, 1.5 mL, 2.4 g, 17.5 mmol). The mixture was gently refluxed for 5 h. After that, the excess  $\text{PCl}_3$  was removed thoroughly *in vacuo* to give the *title compound* as a white foam solid, which was used directly for the next step without further purification.

**1.6.9.7. (*aS,S*)-4-[(*S*)-2-Methoxy-1-phenylethyl]dinaphtho[2,1-*d*:1',2'-*f*][1,3,2]dioxaphosphepine (1.142)****1.142**

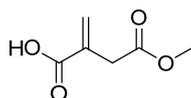
To a solution of the above phosphorous chloride **1.166** in toluene (4 mL) was added  $\text{Et}_3\text{N}$  (0.23 mL, 0.17 g, 1.67 mmol). After cooling to -78 °C, a solution of (*S*)-2-methoxy-1-phenylethanol (0.21 g, 1.40 mmol) in toluene (1 mL) was added to the reaction mixture via cannula dropwise. The reaction was stirred at -78 °C for 1 h, and then warmed to r.t., and stirred at r.t. for 14 h. The reaction mixture was filtered through celite, and the solids were washed with toluene (1 mL). The solvent of the filtrate was removed *in vacuo* to give the *title compound* as a white solid (0.57 g, 87 %), which was used for the next step without further purification. The analytical data were in agreement with literature values.<sup>68</sup>

<sup>1</sup>H NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.97 (d,  $J = 8.9$  Hz, 1 H, Ar*H*), 7.95-7.87 (m, 2 H, Ar*H*), 7.78 (d,  $J = 8.7$  Hz, 1 H, Ar*H*), 7.54 (d,  $J = 8.7$  Hz, 1 H, Ar*H*), 7.46-7.13 (m,

11 H, ArH), 6.87 (d,  $J = 8.8$  Hz, 1 H, ArH), 5.46 (dt,  $J = 9.3, 6.1$  Hz, 1 H, CH), 3.59 (d,  $J = 6.2$  Hz, 2 H, CH<sub>2</sub>), 3.44 (s, 3 H, CH<sub>3</sub>).

<sup>31</sup>P NMR (162 MHz, CDCl<sub>3</sub>)  $\delta$  151.52.

#### 1.6.9.8. 4-Methoxy-2-methylene-4-oxobutanoic acid (1.139)



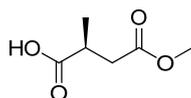
1.139

To a solution of itaconic acid (10 g, 77 mmol) in MeOH (200 mL), Amberlyst 15 (12 g) was added. The mixture was gently stirred at r.t. over 6 days. The brown mixture was filtered through celite, and the solvent was removed *in vacuo*. CH<sub>2</sub>Cl<sub>2</sub> (100 mL) was added to the resulting brown mixture, and was then filtered through celite. The solvent in the filtrate was removed *in vacuo* to give the crude product as a white solid, together with 3 % starting material as estimated by <sup>1</sup>H NMR spectroscopy. Recrystallisation (hexane and EtOAc) gave the *title compound* (10.4 g, 94 %) as white crystals, m.p. 67-68 °C (Lit.<sup>74</sup> m.p. 67-70 °C). The analytical data were in agreement with literature values.<sup>74</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  6.47 (s, 1 H, C=CH<sub>2</sub>), 5.84 (d,  $J = 1.0$  Hz, 1 H, C=CH<sub>2</sub>), 3.71 (s, 3 H, CH<sub>3</sub>), 3.35 (d,  $J = 0.7$  Hz, 2 H, CH<sub>2</sub>).

<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  171.14 (COOH), 170.94 (COOCH<sub>3</sub>), 133.29 (C=CH<sub>2</sub>), 130.97 (C=CH<sub>2</sub>), 52.30 (CH<sub>3</sub>), 37.26 (CH<sub>2</sub>).

#### 1.6.9.9. (S)-4-Methoxy-2-methyl-4-oxobutanoic acid (1.140)



1.140

The preparation of catalyst [Rh(COD)<sub>2</sub>(ligand1.142)<sub>2</sub>]BF<sub>4</sub>: To a solution of [Rh(COD)<sub>2</sub>]BF<sub>4</sub> (70 mg, 0.17 mmol) in (CH<sub>2</sub>Cl)<sub>2</sub> (2 mL) at -25 °C was added

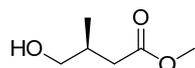
phosphite ligand **1.142** (0.194 g, 0.42 mmol) in (CH<sub>2</sub>Cl)<sub>2</sub> (2 mL) via cannula. The reaction was stirred at -25 °C for 20 min, and then warmed to r.t. and stirred at r.t. for 20 min to give a yellow solution of [Rh(COD)<sub>2</sub>(ligand**1.142**)<sub>2</sub>]BF<sub>4</sub>, and was used immediately.

The mixture of monomethylated acid **1.139** (5 g, 4.7 mmol) in (CH<sub>2</sub>Cl)<sub>2</sub> (60 mL) in a schlenk flask was charged with three vacuum/Ar cycles and three vacuum/H<sub>2</sub> cycles, and then the prepared catalyst [Rh(COD)<sub>2</sub>(ligand**1.142**)<sub>2</sub>]BF<sub>4</sub> solution was added via cannula. The schlenk flask was then charged with H<sub>2</sub> balloon (2 L) and the reaction was stirred at r.t. for 24 h. The solvent was removed *in vacuo*. The resulting yellow oil was purified by Kugelrohr distillations (60-65 °C, 1 mmHg) to give the *title compound* (4.66 g, 92 %) as a clear liquid. The analytical data were in agreement with literature values.<sup>68</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.69 (s, 3 H, OCH<sub>3</sub>), 2.96 (dq, *J* = 14.4, 7.2 Hz, 1 H, CH), 2.74 (dd, *J* = 16.7, 8.0 Hz, 1 H, CH<sub>2</sub>), 2.43 (dd, *J* = 16.7, 6.1 Hz, 1 H, CH<sub>2</sub>), 1.26 (d, *J* = 7.2 Hz, 3 H, CHCH<sub>3</sub>).

**Specific Rotation**  $[\alpha]_{\text{D}}^{22}$  -10.3 (*c* 3.0, CHCl<sub>3</sub>) (Lit.<sup>68</sup>:  $[\alpha]_{\text{D}}^{20}$  -10.5 (*c* 3.9, CHCl<sub>3</sub>))

#### 1.6.9.10. (S)-Methyl 4-hydroxy-3-methylbutanoate (1.141)



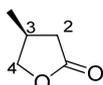
**1.141**

To a solution of the chiral acid **1.140** (5 g, 34.2 mmol) in THF (20 mL) at -30 °C was added BH<sub>3</sub>·SMe<sub>2</sub> (21.4 mL, 2 M in THF, 42.8 mmol). The reaction was warmed to 0 °C and stirred for 5 h. MeOH (40 mL) was added and the mixture was stirred for further 0.5 h. The solvents were removed *in vacuo* to give the *title compound* (4.4, 97 %) as a colourless liquid, which was used for the next step without further purification. The analytical data were in agreement with literature values.<sup>68</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.67 (s, 3 H, OCH<sub>3</sub>), 3.56 (dd, *J* = 10.7, 5.2 Hz, 1 H,

$\text{CH}_2\text{OH}$ ), 3.44 (dd,  $J = 10.7, 6.6$  Hz, 1 H,  $\text{CH}_2\text{OH}$ ), 2.44 (dd,  $J = 14.7, 6.1$  Hz, 1 H,  $\text{CH}_2\text{COO}$ ), 2.25 (dd,  $J = 14.7, 6.5$  Hz, 1 H,  $\text{CH}_2\text{COO}$ ), 2.10 - 2.23 (m, 1 H, CH), 0.95 (d,  $J = 6.6$  Hz, 3 H,  $\text{CHCH}_3$ ).

#### 1.6.9.11. (S)-3-Methylbutyrolactone (1.127)



1.127

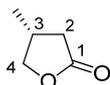
To a solution of chiral alcohol **1.141** (1.32 g, 1 mmol) in  $\text{CH}_2\text{Cl}_2$  (20 mL),  $\text{TsOH} \cdot \text{H}_2\text{O}$  (20 mg, 0.1 mmol) was added. The mixture was stirred at r.t. for 12 h, and then washed by  $\text{NaHCO}_3$  (20 mL), dried over  $\text{Na}_2\text{SO}_4$ , filtered, and the solvent was removed *in vacuo*. The resulting light yellow liquid was purified by flash chromatography (hexane/ $\text{Et}_2\text{O}$  3:1) to give the *title compound* (0.91 g, 91 %) as a colourless liquid. The analytical data were in agreement with literature values.<sup>68</sup>

$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  4.41 (dd,  $J = 8.8, 7.3$  Hz, 1 H, H4a), 3.87 (dd,  $J = 8.8, 6.4$  Hz, 1 H, H4b), 2.69-2.58 (m, 2 H, H3 and H2a), 2.19-2.07 (m, 1 H, H2b), 1.16 (d,  $J = 6.6$  Hz, 3 H,  $\text{CH}_3$ ).

**Specific Rotation**  $[\alpha]_{\text{D}}^{22} -24.3$  ( $c$  1.0, MeOH), (Lit.:  $[\alpha]_{\text{D}}^{20} -24.8$  ( $c$  4.35, MeOH))<sup>68</sup>

### 1.6.10. Preparation of lactones from tigogenin

#### 1.6.10.1. (R)-3-Methylbutyrolactone (1.69)<sup>69</sup>



1.69

(*R*)-3-Methylbutyrolactone was prepared with some modification to the method reported by Cheng et al.<sup>69</sup>

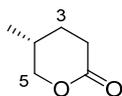
AcOOH was prepared by stirring AcOH (440 mL), H<sub>2</sub>SO<sub>4</sub> (8 mL, 0.16 mol) and H<sub>2</sub>O<sub>2</sub> (30 % w/w, 160 mL, 1.4 mol) in dark for 24 h. Note: AcOOH should not be stored in lab for large quantity as it may explode.

To the mixture of tigogenin (50 g, 0.12 mol) in AcOH (400 mL) was added H<sub>2</sub>SO<sub>4</sub> (5.5 mL, 0.11 mol), and followed by I<sub>2</sub> (4 g, 0.015 mol). Then the prepared AcOOH, was added slowly over 1 h, during which the temperature was kept around 20 °C by cooling with water bath. After addition, the mixture was stirred in dark for 12 hours. A solution of Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> (50 g) in water (200 mL) was added during which the mixture was cooled with ice bath. The solids were removed by filtration, and solvent was removed *in vacuo*. Afterwards, MeOH (200 mL), H<sub>2</sub>O (30 mL), and NaOH (22 g) were added. The mixture was stirred at r.t. for 14 h. Water (100 mL) was added, and the mixture was stirred for another 30 min, and then filtered, and the solids were washed with water (100 mL). The combined filtrates were washed with CH<sub>2</sub>Cl<sub>2</sub> (3 × 250 mL), and the aqueous layer was collected and concentrated *in vacuo* to 100 mL. HCl (35 %) was added drop by drop until pH 2, and then the aqueous mixture was stirred at r.t. for 3 h. The aqueous mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 150 mL), and the combined organic layers were washed with NaHSO<sub>3</sub> solution, saturated NaHCO<sub>3</sub> (250 mL), saturated NH<sub>4</sub>Cl (250 mL), brine, and then the solvent was removed *in vacuo*. The resulting yellow liquid was purified by flash chromatography (hexane/Et<sub>2</sub>O 1:1) to give the *title compound* (4.45 g, 37 %) as a colourless liquid.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 4.41 (dd, *J* = 8.8, 7.2 Hz, 1 H, H4a), 3.87 (dd, *J* = 8.8, 6.3 Hz, 1 H, H4b), 2.72-2.58 (m, 2 H, H3 and H2a), 2.21-2.07 (m, 1 H, H2b), 1.16 (d, *J* = 6.5 Hz, 3 H, CH<sub>3</sub>).

<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 177.37(C=O), 74.82(C4), 36.27(C2), 30.53(C3), 18.07(CH<sub>3</sub>).

**Specific Rotation**  $[\alpha]_D^{22} +24.9$  (*c* 0.9, MeOH) (Lit.<sup>110</sup>:  $[\alpha]_D^{22} +22.5$  (*c* 4.07, MeOH)).

**1.6.10.2. (R)-4-Methyl- $\delta$ -valerolactone (1.146)<sup>111</sup>****1.146**

To a mixture of tigogenin (16.7 g, 40 mmol) in  $\text{CH}_2\text{Cl}_2$  (150 mL) and formic acid (300 mL) was added  $\text{H}_2\text{O}_2$  (30 % w/w, 30 mL, 291 mmol). After heating at 50 °C for 6 h, the mixture was cooled with ice bath, and saturated  $\text{NaHSO}_3$  (50 mL) was added. The organic layer was separated, and the aqueous layer was further extracted with  $\text{CH}_2\text{Cl}_2$  ( $2 \times 150$  mL). The combined organic layers were washed with saturated brine, and the solvent removed *in vacuo*. MeOH (400 mL) and NaOH solution (6 M, 60 mL) was added, and then heated at 65 °C for 2 h. After removing the solvent *in vacuo*, water (300 mL) was added. The precipitates were removed by filtration and the filtrate was extracted with EtOAc (250 mL). The combined aqueous layers were acidified with HCl (5 M) to pH 2, stirred for further 1 h, and extracted with EtOAc ( $3 \times 500$  mL). The combined organic layers were washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , filtered and the solvent removed *in vacuo*. The resulting yellow liquid was purified by flash chromatography (hexane/EtOAc 3:1) to give the *title compound* (1.5 g, 32.8 %) as a colourless liquid. The analytical data were in agreement with literature values.<sup>111</sup>

**$^1\text{H}$  NMR** (300 MHz,  $\text{CDCl}_3$ )  $\delta$  4.31 (ddd,  $J = 11.1, 4.5, 2.1$  Hz, 1 H, H5a), 3.91 (dd,  $J = 11.1, 9.9$  Hz, 1 H, H5b), 2.70-2.46 (m, 2 H,  $\text{CH}_2\text{COO}$ ), 2.14-1.90 (m, 2 H, H3a and CH), 1.59-1.44 (m, 1 H, H3b), 1.01 (d,  $J = 6.6$  Hz, 3 H,  $\text{CH}_3$ ).

**Specific Rotation**  $[\alpha]_{\text{D}}^{22} +16.9^\circ$  ( $c$  1.0,  $\text{CHCl}_3$ ) (Lit.<sup>111</sup>:  $[\alpha]_{\text{D}}^{22} +15.7^\circ$  ( $c$  0.95,  $\text{CHCl}_3$ ))

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## Chapter 2. Enantioselective Syntheses of Selected Chiral Isoprenoid Targets

*In this Chapter, the methodology developed in Chapter 1 was applied to the synthesis of several chiral isoprenoid targets, including a sex pheromone, fragrances, the side chain of zaragozic acid C, an intermediate of tuberculostearic acid, and the side chain of vitamin E. In addition, (R)-2-methylheptylisonicotinate, purportedly a natural product with antibacterial and antifungal activities, was also prepared. Evidence is presented which contradicts both the reported structure as well as its reported bioactivity. More general application of the lactone cleavage reaction to chain elongation is also discussed employing simple, unbranched lactones.*

## 2.1. Syntheses of sex pheromones

### 2.1.1. The significance of optical activity for sex pheromones

Pheromones are chemicals used to convey information within the same species, and such communications are effective even at low concentration. Among all the pheromones, sex pheromones, especially of those species relevant to economically important crops, attract most attention with the aim of insect control.<sup>1</sup>

Insect control is an issue of continuing importance to agriculture. Nowadays, the most widely used approach is through the application of insecticides. However, insecticides not only kill pests, but can also harm through the application of the environment and humans. Some of the insecticides are not decomposed easily, and may result in concentration within organisms higher up the food chain.<sup>2</sup> Despite the fact that insecticides are the major contributor to the increase in agricultural productivity during the last century, nearly all have the potential harm or threaten to the ecosystem.<sup>3</sup> Dichlorodiphenyltrichloroethane, for example, was widely used as pesticide, and effectively controlled the population of pests.<sup>4</sup> At the same time, it reduced the thickness of bird egg shells, causing reductions in bird populations, and concentration in fat through the food chain, resulting in the tendency for concentration in the human body.<sup>4</sup> In addition, pests often develop resistance to insecticides. Considering the long-term effects of insecticide, it is imperative to seek alternative approaches as the substitutions of conventional insecticides.

Sex pheromones are generated and released by organisms for attracting opposite sex to mate with them. Applying sex pheromones would cause mating disruption and consequently control reproduction cycle.<sup>5</sup> Superior to traditional insecticides, sex pheromones are highly effective at low concentration dispersion, and they are species-specific, with no disruption of other organisms or environment, making it a sustainable and desirable substitution for the conventional insecticides, especially with the increasing demand for organic food and environmental friendly farming.<sup>5</sup>

The first identified insect sex pheromone is bombykol, with the structure of (*E,Z*)-10,12-hexadecadienol,<sup>6</sup> isolated from silkworm moth, *Bombyx mori* in 1959.

Since then, this field attracted vast research. Numerous sex pheromones were discovered and identified based on extensive study in thousands of insect species in the past three decades.<sup>6</sup>

The accessibility of sex pheromones is a challenging problem to study their bioactivity and the commercial application. The elucidation of the mechanism for the first sex pheromone, bombykol, consumed half million female abdomens and massive purification work to isolate sex pheromones from Nature over 20 years.<sup>6</sup> Synthetic sex pheromones as another source of sex pheromones, have been used as bait in the traps for detecting the insect populations.<sup>7</sup> Development of cost-efficient total synthetic approaches towards sex pheromones is required for the scale up of sex pheromones to ensure a competitive price in promotion the usage of sex pheromones.

Many sex pheromones are chiral compounds, and the enantiomeric purity has an effect in their bioactivities. For majority of chiral pheromones, only one single enantiomer is bioactive, and the opposite enantiomer does not inhibit the action of the pheromone.<sup>8</sup> However, for some pheromones, only one enantiomer is bioactive, and the enantiomers inhibit the action of the pheromone.<sup>9</sup>

In all the chiral sex pheromones, there is a group of compounds with chiral methyl branches in their structures (**Figure 2.1**). (*S*)-9-Methylnonadecane **2.1** has been identified as the major compound of sex pheromone produced by the female moths and its larvae, cotton leafworm (*Alabama argillacea*), which is considered a pest feeding on the cotton leaves, twigs and buds.<sup>10</sup> (*S*)-7-Methylheptadecane **2.2** is one of the active components of the female-produced sex pheromone of the spring hemlock looper moth (*Lambdina athasaria*) and the pitch pine looper moth (*L. pellucidaria*),<sup>11</sup> both are forest pests in north eastern America.

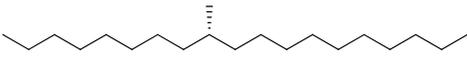
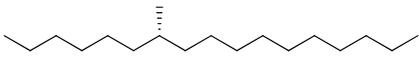
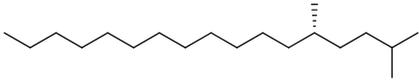
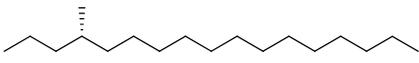
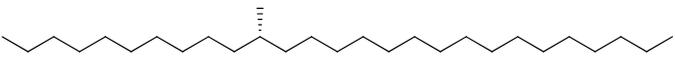
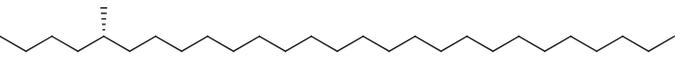
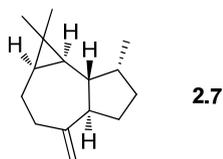
	Sex pheromone of female moths ( <i>Alabama argillacea</i> )
	Sex pheromone of female spring hemlock looper moth ( <i>L. athasaria</i> ) female pitch pine looper moth ( <i>L. pellucidaria</i> )
	Sex pheromone of western hemlock looper ( <i>L. fiscellaria lugubrosa</i> Hulst)
	Sex pheromone of the peach leafminer moth ( <i>Lyonetia clerkella</i> Linne)
	Sex pheromone for Longhorned Beetles ( <i>Tetropium fuscum</i> and <i>T. cinnamopterum</i> )
	Sex pheromone of ants ( <i>Dicamma species</i> )

Figure 2.1: Pheromones with chiral isoprenoid units

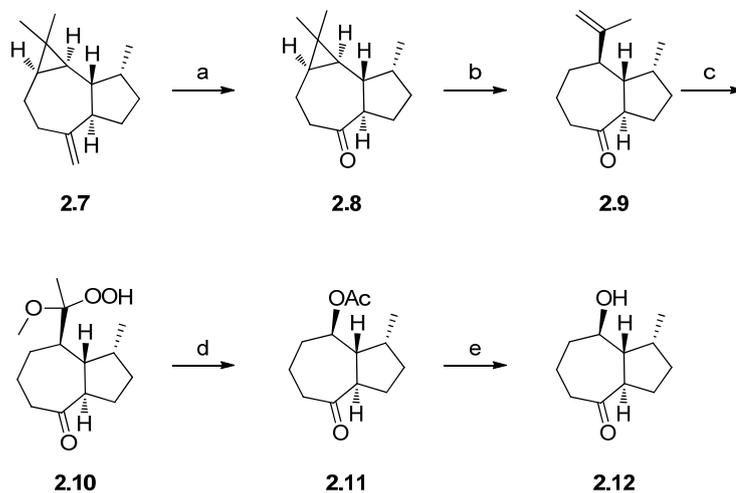
### 2.1.2. Previous synthesis of (*S*)-9-methylnonadecane

(*S*)-9-Methylnonadecane **2.1** was synthesised by Lamers and co-workers in 2003 from (+)-aromadendrene in 15 steps.<sup>12</sup> (+)-Aromadendrene **2.7** (Figure 2.2) is contained in the commercially available distillation tail of *Eucalyptus globulus* leaf oil, making it a cheap starting material for chiral pool synthesis.

Figure 2.2: (+)-Aromadendrene **2.7**

(+)-Aromadendrene **2.7** was converted to keto-alcohol **2.12** over five steps (Scheme 2.1).<sup>13</sup> Ozonolysis of **2.7** gave ketone **2.8** after recrystallisation. Acid catalysed

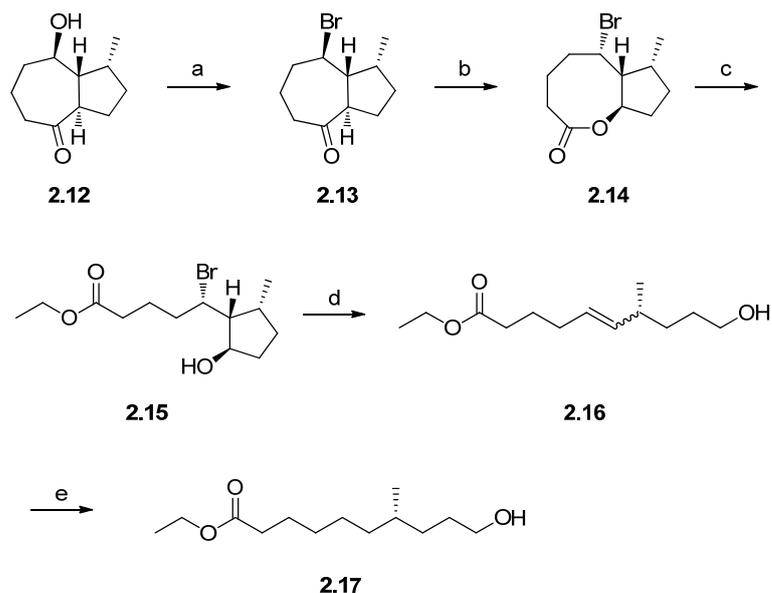
selective cleavage of the cyclopropane ring in **2.8** afforded the alkene **2.9**. Ozonolysis of **2.9** in MeOH gave a peroxide intermediate **2.10**, which was treated with Ac<sub>2</sub>O to give the acetate **2.11**, followed by hydrolysis to give alcohol **2.12**.<sup>13</sup>



Reagents and conditions: a) O<sub>3</sub>, -78 °C to r.t., EtOH; b) HCl, EtOH, reflux 1.5 h; c) O<sub>3</sub>, CCl<sub>4</sub>/MeOH, -30°C; d) Ac<sub>2</sub>O, Et<sub>3</sub>N, DMAP, -30 °C; e) NaOCH<sub>3</sub>, MeOH.

**Scheme 2.1:** Synthesis of chiral intermediates from (+)-aromadendrene **2.7**<sup>13</sup>

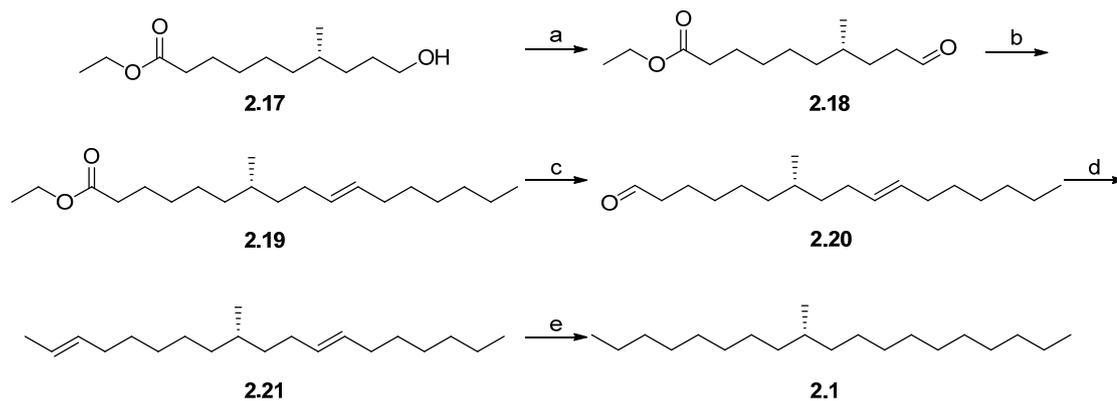
Alcohol **2.12** was brominated to give **2.13** by Appel reaction (**Scheme 2.2**).<sup>12</sup> After Baeyer-Villiger oxidation of **2.13**, lactone **2.14** was obtained, which was ring opened to give the more stable ester **2.15**. Grob fragmentation of **2.15** was carried out using NaOEt in EtOH, and followed by the reduction of the aldehyde intermediate with NaBH<sub>4</sub> *in situ* to give alcohol **2.16**, which was subjected to hydrogenation reaction using Pd/C under H<sub>2</sub>, to give chiral intermediate **2.17**.



Reagents and conditions: a)  $\text{CBr}_4$ ,  $\text{PPh}_3$ ; b) *m*CPBA; c) TsOH, EtOH; d) NaOEt,  $\text{NaBH}_4$ ; e) Pd/C,  $\text{H}_2$ .

**Scheme 2.2:** Synthesis of chiral intermediate **2.17**<sup>12</sup>

As a versatile chiral intermediate, **2.17** could be used for the synthesis of several chiral methyl-branched natural products.<sup>12</sup> The sex pheromone (*S*)-9-methylnonadecane **2.1** was synthesised from **2.17** over five steps by Groot and co-workers (**Scheme 2.3**). **2.17** was firstly oxidised to give aldehyde **2.18**, which was subject to Wittig reaction to give the unsaturated alkene ester **2.19**. **2.19** was reduced to the corresponding aldehyde **2.20** using DIBALH, and followed by Wittig reaction to give the alkene **2.21**. After hydrogenation using Pd/C, (*S*)-9-methylnonadecane **2.1** was obtained in 6.3 % yield over 14 steps from **2.7**.

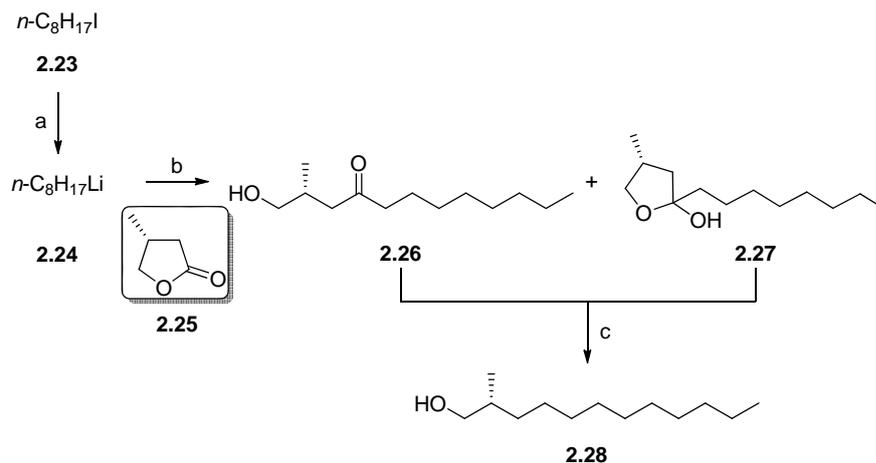


Reagents and conditions: a) PCC; b)  $[\text{C}_7\text{H}_{15}\text{PPh}_3]^+\text{T}^-$ , *n*-BuLi; c) DIBALH; d)  $[\text{C}_2\text{H}_5\text{PPh}_3]^+\text{T}^-$ , *n*-BuLi; e) Pd/C,  $\text{H}_2$ .

**Scheme 2.3:** Synthesis of sex pheromone **2.1** from chiral intermediate **2.17**<sup>12</sup>

### 2.1.3. Synthesis of the sex pheromone, (*S*)-9-methylnonadecane, via the lactone ring cleavage reaction

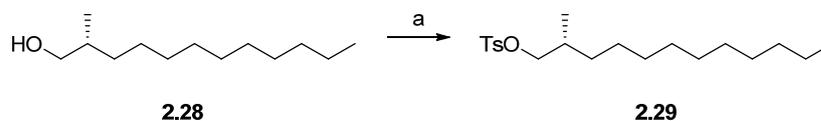
Alcohol **2.28**, an intermediate for synthesis of (*S*)-9-methylnonadecane **2.1**, was synthesised from (*R*)-3-methylbutyrolactone **2.25** via our lactone ring cleavage reaction (**Scheme 2.4**). Lactone **2.25** was reacted with *n*- $\text{C}_8\text{H}_{17}\text{Li}$  (generated *in situ* by reacting *n*- $\text{C}_8\text{H}_{17}\text{I}$  with 2.2 eq. *t*-BuLi at  $-78\text{ }^\circ\text{C}$  for 1 h) in  $\text{Et}_2\text{O}$  at  $-78\text{ }^\circ\text{C}$  for 3 h giving keto-alcohol **2.26** and hemiketal **2.27** in an overall 76 % yield. The identity of **2.26** was confirmed by the appearance of peaks at 3.35-3.60 ppm in the  $^1\text{H}$  NMR spectrum, which accounted for the new hydroxymethylene protons. **2.26** was then reduced by the modified Clemmensen reduction using 10 eq. TMSCl and 10 eq. Zn in MeOH and  $\text{Et}_2\text{O}$  at  $0\text{ }^\circ\text{C}$  for 1 h,<sup>14</sup> giving (*R*)-2-methyldodecanol **2.28** in 96 % yield. The completion of this reaction was detected by the disappearance of the peaks at 2.30-2.60 ppm region in  $^1\text{H}$  NMR spectrum, which accounted for the protons in the ketone  $\alpha$ -methylenes. The enantiomeric purity was confirmed by specific rotation, and was in  $> 99\%$  ee.<sup>15</sup>



Reagents and conditions: a)  $t\text{-BuLi}$  (2.1 eq.),  $\text{Et}_2\text{O}$ ,  $-78\text{ }^\circ\text{C}$ , 1 h; b)  $\text{Et}_2\text{O}$ , 3 h; c)  $\text{TMSCl}$  (10 eq.),  $\text{Zn}$  (10 eq.),  $\text{MeOH}$ ,  $0\text{ }^\circ\text{C}$ , 1 h.

**Scheme 2.4:** Synthesis of chiral intermediate alcohol **2.28** from lactone

(*R*)-2-Methyldodecanol **2.28** could be a useful intermediate for preparing many natural products. **2.28** was treated with 1.5 eq.  $\text{TsCl}$  in the presence of 0.1 eq.  $\text{DMAP}$  and 3 eq. pyridine to give tosylate **2.29** after stirring at r.t. for 14 h (**Scheme 2.5**). The identity of the **2.29** was confirmed by the peaks at 7.30-7.80 ppm in the  $^1\text{H}$  NMR spectrum, which accounted for the protons in the tosylate group. The chemical shift for the  $\text{CH}_2\text{O}$  protons shifted downfield from 3.50 ppm to 3.84 ppm in the  $^1\text{H}$  NMR spectrum.

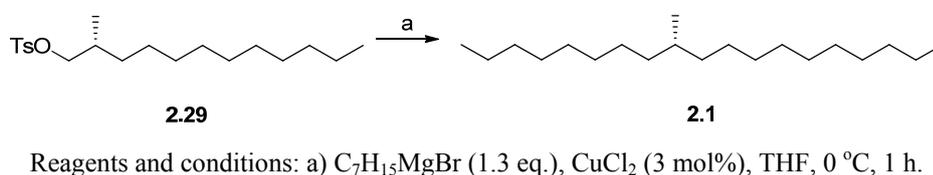


Reagents and conditions: a)  $\text{TsCl}$  (1.5 eq.),  $\text{DMAP}$  (0.1 eq.), pyridine (3 eq.),  $\text{CH}_2\text{Cl}_2$ , r.t., 14 h.

**Scheme 2.5:** Synthesis of tosylate **2.29** from alcohol **2.28**

The tosylate **2.29** was readily coupled with Grignard reagents for the synthesis of chiral alkanes. The Grignard reagents were generated by adding  $n\text{-C}_7\text{H}_{15}\text{Br}$  to 3 eq.  $\text{Mg}$  turning in  $\text{THF}$  using a small  $\text{I}_2$  crystal as activating agent. The mixture was refluxed for 2 h, and then the concentration of the generated Grignard reagent was determined by titration.<sup>16</sup>

Coupling tosylate **2.29** with Grignard reagents in the presence of  $\text{CuCl}_2$  as catalyst giving alkanes has been reported.<sup>17</sup> **2.29** was coupled with  $n\text{-C}_7\text{H}_{15}\text{MgBr}$  in the presence of 3 mol%  $\text{CuCl}_2$  in THF at  $0^\circ\text{C}$  for 1 h and then r.t. for 2 h to give the target sex pheromone (*S*)-9-methylnonadecane **2.1** in 73 % yield (**Scheme 2.6**). The completion of the reaction was indicated by the disappearance of the peaks at 7.30-7.80 ppm and 3.50 ppm in  $^1\text{H}$  NMR spectrum. The product was purified by eluting through a short plug of silica with hexane. The identity of **2.1** was confirmed by the appearance of peaks at 0.84 ppm (doublet) and 0.88 ppm (triplets) in the  $^1\text{H}$  NMR spectrum, which accounted for the  $\text{CHCH}_3$  and two  $\text{CH}_2\text{CH}_3$  respectively.



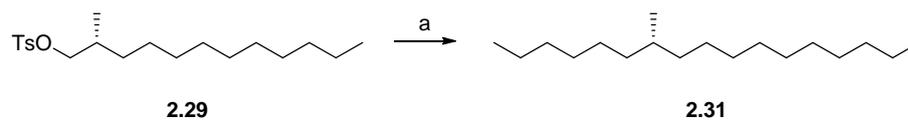
**Scheme 2.6:** Synthesis of sex pheromone **2.1** from tosylate **2.29**

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In conclusion, an asymmetric synthesis of sex pheromone, (*S*)-9-methylnonadecane, was developed.  $\gamma$ -Lactone **2.25** was ring opened by alkyllithium **2.24**, followed by modified Clemmensen reduction to give alcohol **2.28**. **2.28** was converted to tosylate **2.29**, which was then coupled with Grignard reagent to give (*S*)-9-methylnonadecane **2.1**. The total synthesis of **2.1** took four steps from  $\gamma$ -lactone **2.25** in an overall yield of 54 %.

#### 2.1.4. Potential for the synthesis of other sex pheromones

Although time constraints did not permit further exploration of pheromone synthesis, it is clear that this approach could be easily applied to other selected targets, such as (*S*)-7-methylheptadecane **2.31** (**Scheme 2.7**).



Reagents and conditions: a)  $C_5H_{11}MgBr$ ,  $CuCl_2$ , THF.

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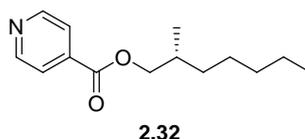
**Scheme 2.7:** Proposed synthesis for another sex pheromone **2.31**

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## 2.2. Synthesis of (*R*)-2-methylheptylisonicotinate, a purported naturally occurring bioactive agent

### 2.2.1. (*R*)-2-Methylheptylisonicotinate

In 2001, Bora and co-workers isolated a “brown solid mass” from tea garden soil in north east of India, and the compound was named as *Streptomyces* sp. 201. It was reported to possess antibacterial and antifungal activity against a variety of fungi and bacteria.<sup>18,19</sup> The structure of this extracted compound was elucidated as 2-methylheptylisonicotinate by NMR, IR, MS, and element analyses. In 2004, Bora and co-workers reported the synthesis of both (*R*)- and (*S*)-2-methylheptylisonicotinates. They compared the data with naturally extracted compound, and claimed the full structure, including absolute stereochemistry of the natural *Streptomyces* sp. 201 as (*R*)-2-methylheptylisonicotinate **2.32** (Figure 2.3). In addition, their antimicrobial experiments showed that besides its previously reported bioactivities, isonicotinate **2.32** also showed strong antimicrobial activity against *Mycobacterium tuberculosis*.<sup>20</sup>



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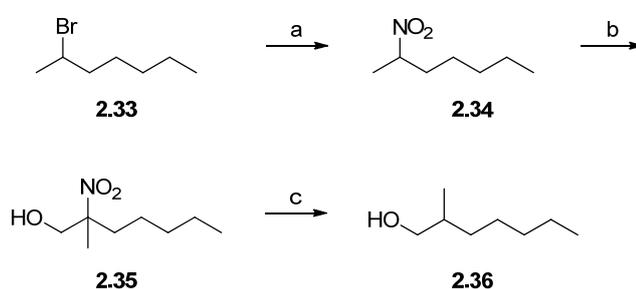
**Figure 2.3:** The claimed antibacterial and antifungal compound<sup>18,19</sup>

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Tuberculosis is a highly infectious disease. According to the World Health Organisation’s report, in 2011, 8.8 million people fell ill with tuberculosis and 1.4 million died it.<sup>21</sup> *M. tuberculosis* is the pathogenic bacterial species, which causes tuberculosis. Since Bora and co-workers reported isonicotinate **2.32** demonstrated antimicrobial activity against *M. tuberculosis*, **2.32** has attracted considerable interest.

### 2.2.2. Bora's reported synthesis of isonicotinate

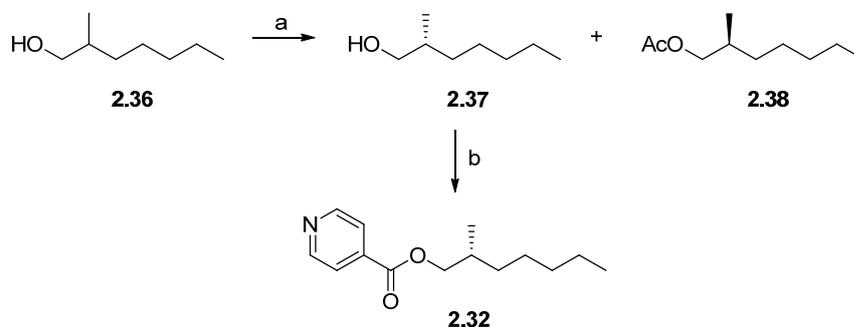
Bora and co-workers reported the synthesis of (*R*)-2-methylheptylisonicotinate **2.32** from racemic 2-methylheptanol **2.36**, which was prepared in three steps from bromide **2.33** (Scheme 2.8).<sup>20</sup> Bromide **2.33** was treated with NaNO<sub>2</sub> to give nitroalkane **2.34**, which was reacted with formaldehyde to give nitro-alcohol **2.35**. After denitration of **2.35** by refluxing in benzene in the presence of azobisisobutyronitrile, racemic 2-methylheptanol **2.36** was obtained.



Reagents and conditions: a) NaNO<sub>2</sub>, DMF; b) HCHO, K<sub>2</sub>CO<sub>3</sub>; c) Azobisisobutyronitrile, reflux.

**Scheme 2.8:** Preparing racemic alcohol **2.36** by Bora<sup>20</sup>

Racemic alcohol **2.36** was resolved by *Candida rogusa* lipase to give chiral alcohol **2.37** in 49 % yield and 96 % ee, together with chiral acetate **2.38** in 49 % yield and 97 % ee (Scheme 2.9).<sup>20</sup> Esterification of chiral alcohol **2.37** with isonicotinoyl chloride·HCl gave (*R*)-2-methylheptylisonicotinate **2.32**.



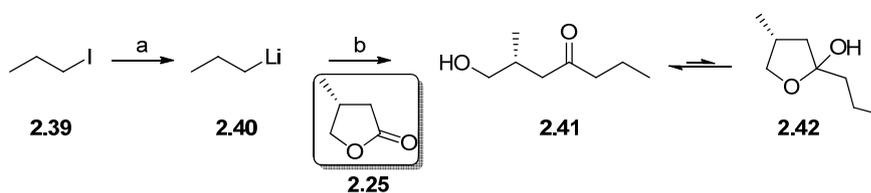
Reagents and conditions: a) *C. rogusa* lipase, vinyl acetate, hexane; b) Isonicotinoyl chloride·HCl, Amberlyst A-21; c) K<sub>2</sub>CO<sub>3</sub>/MeOH, 1 h; d) Isonicotinoyl chloride·HCl, Amberlyst A-21.

**Scheme 2.9:** Synthesis of isonicotinate **2.32** by Bora<sup>20</sup>

### 2.2.3. Synthesis via the lactone ring cleavage reaction

Isonicotinate **2.32** appeared to be an excellent candidate target for our methodology. Alkyl lithium **2.40**, treated with chiral lactone **2.25** gave the keto-alcohol **2.41**, followed by reduction to the alcohol **2.37**, which is the key intermediate of isonicotinate.

Thus,  $n\text{-C}_3\text{H}_7\text{Li}$ , prepared *in situ* by treating  $n\text{-C}_3\text{H}_7\text{I}$  with 2.1 eq.  $t\text{-BuLi}$  in  $\text{Et}_2\text{O}$  at  $-78\text{ }^\circ\text{C}$  for 1 h, was added to 1.1 eq.  $\gamma$ -lactone **2.25** at  $-78\text{ }^\circ\text{C}$ , and reacted for 3 h to give keto-alcohol **2.41** in 72 % yield (**Scheme 2.10**).

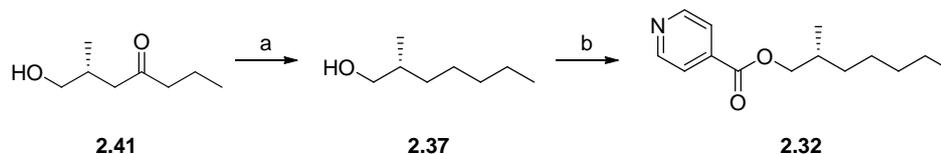


Reagents and conditions: a)  $t\text{-BuLi}$  (2.1 eq.),  $\text{Et}_2\text{O}$ ,  $-78\text{ }^\circ\text{C}$ , 1 h; b) **2.25** (1.1 eq.),  $\text{Et}_2\text{O}$ ,  $-78\text{ }^\circ\text{C}$ , 3 h.

**Scheme 2.10:** Synthesis of keto-alcohol **2.41** via lactone ring cleavage reaction

The ketone group in keto-alcohol **2.41** was reduced as before by modified Clemmensen reduction (**Scheme 2.11**). The completion of this reaction was detected by the disappearance of the peaks at 2.30-2.60 ppm region in  $^1\text{H}$  NMR spectrum, which accounted for the protons in the ketone  $\alpha$ -methylenes. Alcohol **2.37** was obtained in 95 % yield and 98 % ee after aqueous work up and flash chromatography purification.<sup>22</sup>

Esterification **2.37** with 1.1 eq. isonicotinoyl chloride·HCl in pyridine at r.t. for 16 h gave isonicotinate **2.32** as a colourless liquid in 94 % yield (**Scheme 2.11**). The peaks accounted for protons in  $\text{CH}_2\text{O}$  shifted downfield from 3.35-3.60 ppm to 4.10-4.30 ppm in  $^1\text{H}$  NMR spectrum. The whole procedure started from  $\gamma$ -lactone **2.25** and gave isonicotinate **2.32** as a colourless liquid in three steps in an overall yield of 64 %.



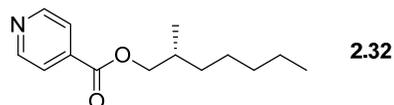
Reagents and conditions: a) TMSCl (10 eq.), Zn (10 eq.), MeOH/CH<sub>2</sub>Cl<sub>2</sub> (3:1), 0 °C; b) Isonicotinoyl chloride·HCl (1.1 eq.), pyridine, r.t., 16 h.

**Scheme 2.11:** Synthesis of chiral isonicotinate **2.32** from the chiral keto-alcohol **2.41**

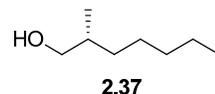
The data for isonicotinate **2.32** synthesised via our lactone ring cleavage reaction were significantly at odds with both the naturally occurring compound and the synthesised one by Bora and co-workers (**Table 2.1**). After careful analysis, it was found that the <sup>1</sup>H NMR and <sup>13</sup>C NMR data for Bora's synthetic, *R*-configured compound were not entirely consistent with that of their isolated natural compound, especially for the chemical shifts for the protons in the isonicotinic ring.<sup>18,20</sup> Besides, the naturally occurring compound was claimed as “a brown solid mass”,<sup>18</sup> while their synthetic compound was claimed as an “oily” substance.<sup>20</sup>

Bora and co-workers used *C. rugosa* lipase for the preparation of 2-methyl alcohol **2.37**,<sup>20</sup> Only specific rotation data were provided for **2.37** by them, which were in agreement with our measurement data and previous literature (**Table 2.2**).<sup>22,23</sup> However, the reference they cited there used a different lipase, *Pseudomonas cepacia* lipase, for resolution.<sup>24</sup> *C. rugosa* lipase was used for resolution of 2-methylalkanoic acids.<sup>25</sup> To the best of our knowledge, no other supporting information showed *C. rugosa* lipase could be used for resolution of 2-methyl alcohols.

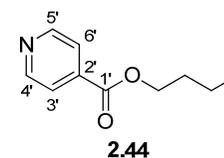
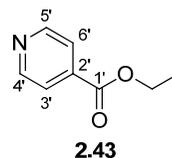
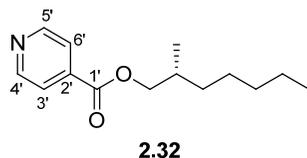
Bora and co-workers claimed the chemical shifts of the protons in isonicotinic ring of both the natural compound and their synthetic compounds were at 7.7 ppm and 7.5 ppm. These shifts are clearly inconsistent with an isonicotinic ring. There is ample evidence in the literature for the chemical shifts for commercially available alkyl isonicotinates (**Table 2.3**),<sup>26</sup> and indeed typical values are around 8.78 ppm (ortho position to N) and 7.84 ppm (meta position to N), which matched with our data perfectly. We are confident that with the evidence from HRMS, NMR, and IR analyses, the compound we synthesised via our lactone ring cleavage reaction is indeed (*R*)-2-methylheptylisonicotinate **2.32**.

**Table 2.1:** Data comparison for (*R*)-2-methylheptylisonicotinate

	Our data	Bora's data for their synthetic compound <sup>20</sup>	Bora's data for the natural extracting product <sup>18</sup>
<sup>1</sup> H NMR	8.78 (dd, <i>J</i> = 4.5, 1.6 Hz, 2H), 7.84 (dd, <i>J</i> = 4.4, 1.6 Hz, 2H), 4.25 (dd, <i>J</i> = 10.7, 5.8 Hz, 1H), 4.14 (dd, <i>J</i> = 10.7, 6.8 Hz, 1H), 2.09-1.77 (m, 1H), 1.51-1.17 (m, 8H), 1.02 (d, <i>J</i> = 6.8 Hz, 3H), 0.89 (t, <i>J</i> = 6.9 Hz, 3H).	7.91 (dd, <i>J</i> = 4, 6 Hz, 2H), 7.51 (dd, <i>J</i> = 4, 6 Hz, 2H), 4.22 (d, <i>J</i> = 6 Hz, 2H), 1.48 (m, 1H), 1.45 (m, 2H), 1.25-1.42 (br, 6H), 0.93 (d, <i>J</i> = 7 Hz, 3H), 0.88 (t, <i>J</i> = 6.15 Hz, 3H).	7.7 (dd, <i>J</i> = 4.6 Hz, 2H), 7.5 (dd, <i>J</i> = 4, 6 Hz, 2H), 4.22 (m, 2H), 1.45 (m, 1H), 1.42 (m, 2H), 1.25-1.41 (m, 6H), 0.93 (d, <i>J</i> = 7 Hz, 3H), 0.88 (t, <i>J</i> = 7 Hz, 3H).
<sup>13</sup> C NMR	165.34, 150.75, 137.83, 122.99, 70.81, 33.51, 32.79, 32.15, 26.65, 22.74, 17.11, 14.18.		168.09, 132.87, 131.23, 129.17, 68.52, 39.16, 30.77, 29.32, 24.17, 23.35, 14.39, 11.83.
MS	[M <sup>+</sup> +H]: 236.1644 (Calcd.: 236.1645)		235
[α] <sub>D</sub>	[α] <sub>D</sub> <sup>22</sup> -2.5 ° ( <i>c</i> 0.6, CHCl <sub>3</sub> )	[α] <sub>D</sub> <sup>22</sup> +8.3 ° ( <i>c</i> 0.6, CHCl <sub>3</sub> ) <sup>20</sup>	

**Table 2.2:** Data comparisons for (*R*)-2-methylheptanol **2.37**

	Our data	Bora's data <sup>20</sup>	Other reference's data <sup>23,27</sup>
<sup>1</sup> H NMR	3.56-3.38 (m, 2 H), 1.67-1.56 (m, 1H), 1.45-1.03 (m, 8 H), 0.96-0.87 (m, 6 H).	None	3.52 (dd, <i>J</i> = 10.4, 5.7 Hz, 1 H), 3.43 (dd, <i>J</i> = 10.4, 5.7 Hz, 1 H), 1.61 (m, 1 H), 1.44-1.20 (m, 8 H), 0.92 (t, <i>J</i> = 6.7 Hz, 3 H), 0.89 (t, <i>J</i> = 7.1 Hz, 3 H).
<sup>13</sup> C NMR	68.61, 35.94, 33.26, 32.30, 26.79, 22.79, 16.73, 14.22.	None	68.4, 35.8, 33.1, 32.1, 26.6, 22.6, 16.55, 14.05.
[α] <sub>D</sub>	+12.8 ° ( <i>c</i> 0.6, CHCl <sub>3</sub> )	+13.8° ( <i>c</i> 0.8, CHCl <sub>3</sub> )	<i>S</i> : -13.1 ° ( <i>c</i> 1.15, CHCl <sub>3</sub> )

**Table 2.3:** Data for alkyl isonicotinates<sup>20,26</sup>

	<b>2.32</b>	<b>2.43</b> <sup>26</sup>	<b>2.44</b> <sup>26</sup>	Bora's data for their synthetic compound <sup>20</sup>
<sup>1</sup> H NMR	8.78 (H4', H5'), 7.84 (H3', H6').	8.77(H4', H5'), 7.85(H3', H6').	8.78(H4', H5'), 7.85(H3', H6').	7.7 (2H), 7.5 (2H).
<sup>13</sup> C NMR	165.34 (C1'), 150.75 (C4', C5'), 137.83 (C2'), 122.99 (C3', C6').	165.01(C1'), 150.63(C4', C5'), 137.68(C2'), 122.84(C3', C6').	165.11(C1'), 150.61(C4', C5'), 137.72(C2'), 122.87(C3', C6').	168.09, 132.87, 131.23, 129.17.

Bora and co-workers claimed both of (*R*)-2-methylheptylisonicotinate **2.32** and octyl isonicotinate **2.45** (Figure 2.4) showed activity against *Bacillus subtilis* and *E. coli* at 16  $\mu\text{g/mL}$  and 30  $\mu\text{g/mL}$  respectively.<sup>20</sup>

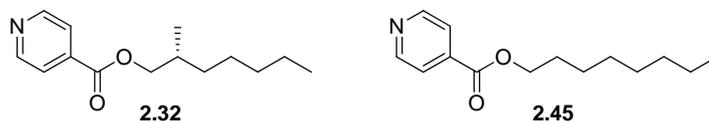


Figure 2.4: Compounds claimed with bioactivity<sup>18,19</sup>

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To verify these claims, **2.45** was synthesised from isonicotinoyl chloride and octanol. Bioactivity tests were carried out by Professor Ben Adler from Monash University. *Bacillus subtilis*, and *E. coli* were grown in nutrient broth medium. A solvent of (*R*)-2-methylheptylisonicotinate was prepared in DMSO at a concentration of 10 mg/mL, and was diluted with culture broth to the concentrations at 32  $\mu\text{g/mL}$ , 63  $\mu\text{g/mL}$ , 125  $\mu\text{g/mL}$ , 250  $\mu\text{g/mL}$  and 500  $\mu\text{g/mL}$ . The antimicrobial tests were carried out with two controls, one without bacteria and the other without isonicotinates **2.32** or **2.45**. It was found that neither **2.32** nor **2.45** showed inhibition of growth at up to 500  $\mu\text{g/mL}$  after incubation at 37 °C for 48 h (Figure 2.5).



Figure 2.5: Bioassays of compound **2.32** and **2.45** carried out by Ben Adler<sup>18,19</sup>

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#### 2.2.4. Summary

In summary, our methodology was applied to the unambiguous synthesis of the purported natural product, (*R*)-2-methylheptylisonicotinate **2.32**. We found that the data for our product differed significantly to those reported by Bora and co-workers. Furthermore, bioassays for our synthetic isonicotinates **2.32** and **2.45** were carried out and no bioactivity to *Bacillus subtilis* or *E. coli* at up to 500 µg/mL was observed.

## 2.3. Synthesis of fragrances and odorants via the “one pot” procedure

### 2.3.1. Fragrances and odorants

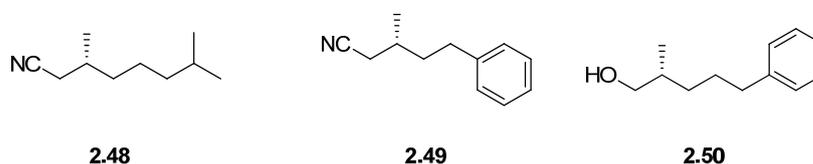
Fragrance is a kind of odorous material that brings us sensory pleasure. Natural product extraction is an important, but usually expensive source of fragrance. Synthetic fragrance plays an important role in this 150 billion USD fragrance market with the development of organic chemistry for the following reasons. Certain notes were not available from Nature, and new synthetic fragrances provided the possibility of creating new fragrances.<sup>28</sup> In addition, fragrance extracted from Nature usually varies by the cultivated times and locations, making it difficult to produce consistent products, while synthetic fragrance is much more reliable in the consistence.<sup>28</sup> More importantly, synthetic fragrance fills the gap of the huge demand from fragrance market and limited expensive natural extracted fragrance.

Some kinds of fragrance are chiral compounds, and the stereochemistry plays important role for their properties and psychological effects. Buchbauer reported that (*S*)-limonene **2.47** was metabolised more slowly than its enantiomer.<sup>29</sup> Heuberger and co-workers carried out the research on the influences of limonene enantiomers on the human autonomic nervous system and self-evaluation system, and found that inhalation of (*R*)-limonene **2.46** would cause higher systolic blood pressure, and subjective alertness and restlessness, while for *S*- enantiomer, only systolic blood pressure increase was observed, without any psychological effects (**Figure 2.6**).<sup>30</sup> The floral notes of chiral fragrances may also be different between enantiomers. For example, (*S*)-(+)-carvone has an aromatic flavour, while its *R*-configured enantiomer possesses minty flavour.<sup>31</sup> Thus, human nerves may perceive different floral notes and intensity from the stereo enantiomers, and present different psychological effects, making preparing enantiomeric pure fragrance desired.<sup>30</sup>



**Figure 2.6:** Structures of (*R*)- and (*S*)-limonene

3,7-Dimethyl octanenitrile,<sup>32</sup> Citralis Nitrile<sup>TM</sup>,<sup>33</sup> and Rosaphen<sup>TM</sup><sup>34</sup> are widely used odorants. Their *R*-configured enantiomers, **2.48**, **2.49** and **2.50** were synthesised via our “one pot” procedure (**Figure 2.7**).



**Figure 2.7:** Fragrances with saturated isoprenoid units

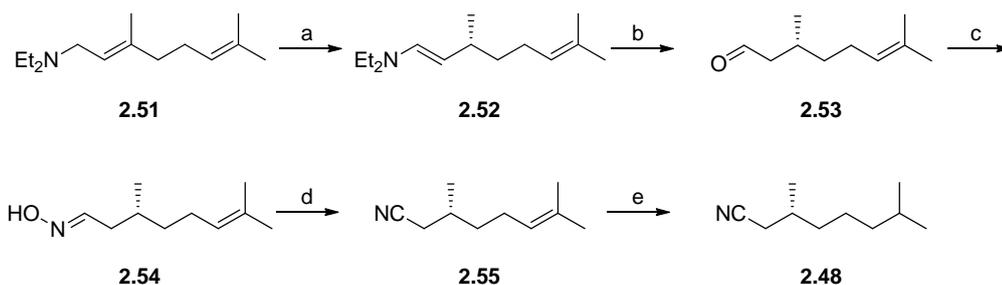
## 2.3.2. Synthesis of the odorant (*R*)-3,7-dimethyloctanenitrile

### 2.3.2.1. 3,7-Dimethyloctanenitrile

3,7-Dimethyloctanenitrile **2.48** is a well known odorant used in cosmetics and personal care products. (*S*)-3,7-Dimethyloctanenitrile has a citrus floral odour with a fatty and herbal top note, while (*R*)-3,7-dimethyloctanenitrile **2.48** has a floral and slightly citrus odour with natty and fatty top note.<sup>32</sup>

### 2.3.2.2. Previous asymmetric synthesis reported by Kanisawa

Kanisawa reported an asymmetric synthesis of nitrile **2.48** employing a readily available aldehyde used in the commercial production of L-menthol (**Scheme 2.12**).<sup>35</sup> Thus asymmetric isomerisation amine **2.51** to the chiral enamine **2.52** followed by hydrolysis gave aldehyde **2.53**. **2.53** was treated with hydroxylamine to give oxime **2.54**, which was converted to nitrile **2.55** after dehydration. Hydrogenation then gave the target nitrile **2.48**.

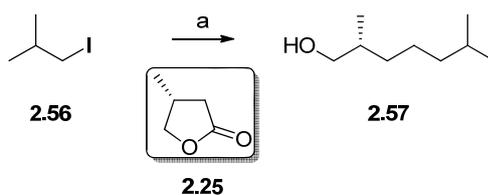


Reagents and conditions: a) (*S*)-Rh(I)-BINAP; b) H<sup>+</sup>; c) NH<sub>2</sub>OH·HCl, Na<sub>2</sub>CO<sub>3</sub>; d) Ac<sub>2</sub>O; e) Pd, H<sub>2</sub>.

**Scheme 2.12:** Synthesis of nitrile odorant **2.48** with Rh(I)-BINAP catalysts<sup>32,35</sup>

### 2.3.2.3. Synthesis of nitrile odorant **2.48** via the “one pot” procedure

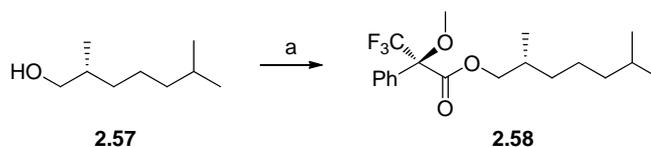
The “one pot” procedure was successfully applied to the synthesis of key intermediate for **2.48**, the alcohol **2.57** (Scheme 2.13). *i*-BuLi was generated *in situ* by treating 1 eq. *i*-BuI with 2.1 eq. *t*-BuLi in Et<sub>2</sub>O at -78 °C for 1 h, and then the generated *i*-BuLi was added to a solution of 1.1 eq. lactone **2.25** in Et<sub>2</sub>O at -78 °C. The reaction was stirred at -78 °C for 3 h, and then 10 eq. TMSCl was added as before. After warming the mixture to 0 °C, 10 eq. Zn dust were added, and followed by adding MeOH over 15 min. The reaction was stirred at 0 °C for 1 h. After acid work up and purification by flash chromatography, alcohol **2.57** was obtained in 79 % yield.



Reagents and conditions: All in “one pot”: a) *i*-*t*-BuLi (2.1 eq.), -78 °C, Et<sub>2</sub>O, -78 °C; ii- **2.25** (1.1 eq.) Et<sub>2</sub>O, 3 h; b) TMSCl (10 eq.), Zn (10 eq.), MeOH, 0 °C, 1 h.

**Scheme 2.13:** Asymmetric synthesis of **2.57** from lactone

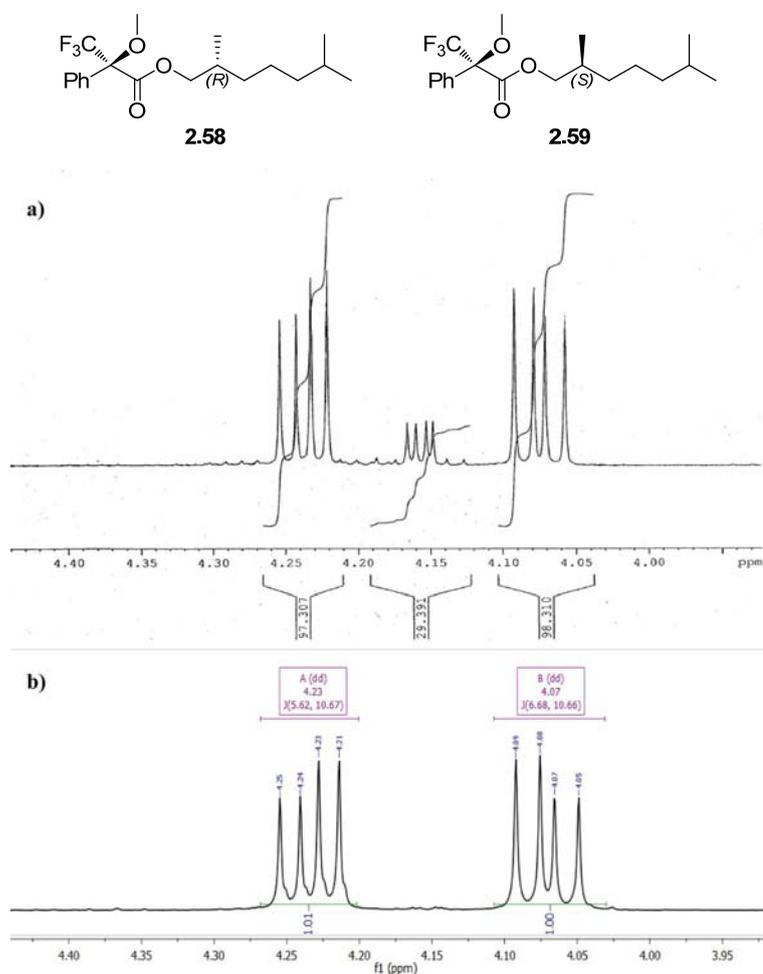
To determine the absolute configuration and enantiomeric purity of the chiral alcohol **2.57**, it was converted to Mosher’s ester **2.58** by treatment with 1.4 eq. Mosher’s acid in CH<sub>2</sub>Cl<sub>2</sub> at r.t. stirring for 2 d in the presence of 1.5 eq. DCC and 0.1 eq. DMAP (Scheme 2.14).<sup>36</sup> The solvent was removed *in vacuo* to give a colourless oil.



Reagents and conditions: a) DCC (1.5 eq.), DMAP (0.1 eq.), Mosher's acid (1.4 eq.), r.t., 2 d.

**Scheme 2.14:** Converting to Mosher's ester for enantiomeric purity test

Negishi reported the  $^1\text{H}$  NMR spectrum of a mixture of Mosher's ester **2.58** (major) and **2.59** (minor) (as spectrum **a** in **Figure 2.8**).<sup>36,37</sup>



a) Spectrum reported by Negishi for the mixture of **2.58** (major) and **2.59** (minor);

b) The spectrum of the crude ester of the alcohol **2.57** synthesised from the "one pot" procedure.

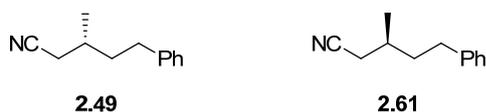
**Figure 2.8:** Enantiomeric purity determination of  $^1\text{H}$  NMR of the Mosher's ester<sup>37</sup>



### 2.3.3. Synthesis of (*R*)-Citralis Nitrile<sup>TM</sup>

#### 2.3.3.1. Citralis Nitrile<sup>TM</sup>

Citralis Nitrile<sup>TM</sup> is a tradename of Innospec Inc for 3-methyl-5-phenyl pentanenitrile. It is a common fragrance used in a wide variety of cosmetics and personal care products. (*S*)-Citralis Nitrile<sup>TM</sup> **2.61** displays fragrance characteristics which are “typical citrus, reminiscent of lemon peel and geranyl nitrile, with fresh, fruity and slightly green nuances”, while (*R*)-Citralis Nitrile<sup>TM</sup> **2.49** is similar in smell, but slightly stronger, and with “additional waxy aspects and a more pronounced green, leafy side” (Figure 2.9).<sup>33</sup>



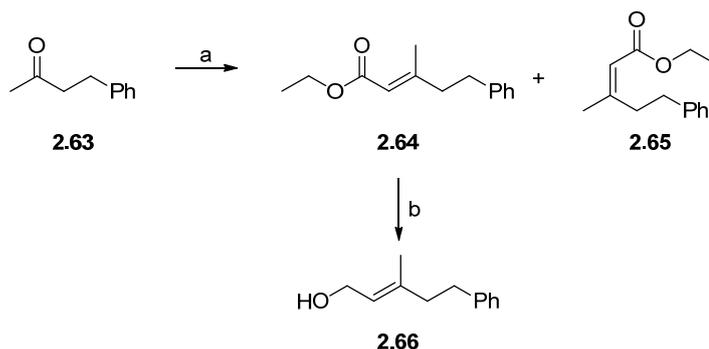
**Figure 2.9:** Structure of (*R*)-Citralis Nitrile<sup>TM</sup> **2.49** and its enantiomer **2.61**

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#### 2.3.3.2. Previous synthesis of (*R*)-Citralis Nitrile<sup>TM</sup>

A benchmark synthesis of (*R*)-nitrile **2.49** by asymmetric hydrogenation of the olefin alcohol **2.66** using Ru(II)-BINAP catalyst was reported by Scrivanti.<sup>20</sup>

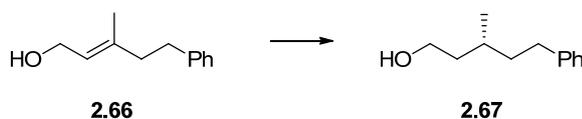
*E*-Configured olefin alcohol **2.66**, the substrate of the asymmetric hydrogenation, was prepared from benzylacetone **2.63** and triethyl phosphonoacetate by Horner-Wadsworth-Emmons reaction (Scheme 2.16), giving a mixture of olefin esters **2.64** and **2.65**. Purification by flash chromatography gave *E*-configured **2.64** in 66 % yield.<sup>20,38</sup> Reduction of the olefin ester **2.64** with DIBALH gave acid **2.66**.



Reagents and conditions: a) Triethyl phosphonoacetate, NaH; b) DIBAL.

**Scheme 2.16:** Synthesis of olefin alcohol **2.66**<sup>20,38</sup>

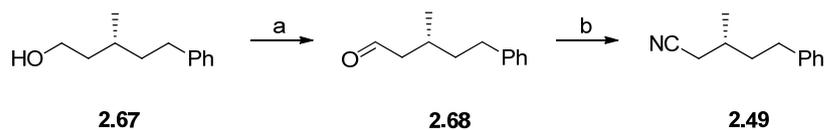
The asymmetric hydrogenation was carried out under 100 atm pressure of H<sub>2</sub> in MeOH with 2 mol% of (*S*)-Ru(II)-BINAP as catalyst, giving alcohol **2.67** in 97 % ee, which was the key intermediate for preparing (*R*)-nitrile **2.49** (Scheme 2.17).<sup>33</sup>



Reagents and conditions: (*S*)-Ru(II)-BINAP (2 mol%), H<sub>2</sub> (100 atm), MeOH.

**Scheme 2.17:** Asymmetric hydrogenation of olefin alcohol **2.67**<sup>33</sup>

The generated alcohol **2.67** was oxidised to aldehyde **2.68**, and then converted to (*R*)-Citralis Nitrile<sup>TM</sup> **2.49** via the oxime (Scheme 2.18). The whole procedure took five steps from benzylacetone **2.63**, and gave (*R*)-Citralis Nitrile<sup>TM</sup> **2.49** in an overall yield of 17 %.<sup>33</sup>

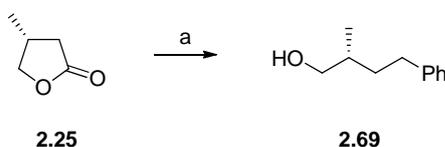


Reagents and conditions: a) PCC, CH<sub>2</sub>Cl<sub>2</sub>; b) NaI, CH<sub>3</sub>CN, NH<sub>2</sub>OH·HCl; Ac<sub>2</sub>O.

**Scheme 2.18:** Scrivanti's synthesis of (*R*)-Citralis Nitrile<sup>TM</sup> **2.49**<sup>33</sup>

**2.3.3.3. Synthesis of (*R*)-Citralis Nitrile<sup>TM</sup> 2.49 via the “one pot” procedure**

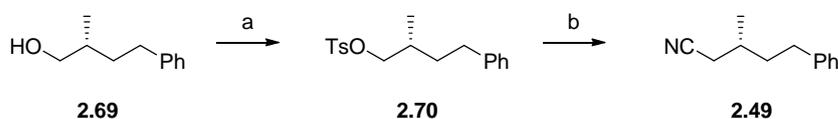
Our “one pot” procedure was applied to the total synthesis of nitrile **2.49**. The key intermediate, chiral alcohol **2.69**, was synthesised from  $\gamma$ -lactone **2.25** (Scheme 2.19). PhLi, generated *in situ* from PhBr and 2.1 eq. *t*-BuLi in Et<sub>2</sub>O at -78 °C for 1 h, was reacted with  $\gamma$ -lactone **2.25** in Et<sub>2</sub>O at -78 °C for 3 h. Sequential treatment with TMSCl, Zn and MeOH as before, followed by acid work up and purification by flash chromatography, gave chiral alcohol **2.69** in 77 % yield.



Reagents and conditions: a) i - PhLi (0.9 eq.), Et<sub>2</sub>O, -78 °C, 3 h; ii - TMSCl (10 eq.), Zn (10 eq.), MeOH, 0 °C, 1 h.

**Scheme 2.19:** Synthesis of chiral alcohol **2.69** via the “one pot” procedure

Again as before, after conversion of alcohol to tosylate **2.70**, displacement of the corresponding tosylate with NaCN in DMSO gave Citralis Nitrile<sup>TM</sup> **2.49** (Scheme 2.20), whose structure was confirmed by the appearance of peaks at 7.10-7.35 ppm region (phenyl protons) and 3.51 ppm (hydroxymethylene protons) in the <sup>1</sup>H NMR spectrum.



Reagents and conditions: a) TsCl (1.5 eq.), DMAP (0.1 eq.), pyridine (3 eq.), CH<sub>2</sub>Cl<sub>2</sub>; b) NaCN (6 eq.), DMSO, r.t., 24 h.

**Scheme 2.20:** Synthesis of (*R*)-Citralis Nitrile<sup>TM</sup> from alcohol **2.69**

The whole synthetic procedure took three steps from lactone **2.25**, giving **2.49** in an overall yield of 71 %, and the enantiomeric purity of > 99 % ee, which was confirmed by specific rotation.<sup>39</sup>

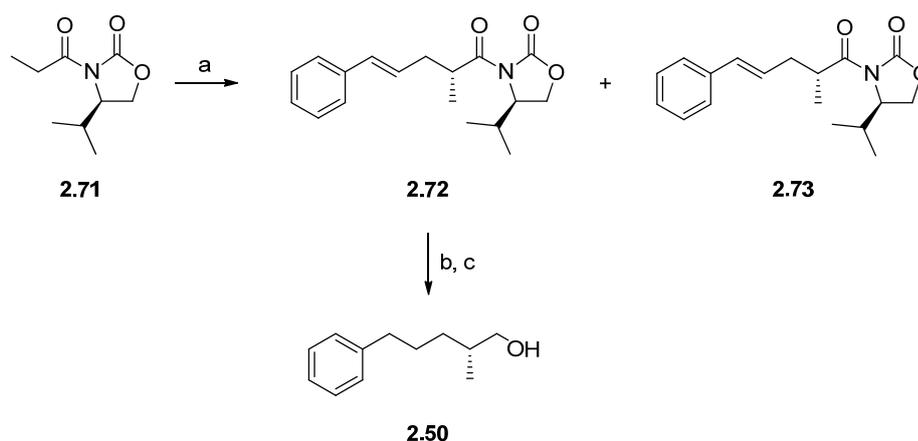
### 2.3.4. Synthesis of (*R*)-Rosaphen<sup>TM</sup>

#### 2.3.4.1. Rosaphen<sup>TM</sup>

Rosaphen<sup>TM</sup> is a tradename of Symrise AG for the compound 2-methyl-5-phenylpentanol. It is a common fragrance used in a wide variety of perfumes and personal care products. (*S*)-Rosaphen<sup>TM</sup> is characterised with a floral rosy odour in the direction of phenylethyl esters, together with green, powdery, sweet, and slightly animalic scent, while (*R*)-Rosaphen<sup>TM</sup> has apparent floral rosy and watery odour.<sup>34</sup>

#### 2.3.4.2. Previous syntheses of Rosaphen<sup>TM</sup>

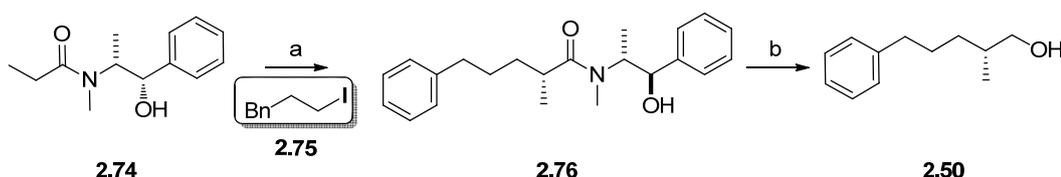
The first asymmetric synthesis of Rosaphen<sup>TM</sup> was reported by Santini in 1994 (Scheme 2.21).<sup>40</sup> The chiral methyl group was introduced via oxazolidinone chiral auxiliary **2.71**, which was converted to sodium enolate using  $\text{NaN}(\text{TMS})_2$  as base, followed by alkylation with cinnamyl bromide to give a mixture of **2.72** and its diastereomer **2.73** (*R/S* 97:3). The desired **2.72** was isolated by flash chromatography purification. After hydrogenation of the olefin **2.72** with Pd/C and  $\text{H}_2$ , the chiral auxiliary group was removed by treatment with  $\text{LiAlH}_4$  in THF to give the (*R*)-Rosaphen<sup>TM</sup> **2.50**. The whole procedure took three steps from oxazolidinone **2.71**.



Reagents and conditions: a)  $\text{NaN}(\text{TMS})_2$ , THF,  $-78\text{ }^\circ\text{C}$ , cinnamyl bromide; b)  $\text{H}_2$ , 10% Pd/C; c)  $\text{LiAlH}_4$ , THF,  $0\text{ }^\circ\text{C}$ .

**Scheme 2.21:** Asymmetric synthesis of Rosaphen<sup>TM</sup> **2.50** reported by Santini<sup>40</sup>

The other asymmetric synthesis was published by Nicewicz in 2008 (**Scheme 2.22**).<sup>41</sup> Similarly, **2.74** was treated with iodide **2.75** in the presence of LDA to give amide **2.76** with a chiral methyl group, which was reduced by  $\text{LiH}_2\text{NBH}_3$  to give (*R*)-Rosaphen<sup>TM</sup> **2.50**. Nicewicz's synthesis took two steps in an overall yield of 87 % from amide **2.74**.

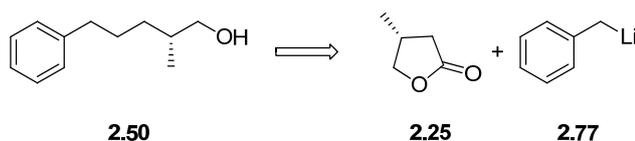


Reagents and conditions: a) LDA, LiCl; b)  $\text{LiH}_2\text{NBH}_3$ .

**Scheme 2.22:** Asymmetric synthesis of Rosaphen<sup>TM</sup> **2.50** reported by Nicewicz<sup>41</sup>

### 2.3.4.3. Synthesis of Rosaphen<sup>TM</sup> from (*R*)-3-methylbutyrolactone via the “one pot” procedure

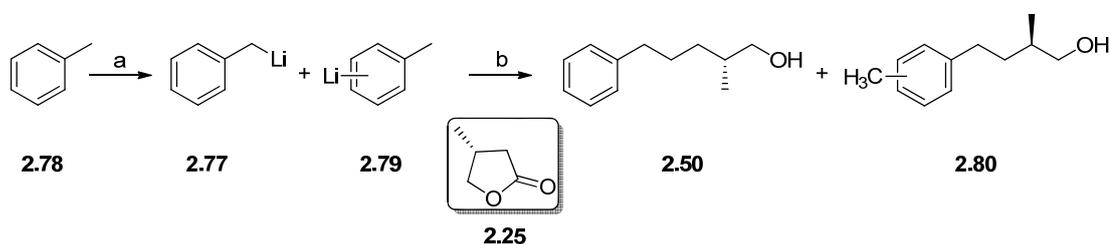
It was clear that (*R*)-Rosaphen<sup>TM</sup> **2.50** could be synthesised from (*R*)-3-methylbutyrolactone **2.25** (**Scheme 2.23**). However, the use of benzyl lithium **2.77** proved somewhat problematic at first.



**Scheme 2.23:** Retrosynthesis of (*R*)-Rosaphen<sup>TM</sup> **2.50** via “one pot” procedure

Metalation of BnBr by *t*-BuLi gave 1,2-diphenylethane as the major product, as the initially formed BnLi could undergo Wurtz coupling with BnBr.<sup>42,43</sup> Metalation of toluene with *n*-BuLi in the presence of tetramethylethylenediamine at 30 °C was attempted next (**Scheme 2.24**).<sup>44</sup> The desired alcohol **2.50** was successfully obtained together with some impurities **2.80** due to competing ring metalation (molar ratio of **2.50:2.80** was 92:8) according to GCMS. The impurities **2.80** were apparent from the appearance of methyl peaks at 2.3-2.35 ppm in the <sup>1</sup>H NMR spectrum. Attempts to remove the impurities by flash chromatography proved unsuccessful. Consequently,

an alternative synthetic approach was chosen to produce (*R*)-Rosaphen<sup>TM</sup> **2.50**.

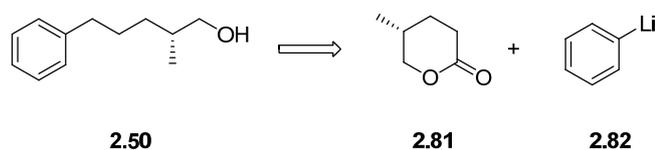


Reagents and conditions: a) *n*-BuLi (1 eq.), tetramethylethylenediamine, 30 °C; b) i - **2.25** (1.1 eq.), Et<sub>2</sub>O, -78 °C, 3 h; ii - TMSCl (10 eq.), Zn (10 eq.), MeOH, 0 °C, 1 h.

**Scheme 2.24:** Synthesis of (*R*)-Rosaphen<sup>TM</sup> from  $\gamma$ -lactone via “one pot” procedure

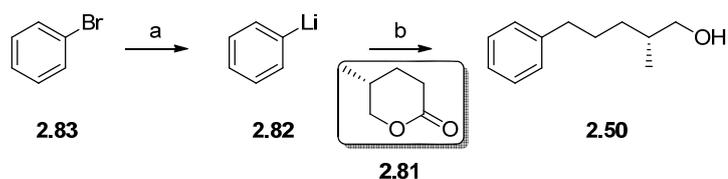
#### 2.3.4.4. Synthesis of (*R*)-Rosaphen<sup>TM</sup> from (*R*)-4-methyl- $\delta$ -valerolactone

Considering PhLi **2.82** could be easily prepared from PhBr and *t*-BuLi to afford clean product (see 2.3.3.3), a retrosynthesis of (*R*)-Rosaphen<sup>TM</sup> **2.50** from  $\delta$ -valerolactone **2.81** and PhLi **2.82** was proposed (Scheme 2.25). The  $\delta$ -valerolactone **2.81** was prepared from tigogenin (see section 1.4.3).



**Scheme 2.25:** Retrosynthesis of (*R*)-Rosaphen<sup>TM</sup> **2.50** from  $\delta$ -valerolactone **2.81**

The same procedure for  $\gamma$ -lactone ring cleavage was applied on  $\delta$ -lactone **2.81** (Scheme 2.26). Thus a solution of PhLi at -78 °C was added to a solution of  $\delta$ -valerolactone **2.81** in Et<sub>2</sub>O via cannula. The mixture was stirred at -78 °C for 3 h, and then the reaction was quenched by adding 10 eq. TMSCl, and allowed to warm to 0 °C. 10 eq. Zn dust was added, and followed by adding MeOH. After the usual work up, (*R*)-Rosaphen<sup>TM</sup> **2.50** was obtained as a colourless liquid in 76 % yield. The identity of **2.50** was confirmed by the appearance of peaks at 7.15-7.35 ppm region (phenyl protons) and 3.47 ppm (hydroxymethylene protons) in the <sup>1</sup>H NMR spectrum. The enantiomeric purity was confirmed by specific rotation, and was in > 99 % ee.<sup>45</sup>



Reagents and conditions: a) *t*-BuLi (2.1 eq.), Et<sub>2</sub>O, -78 °C, 1 h; b) i - **2.81** (1.1 eq.), -78 °C, 3 h; ii - TMSCl (10 eq.), Zn (10 eq.), MeOH, 0 °C, 1 h.

**Scheme 2.26:** Synthesis of (*R*)-Rosaphen<sup>TM</sup> **2.50** from  $\delta$ -valerolactone

Hence (*R*)-Rosaphen<sup>TM</sup> **2.50** was prepared from  $\delta$ -lactone **2.81** in “one pot” in an overall 76 % yield and > 99 % ee, indicating that the “one pot” procedure for  $\delta$ -lactones is as efficient as that for  $\gamma$ -lactones.

### 2.3.5. Summary

In summary, three chiral fragrances (*R*)-3,7-dimethyloctanenitrile **2.48**, (*R*)-Citralis Nitrile<sup>TM</sup> **2.49**, and (*R*)-Rosaphen<sup>TM</sup> **2.50** have been synthesised via the “one pot” procedure from  $\gamma$ -lactone **2.25** or  $\delta$ -lactone **2.81** in good yields and high enantiomeric purities.

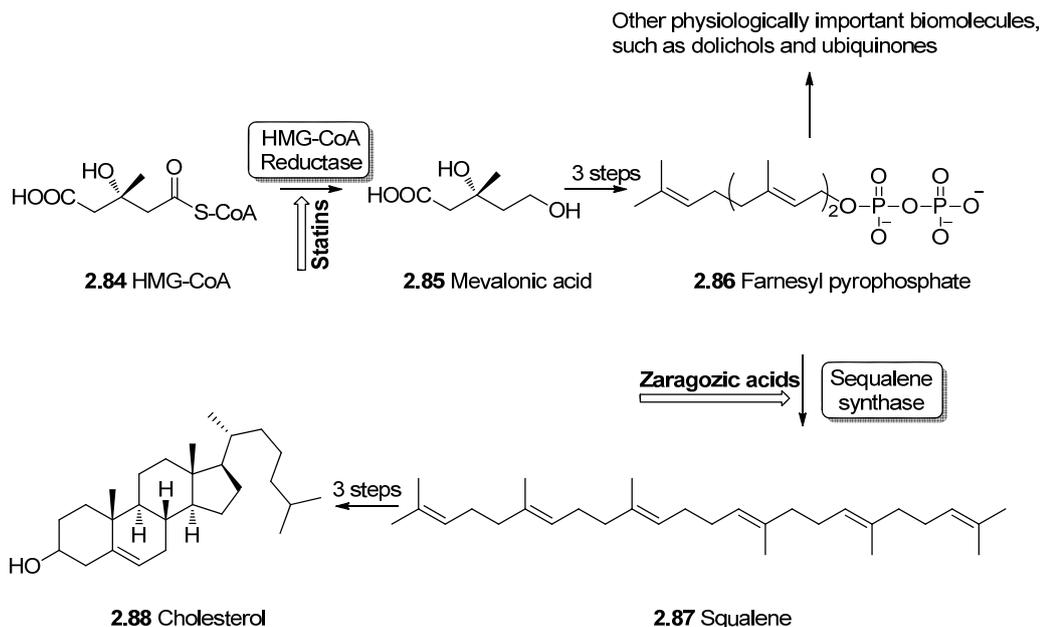
## 2.4. Synthesis of the C6 side chain of zaragozic acid C

### 2.4.1. The zaragozic acids

Coronary heart disease is clinical characterised as blockage of the coronary arteries, and the major cause of the blockage is high concentration of cholesterol in the blood. Coronary heart disease is one of the deadliest diseases in the world. Efficient therapies are required to lower the concentration of the cholesterol.<sup>46</sup>

Biosynthesis of cholesterol in the body starts from the generation of HMG-CoA **2.84** from acetyl CoA and acetoacetyl-CoA catalysed by HMG-CoA synthase (**Scheme 2.27**). With the assistance of HMG-CoA reductase enzyme, HMG-CoA is reduced to mevalonic acid **2.85**.<sup>47</sup> This stage can be controlled by employing HMG-CoA reductase inhibitors, a class of statins drugs to hinder the generation of cholesterol in the following steps. Statins, as major cholesterol-lowering drugs in the market, have some side effects due to the inhibiting the production of mevalonic acid, which is important in the biosynthesis of some other physiologically important compounds, such as dolichols and ubiquinones.<sup>47</sup>

In the biosynthesis pathway of cholesterol, mevalonic acid is then converted to farnesyl pyrophosphate **2.86**. After that, two molecules of farnesyl pyrophosphate are condensed to give squalene **2.87** with the implication of squalene synthase, which was converted to cholesterol **2.88**. If the squalene synthase is inhibited, biosynthesis of cholesterol will be blocked effectively without affecting the biosynthesis of dolichols and ubiquinones.<sup>48</sup>

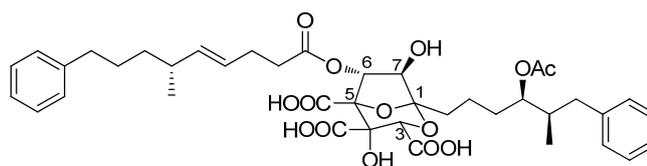
Scheme 2.27: Biosynthetic pathway of cholesterol<sup>47</sup>

The first zaragozic acid, zaragozic acid A, was discovered from three different fungal species by three groups independently.<sup>48</sup> After that, more zaragozic acid family members were discovered (Table 2.4).<sup>49-52</sup> These compounds, all with potent inhibitory activity of squalene syntheses, were characterized with the same structural core, 2,8-dioxabicyclo[3.2.1] octane-3,4,5-tricarboxylic acid **2.89**, differing mainly in the side chains at C1 and C6.

**Table 2.4:** The structures of representative zaragozic acids

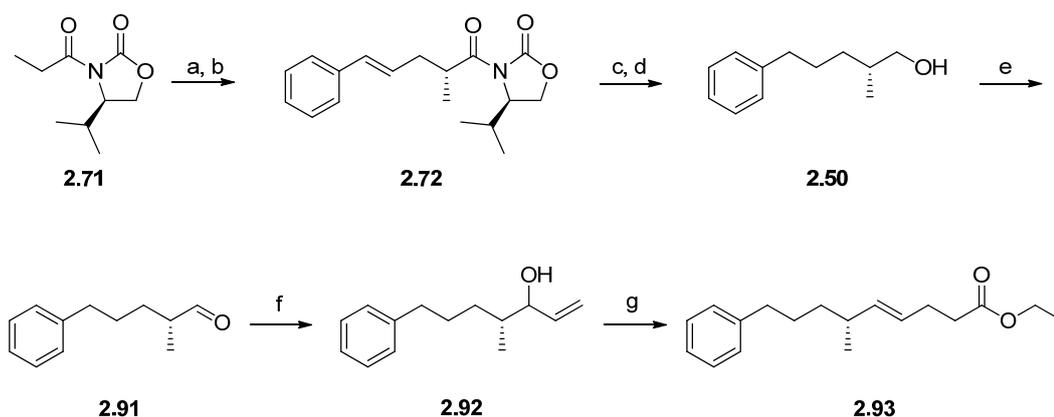
Zaragozic acid	R	R'
	 <b>2.89</b>	
A		
B		
C		
D		

Zaragozic acid C **2.90** (Figure 2.10), isolated from the sterile fungal culture *Leptodontium elatius*, exhibits inhibition of the enzyme squalene synthase even at nanomolar concentration.<sup>49-52</sup>

**2.90****Figure 2.10:** The structure of zaragozic acid C **2.90**

There have been more than ten reported synthetic approaches to the core system of zaragozic acid C through total asymmetric synthesis,<sup>40,41,53-60</sup> and the most recently published benchmark method is by controlled oligomerisation.<sup>41</sup> However, only two similar synthesis of the chiral hydrophobic side chain on C6 have been reported so far,<sup>40,54</sup> and both these two methods synthesised via the key intermediate, (*R*)-Rosaphen<sup>TM</sup> **2.50**.

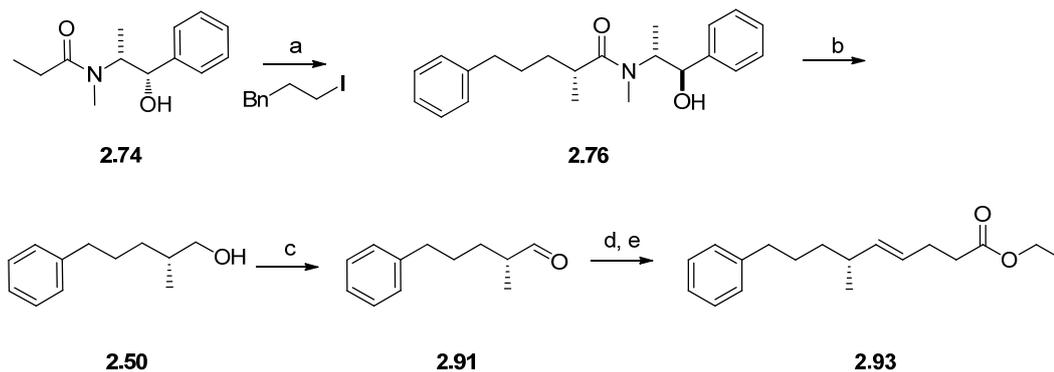
The first asymmetric synthesis of C6 side chain of zaragozic acid C was reported by Santini in 1994 (**Scheme 2.28**).<sup>40</sup> After generating the (*R*)-Rosaphen<sup>TM</sup> **2.50** using oxazolidinone **2.71** as chiral auxiliary, it was oxidised by Swern oxidation to give aldehyde **2.91**. **2.91** was treated with Grignard reagent to give terminal alkene **2.92**, followed by Claisen rearrangement to give single *E*-configured ester product **2.93**. The whole procedure took seven steps in an overall yield of 42 %.<sup>40</sup>



Reagents and conditions: a)  $\text{NaN}(\text{TMS})_2$ , THF,  $-78\text{ }^\circ\text{C}$ ; b) Cinnamyl bromide; c)  $\text{H}_2$ , 10 % Pd/C; d)  $\text{LiAlH}_4$ , THF,  $0\text{ }^\circ\text{C}$ ; e) Swern oxidation; f) Vinyl magnesium bromide; g)  $\text{CH}_3\text{C}(\text{OEt})_3$ .

**Scheme 2.28:** Asymmetric synthesis of C6 side chain of zaragozic acid C by Santini<sup>40</sup>

The other asymmetric synthesis was published by Nicewicz in 2008 (**Scheme 2.29**),<sup>41</sup> from which, the (*R*)-Rosaphen<sup>TM</sup> **2.50** was prepared with the assistance of another chiral auxiliary, the amide **2.74**.



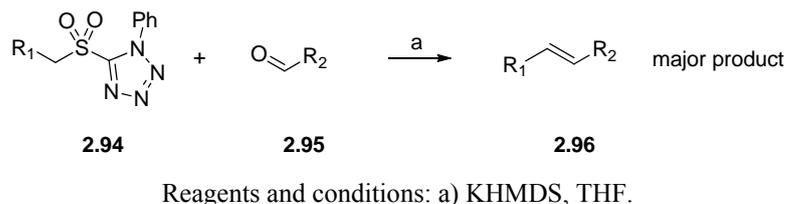
Reagents and conditions: a)  $\text{LiAlH}_4$ ,  $\text{LiCl}$ ; b)  $\text{LiH}_2\text{NBH}_3$ ; c) TEMPO,  $\text{NaOCl}$ ; d) Vinyl magnesium bromide; e)  $\text{CH}_3\text{C}(\text{OEt})_3$ .

**Scheme 2.29:** Synthesis of C6 side chain of zaragozic acid C by Nicewicz<sup>41</sup>

The synthesis of (*R*)-Rosaphen<sup>TM</sup> **2.50** with our “one pot” procedure from  $\delta$ -valerolactone **2.81** has been discussed (see section 2.3.4). The only problem left for synthesis of C6 side chain of zaragozic acid C was the olefination reaction to stereoselectively prepare the olefine ester **2.93** from alcohol **2.50**.

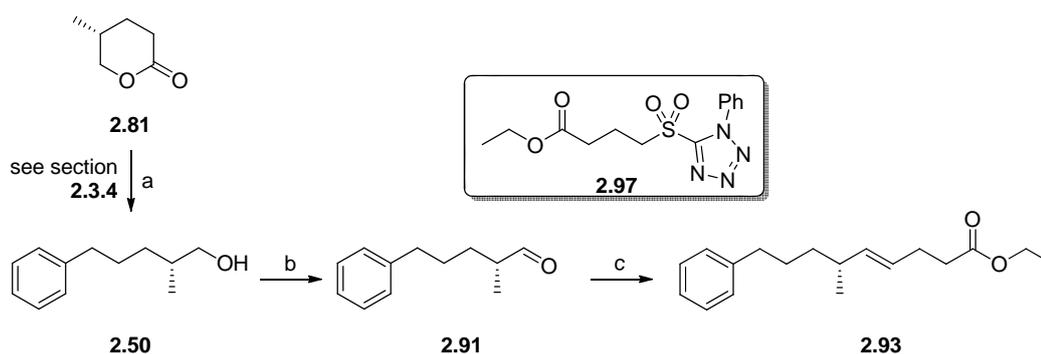
#### 2.4.2. Kocienski modified Julia olefination reactions for the *E*-selective synthesis of olefins

Several olefination reactions were considered for selective synthesis of the *E*-configured olefination ester **2.93**, such as Wittig reaction, Horner-Wittig reaction, Horner-Wadsworth-Emmons reaction, Peterson reaction, Johnson reaction, and Julia olefination reaction.<sup>61</sup> Julia olefination has been used for olefination synthesis via metallated heteroarylsulfones reacting with carbonyl compounds. To improve the stereo-selectivity, several modified Julia olefination reactions have been reported.<sup>61</sup> Kocienski group found using metallated 1-phenyl-1*H*-tetrazol-5-yl sulfones as coupling partners to react with aldehydes would result in high yield of the coupling products.<sup>62</sup> The selectivity of *E*-isomer was depended on the properties of base and solvent involved. When KHMDS and polar solvents like THF were used as base, the *E*-configured product was obtained selectively (**Scheme 2.30**).

Scheme 2.30: Kocienski modified Julia olefination reaction<sup>62,63</sup>

### 2.4.3. Synthesis of the C6 side chain of zaragozic acid C from (*R*)-Rosaphen<sup>TM</sup>

Thus the overall procedure for the asymmetric synthesis of C6 side chain of zaragozic acid C from  $\delta$ -lactone **2.81** is shown in Scheme 2.31.



Reagents and conditions: a) i - PhLi (0.9 eq.), -78 °C, 3 h; ii - TMSCl (10 eq.), Zn (10 eq.), MeOH, 0 °C; b) Dess-Martin periodinane (1.5 eq.), 0 °C, 30 min; c) **2.97**, KHMDS (1.4 eq.), -78 °C to r.t., 14 h.

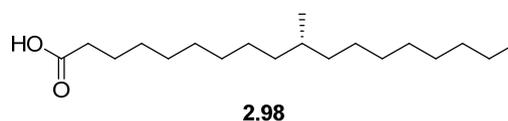
Scheme 2.31: Overall procedure of preparation side chain of zaragozic acid C

Alcohol **2.50** was oxidised with 1.5 eq. Dess-Martin periodinane to give aldehyde **2.91**, which was used directly for the olefination reaction. Olefination with the potassium enolate of sulfone **2.97** in THF at -78 °C, and after stirring at -78 °C for 4 h, and the reaction gave alkene **2.93** in 81 % yield over two steps, together with 4 % of the *Z*-isomer. The whole procedure took three steps, and the product **2.93** was obtained in an overall yield of 58 % and 98 % ee.<sup>40</sup>

## 2.5. A formal synthesis of tuberculostearic acid via the “one pot” procedure

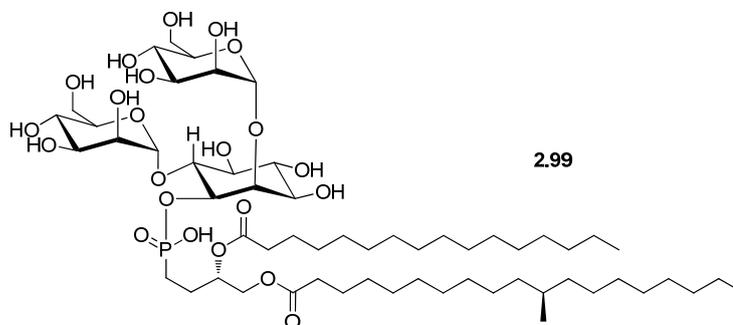
### 2.5.1. Tuberculostearic acid

Tuberculostearic acid, (*R*)-10-methylstearic acid, is a methyl-branched, saturated fatty acid (**Figure 2.11**). It was originally isolated from *Mycobacterium tuberculosis* in 1929 by Anderson and Chargaff.<sup>64</sup>



**Figure 2.11:** Structure of tuberculostearic acid

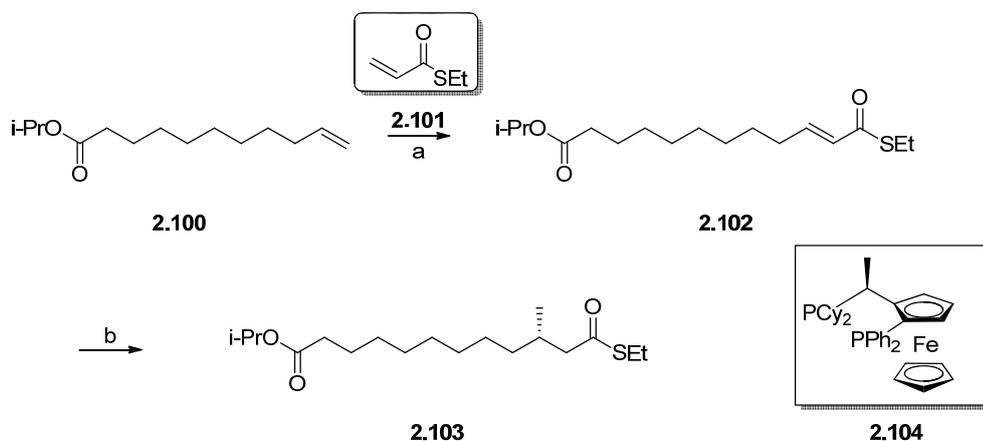
Tuberculostearic acid **2.98** is now used as a marker to help diagnosis of pulmonary tuberculosis, which is regarded as one of the most devastating infectious diseases in the world.<sup>65</sup> **2.98** is also an important intermediate for an antigenic glycolipid, **2.99**, isolated from the cell wall of *M. tuberculosis* (**Figure 2.12**). Reports have shown that **2.99** can be presented by CD1d, and recognised by natural killer T cells, and thereby trigger antigen-specific cytokine production and cell-mediated cytotoxicity.<sup>66-68</sup> The biological activities of **2.99** aroused the interests of its synthesis for assistance of related biological studies in the aim of developing vaccines against tuberculosis.



**Figure 2.12:** Structure of an antigenic glycolipid, **2.99**<sup>68</sup>

## 2.5.2. Previous syntheses of tuberculostearic acid

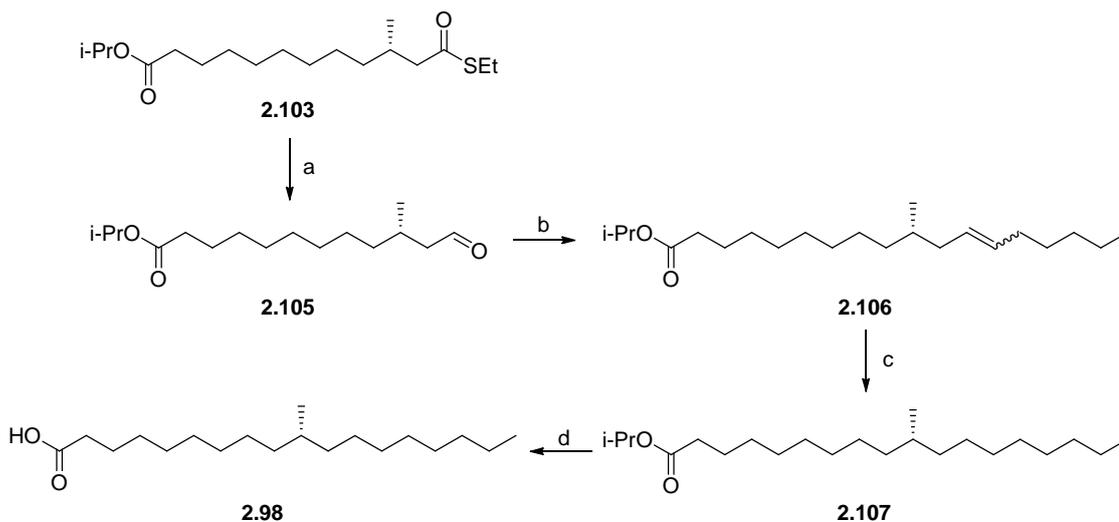
Three synthetic methods were reported for (*R*)-tuberculostearic acid. Feringa and co-workers synthesised tuberculostearic acid via catalytic asymmetric conjugate addition.<sup>69</sup> Using Hoveyda-Grubbs second generation catalyst, terminal alkene **2.100** was coupled with *S*-ethyl thioacrylate **2.101** to give unsaturated thioester **2.102** (Scheme 2.32). After asymmetric conjugate addition with Grignard reagent using **2.104**·CuBr complex as catalyst, chiral methyl-branched thioester **2.103** was obtained.



Reagents and conditions: a) Hoveyda-Grubbs second generation catalyst, CH<sub>2</sub>Cl<sub>2</sub>, reflux; b) **2.104**·CuBr (1 mol%), MeMgBr, *t*-BuOMe, -78 °C.

**Scheme 2.32:** Feringa's asymmetric synthesis of **2.103**<sup>69</sup>

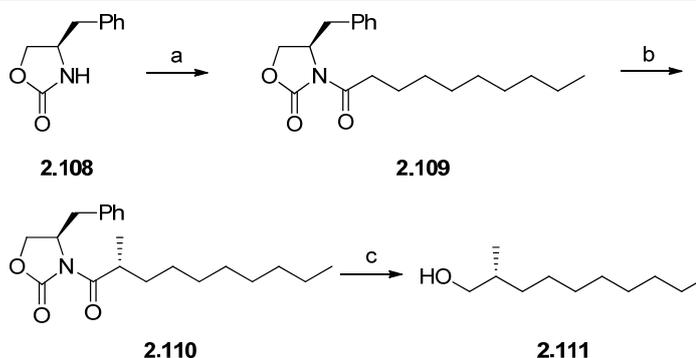
Thioester **2.103** was reduced to aldehyde **2.105**, and followed by Wittig reaction to give the olefin **2.106** (Scheme 2.33). The olefin **2.106** was reduced using NH<sub>2</sub>NH<sub>2</sub>·H<sub>2</sub>O in the presence of flavin catalyst to give saturated ester **2.107**. After hydrolysis with base and then acid work up, (*R*)-tuberculostearic acid **2.98** was obtained in 67 % yield over seven steps with 90 % ee.<sup>69</sup>



Reagents and conditions: a) Pd/C, Et<sub>3</sub>SiH; b) C<sub>5</sub>H<sub>11</sub>CH=PPh<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>/THF; c) Flavin catalyst, NH<sub>2</sub>NH<sub>2</sub>; d) KOH, EtOH, acid work up.

**Scheme 2.33:** Feringa's asymmetric synthesis of **2.98**<sup>69</sup>

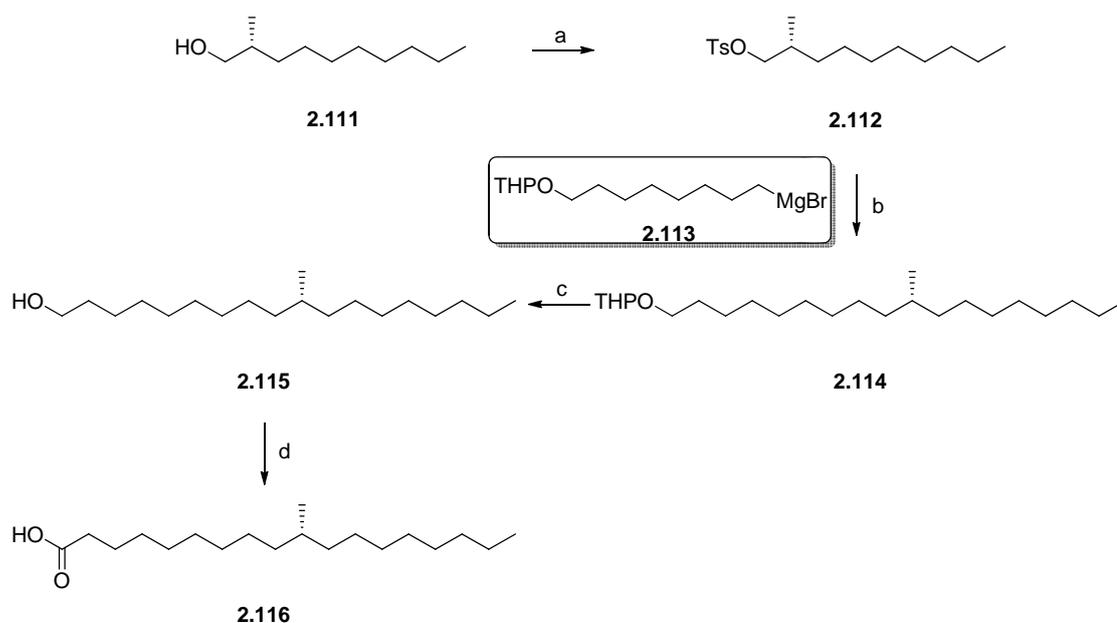
Benjamin Cao and co-workers from Melbourne University reported the synthesis of tuberculostearic acid via the use of chiral oxazolidinone chemistry (**Scheme 2.34**).<sup>70</sup> Acid chloride reacted with oxazolidinones **2.108** to afford imide **2.109**. The steric hindrance of benzyl group in imide **2.109** determined the configuration of the later introduced methyl group to form the imide **2.110** with chiral methyl group, followed by reduction using NaBH<sub>4</sub> to remove the auxiliary group, and chiral 2-methyl branched alcohol **2.111** was obtained stereoselectively in 76 % yield.



Reagents and conditions: a) *n*-BuLi, C<sub>9</sub>H<sub>19</sub>COCl, THF, -78 °C to r.t.; b) NaHMDS, THF, -78 °C, 1 h then MeI, -78 °C, 2 h; c) NaBH<sub>4</sub>, THF/H<sub>2</sub>O, 0 °C, overnight.

**Scheme 2.34:** Benjamin Cao's synthesis of alcohol **2.111**<sup>70</sup>

Alcohol **2.111** was converted to tosylate **2.112** by treatment with TsCl, and then coupled with Grignard **2.113** to form a THP-protected ether **2.114** (Scheme 2.35). The THP protecting group was removed to give the alcohol **2.115**, followed by oxidation to afford (*R*)-tuberculostearic acid **2.116**.



Reagents and conditions: a) TsCl, pyridine, 0 °C; b) Li<sub>2</sub>CuCl<sub>4</sub>, THF, 0 °C to r.t.; c) TsOH, MeOH, reflux; d) KMnO<sub>4</sub>, CH<sub>2</sub>Cl<sub>2</sub>/H<sub>2</sub>O/AcOH, reflux.

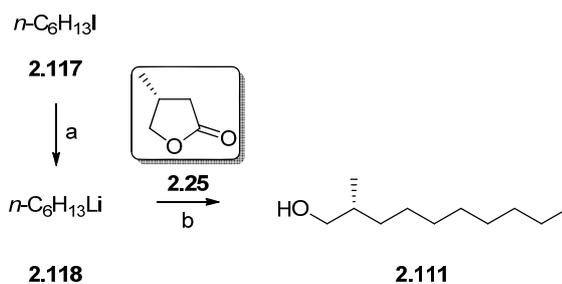
**Scheme 2.35:** Benjamin Cao's synthesis of tuberculostearic acid **2.116** from **2.111**<sup>70</sup>

Other synthetic procedures of tuberculostearic acid **2.116** have also been reported. Seeberger and co-workers reported the synthesis from (*S*)-Roche ester.<sup>71,72</sup> Larsen and co-workers reported a synthesis of chiral methyl-branched alcohols from (*S*)-citronellol.<sup>68</sup>

### 2.5.3. Synthesis of (*R*)-2-methyldecanol, an intermediate for tuberculostearic acid

Our “one pot” procedure was next applied to the synthesis of an intermediate for tuberculostearic acid, (*R*)-2-methyldecanol **2.111** (Scheme 2.36). Thus, *n*-C<sub>6</sub>H<sub>13</sub>Li, generated *in situ* by treating *n*-C<sub>6</sub>H<sub>13</sub>I with 2.1 eq. *t*-BuLi, was reacted with 1.1 eq.

$\gamma$ -lactone **2.25**, followed by the modified Clemmensen reduction. Chiral alcohol **2.111**, was generated in “one pot” in 75 % yield and 96 % ee. The identity of **2.111** could be confirmed by the appearance of the peak at 3.56-3.36 ppm in  $^1\text{H}$  NMR spectrum, which accounted for the hydroxymethylene protons.



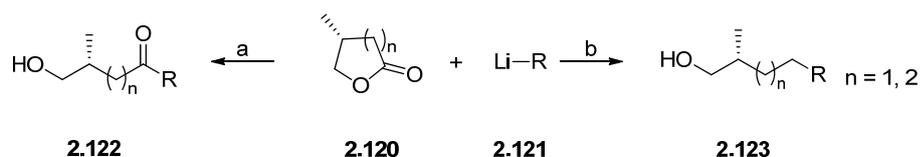
Reagents and conditions: a)  $t\text{-BuLi}$  (2.1 eq.),  $\text{Et}_2\text{O}$ ,  $-78\text{ }^\circ\text{C}$ , 1 h; b) i - **2.25** (1.1 eq.),  $\text{Et}_2\text{O}$ ,  $-78\text{ }^\circ\text{C}$ , 3 h; ii -  $\text{TMSCl}$  (10 eq.),  $\text{Zn}$  (10 eq.),  $\text{MeOH}/\text{Et}_2\text{O}$  (3:1),  $0\text{ }^\circ\text{C}$ , 1 h.

**Scheme 2.36:** Synthesis of intermediate **2.111** with “one pot” procedure from **2.25**

(*R*)-2-Methyldecanol could be used to synthesise tuberculostearic acid over four steps using Benjamin Cao’s method as shown in **Scheme 2.35**.<sup>70</sup> The whole scheme via “one pot” procedure would give tuberculostearic acid in five steps.

## 2.6. Chain elongations

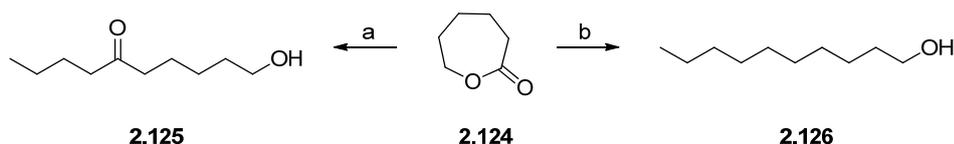
We have shown that alkyllithiums can react with  $\gamma$ -lactones and  $\delta$ -lactones **2.120** to afford either keto-alcohol **2.122** after aqueous work up procedure, or chain extended alcohol **2.123** after modified Clemmensen reduction in one pot (**Scheme 2.37**).



Reagents and conditions: a)  $-78\text{ }^\circ\text{C}$ ,  $\text{Et}_2\text{O}$ , 3 h; acid work up; b) i -  $-78\text{ }^\circ\text{C}$ ,  $\text{Et}_2\text{O}$ , 3 h; ii -  $\text{TMSCl}$  (10 eq.),  $\text{Zn}$  (10 eq.),  $\text{MeOH}$ ,  $0\text{ }^\circ\text{C}$ , 1 h.

**Scheme 2.37:** General procedure for preparing enantiomerically enriched 2-methyl alcohols

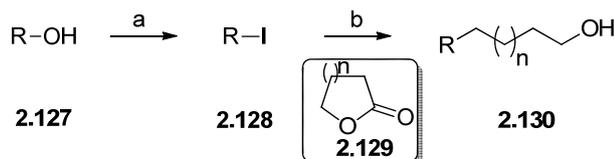
More generally, such carbon chain elongation reactions could be applied to other racemic lactones or unsubstituted lactones.  $\gamma$ -Butyrolactone should be a good substrate for this reaction. It is a widely used solvent and reagent in industry and household products, such as stain remover, superglue remover or paint stripper. However, as  $\gamma$ -butyrolactone is a prodrug for  $\gamma$ -hydroxybutyric acid, which is an illegal drug in many countries,  $\gamma$ -butyrolactone is a border controlled substance in Australia. Because of the issues surrounding  $\gamma$ -butyrolactone, we chose to examine the efficiency of our method with  $\epsilon$ -caprolactone **2.124** (**Scheme 2.38**). In the event, the reaction worked well, producing keto-alcohol **2.125** as a colourless liquid in 80 % yield. Alternatively, the “one pot” procedure gave alcohol **2.126** in 81 % yield.



Reagents and conditions: a)  $n\text{-BuLi}$ ,  $\text{Et}_2\text{O}$ ,  $-78\text{ }^\circ\text{C}$ , 3 h, aqueous work up; b) i -  $n\text{-BuLi}$ ,  $\text{Et}_2\text{O}$ ,  $-78\text{ }^\circ\text{C}$ , 3 h; ii -  $\text{TMSCl}$  (10 eq.),  $\text{Zn}$  (10 eq.),  $\text{MeOH}$ ,  $0\text{ }^\circ\text{C}$ , 1 h.

**Scheme 2.38:** Ring cleavage of caprolactone using  $n\text{-BuLi}$

Thus we have developed a general protocol for the chain extension of primary alcohols, and only a few steps are involved (**Scheme 2.39**). Alcohols **2.127** are converted to halides, which are lithiated to alkyllithiums *in situ* with *t*-BuLi. Afterwards, the generated alkyllithiums could be used to ring open the desired lactones, to give the corresponding chain extend alcohols **2.130**.



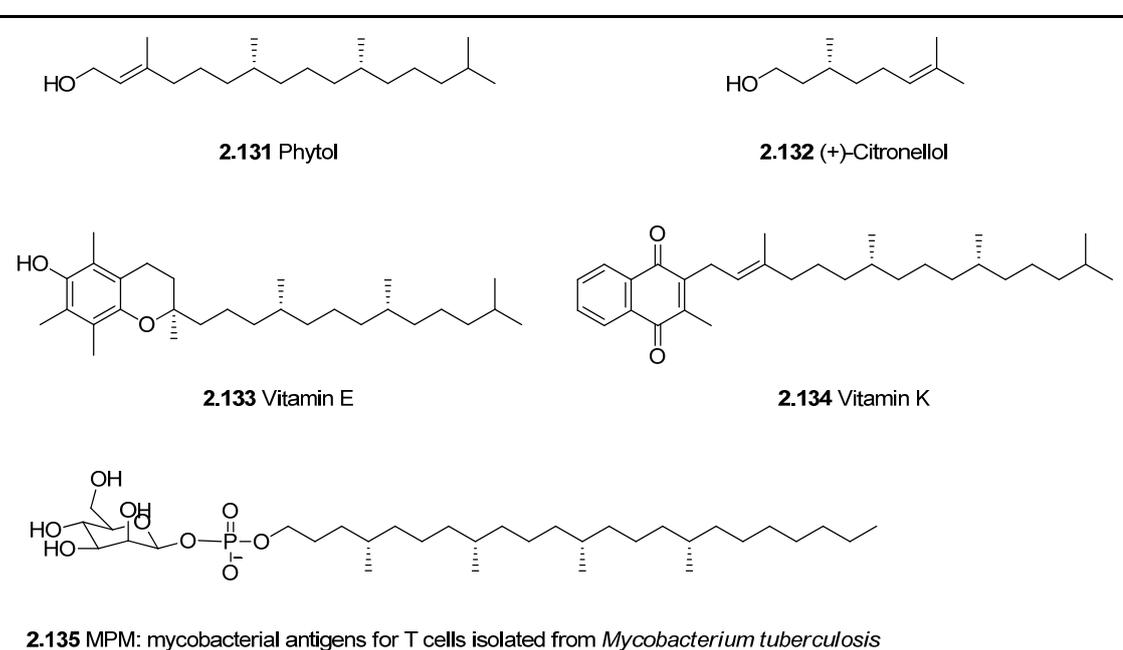
Reagents and conditions: a)  $\text{PPh}_3$  (1.1 eq.),  $\text{I}_2$  (1.1 eq.),  $0\text{ }^\circ\text{C}$ ,  $\text{CH}_2\text{Cl}_2$ ; b) i - *t*-BuLi (2.1 eq.),  $\text{Et}_2\text{O}$ ,  $-78\text{ }^\circ\text{C}$ , 1 h; ii -  $\text{Et}_2\text{O}$ ,  $-78\text{ }^\circ\text{C}$ , 3 h; iii -  $\text{TMSCl}$  (10 eq.),  $\text{Zn}$  (10 eq.),  $\text{MeOH/Et}_2\text{O}$  (3:1),  $0\text{ }^\circ\text{C}$ , 1 h.

**Scheme 2.39:** Chain extension by alkyllithiums ring opening lactones

## 2.7. Synthesis of the side chain of vitamin E

### 2.7.1. Natural products with repeating isoprenoid moieties

In Nature, there is a group of products that contain repeating isoprenoid moieties, such as phytol **2.131**, citronellol **2.132**, vitamins E **2.133** and K **2.134**, and MPM **2.135** (Figure 2.13).



**Figure 2.13:** Natural products with repeating chiral isoprenoid moieties

### 2.7.2. Vitamin E

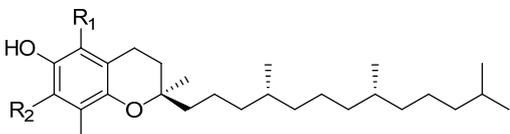
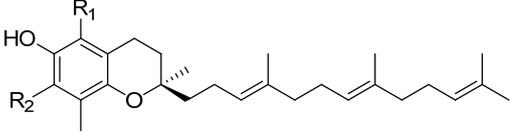
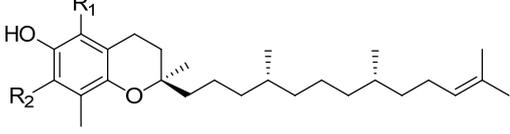
Evans and co-workers first discovered vitamin E in 1922, believing it is a substance which maintains rat fertility.<sup>73</sup> In 1936, his group isolated one pure vitamin E form,  $\alpha$ -tocopherol from wheat-germ oil.<sup>74</sup> The correct chemical structure was identified by Fernholz and co-workers in 1938,<sup>75</sup> followed by the first racemic total synthesis by Karrer and co-workers in the same year.<sup>76</sup> Other family members of vitamin E were discovered and identified over the following years. Vitamin E plays important physiological roles in human and animal bodies. It is an efficient antioxidant in

membranes,<sup>77</sup> and also possesses anti-inflammatory properties.<sup>78</sup>

Vitamin E is not synthesised by humans and animals, making supplementation from diet essential. For animals, vitamin E deficiency may result in anemia, muscle necrosis, and death. For humans, the symptoms of vitamin E deficiency include muscle weakness, reduced sensory perception, scoliosis, and muscle structural abnormality.<sup>79</sup>

There are three groups in the vitamin E family, tocopherols, tocotrienols and the newly discovered tocomonoenol (**Table 2.5**).<sup>80</sup> Each vitamin E family member exhibits slightly different biological activity.  $\gamma$ -Tocopherol is the most prevalent form of vitamin E contained in plant seeds. Cheng and co-workers found that natural  $\gamma$ -tocopherol exhibited estrogenic activity is effective in activation of estrogenic receptors, making it useful for menopausal women.<sup>81</sup>

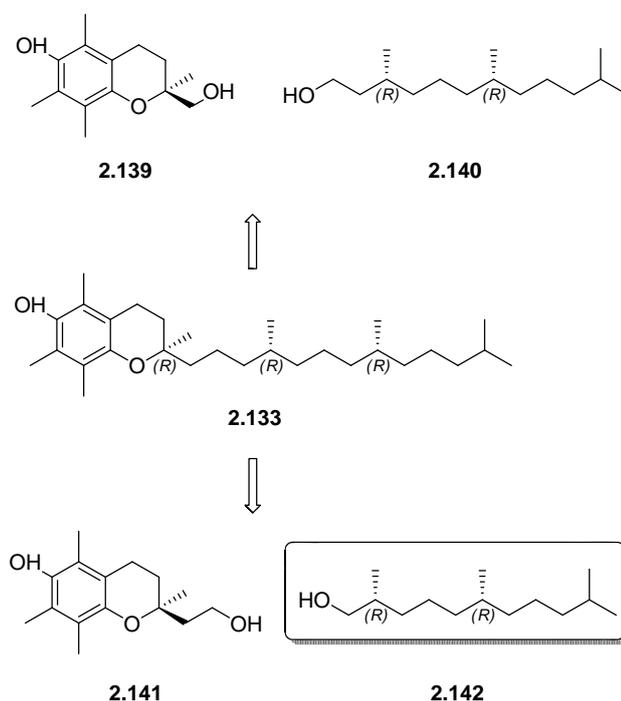
**Table 2.5:** Vitamin E family members and structures

Structures	R <sub>1</sub>	R <sub>2</sub>	Name
 <p><b>2.136</b> Tocopherol</p>	CH <sub>3</sub>	CH <sub>3</sub>	$\alpha$ -Tocopherol
	CH <sub>3</sub>	H	$\beta$ -Tocopherol
	H	CH <sub>3</sub>	$\gamma$ -Tocopherol
	H	H	$\delta$ -Tocopherol
 <p><b>2.137</b> Tocotrienol</p>	CH <sub>3</sub>	CH <sub>3</sub>	$\alpha$ -Tocotrienol
	CH <sub>3</sub>	H	$\beta$ -Tocotrienol
	H	CH <sub>3</sub>	$\gamma$ -Tocotrienol
	H	H	$\delta$ -Tocotrienol
 <p><b>2.138</b> Tocomonoenol</p>	H	H	$\alpha$ -Tocomonoenol

Many thousand tons of vitamin E are produced annually, 90% of which are racemic  $\alpha$ -tocopherol, which is mainly used in animal feed and cosmetic products.<sup>82</sup>

### 2.7.3. Previous syntheses of the side chain of vitamin E

As *R,R,R*- $\alpha$ -tocopherol **2.133** is the natural stereoisomer and the bioactive form, its synthesis will be discussed here. It can be synthesised from two chroman derivatives **2.139** and **2.141** cross coupled with two different side chains **2.140** and **2.142**. Both of the asymmetric synthetic approaches to chromanmethanol **2.139** and chromanethanol **2.141** have been reported (Scheme 2.40).<sup>83-85</sup>

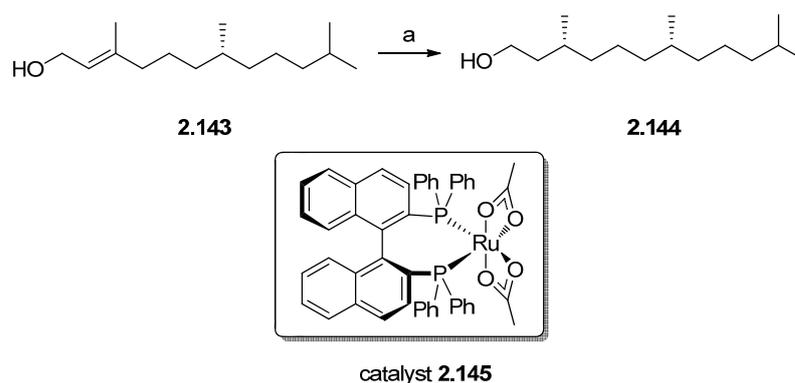


**Scheme 2.40:** Retrosynthesis of  $\alpha$ -tocopherol

Two benchmark synthesis of alcohol **2.140** and **2.142** have been reported by Noyori<sup>86</sup> and Negishi.<sup>37</sup> The total synthesis of **2.142** is the focus of our work described in section 2.7.4.

#### 2.7.3.1. Noyori's method

Noyori reported that pure (*R,E*)-3,7,11-trimethyldodec-2-enol **2.143** can undergo asymmetric hydrogenation by treatment with (*S*)-Ru-BINAP **2.145** as catalyst under 100 atm H<sub>2</sub> in MeOH at 20 °C for 3.5 h (Scheme 2.41).<sup>86</sup> The product **2.144** was obtained in 99 % yield and 99 % ee.

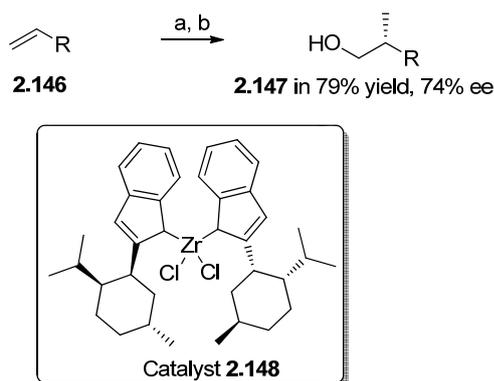


Reagents and conditions: a) **2.145** (0.2 mol%), H<sub>2</sub> (100 atm), MeOH, 20 °C, 3.5 h.

**Scheme 2.41:** Noyori's asymmetric synthesis with Ru-BINAP complex as catalyst<sup>86</sup>

### 2.7.3.2. Negishi's method

Negishi developed a splendid, high yielding protocol for generating repeating isoprenoid moieties via Zr-catalysed asymmetric carboalumination of alkenes (**Scheme 2.42**).<sup>37</sup> With (3-neomenthyl-1-indenyl)<sub>2</sub>ZrCl<sub>2</sub> **2.148** as catalyst, terminal alkenes form carbon-carbon bonds with good levels of enantioselectivity. Alkene polymerisation is minimized by employing no more than 1 eq. alkene.

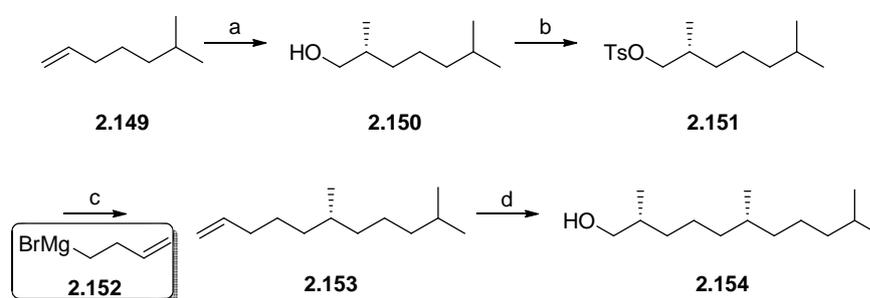


Reagents and conditions: a) Me<sub>3</sub>Al, catalyst **2.148**; b) O<sub>2</sub>.

**Scheme 2.42:** Asymmetric synthesis 2-methyl alcohols with **2.148** as catalyst<sup>37</sup>

Negishi's synthesis of **2.154** began with the treatment of alkene **2.149** with 1 eq. Me<sub>3</sub>Al in the presence of 2 mol% of catalyst **2.148** in CH<sub>2</sub>Cl<sub>2</sub>, followed by oxidation

with O<sub>2</sub> to give alcohol **2.150** in 79 % yield and 74 % ee (**Scheme 2.43**).<sup>37</sup> To introduce a second chiral moiety, alcohol **2.150** was converted to its corresponding tosylate, followed by cross coupling with Grignard **2.152** to give terminal alkene **2.153**. Asymmetric carboalumination reaction gave target alcohol **2.154** with two chiral isoprenoid moieties. Overall, the process took four steps for introducing each chiral methyl group (from alkene to alkene), and the overall yield for one cycle was up to 61 %.<sup>37</sup> This methodology is a direct and efficient synthetic method. However, the enantiomeric purity for each embedded methyl branch is only 74 % ee.

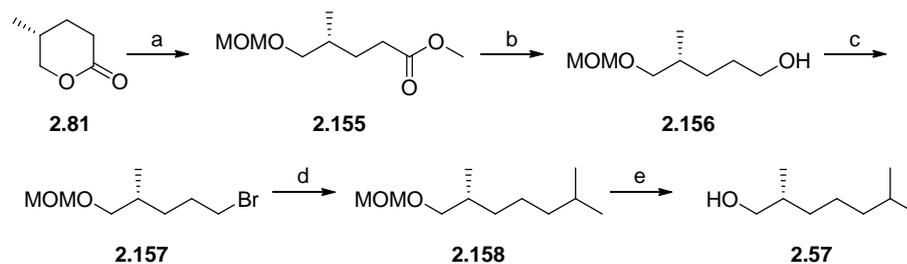


Reagents and conditions: a) i - Me<sub>3</sub>Al, (-)-(3-neomenthyl-1-indenyl)<sub>2</sub>ZrCl<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>; ii - O<sub>2</sub>; b) TsCl, pyridine; c) Li<sub>2</sub>CuCl<sub>4</sub>, THF; d) i - Me<sub>3</sub>Al, (-)-(3-neomenthyl-1-indenyl)<sub>2</sub>ZrCl<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>; ii - O<sub>2</sub>.

**Scheme 2.43:** Negishi's asymmetric synthesis with **2.148** as catalyst<sup>37</sup>

### 2.7.3.3. Tian's method

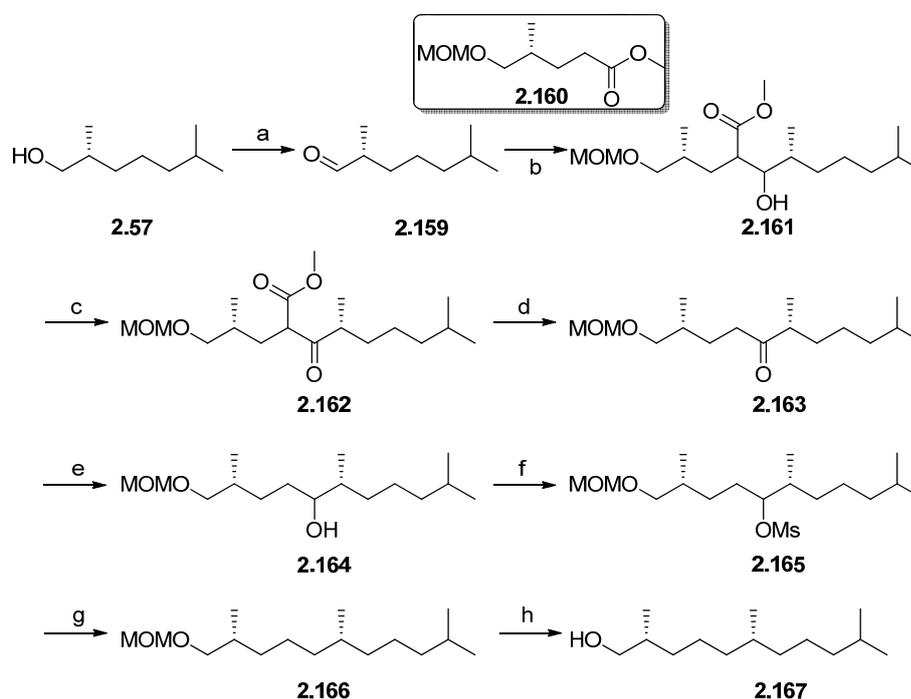
Tian and co-workers have reported a synthesis of the vitamin E side chain from (*R*)-4-methyl- $\delta$ -valerolactone **2.81**.<sup>87</sup> The intermediate (*R*)-2,6-dimethylheptanol **2.57** was prepared over five steps from  $\delta$ -lactone **2.81** (**Scheme 2.44**). **2.81** was treated with dimethoxymethane to give ester **2.155**, followed by reduction to alcohol **2.156** with LiAlH<sub>4</sub>. Cross-coupling of the corresponding bromide **2.157** with Grignard *i*-C<sub>3</sub>H<sub>7</sub>MgBr gave **2.158**, and deprotection then gave (*R*)-2,6-dimethylheptanol **2.57**.



Reagents and conditions: a) Dimethoxymethane, H<sub>2</sub>SO<sub>4</sub>; b) LiAlH<sub>4</sub>, THF; c) CBr<sub>4</sub>, PPh<sub>3</sub>, K<sub>2</sub>CO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>; d) Li<sub>2</sub>CuCl<sub>4</sub>, NMP, *i*-C<sub>3</sub>H<sub>7</sub>MgBr, THF; e) HCl, THF, reflux.

**Scheme 2.44:** Synthesis of (*R*)-2,6-dimethylheptanol by Tian<sup>87</sup>

Chiral alcohol **2.57** was oxidised to aldehyde **2.159**, which then underwent aldol reaction with ester **2.160** (Scheme 2.45).<sup>87</sup> Oxidation and decarboxylation of the resultant β-keto ester gave ketone **2.163**. Clemmensen reduction of **2.163** led to racemisation. Consequently, **2.163** was reduced to alcohol **2.164**, and then converted to mesylate **2.165**. Reduction and deprotection gave target (*2R,6R*)-2,6,10-trimethyl undecanol **2.167**.



Reagents and conditions: a) (COCl)<sub>2</sub>, DMSO; b) *n*-BuLi, *i*-Pr<sub>2</sub>NH, THF; c) Jone's oxidation; d) LiOH, THF, reflux; e) NaBH<sub>4</sub>, Et<sub>2</sub>O/MeOH; f) MsCl, Et<sub>3</sub>N, DCM; g) NaBH<sub>4</sub>, DMSO, 100 °C; h) CH<sub>3</sub>OH, HCl, reflux.

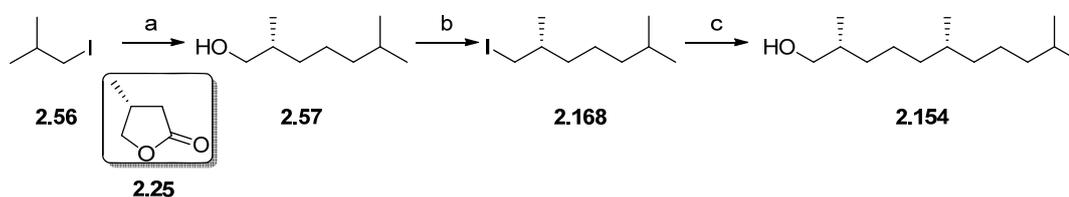
**Scheme 2.45:** Asymmetric synthesis of the side chain of vitamin E by Tian<sup>87</sup>

The whole procedure took 13 steps from  $\delta$ -valerolactone **2.81** in an overall yield of only 5%.<sup>87</sup>

#### 2.7.4. Synthesis of the side chain of vitamin E via the “one pot” procedure

Given the limitation outlined above in current approach, it was interesting to establish whether our developed approach would be effective in preparing a system with two chiral isoprenoid units as is the case for the side chain of vitamin E **2.154** (Scheme 2.46). Thus (*R*)-2,6-dimethylheptanol **2.57** was prepared, as described earlier, from *i*-BuLi and 1.1 eq.  $\gamma$ -lactone **2.25**. **2.57** was then converted to iodide **2.168**. As discussed in Chapter 1, there are options available for such conversion and in this case we selected Appel reaction. Thus alcohol **2.57** was treated with 1.1 eq. I<sub>2</sub>, PPh<sub>3</sub> and imidazole in CH<sub>2</sub>Cl<sub>2</sub> at 0 °C for 1 h. The product was purified by eluting through a short plug of silica with pentane giving the iodide **2.168** as a colourless liquid in 81 % yield. The identity of **2.168** was confirmed by the appearance of peaks at 3.23 ppm, which accounted for the CH<sub>2</sub>I protons.

Lithiation with *t*-BuLi in Et<sub>2</sub>O at -78 °C for 1 h, followed by addition to  $\gamma$ -lactone **2.25** in Et<sub>2</sub>O *in situ* at -78 °C gave the keto-alcohol, which was reduced *in situ* using 20 eq. TMSCl, 20 eq. Zn and MeOH to give (*2R,6R*)-2,6,10-trimethylundecanol **2.154** in 41 % yield.



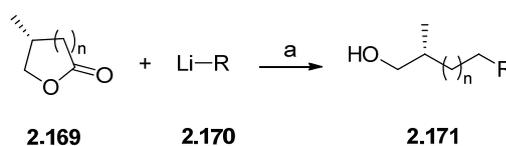
Reagents and conditions: a) i - 2.1 eq. *t*-BuLi, Et<sub>2</sub>O, -78 °C, 1 h; ii - **2.25** (1.1 eq.), -78 °C, 3 h; iii - TMSCl (10 eq.), Zn (10 eq.), MeOH, 0 °C, 1 h; b) PPh<sub>3</sub> (1.1 eq.), I<sub>2</sub> (1.1 eq.), imidazole (1.1 eq.), 0 °C, 1 h; c) i- *t*-BuLi (2.1 eq.), Et<sub>2</sub>O, -78 °C, 1 h; ii - **2.25** (1.1 eq.), -78 °C, Et<sub>2</sub>O, 3 h; iii - TMSCl (10 eq.), Zn (10 eq.), MeOH, 0 °C, 1 h.

**Scheme 2.46:** Asymmetric synthesis of vitamin E side chain from lactone

(*R*)-2,6-Dimethylheptanol **2.57** generated from the first ring opening cycle, has been proved enantiomeric pure by NMR analysis of its Mosher ester. Considering the reaction conditions for each cycle are identical, thus (*2R,6R*)-2,6,10-trimethylundecanol **2.154** should also be enantiomeric pure.

## 2.8. Conclusions

The key intermediates for many chiral natural products, the chiral 2-methyl alcohols **2.171**, were synthesised from ring cleavage of chiral lactones to give the keto-alcohols, which were reduced by a modified Clemmensen reduction *in situ* to give the 2-methyl alcohols **2.171** (Scheme 2.47).



Reagents and conditions: a) i - Et<sub>2</sub>O, -78 °C, 4 h; ii - TMSCl (10 eq.), Zn (10 eq.), MeOH, 0 °C, 1 h.

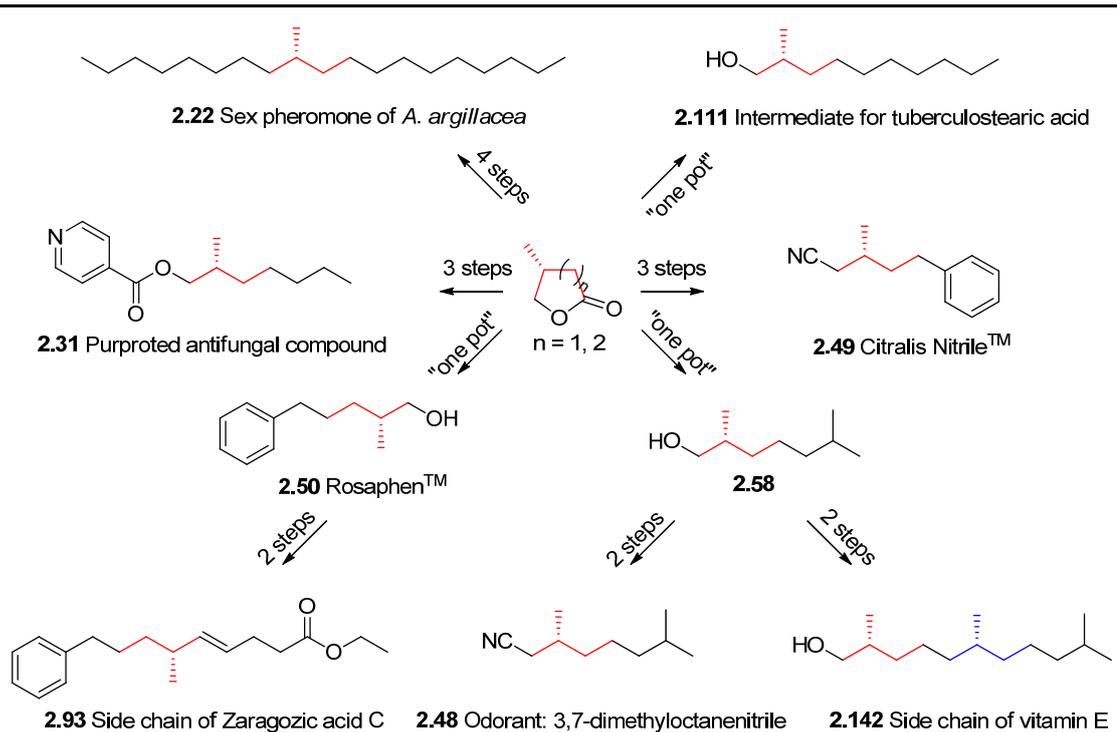
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**Scheme 2.47:** General procedure for preparing chiral 2-methyl alcohols

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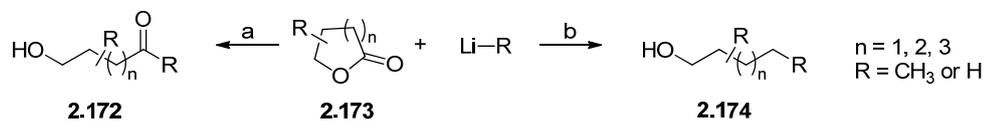
The enantiomerically pure alcohols were employed in the synthesis of several natural products, including the sex pheromone of *A. argillacea* **2.22**, three chiral fragrances **2.48**, **2.49**, and **2.50**, the side chain of zaragozic acid C **2.93**, an intermediate of tuberculostearic acid **2.111**, the side chain of vitamin E **2.142** and (*R*)-2-methylheptylisonicotinate **2.31** (Figure 2.14).

The developed methodology was efficient with good yield, short schemes and convenient reaction conditions. Lactones could be prepared in high enantiomeric purity from degradation of tigogenin, or via asymmetric hydrogenation. The chirality of the starting material lactones was well retained during the reaction procedures to ensure the products with high enantiomeric purity.



**Figure 2.14:** Summary of synthesised structures from chiral lactones

More generally, the ring opening reactions could be applied to racemic or unbranched lactones (**Scheme 2.48**). Alkylolithiums ring opening five, six, and seven member lactones were attempted using  $\gamma$ -,  $\delta$ -, and  $\epsilon$ -lactones to give either keto-alcohols **2.172** after aqueous work up procedure, or chain extended alcohols **2.174** by modified Clemmensen reduction *in situ*. The application for extending more than six carbons using macrolactones could be carried out in future work.

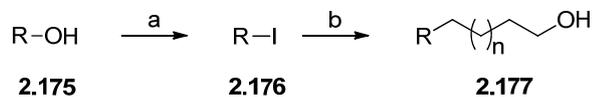


Reagents and conditions: a)  $\text{Et}_2\text{O}$ ,  $-78^\circ\text{C}$ , 3 h, HCl work up; b) i-  $\text{Et}_2\text{O}$ ,  $-78^\circ\text{C}$ , 3 h; ii - TMSCl (10 eq.), Zn (10 eq.), MeOH,  $0^\circ\text{C}$ , 1 h.

**Scheme 2.48:** Lactone ring cleavage reactions using alkylolithiums

Two steps are required to extend carbon chain alcohols (**Scheme 2.49**). Alcohol **2.175** could be converted to iodide **2.176**, which is converted to alkylolithium after treatment

with *t*-BuLi. The generated alkyllithium can be used to ring open desired lactones and followed by reduction *in situ* to give the corresponding chain extend alcohol **2.177**.



Reagents and conditions: a) PPh<sub>3</sub> (1.1 eq.), I<sub>2</sub> (1.1 eq.), CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 1 h; b) i - *t*-BuLi (2.1 eq.), Et<sub>2</sub>O, -78 °C, 1 h; ii - **2.173** (1.1 eq.), Et<sub>2</sub>O, -78 °C, 3 h; iii - TMSCl (10 eq.), Zn (10 eq.), MeOH, 0 °C, 1 h.

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**Scheme 2.49:** Chain extension by alkyllithiums ring opening lactones

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## 2.9. Experimental

### 2.9.1. General procedure

Melting points were measured on Reichert hot stage melting point apparatus (Reichert, Austria).

Optical Rotations were measured on a PolAAR 2001 automatic polarimeter (Optical Activity Ltd., Huntingdon, Cambridgeshire, UK) with 1 dm cell in CHCl<sub>3</sub>, at a wavelength of 589 nm (sodium D line) at r.t., and were assigned as [ $\alpha$ ]<sub>D</sub>, temperature, concentration *c* (g/100 mL) and solvent.

<sup>1</sup>H NMR spectra were determined at 300 MHz with a Bruker Avance DPX300 spectrometer or at 400 MHz with a Bruker Avance DRX400 spectrometer (Bruker BioSpin Corp., Billerica, Massachusetts, USA). The chemical shifts ( $\delta$ , ppm) were referenced to the residual CHCl<sub>3</sub> signal ( $\delta$  7.26 ppm). Each resonance was quoted with chemical shifts measured in parts per million (ppm), multiplicities, coupling constants (*J* Hz), number of protons, and structural assignments. Multiplicities were denoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or b (broad).

<sup>13</sup>C NMR spectra were recorded on 75 MHz with a Bruker Avance DPX300 spectrometer or at 100 MHz with a Bruker Avance DRX400 spectrometer (Bruker BioSpin Corp., Billerica, Massachusetts, USA) in CDCl<sub>3</sub>, referenced with the residual CHCl<sub>3</sub> signal ( $\delta$  77.16 ppm). Each resonance was quoted with chemical shifts measured in parts per million (ppm) and structural assignment.

<sup>19</sup>F NMR spectrum was determined at 377 MHz at a Bruker Avance DRX400 spectrometer (Bruker BioSpin Corp., Billerica, Massachusetts, USA). Trichlorofluoromethane was used as internal reference ( $\delta$  0.00 ppm).

Low resolution electrospray ionisation mass spectrometry was measured on a Micromass Platform spectrometer (Micromass Ltd., Wythenshawe, Manchester, UK). Accurate mass determinations were made on an Agilent G1969A LC-TOF system (Agilent Technologies, Santa Clara, California, USA) with reference and mass

correction at 4000 V capillary voltage for electrospray ionisation.

Infra red spectra were obtained using a Bruker Equinox IFS55 FTIR, fitted with a mercury-cadmium-telluride detector and a Specac Golden Gate single reflection diamond ATR element. The operation condition was 50 scans at a resolution of 4 cm<sup>-1</sup>. The intensity of absorption bands for all samples have been specified as either s (strong), m (medium), w (weak) or b (broad).

Kugelrohr distillations were carried on a Büchi GKR-50 apparatus *in vacuo*.

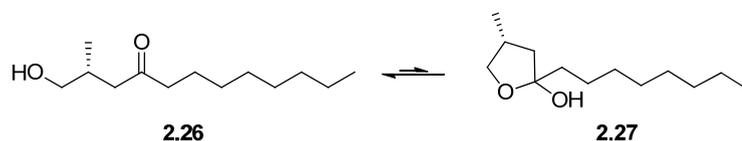
Analytical TLC was performed on Merck Silica Gel 60 F<sub>254</sub>, and visualised under a 254 nm UV lamp and/or stained using an alkaline KMnO<sub>4</sub> solution. Flash chromatography was performed using Silica Gel 60 (230-400 mesh, no. 9385) from Merck or SiliaFlash<sup>®</sup> P60 (230-400 mesh) from Silicycle.

### 2.9.2. Materials

Commercially available reagents and chemicals were purchased from Sigma-Aldrich Company. (Milwaukee, Wisconsin, USA), AK Scientific Inc. (Union City, California, USA), Merck (Darmstadt, Hesse, Germany), Ajax Finechem Pty. Ltd. (Sydney, New South Wales, Australia), or Lancaster Synthesis Ltd. (Morecambe, Lancashire, UK), and are reagent grade unless specified. Anhydrous solvents were freshly distilled under N<sub>2</sub> prior to use as the following procedure: Toluene and Et<sub>3</sub>N were distilled from CaH; CH<sub>2</sub>Cl<sub>2</sub> was distilled from P<sub>2</sub>O<sub>5</sub>; THF and Et<sub>2</sub>O were distilled from Na/benzophenone; (CH<sub>2</sub>Cl)<sub>2</sub> was dried over activated 4 Å molecular sieves. The solvents used in alkyllithiums or Grignard reagents involved reactions were degassed by sparging with Ar for 45 min after distillation from Na.

### 2.9.3. Synthesis of sex pheromone (*S*)-9-methylnonadecane via the lactone ring cleavage reaction

#### 2.9.3.1. (*R*)-Hydroxy-2-methyldodecan-4-one (2.26)

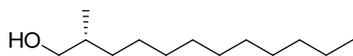


To a solution of *n*-C<sub>8</sub>H<sub>17</sub>I (0.48 g, 2 mmol) in Et<sub>2</sub>O (20 mL) at -78 °C was added *t*-BuLi (1.7 M in pentane, 2.6 mL, 4.4 mmol). The reaction was stirred at -78 °C for 1 h and then warmed gradually to r.t. for 20 min. The mixture was then added via cannula to a solution of  $\gamma$ -lactone **2.25** (0.21 g, 2.1 mmol) in Et<sub>2</sub>O (10 mL) at -78 °C. After stirring at -78 °C for 4 h, the reaction was quenched by adding HCl (5 M, 2 mL), and gradually warmed to r.t.. The mixture was washed with HCl (1 M, 30 mL). The organic layer was collected, and the aqueous phase was extracted with Et<sub>2</sub>O (2  $\times$  25 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and the solvent was removed *in vacuo*. The resulting yellow liquid was purified by flash chromatography (hexane/EtOAc 3:1) to give the **2.26** and **2.27** (0.30 g, 76 % altogether) as a colourless liquid.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  3.59-3.51 (m, 1 H, CH<sub>2</sub>OH), 3.44-3.36 (m, 1 H, CH<sub>2</sub>OH), 2.55 (dd, *J* = 16.6, 6.7 Hz, 1 H, CH<sub>2</sub>C=O), 2.45-2.38 (m, 2 H, CH<sub>2</sub>C=O), 2.34 (dd, *J* = 16.6, 6.3 Hz, 1 H, CH<sub>2</sub>C=O), 2.22 (m, 1 H, CH), 1.65-1.48 (m, 2 H, CH<sub>2</sub>), 1.36-1.18 (m, 10 H, 5  $\times$  CH<sub>2</sub>), 0.93 (d, *J* = 6.8 Hz, 3 H, CHCH<sub>3</sub>), 0.88 (m, 3 H, CH<sub>2</sub>CH<sub>3</sub>).

**ESI-MS:** Calcd. for [C<sub>13</sub>H<sub>26</sub>O<sub>2</sub>+Na<sup>+</sup>]: 237.18, found 237.18.

**Specific Rotation**  $[\alpha]_D^{22}$  +12.4 ° (*c* 0.7, CHCl<sub>3</sub>)

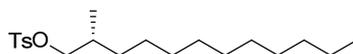
**2.9.3.2. (R)-Methyldodecanol (2.28)****2.28**

To a solution of keto-alcohol **2.26** (0.1 g, 0.47 mmol) in MeOH (12 mL) and CH<sub>2</sub>Cl<sub>2</sub> (4 mL) at 0 °C, was added Zn dust (0.24 g, 3.73 mmol) and TMSCl (0.47 mL, 3.73 mmol). The reaction was stirred at 0 °C for 1 h, and quenched by adding NaHCO<sub>3</sub> (0.39 g, 4.67 mmol). After stirring for 5 min at r.t., the suspension was filtered through celite, and the solvent was removed *in vacuo*. HCl (1 M, 30 mL) was added and the mixture was extracted with Et<sub>2</sub>O (3 × 30 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent removed *in vacuo*. The crude product was purified by flash chromatography (Hexane/Et<sub>2</sub>O 3:1) to give the *title compound* (90 mg, 96 %) as a colourless liquid. The analytical data were in agreement with literature values.<sup>15</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.50 (dd, *J* = 10.5, 5.8 Hz, 1 H, CH<sub>2</sub>OH), 3.41 (dd, *J* = 10.5, 6.6 Hz, 1 H, CH<sub>2</sub>OH), 1.68-1.51 (m, 1 H, CH), 1.45-1.18 (m, 17 H, CH<sub>2</sub>), 1.11 (dd, *J* = 13.8, 7.0 Hz, 1 H, CHCH<sub>2</sub>), 0.91 (d, *J* = 6.7 Hz, 3 H, CH<sub>3</sub>), 0.88 (t, *J* = 6.9 Hz, 3 H, CH<sub>3</sub>).

<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 68.58(CH<sub>2</sub>OH), 35.91(CH), 33.29(CH<sub>2</sub>), 32.06(CH<sub>2</sub>), 30.09(CH<sub>2</sub>), 29.81(CH<sub>2</sub>), 29.79(CH<sub>2</sub>), 29.49(CH<sub>2</sub>), 27.13(CH<sub>2</sub>), 22.83(CH<sub>2</sub>), 16.72(CHCH<sub>3</sub>), 14.26(CH<sub>2</sub>CH<sub>3</sub>).

**Specific Rotation**  $[\alpha]_{\text{D}}^{22} +9.1^\circ$  (*c* 0.8, CHCl<sub>3</sub>) (*S*: Lit.<sup>15</sup>:  $[\alpha]_{\text{D}}^{22} -8.4^\circ$  (*c* 1, CHCl<sub>3</sub>))

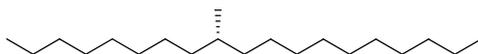
**2.9.3.3. (R)-2-Methyldodecyl 4-methylbenzenesulfonate (2.29)****2.29**

To a solution of alcohol **2.28** (0.30 g, 1.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was added TsCl (0.43 g, 2.2 mmol), DMAP (18 mg, 0.15 mmol) and pyridine (0.36 mL, 4.5 mmol). The reaction was stirred at r.t. for 24 h. The solvent was removed *in vacuo*, and the resulting yellow liquid was purified by eluting through a short plug of silica (hexane/Et<sub>2</sub>O 3:1) to give the *title compound* (0.53 g, quantitative yield) as a

colourless liquid.

$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.79 (d,  $J = 8.3$  Hz, 2 H, ArH), 7.34 (d,  $J = 8.0$  Hz, 2 H, ArH), 3.84 (ddd,  $J = 32.7, 9.4, 6.1$  Hz, 2 H,  $\text{CH}_2\text{O}$ ), 2.45 (s, 3 H,  $\text{CH}_3$  for Ts), 1.76 (td,  $J = 12.7, 6.5$  Hz, 1 H, CH), 1.37-1.00 (m, 18 H,  $9 \times \text{CH}_2$ ), 0.88 (t,  $J = 6.6$  Hz, 6 H,  $2 \times \text{CH}_3$ ).

#### 2.9.3.4. (S)-9-Methylnonadecane (2.1)



**2.22**

$n\text{-C}_7\text{H}_{15}\text{MgBr}$  was generated *in situ* with following procedure: To a solution of a small  $\text{I}_2$  crystal in THF (1 mL) in a round bottom flask equipped with a condenser was added fresh Mg turning (0.08 g, 3.4 mmol). The mixture was initially heated to reflux until the pink solution turned colourless, and then the oil bath was removed, and  $n\text{-C}_7\text{H}_{15}\text{Br}$  (0.3 g, 1.68 mmol) in THF (1 mL) was added in with a controlled speed to keep the mixture moderate reflux. After being refluxed for 3 h, the mixture was cool to r.t., and the Grignard reagent was titrated with salicylaldehyde phenylhydrazine.<sup>16</sup> The concentration was determined as 0.71 mol/L.

To a solution of tosylate **2.29** (40 mg, 0.11 mmol) in THF (0.5 mL) was added  $\text{CuCl}_2$  (0.5 mg, 3.7  $\mu\text{mol}$ ). The mixture was cooled to  $-10$  °C, and the freshly prepared  $n\text{-C}_7\text{H}_{15}\text{MgBr}$  (0.19 mL, 0.71 mol/L, 0.135 mmol) was added dropwise via cannula. After addition, the reaction was kept at  $0$  °C for 1 h, and then r.t. for 2 h. The resulting brown liquid was purified by eluting through a short plug of silica with hexane to give the *title compound* (23 mg, 73%) as a colourless film. The analytical data were in agreement with literature values.<sup>12</sup>

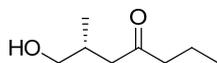
$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.58-1.02 (m, 33 H, CH and  $16 \times \text{CH}_2$ ), 0.88 (t,  $J = 6.9$  Hz, 6 H,  $2 \times \text{CH}_3$ ), 0.84 (d,  $J = 6.5$  Hz, 3 H,  $\text{CH}_3$ ).

**IR**  $\nu_{\text{max}}$  2956 (m), 2923 (s), 2853 (m)  $\text{cm}^{-1}$ .

**Specific Rotation**  $[\alpha]_{\text{D}}^{22} -4.1$  ° ( $c$  0.3,  $\text{CHCl}_3$ )

## 2.9.4. Synthesis of (*R*)-2-methylheptylisonicotinate, a purported antifungal compound, via the lactone ring cleavage reaction

### 2.9.4.1. (*R*)-Hydroxy-2-methylheptan-4-one (2.41)

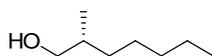


2.41

To a solution of *n*-C<sub>3</sub>H<sub>7</sub>I (0.26 g, 1.53 mmol) in Et<sub>2</sub>O (15 mL) at -78 °C was added *t*-BuLi (1.7 M in pentane, 1.98 mL, 3.36 mmol). The reaction was stirred at -78 °C for 1 h and then warmed to r.t. for 20 min. The mixture was then added via cannula to a solution of  $\gamma$ -lactone **2.25** (0.15 g, 1.50 mmol) in Et<sub>2</sub>O (10 mL) at -78 °C and the reaction stirred for 4 h. TMSCl (1.0 mL, 7.88 mmol) was added, and the reaction was allowed to warm to r.t. The mixture was washed with HCl (1 M, 25 mL), and the aqueous phase extracted with Et<sub>2</sub>O (3  $\times$  25 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and the solvent was removed *in vacuo*. The product was purified by flash chromatography (hexane/EtOAc 3: 1) to give the *title compound* (0.16 g, 72 %) as a colourless liquid.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  3.59-3.37 (m, 2 H, CH<sub>2</sub>O), 2.55 (dd, *J* = 16.6, 6.7 Hz, 1 H, CH<sub>2</sub>C=O), 2.40 (t, *J* = 7.3 Hz, 2 H, CH<sub>2</sub>C=O), 2.33 (dd, *J* = 16.6, 6.4 Hz, 1 H, CH<sub>2</sub>C=O), 2.22 (m, 1 H, CH), 1.82 (br s, 1 H, OH), 1.64-1.59 (m, 2 H, CH<sub>2</sub>CH<sub>3</sub>), 0.92 (m, 6 H, 2  $\times$  CH<sub>3</sub>).

### 2.9.4.2. (*R*)-2-Methylheptanol (2.37)



2.37

To a solution of keto-alcohol **2.41** (0.18 g, 1.25 mmol) in MeOH (15 mL) and CH<sub>2</sub>Cl<sub>2</sub> (5 mL) at 0 °C was added Zn dust (0.41 g, 6.24 mmol) and TMSCl (0.79 mL, 6.24 mmol). The reaction was stirred at 0 °C for 1 h, and then quenched by adding NaHCO<sub>3</sub> (0.8 g, 9.52 mmol). After stirring for 10 min at r.t., the mixture was filtered through celite, and the solvent was removed *in vacuo*. HCl (1 M, 30 mL) was added and then extracted with Et<sub>2</sub>O (3  $\times$  30 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent removed *in vacuo*. The crude product was purified by flash chromatography (pentane/Et<sub>2</sub>O 2:1) to give the *title compound*

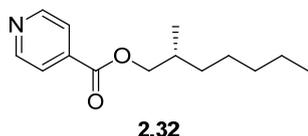
(0.15 g, 95%) as a colourless liquid. The analytical data were in agreement with literature values.<sup>27</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.56-3.38 (m, 2 H, CH<sub>2</sub>OH), 1.67-1.56 (m, 1 H, CH), 1.45-1.03 (m, 8 H, 4 × CH<sub>2</sub>), 0.96-0.87 (m, 6 H, 2 × CH<sub>3</sub>).

<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 68.61(CH<sub>2</sub>OH), 35.94(CHCH<sub>3</sub>), 33.26(CH<sub>2</sub>), 32.30(CH<sub>2</sub>), 26.79(CH<sub>2</sub>), 22.79(CH<sub>2</sub>), 16.73(CHCH<sub>3</sub>), 14.22(CH<sub>2</sub>CH<sub>3</sub>).

**Specific Rotation**  $[\alpha]_{\text{D}}^{22} +12.8^{\circ}$  (*c* 1.2, CHCl<sub>3</sub>) (*S*: Lit.<sup>27</sup>:  $[\alpha]_{\text{D}}^{20} -13.1^{\circ}$  (*c* 1.15, CHCl<sub>3</sub>))

#### 2.9.4.3. (*R*)-2-Methylheptylisonicotinate (2.32)



To a solution of alcohol **2.37** (0.10 g, 0.77 mmol) in pyridine (10 mL) was added isonicotinoyl chloride·HCl (0.15 g, 0.85 mmol). The mixture was stirred at r.t. for 16 h. The crude product was purified by flash chromatography (hexane/EtOAc 3:1) to give the *title compound* (0.17 g, 94 % yield) as a colourless liquid.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.78 (dd, *J* = 4.5, 1.6 Hz, 2 H, 2 × NCH), 7.84 (dd, *J* = 4.4, 1.6 Hz, 2 H, 2 × CCH), 4.25 (dd, *J* = 10.7, 5.8 Hz, 1 H, CH<sub>2</sub>O), 4.14 (dd, *J* = 10.7, 6.8 Hz, 1 H, CH<sub>2</sub>O), 2.09-1.77 (m, 1 H, CH), 1.51-1.17 (m, 8 H, 2 × CH<sub>2</sub>), 1.02 (d, *J* = 6.8 Hz, 3 H, CH<sub>3</sub>), 0.89 (t, *J* = 6.9 Hz, 3 H, CH<sub>3</sub>).

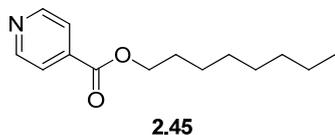
<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 165.34 (C=O), 150.75 (NCH), 137.83 (CCOO), 122.99 (CCH), 70.81 (CH<sub>2</sub>O), 33.51 (CH<sub>2</sub>), 32.79 (CH), 32.15 (CH<sub>2</sub>), 26.65 (CH<sub>2</sub>), 22.74 (CH<sub>2</sub>), 17.11 (CH<sub>3</sub>), 14.18 (CH<sub>3</sub>).

**IR**  $\nu_{\text{max}}$  3441, 3032, 2957, 2927, 2873, 2855, 1727, 1596, 1562, 1492, 1466, 1407, 1389, 1376, 1323, 1275, 1251, 1212, 1117, 1063, 992, 973, 918, 850, 756, 706, 675 cm<sup>-1</sup>.

**HRMS**: Calcd. for [C<sub>14</sub>H<sub>21</sub>NO<sub>2</sub>+H<sup>+</sup>]: 236.1645, found 236.1644.

**Specific Rotation**  $[\alpha]_{\text{D}}^{22} -2.46^{\circ}$  ( $c$  0.6,  $\text{CHCl}_3$ ).

#### 2.9.4.4. Octyl isonicotinate (2.45)



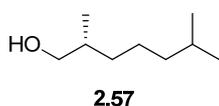
To a solution of *n*-octanol (0.3 g, 2.30 mmol) in pyridine (15 mL) was added isonicotinoyl chloride·HCl salt (0.45 g, 2.53 mmol). The mixture was stirred at r.t. for 16 h and then the solvent removed *in vacuo*. The crude product was purified by flash chromatography (hexane/EtOAc 3:1) to give the *title compound* (0.50 g, 92%) as a colourless liquid. The analytical data were in agreement with literature values.<sup>26</sup>

**<sup>1</sup>H NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.78 (dd,  $J = 4.4, 1.6$  Hz, 2 H,  $2 \times \text{NCH}$ ), 7.85 (dd,  $J = 4.4, 1.6$  Hz, 2 H,  $2 \times \text{CCH}$ ), 4.35 (t,  $J = 6.7$  Hz, 2 H,  $\text{CH}_2\text{O}$ ), 1.84-1.72 (m, 2 H,  $\text{CH}_2$ ), 1.51-1.21 (m, 10 H,  $5 \times \text{CH}_2$ ), 0.89 (t,  $J = 6.9$  Hz, 3 H,  $\text{CH}_3$ ).

**IR**  $\nu_{\text{max}}$  3033, 2956, 2926, 2856, 1729, 1597, 1563, 1467, 1407, 1324, 1280, 1213, 1120, 1065, 992, 951, 851, 758, 708, 676  $\text{cm}^{-1}$ .

#### 2.9.5. Synthesis of a nitrile odorant, (*R*)-3,7-dimethyl octanenitrile, via the “one pot” procedure

##### 2.9.5.1. (*R*)-2,6-Dimethylheptanol (2.57)



To a solution of *i*-BuI (0.56 g, 3.0 mmol) in  $\text{Et}_2\text{O}$  (25 mL) at  $-78^{\circ}\text{C}$  was added *t*-BuLi (1.7 M in pentane, 3.9 mL, 6.6 mmol) dropwise. The reaction was stirred at  $-78^{\circ}\text{C}$  for 1 h and then warmed to r.t. over 10 min and stirred at r.t. for 20 min. The reaction mixture was then added via cannula to a solution of  $\gamma$ -lactone **2.25** (0.33 g, 3.3 mmol) in  $\text{Et}_2\text{O}$  (10 mL) at  $-78^{\circ}\text{C}$  and the reaction was stirred at  $-78^{\circ}\text{C}$  for 3 h. TMSCl (3.8 mL, 30 mmol) was added and the reaction mixture was allowed to warm to  $0^{\circ}\text{C}$ . After stirring at  $0^{\circ}\text{C}$  for 10 min, Zn dust (2.2 g, 33 mmol) was added, and followed by adding MeOH (90 mL) over 15 min. After stirring at  $0^{\circ}\text{C}$  for 1 h, the reaction

mixture was filtered and solvent removed *in vacuo*. Et<sub>2</sub>O (30 mL) was added, washed with HCl (1 M, 30 mL) and saturated NaHCO<sub>3</sub> (30 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and the solvent removed *in vacuo*. The resulting yellow liquid was purified by flash chromatography (hexane/Et<sub>2</sub>O 3:2) to give the *title compound* (0.34 g, 79 %) as a colourless liquid. The analytical data were in agreement with literature values.<sup>87</sup>

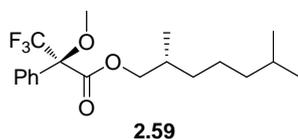
<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.51 (dd, *J* = 10.5, 5.8 Hz, 1 H, CH<sub>2</sub>OH), 3.41 (dd, *J* = 10.5, 6.6 Hz, 1 H, CH<sub>2</sub>OH), 1.68-1.31 (m, 2 H, 2 × CH), 1.45-1.02 (m, 6 H, 3 × CH<sub>2</sub>), 0.91 (d, *J* = 6.7 Hz, 3 H, CH<sub>3</sub>), 0.86 (d, *J* = 6.7 Hz, 6 H, 2 × CH<sub>3</sub>).

<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 68.57(CH<sub>2</sub>OH), 39.38(CH), 35.91(CH<sub>2</sub>), 33.49(CH<sub>2</sub>), 28.07(CH), 24.84(CH<sub>2</sub>), 22.83(CH<sub>3</sub>), 22.72(CH<sub>3</sub>), 16.73(CH<sub>3</sub>).

**Specific Rotation**  $[\alpha]_{\text{D}}^{22} +9.9^{\circ}$  (*c* 2.3, C<sub>6</sub>H<sub>6</sub>) (Lit.<sup>87</sup>:  $[\alpha]_{\text{D}}^{23} +10.37^{\circ}$  (*c* 1.48, C<sub>6</sub>H<sub>6</sub>))

**IR**  $\nu_{\text{max}}$  3333.8(s), 2952.9(s), 2927.5(s), 2868.7(s), 1466.3(m), 1364.3(m), 1034.7(m) cm<sup>-1</sup>.

### 2.9.5.2. (*R,R*)-2,6-Dimethylheptyl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (2.59)



To a solution of alcohol **2.57** (4.1 mg, 0.028 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.3 mL) was added DCC (7 mg, 0.03 mmol), DMAP (0.5 mg, 4.1 μmol) and (*R*)- $\alpha$ -methoxy- $\alpha$ -trifluoromethyl phenylacetic acid (10 mg, 0.043 mmol). The mixture was stirred at r.t. for 2 days. The solvent was removed *in vacuo* to give a colourless oil and NMR experiments were carried on crude product for calculation of enantiomeric excess. The analytical data were in agreement with literature values.<sup>37</sup>

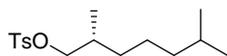
<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.60-7.37 (m, 5 H, ArH), 4.24 (dd, *J* = 10.7, 5.6 Hz, 1 H, CH<sub>2</sub>O), 4.07 (dd, *J* = 10.7, 6.7 Hz, 1 H, CH<sub>2</sub>O), 3.57(s, 3 H, OCH<sub>3</sub>), 1.92-1.77 (m, 1 H, CHCH<sub>2</sub>O), 1.58-1.44 (m, 1 H, CH(CH<sub>3</sub>)<sub>2</sub>), 1.38-1.05 (m, 6 H, 2 × CH<sub>2</sub>), 0.90 (t, *J* = 5.9 Hz, 3 H, CH<sub>3</sub>CH), 0.86 (d, *J* = 6.6 Hz, 6 H, CH(CH<sub>3</sub>)<sub>2</sub>).

<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 166.84(C=O), 132.54(ArC), 129.71(ArC),

128.53(ArC), 127.51(ArC), 124.93(CF<sub>3</sub>), 122.07(CCF<sub>3</sub>), 71.42(CH<sub>2</sub>O), 55.56(OCH<sub>3</sub>), 39.19(CH<sub>2</sub>), 33.44(CH<sub>2</sub>), 32.55(CHCH<sub>3</sub>), 28.00(CH(CH<sub>3</sub>)<sub>2</sub>), 24.56(CH<sub>2</sub>), 22.76 and 22.69(CH(CH<sub>3</sub>)<sub>2</sub>), 16.91(CHCH<sub>3</sub>).

<sup>19</sup>F NMR (377 MHz, CDCl<sub>3</sub>) δ -72.05.

### 2.9.5.3. (R)-2,6-Dimethylheptyl 4-methylbenzenesulfonate (2.61)

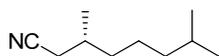


2.61

To a solution of alcohol **2.57** (0.1 g, 0.69 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (8 mL) was added TsCl (0.20 g, 1.1 mmol) and pyridine (0.11 mL, 1.4 mmol). The reaction mixture was stirred at r.t. for 24 h. The mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (10 mL), washed with HCl (1 M, 15 mL) and saturated NaHCO<sub>3</sub> (15 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent removed *in vacuo* to give the *title compound* (0.20 g, quantitative yield) as a colourless liquid.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.79 (d, *J* = 8.3 Hz, 2 H, ArH), 7.34 (d, *J* = 8.0 Hz, 2 H, ArH), 3.88 (dd, *J* = 9.3, 5.7 Hz, 1 H, CH<sub>2</sub>OTs), 3.81 (dd, *J* = 9.3, 6.5 Hz, 1 H, CH<sub>2</sub>OTs), 2.45 (s, 3 H, CH<sub>3</sub> for Ts), 1.83-1.70 (m, 1 H, CH), 1.53-1.41 (m, 1 H, CH(CH<sub>3</sub>)<sub>2</sub>), 1.34-1.01 (m, 6 H, 3 × CH<sub>2</sub>), 0.88 (d, *J* = 6.7 Hz, 3 H, CHCH<sub>3</sub>), 0.83 (d, *J* = 6.6 Hz, 6 H, CH(CH<sub>3</sub>)<sub>2</sub>).

### 2.9.5.4. (R)-3,7-Dimethyloctanenitrile (2.62)



2.62

To a solution of tosylate **2.61** (0.18 g, 0.60 mmol) in DMSO (2 mL) was added NaCN (0.12 g, 2.4 mmol). The mixture was stirred at r.t. for 24 h. To work up, Et<sub>2</sub>O (20 mL) was added, and washed with water (2 × 20 mL). The resulting organic layer was concentrated by distilling to remove most of the Et<sub>2</sub>O, giving the *title compound* (90 % yield) as a colourless solution in Et<sub>2</sub>O (4.5 mL, 0.12 mol/L). The analytical data were in agreement with literature values.<sup>35</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 2.27 (qd, *J* = 16.7, 6.3 Hz, 2 H, CH<sub>2</sub>CN), 1.91-1.78 (m, 1 H, CHCH<sub>3</sub>), 1.53-1.41 (m, 1 H, CH(CH<sub>3</sub>)<sub>2</sub>), 1.44-1.11 (m, 6 H, 3 × CH<sub>2</sub>), 1.07 (d, *J*

= 6.7 Hz, 3 H, CHCH<sub>3</sub>), 0.87 (d, *J* = 6.7 Hz, 6 H, CH(CH<sub>3</sub>)<sub>2</sub>).

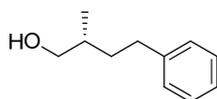
<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 39.04(CH<sub>2</sub>), 36.35(CH<sub>2</sub>), 30.73(CH<sub>2</sub>), 28.10(CH), 24.84(CH<sub>2</sub>), 24.75(CH<sub>2</sub>), 22.84 and 22.78(CH(CH<sub>3</sub>)<sub>2</sub>), 19.73(CHCH<sub>3</sub>).

**Specific Rotation**  $[\alpha]_{\text{D}}^{22}$  -2.3 ° (*c* 1.9, Et<sub>2</sub>O)

**HRMS:** Calcd. for [C<sub>10</sub>H<sub>19</sub>N+NH<sub>4</sub><sup>+</sup>]: 171.1856, found 171.1859.

## 2.9.6. Synthesis of (*R*)-Citralis Nitrile<sup>TM</sup> via the “one pot” procedure

### 2.9.6.1. (*R*)-2-Methyl-4-phenylbutanol (2.69)



**2.69**

To a solution of BnBr (0.236 g, 1.5 mmol) in Et<sub>2</sub>O (10 mL) at -78 °C was added *t*-BuLi (1.7 M in pentane, 2 mL, 3.4 mmol) dropwise. The reaction was stirred at -78 °C for 1 h and then warmed to r.t. for 20 min. The mixture was then added via cannula to a solution of  $\gamma$ -lactone **2.25** (0.165 g, 1.65 mmol) in Et<sub>2</sub>O (10 mL) at -78 °C and the reaction was stirred for 3 h. TMSCl (1.6 mL, 12.61 mmol) was added and the reaction was allowed to warm to 0 °C. After stirring at 0 °C for 10 min, Zn dust (0.82 g, 12.60 mmol) was added, and followed by adding MeOH (30 mL) over 15 min. After stirring at 0 °C for 1 h, the mixture was filtered through celite and the solvent was removed *in vacuo*. Et<sub>2</sub>O (20 mL) was added, and then was washed with HCl (1 M, 20 mL) and saturated NaHCO<sub>3</sub> solution (20 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and the solvent removed *in vacuo*. The product was purified by flash chromatography (hexane/Et<sub>2</sub>O 2:1) to give the *title compound* (0.185 g, 77 %) as a colourless liquid. The analytical data were in agreement with literature values.<sup>39,88</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.32-7.14 (m, 5 H, ArH), 3.51 (ddd, *J* = 28.1, 10.5, 6.1 Hz, 2 H, CH<sub>2</sub>OH), 2.66 (dddd, *J* = 43.7, 13.7, 10.0, 6.0 Hz, 2 H, PhCH<sub>2</sub>), 1.82-1.72 (m, 1 H, CH), 1.68 (dt, *J* = 19.2, 6.4 Hz, 1 H, CH<sub>2</sub>CH), 1.51-1.39 (m, 1 H, CH<sub>2</sub>CH), 1.25 (br s, 1 H, OH), 0.99 (d, *J* = 6.7 Hz, 3 H, CH<sub>3</sub>).

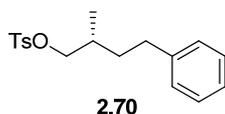
$^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  142.74 (ArC), 128.48 (ArC), 125.87 (ArC), 68.35 ( $\text{CH}_2\text{OH}$ ), 35.53 (CH), 35.12 ( $\text{CH}_2\text{CH}$ ), 33.44 ( $\text{PhCH}_2$ ), 16.65 ( $\text{CH}_3$ ).

**EI-MS:** Calc. for  $[\text{C}_{11}\text{H}_{16}\text{O}]$ : 164.1, found 164.2.

**IR**  $\nu_{\text{max}}$  3333, 3085, 3062, 3026, 2923, 2871, 1603, 1495, 1454, 1379, 1902, 1032, 989, 903, 745, 698.

**Specific Rotation**  $[\alpha]_{\text{D}}^{22} +16.8^\circ$  ( $c$  1.0,  $\text{CHCl}_3$ ) (Lit.<sup>39</sup>:  $+14.5^\circ$  ( $c$  0.97,  $\text{CHCl}_3$ ))

### 2.9.6.2. (*R*)-2-Methyl-4-phenylbutyl 4-methylbenzenesulfonate (2.70)



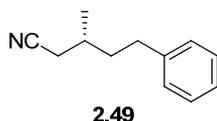
To a solution of alcohol **2.69** (0.17 g, 1.04 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) was added DMAP (1.3 mg, 0.106 mmol), TsCl (0.30 g, 1.5 mmol) and pyridine (0.25 mL, 3.1 mmol). The mixture was stirred at r.t. for 24 h.  $\text{CH}_2\text{Cl}_2$  (20 mL) was added, and then washed with 1 M HCl (20 mL), dried over  $\text{Na}_2\text{SO}_4$ , filtered, and the solvent removed *in vacuo*. The resulting yellow liquid was purified by flash chromatography (hexane/ $\text{Et}_2\text{O}$  3:1) to give the *title compound* (0.32 g, 97 % yield) as a colourless liquid.

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.81-7.75 (m, 2 H, ArH), 7.37-7.07 (m, 7 H, ArH), 3.89 (qd,  $J = 9.4, 5.9$  Hz, 2 H,  $\text{CH}_2\text{SO}_3$ ), 2.65-2.46 (m, 2 H,  $\text{PhCH}_2$ ), 2.44 (s, 3 H,  $\text{CH}_3$  for Ts), 1.87-1.78 (m, 1 H,  $\text{CH}_2\text{CH}$ ), 1.73-1.62 (m, 1 H, CH), 1.49-1.40 (m, 1 H,  $\text{CH}_2\text{CH}$ ), 0.95 (d,  $J = 6.8$  Hz, 3 H,  $\text{CH}_3$ ).

**IR**  $\nu_{\text{max}}$  3853, 3745, 3675, 3649, 3062, 3027, 2964, 2930, 2362, 2336, 1653, 1599, 1496, 1455, 1358, 1307, 1292, 1189, 1176, 1097, 1020, 963, 938, 834, 814, 748, 700, 667.

**Specific Rotation**  $[\alpha]_{\text{D}}^{22} -2.97^\circ$  ( $c$  7.8,  $\text{CHCl}_3$ )

### 2.9.6.3. (*R*)-3-Methyl-5-phenylpentanenitrile (2.49)



To a solution of tosylate **2.70** (0.25 g, 0.79 mmol) in DMSO (5 mL) was added NaCN (0.23 g, 4.71 mmol). The mixture was stirred at r.t. for 24 h. Et<sub>2</sub>O (50 mL) was added, and washed with H<sub>2</sub>O (50 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent removed *in vacuo*. The resulting colourless liquid was purified by flash chromatography (hexane/Et<sub>2</sub>O 3:1) to give the *title compound* (0.13 g, 95 % yield) as a colourless liquid. The analytical data were in agreement with literature values.<sup>33,89</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.47-7.09 (m, 5 H, ArH), 2.82-2.52 (m, 2 H, CH<sub>2</sub>Ph), 2.31 (qd, *J* = 16.7, 6.2 Hz, 2 H, CH<sub>2</sub>CN), 1.89 (qd, *J* = 12.7, 6.6 Hz, 1 H, CH), 1.78 (ddt, *J* = 13.5, 9.5, 6.3 Hz, 1 H, CH<sub>2</sub>CH), 1.64 (dddd, *J* = 13.8, 9.5, 7.7, 6.2 Hz, 1 H, CH<sub>2</sub>CH), 1.13 (d, *J* = 6.7 Hz, 3 H, CH<sub>3</sub>).

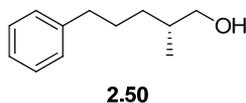
<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 141.49 (ArC), 128.64 (ArC), 128.42 (ArC), 126.20 (ArC), 118.80 (CN), 37.62 (CHCH<sub>2</sub>), 33.23 (PhCH<sub>2</sub>), 30.05 (CH), 24.64 (CH<sub>2</sub>CN), 19.48 (CH<sub>3</sub>).

IR  $\nu_{\max}$  3085, 3062, 3027, 2960, 2929, 2859, 2245, 1603, 1496, 1455, 1424, 1384, 1178, 1061, 1031, 747, 700 cm<sup>-1</sup>.

Specific Rotation  $[\alpha]_{\text{D}}^{22}$  -3.74 ° (*c* 1.4, EtOH) (Lit.<sup>33</sup>:  $[\alpha]_{\text{D}}^{22}$  -2.3 ° (*c* 2.2, EtOH))

## 2.9.7. Total synthesis of side chain of (R)-Rosaphen<sup>TM</sup> via the “one pot” procedure

### 2.9.7.1. (R)-2-Methyl-5-phenylpentanol (2.50)



To a solution of PhBr (0.31 g, 2 mmol) in Et<sub>2</sub>O (20 mL) at -78 °C was added *t*-BuLi (1.7 M in pentane, 2.5 mL, 4.2 mmol) dropwise. The reaction was stirred at -78 °C for 1 h and then warmed to r.t. for 10 min. The mixture was then added via cannula to a solution of (R)-4-methyl-δ-valerolactone **2.81** (0.23 g, 2 mmol) in Et<sub>2</sub>O (20 mL) at -78 °C and the reaction was stirred at -78 °C for 4 h. TMSCl (2.54 mL, 20.0 mmol) was added and the reaction was allowed to warm to 0 °C. After stirring at 0 °C for

10 min, Zn dust (01.3 g, 20.0 mmol) was added, and followed by adding MeOH (30 mL) over 15 min. After stirring at 0 °C for 1 h, the reaction mixture was filtered through celite and the solvent was removed *in vacuo* to give a yellow liquid. Et<sub>2</sub>O (20 mL) was added, and then was washed with 1 M HCl (20 mL) and saturated NaHCO<sub>3</sub> solution (20 mL), and then dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and the solvent removed *in vacuo*. The resulting yellow liquid was purified by flash chromatography (hexane/EtOAc 3:1) to give the *title compound* (0.27 g, 76 %) as a colourless liquid. The analytical data were in agreement with literature values.<sup>45</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.34-7.15 (m, 5 H, ArH), 3.47 (ddd, *J* = 32.3, 10.4, 6.2 Hz, 2 H, CH<sub>2</sub>OH), 2.70-2.53 (m, 2 H, PhCH<sub>2</sub>), 1.79-1.57 (m, 3 H, CH and PhCH<sub>2</sub>CH<sub>2</sub>), 1.53-1.41 (m, 1 H, CHCH<sub>2</sub>), 1.32-1.11 (m, 2 H, CHCH<sub>2</sub> and OH), 0.92 (d, *J* = 6.7 Hz, 3 H, CH<sub>3</sub>).

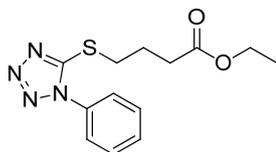
<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 142.76 (ArC), 128.53 (ArC), 128.43 (ArC), 125.83 (ArC), 68.45 (CH<sub>2</sub>OH), 36.37 (CH<sub>2</sub>Ph), 35.84 (CH), 32.94 (CHCH<sub>2</sub>), 29.05 (PhCH<sub>2</sub>CH<sub>2</sub>), 16.67 (CH<sub>3</sub>).

IR  $\nu_{\max}$  3349.5(s), 3084.7(w), 3062.1(w), 3026.1(m), 2930.5(s), 2858.5(s), 1603.5(w), 1495.7(m), 1453.1(m), 1379.4(w), 1031.2(m), 747.4(m), 698.2(m) cm<sup>-1</sup>.

**Specific Rotation:**  $[\alpha]_{\text{D}}^{22}$  +12.8 ° (*c* 1.8, EtOH) (Lit.<sup>45</sup>:  $[\alpha]_{\text{D}}^{22}$  +10.9 ° (*c* 1, EtOH))

## 2.9.8. Total synthesis of the C6 side chain of (+)-zaragozic acid C via the “one pot” procedure

### 2.9.8.1. Ethyl 4-((1-phenyl-1H-tetrazol-5-yl)thio)butanoate (2.178)



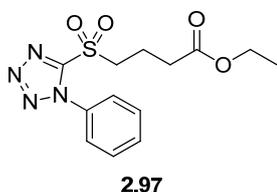
2.178

To a solution of 1-phenyl-1H-tetrazole-5-thiol sodium salt (0.49 g, 2.45 mmol) in acetone (15 mL) was added ethyl 4-bromobutyrate (0.5 g, 2.56 mmol) in acetone (15 mL). White precipitates were formed immediately and the mixture was reflux for

2 h. The reaction was then cooled to r.t., filtered and the solvent removed *in vacuo*. The resulting colourless liquid was purified by eluting through a short plug of silica (hexane/Et<sub>2</sub>O 3:1) to give the *title compound* (0.71 g, 100 %) as a colourless oil. The analytical data were in agreement with literature values.<sup>90</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.61-7.51 (m, 5 H, ArH), 4.14 (q, *J* = 7.1 Hz, 2 H, OCH<sub>2</sub>CH<sub>3</sub>), 3.46 (t, *J* = 7.2 Hz, 2 H, SCH<sub>2</sub>), 2.48 (t, *J* = 7.2 Hz, 2 H, CH<sub>2</sub>COO), 2.26-2.10 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.25 (t, *J* = 7.1 Hz, 3 H, CH<sub>3</sub>).

### 2.9.8.2. Ethyl 4-((1-phenyl-1H-tetrazol-5-yl)sulfonyl)butanoate (2.97)

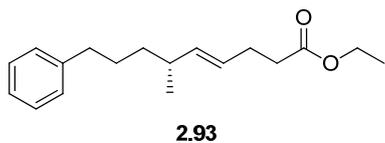


To the solution of thiol **2.178** (0.71 g, 2.43 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) was added *m*CPBA (2.6 g, 12.1 mmol). The mixture was stirred at r.t. for 14 h. Then it was filtered through celite, and washed with saturated Na<sub>2</sub>SO<sub>3</sub> and NaHCO<sub>3</sub>. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent was removed *in vacuo*. The resulting yellow solids were purified by flash chromatography (hexane/Et<sub>2</sub>O 3:2) to give the *title compound* (0.70 g, 89%) as white needle crystals, m.p. 58-60 °C. The analytical data were in agreement with literature values.<sup>90</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.80-7.52 (m, 5 H, ArH), 4.16 (q, *J* = 7.1 Hz, 2 H, CH<sub>2</sub>CH<sub>3</sub>), 3.93-3.78 (m, 2 H, SO<sub>2</sub>CH<sub>2</sub>), 2.56 (t, *J* = 7.0 Hz, 2 H, CH<sub>2</sub>COO), 2.36-2.21 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.27 (t, *J* = 7.2 Hz, 3 H, CH<sub>3</sub>).

<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 171.83 (C=O), 153.35 (CSO<sub>2</sub>), 133.00 (ArC), 131.65 (ArC), 129.89 (ArC), 125.24 (ArC), 61.08 (CH<sub>2</sub>CH<sub>3</sub>), 55.21 (SO<sub>2</sub>CH<sub>2</sub>), 32.08 (CH<sub>2</sub>COO), 18.03 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 14.32 (CH<sub>2</sub>CH<sub>3</sub>).

IR  $\nu_{\max}$  2987.8(m), 2962.8(m), 2921.5(m), 2907.7(m), 1721.5(s), 1592.9(w), 1498.5(m), 1477.2(w), 1457.4(w), 1416.5(w), 1394.7(w), 1381.3(w), 1339.4(s), 1320.5(s), 1304.1(m), 1239.9(s), 1191.5(s), 1153.3(s), 1132.5(m), 1105.5(w), 1069.1(w), 1052.5(w), 1041.0(w), 1031.7(m), 1014.8(w), 976.6(w), 948.4(w), 920.9(w), 871.0(w), 841.1(w), 766.3(s), 737.3(s), 723.8(w), 688.4(s) cm<sup>-1</sup>.

**2.9.8.3. (6R, 4E)-Ethyl 6-methyl-9-phenylnon-4-enoate (2.93)** <sup>40</sup>

To a solution of alcohol **2.50** (44.2 mg, 0.25 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) at 0 °C was added Dess-Martin periodinane (0.2 g, 0.37 mmol). The reaction was stirred at 0 °C for 30 minutes, and then washed with saturated  $\text{NaHCO}_3$ , dried over  $\text{Na}_2\text{SO}_4$ , filtered and the solvent removed *in vacuo*. The resulting colourless liquid was purified by running through a short plug (hexane/ $\text{Et}_2\text{O}$  2:1) to give the aldehyde as a colourless liquid, which was used immediately for the next step.

To a solution of sulfone **2.97** (0.11 g, 0.34 mmol) in THF (10 mL) at -78 °C was added KHMDS (0.5 M, 0.6 mL, 0.3 mmol). The mixture was stirred at -78 °C for 30 min. The prepared aldehyde in THF (10 mL) was added, and the mixture was stirred at -78 °C for 4 h. The reaction was warmed slowly to r.t. over 2 h, and then stirred at r.t. for 14 h. The solvent was removed *in vacuo*, and  $\text{CH}_2\text{Cl}_2$  (15 mL) was added, washed with saturated  $\text{NH}_4\text{Cl}$  (15 mL), dried over  $\text{Na}_2\text{SO}_4$ , filtered, and the solvent removed *in vacuo*. The resulting colourless oil was purified by flash chromatography (hexane/ $\text{Et}_2\text{O}$  30:1) to give the *title compound* (61 mg, 81 % over two steps) as colourless oil. The analytical data were in agreement with literature values.<sup>40</sup>

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.30-7.14 (m, 5 H, ArH), 5.73-5.21 (m, 2 H, CH=CH), 4.11 (q,  $J = 7.1$  Hz, 2 H,  $\text{OCH}_2\text{CH}_3$ ), 2.57 (t,  $J = 7.3$  Hz, 2 H,  $\text{PhCH}_2$ ), 2.44-2.24 (m, 4 H,  $\text{CH}_2$ ), 2.08 (dt,  $J = 13.6, 6.7$  Hz, 1 H, CH), 1.67-1.50 (m, 2 H,  $\text{CH}_2$ ), 1.34-1.22 (m, 2 H,  $\text{CH}_2\text{CH}$ ), 1.24 (t,  $J = 7.1$  Hz, 3 H,  $\text{OCH}_2\text{CH}_3$ ), 0.94 (d,  $J = 6.7$  Hz, 3 H,  $\text{CHCH}_3$ ).

**$^{13}\text{C}$  NMR** (100 MHz,  $\text{CDCl}_3$ )  $\delta$  173.40 (COO), 142.95 (ArC), 137.69 (CH=CH), 128.54 (ArC), 128.37 (ArC), 126.50 (CH=CH), 125.73 (ArC), 60.36 ( $\text{OCH}_2\text{CH}_3$ ), 36.80 ( $\text{CH}_2\text{CH}$ ), 36.75 (CH), 36.20 ( $\text{PhCH}_2$ ), 34.66 (CH=CH $\text{CH}_2$ ), 29.35 ( $\text{CH}_2$ ), 28.08 ( $\text{CH}_2$ ), 20.92 ( $\text{CH}_3$ ), 14.40 ( $\text{CH}_3$ ).

**IR**  $\nu_{\text{max}}$  3048.8 (w), 3025.8 (w), 2955.7 (m), 2927.4 (s), 2856.0 (m), 1733.4 (s), 1603.1 (w), 1495.6 (w), 1452.3 (m), 1370.7 (m), 1344.4 (w), 1299.8 (w), 1245.3 (m),

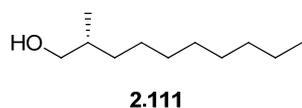
1174.9 (m), 1158.4 (m), 1097.0 (w), 1030.9 (m), 968.6 (m), 906.2 (w), 856.2 (w), 746.6 (m), 697.6 (m)  $\text{cm}^{-1}$ .

**EI-MS:** Calcd. for  $[\text{C}_{18}\text{H}_{26}\text{O}_2]$ : 274.2, found, 274.1.

**Specific Rotation**  $[\alpha]_{\text{D}}^{22}$   $-12.8^\circ$  ( $c$  2.1, EtOH) (Lit.<sup>40</sup>:  $[\alpha]_{\text{D}}^{22}$   $-13.2^\circ$  ( $c$  27.2, EtOH))

## 2.9.9. Synthesis an intermediate for tuberculostearic acid via the “one pot” procedure

### 2.9.9.1. (*R*)-2-Methyldecanol (2.111)



To a solution of  $n\text{-C}_6\text{H}_{13}\text{I}$  (0.64 g, 3 mmol) in  $\text{Et}_2\text{O}$  (20 mL) at  $-78^\circ\text{C}$  was added  $t\text{-BuLi}$  (1.7 M in pentane, 3.9 mL, 6.6 mmol). The reaction was stirred at  $-78^\circ\text{C}$  for 1 h and then warmed gradually to r.t. over 10 min and stir at r.t. for 20 min. The mixture was then added via cannula to a solution of  $\gamma$ -lactone **2.25** (0.3 g, 3 mmol) in  $\text{Et}_2\text{O}$  (10 mL) at  $-78^\circ\text{C}$  and the reaction was stirred for 3 h at  $-78^\circ\text{C}$ .  $\text{TMSCl}$  (7.6 mL, 60 mmol) was added and the reaction was allowed to warm to  $0^\circ\text{C}$ . After stirring at  $0^\circ\text{C}$  for 10 min, Zn dust (4.3 g, 66 mmol) was added, and followed by MeOH (90 mL) over 15 min. After stirring at  $0^\circ\text{C}$  for 1 h, the reaction mixture was filtered over celite and concentrated *in vacuo*.  $\text{Et}_2\text{O}$  (30 mL) was added for dilution, and then was washed with 1 M HCl (30 mL). The aqueous phase was extracted with  $\text{Et}_2\text{O}$  ( $2 \times 30$  mL). The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , filtered and the solvent removed *in vacuo*. The resulting yellow liquid was purified by flash chromatography (hexane/ $\text{Et}_2\text{O}$  3:1) to give the *title compound* (0.39 g, 75 %) as a colourless liquid. The analytical data were in agreement with literature values.<sup>91</sup>

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  3.56-3.36 (dtd,  $J = 16.2, 10.7, 5.7$  Hz, 2 H,  $\text{CH}_2\text{OH}$ ), 1.67-1.53 (m, 1 H, CH), 1.46-1.03 (m, 14 H,  $7 \times \text{CH}_2$ ), 0.91 (d,  $J = 6.7$  Hz, 3 H,  $\text{CHCH}_3$ ), 0.88 (t,  $J = 6.9$  Hz, 3 H,  $\text{CH}_2\text{CH}_3$ ).

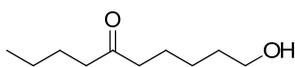
**$^{13}\text{C}$  NMR** (100 MHz,  $\text{CDCl}_3$ )  $\delta$  68.60 ( $\text{CH}_2\text{OH}$ ), 35.91 (CH), 33.29 ( $\text{CH}_2$ ), 32.05 ( $\text{CH}_2$ ), 30.09 ( $\text{CH}_2$ ), 29.75 ( $\text{CH}_2$ ), 29.48 ( $\text{CH}_2$ ), 27.13 ( $\text{CH}_2$ ), 22.83 ( $\text{CH}_2$ ), 16.73

(CHCH<sub>3</sub>), 14.27 (CH<sub>2</sub>CH<sub>3</sub>).

**Specific Rotation**  $[\alpha]_{\text{D}}^{23} +9.0^{\circ}$  (*c* 1.9, CH<sub>2</sub>Cl<sub>2</sub>) (S: it.<sup>92</sup>:  $[\alpha]_{\text{D}}^{21} -9.4^{\circ}$  (*c* 1.0, CH<sub>2</sub>Cl<sub>2</sub>))

## 2.9.10. Chain elongations

### 2.9.10.1. 10-Hydroxydecan-5-one (2.125)

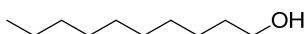


2.125

To a solution of caprolactone (0.4 g, 3.5 mmol) in Et<sub>2</sub>O (30 mL) at -78 °C was added *n*-BuLi (2 mL, 1.6 M in hexane, 3.2 mmol) dropwise. The reaction was stirred at -78 °C for 3 h. TMSCl (0.8 mL, 6.4 mmol) was added to quench the reaction, and the reaction was warmed slowly to r.t.. The mixture was washed with HCl (1 M, 30 mL) and saturated NH<sub>4</sub>Cl (30 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent removed *in vacuo* to give a colourless liquid. Purification by flash chromatography (hexane/Et<sub>2</sub>O 2:1) gave the *title compound* (0.44 g, 80 %) as a colourless liquid.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.64 (t, *J* = 5.7 Hz, 2 H, CH<sub>2</sub>OH), 2.48-2.35 (m, 4 H, 2 × CH<sub>2</sub>C=O), 1.64-1.48 (m, *J* = 15.2, 9.8, 5.9 Hz, 6 H, 3 × CH<sub>2</sub>), 1.40-1.22 (m, 4 H, 2 × CH<sub>2</sub>), 0.90 (t, *J* = 7.3 Hz, 3 H, CH<sub>3</sub>).

### 2.9.10.2. Decanol (2.126)



2.126

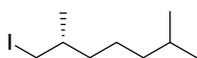
To a solution of caprolactone (0.4 g, 3.5 mmol) in Et<sub>2</sub>O (30 mL) at -78 °C was added *n*-BuLi (2 mL, 1.6 M in hexane, 3.2 mmol) dropwise. The reaction was stirred at -78 °C for 3 h. TMSCl (4 mL, 32 mmol) was added to the reaction, and the mixture was allowed to warm to 0 °C. Zn dust (2.1 g, 32 mmol) and MeOH (90 mL) was added, and the reaction was stirred at 0 °C for 1 h. The mixture was filtered through celite, and the solvent removed *in vacuo*. Et<sub>2</sub>O (30 mL) was added to the resulting colourless liquid, and was washed with HCl (1M, 30 mL), saturated NaHCO<sub>3</sub> (30 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent removed *in vacuo* to give a colourless

liquid. Purification by flash chromatography (hexane/Et<sub>2</sub>O 3:1) gave the *title compound* (0.42 g, 83 %) as a colourless liquid. The analytical data were in agreement with literature values.<sup>26</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.79 (t, *J* = 6.8 Hz, 2 H, CH<sub>2</sub>OH), 1.70-1.57 (m, 2 H, CH<sub>2</sub>), 1.39-1.18 (m, 14 H, 7 × CH<sub>2</sub>), 0.88 (t, *J* = 6.9 Hz, 3 H, CH<sub>3</sub>).

## 2.9.11. Total synthesis of the vitamin E side chain

### 2.9.11.1. (*R*)-Iodo-2,6-dimethylheptane (2.168)



**2.168**

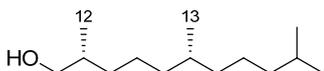
To a solution of alcohol **2.57** (0.2 g, 1.4 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) at 0 °C was added PPh<sub>3</sub> (0.4 g, 1.5 mmol), I<sub>2</sub> (0.38 g, 1.5 mmol) and imidazole (0.39 g, 1.5 mmol). The mixture was stirred at 0 °C for 1 h. The solvent was removed *in vacuo*, and the resulting yellow liquid was purified by eluting through a short plug of silica with pentane to give the *title compound* (0.28 g, 79 %) as a colourless liquid. The analytical data were in agreement with literature values.<sup>37</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.23 (dd, *J* = 9.6, 4.7 Hz, 1 H, CH<sub>2</sub>I), 3.15 (dd, *J* = 9.6, 6.1 Hz, 1 H, CH<sub>2</sub>I), 1.61-1.11 (m, 8 H, 2 × CH and 3 × CH<sub>2</sub>), 0.98 (d, *J* = 6.5 Hz, 3 H, CH<sub>3</sub>), 0.91-0.83 (m, 6 H, 2 × CH<sub>3</sub>).

IR  $\nu_{\max}$  2958(s), 2928(s), 2870(m), 1463(m), 1381(m), 1263(m), 1196(m), 1099(m), 1020(m), 911(m), 806(m), 737(m).

EI-MS: Calcd. for [C<sub>9</sub>H<sub>19</sub>I]: 254.1, found: 254.1.

### 2.9.11.2. (2*R*,6*R*)-2,6,10-Trimethylundecanol (2.154)



**2.154**

To a solution of iodide **2.168** (0.2 g, 0.79 mmol) in Et<sub>2</sub>O (8 mL) at -78 °C was added *t*-BuLi (1.73 mmol, 1.7 M, 1.0 mL). The mixture was stirred at -78 °C for 1 h, and

then warmed to r.t. over 10 minutes, and stirred at r.t. for 20 min. After that, the mixture was added via cannula dropwise to a solution of lactone **2.25** (79 mg, 0.79 mmol) in Et<sub>2</sub>O (10 mL) at -78 °C. The mixture was stirred at -78 °C for 4 h. TMSCl (2.0 mL, 15.7 mmol) was added, and the mixture was warmed to 0 °C and stirred for 5 min. Then Zn dust (1.0 g, 15.7 mmol) was added, followed by adding MeOH (30 mL). The reaction was stirred at 0 °C for 1 h. The mixture was filtered through celite, and then the solvent was removed *in vacuo*. HCl (1 M, 40 mL) was added, and the mixture was extracted with Et<sub>2</sub>O (40 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent removed *in vacuo*. The resulting yellow liquid was purified by flash chromatography (hexane/Et<sub>2</sub>O 3:1) to give the *title compound* (69 mg, 41 %) as a colourless liquid. The analytical data were in agreement with literature values.<sup>37</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.47 (ddd, *J* = 38.4, 10.4, 6.1 Hz, 2 H, CH<sub>2</sub>OH), 1.69-1.00 (m, 15 H, 3 × CH and 6 × CH<sub>2</sub>), 0.92 (d, *J* = 6.7 Hz, 3 H, CH<sub>3</sub>), 0.86 (t, *J* = 6.8 Hz, 9 H, 3 × CH<sub>3</sub>).

<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 68.59 (CH<sub>2</sub>OH), 39.52 (CH<sub>2</sub>), 37.52 (CH<sub>2</sub>), 37.42 (CH<sub>2</sub>), 35.96 (CH), 33.65 (CH<sub>2</sub>), 32.91 (CH), 28.13 (CH), 24.93 (CH<sub>2</sub>), 24.57 (CH<sub>2</sub>), 22.86 (CH<sub>3</sub>), 22.77 (CH<sub>3</sub>), 19.88(C13), 16.79 (C12).

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## Chapter 3. Synthesis of a Mycobacterial Antigen for the Study of CD1-Antigen-T cell Interactions

*To investigate the underlying mechanisms involved in CD1-antigen-T cell interactions,  $\alpha$ -galactosyl diacylglycerol 3.3 (1-O-oleoyl-2-O-palmitoyl-3-O- $\alpha$ -D-galactopyranosyl-sn-glycerol), a potent mycobacterial antigen, was synthesised. Our initial attempts aimed to achieve the synthesis of this glycolipid by coupling an acetyl-protected galactosyl iodide to acetone-protected glycerol, with the required acyl groups installed following hydrolysis of the acetone group. This route, however, failed because no effective conditions for hydrolysis of the acetone protecting groups without affecting glycosidic acetyl bond were found, despite various attempts. At last, the method developed by Gervay-Hague and co-workers was adopted, which involved coupling a TMS-protected galactosyl iodide with an appropriate diacylglycerol to give the required product.<sup>1</sup> Biological studies showed that this synthetic galactosyl diacylglycerol could activate NKT cells to licence DCs, and resulted in CCL17 expression in splenic DCs.<sup>2</sup>*

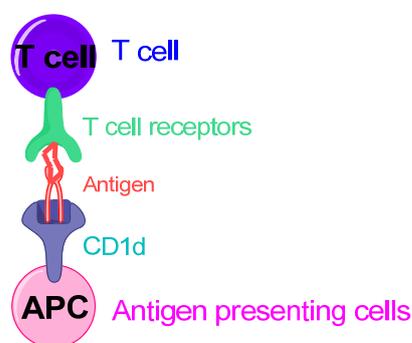
### 3.1. Background on CD1-antigen-T cell interactions

T cells are a group of lymphocytes that play a central role in cell-mediated immunity. As a T lymphocyte sub-lineage, NKT cells are related to immune responses associated with a broad range of diseases, including autoimmunity, infectious diseases, and cancer.<sup>3</sup> In contrast with conventional T cells, that recognise antigens like peptides, NKT cells are activated by glycolipids.

CD1 are a group of proteins, which present antigens to T cell receptors for recognition, which results in a T cell response. The CD1 family of proteins (including CD1a-e) are expressed on the surface of antigen presenting cells.

The investigation of the interactions between CD1, antigen and T cells may provide important insights of T cell-mediated immunity and pathogenesis of human diseases, which could contribute to the design of vaccines.

Glycolipids are a group of important antigens which are recognised by NKT cells when presented by CD1d. The hydrophobic regions of the glycolipids are retained within the grooves of CD1d.<sup>4,5</sup> Exposed hydrophilic parts, such as galactosyl moieties, stick out from the grooves to T cell receptors, whose recognition leads to production of various cytokines or cytotoxic molecules (**Figure 3.1**).<sup>6-10</sup> Previous research has shown that these glycolipids are pivotal for activating NKT cells during immune responses, including development of autoimmunity, maintenance of self tolerance, and responses to tumours and infectious agents.<sup>11,12</sup>

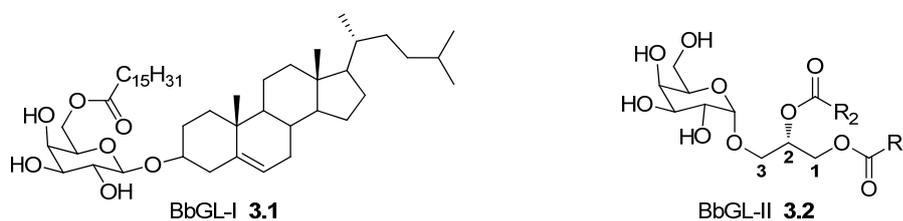


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**Figure 3.1:** T cells and TCR binding to CD1 and antigen

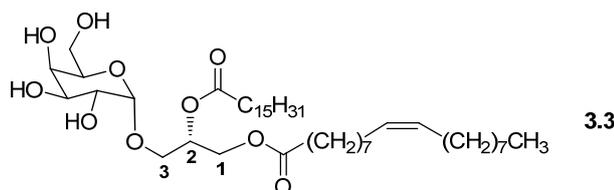
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With more than 25,000 newly reported cases in 2008 in the United States, Lyme disease is an inflammatory condition caused by the spirochete bacterium *Borrelia burgdorferi*.<sup>13</sup> This widespread vector-borne disease was first found in the United States, and gradually became a growing worldwide problem.<sup>14</sup> *B. burgdorferi* is transmitted to humans by the bite of infected ticks. These immunogenic reactive glycolipids acting as antigens are presented by CD1d and recognised by NKT cells. In 2001, a number of glycolipids were isolated from *B. burgdorferi* by Hossain and co-workers,<sup>15</sup> in particular two groups of compounds. Their structures were determined in 2003 by Ben-Menachem and co-workers, and named as *B. burgdorferi* glycolipid I (BbGL-I **3.1**) and *B. burgdorferi* glycolipids II (BbGL-II **3.2**, also known as  $\alpha$ -GalDAG) (**Figure 3.2**).<sup>16</sup> BbGL-II **3.2** compounds are a group of  $\alpha$ -linked mono-galactosyl diacylglycerol analogues with a mixture of fatty acids ester at the C1 and C2 glycerol positions. Kinjo and co-workers suggested that the acyl chain length and saturation have substantial influence the potency of these glycolipids for their role as antigens.<sup>17</sup>



**Figure 3.2:** Structures of BbGL-I **3.1** and BbGL-II **3.2**

Among all BbGL-II **3.2** analogues, BbGL-IIc **3.3**, with an oleate at C1 position and a palmitate residue at C2, was one of the most potent antigens (**Figure 3.3**).<sup>17,18</sup>



**Figure 3.3:** Structure of BbGL-IIc **3.3**

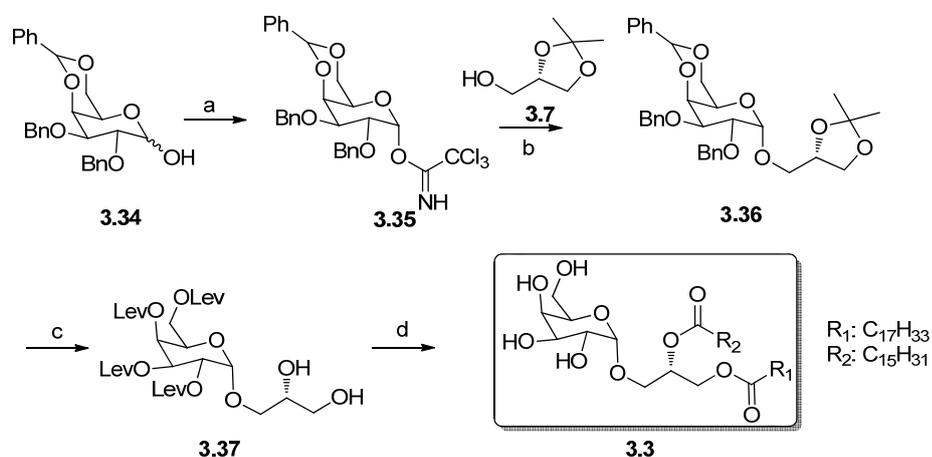
Because of the complaints about autoimmune side effects by vaccine recipients, the LYMERix<sup>®</sup> vaccine, previously marketed by GlaxoSmithKline was withdrawn from sale in the United States in 2002.<sup>19</sup> As there is currently no human vaccine against Lyme disease, a better understanding of the interactions between NKT cells and these recognised diacylglycerol antigens is extremely important for the improvement of vaccine design.

BbGL-IIc **3.3** was our first target molecule in cooperation with Jamie Rossjohn's group in the Department of Biochemistry and Molecular Biology (Monash University, Clayton, Australia) and Christian Kurts's group in the Institutes of Molecular Medicine and Experimental Immunology (Friedrich-Wilhelms-Universität, Bonn, Germany) for further investigation of the underlying immunogenic mechanisms among CD1-antigen-T cells interactions. Although BbGL-IIc **3.3** had previously been synthesised by three different groups,<sup>17,20,21</sup> the reported analytical data for the products were not entirely consistent with the natural compounds, as recently reported by Pozsgay and co-workers.<sup>21</sup> For example, the reported coupling constants between two alkene protons in oleic acyl chain by Kinjo and co-workers were 20.4, 11.4, 6.0,<sup>17</sup> which were not likely for *Z*-configured alkene protons. The coupling constants for H<sub>1'-2'</sub> and H<sub>1a-2</sub> reported by Kinjo<sup>17</sup> and Du<sup>1</sup> were very different from that of natural compound.<sup>16</sup> According to the data reported by Gervay-Hague,<sup>1</sup> there was one proton shorter at the region of 3.87-3.78 than the natural compound.<sup>16</sup> Besides, for both the natural compound and that recently synthesised by Pozsgay,<sup>21</sup> only partial of NMR data were given. Our synthetic characterisation of the target compound would provide some additional information about BbGL-IIc.

## 3.2. Previously reported syntheses of BbGL-IIc

### 3.2.1 Kinjo's synthesis

BbGL-IIc was first synthesised by Kinjo and co-workers in 11 steps from D-galactose and alcohol **3.7** (Scheme 3.1).<sup>17</sup> A benzylidene group was used to protect the C-4 and C-6 hydroxyl groups, and benzyl groups were used to protect the C-2 and C-3 hydroxyl groups to give 2,3,4,6-protected galactose **3.34**. Trichloroacetimidate **3.35** was generated from  $\text{CCl}_3\text{CN}$  and **3.34** in the presence of DBU.<sup>22</sup> Coupling trichloroacetimidate **3.35** and **3.7** gave  $\alpha$ -configured **3.36**. The benzylidene and benzyl protecting groups were removed by hydrogenation using 10 % Pd/C in  $\text{H}_2$ , and then levulinate ester protecting groups were introduced by reacting in levulinic anhydride with the presence of DMAP and pyridine. Acetonide protecting group was hydrolysed with aqueous AcOH (80 %) to give diol **3.37**. Oleate and palmitate residues were then introduced, and the levulinyl protecting groups were then selectively removed with  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$  in aqueous AcOH to give BbGL-IIc **3.3**.

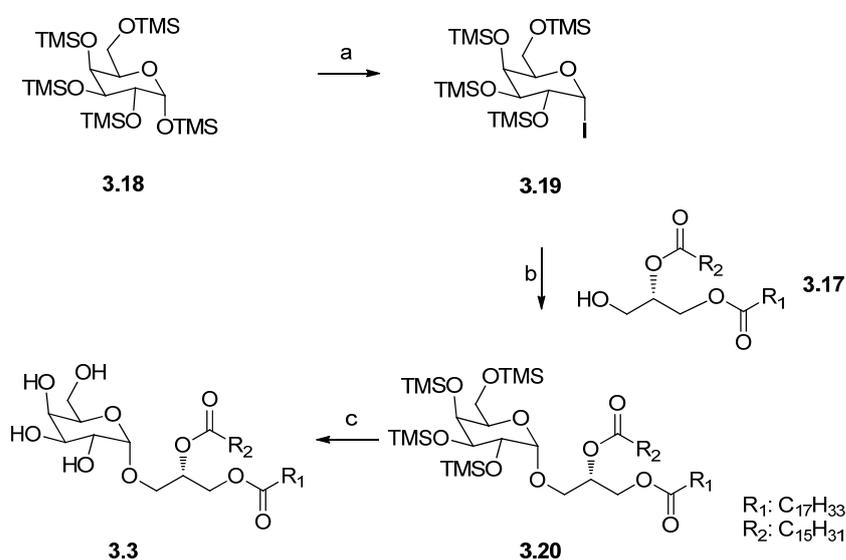


Reagents and conditions: a)  $\text{CCl}_3\text{CN}$ , DBU,  $\text{CH}_2\text{Cl}_2$ ; b)  $\text{BF}_3 \cdot \text{Et}_2\text{O}$ ,  $\text{Et}_2\text{O}$ , THF; c) i -  $\text{H}_2$ , Pd/C (10 %), DMF; ii - Lev<sub>2</sub>O, DMAP, pyridine; iii - AcOH (80 %), 70 °C; d) i -  $\text{R}_1\text{COOH}$ , DCC; ii -  $\text{R}_2\text{COOH}$ , DCC; iii -  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ , AcOH.

Scheme 3.1: Synthetic scheme reported by Kinjo<sup>17</sup>

### 3.2.2 Gervay-Hague's synthesis

Gervay-Hague and co-workers<sup>1</sup> reported a synthetic method requiring just 9 steps from D-galactose and the alcohol **3.7** via TMS-protected iodide **3.19**. This key intermediate shows high reactivity and gives excellent selectivity for the  $\alpha$ -configured product in the glycosylation reaction (**Scheme 3.2**). **3.19** was prepared from per-TMS-protected galactose with TMSI. Oleate and palmitate residues were then introduced in succession to give **3.17**. Coupling with iodide **3.19** gave TMS-protected diacyl **3.20**, which was hydrolysed with acid resin to give BbGL-IIc **3.3**.



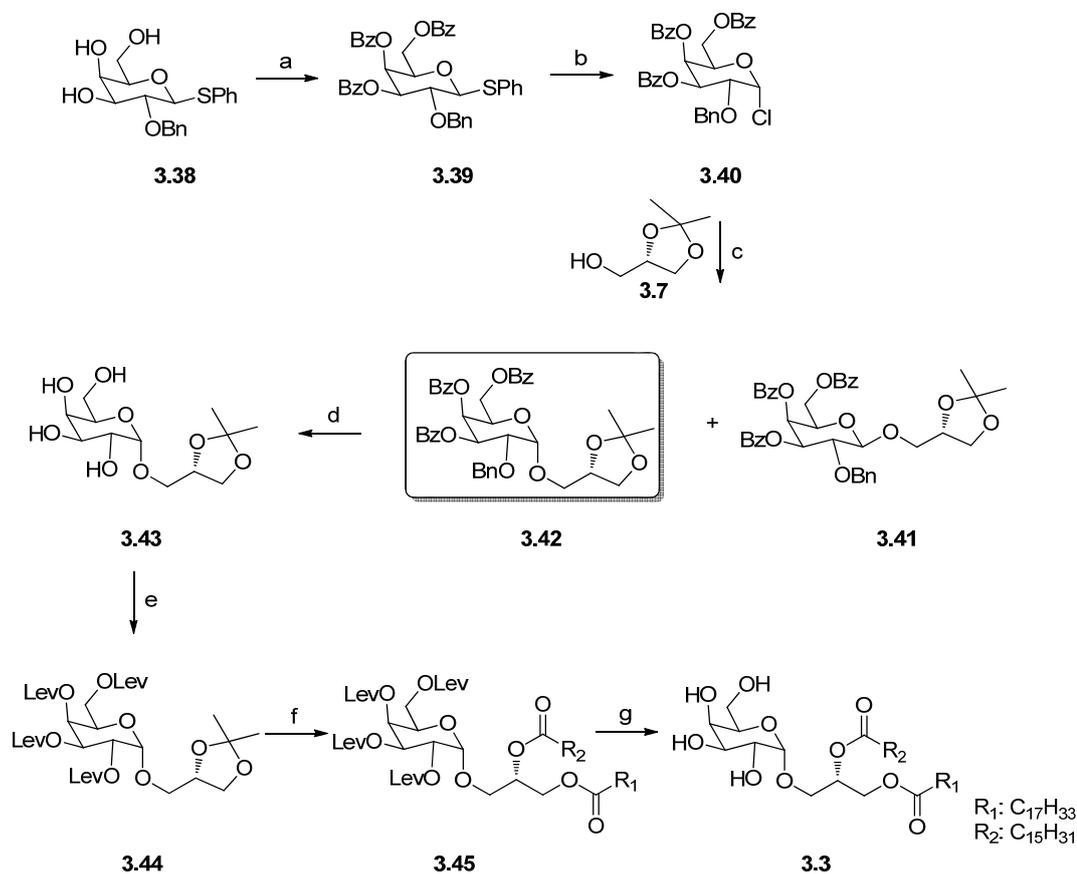
Reagents and conditions: a) TMSI,  $CH_2Cl_2$ , 0 °C; b) TBAI, DIPEA,  $CH_2Cl_2$ , r.t.; c) Dowex  $H^+$  form resin, MeOH, r.t..

**Scheme 3.2:** Synthetic method reported by Gervay-Hague using TMS as protecting group

### 3.2.3 Pozsgay's synthesis

The method reported by Pozsgay in 2011 employed a benzyl- and benzoyl-protected chloride galactosyl as intermediate for the glycosylation reaction (**Scheme 3.3**).<sup>21</sup> The synthesis starting from thioacetal **3.38**,<sup>23,24</sup> and then the C-3, 4, and 6 hydroxyl groups were esterified with benzoyl chloride to give **3.39**. After chlorination with  $Cl_2$ , chloride **3.40** was glycosylated with alcohol **3.7**, giving a mixture of **3.42** and **3.41**, which were separated by flash chromatography. After removing the benzoyl and benzyl protecting groups, the acetonide **3.43** was protected by esterification with

levulinic acid to give acetonide **3.44**. The acetonide group was hydrolysed with aqueous AcOH, and then the oleate and palmitate residues were introduced to give diacyl **3.45**, which was deprotected using  $N_2H_4 \cdot H_2O$  to give BbGL-IIc **3.3**.



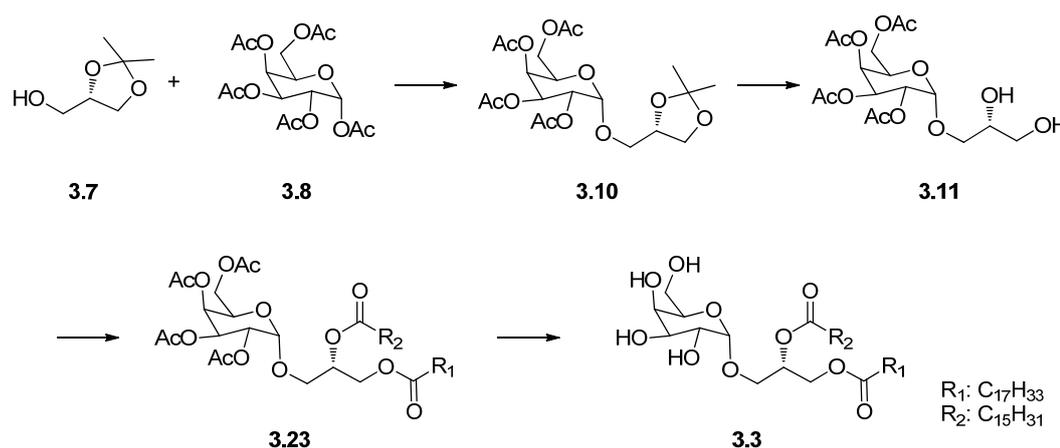
Reagents and conditions: a) i -  $Ac_2O$ , DMAP, pyridine; ii - PhSH,  $BF_3 \cdot Et_2O$ ; iii -  $PhCH_2Br$ , NaH, DMF; iv - BzCl,  $CH_2Cl_2$ , DMAP, pyridine; b)  $Cl_2$  in  $CCl_4$ ,  $CH_2Cl_2$ ,  $0^\circ C$ ; c) 2,4,6-tri-*tert*-butyl pyrimidine, AgOTf,  $-40^\circ C$ , 15 min; d) NaOMe,  $CH_2Cl_2$ ;  $H_2$ , Pd/C, EtOAc; e) Levulinic acid, DCC, DMAP, EtOAc; f) i - AcOH, reflux; ii - oleic acid, DCC, DMAP; iii - palmitic acid, DCC, DMAP; g)  $N_2H_4 \cdot H_2O$ , AcOH r.t., 3 h.

**Scheme 3.3:** Synthetic method reported by Pozsgay using chloride as intermediate

### 3.3. Results and discussion

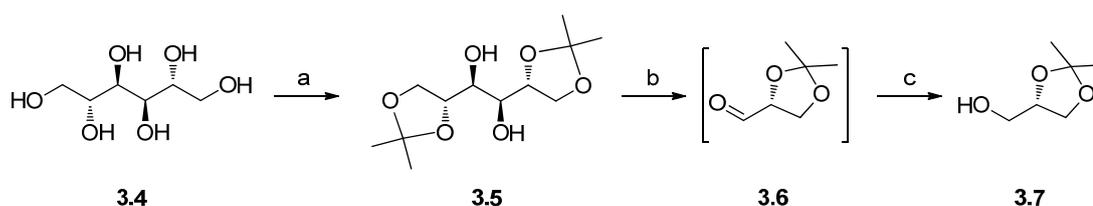
#### 3.3.1 Early synthetic attempts from peracetylated galactose

A synthetic scheme was proposed, using acetyl-protected galactose as a key intermediate, for the preparation of galactosyl diacylglycerol (**Scheme 3.4**).  $\alpha$ -Galactosylation of 1,2-isopropylidene-*sn*-glycerol **3.7** with peracetylated D-galactose **3.8**, followed by hydrolysis of the acetonide protecting group. This was expected to give the diol **3.11**. Subsequent of introduction of the desired acyl groups to give **3.23**, and after hydrolysis, to give **3.3** as product, was expected to provide a feasible route to the desired  $\alpha$ -galactosyl diacylglycerols, which would have the advantage of allowing for variation of the acyl chain at a late stage in the synthesis.



**Scheme 3.4:** Initial synthetic proposal

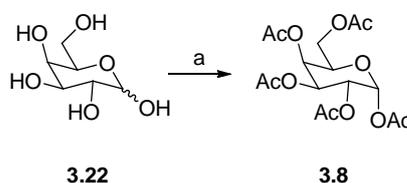
Diacetonide-protected diol **3.5** could be prepared in 54 % yield from inexpensive D-mannitol **3.4** and acetone, using ZnCl<sub>2</sub> as Lewis acid and reacting at r.t. for 12 h. The appearance of two singlets at 1.42 ppm and 1.36 ppm in the <sup>1</sup>H NMR spectrum was consistent with the methyl groups of the acetonide protecting groups. The oxidative cleavage of diacetonide **3.5** was achieved by stirring with NaIO<sub>4</sub> in water at r.t. for 2 h to give the aldehyde **3.6**, which was reduced *in situ* by NaBH<sub>4</sub> in EtOH at r.t. for 3 h to acetonide-protected alcohol **3.7** in 82 % yield (**Scheme 3.5**). The purified acetonide-protected glycerol alcohol **3.7** was obtained by Kugelrohr distillation (75-80 °C, 10 mmHg) as a colourless liquid, and its enantiomeric purity was confirmed by measuring specific rotation.<sup>25</sup>



Reagents and conditions: a)  $\text{ZnCl}_2$ , acetone, r.t., 12 h; b)  $\text{NaIO}_4$ ,  $\text{H}_2\text{O}$ , r.t., 2 h; c)  $\text{NaBH}_4$ , EtOH, r.t., 3h.

**Scheme 3.5:** Preparing 1,2-isopropylidene-*sn*-glycerol **3.7** from manitol

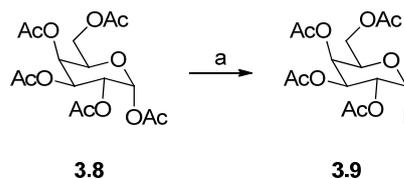
The peracetylated D-galactose **3.8** was prepared from D-galactose **3.22** and  $\text{Ac}_2\text{O}$  in pyridine and with DMAP as catalyst. The reaction was stirred at r.t. 12 h, and the peracetylated product **3.8** was obtained as white crystals in 90 % yield (**Scheme 3.6**). The signal arising from the anomeric proton in  $^1\text{H}$  NMR spectrum was a doublet at 6.38 ppm, and was consistent with the reported data for  $\alpha$ -anomer.<sup>26</sup> The absence of peaks at 5.70 ppm, which would arise from the anomeric proton of the  $\beta$ -anomer, demonstrated that no  $\beta$ -anomer was presented in the product.<sup>26</sup>



Reagents and conditions: a)  $\text{Ac}_2\text{O}$ , DMAP, pyridine, r.t., 12 h.

**Scheme 3.6:** Synthesis of peracetyl galactose **3.8**

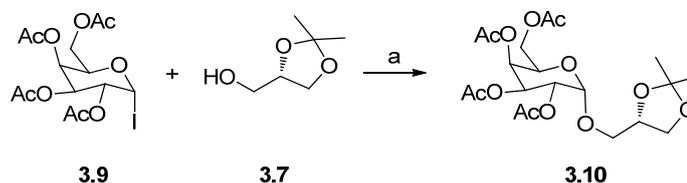
There are several candidate methods for the glycosylation.<sup>22,27</sup> Glycosyl iodides, such as **3.9**, are preferred because of the high  $\alpha$ -stereoselectivity of the reaction.<sup>28</sup> The galactosyl iodide **3.9** was prepared by stirring acetyl-protected galactose **3.8**,  $\text{TMSCl}$  and  $\text{NaI}$  at r.t. for 14 h, via *in situ* formation of TMSI (**Scheme 3.7**). The  $\text{TMSOAc}$  by-product was removed *in vacuo*. The resulting galactosyl iodide **3.9** was used in the next step without further purification. A small portion of the crude product was purified by flash chromatography for characterisation. The signal arising from the anomeric proton in  $^1\text{H}$  NMR spectrum was a doublet at 7.08 ppm, and was consistent with the literature.<sup>29</sup> No signal at 5.81 ppm, which would arise from the  $\beta$ -anomer.<sup>29</sup>



Reagents and conditions: a) TMSCl, NaI, DCM, r.t., 14 h.

**Scheme 3.7:** Synthesis of galactosyl iodide **3.9**

Galactosyl iodide **3.9** was coupled with 1,2-isopropylidene-*sn*-glycerol **3.7** in the presence of DIPEA and TBAI. After stirring for 36 h at r.t., the solvent was removed *in vacuo* and product was purified by passing through a plug of silica (hexane/Et<sub>2</sub>O 1:1 with 2 % Et<sub>3</sub>N, silica flushed with the solvent system before loading the sample) to remove DIPEA and TBAI, and the galactosyl ether **3.10** was obtained in 49 % yield (**Scheme 3.8**). The signal arising from the anomeric proton in <sup>1</sup>H NMR spectrum was a doublet at 5.73 ppm, and was consistent with the repeated data for  $\alpha$ -configured isomer.<sup>30</sup> None of the  $\beta$ -anomer<sup>31</sup> was observed.

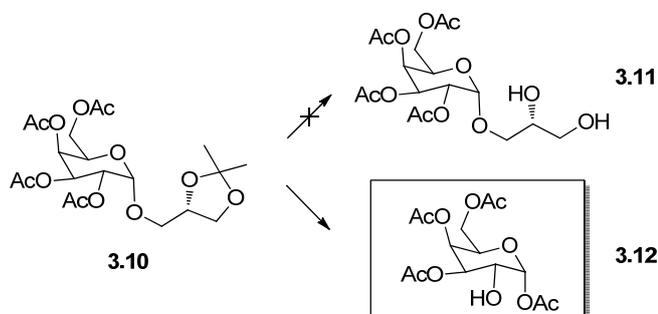


Reagents and conditions: a) DIPEA, TBAI, CH<sub>2</sub>Cl<sub>2</sub>, r.t., 36 h.

**Scheme 3.8:** Glycosylation via iodide as intermediate

After glycosylation, hydrolysis of the acetonide group in galactosyl ether **3.10** was attempted (**Scheme 3.9**). The galactosyl ether **3.10** was treated with 70 % AcOH at 60 °C for 4 h, but none of the expected diol product was obtained. After further analysis of the product and comparison with the literature, the product was identified as tetra-acetyl 2-hydroxyl galactopyranose **3.12**, and was isolated in a yield of 62 %.<sup>32</sup> Milder conditions, using 35 % AcOH and lower temperature (0 °C), were then tried. After stirring for 3 h, no starting material remained, and the same unwanted product **3.12** was isolated by flash chromatography in 60 % yield. FeCl<sub>3</sub> adsorbed on silica gel was also tried as Lewis acid for the hydrolysis. After stirring with 0.2 eq. FeCl<sub>3</sub> in CH<sub>2</sub>Cl<sub>2</sub> at r.t. for 3 h, the same by-product **3.12** was observed by <sup>1</sup>H NMR

spectroscopy, along with a large amount of unreacted galactosyl ether **3.10**. The molar ratio of galactosyl ether **3.10** to alcohol **3.12** was 3:1, according to the integration of the appropriate peaks in the  $^1\text{H}$  NMR spectrum.



Reagents and conditions been tried:

AcOH (70 %), 60 °C, 4 h, all decomposed, yield of **3.12**: 62 %;

AcOH (35 %), 0 °C, 2 h, all decomposed, yield of **3.12**: 60 %;

0.2 eq.  $\text{FeCl}_3$  on silica, r.t., 3 h., giving **3.10** and **3.12** (3:1).

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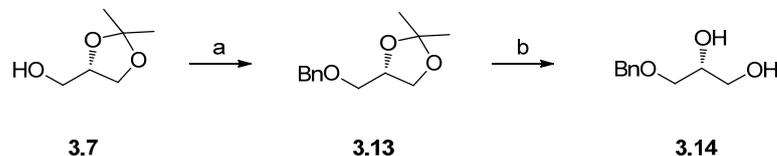
**Scheme 3.9:** Attempts to hydrolysis acetone protecting groups

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A mechanism was proposed for the decomposition of galactosyl ether **3.10** to formation of tetra-acetyl 2-hydroxyl galactopyranose **3.12** (**Figure 3.4**). Firstly, the oxygen in the glycosidic bond is protonated to form oxonium ion **3.46**. After breaking the glycosidic bond, an oxocarbenium ion **3.47** is formed. The lone pair electrons of the oxygen in the neighbouring acetyl group attack the anomeric carbon to form a stabilised oxocarbenium ion **3.48**. Attacked by water results in the formation of oxonium intermediate **3.49**, which equilibrates with oxonium intermediate **3.50** and finally decomposes to give **3.12**.



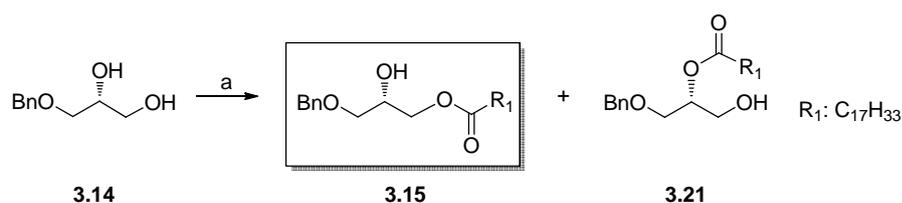
reacting at 80 °C in aqueous AcOH (70 %) to generate the benzyl-protected diol **3.14** in 80 % yield (**Scheme 3.11**).



Reagents and conditions: a) NaH, BnBr, DMF, r.t., 12 h; b) AcOH (70 %), 80 °C, 30 min.

**Scheme 3.11:** Preparation of benzyl-protected diol **3.14**

Because of the high reactivity of the primary hydroxyl group compared to the secondary one, the primary hydroxyl group in the mono benzyl-protected glycerol **3.14** could be selectively acylated after stirring with DCC and DMAP at r.t. for 14 h, to give **3.15** after purification by flash chromatography in 61 % yield (**Scheme 3.12**). The acylation at the secondary hydroxyl group was also observed in the crude product as a by-product **3.21**, in 6 % yield. The chemical shift signal of  $CH_2OBn$  could be used to distinguish the undesired acylated alcohol **3.21** from **3.15**. If the secondary alcohol was acylated to give **3.21**, the signal of  $CH_2OBn$  would shift downfield from 3.90 ppm to 5.05 ppm; otherwise, if the primary alcohol was acylated to give **3.15**, the chemical shift signal of  $CH_2OBn$  would be at 4.03 ppm.

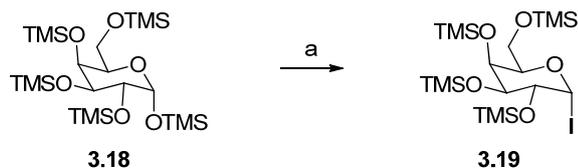


Reagents and conditions: a) Oleic acid, DCC, DMAP,  $CH_2Cl_2$ , r.t., 14 h.

**Scheme 3.12:** Selectively introduce oleate to primary alcohol<sup>1</sup>

The acylation of the secondary hydroxyl group with palmitic acid in the presence of DCC and DMAP at r.t. for 24 h gave the mixed diacyl glycerol **3.16** in 93% yield. To remove the benzyl protecting group without affecting the olefin in the oleate ester, the benzyl ether was treated with  $BCl_3$  in  $CH_2Cl_2$  at -78 °C for 30 min to give the alcohol **3.17** in 57 % yield (**Scheme 3.13**).

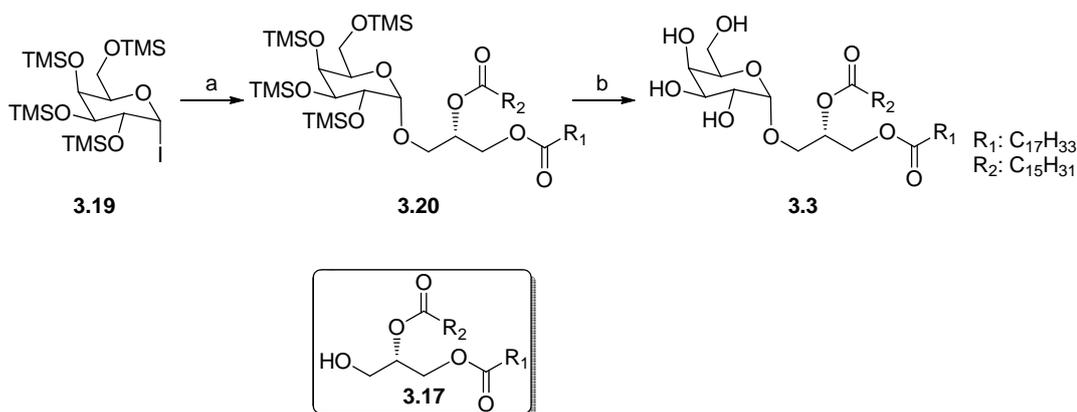




Reagents and conditions: a) TMSI, DCM, 0 °C, 4 h.

**Scheme 3.15:** Preparation of iodide **3.19** from TMS-protected D-galactose<sup>1</sup>

The iodide **3.19** was coupled with diol **3.17** with the presence of TBAI and DIPEA at r.t. for 3 h to form TMS-protected galactosyl diacylglycerol **3.20** without purification. This was hydrolysed with Dowex H<sup>+</sup> form resin to give the target molecule BbGL-IIc **3.3** as a colourless liquid in 60 % yield over 3 steps (**Scheme 3.16**).<sup>1</sup>



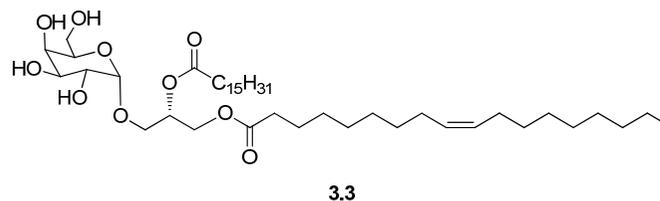
Reagents and conditions: a) **3.17**, TBAI, DIPEA, CH<sub>2</sub>Cl<sub>2</sub>, r.t., 30 h; b) Dowex 50WX2-200-400 mesh H<sup>+</sup> form resin, MeOH, r.t., 4 h.

**Scheme 3.16:** Preparation of BbGL-IIc **3.3** by coupling the glycosyl iodide with diacylglycerol<sup>1</sup>

The coupling constant of  $J_{1',2'}$  in BbGL-IIc **3.3** was  $J$  3.7 Hz, which was consistent with the values of the naturally isolated compound reported by Ben-Menachem and co-workers ( $J$  3.8 Hz)<sup>16</sup>, and the values of the synthesised compound prepared by Pozsgay and co-workers ( $J$  3.8 Hz)<sup>35</sup>, compared to values of the synthesised compounds reported by Gervay-Hague ( $J$  2.95 Hz) and Kinjo ( $J$  2.4 Hz),<sup>17,20</sup> and was significantly different with  $\beta$ -configured glycolipids ( $J$  7.7 Hz),<sup>1</sup> indicating our product was the correct  $\alpha$ -anomer (**Table 3.1**).

In summary, BbGL-IIc **3.3** was synthesised from D-mannitol and D-galactose over 12

steps in overall 6.7 % yield according to the literature.<sup>1</sup> The structure of the product was further characterized and confirmed by <sup>1</sup>H NMR, <sup>13</sup>C NMR, Cosy (H-H), HRMS, IR, and specific rotation analysis, and the data were consistent with the naturally isolated compound.<sup>16</sup>

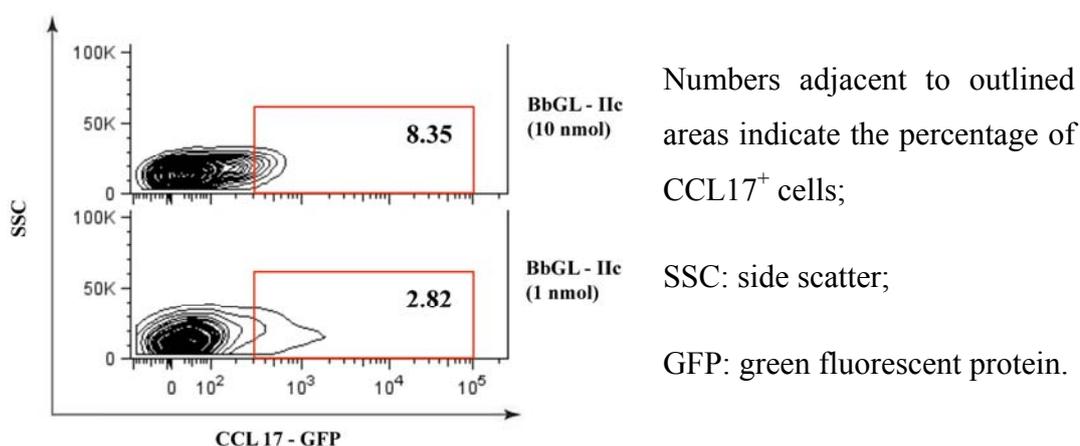
**Table 3.1:** <sup>1</sup>H NMR spectrum data of our synthetic BbGL-IIc **3.3** comparing with references

2006 (Kinjo et al.) <sup>17</sup>	2007 (Gervay-Hague et al.) <sup>1</sup>	2011 (Pozsgay et al.) <sup>35</sup>	Naturally isolated compound by Ben-Menachem et al. <sup>16</sup>	Our data
<b>5.34 (ddd, <i>J</i> 20.4, 11.4, 6.0 Hz, 2 H)</b>	5.39-5.30 (m, 2 H)	5.34 (2 H)	Data not given	5.40-5.29 (m, 2 H, H8, H9)
<b>4.92(d, <i>J</i> 2.4 Hz, 1 H)</b>	<b>4.90 (d, <i>J</i> 2.95 Hz, 1 H)</b>	<b>4.94 (d, <i>J</i> 3.8 Hz, 1 H)</b>	<b>4.94 (d, <i>J</i> 3.8 Hz, 1 H)</b>	<b>4.94 (d, <i>J</i> 3.7 Hz, 1 H, H1')</b>
<b>4.36 (dd, <i>J</i> 12.0, 2.4 Hz, 1 H)</b>	<b>4.35 (dd, <i>J</i> 11.83, 2.99 Hz, 1 H)</b>	<b>4.37 (<i>J</i> 12.0, 3.9 Hz, 1 H)</b>	<b>4.38(dd, <i>J</i> 11.9, 4.1 Hz, 1 H)</b>	<b>4.37(dd, <i>J</i> 11.9, 4.1 Hz, 1 H, H1a)</b>
3.94-3.74 (m, 6 H)	<b>3.87-3.78 (m, 5 H)</b>	3.91, 1 H ( <i>J</i> 11.3, 5.0 Hz); 3.86, 1 H; 3.83, 2 H; 3.81, 1 H; 3.77, 1 H.	3.93 (dd, <i>J</i> 11.5, 5.1, 1 H); 3.84, 1 H; 3.82, 2 H; 3.81, 1 H; 3.77, 1 H.	3.93(dd, <i>J</i> 11.4, 5.2, 1 H, H6a); 3.88-3.74 (m, 5 H, H2', H3', H5', H6'b, H1a)

### 3.3.3 Biological research results

The immunologic study was carried out by Dr Verena Semmling, Christoph Thaiss and co-workers (Friedrich-Wilhelms-Universität, Bonn, Germany).

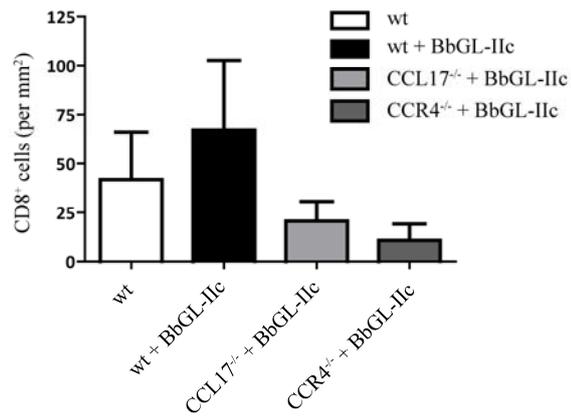
CCL17, as an inflammatory chemokine, is associated with allergic disease of the skin and lung.<sup>36-39</sup> Generally, no CCL17 expression was observed in splenic DCs. However, Verena Semmling, Christoph A. Thaiss and co-workers showed that antigens like BbGL-IIc could activate NKT cells to licence DCs, and such activation is important for DC licensing, including CCL17 expression by DCs (**Figure 3.5**).<sup>2</sup>



**Figure 3.5:** Flow cytometry of mice spleen cells from CCL17-GFP in 20 h after injection of BbGL-IIc

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It was also discovered that there was an accumulation of CD8<sup>+</sup> T cells in the splenic white pulp in 8 h after injection BbGL-IIc (**Figure 3.6**),<sup>2,40</sup> which indicates that CCL17 produced by licensed DCs plays important roles or efficient cross-priming.



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**Figure 3.6:** CD8<sup>+</sup> T cells accumulation in splenic white pulp 8 h after injection of BbGL-IIc

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Other biology experiments are under way in Jamie Rossjohn's group (Monash University, Clayton, Australia) relating to NKT cells and CD1d immunological reactivity.

## 3.4. Experimental

### 3.4.1 General

Melting points were measured on Reichert hot stage melting point apparatus (Reichert, Austria).

Optical rotations were measured on a PolAAR 2001 automatic polarimeter (Optical Activity Ltd., Huntingdon, Cambridgeshire, UK) with 1 dm cell, at a wavelength of 589 nm (sodium D line), and were assigned as  $[\alpha]_D$ , temperature, concentration  $c$  (g/100 mL) and solvent.

$^1\text{H}$  NMR spectra were determined at 300 MHz with a Bruker Avance DPX300 spectrometer or at 400 MHz with a Bruker Avance DRX400 spectrometer (Bruker BioSpin Corp., Billerica, Massachusetts, USA). The chemical shifts ( $\delta$ , ppm) were referenced to the residual  $\text{CHCl}_3$  signal ( $\delta$  7.26 ppm). Each resonance was quoted with chemical shifts measured in parts per million (ppm), multiplicity, coupling constant ( $J$  Hz), number of protons, and structural assignment. Multiplicities were denoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or br s (broad singlet).

$^{13}\text{C}$  NMR spectra were recorded at 100 MHz on a Bruker Avance DRX400 spectrometer (Bruker BioSpin Corp., Billerica, Massachusetts, USA) in  $\text{CDCl}_3$ , referenced to the residual  $\text{CHCl}_3$  signal ( $\delta$  77.16 ppm). Each resonance was quoted as chemical shift measured in parts per million (ppm) and structural assignment.

Low resolution electrospray ionisation mass spectrometry was measured on a Micromass Platform spectrometer (Micromass Ltd., Wythenshawe, Manchester, UK). Accurate mass determinations were made on an Agilent G1969A LC-TOF system (Agilent Technologies, Santa Clara, California, USA) with reference and mass correction at 4000 V capillary voltage for electrospray ionisation.

Infra red spectra were obtained using a Bruker Equinox IFS55 FTIR, fitted with a mercury-cadmium-telluride detector and a Specac "Golden Gate" single reflection diamond ATR element. The operation condition was 50 scans at a resolution of  $4\text{ cm}^{-1}$ .

The intensity of absorption bands for all samples have been specified as either s (strong), m (medium), w (weak) or b (broad).

Kugelrohr distillations were carried out using a Büchi GKR-50 apparatus *in vacuo*.

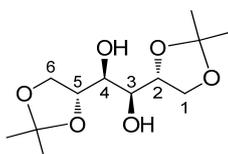
Analytical TLC was performed on Merck Silica Gel 60 F<sub>254</sub>, and visualised under a 254 nm UV lamp and/or stained using an alkaline KMnO<sub>4</sub> solution. Flash chromatography was performed using Silica Gel 60 (230-400 mesh, no. 9385) from Merck or SiliaFlash<sup>®</sup> P60 (230-400 mesh) from Silicycle.

### 3.4.2 Materials

Commercially available reagents and chemicals were purchased from Sigma-Aldrich (Milwaukee, Wisconsin, USA), AK Scientific Inc. (Union City, California, USA), Merck (Darmstadt, Hesse, Germany), Ajax Finechem Pty. Ltd. (Sydney, New South Wales, Australia), Oakwood Chemical (West Columbia, South Carolina, USA), ChemSupply (Gillman, South Australia, Australia) or Lancaster Synthesis Ltd. (Morecambe, Lancashire, UK), and were reagent grade unless specified. Anhydrous solvents were freshly distilled under nitrogen prior to use: toluene and Et<sub>3</sub>N were distilled from CaH; CH<sub>2</sub>Cl<sub>2</sub> was distilled from P<sub>2</sub>O<sub>5</sub>; THF and Et<sub>2</sub>O were distilled from Na/benzophenone.

### 3.4.3 Synthetic attempts via acetyl-protected intermediates

#### 3.1.1.1. 1,2:5,6-Di-*O*-isopropylidene-D-mannitol (3.5)



3.5

Anhydrous ZnCl<sub>2</sub> was prepared according to the following procedure: commercial ZnCl<sub>2</sub> was heated to melt in a porcelain cup and careful heating was continued while stirring with a glass rod until no more bubbles were generated. The molten mass was poured into a mortar, allowed to cool down, crushed and used directly in the reaction.

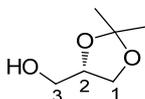
Anhydrous ZnCl<sub>2</sub> (14 g, 0.1 mol) was added to acetone (70 mL) and stirred for 30 min.

D-Mannitol (7.2 g, 39.6 mmol) was added, and the reaction mixture was stirred at r.t. for 12 h. The mixture was poured into a solution of  $K_2CO_3$  (18 g, 0.14 mol) in water (18 mL), and stirred for 30 min. After removing acetone *in vacuo*,  $CH_2Cl_2$  (100 mL) was added and the mixture was filtered through celite. The solids were washed with  $CH_2Cl_2$  ( $3 \times 50$  mL), and the combined filtrates were washed with water (200 mL), dried over  $Na_2SO_4$ , filtered and then solvent removed *in vacuo* to give a white solid. Recrystallisation (1: 8  $CHCl_3$ /hexane) gave the *title compound* (5.6 g, 54 %) as white crystals, m.p. 120-121 °C (Lit.<sup>41</sup> m.p. 116-119 °C). The analytical data were in agreement with literature values.<sup>41,42</sup>

<sup>1</sup>H NMR (400 MHz,  $CDCl_3$ )  $\delta$  4.22-4.10 (m, 4 H, H1 and H6), 3.97 (dd,  $J = 8.6$ , 5.7 Hz, 2 H, H2 and H5), 3.75 (t,  $J = 6.3$  Hz, 2 H, H3 and H4), 2.52 and 2.53 (2 s, 2 H,  $2 \times OH$ ), 1.42 and 1.36 (2 s, 6 H each,  $CH_3$ ).

<sup>13</sup>C NMR (100 MHz,  $CDCl_3$ ): 109.4( $C(CH_3)_2$ ), 76.9 (C1, C6), 71.6 (C2, C5), 66.7 (C3, C4), 26.7 ( $CH_3$ ), 25.2 ( $CH_3$ ).

### 3.1.1.2. (S)-(+)-1,2-Isopropylidenglycerol (3.7)



3.7

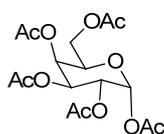
To a solution of  $NaIO_4$  (4 g, 18.7 mmol) in water (50 mL) at 0 °C was slowly added diol **3.5** (3.42 g, 13.0 mmol), and the reaction mixture was stirred at r.t. for 2 h. After addition of EtOH (100 mL), the mixture was cooled to 5 °C and allowed to stir for another 30 min. The solids were removed by filtering, and washed with cold EtOH (100 mL). To the filtrate a solution of  $NaBH_4$  (1.5 g, 39.4 mmol) in water (3 mL) was added at 0 °C dropwise. After stirring at r.t. for 3 h, the reaction was quenched by slowly addition of AcOH to pH 7. The solvent was removed *in vacuo* to give a turbid mixture, which was diluted with water (100 mL) and extracted with  $CH_2Cl_2$  ( $3 \times 100$  mL). The combined organic layers were dried over  $Na_2SO_4$ , filtered, and the solvent removed *in vacuo* to give a colourless oil, which was purified by Kugelrohr distillation (75-80 °C, 10 mmHg) to give the *title compound* (1.4 g, 82 %) as a colourless liquid. The analytical data were in agreement with literature values.<sup>42,43</sup>

$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  4.19 (tdd,  $J = 6.5, 5.4, 3.9$  Hz, 1 H, H2), 4.00 (dd,  $J = 8.2, 6.6$  Hz, 1 H, H1a), 3.74 (dd,  $J = 8.2, 6.5$  Hz, 1 H, H1b), 3.70-3.51 (m, 2 H, H3), 2.52 (br s, 1 H, OH); 1.40 and 1.33 (2 s, 3 H each,  $\text{CH}_3$ ).

$^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ )  $\delta$  109.5 ( $\text{C}(\text{CH}_3)_2$ ), 76.3 (CH), 65.8 (C1), 63.1 (C3), 26.7 and 25.3 ( $\text{C}(\text{CH}_3)$ ).

**Specific Rotation**  $[\alpha]_{\text{D}}^{22} +14.5^\circ$  ( $c$  5, MeOH) (Lit.<sup>25</sup>  $[\alpha]_{\text{D}}^{22} +11.3^\circ$  ( $c$  5.2, MeOH))

### 3.1.1.3. 1,2,3,4,6-Penta-*O*-acetyl- $\alpha$ -D-galactopyranose (3.8)

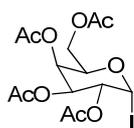


**3.8**

To a solution of D-galactose (5 g, 27.8 mmol) in pyridine (dried over activated 4 Å molecular sieves, 143 mL, 1.78 mol) was added  $\text{Ac}_2\text{O}$  (165 mL, 1.78 mol) and DMAP (0.5 g, 4.4 mmol) at  $0^\circ\text{C}$ . The reaction mixture was stirred at r.t. for 12 h, and then the reaction was quenched by pouring the reaction mixture into ice water (600 mL). The aqueous solution was extracted with EtOAc ( $2 \times 600$  mL). The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , filtered and the solvent removed *in vacuo*. The resulting solid was purified by recrystallisation from MeOH to give the *title compound* (9.7 g, 90 %) as white crystals, m.p.  $89\text{--}91^\circ\text{C}$  (Lit.<sup>44</sup> m.p.  $88\text{--}90^\circ\text{C}$ ). The analytical data were in agreement with literature values.<sup>26</sup>

$^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  6.38 (d,  $J = 1.5$  Hz, 1 H, H1), 5.50 (d,  $J = 1.2$  Hz, 1 H, CH), 5.40-5.30 (m, 2 H, CH), 4.40-4.29 (m, 1 H, CH), 4.10 (dd,  $J = 6.7, 2.7$  Hz, 2 H, H6); 2.16, 2.10, 2.04, 2.02 and 2.00 (5 s, 3 H each, acetyl  $\text{CH}_3$ ).

### 3.1.1.4. 2,3,4,6-*O*-Acetyl- $\alpha$ -galactopyranosyl iodide (3.9)



**3.9**

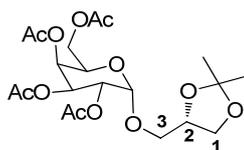
To a mixture of  $\text{TMSCl}$  (0.4 mL, 3.1 mmol) and  $\text{NaI}$  (0.54 g, 3.6 mmol) in  $\text{CH}_2\text{Cl}_2$  (3 mL), a solution of peracetyl-protected galactose **3.8** (0.9 mmol, 0.35 g) in  $\text{CH}_2\text{Cl}_2$

(5 mL) was added at 0 °C under Ar. The reaction was stirred at r.t. for 14 h, and then toluene (10 mL) was added and the mixture was filtered through celite. The solvent was removed *in vacuo* to give the *title compound* as a slightly red liquid (0.28 g, 68 %) which was used in the next step without further purification. A small portion of the product was purified by flash chromatography (hexane/Et<sub>2</sub>O 2:1) to give a colourless liquid. The analytical data were in agreement with literature values.<sup>45</sup>

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.08 (d, *J* = 4.25 Hz, 1 H, H1'), 5.50 (d, *J* = 2.1 Hz, 1 H, CH), 5.33 (dd, *J* = 10.5, 3.2 Hz, 1 H, CH), 4.36 (dd, *J* = 10.6, 4.2 Hz, 1 H, CH), 4.26-4.02 (m, 3 H, CH and CH<sub>2</sub>); 2.15, 2.11, 2.06 and 2.00 (4 s, 3 H each, CH<sub>3</sub>).

ESI-MS: Calcd. for [C<sub>14</sub>H<sub>19</sub>IO<sub>9</sub>+Na<sup>+</sup>]: 481.0, found 480.7.

### 3.1.1.5. 1,2-Isopropylidene-3-*O*-(2,3,4,6-*O*-acetyl- $\alpha$ -galactopyranosyl)-*sn*-glycerol (3.10)



**3.10**

Galactosyl iodide **3.9** (0.26 g, 0.57 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was added via cannula to a mixture of DIPEA (0.22 g, 1.7 mmol), glycerol **3.7** (75 mg, 0.57 mmol), TBAI (0.21 g, 0.57 mmol) and 4Å molecular sieves (0.5 g) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) at 0 °C under Ar. After stirring at r.t. for 36 h, the solvent was removed *in vacuo*, and the crude product was purified by eluting through short plug of silica (hexane/Et<sub>2</sub>O 1:1 with 2 % Et<sub>3</sub>N, silica was washed with the solvent system before loading sample) to remove DIPEA and TBAI. The solvent was removed *in vacuo* to give the *title compound* (0.13 g, 49 %) as a colourless liquid. The analytical data were in agreement with literature values.<sup>30,46</sup>

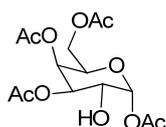
<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 5.73 (d, *J* = 4.8 Hz, 1 H, H1'), 5.34 (dd, *J* = 3.2, 2.7 Hz, 1 H, H4'), 4.97 (dd, *J* = 6.7, 3.4 Hz, 1 H, H6'a), 4.23 (dt, *J* = 6.7, 4.0 Hz, 2 H, H2' and H6'b), 4.17-3.90 (m, 3 H, H5', H3' and H2), 3.96 (dd, *J* = 8.3, 6.3 Hz, 1 H, H1a), 3.66 (dd, *J* = 8.3, 5.7 Hz, 1 H, H1b), 3.53 (dd, *J* = 9.3, 5.4 Hz, 1 H, H3a), 3.38 (dd, *J* = 9.4, 6.4 Hz, 1 H, H3b); 2.13, 2.02, 1.98 and 1.97 (4 s, 3 H each, acetyl

$CH_3$ ); 1.32 and 1.26 (2 s, 3 H each, acetonide  $CH_3$ ).

**ESI-MS:** Calcd. for  $[C_{20}H_{30}O_{12}+Na^+]$ : 485.2, found 485.0.

### 3.1.1.6. Attempted deacetonisation of 1,2-isopropylidene-3-O-(2,3,4,6-O-acetyl- $\alpha$ -galactopyranosyl)-*sn*-glycerol

#### 1,3,4,6-Tetra-O-acetyl-2-hydroxyl- $\alpha$ -d-galactopyranose (3.12)



**3.12**

(1) Hydrolysis with 70% aqueous AcOH at 60 °C<sup>30</sup>

$\alpha$ -Galactosyl ether **3.10** (0.1 g, 0.22 mmol) was dissolved in aqueous AcOH (70%, 3 mL), and the reaction mixture was stirred at 60 °C for 4 h, then the solvent was removed *in vacuo* by azeotropic distillation with toluene (3  $\times$  5 mL). The resulting white solid was purified by flash chromatography (hexane/Et<sub>2</sub>O 2:1) to give the *title compound* (47 mg, 62 %) as a white powder, m.p. 145-146 °C (Lit.<sup>32</sup> m.p. 145-147 °C). The analytical data were in agreement with literature values.<sup>32,47</sup>

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  6.28 (d,  $J$  = 3.8 Hz, 1 H, H1), 5.43 (dd,  $J$  = 3.3, 1.3 Hz, 1 H, CH), 5.16 (dd,  $J$  = 10.6, 3.3 Hz, 1 H, CH), 4.26 (td,  $J$  = 6.7, 0.9 Hz, 1 H, CH), 4.19-4.11 (m, 1 H, CH), 4.06 (dd,  $J$  = 6.7, 3.1 Hz, 2 H, H6), 2.38-2.26 (br s, 1 H, OH); 2.16, 2.12, 2.04 and 2.01 (4 s, 3 H each, acetyl  $CH_3$ ).

**ESI-MS:** Calcd. for  $[C_{14}H_{20}O_{10}+Na^+]$ : 371.1, found 371.0.

(2) Hydrolysis with 35 % AcOH at 0 °C

A solution of acetonide-protected galactosyl ether **3.10** (0.1 g, 0.22 mmol) in aqueous AcOH (35 %, 15 mL) was stirred at 0 °C for 2 h, and then the solvent was removed *in vacuo*. The resulting white solid was purified by flash chromatography (hexane/Et<sub>2</sub>O 2:1) to give the *title compound* (45 mg, 60 %).

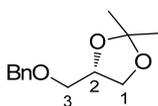
(3) Hydrolysis using FeCl<sub>3</sub> adsorbed on silica gel<sup>48</sup>

FeCl<sub>3</sub> adsorbed on silica gel was prepared by the following procedure: FeCl<sub>3</sub>·6H<sub>2</sub>O (0.13 g, 0.1 mmol) in acetone (3 mL) was added to silica (1.1 g) and allowed to stir until evenly dispersed. The solvent was then removed *in vacuo*.

A mixture of FeCl<sub>3</sub> on silica gel (62 mg, 0.05 mmol) and acetonide-protected galactosyl ether **3.10** (0.11 g, 0.25 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was stirred at r.t. for 3 h, and then filtered. The solvent was removed *in vacuo* to give a white solid, which contained a 3:1 molar ratio of starting material and the *title compound* according to the integration of the appropriate peaks in the <sup>1</sup>H NMR spectrum.

### 3.4.4 Synthesis of BbGL-IIc through TMS-protected intermediates

#### 3.1.1.7. 3-O-Benzyl-(S)-(+)-1,2-isopropylidenglycerol (3.13)

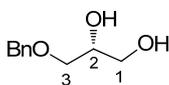


**3.13**

To a solution of (*S*)-(+)-1,2-isopropylidenglycerol **3.7** (0.3 g, 2.27 mmol) in DMF (6 mL) was slowly added NaH (0.08 g, 3.33 mmol), followed by addition of BnBr (0.43 g, 2.5 mmol). The reaction mixture was stirred at r.t. for 12 h, and then MeOH (0.5 mL) was added. The reaction mixture was diluted with EtOAc (20 mL) and then washed with water (20 mL). The aqueous layer was extracted with EtOAc (2 × 20 mL) and the combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent removed *in vacuo* to give the *title compound* (0.49 g, 97 %) as a colourless oil. The analytical data were in agreement with literature values.<sup>49</sup>

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.38-7.13 (m, 5 H, ArH), 4.59-4.41 (m, 2 H, OCH<sub>2</sub>Ph), 4.23 (p, *J* = 6.0 Hz, 1 H, H<sub>2</sub>), 3.98 (dd, *J* = 8.2, 6.4 Hz, 1 H, H<sub>3a</sub>), 3.68 (dd, *J* = 8.2, 6.3 Hz, 1 H, H<sub>3b</sub>), 3.45 (ddd, *J* = 26.7, 9.8, 5.7 Hz, 2 H, H<sub>1</sub>); 1.35 and 1.29 (2 s, 3 H each, CH<sub>3</sub>).

#### 3.1.1.8. 3-O-Benzyl-*sn*-glycerol (3.14)

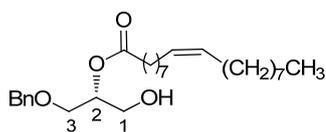


**3.14**

A solution of acetonide-protected diol **3.13** (0.3 g, 1.35 mmol) in aqueous AcOH (70%, 10 mL) was heated at 80 °C for 30 min and then solvent was removed *in vacuo* to obtain a light yellow oil. Purification by flash chromatography (hexane/EtOAc 1:1) gave the *title compound* (0.2 g, 80 %) as a colourless oil. The analytical data were in agreement with literature values.<sup>49</sup>

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.18-7.38 (m, 5 H, ArH), 4.56 (s, 2 H, OCH<sub>2</sub>Ph), 3.93-3.85 (m, 1 H, H2), 3.68 (dd, *J* = 3.7, 12.0 Hz, 1 H, H1a), 3.60 (dd, *J* = 6.0, 12.1 Hz, 1 H, H1b), 3.55 (dd, *J* = 5.4, 9.6 Hz, 1 H, H3a), 3.46 (dd, *J* = 6.5, 9.6 Hz, 1 H, H3b), 2.47 and 2.48 (2 s, 2H, 2 × OH).

### 3.1.1.9. Mono-acyl glycerol (3.21)



**3.21**

To a solution of 3-*O*-benzyl-*sn*-glycerol **3.14** (0.25 g, 1.37 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) at 0 °C was added DCC (0.57 g, 2.74 mmol) and DMAP (8.4 mg, 0.07 mmol), followed by addition of a solution of oleic acid (0.35 mg, 1.24 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) dropwise. The reaction mixture was stirred at r.t. 14 h, and then filtered through celite, washed with saturated NaHCO<sub>3</sub> solution (15 mL) and brine (15 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and the solvent removed *in vacuo* to give a light yellow oil. Purification by flash chromatography (hexane/EtOAc 5:1) gave the *title compound* **3.15** (0.37 g, 61 %) as a colourless oil, and 3-*O*-benzyl-2-*O*-oleyl-*sn*-glycerol **3.21** (37 mg, 6 %) as a colourless oil. The analytical data were in agreement with literature values.<sup>1</sup>

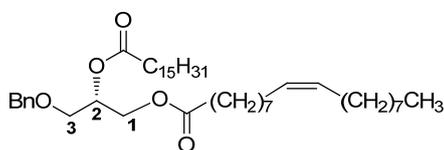
### 3-*O*-Benzyl-1-*O*-oleyl-*sn*-glycerol (3.15):

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.41-7.27 (m, 5 H, ArH), 5.43-5.27 (m, 2 H, CH=CH), 4.56 (s, 2 H, OCH<sub>2</sub>Bn), 4.24-4.09 (m, 2 H, H1), 4.09-3.97 (m, 1 H, H2), 3.53 (qd, *J* = 9.6, 5.2 Hz, 2 H, H3), 2.48 (br s, 1 H, OH), 2.32 (t, *J* = 7.5 Hz, 2 H, CH<sub>2</sub>C=O), 2.09-1.91 (m, 4 H, CH<sub>2</sub>CH=CH), 1.70-1.51 (m, 2 H, CH<sub>2</sub>), 1.43-1.16 (m, 20 H, CH<sub>2</sub>), 0.88 (t, *J* = 6.7 Hz, 3 H, CH<sub>3</sub>).

**3-O-Benzyl-1-O-oleyl-sn-glycerol (3.21):**

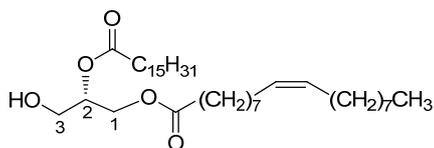
$^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.33 (dd, 5 H, ArH), 5.41-5.27 (m, 2 H, CH=CH), 5.05 (p,  $J = 4.9$  Hz, 1 H, H2), 4.55 (d,  $J = 3.6$  Hz, 2 H,  $\text{OCH}_2\text{Ph}$ ), 3.82 (t,  $J = 5.5$  Hz, 2 H, H1), 3.66 (dd,  $J = 5.1, 3.0$  Hz, 2 H, H3), 2.41-2.31 (m, 2 H,  $\text{COOCH}_2$ ), 2.10-1.90 (m, 4 H,  $2 \times \text{CH}_2\text{CH}=\text{CH}$ ), 1.72-1.57 (m, 2 H,  $\text{COOCH}_2\text{CH}_2$ ), 1.43-1.15 (m, 20 H, alkyl chain  $\text{CH}_2$ ), 0.95-0.81 (m, 3 H,  $\text{CH}_3$ ).

**ESI-MS:** Calcd. for  $[\text{C}_{28}\text{H}_{46}\text{O}_4 + \text{Na}^+]$ : 469.3, found 469.1.

**3.1.1.10. 3-O-Benzyl-1-O-oleyl-2-O-palmitoyl-sn-glycerol (3.16)****3.16**

A solution of DCC (0.27 g, 1.32 mmol), DMAP (4 mg, 0.03 mmol) and palmitic acid (0.25 g, 0.99 mmol) in  $\text{CH}_2\text{Cl}_2$  (4 mL) was cooled to  $0^\circ\text{C}$ . Mono-acylglycerol **3.15** (0.3 g, 0.66 mmol) in  $\text{CH}_2\text{Cl}_2$  (6 mL) was added slowly and the mixture was stirred at r.t. for 24 h. The reaction mixture was filtered through celite and washed with saturated  $\text{NaHCO}_3$ , dried over  $\text{Na}_2\text{SO}_4$ , filtered, and the solvent removed *in vacuo* to give a yellow oil. Purification by flash chromatography (hexane/ $\text{Et}_2\text{O}$  6:1) gave the *title compound* (0.42 g, 93 %) as colourless liquid. The analytical data were in agreement with literature values.<sup>1</sup>

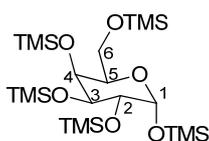
$^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.39-7.27 (m, 5 H, ArH), 5.40-5.28 (m, 2 H, CH=CH), 5.30-5.17 (m, 1 H, H2), 4.48 (dd,  $J = 11.9, 3.3$  Hz, 2 H,  $\text{OCH}_2\text{Ar}$ ), 4.35 (dd,  $J = 11.9, 3.9$  Hz, 1 H, H1a), 4.19 (dd,  $J = 11.9, 6.4$  Hz, 1 H, H1b), 3.59 (d,  $J = 5.3$  Hz, 2 H, H1 and H3), 2.30 (dt,  $J = 12.1, 7.5$  Hz, 4 H,  $\text{C}=\text{OCH}_2$ ), 2.01 (dd,  $J = 12.4, 6.7$  Hz, 4 H,  $\text{CH}=\text{CHCH}_2$ ), 1.60 (dd,  $J = 14.4, 7.2$  Hz, 4 H,  $2 \times \text{CH}_2$ ), 1.40-1.16 (m, 44 H), 0.95-0.81 (m, 6 H,  $2 \times \text{CH}_3$ ).

3.1.1.11. 1-*O*-Oleyl-2-*O*-palmitoyl-*sn*-glycerol (3.17)

3.17

To a solution of benzyl-protected diacylglycerol **3.16** (0.4 g, 0.58 mmol) in  $\text{CH}_2\text{Cl}_2$  (6 mL) at  $-78^\circ\text{C}$ ,  $\text{BCl}_3$  (1.2 mL, 1.2 mmol, 1 M in  $\text{CH}_2\text{Cl}_2$ ) was added dropwise, and then the mixture was stirred for 30 min. The reaction mixture was quenched by pouring into ice water, the aqueous layer was extracted with  $\text{CH}_2\text{Cl}_2$  ( $3 \times 10$  mL), and the combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , filtered, and the solvent removed *in vacuo* to give a light yellow oil. Purification by flash chromatography (hexane/ $\text{Et}_2\text{O}$  8:1) gave the *title compound* (0.2 g, 57 %) as a colourless oil. The analytical data were in agreement with literature values.<sup>1</sup>

$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.43-5.28 (m, 2 H,  $\text{CH}=\text{CH}$ ), 5.08 (dt,  $J = 12.7, 5.0$  Hz, 1 H, H2), 4.32 (dd,  $J = 11.9, 4.6$  Hz, 1 H, H1a), 4.24 (dd,  $J = 11.9, 5.7$  Hz, 1 H, H1b), 3.73 (ddd,  $J = 6.4, 5.0, 1.2$  Hz, 2 H, H3), 2.38-2.28 (m, 4 H,  $\text{C}=\text{OCH}_2$ ), 2.06-1.92 (m, 4 H,  $\text{CH}_2\text{CH}=\text{CH}$ ), 1.71-1.56 (m, 4 H,  $\text{CH}_2$ ), 1.40-1.19 (m, 44 H,  $22 \times \text{CH}_2$ ), 0.95-0.81 (m, 6 H,  $2 \times \text{CH}_3$ ).

3.1.1.12. 1,2,3,4,6-Penta-*O*-trimethylsilyl- $\alpha$ -D-galactopyranose (3.18)

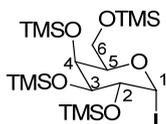
3.18

To a solution of D-galactose (1.0 g, 5.6 mmol) in DMF (50 mL) at  $0^\circ\text{C}$  was slowly added  $\text{Et}_3\text{N}$  (5.4 mL, 39.0 mmol) and  $\text{TMSCl}$  (4.2 mL, 33.3 mmol). The reaction mixture was stirred at r.t. for 7 h, and the solvent removed *in vacuo*. The crude product was purified by flash chromatography (hexane/ $\text{Et}_2\text{O}$  10:1) to give the *title compound* (2.5 g, 83.0 %) as a colourless liquid. The analytical data were in agreement with literature values.<sup>33</sup>

$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.05 (d,  $J = 2.4$  Hz, 1 H, H1), 3.96 – 3.74 (m, 4 H, H2,

H3, H4 and H5), 3.63 (dd,  $J = 9.7, 7.7$  Hz, 1 H, H6a), 3.53 (dd,  $J = 9.6, 5.5$  Hz, 1 H, H6b), 0.14 (d,  $J = 2.8$  Hz, 27 H,  $3 \times \text{Si}(\text{CH}_3)_3$ ), 0.11 (d,  $J = 2.9$  Hz, 18 H,  $2 \times \text{Si}(\text{CH}_3)_3$ ).

### 3.1.1.13. Tetra-TMS-protected glycosyl iodide (3.19)



3.19

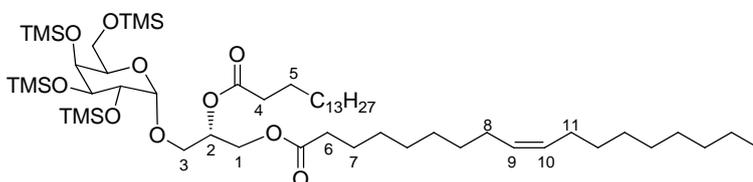
(1) Attempted preparation of galactosyl iodide from TMSCl and NaI:

A solution of TMS-protected D-galactose **3.18** (0.1 g, 0.20 mmol) in  $\text{CH}_2\text{Cl}_2$  (1.5 mL) was added to a mixture of TMSCl (38  $\mu\text{L}$ , 0.3 mmol) and NaI (45 mg, 0.3 mmol) in  $\text{CH}_2\text{Cl}_2$  (1.5 mL) at 0 °C. After stirring for 4 h at r.t., no expected iodide product was detected by  $^1\text{H}$  NMR spectrum or mass spectrometry.

(2) Preparing galactosyl iodide from TMSI<sup>1</sup>

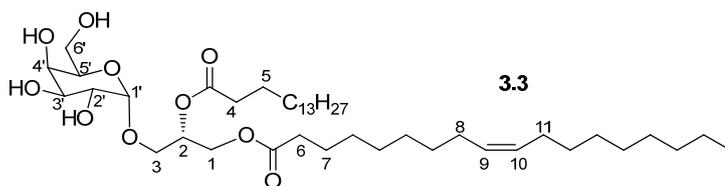
To a solution of TMS-protected D-galactose **3.18** (1.1 g, 2 mmol) in  $\text{CH}_2\text{Cl}_2$  (30 mL) under Ar at 0 °C was added TMSI (0.4 g, 2.2 mmol) dropwise. The reaction mixture was stirred at 0 °C for 4 h, and then the solvent was removed *in vacuo*. Toluene ( $2 \times 20$  mL) was added, and then removed *in vacuo* to give the iodide **3.19** as a colourless liquid, which was used immediately.<sup>1</sup>

### 3.1.1.14. (S)-2-(Palmitoyloxy)-3-(TMS-O-D-galactopyranose) propyl oleate (3.51)



3.51

To a mixture of 4Å molecular sieves (0.1 g), TBAI (0.23 g, 0.6 mmol), 1-*O*-oleyl-2-*O*-palmitoyl-*sn*-glycerol (0.04 g, 0.07 mmol) and DIPEA (0.08 mg, 0.60 mmol) in  $\text{CH}_2\text{Cl}_2$  (3 mL) was added iodide **3.19** in  $\text{CH}_2\text{Cl}_2$  (2 mL) dropwise. After stirring at r.t. for 30 h, the solvent was removed *in vacuo* to give the *title compound* as a colourless liquid, and was used in next step without any purification.

3.1.1.15. 1-*O*-Oleoyl-2-*O*-palmitoyl-3-*O*- $\alpha$ -D-galactopyranosyl-*sn*-glycerol (3.3)

To the solution of the prepared diacyl **3.51** in MeOH (25 mL) Dowex 50WX2 200-400 mesh H<sup>+</sup> form ion exchange resin (1 g) was added. The reaction mixture was stirred at r.t. for 4 h. After filtering to remove the resin, the solvent was removed *in vacuo* and the resulting residue was purified by flash chromatography (CHCl<sub>3</sub>/MeOH 10:1) to give the *title compound* (0.03 g, 60 %) as a colourless film. The analytical data were in agreement with the values of the naturally isolated compound by Ben-Menachem and the values of the synthesised compound by Pozsgay.<sup>16,21</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.40-5.29 (m, 2 H, H8 and H9), 5.29-5.20 (m, 1 H, H2), 4.94 (d,  $J = 3.7$  Hz, 1 H, H1'), 4.37 (dd,  $J = 11.9, 4.1$  Hz, 1 H, H1a), 4.13 (dd,  $J = 11.9, 5.9$  Hz, 1 H, H1b), 4.09 (br d,  $J = 2.7$  Hz, 1 H, H4'), 3.93 (dd,  $J = 11.4, 5.2$ , 1 H, H6'a), 3.88-3.74 (m, 5 H, H2', H3', H5', H6'b and H1a), 3.63 (dd,  $J = 11.0, 6.2$  Hz, 1 H, H1b); 3.08, 2.97 and 2.47 (3 br s, 1 H, OH); 2.32 (td,  $J = 7.6, 3.0$  Hz, 4 H, H4 and H6), 2.01 (dd,  $J = 12.2, 6.4$  Hz, 4 H, H8 and H11), 1.60 (td,  $J = 14.3, 7.0$  Hz, 4 H, H5 and H7), 1.37-1.20 (m, 44 H, 22  $\times$  CH<sub>2</sub>), 0.88 (t,  $J = 6.9$  Hz, 6 H, 2  $\times$  CH<sub>3</sub>).

<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  173.83 and 173.48 (C=O), 130.13 and 129.80 (C9 and C10), 99.53 (C1'), 71.01 (C3'), 70.44 (C4'), 70.18 (C5'), 70.03 (C2), 69.51 (C2'), 66.56 (C3), 63.18 (C6'), 62.27 (C1), 34.42 (CH<sub>2</sub>), 34.23 (CH<sub>2</sub>), 32.05 (CH<sub>2</sub>), 32.03 (CH<sub>2</sub>), 29.89 (CH<sub>2</sub>), 29.87 (CH<sub>2</sub>), 29.85 (CH<sub>2</sub>), 29.82 (CH<sub>2</sub>), 29.79 (CH<sub>2</sub>), 29.68 (CH<sub>2</sub>), 29.65 (CH<sub>2</sub>), 29.62 (CH<sub>2</sub>), 29.49 (CH<sub>2</sub>), 29.45 (CH<sub>2</sub>), 29.43 (CH<sub>2</sub>), 29.40 (CH<sub>2</sub>), 29.36 (CH<sub>2</sub>), 29.31 (CH<sub>2</sub>), 29.28 (CH<sub>2</sub>), 27.35 and 27.32 (C8 and C11), 25.05 (CH<sub>2</sub>), 25.00 (CH<sub>2</sub>), 22.82 and 22.80 (CH<sub>2</sub>CH<sub>3</sub>), 14.21 (CH<sub>3</sub>).

**HRMS:** Calcd. for [C<sub>43</sub>H<sub>80</sub>O<sub>10</sub>+Na<sup>+</sup>]: 779.5644, found 779.5642.

**IR**  $\nu_{\max}$  3437 (br, m), 2922 (s), 2852 (m), 1735 (m), 1466 (w), 1379 (w), 1153 (m), 1078 (m), 908 (m), 734 (m), 650 (w).

**Specific Rotation**  $[\alpha]_{\text{D}}^{25} + 39.4^{\circ}$  ( $c$  1, CHCl<sub>3</sub>) (Lit.<sup>1</sup>  $[\alpha]_{\text{D}}^{22} + 46^{\circ}$  ( $c$  1.1, CHCl<sub>3</sub>))

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## ***Appendix: Publications***

The following publication resulted from the research conducted during the author's Doctor of Philosophy candidature:

Alternative cross-priming through CCL17-CCR4-mediated attraction of CTLs toward NKT cell-licensed DCs

Semmling, V.; Lukacs-Kornek, V.; Thaiss, C. A.; Quast, T.; Hochheiser, K.; Panzer, U.; Rossjohn, J.; Perlmutter, P.; Cao, J.; Godfrey, D. I.; Savage, P. B.; Knolle, P. A.; Kolanus, W.; Forster, I.; Kurts, C. *Nature Immunology*, **2010**, 313-320.

# Alternative cross-priming through CCL17-CCR4-mediated attraction of CTLs toward NKT cell-licensed DCs

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Cross-priming allows dendritic cells (DCs) to induce cytotoxic T cell (CTL) responses to extracellular antigens. DCs require cognate 'licensing' for cross-priming, classically by helper T cells. Here we demonstrate an alternative mechanism for cognate licensing by natural killer T (NKT) cells recognizing microbial or synthetic glycolipid antigens. Such licensing caused cross-priming CD8 $\alpha^+$  DCs to produce the chemokine CCL17, which attracted naive CTLs expressing the chemokine receptor CCR4. In contrast, DCs licensed by helper T cells recruited CTLs using CCR5 ligands. Thus, depending on the type of antigen they encounter, DCs can be licensed for cross-priming by NKT cells or helper T cells and use at least two independent chemokine pathways to attract naive CTLs. Because these chemokines acted synergistically, this can potentially be exploited to improve vaccinations.

Cross-presentation permits specialized dendritic cells (DCs) to present extracellular antigens to cytotoxic CD8<sup>+</sup> T cells (cytotoxic T lymphocytes (CTLs))<sup>1–3</sup>. Cross-presentation of autoantigens or innocuous circulating antigens usually results in cross-tolerance<sup>4,5</sup>. Immunogenic cross-presentation, also known as cross-priming, requires the presence of pathogen-associated molecular patterns, such as Toll-like receptor (TLR) ligands, and/or of specific CD4<sup>+</sup> helper T cells<sup>6,7</sup> that 'license' the cross-presenting DC for cross-priming<sup>1,8</sup>. The underlying molecular mechanisms that result from such licensing are under intense investigation and include enhanced costimulatory signals and diminished proapoptotic signals by the DCs<sup>9,10</sup>.

The necessity of DC licensing in cross-priming indicates that several rare cells of the immune response, that is, antigen-specific CTLs, specific helper T cells and cross-presenting DCs, must physically interact. Such interactions are usually governed by chemokines and their receptors<sup>11</sup>, which suggests that these molecules might be involved in cross-priming. In support of this idea, it has been shown that the interaction of helper T cells with TLR ligand-stimulated DCs causes the production of ligands for the chemokine receptor CCR5, which attract naive CTLs and increase the likelihood of their encounter with licensed DCs<sup>12,13</sup>. Activated CTLs themselves can then produce CCR5 ligands that attract further CTLs to DCs that have successfully

cross-primed<sup>14</sup>. Also, natural killer T cells (NKT cells) can activate DCs for cross-priming<sup>15–17</sup>, but the underlying mechanisms are unclear. As NKT cell-dependent cross-priming by DCs also requires the encounter of three immune cell types, a role for chemokines is likely but has not yet been studied.

NKT cells are thought to link innate and adaptive immunity of both the T helper type 1 (T<sub>H</sub>1) and T<sub>H</sub>2 type and have been associated with antimicrobial, autoimmune and antitumor immunity<sup>18–20</sup>. Most NKT cells express a semi-invariant T cell antigen receptor repertoire through which they recognize glycolipid antigens presented by the nonpolymorphic major histocompatibility complex (MHC) class I-like protein CD1d<sup>21</sup>. Bacterial ligands include  $\alpha$ -galactosyldiacylglycerol ( $\alpha$ -GalDAG) from *Borrelia burgdorferi* and  $\alpha$ -glycuronosylceramides such as GSL-1' from *Sphingomonas* species<sup>22–24</sup>. Isoglobotrihexosylceramide (iGb3) is an endogenous ligand that has been linked to NKT cell activation after infection with Gram-negative bacteria<sup>23</sup>. The glycosphingolipid  $\alpha$ -galactosylceramide ( $\alpha$ -GC) has been widely used as model NKT cell antigen<sup>15,18,19</sup>. OCH is a synthetic  $\alpha$ -GC derivative with a truncated sphingosine chain that induces T<sub>H</sub>2-like responses<sup>25</sup>. Mutagenesis studies indicate that CD1d binds  $\alpha$ -GC and other ligands, including iGb3 and  $\alpha$ -GalDAG, in a similar manner<sup>26,27</sup>. NKT cell activation causes rapid production of T<sub>H</sub>1, T<sub>H</sub>2 and T<sub>H</sub>17 cytokines and various chemokines and chemokine

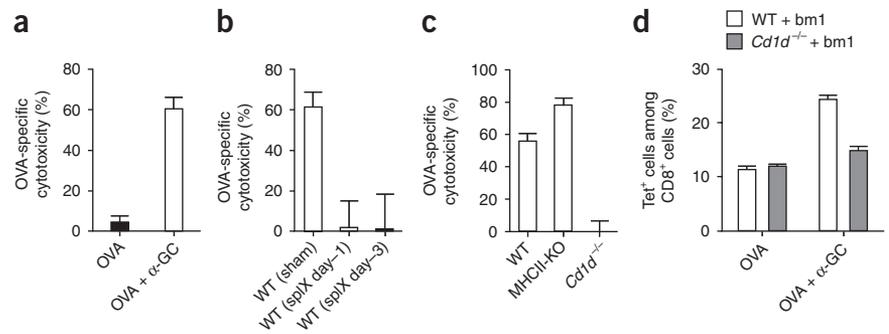
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**Figure 1** Cognate NKT cell licensing of splenic DCs for cross-priming. (a–c) *In vivo* OVA-specific cytotoxic response in the spleen (a,c) or the blood (b) 5 d after priming of wild-type (WT) mice with soluble OVA with or without  $\alpha$ -GC (a), 5 d after priming of wild-type mice with OVA plus  $\alpha$ -GC at 1 d or 3 d after sham operation or splenectomy (splX) (b) or after priming of MHC class II-deficient mice (MHCII-KO) or CD1d-deficient mice (*Cd1d*<sup>-/-</sup>) with OVA plus  $\alpha$ -GC (c). (d) Flow cytometry analysis of OT-I cells among splenic CD8<sup>+</sup> cells from bm1 mice reconstituted with 50% bm1 bone marrow and 50% CD1d-deficient or wild-type bone marrow 8 weeks before, analyzed 3 d after adoptive transfer of  $1 \times 10^6$  OT-I cells and priming with OVA plus  $\alpha$ -GC. Tet<sup>+</sup> OT-I cells identified by staining with specific tetramers. Data are representative of two individual experiments with four to five mice per group in each (mean and s.d.).



receptors, as well CD40L upregulation and cytotoxic activity<sup>19,28,29</sup>. In turn, DCs are activated by NKT cells and respond by upregulating MHC class II and costimulatory molecules and by secreting interleukin 12 (IL-12)<sup>19,20,30,31</sup>.

One chemokine receptor expressed by NKT cells is CCR4 (refs. 32–34). This receptor and its two ligands, CCL17 (also called thymus- and activation-regulated chemokine) and CCL22 (also called macrophage-derived chemokine) have been linked to memory CD4<sup>+</sup> helper T cell responses<sup>35,36</sup>, especially of the T<sub>H</sub>2 type<sup>37</sup>, and to allergic conditions of the airways and the skin<sup>38–42</sup>. CCL17 expression is highly restricted to DCs, which suggests a role in the unique ability of DCs to prime T cells. Little is known about CCR4 and its ligands in immune responses in the spleen or about their roles in CTL responses. Here we report an unexpected role for CCR4 and CCL17 in NKT cell-licensed cross-priming.

## RESULTS

### Cognate NKT cell licensing of splenic DCs

NKT cells can enhance CTL cross-priming by DCs<sup>17,30,31</sup>. We studied the underlying mechanism in a mouse model of cross-priming against the model antigen ovalbumin (OVA) in mice also injected with CD1d-restricted NKT cell ligand  $\alpha$ -GC. Consistent with published findings<sup>30</sup>,  $\alpha$ -GC augmented CTL activation in the spleen, as shown by greater cytotoxic activity (Fig. 1a), production of interferon- $\gamma$  (IFN- $\gamma$ ) and more OVA-specific CTLs (Supplementary Fig. 1). In mice lacking spleens,  $\alpha$ -GC did not enhance cytotoxicity (Fig. 1b),

which identified the spleen as the site where  $\alpha$ -GC enhanced CTL cross-priming. We found that  $\alpha$ -GC was also operative in MHC class II-deficient mice but not in CD1d-deficient mice (Fig. 1c), which confirmed the dependence on NKT cells and independence of helper T cells in this system.

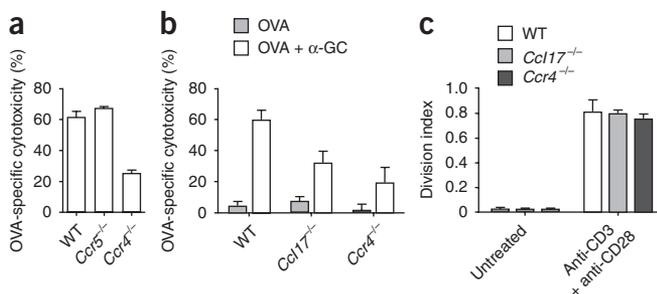
Classical cross-priming requires that the same DC that cross-primed CTLs receives stimulating signals from helper T cells, which has been called ‘cognate licensing’<sup>6,8</sup>. We determined whether this is also necessary for NKT cell licensing of DCs by using mixed-bone marrow chimeras that received bm1 and CD1d-deficient bone marrow at a ratio of 1:1. DCs from bm1 mice interacted via CD1d with NKT cells but not with OVA-specific CTLs, whereas DCs from CD1d-deficient mice interacted with those CTLs but not with NKT cells (Supplementary Fig. 2). In such mixed-bone marrow chimeras,  $\alpha$ -GC did not enhance the cross-priming of adoptively transferred OVA-specific CTLs from OT-I transgenic mice, in contrast to results obtained with control chimeras that received bm1 plus wild-type bone marrow, which had DCs that were able to interact with both NKT cells and CTLs (Fig. 1d). Thus, NKT cell licensing requires that the same DC physically interacts with NKT cells and CTLs.

### NKT cell-licensed cross-priming requires CCL17 and CCR4

Helper T cells enhance cross-priming by inducing CCR5 ligands that attract naive CTLs toward cross-presenting DCs<sup>12</sup>. To determine whether CCR5 was also important in NKT cell-licensed cross-priming, we challenged CCR5-deficient mice with OVA plus  $\alpha$ -GC. We found that  $\alpha$ -GC-induced enhancement of cross-priming was unchanged in these mice, whereas it was 65% lower in CCR4-deficient mice (Fig. 2a,b) and was 50% lower in mice deficient in CCL17 (Fig. 2b), a DC-restricted CCR4 ligand<sup>40</sup>. As CCL17-deficient mice, we used homozygous CCL17-eGFP knock-in mice in which both *Ccl17* genes had been replaced with a gene encoding enhanced green fluorescent protein (eGFP), leaving the *Ccl17* promoter in place<sup>40</sup>. We also found such dependency on CCR4 and CCL17 when we counted OVA-specific CTLs and IFN- $\gamma$ -producing cells as alternative ‘readouts’ (Supplementary Fig. 3). This result was not due to intrinsic CTL defects in CCR4 and CCL17-deficient mice, because naive polyclonal CTLs from CCR4- and CCL17-deficient mice responded like wild-type cells to stimulation *in vitro* with antibody to CD3 (anti-CD3) and anti-CD28 (Fig. 2c). These data showed that NKT cell-licensed cross-priming did not depend on CCR5 but instead depended on CCR4. Of the two CCR4 ligands, CCL17 mediated most of the CCR4-dependent enhancement.

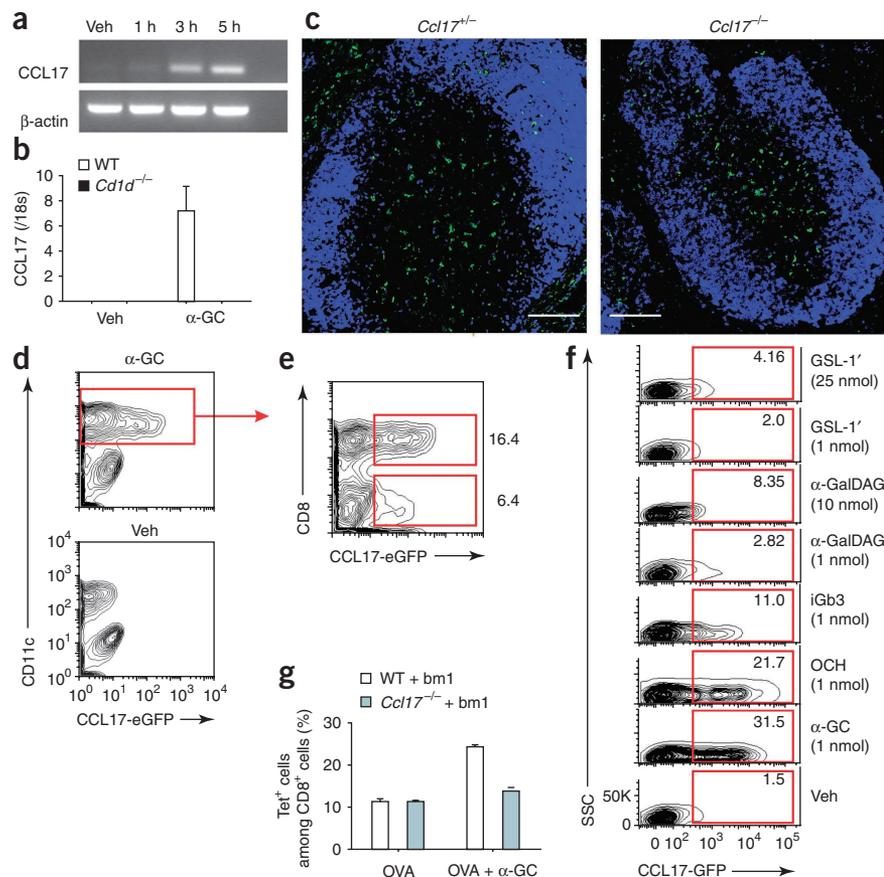
### NKT cells induce CCL17 in splenic DCs

The role of CCL17 in  $\alpha$ -GC-enhanced cross-priming in the spleen was unexpected, because splenic DCs have been shown to be unable



**Figure 2** NKT cell-licensed cross-priming requires CCL17 and CCR4. (a,b) OVA-specific cytotoxicity 5 d after priming of wild-type mice, CCR5-deficient mice (*Ccr5*<sup>-/-</sup>) or CCR4-deficient mice (*Ccr4*<sup>-/-</sup>) with soluble OVA plus  $\alpha$ -GC (a) or wild-type mice, CCL17-deficient mice (*Ccl17*<sup>-/-</sup>) or CCR4-deficient mice with soluble OVA with or without  $\alpha$ -GC (b). (c) Division indices of endogenous CTLs, labeled with the cytosolic dye CFSE, obtained from wild-type, CCL17-deficient or CCR4-deficient mice and left untreated or stimulated with anti-CD3 plus anti-CD28. Data are representative of two individual experiments with four mice (a,b) or four individual samples (c) per group in each (mean and s.d.).

**Figure 3** NKT cells induce CCL17 in splenic DCs. (a) RT-PCR analysis of CCL17 RNA in splenic CD11c<sup>+</sup> cells from mice at 1, 3 or 5 h after injection of  $\alpha$ -GC or vehicle (Veh).  $\beta$ -actin, loading control. (b) CCL17 mRNA expression in wild-type or CD1d-deficient mice 5 h after injection of  $\alpha$ -GC or vehicle alone, presented relative to 18S RNA. (c) Immunofluorescence staining of B cell zones (blue; B220) in heterozygous CCL17-eGFP reporter mice (*Ccl17*<sup>+/-</sup>) and CCL17-deficient mice injected with  $\alpha$ -GC, presented with the CCL17-eGFP signal (green). Scale bars, 200  $\mu$ m. (d,e) Flow cytometry of spleen cells from CCL17-eGFP reporter mice 5 h after injection of vehicle or  $\alpha$ -GC, with gating of CD11c<sup>+</sup>CCL17<sup>+</sup> cells (red box, d) followed by analysis of the expression of CD8 and CCL17 (e). Numbers adjacent to outlined areas (e) indicate percent CD8<sup>+</sup>CCL17<sup>+</sup> cells (top) or CCL17<sup>+</sup> cells (bottom). (f) Flow cytometry analysis of CCL17-eGFP in CD11c<sup>+</sup> splenocytes 20 h after injection of NKT cell ligands or vehicle (right margin). Numbers adjacent to outlined areas indicate percent CCL17<sup>+</sup> cells. SSC, side scatter. (g) Flow cytometry analysis of the proportion of OT-I cells in splenic CD8<sup>+</sup> cells of bm1 mice reconstituted with 50% bm1 bone marrow and 50% CCL17-deficient or wild-type bone marrow 8 weeks before, analyzed on day 3 after adoptive transfer of  $1 \times 10^6$  OT-I cells and priming with OVA plus  $\alpha$ -GC. Data are representative of two experiments (mean and s.d. of three to four mice per group in each).



to express this chemokine, even after systemic TLR stimulation<sup>40</sup>. To determine whether  $\alpha$ -GC can induce CCL17 expression, we used RT-PCR to analyze splenic CD11c<sup>+</sup> DCs isolated from mice injected with this agent. Indeed, CCL17 mRNA was detectable after 3 h and continued to increase until 5 h after injection (Fig. 3a,b). We did not obtain this result with CD1d-deficient mice (Fig. 3b), which confirmed that NKT cells were required for CCL17 upregulation.

To identify the cell that expressed CCL17, we used heterozygous CCL17-eGFP knock-in mice in which eGFP fluorescence represents a reporter of CCL17 protein expression (called 'CCL17-eGFP reporter mice' here)<sup>40</sup>. Histological analysis of splenic tissue located many CCL17<sup>+</sup> cells in the T cell–DC areas and a sparse presence of these cells in B cell zones and red pulp (Fig. 3c). The lack of CCL17 in homozygous CCL17-eGFP knock-in mice did not alter this pattern (Fig. 3c). Flow cytometry showed that only CD11c<sup>+</sup> cells expressed CCL17-eGFP (Fig. 3d) and that these cells also expressed MHC class II and CD80 (data not shown), which identified them as DCs. CCL17 was produced mainly by CD8 $\alpha$ <sup>+</sup> DCs (Fig. 3e), which are able to cross-present<sup>3</sup>. Notably, other NKT cell ligands (including more physiological ligands) induced CCL17-eGFP expression in splenic DCs in the following order of potency:  $\alpha$ -GC > OCH > iGb3 >  $\alpha$ -GalDAG > GSL-1' (Fig. 3f).

To determine whether the DCs that cross-primed CTLs needed to produce CCL17, we created mixed-bone marrow chimeras that received bm1 and CCL17-deficient bone marrow at a ratio of 1:1 (Supplementary Fig. 4). NKT cells did not enhance cross-priming of OT-I cells adoptively transferred into these mixed-bone marrow chimeras, whereas they did so in control chimeras that had received bm1 plus wild-type bone marrow and thus contained DCs that could both produce CCL17 and interact with CTLs (Fig. 3g). This indicated that CCL17 was effective

only when produced by the same DC that activated CTLs. Given that CD1d expression by this DC was also required (Fig. 1d), it follows that only CD1d-expressing DCs produce CCL17, which confirms that NKT cells induce CCL17 only in those DCs they physically interact with.

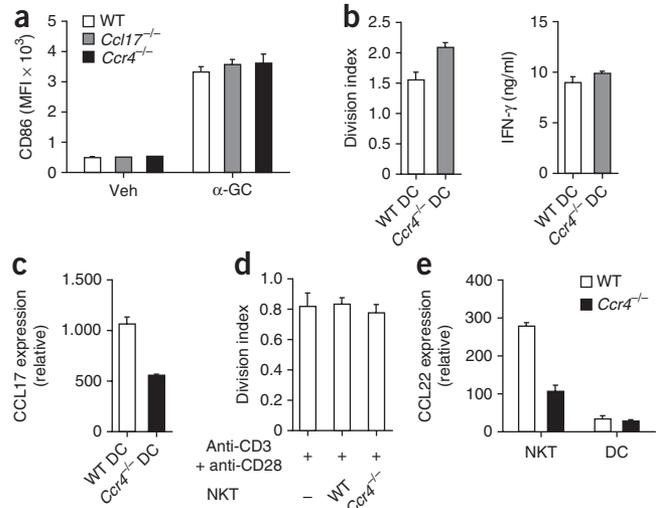
### CCL17 effects on DCs and NKT cells are not responsible

We next sought to identify the cell types that responded to CCL17 in cross-priming. The CCL17 receptor CCR4 is expressed by activated CD4<sup>+</sup> T cells, Langerhans cells, NK cells and NKT cells<sup>32–34,37,43</sup>. We first focused on DCs, because the boosting effect of  $\alpha$ -GC is usually ascribed to DC activation after contact with NKT cells<sup>17,30</sup>. We hypothesized that autocrine stimulation of DCs via CCL17 might upregulate costimulatory molecules, resulting in improved CTL activation (the possibility of paracrine stimulation was excluded by the experiment in Fig. 3g). Injection of  $\alpha$ -GC indeed upregulated the expression of CD80, CD86, CD40 and CD70 by DCs, but this was dependent on neither CCR4 nor CCL17 (Fig. 4a and Supplementary Fig. 5), which indicated that CCL17 did not augment DC activation. Nevertheless, such a CCR4-independent effect of NKT cells on DC activation may explain the CCR4-independent, quantitatively smaller component of NKT cell-enhanced cross-priming (Fig. 2a,b). Although  $\alpha$ -GC induced CCR4-dependent production of IL-12 *in vivo* (Supplementary Fig. 6), this cytokine was irrelevant in our system, because the  $\alpha$ -GC-enhanced CTL response to OVA was similar in IL-12p35-deficient and wild-type mice (Supplementary Fig. 7), which confirmed published studies of mice lacking both IL-12 and IL-23 (ref. 17). To directly investigate whether CCL17 increased the stimulatory capacity of DCs, we loaded splenic DCs from  $\alpha$ -GC-injected mice with the OVA peptide SIINFEKL and cultured them together with OT-I cells. Both proliferation and IFN- $\gamma$  production were similar

**Figure 4** CCL17 enhances cross-priming neither by activating DCs nor by recruiting NKT cells. **(a)** Flow cytometry analysis of CD86 expression on splenic DCs from wild-type, CCL17-deficient and CCR4-deficient mice injected 14 h before with  $\alpha$ -GC or vehicle. MFI, mean fluorescent intensity. **(b)** Division index (left) and IFN- $\gamma$  concentration in supernatants (right) of  $1.5 \times 10^5$  OT-I cells cultured for 2 d together with  $0.5 \times 10^5$  wild-type or CCR4-deficient splenic DCs coated with OVA peptide (SIINFEKL). **(c)** CCL17 mRNA expression in wild-type and CCR4-deficient DCs 5 h after injection of  $\alpha$ -GC, presented relative to 18S RNA expression. **(d)** Division indices of splenic CD8 $^+$  T cells obtained from  $\alpha$ -GC-injected wild-type mice, then stimulated with anti-CD3 plus anti-CD28 and cultured in the presence or absence of splenic NKT cells from mice injected with  $\alpha$ -GC 5 h before. **(e)** CCL22 mRNA expression in wild-type and CCR4-deficient NKT cells (left) and DCs (right) 5 h after injection of  $\alpha$ -GC, presented relative to 18S RNA expression. Data are representative of two experiments (mean and s.d. of three to four mice per group in each).

when we used wild-type and CCR4-deficient DCs (**Fig. 4b**). Moreover, neither the absolute number of splenic DCs nor the ratio of cross-presenting CD8 $\alpha^+$  DCs to non-cross-presenting CD8 $^-$  DCs differed in wild-type, CCL17- and CCR4-deficient mice (**Supplementary Fig. 8**), which demonstrated that CCL17 did not render cross-priming DCs more numerous or immunostimulatory.

Notably, message for CCL17 itself was lower in DCs from  $\alpha$ -GC-injected CCR4-deficient mice (**Fig. 4c**), which indicated that CCL17 positively regulated its own production by CCR4-expressing DCs either in an autocrine way or indirectly by recruiting NKT cells. Hence, we next focused our attention on NKT cells, hypothesizing that CCL17 in our system might attract additional NKT cells to DCs to stimulate CTLs. This did not occur indirectly by further activation of DCs, as this possibility was excluded by the experiments described above (**Fig. 4a,b** and **Supplementary Figs. 5** and **8**). To test whether hypothetically recruited NKT cells directly stimulated CTLs, we activated CTLs with anti-CD3 and anti-CD28 and cultured them in the presence or absence of NKT cells isolated from  $\alpha$ -GC-injected wild-type or CCR4-deficient mice. Neither CTL proliferation (**Fig. 4d**) nor IFN- $\gamma$ -production (data not shown) was greater in the presence of NKT cells. Thus, even if CCL17 recruited additional NKT cells in our system, these cells were unlikely to further stimulate CTLs. Message for CCR5 ligands was not more abundant in sorted NKT cells from  $\alpha$ -GC-injected mice (data not shown), which confirmed that NKT cells did not recruit CTLs similar to helper T cells in classical cross-priming<sup>12</sup> (**Fig. 2a**). Likewise, CCL17 message was not more abundant, which confirmed our finding that among splenocytes, only DCs expressed CCL17 (**Fig. 3d,e**). For completeness, we also examined

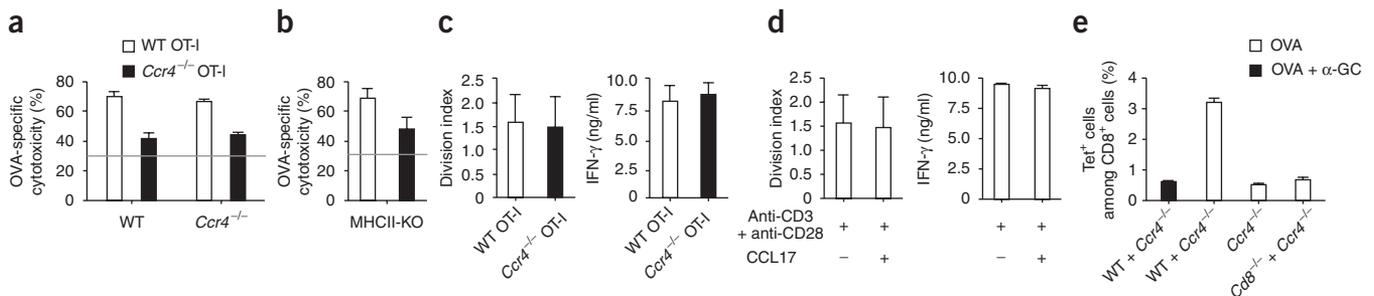


the other CCR4 ligand, CCL22. Although the abundance of CCL22 message in DCs was very low, sorted NKT cells from  $\alpha$ -GC-injected mice unexpectedly expressed CCL22 message in a CCR4-dependent way (**Fig. 4e**).

Together these experiments challenge the idea of CCL17 action on DCs and NKT cells. The only relevant mediators produced by these cells in a CCR4-dependent way were the CCR4 ligands themselves. This raises the possibility that these ligands targeted the CTLs directly, despite the lack of published evidence of a function for CCR4 in CTL responses.

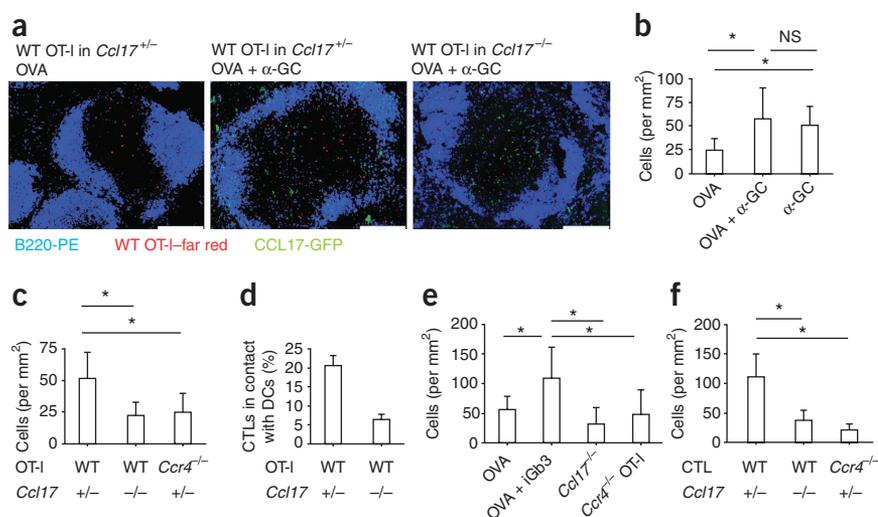
#### DC-derived CCL17 acts directly on CTLs

To investigate whether CCR4 ligands acted directly on CTLs in our system, we crossed OT-I mice with CCR4-deficient mice. We injected CCR4-sufficient or CCR4-deficient OT-I cells into recipient mice, followed by OVA plus  $\alpha$ -GC. CCR4-deficient OT-I cells added little to the background cytotoxicity on day 4, which resulted from endogenous polyclonal CTLs, whereas CCR4-sufficient OT-I cells caused substantially more cytotoxicity (**Fig. 5a**), which demonstrated that CCR4 expression by CTLs was necessary for CCL17-mediated enhanced cytotoxicity. Transfer of CCR4-sufficient OT-I cells into CCR4-deficient mice restored cytotoxicity to amounts observed in wild-type mice (**Fig. 5a**), which demonstrated that CCR4 expression by CTLs was sufficient for enhancing cytotoxicity. CCR4-expressing OT-I cells



**Figure 5** Splenic DC-derived CCL17 acts directly on CTLs. **(a,b)** *In vivo* cytotoxicity on day 4 in wild-type mice, CCR4-deficient mice **(a)** and MHC class II-deficient mice **(b)** given  $5 \times 10^3$  CCR4-sufficient or CCR4-deficient OT-I cells and primed with OVA plus  $\alpha$ -GC 1 d later. Horizontal lines, average background cytotoxicity in wild-type mice on day 4 due to endogenous CTLs. **(c,d)** Division index (left) and IFN- $\gamma$  content in supernatants (right) of  $2 \times 10^5$  OT-I cells stimulated for 2 d with anti-CD3 plus anti-CD28 **(c)** or stimulated with anti-CD3 plus anti-CD28 with or without recombinant CCL17 (600 ng/ml; **d**). **(e)** Flow cytometry analysis of endogenous OVA-specific CTLs among splenic CD8 $^+$  cells of wild-type mice reconstituted with 50% CCR4-deficient bone marrow and 50% wild-type, CCR4-deficient or CD8-deficient bone marrow, assessed 2 d after priming with OVA plus  $\alpha$ -GC or OVA alone. Data are representative of two experiments (mean and s.d. of four to five mice per group in each).

**Figure 6** DC-derived CCL17 recruits CTLs into the splenic T cell–DC zone. **(a)** Immunofluorescence staining of spleen cryosections from CCL17-eGFP reporter mice or CCL17-deficient mice injected with  $2.5 \times 10^6$  far red fluorochrome-labeled CCR4-sufficient OT-I cells on day –1 and then injected with OVA with or without  $\alpha$ -GC on day 0. Blue staining indicates B220<sup>+</sup> cells (defines B cell zones). Scale bars, 200  $\mu$ m. **(b,c)** Absolute number of CCR4-sufficient or CCR4-deficient OT-I cells in CCL17-deficient or CCL17-eGFP reporter mice 10 h after injection with  $\alpha$ -GC, presented as cells per mm<sup>2</sup> of the T cell–DC zone enclosed by the (blue) B cell zone. **(d)** Proportion of OT-I cells in **c** positioned adjacent to GFP<sup>+</sup> DCs. **(e)** Absolute number of CCR4-sufficient or CCR4-deficient OT-I cells in CCL17-deficient or CCL17-sufficient mice 10 h after injection of iGb3, presented as described in **b,c**. **(f)** Absolute number of far red fluorochrome-labeled polyclonal wild-type or CCR4-deficient CTLs transferred into  $\alpha$ -GC-injected CCL17-deficient mice or CCL17-eGFP reporter mice as described in **a–c**. NS, not significant; \* $P < 0.0005$  (Kruskal–Wallis and Dunn's post-test). Data are representative of two individual experiments with at least 25 T cell–DC zones per group derived from three to four nonconsecutive sections from three mice each (mean and s.d.).



were also effective when transferred into MHC class II-deficient mice injected with OVA plus  $\alpha$ -GC (Fig. 5b), which confirmed that the CCR4 effect was helper T cell independent. We ruled out the possibility of intrinsic deficiencies in CCR4-deficient OT-I cells, because their proliferative response to stimulation with anti-CD3 and anti-CD28 was equal to that of CCR4-sufficient OT-I cells (Fig. 5c). Without injection of OVA plus  $\alpha$ -GC, CCR4-sufficient and CCR4-deficient OT-I cells were present in similar numbers (Supplementary Fig. 9), which ruled out the possibility that these cells entered the T cell pool differently after intravenous injection. CCL17 did not act like a growth cytokine, because adding recombinant CCL17 did not enhance OT-I cell activation *in vitro* (Fig. 5d).

To confirm the findings reported above in an endogenous CTL repertoire, we created mixed-bone marrow chimeras that received CD8-deficient and CCR4-deficient bone marrow at a ratio of 1:1. The CD8-deficient bone marrow can generate CCR4<sup>+</sup> cells, including NKT cells and DCs, whereas all CD8<sup>+</sup> cells are derived from CCR4-deficient bone marrow (Supplementary Fig. 10). In these chimeras, the addition of  $\alpha$ -GC failed to result in more CD8<sup>+</sup> OVA-specific CTLs, in contrast to results obtained with control chimeras generated with wild-type plus CCR4-deficient bone marrow (Fig. 5e), which could generate CCR4<sup>+</sup> CD8<sup>+</sup> cells. Instead, CTL numbers equaled those in control chimeras that had received only CCR4-deficient bone marrow (Fig. 5e). Thus, CCR4 had to be expressed by endogenous CTLs, which confirmed that CCL17 enhanced cross-priming by acting directly on CTLs.

### DC-derived CCL17 recruits CTLs

As CCL17 did not act like a growth cytokine (Fig. 5d), we hypothesized that it might affect CTL migration. To test our hypothesis, we labeled OT-I cells with a far red fluorochrome and injected the cells into heterozygous or homozygous CCL17-eGFP knock-in mice, which we subsequently challenged with OVA plus  $\alpha$ -GC. After 10 h, we counted the labeled OT-I cells by immunofluorescence microscopy (Fig. 6a and Supplementary Fig. 11) in the splenic T cell–DC zone, where CCL17 was expressed (Fig. 3c). We distinguished this zone from the B cell zone by costaining with anti-B220 and by the GFP fluorescence of NKT cell-licensed DCs (Fig. 3c). More OT-I cells were present in the T cell–DC zone after injection of OVA plus

$\alpha$ -GC than after injection of OVA alone (Fig. 6a,b). Injection of  $\alpha$ -GC alone was sufficient to produce this result (Fig. 6b), which demonstrated that OT-I cell activation was not necessary. In other words, naive CTLs were recruited. This depended on CCL17–CCR4, because fewer OT-I cells were present in the T cell–DC zone when DCs could not produce CCL17 (in experiments with homozygous CCL17-eGFP knock-in mice), when DCs were not licensed for CCL17 production (in experiments with no  $\alpha$ -GC injection) or when CTLs could not respond to CCL17 (in experiments with CCR4-deficient OT-I cells; Fig. 6a–c and Supplementary Fig. 11a). In particular, more CTLs were located adjacent to CCL17-expressing DCs than to CCL17-deficient DCs (Fig. 6d), which demonstrated that CTL recruitment targeted individual DCs.

We also sought to test one of the other NKT cell ligands that induced splenic CCL17 expression (Fig. 3f). We chose iGb3, because OCH is too close to  $\alpha$ -GC and because the responses induced by  $\alpha$ -GalDAG and GSL-1' were too small. Furthermore, iGb3 is an example of an endogenous ligand that NKT cells are likely to encounter. We found that 1 nmol iGb3 and 1 nmol  $\alpha$ -GC enhanced the cross-priming of endogenous CTLs equally well (Supplementary Fig. 12). Indeed, iGb3 also resulted in more recruitment of OT-I cells into the T cell–DC zone in a strictly CCR4-dependent way (Fig. 6e), which demonstrated that this NKT cell ligand engaged the same CTL-recruitment mechanism.

The ability of CCL17 to attract naive OT-I cells (Fig. 6b) indicated that CTL specificity was irrelevant for recruitment. If so, then our *in vivo* recruitment assay should yield similar results with polyclonal CTLs. We tested this by transferring far red fluorochrome-labeled endogenous CTLs from wild-type and CCR4-deficient mice into CCL17 reporter mice or CCL17-deficient mice. Polyclonal CTLs were recruited more effectively into the T cell–DC zone when DCs produced CCL17, DCs were licensed by NKT cells and CTLs could respond to CCL17 (Fig. 6f and Supplementary Fig. 11b). These findings demonstrated that NKT cell-licensed DCs produced CCL17 to recruit naive CCR4<sup>+</sup> CTLs to the splenic T cell–DC zone.

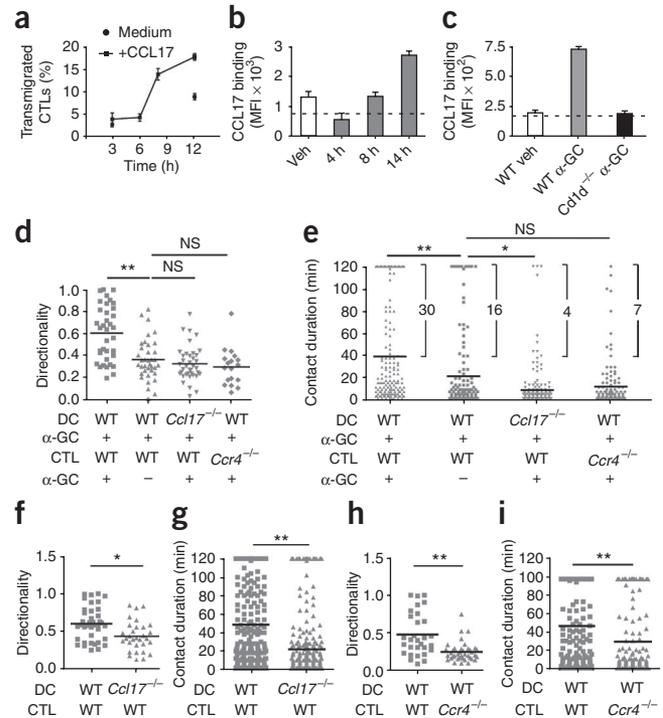
### Directional CTL migration and more contact time

As CCL17-mediated recruitment of CCR4<sup>+</sup> CTLs has not been reported before, to our knowledge, we used an *in vitro* Transwell

**Figure 7** CCL17 improves the directional migration of CTLs toward CCL17-producing DCs and increases their contact time. **(a)** Transwell assay of the migration of polyclonal CTLs toward CCL17 (800 ng/ml); cells were from mice injected with  $\alpha$ -GC 3, 6, 8 or 12 h before analysis. **(b,c)** Flow cytometry of the binding of fluorescein isothiocyanate-labeled CCL17 to CTLs from spleens of vehicle- or  $\alpha$ -GC-injected wild-type mice after 4, 8 and 14 h **(b)** or CD11d-deficient mice after 14 h **(c)**, presented as mean fluorescence intensity. Dashed lines, background fluorescence of control-stained CTLs. **(d,e)** *In vitro* migration of CTLs with or without CCR4 expression toward DCs with or without CCL17 production, recorded by time-lapse videomicroscopy over 2–3 h and presented as CTL directionality before physical contact with DCs **(d)** or subsequent duration of CTL-DC contact **(e)**. Below graphs:  $\alpha$ -GC indicates DCs or CTLs from donor mice injected with  $\alpha$ -GC 14 h before (+) or not (-). Numbers adjacent to vertical brackets **(e)** indicate percent contacts lasting longer than 40 min. **(f,g)** CTL directionality **(f)** and contact duration **(g)** of mixed populations of DCs with or without CCL17 production, recorded by time-lapse videomicroscopy over 2–3 h. **(h,i)** CTL directionality **(h)** and contact duration **(i)** of mixed populations of CTLs with or without CCR4 expression, recorded by time-lapse videomicroscopy over 2–3 h. In **d–i**, each symbol represents an individual cell ( $n = 30–40$  cells (directionality) or  $n = 100–300$  cells (contact duration)); small horizontal lines indicate the mean. \* $P < 0.005$  and \*\* $P < 0.0005$  (Kruskal-Wallis and Dunn's post-test **(d,e)** or Mann-Whitney **(f–i)**). Data are representative of three experiments with three to four mice per group each (mean and s.d.).

assay to demonstrate this directly. Although polyclonal CTLs from untreated donor mice failed to migrate toward recombinant CCL17 (data not shown), some CTLs from donor mice injected with  $\alpha$ -GC 8–12 h earlier did migrate (Fig. 7a). This indicated that NKT cells not only acted by inducing CCL17 production in splenic DCs but also made naive CTLs responsive to this chemokine. In confirmation of that interpretation, CTLs from  $\alpha$ -GC-injected wild-type mice bound fluorescence-labeled recombinant CCL17 with kinetics similar to those in Transwell migration (Fig. 7a,b). We did not observe such binding in CD11d-deficient mice (Fig. 7c), which indicated that NKT cells induced CCR4 expression on CTLs. Notably, we observed such expression on only 15–20% of the CTLs (Supplementary Fig. 13), which matched the proportion of CTLs that migrated in the Transwell assays (Fig. 7a).

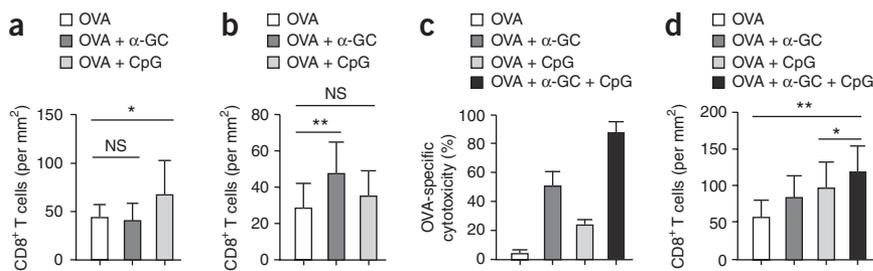
We next did live-cell imaging of splenic DCs from  $\alpha$ -GC-injected wild-type mice cultured together with polyclonal splenic CTLs from  $\alpha$ -GC-injected or uninjected donor mice. CTLs from  $\alpha$ -GC-injected wild-type mice showed more directionality toward DCs (Fig. 7d,e and Supplementary Fig. 14) and longer contact time with DCs (Fig. 7e and Supplementary Movies 1 and 2) compared with CTLs from



uninjected donor mice, unless we used CCL17-deficient DCs or CCR4-deficient CTLs (Fig. 7d,e and Supplementary Movies 3 and 4). We confirmed those results when we examined CTL migration in three-part mixed cultures with CCL17-producing or CCL17-deficient DCs (Fig. 7f,g) and in three-part cultures of CCL17-competent DCs with CCR4-deficient or CCR4-sufficient CTLs (Fig. 7h,i and Supplementary Movie 5). These findings directly demonstrated that DC-derived CCL17 attracted CTLs that had been conditioned by NKT cells to express CCR4.

**Synergy of helper T cell- and NKT cell-licensed cross-priming** CCL17 and CCR4 seem to regulate  $\alpha$ -GC-enhanced cross-priming, in contrast to the CCR5-mediated regulation of classical cross-priming in the presence of TLR ligands<sup>12</sup>. To confirm that different chemokines were involved, we transferred CCR4- or CCR5-deficient CTLs into OVA-primed mice also injected with either  $\alpha$ -GC or the TLR9 ligand CpG. Only CpG enhanced the recruitment of CCR4-deficient CTLs (Fig. 8a), whereas  $\alpha$ -GC enhanced only the recruitment of CCR5-deficient CTLs (Fig. 8b).

If helper T cells and NKT cells use distinct chemokine mechanisms to regulate cross-priming, then activating both cell types should be synergistic. Indeed, the use of maximally effective doses of CpG and  $\alpha$ -GC enhanced cytotoxicity beyond amounts achieved by these agents alone (Fig. 8c). CpG and  $\alpha$ -GC together also enhanced the recruitment of adoptively transferred far red fluorochrome-labeled endogenous CTLs into the splenic T cell-DC zone (Fig. 8d). Mice primed subcutaneously with OVA together with both  $\alpha$ -GC and CpG had many more specific CTLs in the draining lymph node than did mice primed with OVA plus  $\alpha$ -GC



**Figure 8** Helper T cell- and NKT cell-licensed cross-priming are synergistically regulated by distinct chemokines. **(a,b)** Absolute number of adoptively transferred CCR4-deficient T cells **(a)** or CCR5-deficient T cells **(b)** in OVA-primed wild-type mice injected with  $\alpha$ -GC or CpG, assessed by histology as described in Figure 6 (presented as cells per mm<sup>2</sup> of the T cell-DC zone). **(c)** *In vivo* cytotoxicity on day 5 in OVA-primed wild-type mice injected with  $\alpha$ -GC, CpG or both agents. **(d)** Absolute number of adoptively transferred polyclonal T cells, assessed as described in **a,b**. \* $P < 0.05$  and \*\* $P < 0.0005$  (Kruskal-Wallis and Dunn's selected columns).

or CpG alone (**Supplementary Fig. 15**). These findings verified that CCR4 and CCR5 mediated distinct and synergistic CTL-recruitment mechanisms that were operative in cross-priming licensed by NKT cells and in cross-priming licensed by helper T cells and enhanced by TLR ligands, respectively (**Supplementary Fig. 16**).

## DISCUSSION

Cross-priming requires physical contact among at least three rare cells of the immune response: antigen-specific CTLs, cross-presenting DCs and antigen-specific helper T cells that license the DCs for cross-priming. Alternatively, NKT cells can license DCs, but little is known about the underlying mechanisms, except that DCs mature and upregulate costimulatory molecules<sup>15,17,31</sup>. Here we have reported an additional, quantitatively stronger effect that NKT cells exert on DCs, mediated by the induction of CCL17 that acts on CTLs expressing CCR4.

CCL17 is an inflammatory chemokine with a highly organ- and DC-restricted expression profile<sup>40,44</sup> and has been linked to allergic diseases of the skin and lung<sup>39–41</sup>. Notably, splenic DCs normally completely lack CCL17 expression, even after systemic injection of various TLR ligands<sup>40,44</sup>. However, we found here that NKT cells stimulated cross-priming CD8 $\alpha^+$  splenic DCs to secrete large amounts of CCL17. Moreover, interaction with DCs induced NKT cells to upregulate mRNA for CCL22, a second CCR4 ligand that is known mainly as a macrophage-derived chemokine. Its role in our system, however, was small.

CCR4 is expressed by activated T<sub>H</sub>2 cells<sup>37</sup>, especially in the lung and skin<sup>45,46</sup>, and has been linked to allergic diseases of these organs<sup>38,39,42</sup>. However, the association with T<sub>H</sub>2 responses is not strict, and it has been proposed that CCR4 is expressed by all memory CD4<sup>+</sup> helper T cell types, except those homing to the intestine<sup>35,36,38</sup>. CCR4 is expressed also by antigen-presenting cells and NKT cells<sup>32–34,37,43</sup>. In our model, we found no evidence that CCL17 improved cross-priming by stimulating CCR4-expressing DCs or NKT cells. Even if this were the case, these cells would have to stimulate CTLs by another signal, for example, by more costimulatory molecules on DCs. However, expression of such molecules was independent of both CCR4 and CCL17. We found only two DC-derived signals that resulted from interaction with NKT cells that depended on CCL17 and its receptor: IL-12 and CCL17. Using mice deficient in IL-12p35, we ruled out the possibility that NKT cell-licensed DCs used IL-12 to stimulate CTLs, consistent with a published study of mice deficient in both IL-12 and IL-23 (ref. 17). Initially, involvement of CCL17 seemed unlikely because neither this chemokine nor its receptor, CCR4, has been linked to CTL responses. A subset of circulating CCR4-expressing human memory CTLs has been characterized that migrate toward CCR4 ligands *in vitro*, but the functional relevance of this is still unclear<sup>47</sup>. Nevertheless, CCL17 attracted naive CCR4<sup>+</sup> CTLs both *in vitro* and *in vivo*. It guided naive CCR4<sup>+</sup> CTLs into the splenic T cell–DC zone, in which the cross-priming CCL17<sup>+</sup> DCs resided, thereby locally increasing the number of CTL precursors available for activation. This was true not only for OT-I cells but also for endogenous polyclonal CTLs.

Moreover, CCL17 guided CTLs into the splenic T cell–DC zone precisely toward those DCs that had been licensed by NKT cells. Histological analysis showed that there were more CTLs adjacent to CCL17-producing DCs. Moreover, this conclusion can be extrapolated from our experiments with mixed–bone marrow chimera, which showed that the same DC that cross-primed CTLs also interacted with NKT cells in a CD1d-restricted manner and produced CCL17. In other words, CCL17 produced by neighboring DCs was ineffective,

which indicates that CCL17 operated not only on a tissue-specific level but also on a DC-specific level. Selective recruitment toward relevant DCs probably accelerates CTL priming better than mere attraction into a larger area such as the T cell–DC zone, because CTLs will not lose time examining unlicensed DCs. Furthermore, CCL17 increased the DC–CTL contact time, which may be important for cross-priming, as sustained T cell antigen receptor signaling is necessary for immunogenicity<sup>48,49</sup>.

The dependency of CCL17 production on its receptor CCR4 indicated that this chemokine was regulated by a positive feedback loop. Although this loop did not increase the stimulatory activity of DCs for CTLs *per se*, it may nevertheless be relevant in our system, because CCL17-expressing DCs might attract further CCR4-expressing NKT cells to upregulate CCL17 to maximal amounts, thereby permitting faster attraction of CCR4<sup>+</sup> CTLs.

Our findings have identified CCR4–CCL17 as the second chemokine–chemokine receptor pair that regulates cross-priming. It differs from CCR5 and its three ligands, which facilitate helper T cell-licensed classical cross-priming<sup>12,14</sup>. Our data have therefore demonstrated that at least two independent mechanisms exist that recruit naive CTLs for cross-priming and that these discriminate between helper T cell- and NKT cell-licensed DCs. This might permit an additional level of selectivity in CTL attraction for cross-priming. For example, if during an infection DCs presented a microbial antigen such as GSL-1' or  $\alpha$ -GalDAG<sup>22–24</sup> or a self antigen such as iGb3 (ref. 23), then NKT cells would induce CCL17 expression and thereby render the DCs especially attractive for naive CTLs, enhancing the likelihood that these DCs would be scanned earlier than unlicensed DCs or DCs licensed by helper T cells. In contrast, CTLs may locate DCs licensed by helper T cells and TLR ligands faster by following CCR5 ligands. When we engaged both pathways simultaneously, the recruitment of naive CTLs was even faster and their resulting cytotoxic response was higher. This synergism may serve to 'preferentially' scan DCs that have successfully presented antigenic epitopes to both helper T cells and NKT cells, as such DCs evidently contain relevant foreign antigen and moreover will provide optimal costimulation because they have been licensed by two lymphocyte types.

In our studies, only CTLs from  $\alpha$ -GC-injected mice expressed CCR4, reminiscent of published findings showing that injection of lipopolysaccharide or CpG is necessary to render naive CTLs responsive to CCR5 ligands<sup>12</sup>. Hence, signals must exist that induce expression of these chemokine receptors, which obviously must operate *in trans* (that is, before CTLs encounter DCs); this suggests that soluble cytokines may be candidates. Further studies are needed to identify the signals that condition naive CTLs to express CCR4 and CCR5.

In summary, we have described an unexpected role for CCL17 and CCR4: they facilitate an NKT cell-dependent way of licensing DCs for cross-priming, which may be important for cross-priming against microbes bearing NKT cell glycolipid antigens. Our findings support the theory that DC–T cell encounters are not random but are highly coordinated through chemokines<sup>12</sup> and extend that theory by showing that different chemokines coordinate the recruitment of naive CTLs toward the relevant DCs. The synergism between these two mechanisms may be exploited to improve vaccinations aimed at inducing CTLs, for example, by combining adjuvants that elicit both helper T cell and NKT cell licensing.

## METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/natureimmunology/>.

Note: Supplementary information is available on the Nature Immunology website.

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#### AUTHOR CONTRIBUTIONS

V.S. and V.L.-K. designed and did most experiments, analyzed and interpreted data and contributed to the writing of the manuscript; C.A.T., T.Q. and K.H. designed, did and analyzed individual experiments; U.P., J.R., P.P., J.C., D.I.G., P.B.S., P.A.K., W.K. and I.F. contributed tools, discussed and interpreted results and edited the manuscript; and C.K. conceived the project, designed and interpreted experiments and wrote the manuscript.

#### COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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## ONLINE METHODS

**Reagents and mice.** All reagents were from Sigma-Aldrich unless specified otherwise. All mice had been backcrossed to C57BL/6 at least ten times, were bred under specific pathogen-free conditions at the central animal facility of the University Clinic of Bonn and were used at 8–12 weeks of age. Animal experiments were approved by the Bezirksregierung Köln of the German state of North Rhine-Westphalia. Soluble OVA (10 µg per gram body weight in 200 µl buffer) was injected intravenously, accompanied by 0.2 µg of α-GC (1 nmol; Axxora), iGb3 (1 nmol) or OCH (Alexis) or 20 µg CpG (TIB MolBiol), where appropriate. GSL-1<sup>+</sup> was synthesized as described<sup>50</sup>. For the preparation of α-GalDAG, globally trimethylsilyl-protected α-galactosyl iodide was coupled to 1-*O*-oleoyl-2-*O*-palmitoyl-*sn*-glycerol by an adaptation of a published method<sup>51</sup>.

**Mixed-bone marrow chimeras.** Bone marrow cells were obtained from femurs of donor mice. Mice were irradiated with 9 Gy from a <sup>137</sup>Cs source and then were injected intravenously with a 1:1 mixture of a total of  $1 \times 10^7$  bone marrow cells. The ratio of bone marrow types used was always 50% ± 10% of each type at 8 weeks after transplantation.

**Isolation and fluorochrome labeling of T cells.** OT-I cells and polyclonal CD8<sup>+</sup> T cells were isolated from OT-I and wild-type C57BL/6 mice, respectively, as described<sup>4</sup>. Single-cell suspensions of spleen and lymph node cells were treated with erythrolysis buffer (146 mM NH<sub>4</sub>Cl, 10 mM NaHCO<sub>3</sub> and 2 mM EDTA) for the removal of red blood cells and were further purified with a CD8<sup>+</sup> T cell isolation kit (Miltenyi). For CFSE labeling,  $10 \times 10^6$  to  $20 \times 10^6$  cells per ml were resuspended in PBS, followed by the addition of CFSE (carboxyfluorescein diacetate succinimidyl ester; Invitrogen) or Far Red DDAO-SE (7-hydroxy-9H-(1,3-dichloro-9,9-dimethylacridin-2-one, succinimidyl ester; Invitrogen) to a final concentration of 5 µM, for 10 min at 37 °C, as described<sup>4,5</sup>. Purity was always >85% of viable lymphocytes. DCs and NKT cells were sorted from splenic single-cell suspensions on a FACSVantage DiVa as described<sup>5</sup>.

**In vitro T cell activation assay.** Purified OT-I or polyclonal CD8<sup>+</sup> T cells ( $1.5 \times 10^5$ ) were cultured for 2 d together with anti-CD3 plus anti-CD28 beads according to the manufacturer's recommendation (Invitrogen) or with  $0.5 \times 10^5$  splenic DCs from wild-type or *Ccr4*<sup>-/-</sup> mice. At 5 h after injection of α-GC, DCs were isolated by digestion for 20 min with collagenase plus DNase followed by further purification with a CD11c<sup>+</sup> isolation kit (Miltenyi). DCs were pulsed for 20 min with OVA peptide (SIINFEKL; 20 µg/ml) before coculture.

**Antibodies and flow cytometry.** Cells were stained with the following fluorochrome-conjugated antibodies from BD Pharmingen and eBioscience: anti-CD8α (53-6.7), anti-B220 (RA36B2), anti-NK1.1 (PK136), anti-CD11b (M1/70), anti-CD11c (N418), anti-CD80 (16-10A1), anti-CD86 (GL1), anti-CD40 (3/23), anti-CD70 (FR70), anti-I-A<sup>b</sup> (AF6-120.1), anti-CD3 (145-2C11), anti-IFN-γ (XMG1.2), anti-Vα2 (B20.1) and anti-Vβ5 (MR9-4).

OVA-specific CTLs were detected by allophycocyanin-conjugated iTag MHC class I mouse tetramers (Beckman Coulter). For the measurement of CCR4, CCL17 was biotinylated with a fluorokine kit according to the manufacturer's recommendations (R&D Systems). Splenocytes ( $0.1 \times 10^6$ ) were incubated for 1 h at 4 °C with biotinylated recombinant mouse CCL17 (R&D systems) before the addition of avidin-fluorescein isothiocyanate with anti-CD3 and anti-CD28 Dynabeads (Invitrogen) for 30 min at 4 °C. Fc receptors were blocked with 24G2 hybridoma supernatant. Dead cells were excluded by Hoechst 33342 or 7-amino-actinomycin D dye and only events that appeared single in forward-scatter width were analyzed. A FACSCanto II and FACSDiva software (BD) were used for flow cytometry and data were analyzed with FlowJo software (TreeStar).

**Cytokine analysis.** For intracellular cytokine analysis, single-cell suspensions were restimulated for 4 h with OVA peptide (SIINFEKL) or anti-CD3 plus anti-CD28 beads (Invitrogen) in the presence of GolgiPlug (BD Bioscience). Cells were fixed for 15 min at 4 °C with 2% (vol/vol) paraformaldehyde in PBS, then were washed and incubated for 20 min in a solution of 0.1% (wt/vol) BSA and 0.5% (wt/vol) saponin in PBS, followed by an additional 30 min of incubation with fluorochrome allophycocyanin-conjugated anti-IFN-γ (BD) in a solution of 0.1% (wt/vol) BSA and 0.5% (wt/vol) saponin in PBS and unconjugated rat immunoglobulin G. For cytokine analysis in cell culture supernatants, commercial enzyme-linked immunosorbent assay kits were used (Invitrogen).

**Real-time reverse-transcription PCR.** RNA was isolated with the RNeasy Micro Kit (Qiagen), then cDNA was synthesized with random hexamer primers. An ABI Prism 7000 Sequence Detection system (Applied Biosystems) was used for RT-PCR with the following settings: 40 cycles of 15 s of denaturation at 95 °C, and 1 min of primer annealing and elongation at 60 °C as described<sup>13</sup>. RT-PCR was done with 1.5 µl cDNA plus 2.5 µl (0.9 µM) specific primers and 12.5 µl of 2× Platinum SYBR Green qPCR SuperMix (Invitrogen), and the following primers: mCCL17 1 (5'-TGGTATAAGACCTCAGTGGAG TGTTC-3') and mCCL17 2 (5'-GCTTGCCCTGGACAGTCAGA-3'); and mCCL22 1 (5'-GAGTTCTTCTGGACCTCAAATCC-3') and mCCL22 2 (5'-TCTCGGTTCTTGACGGTTATCA-3'). All samples were run in duplicate and results were normalized to those of 18S rRNA.

**In vivo cytotoxicity assay.** *In vivo* cytotoxicity assays were done as described<sup>5</sup>. Spleen cells were pulsed for 15 min at 37 °C with OVA peptide (SIINFEKL; 2 µg/ml) and labeled with 1 µM CFSE (CFSE<sup>hi</sup> cells) or were not pulsed with peptide and were labeled with 0.1 µM CFSE (CFSE<sup>lo</sup> cells). Both target cell types ( $1 \times 10^7$ ) were injected intravenously. After 4 h, the survival of target cells in the spleen was analyzed by flow cytometry. Specific lysis was calculated with the following formula: % specific cytotoxicity =  $100 - (100 \times (\text{CFSE}^{\text{hi}} / \text{CFSE}^{\text{lo}}) \text{ primed} / (\text{CFSE}^{\text{hi}} / \text{CFSE}^{\text{lo}}) \text{ control})$ .

**Immunofluorescence.** Cryosections 5 µm in thickness from shock-frozen spleens were prepared; at least two sections between individual sections were discarded to avoid staining of the same T cells in different sections. Sections were fixed with iced acetone and blocked for 1 h with 1% (wt/vol) BSA in PBS. B cell zones were visualized by staining with phycoerythrin-conjugated anti-B220 (eBioscience). Sections were viewed with an Olympus IX71 with a 10× objective. Images were captured and T cells per mm<sup>2</sup> T cell area were counted with Cell F software (Olympus).

**Transwell cell-migration assay.** Splenocytes ( $1 \times 10^6$ ) from α-GC-injected wild-type mice were loaded into the top chamber of Transwell inserts (pore size, 5 µm; Costar). Bottom wells were filled with RPMI medium containing 2% (vol/vol) FCS with or without CCL17 (R&D Systems). Cells in the lower chambers were collected after 6 h at 37 °C and transmigrated CTLs were counted by flow cytometry with antibody staining and the addition of constant numbers of CaliBRITE beads (BD Biosciences).

**In vitro analysis of CTL recruitment by DCs.** CTLs and DCs obtained from the spleen were labeled with different fluorochromes and mixed at a ratio of 4:1 in RPMI medium containing 2% (vol/vol) FCS and then were placed on plastic channel slides (µ-slide; Ibidi) coated with fibronectin (Harbour Bio-Products). Time-lapse series were recorded with a fully automated inverted Olympus Fluoview 1000 confocal microscope equipped with motorized xyz stage (Märzhäuser) and a climate chamber (37 °C and 5% CO<sub>2</sub> with humidity). Up to eight samples were analyzed simultaneously with the multipoint function of the microscope, which allowed use of the same conditions for all probes. Cell motility and cell-cell interactions were monitored over a period of 2 h by capture of fluorescence and differential interference contrast images every 2 min with a 0.75 Plan S Apo 20× objective (Olympus). In each experiment, 100–300 motile CTLs were tracked with the Manual Tracking plug-in of ImageJ software (National Institutes of Health). The directionality of migrating CTLs before physical contact with DCs, as well as the duration of cell-cell interactions, was calculated with the Chemotaxis and Migration Tool plug-in (Ibidi) of ImageJ software. For analysis of directionality, the ratio of euclidean to accumulated distance of individual cell tracks was calculated (sample of analysis, **Supplementary Fig. 11**). CTL directionality and CTL-DC contact in high resolution was also analyzed as a time series with a 0.9 ultraviolet-transmitting Plan Apo 40× objective (Olympus).

**Statistical analysis.** Comparisons were made with the Mann-Whitney or Kruskal-Wallis test and Dunn's post-test using Prism 5 software (Graphpad Software).

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