UNIVERSITY OF SOUTHAMPTON

FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCES

School of Chemistry

Total Syntheses of (±)-Vibralactone and ^{13}C-Labelled All-*Trans*-Retinals

by

Alexander James Leeder

Thesis for the degree of Doctor of Philosophy

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UNIVERSITY OF SOUTHAMPTON

ABSTRACT

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TOTAL SYNTHESES OF (±)-VIBRALACTONE AND ^{13}C-LABELLED ALL-*TRANS*-RETINALS

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Vibralactone is a potent inhibitor of pancreatic lipase and a potential candidate for a new anti-obesity therapeutic. Not only is vibralactone an interesting molecule due to its topical biological activity, but its unique fused bicyclic β-lactone structure also presents a challenging target for synthetic investigation. Herein two novel and highly diastereoselective total syntheses of (±)-vibralactone will be presented along with efforts towards an asymmetric synthesis of (–)-vibralactone.

Highly functionalised intermediates have been accessed by a unique titanium-induced acetal addition to malonate precursors, followed by a highly diastereoselective allylation to set the relative stereochemistry of the adjacent chiral centres of vibralactone. Two synthetic routes to vibralactone have been completed from these novel scaffolds in 17 and 11 steps with overall yields of 4.5% and 16% respectively. Both routes exploit aldol condensations to prepare the cyclopentene ring of vibralactone, and the second-generation synthesis employs a novel and very efficient deallylation/cyclisation reaction to form the β-lactone ring.

Two all-*trans*-retinals ([10-18-$^{13}C_9$] and [12,15-$^{13}C_2$]) containing multiple ^{13}C labels have been synthesised to facilitate DNP enhanced solid-state MAS NMR investigations into the ring orientation and retinal interaction with transmembrane retinylidene proteins, proteorhodopsin, channelrhodopsin and KR2. Channelrhodopsin and KR2 have very important applications in optogenetics; a technique that can be used to probe how the brain functions and has the potential to relieve symptoms of neurological conditions.

The labelled retinal isotopomers have been accessed from simple and readily available ^{13}C-enriched starting materials using a synthetic route that will ultimately permit access to other labelling patterns as required.

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I, Alexander James Leeder

declare that this thesis entitled

TOTAL SYNTHESES OF (±)-VIBRALACTONE AND ^{13}C-LABELLED ALL-*TRANS*-RETINALS

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Definitions and Abbreviation

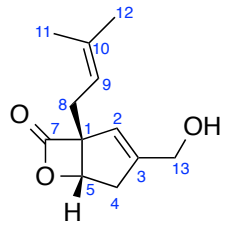
aq.	Aqueous
atm	Atmosphere
BINAP	(1,1'-Binaphthalene-2,2'-diyl)bis(diphenylphosphine)
BPR	Blue absorbing proteorhodopsin
BMI	Body Mass Index
br	Broad
d	Doublet
Da	Dalton
dba	Dibenzylideneacetone
DCC	*N*,*N*'-dicyclohexyl-carbodiimide
DCM	Dichloromethane
DIBAL	Diisobutylaluminium hydride
DIPEA	*N*,*N*'-Diisopropylethylamine
DMAP	4-(Dimethylamino)-pyridine
DME	Dimethoxyethane
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DNP	Dynamic Nuclear Polarization
DQ	Double Quantum
DQF	Double Quantum Filtering
ee	Enantiomeric excess
EI	Electron Impact
m (NMR)	Multiplet
min	Minute(s)
Ms	Mesyl
MS	Mass Spectrometry

MW	Molecular Weight
NBS	*N*-Bromosuccinimide
q	Quartet
RCM	Ring Closing Metathesis
rt	Room temperature
s (IR)	Strong
s (NMR)	Singlet
Sat.	Saturated
Ser	Serine
spt	Septet
sxt	Sextet
t	Triplet
t	Tertiary
TBAF	Tetrabutylammoniumfluoride
TBS	*t*-butyldimethylsilyl
ESI	Electrospray Ionisation
eq.	Molar equivalents
FT	Fourier Transform
GC	Gas Chromatography
Gly	Glycine
GPR	Green absorbing proteorhodopsin
h	Hour(s)
His	Histidine
HMD	Hexamethyldisilazane
HPLC	High-Performance Liquid Chromatography
HRMS	High-Resolution Mass Spectrometry
IC_{50}	Half maximal inhibitory concentration

IR	Infrared
J	Coupling constant
dr	Diastereomeric ratio
LDA	Lithium diisopropylamide
LRMS	Low-Resolution Mass Spectrometry
m (IR)	Medium
TFA	Trifluoroacetic Acid
THF	Tetrahydrofuran
NMR	Nuclear Magnetic Resonance
NMO	*N*-Methylmorpholine *N*-Oxide
NOESY	Nuclear Overhauser Effect Spectroscopy
TMS	Trimethylsilyl
PL	Pancreatic Lipase
ppm	Parts per million
RBF	Round Botton Flask
TIPS	Triisopropylsilyl
TLC	Thin Layer Chromatography
Py	Pyridine
TTC	*Trans*-2-tritylcyclohexanol
UV	Ultraviolet
w	Weak
9-BBN	9-Borabicyclo[3.3.1]-nanone

Chapter 1: Introduction to Vibralactone

(−)-Vibralactone (**1.01**), extracted from *Boreostereum Vibrans*,[1] is a potent inhibitor of pancreatic lipase (IC_{50} 0.4 μg ml^{-1}) and a potential anti-obesity lead candidate. The first section of this thesis concerns a new approach to the total synthesis of vibralactone (**1.01**) that improves upon the first published synthesis by Snider *et al*.[2,3] and provides a platform for derivatisation and potential structure-activity relationship (SAR) studies. The broader objective is to enhance knowledge in the field of anti-obesity therapeutics.



1.01

Figure 1.1: Structure of Vibralactone (**1.01**)

1.1 Obesity

The World Health Organisation (WHO) estimated in 2014 that globally, more than 1.9 billion adults were overweight and over 600 million of these were clinically obese.[4] Obesity is defined as a BMI (body mass index) of >30 kg m^{-2} and contributes significantly to chronic illnesses and disability.[5] It has been recognised as the second most influential contributor to preventable death after tobacco smoking.[6,7] Obesity has been associated with an increased incidence of several cancers,[8] diabetes mellitus,[9] hypertension,[10] heart disease,[11] degenerative joint disease[12] and respiratory disorders,[12] and its contribution to mortality is more significant than under-nutrition and infectious disease.[13] It has been estimated that this accounts for 2-6% of the total health care costs in developing countries[14] and, with the continued increase in obesity, the strain on economies is becoming more prevalent. With this increasing epidemic, the market for anti-obesity drugs has been subsequently growing.[14]

Obesity is the result of a routine imbalance of energy intake and energy expenditure that eventually leads to increased adipose tissue mass.[15] Although overeating and a sedentary lifestyle are key contributors to weight gain, health conditions such Cushing's syndrome,[16] polycystic ovarian syndrome[17] and hypothyroidism[18] have also been linked

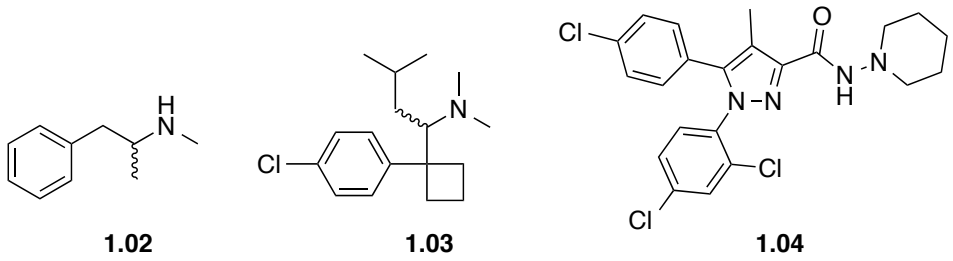
to obesity. In addition, there is evidence that exposure of the foetus to toxic chemicals, for example from smoking, can lead to childhood obesity and health complications in later life.[19]

Although diet regulation and increased physical activity can aid weight loss, it is often found that this is not sustainable without a complete change of lifestyle. In a lot of cases a period of steady weight loss is followed by relapse and substantial weight gain. Bariatric surgery such as a gastric bypass, which leads to a reduction in functional volume of the stomach, is becoming increasingly popular in combating obesity[20] however it carries significant risks.[20]

1.2 Obesity Therapeutics

The National Institute of Health developed clinical guidelines for identification, evaluation and treatment of overweight and obese adults.[21] This document suggests that increased physical activity and reduced calorific intake should form the foundation of treatment, however it acknowledges the potential benefit of therapeutics to assist with long-term weight management.[6]

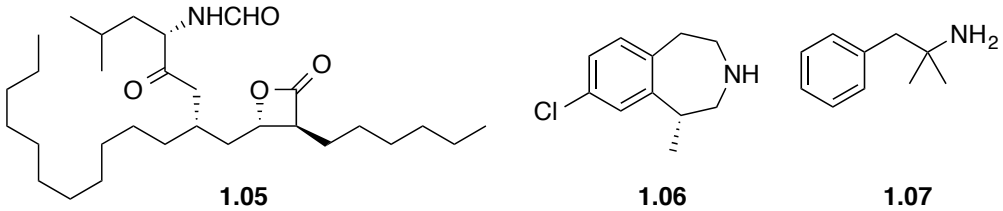
There are many other anti-obesity therapeutics that have been utilised in the past, however serious safety concerns have led to either their withdrawal or restricted use. The majority of obesity drugs, including those in **Figure 1.2**, target neurological pathways to suppress appetite. In 1947, methamphetamine (**1.02**) became the first Food and Drug Administration (FDA) approved obesity drug.[22] This drug is extremely addictive and includes side effects such as hallucinations, heart palpitations and seizures. The FDA limited the suggested course of methamphetamine in 1973 and began regulating it under the Controlled Substances Act, following the start of an epidemic of amphetamine abuse in the US.[22] Sibutramine (**1.03**) is a norepinephrine and serotonin reuptake inhibitor that was approved in 1997, with patients seeing 4.3% increased weight loss when taking the drug.[23] Unfortunately, a study later showed that sibutramine results in an 11.4% increased chance of serious, nonfatal cardiovascular issues and was therefore withdrawn from the market.[24] Similarly, rimonabant (**1.04**), a CB1r (neuroreceptor) antagonist, showed good weight loss results when compared to a placebo,[25] but was later prohibited due to psychiatric side effects such as panic attacks and insomnia.[26]



1.02 **1.03** **1.04**

Figure 1.2: Structures of methamphetamine (**1.02**), sibutramine (**1.03**) and rimonabant (**1.04**).

There are currently three FDA approved long-term obesity treatments: Orlistat (tetrahydrolipsitatin, **1.05**), lorcaserin (**1.06**) and phentermine/topiramate-extended release (ER) (**1.07**) (**Figure 1.3**).[27] Locaserin, phentermine and topiramate all act upon the central nervous system to suppress appetite. Orlistat inhibits pancreatic and gastric lipases to reduce the absorption of free fatty acids and monoglycerides (**section 1.3**).[28,29] This technique of targeting nutrient absorption is becoming a promising new strategy for obesity drugs as fewer serious side effects are usually observed.[14] For a comprehensive review of all current and future drug targets in weight management see the 2011 pharmaceutical research paper by Renger F. Witkamp.[30]



1.05 **1.06** **1.07**

Figure 1.3: Obesity therapeutics: Orlistat (**1.05**), lorcaserin (**1.06**) and phentermine (**1.07**).

There are a wealth of natural products that have been discovered with the potential of being used to treat obesity. An excellent review by Jong Won Yun examines naturally-occurring compounds with anti-obesity activity and details scientific data including active components and mode of action against obesity.[5] It is believed that in the future the continued exploration of active natural products will lead to new, more effective obesity therapeutics.[5]

1.3 Pancreatic Lipase

Pancreatic lipase (PL) is a gastrointestinal lipolytic enzyme synthesised in the pancreas that is responsible for the breakdown of 50-70% of total dietary fats.[14] PL hydrolyses two of the three esters in a triglyceride to afford a mono-glyceride and two fatty acid molecules (**Figure 1.4**).[31,32] The lipolytic products then form micelles with cholesterol, lysophophatidic acid (LPA) and bile acids, which are absorbed into the intestinal wall. Here they are resynthesized into triglycerides and stored as fat. A large build-up of excess fat can lead to obesity.

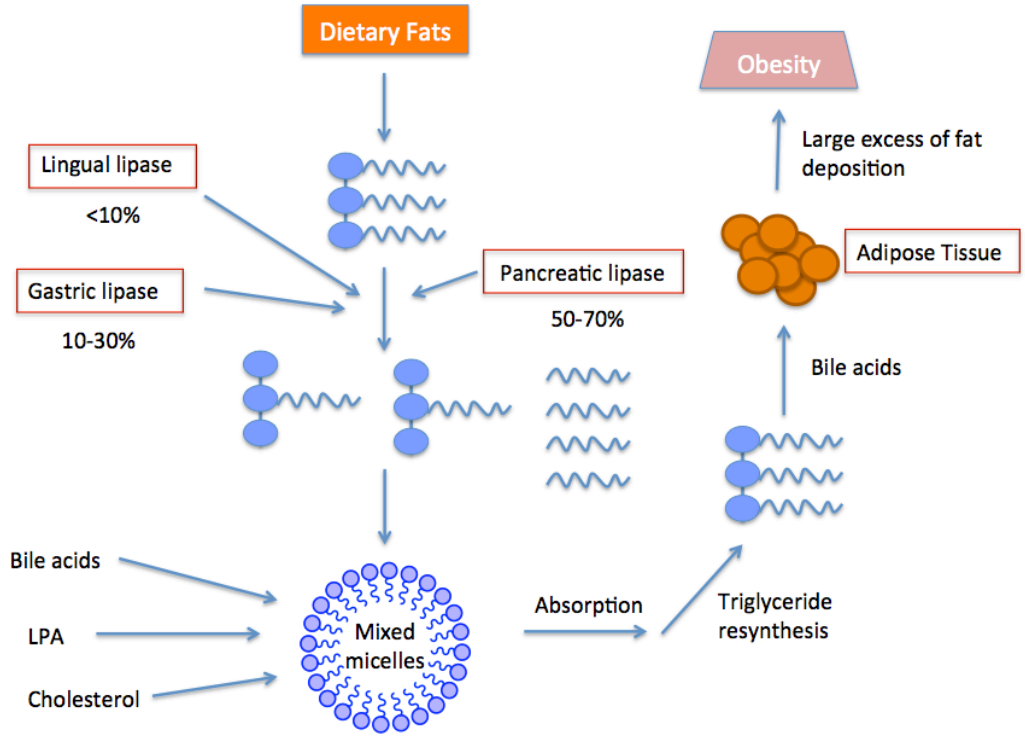
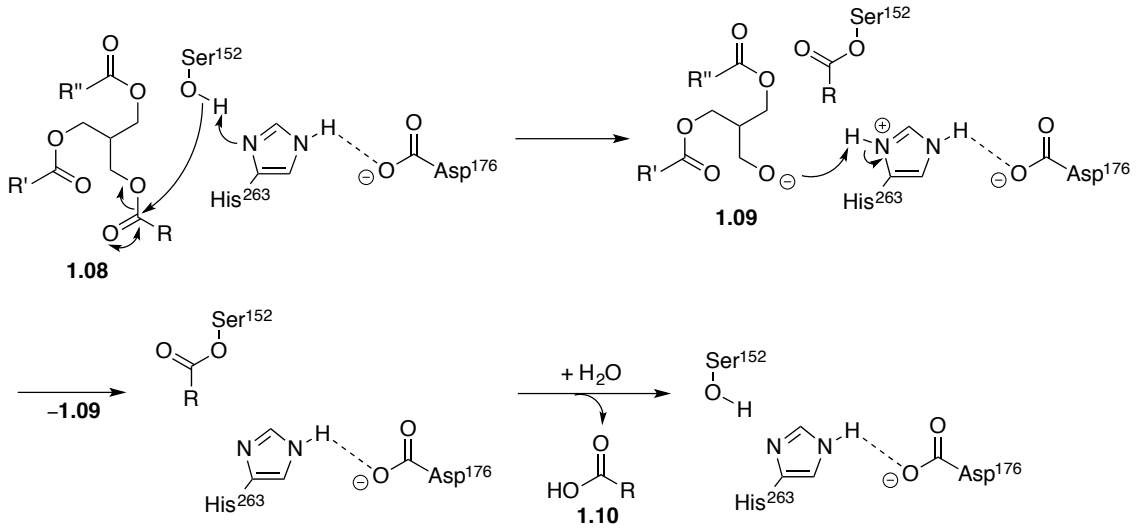


Figure 1.4: Processing of triglycerides from dietary fats adapted from Birari *et al.*[14]

It has been shown that serine-152 is part of a catalytic triad and is the key amino acid residue within the active site of PL that is responsible for the hydrolysis of triglycerides.[33] The mechanism is comparable to that of serine proteases.[34] Ser-152 is activated by His-263, which is in turn activated by Asp-176 (**Scheme 1.1**). The increased nucleophilicity of the serine residue results in a transesterification with one of the esters of triglyceride **1.08**. The resulting serine ester is hydrolysed, affording diglyceride **1.09** and fatty acid **1.10**. The same hydrolysis mechanism is repeated with diglyceride **1.09** to afford a monoglyceride.



Scheme 1.1: Enzymatic hydrolysis of triglyceride **1.08** by the catalytic triad of pancreatic lipase (PL).

Tetrahydrolipstatin (Orlistat, **1.05**) is a natural product derivative formed by the catalytic hydrogenation of lipstatin (**1.11**, **Figure 1.5**),[28] which is extracted from actinobacteria *streptomyces toxytricini*.[35] Both lipstatin (**1.11**) and tetrahydrolipstatin (**1.05**) are pancreatic lipase inhibitors with an IC_{50} of 0.14 μM and 0.36 μM respectively. Although tetrahydrolipstatin (**1.05**) has a lower activity, it is more attractive as a pharmaceutical agent due to its greater stability.[36] Both compounds act as irreversible inhibitors of PL by forming a covalent bond with the active serine site through an acylation reaction with the β-lactone group.[29]

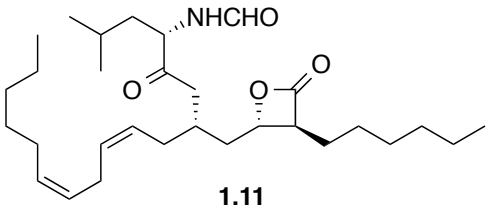


Figure 1.5: Structure of PL inhibitor lipstatin (**1.11**)

Orlistat was approved by the FDA in 1998[37] and in clinical trials it was found that 50-60% of patients benefitted from a 5-10% reduction in body-weight, which they were able to maintain for up to four years.[30] Orlistat forms the largest proportion of anti-obesity drugs worldwide[38] and, as it is predicted that the size of the market will increase from $750 million in 2012 to $2.6 billion in 2019,[39] there is currently a substantial effort to improve production efficiency from natural sources. Despite its increasing clinical

usage, Orlistat can cause a number of unpleasant side effects including diarrhoea, abdominal pain, dyspepsia, faecal spotting and steatorrhoea.[38,40]

1.4 Vibralactone as a Pancreatic Lipase Inhibitor

Vibralactone (**1.01**) was isolated from the culture broth of the cortocioid fungi *Boreosterum vibrans* by extraction with ethyl acetate and then silica gel chromatography of the resulting crude extract.[1] The chemical structure was elucidated by NMR, IR and MS, and the cis stereochemistry tentatively assigned due to the inherent instability of trans-fused species due to high strain energy.[1,41] MM2 calculations for the *cis* and *trans* steric energies of 34.1 and 68.7 kcal mol^{-1} respectively (CS Chem3D Pro) initially supported the stereochemical assignment until it was later confirmed by the first racemic and asymmetric syntheses of vibralactone (**1.01**).[2,3] Vibralactone is of particular interest due to its similar PL inhibition activity of 0.4 μg ml^{-1} compared to 0.36 μg ml^{-1} of tetrahydrolipstatin (**1.05**), which shares the same β-lactone functionality. It is assumed vibralactone (**1.01**) inhibits PL by the same mechanism as tetrahydrolipstatin (**1.05**).

1.4.1 Derivatives of Vibralactone

Since the discovery of vibralactone (**1.01**), a range of derivatives have also been isolated from *Boreosterum vibrans*.[42–44] Vibralactone D (**1.16**), E (**1.17**) and F (**1.18**) have been tested for their inhibitory effects on the human and mouse 11-β hydroxysteriod dehydrogenases HSD1 and HSD2. These enzymes are involved in the reversible conversion of cortisol to sortisone[45] and are linked to insulin resistance, obesity and hypertension.[46] Vibralactone D (**1.16**) has IC_{50} values of: 11β-HSD1 human = 85.7 μM, mouse 295.2 μM and 11β-HSD2 = 87.1 μM. Vibralactone E (**1.17**) and (**1.18**) displayed weak activity against both human 11β-HSDI (43.6% and 31.2% inhibition at 150 μgml^{-1}) and mouse 11β-HSD1 (37.7% and 24.8% inhibition at 150 μgml^{-1}).[42] No biological data has been published for the other derivatives.

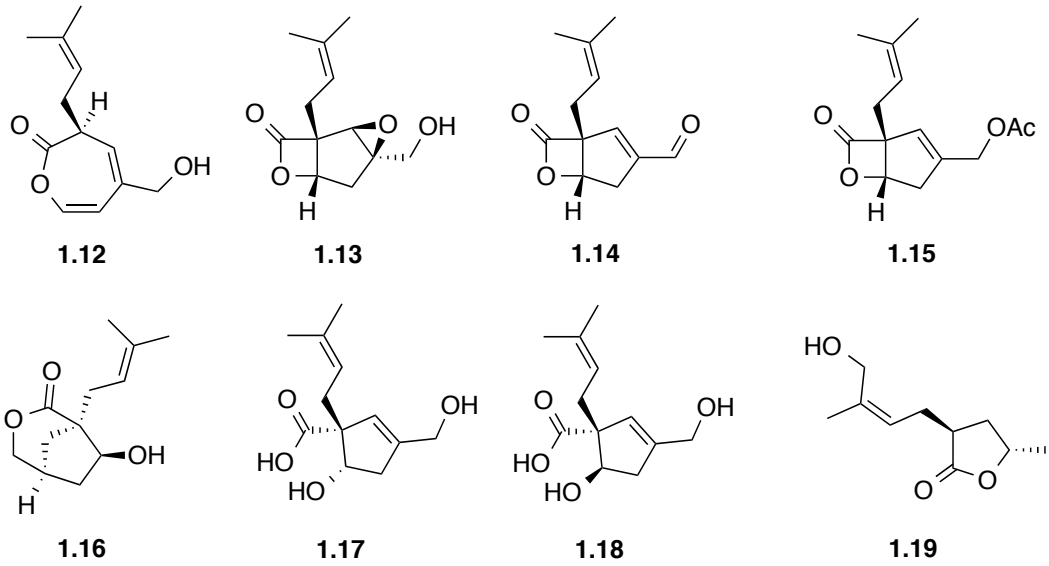
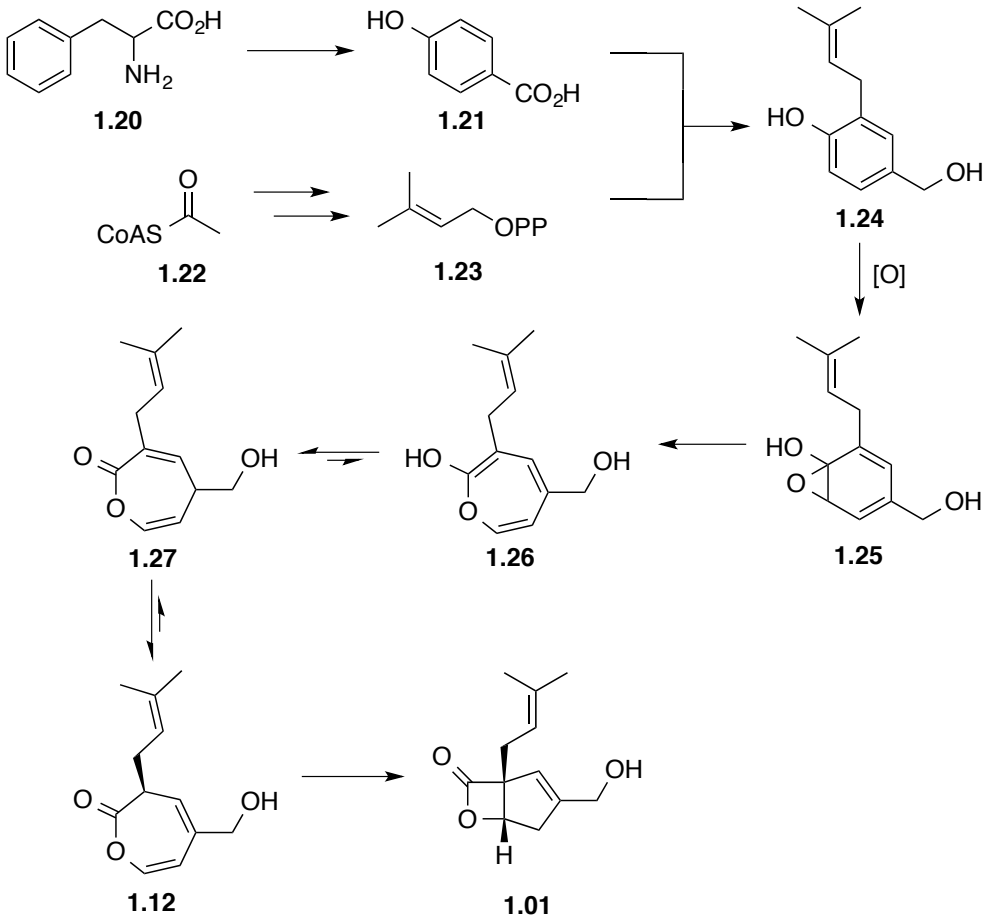


Figure 1.6: Structures of 1,5-seconvibralactone (**1.12**), vibralactone B (**1.13**), vibralactone C (**1.14**), acylated vibralactone (**1.15**), vibralactone D (**1.16**), vibralactone E (**1.17**), vibralactone F (**1.18**) and vibralactone G (**1.19**).

1.4.2 Biosynthesis of Vibralactone

The biosynthetic pathway to vibralactone (**1.01**) was proposed by Zhao *et al.* in 2013 (**Scheme 1.2**).[47] Benzoic acid **1.21**, derived from phenylalanine (**1.20**), is prenylated with **1.23** before oxidation to epoxide **1.25**. Ring expansion forms cyclic intermediate **1.26**, which tautomizes to the stable natural product 1,5-secovibralactone (**1.12**), which has been isolated.[44] An intramolecular ring contraction then forms the fused β-lactone bicycle of vibralactone (**1.01**).



Scheme 1.2: Proposed biosynthesis of vibralactone (**1.01**) by Zhao *et al.*[47]

It has been tentatively proposed that the ring contraction of 1,5-secovibralactone (**1.12**) proceeds via a radical mechanism initiated by a single electron oxidation[48] or by a base catalysed anion pathway (**Figure 1.7**).

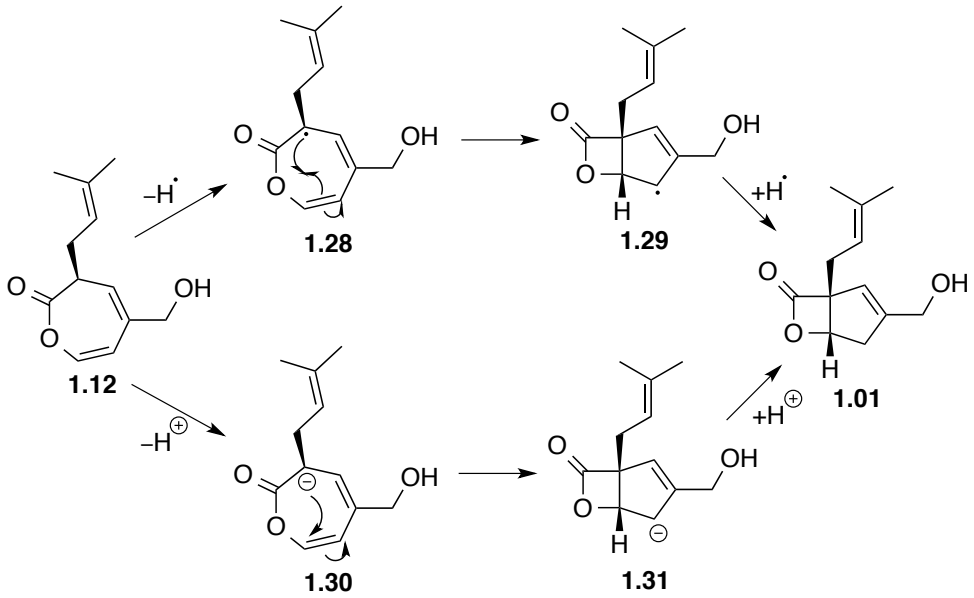
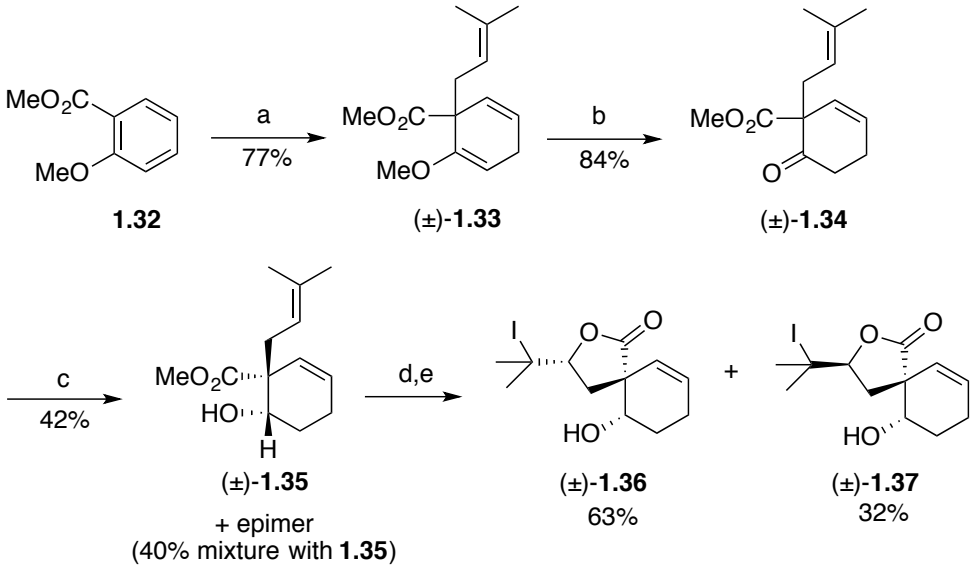


Figure 1.7: Proposed radical and anionic ring contraction of 1,5-secovibralactone (**1.12**) to form the fused bicyclic core of vibralactone (**1.01**).

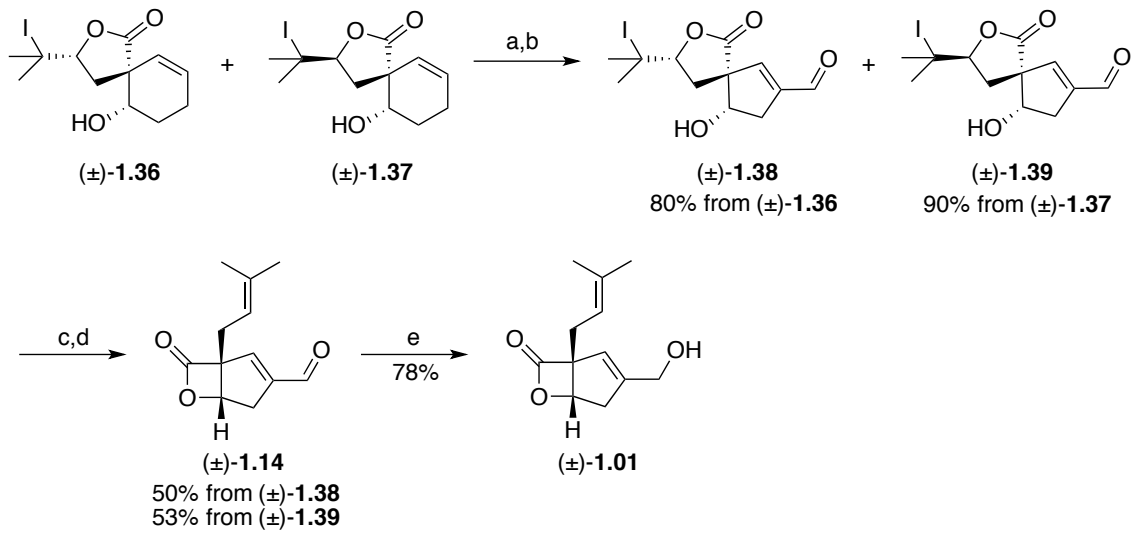
1.4.3 Snider's Total Synthesis of (±)-Vibralactone

The first total synthesis of (±)-vibralactone (**1.01**) was reported by Snider *et al.* in 2008.[2] Starting from methyl methoxybenzoate (**1.32**), a Birch reductive alkylation afforded prenyl derivative **1.33**, which was hydrolysed to provide β-keto ester **1.34** (**Scheme 1.3**). Reduction with Me_4NBH_4 gave **1.35** in 42% yield, alongside 40% yield of the other diastereoisomer, which could be recycled by oxidation to ketone **1.34**. Hydrolysis of *cis* ester **1.35** followed by treatment with $NaHCO_3$, I_2 and KI afforded iodolactones **1.36** and **1.37**. The diastereoisomers were separated so that the relative *cis* stereochemistry of hydroxy ester **1.35** could be confirmed by X-ray crystallography.



Scheme 1.3: *Reagents and conditions:* a) i) K, NH_3, ^tBuOH, −78 °C; ii) LiI, −78 °C; iii) prenyl bromide, −78 °C to rt; b) MeOH, 5% aq. HCl; c) Me_4NBH_4, THF/MeOH, rt; d) KOH, MeOH, 60 °C; e) $NaHCO_3$, I_2, KI, THF/H_2O.

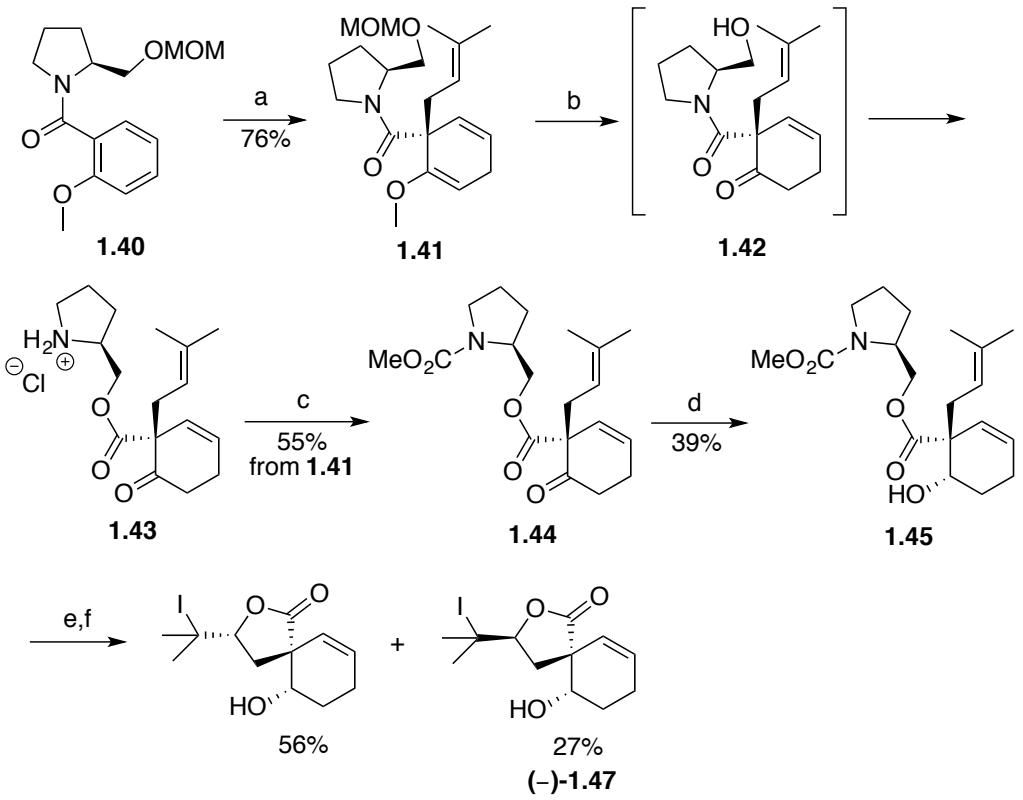
Each iodolactone isomer was ozonized separately and, after workup, the intermediate dialdehydes were subjected to an intramolecular aldol condensation with Bu_2NH•TFA[49] to afford cyclopentenals **1.38** and **1.39** in good yield (**Scheme 1.4**). Deprotection of each diastereoisomer with zinc, followed by lactonisation, provided (±)-vibralactone C (**1.14**). Finally, aldehyde reduction completed the racemic total synthesis of (±)-vibralactone (**1.01**) in 9% over 10 steps.



Scheme 1.4: *Reagents and conditions:* a) i) O_3, CH_2Cl_2/MeOH, −78 °C ii) PPh_3, −78 °C to rt; b) Bu_2NH•TFA, benzene, rt; c) Activated Zn, 4:1 THF/HOAc, 0 °C; d) *p*-TsCl, pyridine; e) $NaBH_4$, 100:1 DME/H_2O.

1.4.4 Snider's Total Synthesis of (−)-Vibralactone

Snider *et al.* later applied their synthetic approach to the asymmetric synthesis of (−)-vibralactone (**1.01**) (**Scheme 1.5**).[3] Cyclohexadiene **1.41** was accessed as a single diastereoisomer by a Birch reductive alkylation of chiral amide **1.40**. The synthesis of this amide precursor was not included in the step count and overall yield of the total synthesis. MOM deprotection and subsequent rearrangement provided ammonium ester **1.43**, which was treated with methyl chloroformate to afford carbamate **1.44**. Keto-reduction furnished a 3:2 mixture of *cis*-**1.45** and *trans*-**1.45**, giving 39% of *cis*-**1.45** after purification. The remaining 1:3 mixture of *cis*-**1.45** and *trans*-**1.45** was recycled with Dess-Martin Periodinane. Hydrolysis and iodolactonisation then provided diastereoisomers (−)-**1.46** and (−)-**1.47** as single enantiomers. The remainder of the total synthesis then mirrored the racemic synthesis of (±)-vibralactone (**1.01**) (**Scheme 1.4**), leading to (−)-vibralactone (**1.01**) in 4.8% over 11 steps from amide **1.40**.



Scheme 1.5: *Reagents and conditions:* a) i) K, NH_3, ^{t}BuOH, −78 °C; ii) prenyl bromide, −78 °C to rt; b) MeOH, 10% aq. HCl, microwave, 60 °C; c) $NaHCO_3$, $ClCO_2Me$, CH_2Cl_2, rt; d) $Me_4N{\bullet}BH_4$, MeOH, rt; e) MeOH, 3.5 M aq. KOH, 60 °C; f) $NaHCO_3$, I_2, KI, THF, H_2O, rt.

1.5 Brown Group Approach to Vibralactone

The Brown group approach to vibralactone (**1.01**) is based around cheap malonate starting materials, using alkylation and redox procedures with the intent to develop a synthetic route that is easily applicable to derivatisation. A significant body of work towards the synthesis of vibralactone (**1.01**) has been performed by Dr Robert Heap during his PhD studies and his key results will be summarised.[50]

Initial investigations focused on the construction of vibralactone's cyclopentene ring by a ring closing metathesis (RCM) approach. Due to limited success, efforts later shifted towards utilizing an aldol condensation to complete the ring closure.

1.5.1 Ring Closing Metathesis Route

It was envisioned that vibralactone (**1.01**) could be prepared by lactonisation of *cis* hydroxy-ester **1.48** as described by Snider *et al* (**Figure 1.8**).[2,3] Cyclopentene **1.48** may be completed from selective RCM of triene **1.49**, which would be accessed by a stereoselective allylation of aldehyde **1.50**. Aldehyde **1.50** could be derived from malonate **1.51** utilising a chiral auxiliary to control the stereochemistry of the all-carbon quaternary centre.

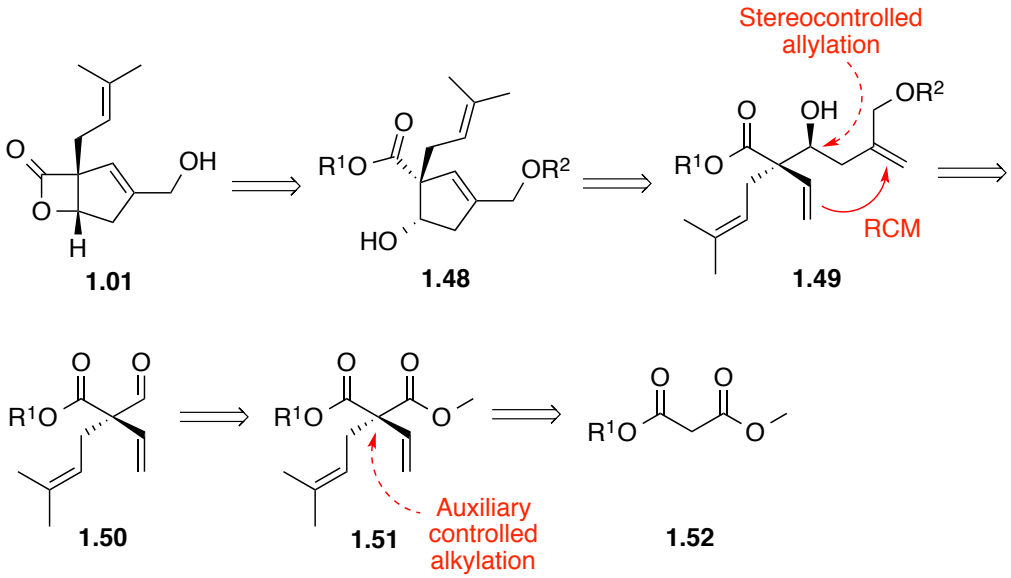
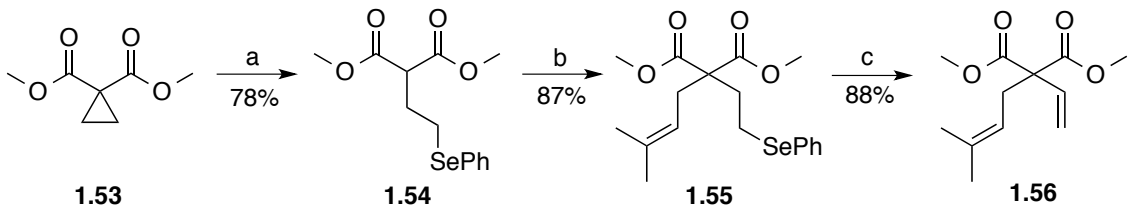


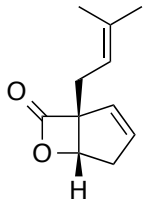
Figure 1.8: Brown group synthetic plan to access vibralactone (**1.01**), using a RCM strategy.

The racemic synthesis of substituted malonate **1.56** was completed in 60% yield over three steps (**Scheme 1.6**). Cyclopropylmalonate **1.53** was selenated with PhSeSePh in the presence of $NaBH_4$ and then alkylated with prenyl bromide to afford selenide **1.55**. Treatment with H_2O_2 gave a selenoxide, which eliminated to provide vinyl malonate **1.56**.



Scheme 1.6: *Reagents and conditions:* a) PhSeSePh, $NaBH_4$, MeOH, rt; b) NaH, prenyl bromide, THF, rt; c) H_2O_2, THF, rt.

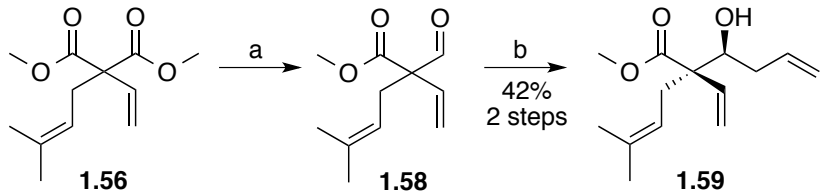
At this stage, a model system was synthesised to see if a RCM would be applicable to the total synthesis of vibralactone (**1.01**). Simplified target **1.57** (**Figure 1.9**) was chosen with the absence of the hydroxymethyl group to enable rapid synthetic access to the RCM precursors.



1.57

Figure 1.9: Vibralactone model system **1.57**.

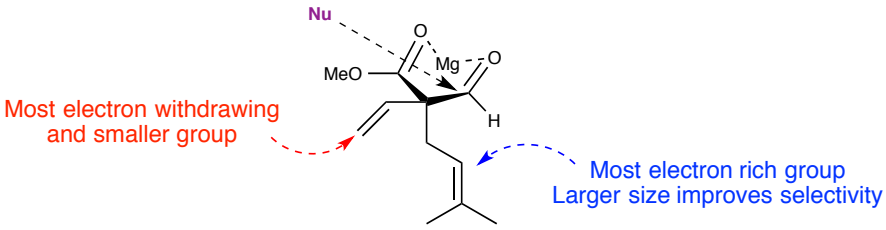
Reduction of diester **1.56** by slow addition of DIBAL at −78 °C afforded aldehyde **1.58** (**Scheme 1.7**). After workup, **1.58** was subjected to a diastereoselective allylation adapted from a procedure developed by Linclau *et al*.[51] Alcohol **1.59** was afforded in moderate yield over two steps but with excellent 15:1 selectivity.



1.56 **1.58** **1.59**
~15:1 dr

Scheme 1.7: *Reagents and conditions:* a) DIBAL, CH_2Cl_2, −78 °C; b) $MgBr_2 \bullet OEt_2$, allyltributyltin, CH_2Cl_2, −78 °C to rt.

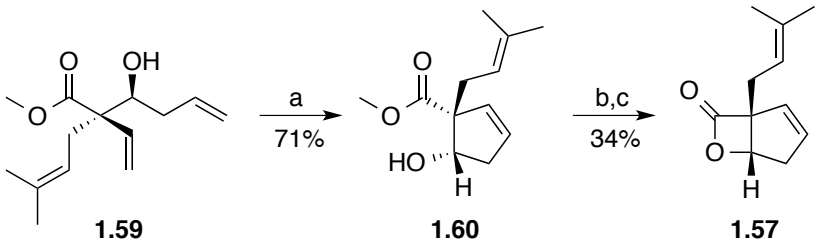
Linclau *et al.* presented a flattened boat model to explain the diastereocontrol observed for related addition reactions.[51] If applied to aldehyde **1.58**, the more electron rich prenyl group adopts a pseudoaxial position while the smaller vinyl group is pseudoequatorial (**Figure 1.10**). The pseudoaxial prenyl group reduces the chance of nucleophilic attack from the *si*-face, leading to the observed selectivity.



1.58

Figure 1.10: Flattened boat model for the diastereoselective allylation of **1.58**.

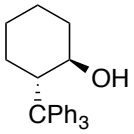
RCM of triene **1.59** proceeded smoothly with Grubbs 2nd generation catalyst (**Figure 1.12**) to form cyclopentene **1.60** (**Scheme 1.8**). Ester hydrolysis followed by lactonisation with retention of stereochemistry afforded model compound **1.57** in 6% yield over 8 steps. The yield for the final step was quite low, but this was largely due to the volatile nature of vibralactone analogue **1.57**.



1.59 **1.60** **1.57**

Scheme 1.8: *Reagents and conditions:* a) Grubbs II, CH_2Cl_2, rt; b) NaOH, MeOH, 60 °C; c) *p*-TsCl, pyridine, 0 °C to 5 °C.

An asymmetric synthesis of vibralactone analogue **1.57** was explored using the chiral auxiliary *trans*-2-tritylcyclohexanol (TTC) (**1.61**, **Figure 1.9**).[52] This auxiliary has been employed within the group in the synthesis of (+)-linalool oxide,[53] and is a bulky version of the better known 8-phenylmenthol auxiliaries.

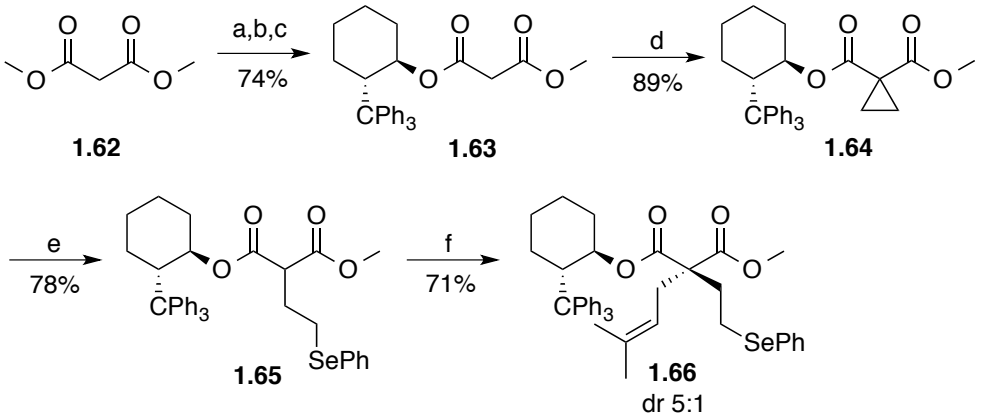


(+)-1.61

Figure 1.11: Structure of (+)-TTC (**1.61**).

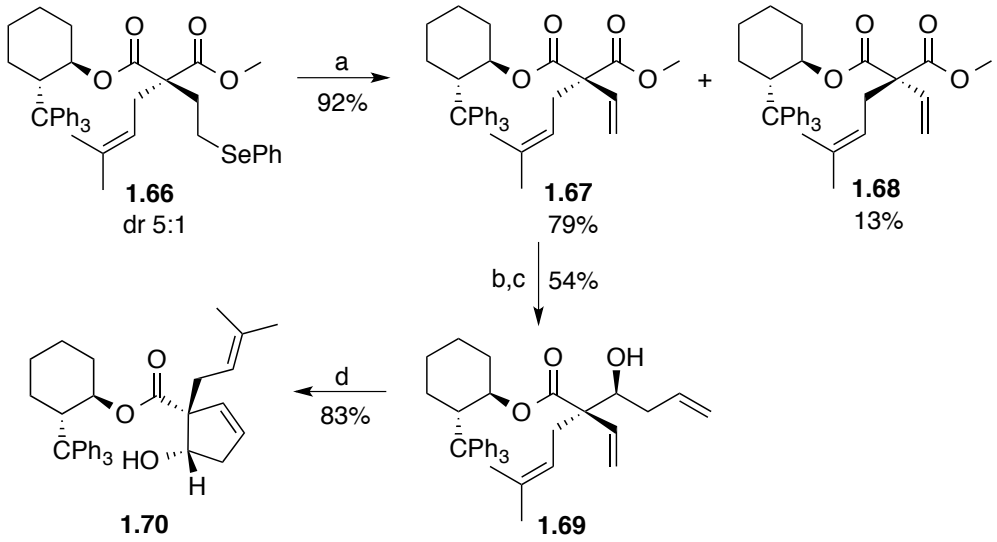
Mono-hydrolysis of dimethyl malonate (**1.62**) followed by acid chloride formation and coupling with (+)-TTC (**1.61**) provided diester **1.63** in good yield (**Scheme 1.9**).

Formation of the cyclopropane ring and selenation gave **1.65** as a mixture of diastereoisomers. Prenylation with ^{n}BuLi as a base afforded **1.66** with 5:1 selectivity. The diastereoisomers were difficult to separate by column chromatography.



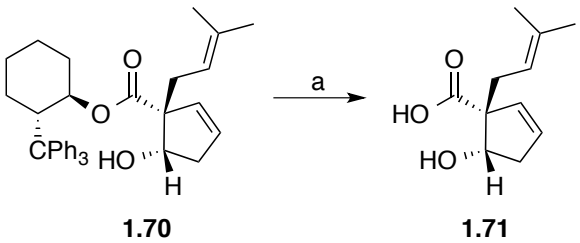
Scheme 1.9: *Reagents and conditions:* a) KOH, MeOH, 65 °C; b) Oxalyl chloride, DMF, CH_2Cl_2, rt; c) (+)-TTC (**1.61**), DMAP, NEt_3, CH_2Cl_2, reflux; d) Dibromoethane, K_2CO_3, TBAB, DMF, 50 °C; e) PhSeSePh, $NaBH_4$, MeOH, reflux; f) ^{n}BuLi, DIPA, prenyl bromide, THF, −78 °C to rt.

Treatment of **1.66** with H_2O_2 provided the selenoxide elimination products **1.67** and **1.68**, which were easily separated (**Scheme 1.10**). The major isomer was reduced with DIBAL and subjected to the diastereoselective allylation to afford **1.69** as a single diastereoisomer in 54% yield. RCM was successfully applied leading to cyclopentene **1.70** in high yield.



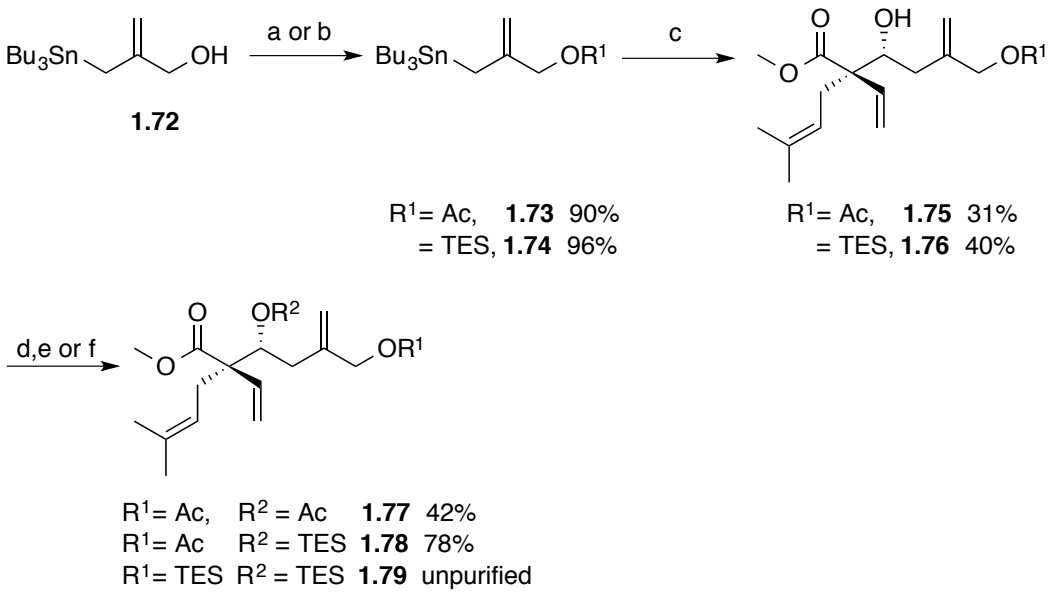
Scheme 1.10: *Reagents and conditions:* a) H_2O_2, THF, rt; b) DIBAL, CH_2Cl_2, −78 °C; c) $MgBr_2$•OEt_2, allyltributyltin, CH_2Cl_2, −78 °C to −25 °C; d) Grubbs 2, CH_2Cl_2, rt.

A number of conditions were investigated for the hydrolysis of **1.70** and only a refluxing 0.3 M solution of KOH in MeOH/H_2O proved effective in cleaving the bulky TTC auxiliary (**Scheme 1.11**). Unfortunately, due to the small quantity of **1.70** obtained from the reaction sequence, none of acid **1.71** was isolated. If more time were available, the route would have been repeated to obtain a large enough quantity of hydroxy-acid **1.71** to allow for completion of the asymmetric synthesis.



Scheme 1.11: *Reagents and conditions*: a) KOH, MeOH/H_2O, 65 °C.

With the racemic synthesis completed for model system **1.57**, the synthesis of (±)-vibralactone (**1.01**) was explored. Primary alcohols have previously been shown to degrade Grubbs I and II catalysts by formation of hydride species.[54,55] Therefore protected triene analogues **1.77**, **1.78** and **1.79** were prepared (**Scheme 1.12**). Tin reagent **1.72** was protected with both an acyl and TES group in good yield. The diastereoselective allylation procedure was then applied to aldehyde **1.58** using tin reagents **1.73** and **1.74** to obtain trienes **1.75** and **1.76** in fairly low yield. Protection of the secondary alcohol then afforded protected triene analogues **1.77**, **1.78** and **1.79**.



1.72

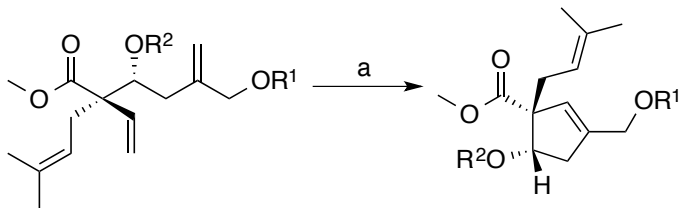
R^1= Ac, **1.73** 90%
= TES, **1.74** 96%

R^1= Ac, **1.75** 31%
= TES, **1.76** 40%

R^1= Ac, R^2 = Ac **1.77** 42%
R^1= Ac R^2 = TES **1.78** 78%
R^1= TES R^2 = TES **1.79** unpurified

Scheme 1.12: *Reagents and conditions:* a) AcCl, NEt_3, CH_2Cl_2, 0 °C; b) TESCl, NEt_3, CH_2Cl_2, 0 °C; c) aldehyde **1.58**, $MgBr_2$•OEt_2, −78 °C to −40 °C; d) AcCl, DMAP, NEt_3, CH_2Cl_2, 0 °C; e) TESOTF, NEt_3, CH_2Cl_2, 0 °C to rt; f) TESOTf, NEt_3, DMAP, CH_2Cl_2, 0 °C to rt.

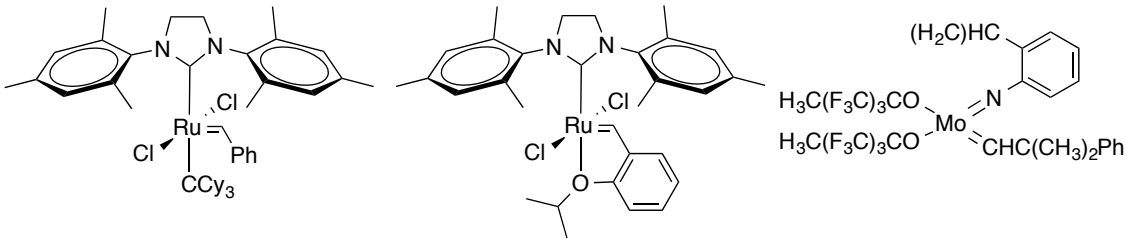
Unfortunately, after significant investigation, no suitable conditions for the RCM of **1.77-1.79** were discovered (**Scheme 1.13**). Grubbs II, Hoveyda-Grubbs II and Schrock's catalyst (**Figure 1.12**) were tested along with a range of different temperatures. All conditions showed poor conversion and some also experienced significant degradation.



R^1= Ac, R^2 = Ac **1.77**
R^1= Ac R^2 = TES **1.78**
R^1= TES R^2 = TES **1.79**

R^1= Ac, R^2 = Ac **1.80**
R^1= Ac R^2 = TES **1.81**
R^1= TES R^2 = TES **1.82**

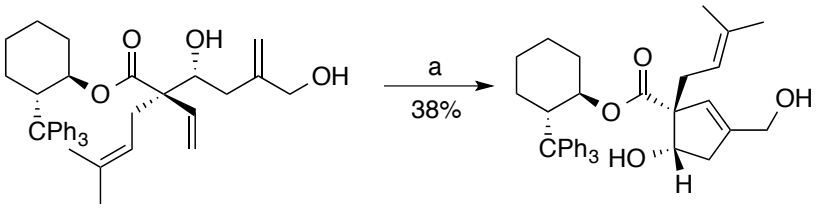
Scheme 1.13: *Reagents and conditions:* a) Metathesis cat., CH_2Cl_2, rt or reflux.



1.83 **1.84** **1.85**

Figure 1.12: Metathesis catalysts: Grubbs 2nd generation catalyst (**1.83**), Hoveyda-Grubbs 2nd generation catalyst (**1.84**) and Schrock's catalyst (**1.85**).

The RCM was achieved with TTC analogue **1.86**, however the yield was too low to provide an efficient route to vibralactone (**1.01**). Due to the difficulty completing the total synthesis of vibralactone using a RCM strategy, efforts transferred to an aldol condensation approach.



1.86 **1.87**

Scheme 1.14: *Reagents and conditions:* a) Grubbs II, CH_2Cl_2, reflux.

1.5.2 Aldol Condensation Approach to (±)-Vibralactone

Snider *et al.* demonstrated in their total synthesis of vibralactone that an aldol condensation was a suitable method for ring closure.[2,3] It was therefore decided to replace the RCM reaction with an aldol condensation in the Brown group's developed sequence (**Figure 1.13**). The vibralactone precursor **1.88** could be accessed from dialdehyde **1.89**. **1.89** could be provided by the appropriate oxidation of allyl derivative **1.90**, from which the relative stereochemistry could be defined using the diastereoselective allylation procedure described previously (**section 1.5.1**).

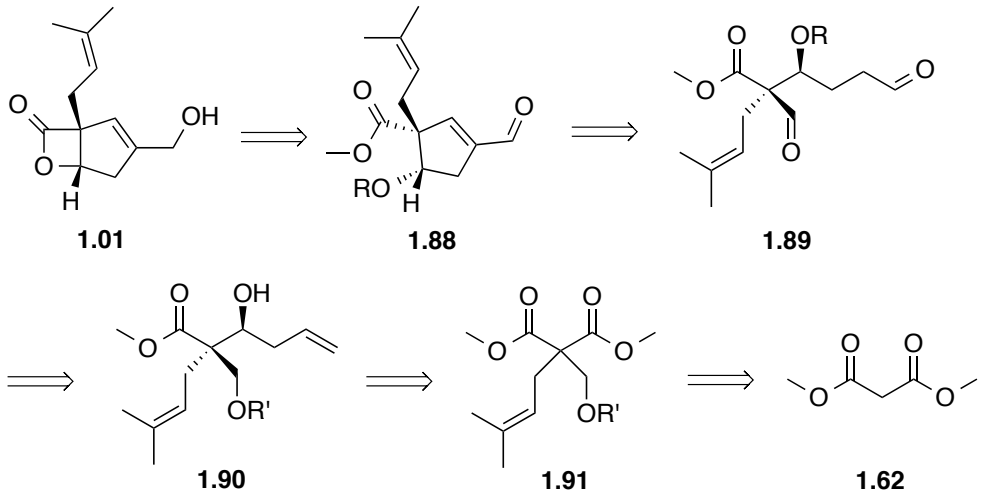
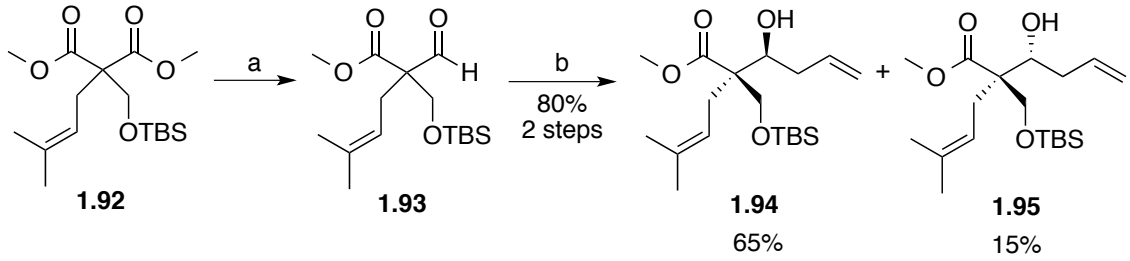


Figure 1.13: Retrosynthesis of vibralactone (**1.01**) using an aldol strategy.

The diastereoselective allylation was applied to aldehyde **1.93** to afford **1.94** and **1.95** as a separable mixture of diastereoisomers (3.8:1 by ^{1}H NMR) in high yield (**Scheme 1.15**). The selectivity of the reaction can be explained using the same flattened boat model presented previously, where the silyl hydroxy ether adopts a pseudoequatorial position (**Figure 1.14**).



Scheme 1.15: *Reagents and conditions:* a) DIBAL, CH_2Cl_2, −78 °C, b) $MgBr_2$•OEt_2, allyltributyltin, CH_2Cl_2, −78 °C.

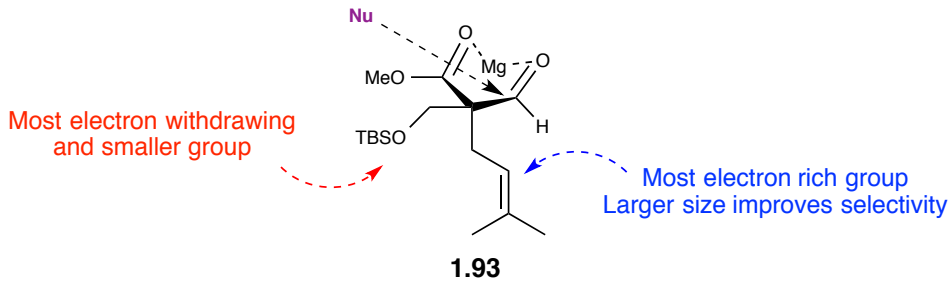
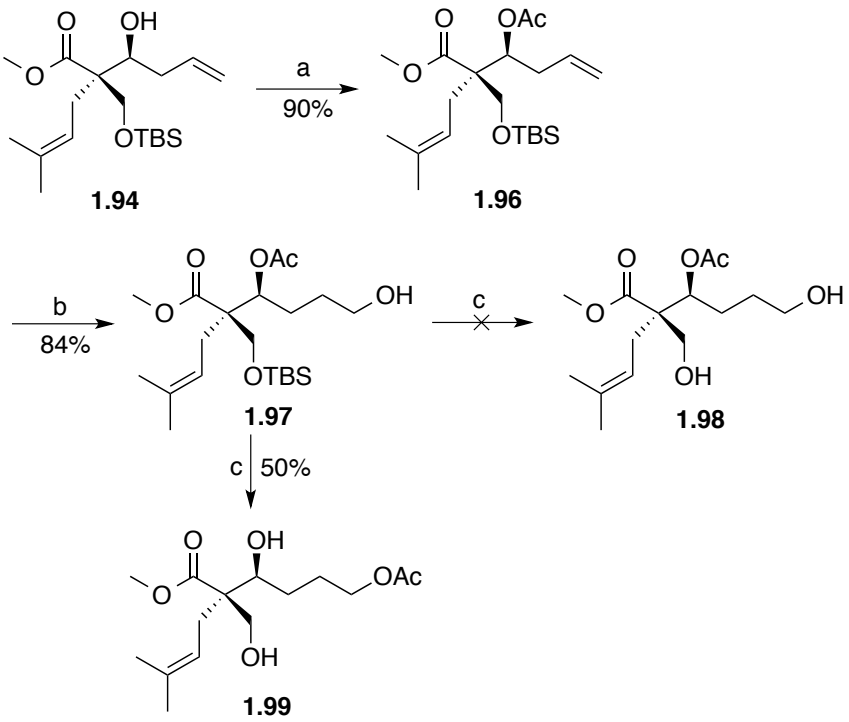


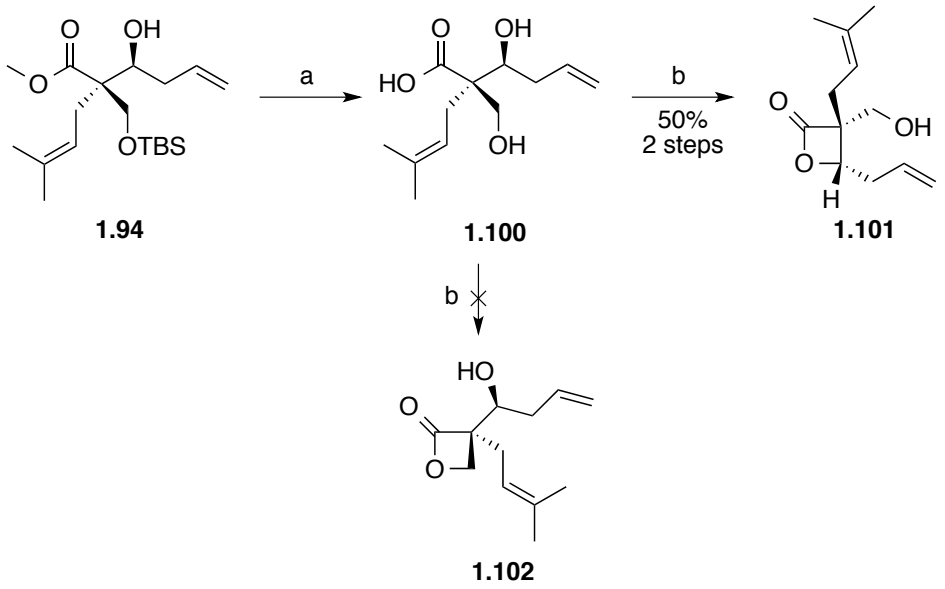
Figure 1.14: Flattened boat model for the diastereoselective allylation of **1.93**.

With allylation product **1.94** in hand, secondary alcohol protection and hydroboration of the terminal alkene afforded **1.97** in high yield (**Scheme 1.16**). The TBS group was successfully removed with TBAF, however acyl migration product **1.99** was isolated. Alternative conditions such as TFA, HF•pyridine and HF in acetonitrile all proved unsuccessful in preventing the acyl migration.



Scheme 1.16: *Reagents and conditions:* a) Ac_2O, DMAP, DIPEA, CH_2Cl_2, 0 °C to rt; b) i) 9-BBN, THF, 0 °C to rt; ii) NaOAc, H_2O_2, H_2O, 0 °C to rt; c) TBAF, THF 0 °C to rt.

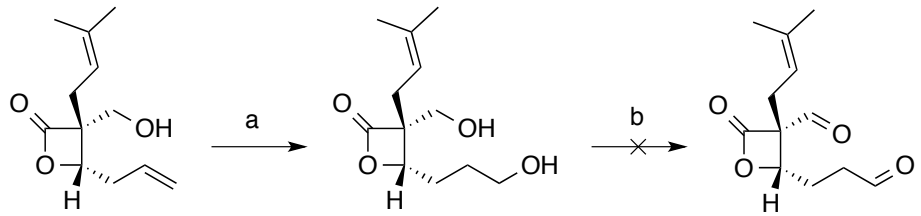
An internal 'protection' strategy was used in which the secondary alcohol was converted to β-lactone **1.101** (**Scheme 1.17**). Hydrolysis of methyl ester **1.94** proceeded efficiently, with TBS deprotection also observed. A regioselective lactonisation of **1.100** gave lactone **1.101** in high yield, with no evidence of alternatively cyclised product **1.102**. The reason for this observed selectivity is currently unknown.



1.94 **1.100** **1.101** **1.102**

Scheme 1.17: *Reagents and conditions:* a) LiOH.H_2O, 50 °C; b) *p*-TsCl, pyridine, 0 °C to 5 °C.

Hydroboration of lactone **1.101** seemed promising by analysis of the crude product however, due to difficulty separating **1.103** from a hydroboration by-product and the instability of the diol, purification was unsuccessful. Unfortunately Swern oxidation of crude diol **1.103** to dialdehyde **1.104** formed a complex mixture of products.



1.101 **1.103** **1.104**

Scheme 1.18: *Reagents and conditions:* a) i) 9-BBN, THF, 0 °C to rt; ii) NaOAc, H_2O_2, H_2O, 0 °C to rt; b) Oxalyl chloride, DMSO, NEt_3, CH_2Cl_2, −78 °C to rt.

Chapter 2: Total Syntheses of (±)-Vibralactone

2.1 Synthetic Strategy

Our strategy for completing the total synthesis of vibralactone (**1.01**) was to employ previously successful reactions from Robert Heap's studies, whilst avoiding known problems such as protecting group migrations and the instability of certain scaffolds. Previous work had shown that advanced lactone intermediate **1.101** could be created in high overall yield, however initial attempts at converting this to dialdehyde **1.104** were unsuccessful. If this dialdehyde could be accessed, it was expected that the total synthesis could be completed by an aldol condensation and reduction of the resulting aldehyde. It was therefore proposed that an alternative synthesis of dialdehyde **1.104** could come from alkyne analogue **2.02** (**Figure 2.1**), which could be accessed by altering the synthesis of **1.94** (**section 1.5.2**). The stereochemistry of the all-carbon quaternary centre could be controlled by a chiral auxiliary and the secondary alcohol centre would be addressed by a diastereoselective propargylation. Installing an alkyne would allow for the direct conversion to the aliphatic aldehyde of **1.104** by a hydroboration. A final aldol condensation of dialdehyde **1.104**, followed by reduction of the resulting aldehyde, would complete the synthesis of vibralactone (**1.01**).

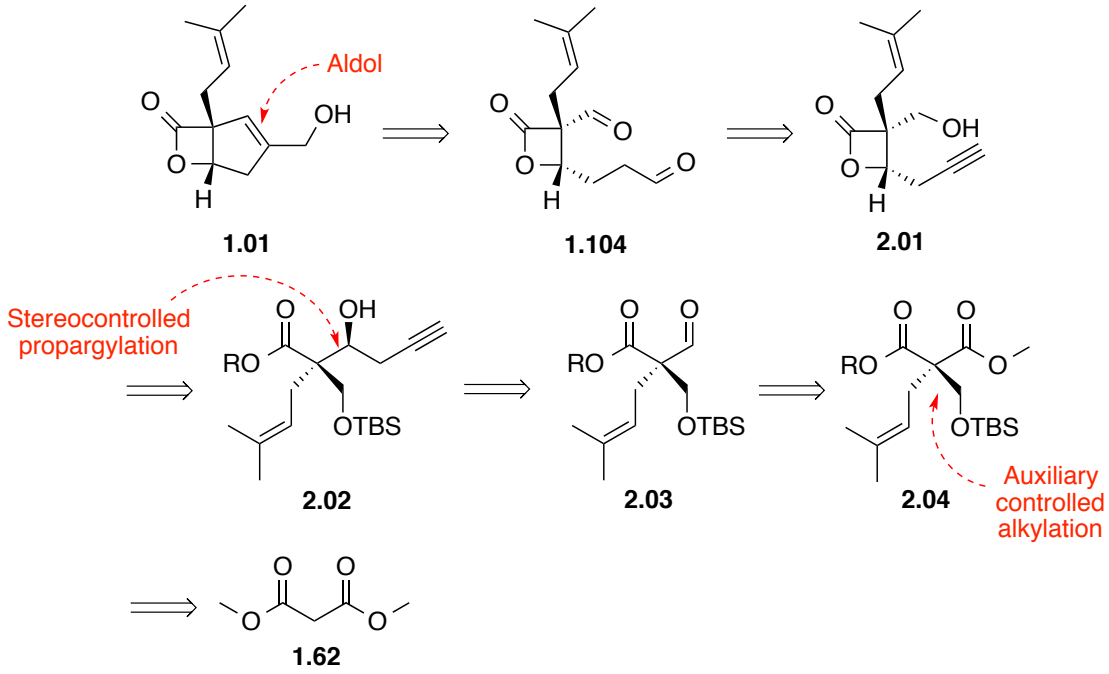
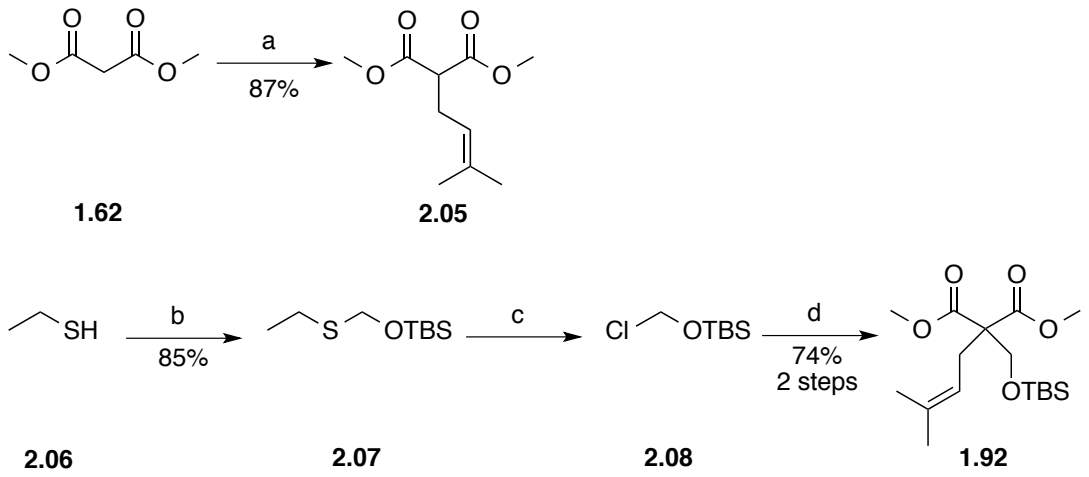


Figure 2.1: Initial synthetic strategy for vibralactone (**1.01**).

2.2 Synthesis of Racemic Alcohol Scaffold 2.09

Preliminary investigations focussed on the preparation of racemic alkyne scaffold **2.09** (**Figure 2.1**). Silyl protection of the primary alcohol was chosen as similar substrates have been investigated by Linclau *et al*.[51] Dimethyl malonate **1.62** was first alkylated with prenyl bromide in good yield using K_2CO_3 (**Scheme 2.1**). An excess of dimethyl malonate **1.62** was used to avoid over-alkylation, which was seen extensively when using NaH as a base. The second alkylation was then achieved with unstable chloromethyl electrophile **2.08**, prepared from paraformaldehyde and ethanethiol (**2.06**).[56]



Scheme 2.1: *Reagents and conditions:* a) K_2CO_3, prenyl bromide, acetone, rt; b) i) paraformaldehyde, NaOMe, 40 °C; ii) TBSCl, imidazole, CH_2Cl_2, rt; c) SO_2Cl_2, CH_2Cl_2, 0 °C; d) **2.08**, NaH. THF, 0 °C to rt.

The reduction of malonate **1.92** by very slow addition of DIBAL at −78 °C provided unstable aldehyde **1.93** (**Scheme 2.2**), which was immediately subjected to the propargylation reaction. A 4:1 mixture of alcohol isomers **2.09** and **2.10** was afforded in moderate yield, however only major isomer **2.09** was isolated successfully. NOESY NMR could be used to prove the stereochemistry of **2.09** once the lactone has been formed, although the stereochemical outcome of the reaction was tentatively assigned based upon the results described in **Scheme 1.15**.

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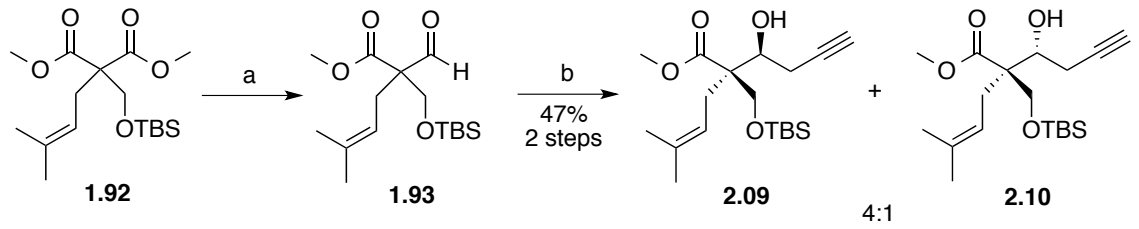
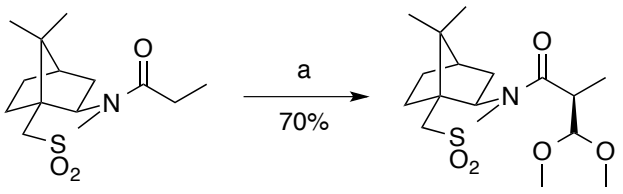


Scheme 2.2: *Reagents and conditions:* a) DIBAL, CH_2Cl_2, −78 °C; b) $MgBr_2 \bullet OEt_2$, allenyltributyltin, CH_2Cl_2. −78 °C.

Unfortunately on a larger scale, the sequence of steps (**Scheme 2.2**) resulted in a very poor overall yield of 20-25%, which was mainly due to the formation of an unidentified by-product during the reduction. Attempted optimisation of the reduction reaction, by varying the number of equivalents of DIBAL used and the quenching conditions, proved unsuccessful in improving the overall yield of alcohol **2.09**. Although it has not been identified, the by-product is thought to originate from some TBS cleavage and as a result this makes **1.92** an unsuitable substrate for desymmetrization with DIBAL.

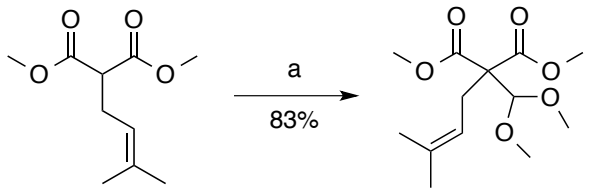
2.3 Synthesis of Racemic Alcohol Scaffolds 2.15 and 2.16

Due to the difficulties with malonate derivative **1.92** (**Scheme 2.2**), an alternative to the TBS-hydroxymethyl group was explored. The unique addition of a dimethoxy acetal by soft enolisation with $TiCl_4$ and DIPEA was reported by Evans *et al.* in 1990 (**Scheme 2.3**).[57] This reaction is particularly interesting as a masked aldehyde was required for the synthesis of vibralactone.



Scheme 2.3: *Reagents and conditions:* a) $TiCl_4$, DIPEA, $CH(OMe)_3$, CH_2Cl_2, 0 °C.

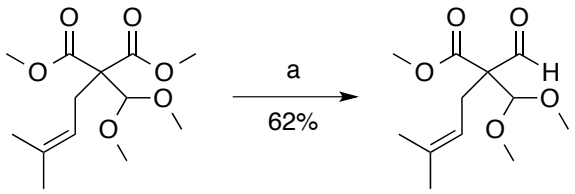
Prenyl malonate **2.05** reacted rapidly with trimethylorthoformate in the presence of $TiCl_4$ and NEt_3 to afford the novel substituted malonate **2.12** in good yield (**Scheme 2.4**). To the best of our knowledge this is the first time that this reaction has been used in malonic systems, and provides a highly functionalised intermediate for further elaboration.



2.05 **2.13**

Scheme 2.4: *Reagents and conditions:* a) $TiCl_4$, DIPEA, $CH(OMe)_3$, CH_2Cl_2, 0 °C to rt.

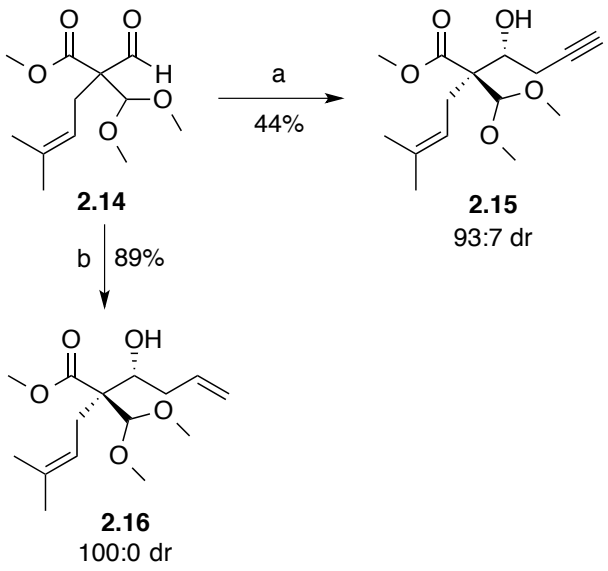
With substituted malonate **2.13** in hand, mono-reduction to aldehyde **2.14** with DIBAL was achieved cleanly and in moderate isolated yield (**Scheme 2.5**). Compared to TBS ether analogue **1.92**, aldehyde **2.14** was fairly stable and purification was possible by silica gel chromatography. In addition, it is important to note that an additional equivalent of the reducing agent was required in the reaction, which was attributed to coordination of DIBAL to the dimethoxyacetal. This coordination apparently made the first equivalent of DIBAL unavailable for reaction with the ester.



2.13 **2.14**

Scheme 2.5: *Reagents and conditions:* a) DIBAL, CH_2Cl_2, −78 °C.

The diastereoselective propargylation with allenyltributyltin (**Scheme 2.6**) afforded alkyne **2.15** in moderate to low yield but with good stereoselectivity (dr = 93:7 – major isomer isolated only). No reaction was observed at −78 °C so it was allowed to warm to −41 °C in a MeCN/cardice bath and the starting material was fully consumed after 3 h. Due to the low yield of the propargylation, an allylation was attempted with great success. Alkene **2.16** was afforded in excellent yield and selectivity, with rapid conversion to the product at −78 °C.



Scheme 2.6: *Reagents and conditions:* a) $MgBr_2 \bullet OEt_2$, allenyltributyltin, CH_2Cl_2, −78 °C to −41 °C; b) $MgBr_2 \bullet OEt_2$, allyltributyltin, CH_2Cl_2, −78 °C.

Interestingly the stereochemistry of **2.15** and **2.16** was reversed compared to TBS ether analogue **2.09**. The relative configuration of the alcohol and acetal was proved by NOESY NMR of the cyclic intermediates formed later in the synthesis (**Figure 2.8**). The stereoselectivity can be explained by chelation of the acetal group after addition of $MgBr_2$ (**Figure 2.2**).[51] This coordination blocked the *si*-face so that attack of the bulky tin reagent occured predominately from the *re*-face.

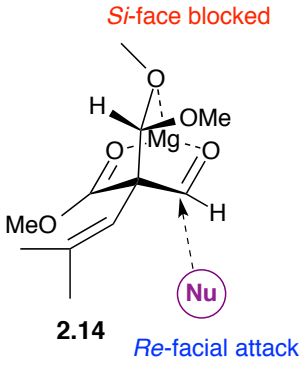
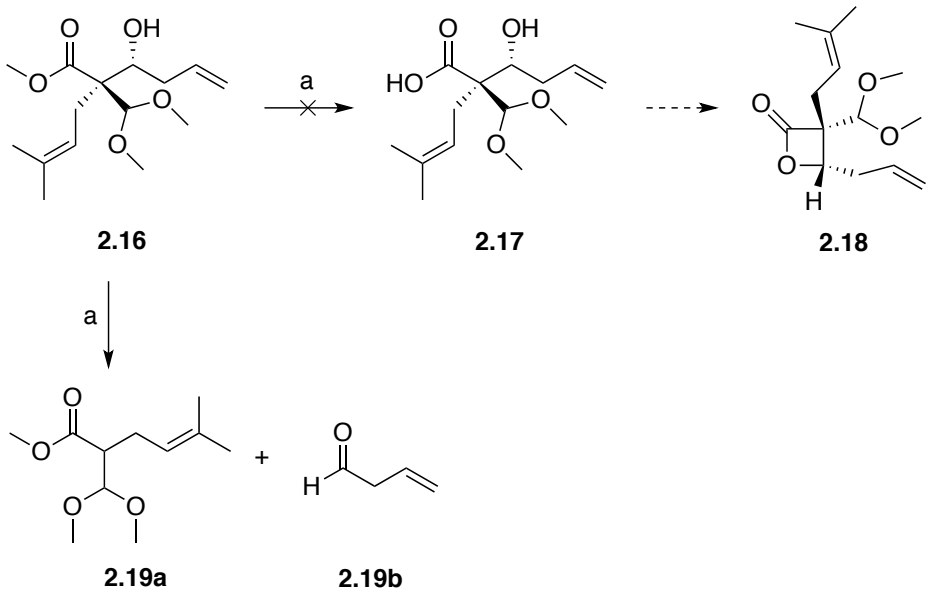


Figure 2.2: Proposed chelation model to account for the observed stereochemistry.[51]

2.4 Closure of the [3.2.0] Bi-cycle

2.4.1 Lactone Closure from Methyl Ester 2.16

With alcohols **2.15** and **2.16** in hand, completion of the bicyclic system of (±)-vibralactone (**1.01**) was explored. To form lactone **2.18**, hydroxy-acid **2.17** first had to be accessed (**Scheme 2.7**). Unfortunately the base-catalysed hydrolysis of methyl ester **2.16** resulted in retro-aldol product **2.19a**, which proved difficult to purify (**Figure 2.3**). Aldehyde fragment **2.19b** was not observed.



2.16 **2.17** **2.18**

2.19a **2.19b**

Scheme 2.7: *Reagents and conditions:* a) $LiOH.H_2O$, $MeOH/H_2O$, rt.

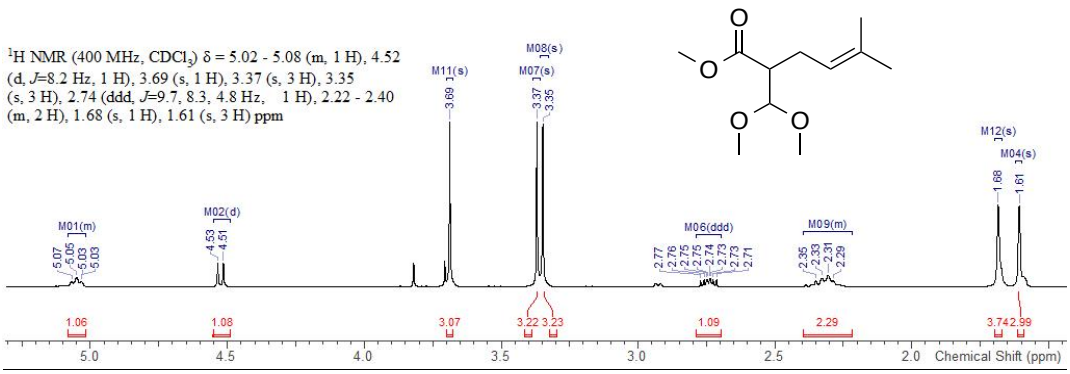
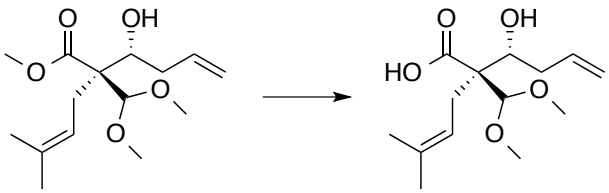


Figure 2.3: ^1H NMR of impure retro-aldol product **2.19**.

Mild hydrolysis methods were investigated without success (**Scheme 2.8**). Hydrolysis with LiBr and NEt_3,[58] Me_3SnOH[59] and various enzymes resulted in no conversion to hydroxy-acid **2.17**. As an alternative to hydrolysis, a demethylation with $AlBr_3$ in

tetrahydrothiophene[60] was employed. Although trace hydroxy-acid product **2.17** was observed by HPLC-MS, the reaction yielded a complex mixture of products.



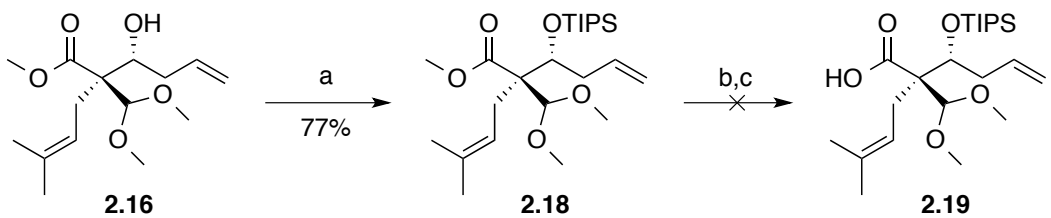
2.16 **2.17**

Scheme 2.8: See **Table 2.1** for reagents and conditions

Entry	Reagent	Solvent	Temp/ºC	Time/h	Product yield/%
1	LiBr, NEt_3	MeCN (2 vol % H_2O)	25	96	0
2	Me_3SnOH	1,2-Dichloroethane	60	96	0
3	Lipase B from *Candida Antarctica*	1,4-Dioxane/ ^{t}BuOH	25	72	0
4	Lipase from Porcine Pancreas	Acetone/Phosphate Buffer (pH 7.4)	25	24	0
5	Pig Liver Esterase	Acetone/Phosphate Buffer (pH 7.4)	25	24	0
6	$AlBr_3$	THP	25	72	~5

Table 2-1: Table of alternative reaction conditions for the formation of hydroxy-acid **2.17** (**Scheme 2.8**).

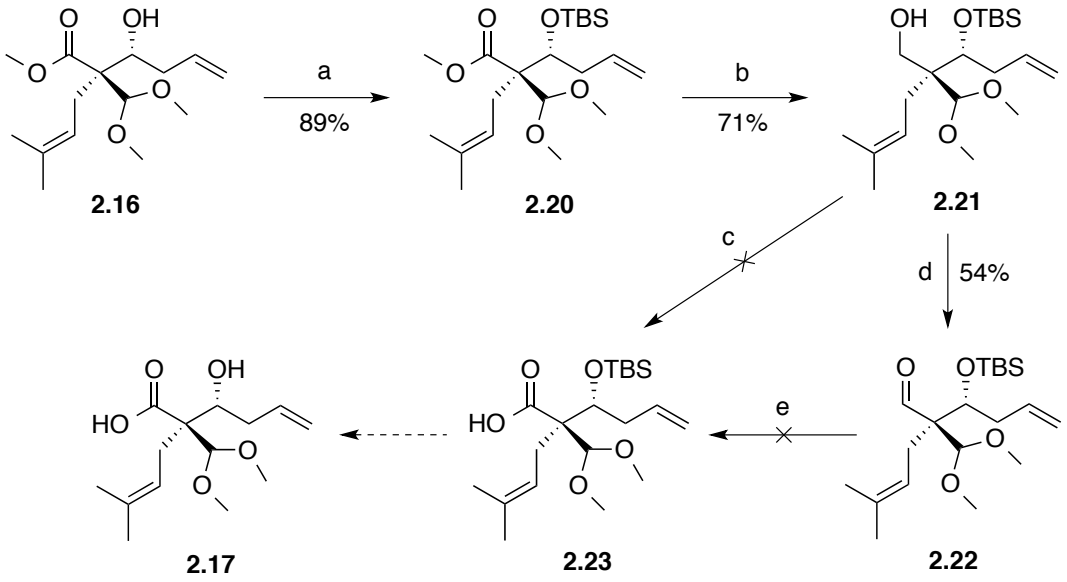
As mild hydrolysis conditions were unsuitable, more forcing conditions were explored. Alcohol **2.16** was first protected with TIPS to prevent a retro-aldol reaction (**Scheme 2.9**). **2.18** was then heated with KOH and LiOOH (**Figure 2.10**), which resulted in no hydrolysis and only starting material was recovered. It became apparent that the system was too hindered for the ester to be cleaved at the carbonyl position under practical conditions.



2.16 **2.18** **2.19**

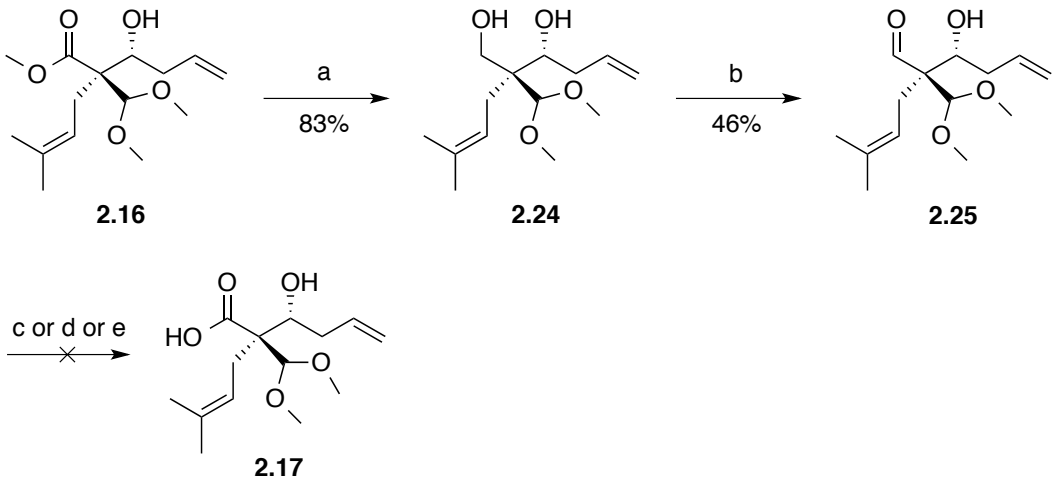
Scheme 2.9: *Reagents and conditions:* a) TIPS-OTf, NEt_3, DMAP, CH_2Cl_2, 0 °C to rt; b) KOH, THF/H_2O, 60 °C; c) LiOH, H_2O_2, THF/H_2O, 60 °C.

Finally a reduction and re-oxidation route to hydroxy-acid **2.17** was considered (**Scheme 2.10**). After protecting alcohol **2.16**, the ester was reduced with DIBAL to afford primary alcohol **2.20**. It is important to note that reduction with $LiAlH_4$ resulted in TBS deprotection. A direct oxidation to carboxylic acid **2.23** with NMO monohydrate and TPAP[61] was unsuccessful so a stepwise approach was investigated. A Ley-Griffith oxidation provided aldehyde **2.22** in moderate un-optimised yield. Pinnick oxidation to carboxylic acid **2.23** unfortunately resulted in a complex mixture of products.



Scheme 2.10: *Reagents and conditions:* a) TBS-OTf, NEt_3, DMAP, CH_2Cl_2, 0 °C to rt; b) DIBAL, CH_2Cl_2, 0 °C to rt.; c) NMO•H_2O, TPAP, MeCN; d) TPAP, NMO, 4 Å, CH_2Cl_2, rt; e) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4, $^tBuOH/H_2O$, 0 °C to rt.

It was thought that the TBS group of **2.22** may be causing problems with the Pinnick oxidation. Therefore hydroxy-aldehyde **2.25** was prepared. Ester **2.16** was reduced to diol **2.24** and a selective oxidation of the primary alcohol using TEMPO and $PhI(OAc)_2$[62] gave access to **2.25** in moderate un-optimised yield. Again, however, oxidation to carboxylic acid **2.17** was hampered due to significant degradation under Pinnick conditions (2-methyl-2-butene and H_2O_2 scavengers) and silver oxide.



Scheme 2.11: *Reagents and conditions:* a) $LiAlH_4$, THF, 0 °C to rt; b) TEMPO (10 mol %), $PhI(OAc)_2$, CH_2Cl_2, rt; c) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4, $^tBuOH/H_2O$, 0 °C to rt; d) $NaClO_2$, H_2O_2, NaH_2PO_4, $MeCN/H_2O$, 0 °C to rt; e) Ag_2O, NaOH, ethanol/H_2O, rt.

2.4.2 Synthesis of Aldehyde Scaffold 2.26

Difficulty accessing lactone **2.18** was impeding completion of the total synthesis via the originally proposed route (**Figure 2.1**). It was therefore proposed that the cyclopentene ring could be formed first, prior to the β-lactone synthesis, from aldehyde intermediate **2.26** (**Figure 2.4**). An aldol condensation may be possible from **2.26** or the corresponding dialdehyde.

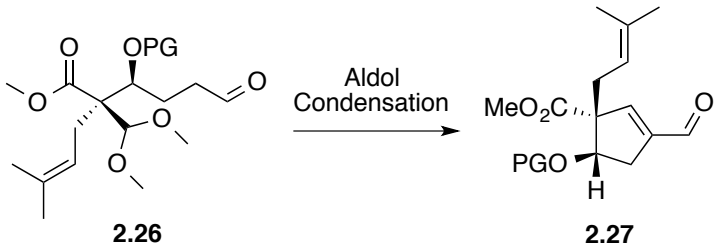
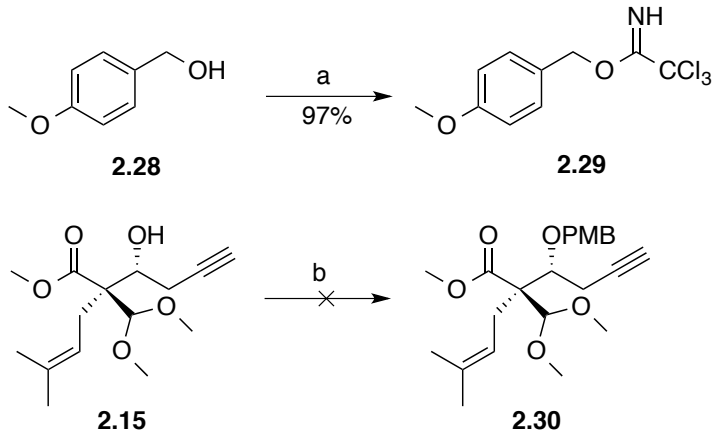


Figure 2.4: Proposed aldol condensation from aldehyde intermediate **2.26**.

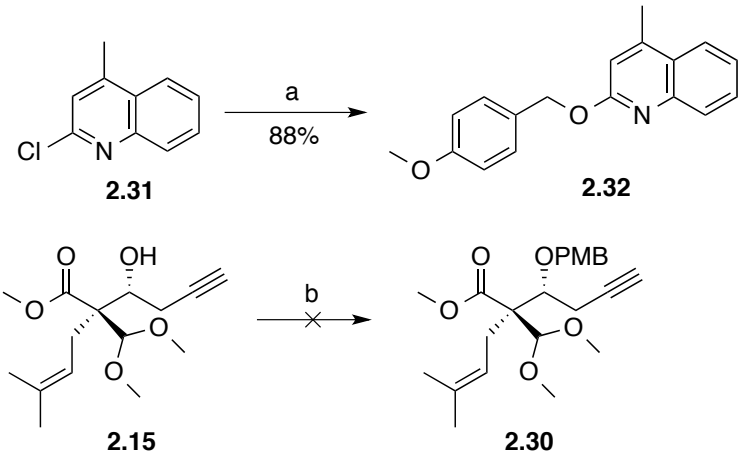
Aldehyde **2.26** could potentially be accessed by the hydroboration of previously synthesised alkyne intermediate **2.15**. It was first necessary to protect the secondary alcohol to avoid a possible lactol ring closure after hydroboration. A *p*-methoxybenzyl (PMB) protecting group was first explored due to the mild deprotection conditions (DDQ) required later in the synthesis. Alkylation with PMB-Cl and strong base was not appropriate for the protection as this would result in the retro-aldol reaction seen previously (**Scheme 2.7**). Acid-catalysed protection using the trichloroacetimidate **2.29** resulted in a complex mixture of products with no protected alcohol **2.30** observed

(**Scheme 2.12**). This was possibly due to deprotection of the acetal group, which led to side reactions.



Scheme 2.12: *Reagents and conditions:* a) i) NaH, Et_2O, 0 °C to rt ii) $NCCl_3$, Et_2O, 0 °C to rt; b) **2.15**, CSA (10 mol %), CH_2Cl_2.

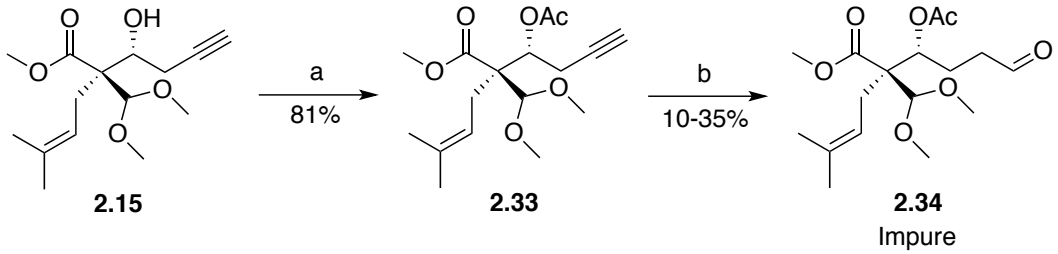
As both acidic and basic conditions were unsuitable for PMB protection, a procedure by Nwoye *et al.*, which uses neutral conditions, was examined.[63] It is reported that quinoline **2.32**, prepared by a condensation reaction between 2-chlorolepidine (**2.31**) and 4-methoxybenzylalcohol (**2.28**) (**Scheme 2.13**), is methylated by MeOTf and then reacts with alcohols in a similar manner to that observed with trichloroacetimidates. Unfortunately no reaction was observed with alcohol **2.15**.



Scheme 2.13: *Reagents and conditions:* a) 4-methoxybenzylalcohol, KOH, 18-crown-6, toluene, Δ, Dean & Stark; b) **2.32**, MgO, MeOTf, toluene.

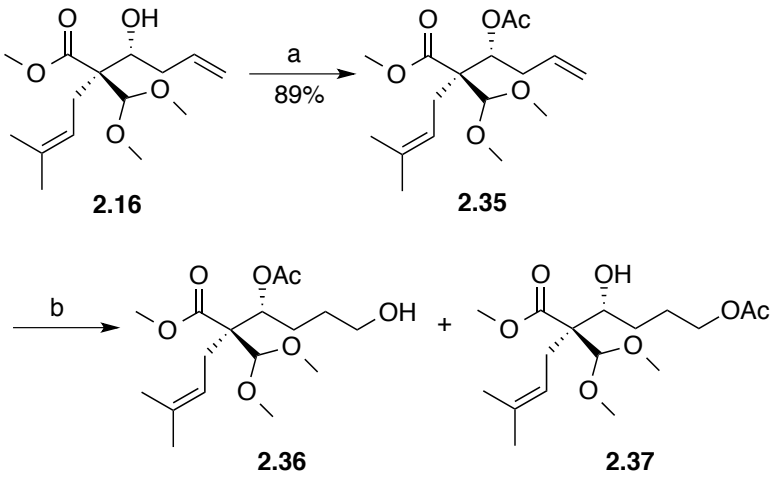
As alcohol **2.15** could not be protected with PMB, an acyl group was utilised. Acylation of alcohol **2.15** with acetic anhydride afforded protected intermediate **2.33** in good yield (**Scheme 2.14**). The hydroboration of terminal alkynes typically requires bulky di-substituted boranes to prevent a second borane addition to the boron-substituted alkene

intermediate. The hydroboration of alkyne **2.33** was performed with disamylborane followed by oxidation with sodium perborate. Unfortunately, aldehyde **2.34** was obtained in poor yield (~10-35%) and a pure sample could not be obtained. Sodium perborate, a mild oxidising reagent,[64] was required for the oxidation step as H_2O_2 and NaOH resulted in significant degradation.



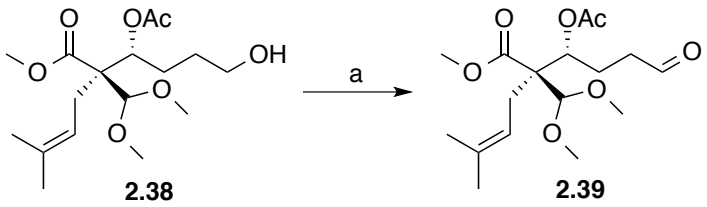
Scheme 2.14: *Reagents and conditions:* a) Ac_2O, DMAP, NEt_3, CH_2Cl_2, 40 °C; b) i) disamylborane, THF, 0 °C to rt; ii) $NaBO_3.4H_2O$, H_2O, 50 °C.

As the hydroboration of alkyne **2.15** proved unworkable, synthesis of aldehyde **2.34** was explored from alkene **2.16** (**Scheme 2.15**). Hydroboration of **2.35** afforded an inseparable mixture of desired alcohol **2.36** (major product) and ~10% of migration product **2.37**. Alcohol **2.36** was highly acid sensitive and yields (taking into account the purity of the isolated product) ranged from 10-48%.



Scheme 2.15: *Reagents and conditions:* a) Ac_2O, NEt_3, DMAP, CH_2Cl_2, rt; b) i) 9-BBN, THF, 0 °C to rt; ii) H_2O_2, NaOAc, H_2O, 0 °C to rt.

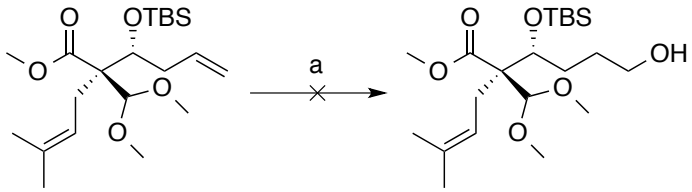
Impure alcohol **2.38** was oxidised to aldehyde **2.39** in approximately 35% yield, although it was not possible to fully separate **2.39** from impurities generated during the oxidation and the previous hydroboration step (**Scheme 2.16**).



2.38 **2.39**

Scheme 2.16: *Reagents and conditions:* a) TPAP, NMO, 4 Å sieves, CH_2Cl_2, rt.

Finally the hydroboration of TBS protected analogue **2.20** was attempted with BH_3, however it resulted in a complex mixture of products (**Scheme 2.17**). No reaction was seen with 9-BBN. As access to aldehyde **2.39** in reasonable yield and purity was not possible, the synthetic route was abandoned, and a re-evaluation of our strategy took place.



2.20 **2.40**

Scheme 2.17: *Reagents and conditions:* a) i) BH_3.THF, THF, 0 °C to rt; ii) H_2O_2, NaOAc, H_2O, 0 °C to rt.

This section has focussed on the closure of the [3.2.0] bi-cycle of vibralactone (**1.01**) from highly functionalised intermediate **2.16**. Cleavage of the methyl ester of **2.16** was unsuccessful, preventing access to hydroxy-acid **2.17** and subsequent β-lactone closure with inversion of stereochemistry (**Figure 2.5**). An alternative pathway was pursued by an aldol condensation of **2.39**, or its dialdehyde equivalent, to close the cyclopentene ring of vibralactone (**1.01**). Unfortunately, low yielding reactions and the instability of intermediates prevented an efficient synthesis of **2.39** from **2.16**. At this point a strategic re-revaluation of the synthetic route was required.

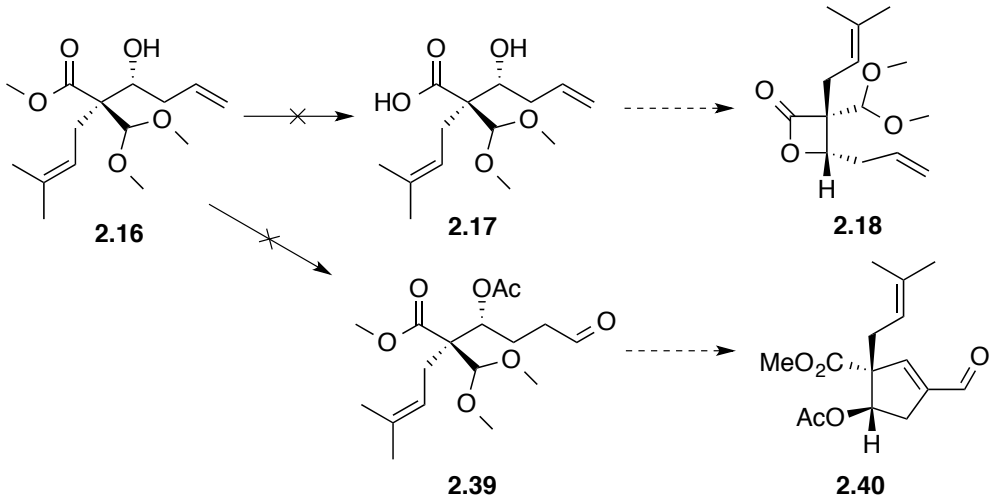


Figure 2.5: Evaluation of the unsuccessful syntheses of hydroxy-acid **2.17** and aldehyde **2.39**, which are acyclic precursors to key cyclised intermediates **2.18** and **2.40**.

2.4.3 Strategic Re-Evaluation Leading to the Synthesis of (±)-Vibralactone

The originally proposed cyclisation pathway focused on closure of the cyclopentene ring from the alkene and acetal of **2.16** (**Figure 2.6**). As the ester and protected aldehyde moieties of **2.16** are bound to the same chiral centre, either of these carbonyl derivatives could be used to form the lactone and cyclopentene. Therefore it is also possible to form the cyclopentene ring from the alkene and ester group of **2.16** and the lactone from the secondary alcohol and acetal. However, this would require consideration of the relative stereochemical relationships within the intermediates: one route requires lactonisation with inversion of stereochemistry (blue) and the other with retention (red) (**Figure 2.6**). Each route would form a different enantiomer of vibralactone from a common enantiopure intermediate such as **2.16**. However preliminary investigations using racemic material will converge and therefore the two pathways are equivalent.

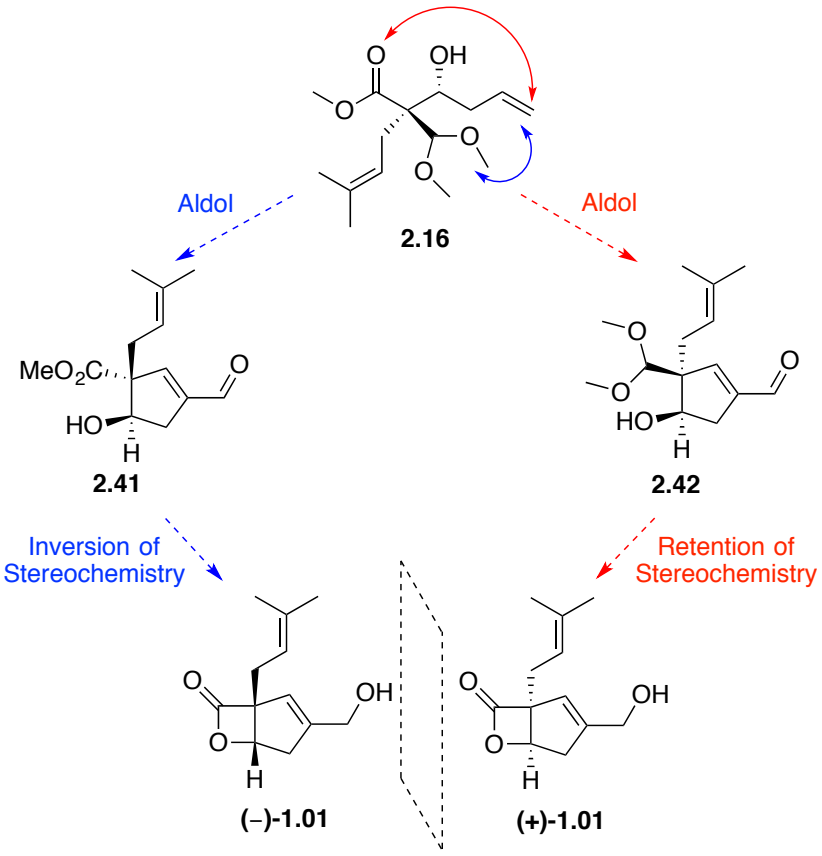


Figure 2.6: Original cyclisation strategy (blue) and re-evaluated strategy (red).

The β-lactone would be formed by a series of functional group interconversions from cyclopentene **2.43** (**Figure 2.7**). The relative stereochemistry of the secondary alcohol would be suitable for lactonisation with retention of stereochemistry using *p*-TsCl. The cyclopentene ring could be fashioned by an aldol condensation of dialdehyde **2.44**, which is accessible from the previously synthesised intermediate **2.16**.

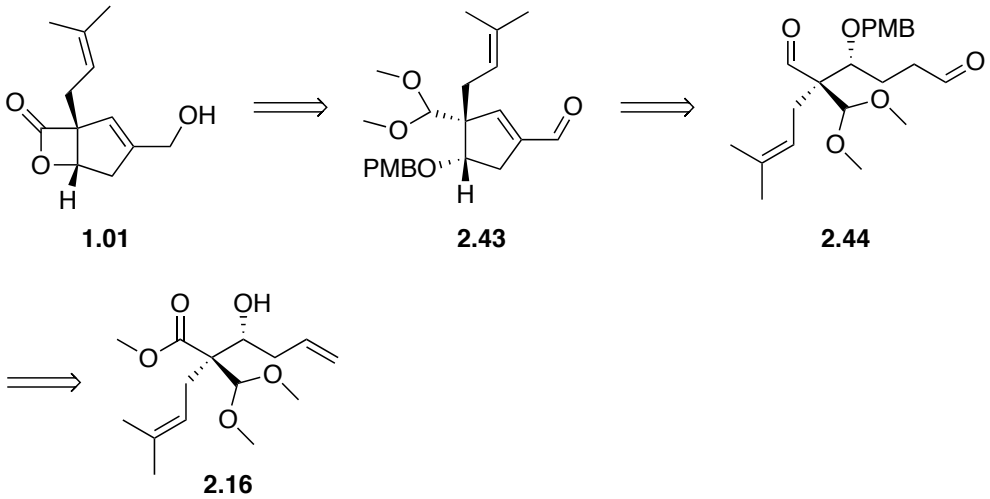
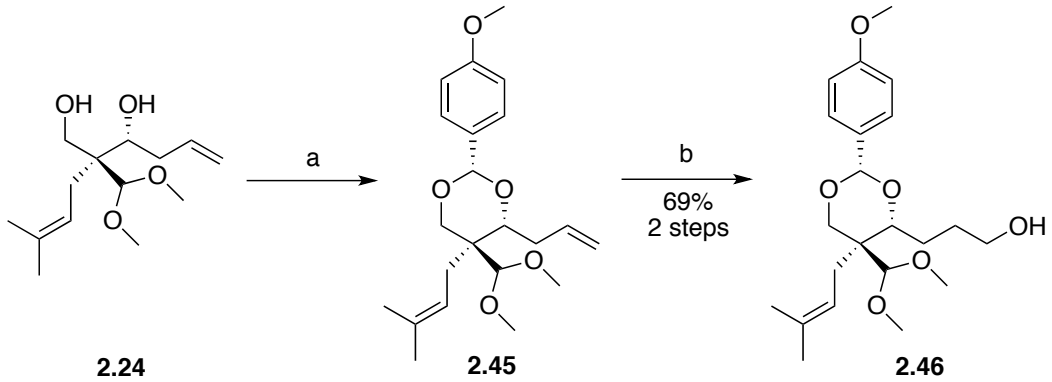


Figure 2.7: Retrosynthetic analysis of an alternative cyclisation pathway.

The synthesis of key dialdehyde intermediate **2.44** began from the previously synthesised diol **2.24** (**Scheme 2.18**). Benzylidene protection afforded acetal **2.45** in quantitative yield. Hydroboration of the terminal alkene then provided alcohol **2.46**. The increased stability conferred upon the system by benzylidene protection gave rise to a far cleaner reaction than with the acetate protected intermediate **2.35**. It is important to note however, that a small amount of hydroboration was observed on the prenyl group double bond, although it was not possible to isolate this side-product.



2.24 **2.45** **2.46**

Scheme 2.18: *Reagents and conditions:* a) Anisaldehyde dimethyl acetal, CSA, CH_2Cl_2, rt; b) i) 9-BBN, THF, 0 °C to rt; ii) H_2O_2, 3 M NaOH, 0 °C to rt.

Two diastereoisomers of cyclic benzylidene intermediate **2.46** could be accessed from **2.24** with the correct relative stereochemistry of the adjacent tertiary and quaternary centres. There are also two possible conformations of each diastereoisomer; **2.46b** and **2.46d** being the most stable (**Figure 2.8**). NOESY NMR data provided evidence for the indicated relative stereochemistry (**2.46b**).

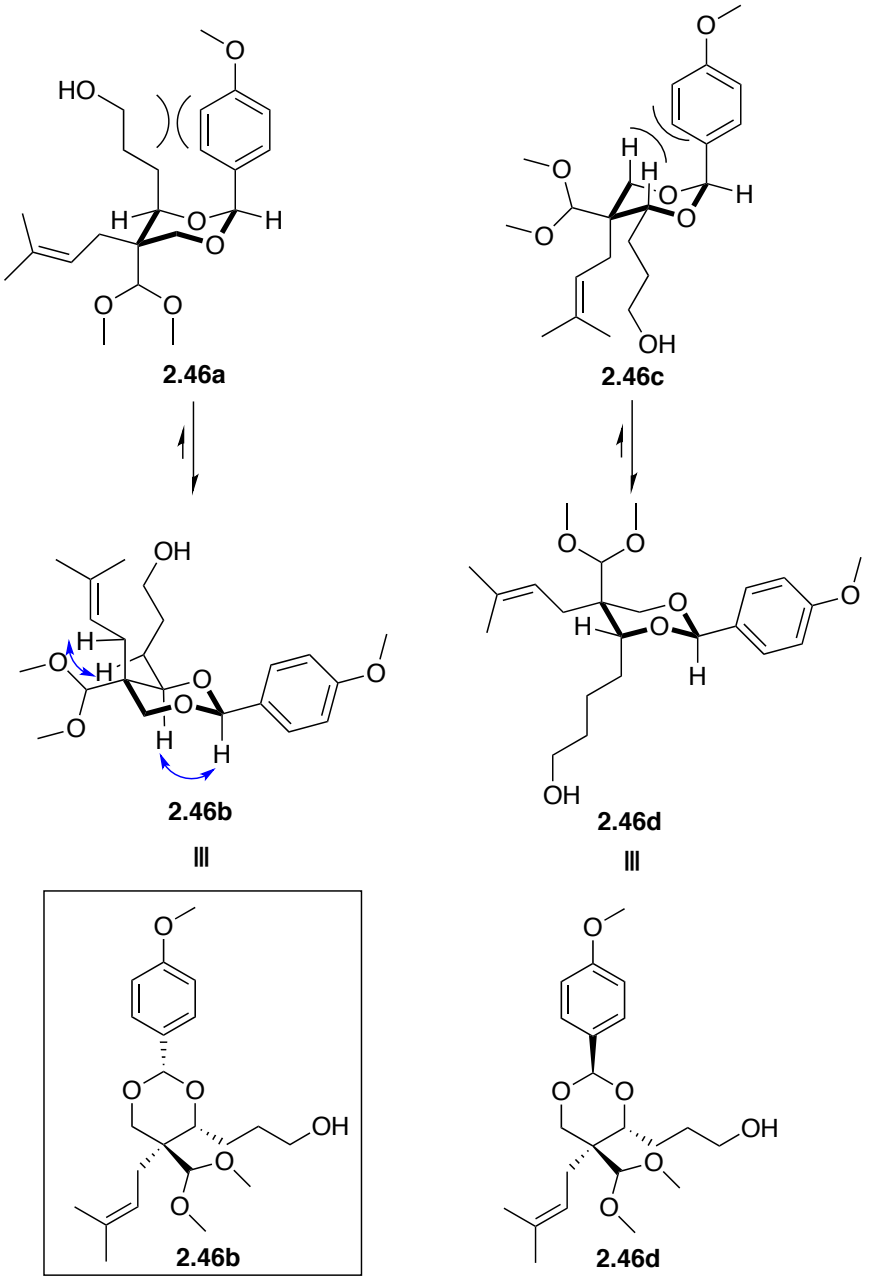
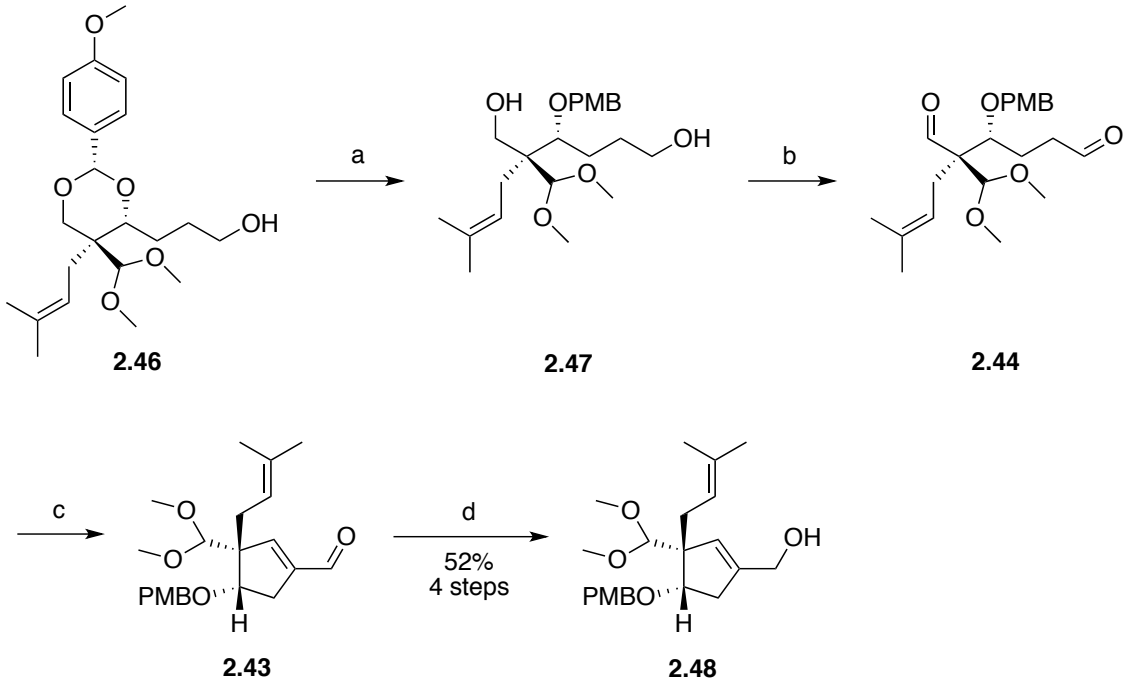


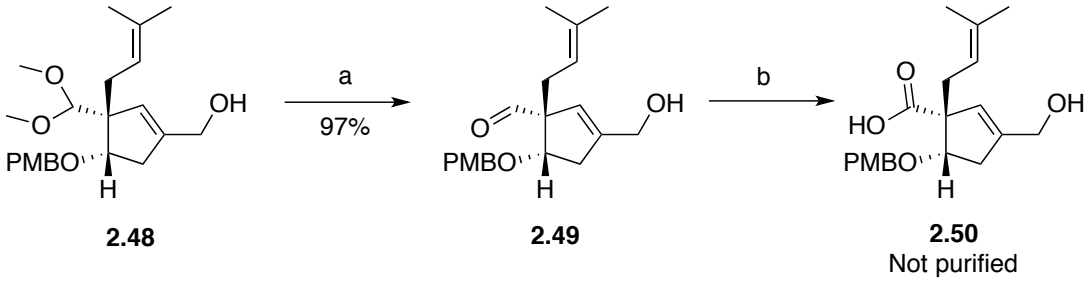
Figure 2.8: Confirmation of the relative stereochemistry of benzylidene **2.46** by NOESY NMR data.

Reduction of the benzylidene acetal furnished diol **2.47** (**Scheme 2.19**), which was subjected to a double Swern oxidation. The resulting dialdehyde **2.44**, which was believed to be unstable, was immediately taken forward to the aldol condensation without purification. After stirring dialdehyde **2.44** overnight with Bn_2NH•TFA in toluene (a method developed by Corey *et al.*[49]), cyclic aldehyde **2.43** was realised. Purification of **2.43** was difficult by column chromatography so the impure product was reduced to alcohol **2.48**, which was isolated in 52% yield over 4 steps.



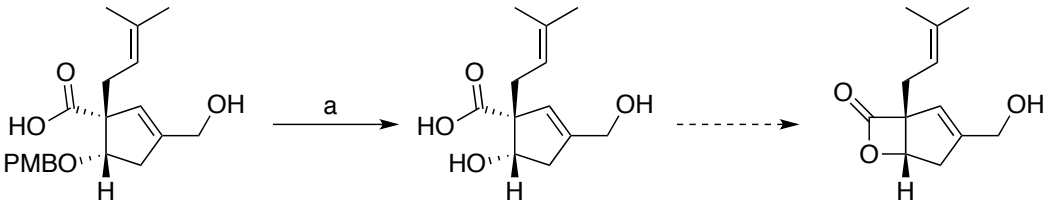
Scheme 2.19: *Reagents and conditions:* a) DIBAL, CH_2Cl_2, 0 °C to rt; b) Oxalyl chloride, DMSO, NEt_3, −78 °C to rt; c) Bn_2NH•TFA, toluene, rt.; d) $NaBH_4$, THF/H_2O, 0 °C to rt.

With the key cyclopentene intermediate **2.48** in hand, closure of the β-lactone with retention of stereochemistry was required to complete the total synthesis of (±)-vibralactone (**1.01**). Acetal deprotection was achieved in quantitative yield (**Scheme 2.20**) and the resulting aldehyde **2.49** was oxidised to carboxylic acid **2.50** using Pinnick conditions. Due to the reaction being on a small scale (<10 mg) and the high polarity of **2.50**, no purification was attempted.



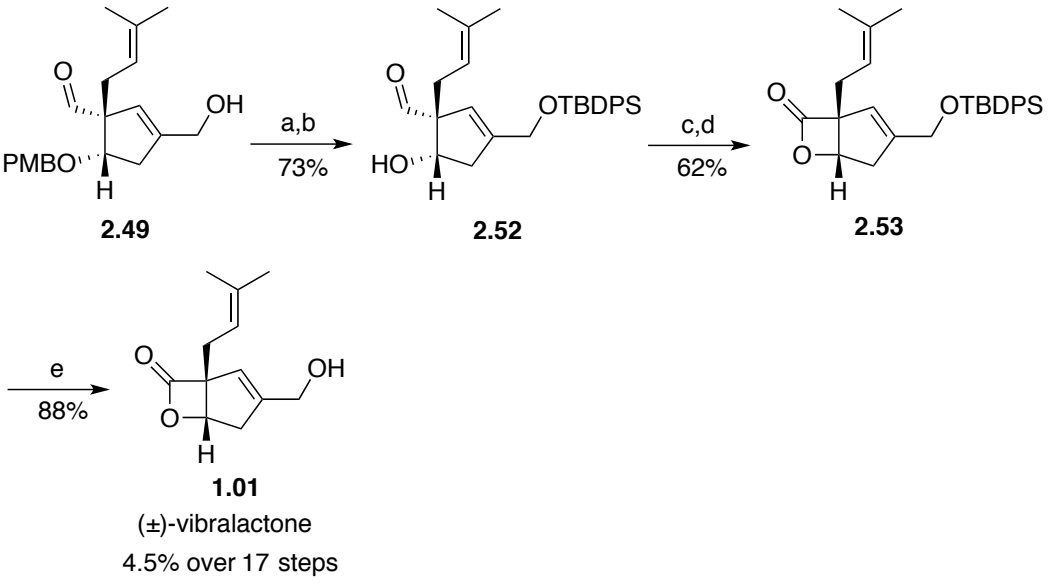
Scheme 2.20: *Reagents and conditions:* a) AcOH, THF/H_2O, 60 °C; b) $NaClO_2$, H_2O_2, NaH_2PO_4, MeCN/H_2O, 0 °C.

PMB deprotection of **2.50** appeared to proceed cleanly by HPLC-MS (**Scheme 2.21**), however extraction of the resulting hydroxy-acid **2.51** from the aqueous phase proved to be very difficult. The small amount of **2.51** that was extracted contained a significant proportion of DDQ.



Scheme 2.21: *Reagents and conditions:* a) DDQ, CH_2Cl_2/H_2O, rt.

Due to the difficulty purifying hydroxy-acid **2.51**, a TBDPS protecting group was chosen to mask the primary alcohol and decrease the overall polarity of the molecule. Silylation of **2.49** with TBDPS-Cl and subsequent PMB deprotection afforded hydroxy-aldehyde **2.52** in 73% yield over 2 steps (**Scheme 2.22**). Pinnick oxidation gave access to the hydroxy-acid, which this time could be extracted from the aqueous phase. Treatment of the crude hydroxy-acid with *p*-TsCl in pyridine resulted in lactone ring closure to afford silyl-protected vibralactone **2.53**. Finally, TBDPS deprotection provided (±)-vibralactone (**1.01**) in high yield. Overall the total synthesis of (±)-vibralactone (**1.01**) was completed in 4.5% yield over 17 steps from dimethyl malonate (**1.62**). All spectroscopic data were consistent with those reported for the isolated natural product[1] and for first total synthesis of (±)-vibralactone by Snider *et al*.[2]



Scheme 2.22: *Reagents and conditions:* a) TBDPS-Cl, NEt_3, DMAP, CH_2Cl_2, rt; b) DDQ, CH_2Cl_2/H_2O, rt; c) $NaClO_2$, H_2O_2, NaH_2PO_4, $MeCN/H_2O$, 0 °C to rt; d) *p*TsCl, pyridine, 5 °C; e) TBAF, THF, 0 °C to rt.

2.5 Streamlining the Synthetic Route

While being delighted by the successful total synthesis of (±)-vibralactone (**1.01**), we were keen to reduce the step-count and increase the overall elegance and yield of the synthesis. Therefore, we re-examined the synthetic route to identity where improvements could be made. Lactone closure was originally explored from methyl ester **2.16** (**section 2.4.1**), however access to hydroxy-acid **2.17** was not possible due to the large steric hindrance about the methyl ester carbonyl and the sensitivity of the intermediate to a base catalysed retro aldol reaction. Due to this, an alternative pathway was explored, which led to the completion of (±)-vibralactone (**1.01**) over 17 steps. The high step count was due to the requirement of protecting groups and many sequential functional group interconversions. If an alternative method could be developed for the synthesis of lactone **2.18** (**Figure 2.9**), a more streamlined synthetic route could be explored without the use of protecting groups.

The less hindered C-O ester bond of **2.54** provides an alternative site for ester cleavage (**Figure 2.9**). If the carboxylic acid **2.55** could be accessed with the mesylated alcohol, it was believed that treatment with a weak base would lead to lactone **2.18** with inversion of stereochemistry.

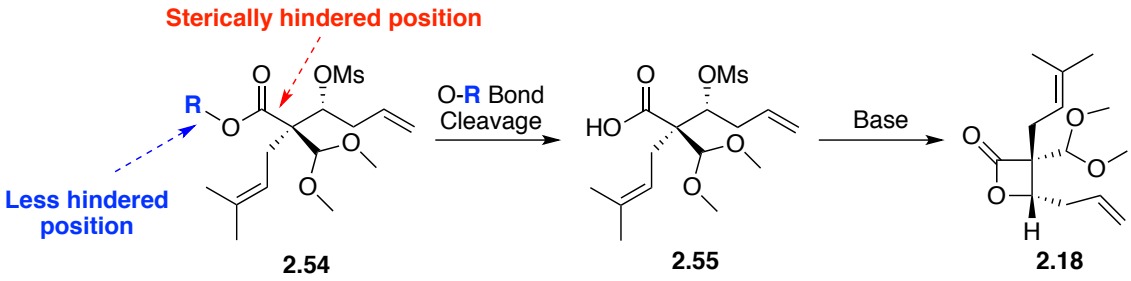
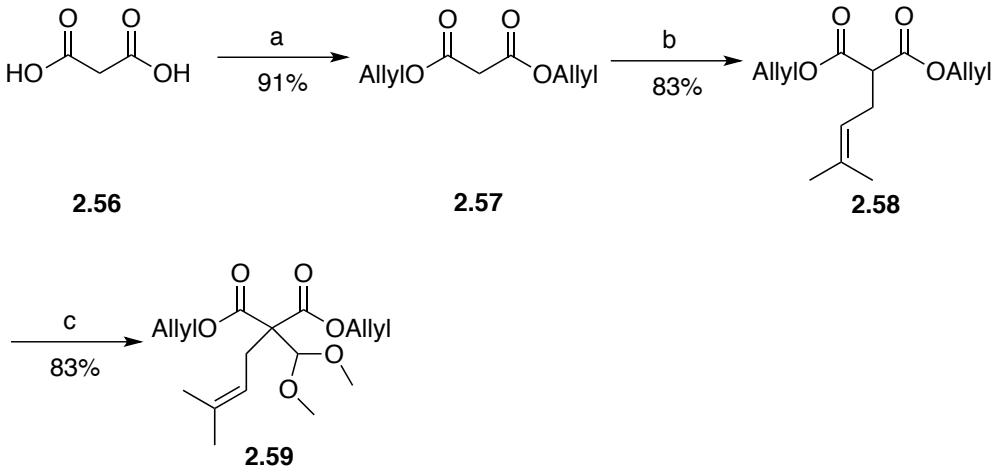


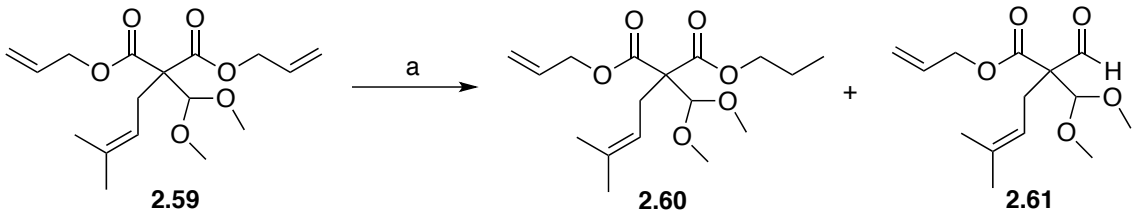
Figure 2.9: The less hindered C-O ester bond provides an alternative site for ester cleavage.

An allyl ester analogue derivative of **2.16** was investigated as this had the potential to be cleaved with Pd catalysis, thus avoiding the problematic ester hydrolysis. Coupling of malonic acid (**2.56**) with allyl alcohol afforded malonate **2.57**, which was purified by Kugelrohr distillation (**Scheme 2.23**).[65] Alkylations with prenyl bromide and trimethyl orthoformate gave access to substituted malonate **2.59** in high yield.



Scheme 2.23: *Reagents and conditions:* a) Allyl alcohol, DCC, MeCN, rt; b) prenyl bromide, K_2CO_3, acetone, rt; c) $TiCl_4$, $CH(OMe)_3$, NEt_3, CH_2Cl_2, 0 °C to rt.

Allyl ester **2.59** showed unexpected reactivity towards DIBAL, resulting in an inseparable mixture of propyl ester **2.60** and aldehyde **2.61** (**Scheme 2.24**). The reaction was repeated, adding DIBAL in 0.2 equivalent portions and monitoring the outcome by HPLC-MS. It was discovered that the terminal double bond of **2.59** was first reduced with DIBAL by an apparent hydroalumination reaction. The resulting propyl ester **2.60** was then reduced to aldehyde **2.61** by a second equivalent of DIBAL.



Scheme 2.24: *Reagents and conditions:* a) DIBAL, CH_2Cl_2, −78 °C.

The preferential reaction with the allyl double bond is believed to be due to the high steric congestion about the ester carbonyl. As a result the hydroalumination reaction outcompetes the expected ester reduction. It is tentatively proposed that the first equivalent of DIBAL reacts at the double bond to form chelated intermediate **2.62**. Coordination of the Lewis acidic aluminium species activates the carbonyl and makes it more reactive to DIBAL than the remaining allyl ester. As a result this activated ester is then reduced to desired aldehyde **2.61**. If it were possible to separate the mixture of **2.60** and **2.61**, the reaction would have been quenched with D_2O to provide evidence for the addition of DIBAL across the allyl double bond.

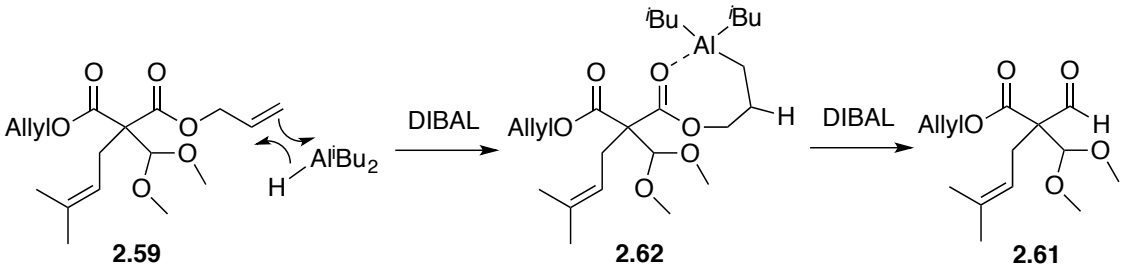
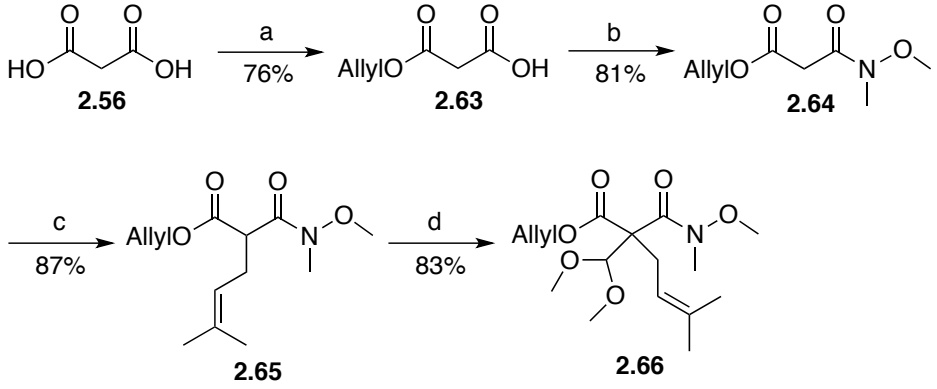


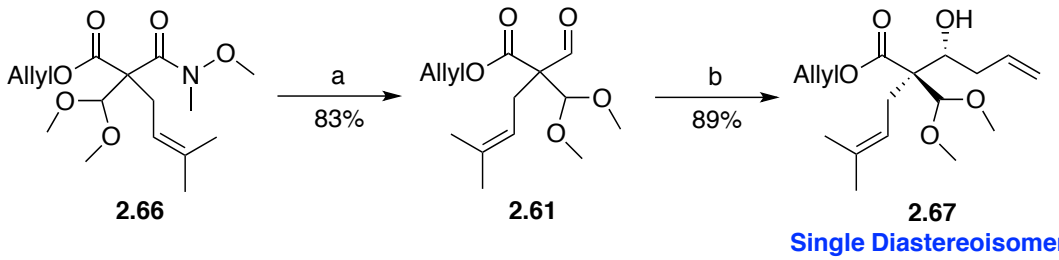
Figure 2.10: Proposed mechanistic pathway for the DIBAL reduction of **2.59**.

An example of the selective reduction of a Weinreb amide in the presence of a bulky *tert*-butyl ester was found in a paper by Gilon *et al.*[66], which led to the idea that it may be possible to reduce Weinreb amide **2.66** (**Scheme 2.25**) in the presence of the allyl ester. Coupling of malonic acid (**2.56**) with allyl alcohol afforded mono-acid **2.63** in good yield. Conversion to the acid chloride and a Schotten-Baumann coupling with *N*,*O*-dimethylhydroxylamine gave access to amide **2.64**. This bi-phasic reaction provided a good alternative to the direct coupling of acid **2.63** with the Weinreb amine salt using DCC, which saw no conversion to **2.64**. Alkylation with prenyl bromide required a strong base (NaHMDS) due to the reduced acidity of **2.64** compared to diallylmalonate (**2.57**). Finally the acetal addition proceeded cleanly to afford the novel scaffold **2.66**.



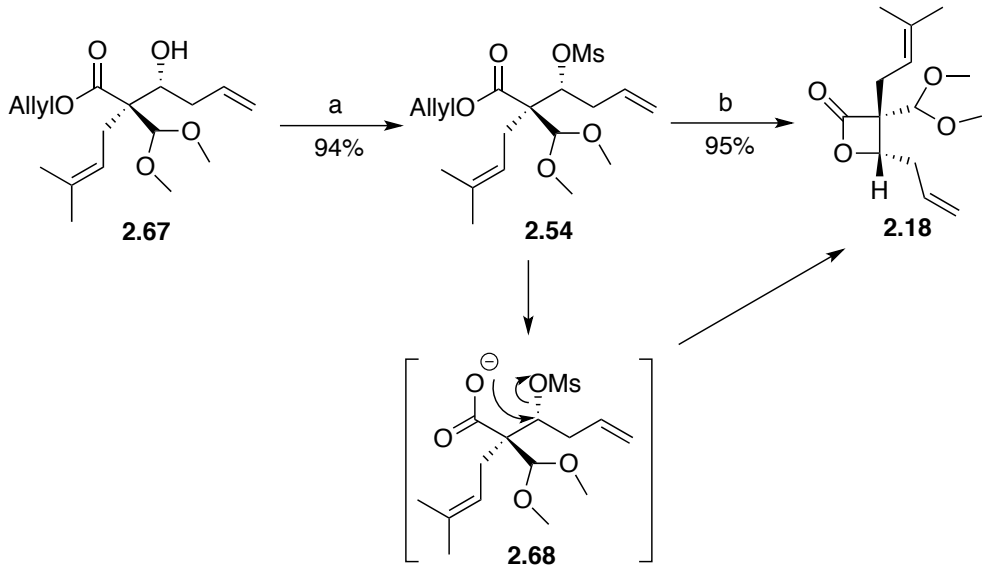
Scheme 2.25: *Reagents and conditions:* a) Allyl alcohol, DCC, MeCN, rt; b) i) $(COCl)_2$, DMF, CH_2Cl_2, 0 °C to rt; ii) MeNH(OMe).HCl, K_2CO_3, Et_2O/H_2O, 0 °C to rt; c) NaHMDS, prenyl bromide, THF, −78 °C to rt; d) $TiCl_4$, NEt_3, $CH(OMe)_3$, CH_2Cl_2, 0 °C to rt.

To our delight, treatment of **2.66** with DIBAL at −78 °C resulted in clean reduction to aldehyde **2.61** (**Scheme 2.26**) with no sign of the troublesome hydroalumination reaction observed for diallyl analogue **2.59**. The subsequent allylation afforded alcohol **2.67** with complete diastereoselectivity and in excellent yield.



Scheme 2.26: *Reagents and conditions:* a) DIBAL, CH_2Cl_2, –78 °C; b) $MgBr_2 \bullet OEt_2$, allyltributyltin, CH_2Cl_2, –78 °C.

With an efficient route to alcohol **2.67** in hand, the ester cleavage was investigated. First the alcohol had to be converted to a leaving group so that the correct lactone isomer could be created by inversion of stereochemistry. Mesylation of alcohol **2.67** with Ms_2O afforded **2.54** in excellent yield (**Scheme 2.27**). The de-allylation reaction was performed by treating a mixture **2.54** and $[Pd(PPh_3)_4]$ with pyrrolidine. Excitingly, the allyl group was cleaved and the resulting carboxylate **2.68** underwent an intramolecular substitution with the mesylated alcohol to afford desired β-lactone **2.18** in almost quantitative yield. A TLC of the reaction after 20 s indicated complete conversion to the product and shows the extraordinary efficiency of the reaction. To the best of our knowledge this is a novel approach to the synthesis of lactones and, in particular, β-lactones. It would be interesting to expand the scope of this reaction to other natural products containing lactones and also to investigate its application in the synthesis of β-lactams.



Scheme 2.27: *Reagents and conditions:* a) Ms_2O, pyridine, DMAP, CH_2Cl_2; b) $[Pd(PPh_3)_4]$ (1 mol %), pyrrolidine, CH_2Cl_2, rt.

It was envisioned that β-lactone **2.18** could be used as a precursor to aldehyde **2.69** which, upon activation by a Lewis acid, would potentially cyclise to form vibralactone C (**1.14**) (**Figure 2.11**).

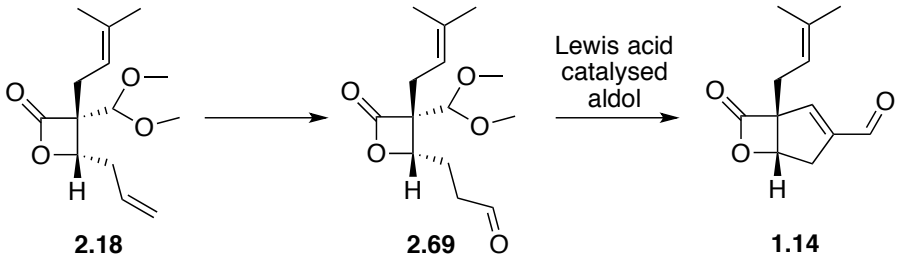
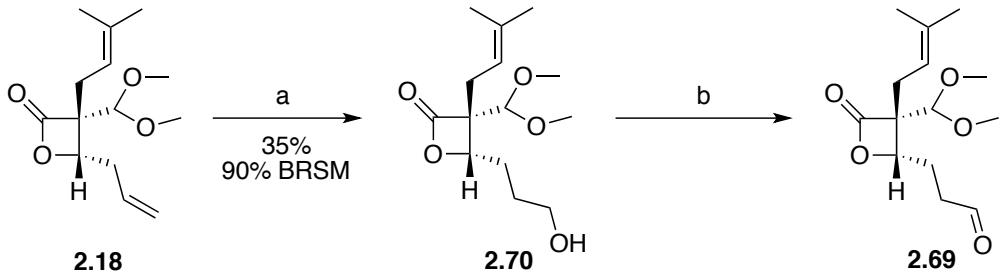


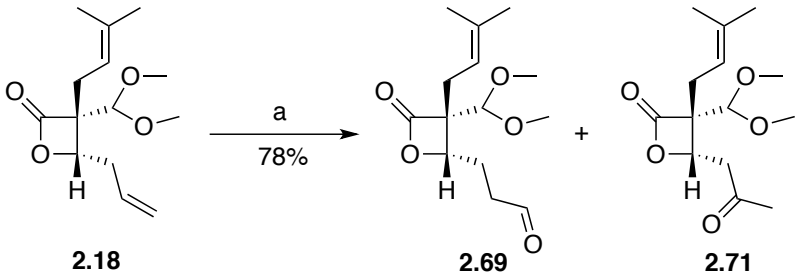
Figure 2.11: Proposed route to vibralactone C (**1.14**) from β-lactone **2.18**.

Hydroboration of alkene **2.18** with 9-BBN and oxidation with $NaBO_3$ afforded alcohol **2.70** in poor isolated yield, but 90% based on recovered starting material (**Scheme 2.28**). The poor conversion of alkene **2.18** to the intermediate organoborane was likely due to steric congestion about the double bond. It may have been possible to improve the yield by heating the reaction for longer, however 3 days were required to reach 35% conversion. Reaction with BH_3•THF unfortunately resulted in hydroboration of the prenyl group. Swern oxidation of alcohol **2.70** appeared successful by crude NMR, however the small scale of the reaction and volatility of **2.69** made purification difficult.



Scheme 2.28: *Reagents and conditions:* a) i) 9-BBN, THF, Δ; ii) $NaBO_3.4H_2O$, H_2O; b) $(COCl)_2$, DMSO, NEt_3, CH_2Cl_2, −78 °C.

To avoid the troublesome hydroboration, an aldehyde selective Wacker-type oxidation developed by Grubbs *et al*. was employed.[67,68] Alkene **2.18** was rapidly oxidised, affording an inseparable 9:1 mixture of anti-Markovnikov aldehyde **2.69** and ketone **2.71** (**Scheme 2.29**). Exchanging silver nitrite for sodium nitrite lowered the selectivity to 4.6:1. This reaction provided an excellent alternative route to key aldehyde intermediate **2.69**.



Scheme 2.29: *Reagents and conditions:* a) $PdCl_2(PhCN)_2$, $CuCl_2.2H_2O$, $AgNO_2$, O_2, rt.

Grubbs *et al.* proposed a model to explain the anti-Markovnikov selectivity of the aldehyde selective Wacker oxidation (**Scheme 2.29**).[67] The metal-mediated addition of an NO_2 radical species to the terminal position of the alkene was suggested, leading to a more stable secondary radical intermediate (**Figure 2.12**). This is in direct contrast to the conventional Wacker oxidation where attack by water on the alkene is controlled by Markovnikov's rule.[69] By performing the reaction within an oxygen-free atmosphere with stoichiometric ^{18}O-labelled $NaNO_2$, ^{18}O incorporation showed that the aldehyde oxygen atom originated from the nitrite salt. Under catalytic conditions the resulting NO species was aerobically re-oxidised back to NO_2.

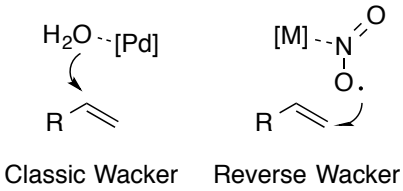
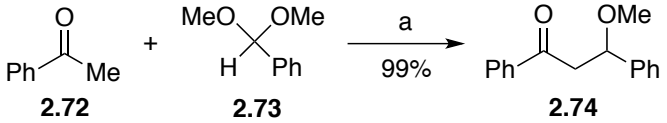


Figure 2.12: Proposed radical model to rationalise the anti-Markovnikov selectivity.[67]

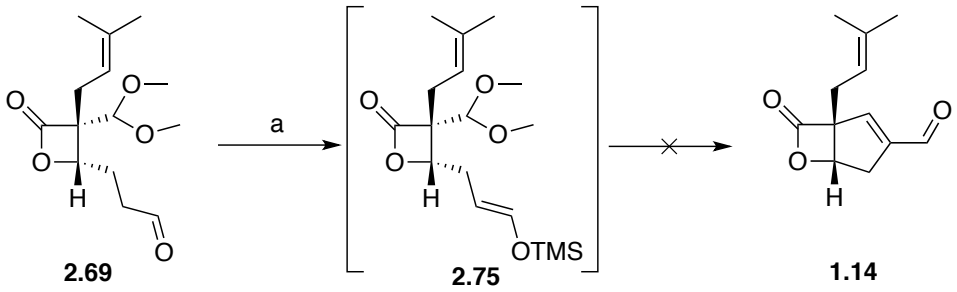
With the advanced intermediate **2.69** in-hand, the aldol condensation step was explored. Downey *et al*. reported a one-pot enol silane formation-Mukaiyama aldol addition (**Scheme 2.30**),[70] which provided great precedent for the aldol condensation of aldehyde **2.69**. The paper proposed a mechanism whereby the enol silane of **2.72** is formed *in situ* from stoichiometric TMS-OTf and DIPEA, and the remaining unreacted TMS-OTf activates the dimethyl acetal to generate an oxocarbenium ion. Finally a Mukaiyama aldol provides product **2.74**.



Scheme 2.30: *Reagents and conditions:* a) TMS-OTf, DIPEA, CH_2Cl_2, 0 °C to rt.[70]

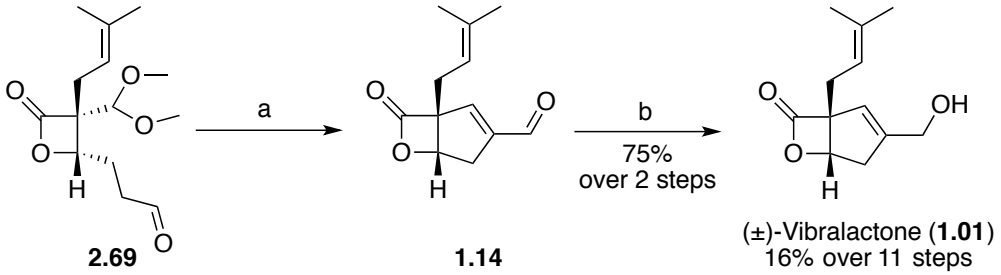
Unfortunately, the unstable silyl enol **2.75** (**Scheme 2.31**) appeared to be formed but no evidence was found of the subsequent aldol condensation. HPLC-MS of an aliquot taken

from the reaction mixture showed the correct mass for enol ether **2.75**, however after an aqueous workup the ether decomposed. The failed cyclisation suggested that TMS-OTf was not able to activate the dimethyl acetal within this system. The reaction was repeated with the addition of BF_3•OEt_2, although this unfortunately led to significant degradation. It is likely that the Lewis acid caused β-lactone ring opening.



Scheme 2.31: *Reagents and conditions:* a) TMS-OTf, DIPEA, CH_2Cl_2, 0 °C to rt.

Aldehyde **2.69** was stirred with Bn_2NH•TFA in toluene using the same procedure as **Scheme 2.19** but no aldol condensation was observed. By substituting trifluoroacetic acid with triflic acid in the amine salt, the dimethyl acetal was successfully activated and the cyclopentene ring was formed (**Scheme 2.32**). Vibralactone C (**1.14**) appeared to be slightly sensitive to silica and a pure sample could not be obtained. The resulting impure aldehyde **1.14** was reduced to (±)-vibralactone (**1.01**) and isolated in 75% yield over the two steps. All spectroscopic data were consistent with those reported for the isolated natural product,[1] the first total synthesis of (±)-vibralactone by Snider *et al*.[2] and for the first generation total synthesis described in **2.4.3**. Overall the second generation total synthesis of (±)-vibralactone (**1.01**) was completed in 16% over 11 steps, representing a great improvement over the first synthesis.



Scheme 2.32: *Reagents and conditions:* a) Bn_2NH•TfOH, toluene, rt; b) $NaBH_4$, DME/H_2O, 0 °C.

2.6 Towards the Asymmetric Synthesis of (−)-Vibralactone

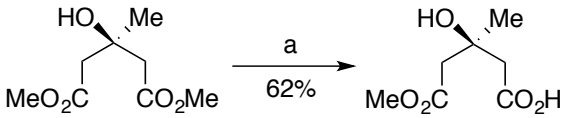
With two synthetic routes to (±)-vibralactone (**1.01**) completed, a method of forming the all-carbon quaternary centre in an enantioselective fashion was explored so that enantiomerically pure (−)-vibralactone (**1.01**) could be prepared. Two main strategies could potentially be employed: the enantioselective desymmetrization of a pro-chiral malonate or an auxiliary mediated alkylation.

2.6.1 Enantioselective Desymmetrization

Desymmetrization strategies can allow for the creation of chiral centres rapidly and with great efficiency, and usually with good atom economy (unlike with chiral auxiliaries). There has been a recent growth in the development of new desymmetrization strategies, in particular for the application in natural product total synthesis.[71] For a comprehensive review on enantioselective desymmetrization reactions to all-carbon quaternary stereocentres see Zeng *et al*.[72]

2.6.1.1 Enzymatic Hydrolysis

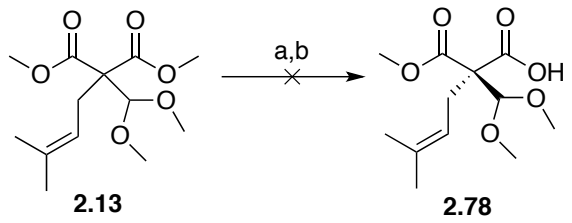
A traditional and widely used enantioselective desymmetrization technique is the enzymatic hydrolysis of pro-chiral diesters.[73] Pig liver esterase (PLE) is a common broad spectrum enzyme used for the hydrolysis of esters, such as in the synthesis of (*R*)- and (*S*)-mavalonic acids, which gives extremely high enantioselectivity (**Scheme 2.33**).[74]



2.76 **2.77**
99% ee

Scheme 2.33: *Reagents and conditions:* a) PLE, 0.1 M phosphate buffer (pH 8.0), rt.

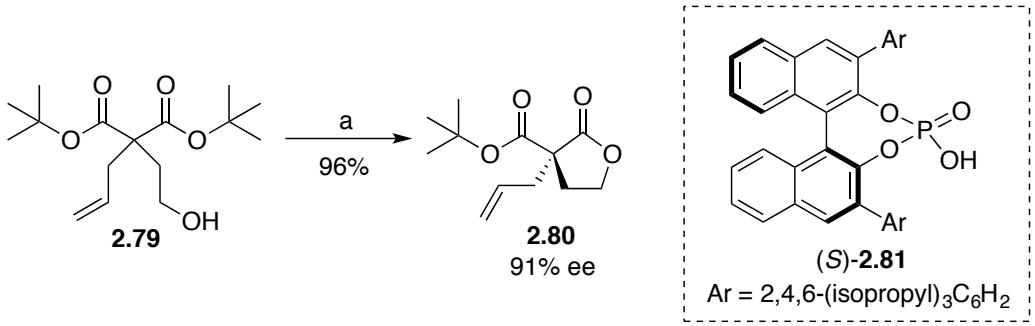
Unfortunately PLE and another broad-spectrum enzyme, porcine pancreatic lipase (PPL), were unable to hydrolyse the methyl ester of substituted malonate **2.13** (**Scheme 2.34**). This result was not entirely unexpected due to the high steric congestion about the quaternary centre.



Scheme 2.34: *Reagents and conditions:* a) PLE, acetone/phosphate buffer (pH 7.4), 30 °C b) PPL, acetone/phosphate buffer (pH 7.4), 30 °C.

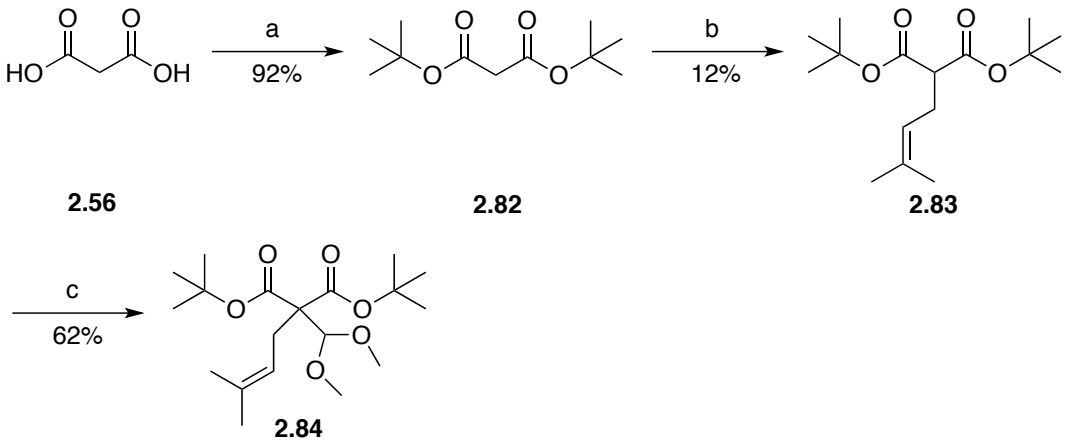
2.6.1.2 Chiral Brønsted acid Desymmetrization

A highly topical desymmetrization of diesters has recently been published by Petersen *et al.*[75] Substituted di-*tert*-butyl malonate **2.79** has been converted to lactone **2.80** in the presence of chiral Brønsted acid **2.81** in high yield and enantioselectivity (**Scheme 2.35**). It is presumed that selective activation of one of the esters by the chiral acid results in an intramolecular transesterification,[76] however there is currently no evidence for this mechanism.



Scheme 2.35: *Reagents and conditions:* a) **2.81** (5 mol %), CH_2Cl_2, 35 °C.

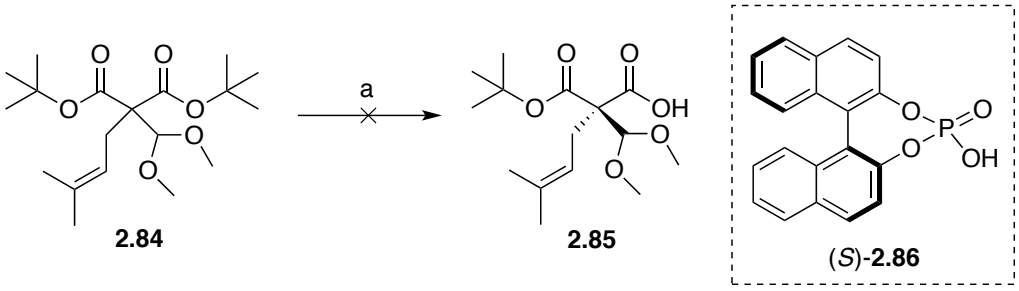
The application of this desymmetrization reaction (**Scheme 2.35**) in the hydrolysis of **2.84** was a worthwhile investigation despite the absence of a hydroxyl group. Di-*tert*-butyl malonate (**2.82**) was accessed efficiently from malonic acid (**2.56**) and then alkylated with prenyl bromide to afford **2.83** in very poor yield (**Scheme 2.36**). This was due to the very slow conversion as a result of **2.82** being sterically encumbered by the bulky *tert*-butyl esters. No optimisation was performed for this reaction. Finally, the acetal addition proceeded cleanly to afford substituted malonate **2.84**.



2.56 **2.82** **2.83** **2.84**

Scheme 2.36: *Reagents and conditions:* a) ^{t}BuOH, DCC, MeCN, rt; b) prenyl bromide, K_2CO_3, acetone, rt; c) $TiCl_4$, $CH(OMe)_3$, NEt_3, CH_2Cl_2, 0 °C to rt.

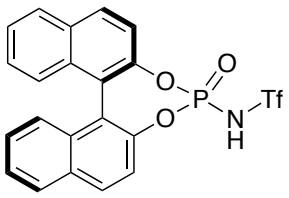
Disappointedly, after heating di-*tert*-butyl malonate **2.84** with chiral acid **2.86** in a sealed vial at 80 °C for 7 days, only starting material was recovered (**Scheme 2.37**). The reaction was also performed in MeOH to potentially facilitate a trans-esterification but this was also unsuccessful. The stability of the substrate under these conditions was either due to the absence of a hydroxyl group required to perform a transesterification, or the steric hindrance that appears innate in the majority of these functionalised malonates.



2.84 **2.85** (*S*)-**2.86**

Scheme 2.37: *Reagents and conditions:* a) **2.86** (10 mol %), CH_2Cl_2, 80 °C, 70 days.

If more time were available, a stronger chiral acid such as **2.87** (**Figure 2.13**) would be prepared[77] and tested in the desymmetrization of **2.84**.



(*R*)-**2.87**

Figure 2.13: Chiral Brønstead acid **2.87**.

2.6.1.3 Enantioselective Deallylation

Considering the effectiveness of the palladium catalysed deallyation within the sterically encumbered intermediate **2.54** (**Scheme 2.27**), it was interesting to explore a possible enantioselective deallylation to gain chiral enrichment of the all-carbon quarternary centre. By subjecting diallyl malonate **2.59** to the same reaction conditions used previously, but with a chiral ligand, it may be possible to differentiate between the enantiotopic allyl ester groups and achieve an enantioselective desymmetrization (**Figure 2.14**). This would allow for chiral enrichment of the resulting mono-acid **2.88**.

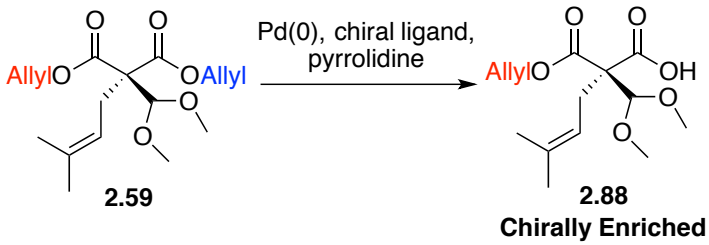
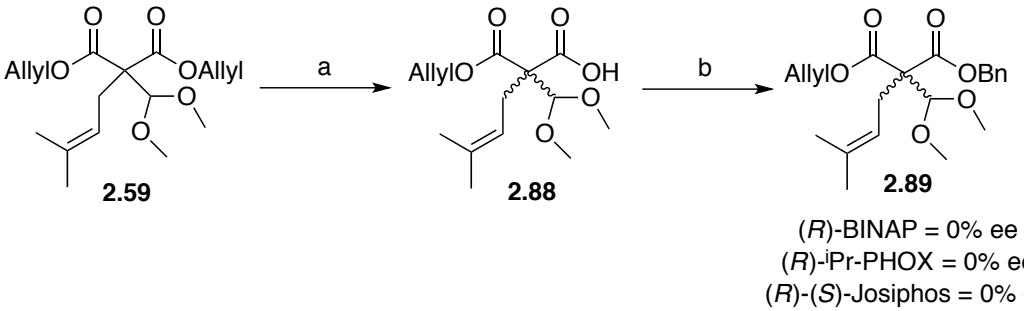
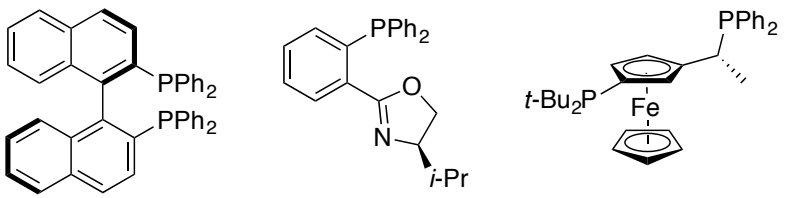


Figure 2.14: Proposed enantioselective deallylation reaction.

Diallyl malonate **2.59** was subjected to the deallylation conditions with (*R*)-BINAP (**2.90**), (*R*)-^{i}Pr-PHOX (**2.91**) and (*R*)-(*S*)-Josiphos (**2.92**) (**Figure 2.15**) and a sample of the resulting acid **2.88** was benzylated to afford **2.89** (**Scheme 2.38**). Unfortunately analysis by chiral HPLC showed no enantiomeric excess of mixed ester **2.89** with any of the chiral ligands.



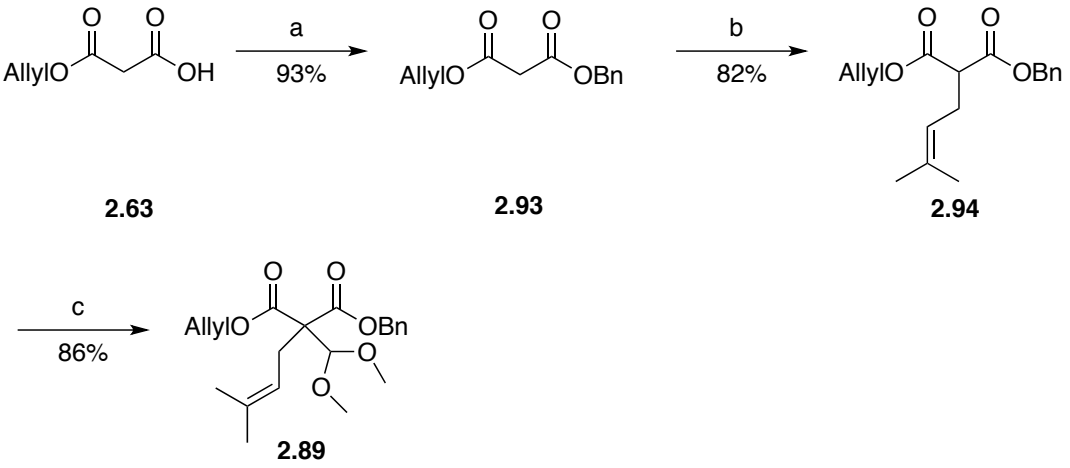
Scheme 2.38: *Reagents and conditions:* a) Pd_2dba_3 (1 mol %), chiral ligand, pyrrolidine, CH_2Cl_2, rt; b) benzyl bromide, K_2CO_3, acetone, rt.



(*R*)-BINAP (**2.90**) (*R*)-^{i}Pr-PHOX (**2.91**) (*R*)-(*S*)-Josiphos (**2.92**)

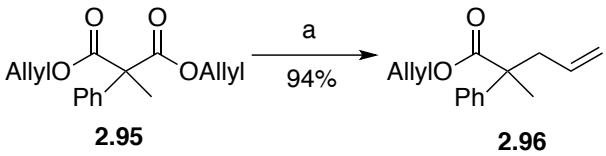
Figure 2.15: Structures of the chiral ligands **2.90**, **2.91** and **2.92**.

The ratio of the two enantiomers of **2.89** (**Scheme 2.38**) observed by chiral HPLC was confirmed by comparing it to the racemic compound prepared by the standard alkylation route (**Scheme 2.39**).



Scheme 2.39: *Reagents and conditions:* a) Benzyl alcohol, DCC, MeCN, rt; b) prenyl bromide, K_2CO_3, acetone, rt; c) $TiCl_4$, $CH(OMe)_3$, NEt_3, CH_2Cl_2, 0 °C to rt.

Although no enatiomeric excess of **2.89** was observed with the three chiral ligands that were tested, an interesting result from the investigation was that no decarboxylation of carboxylic acid **2.88** was seen. The decarboxylative allyation reaction is a very popular reaction that has been studied extensively[78] since its discovery by Tsuji *et al*.[79] and Saegusa *et al.*[80] One such example is the decarboxylative allylation of diallyl malonates with benzylic quaternary carbon centres reported by Imao *et al.* (**Scheme 2.40**).[78] Diallyl malonate **2.95** has a very similar structure to acetal substuted malonate **2.59** so it was a surprise to see that the same reaction does not occur with **2.59**.



Scheme 2.40: *Reagents and conditions:* a) $[Pd(PPh_3)_4]$, 1,4-dioxane, 1 h, rt.

Imao *et al*. suggested a mechanism for the decarboxylative allylation of **2.95** (**Figure 2.16**).[78] Oxidative addition of **2.97** with the palladium species forms complex **2.98**. The π-allylpalladium species is released to generate ion pair **2.99** followed by decarboxylation to form the reactive carbanion/enolate pair **2.100**. Attack of the π-allylpalladium by the enolate finally affords the alkylated product **2.96**.

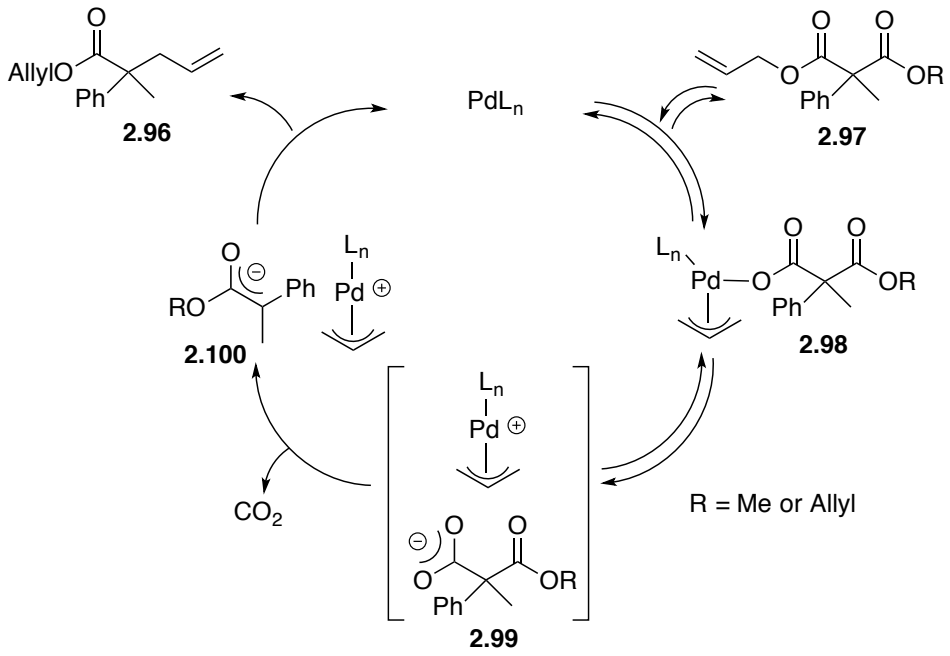
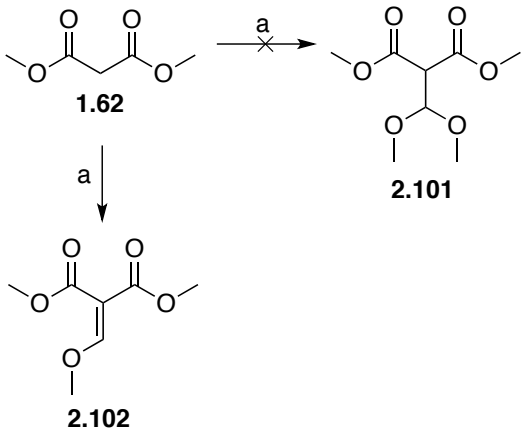


Figure 2.16: Proposed catalytic mechanism for the decarboxylative allylation of **2.94** by Imao *et al*.[78]

Tsuji *et al*. showed that malonate derivatives usually require elevated temperatures to undergo decarboxylative allylation.[81] The lower temperature required for diallyl malonate **2.95** is believed to be due to the lower pk_{aH} of the benzylic enolate **2.100** compared to aliphatic enolates (by ca. 6-7 pK_a units).[82] The greater enolate stability allows for facile decarboxylation. This could explain why acetal malonate **2.59** (**Scheme 2.38**) does not undergo decarboxylation, as the acetal and prenyl groups do not stabilise the enolate intermediate. It is more likely that the π-allylpalladium species is not released after oxidative additive and is instead attacked by pyrrolidine, resulting in deallylation and formation of acid product **2.88** after aqueous workup.

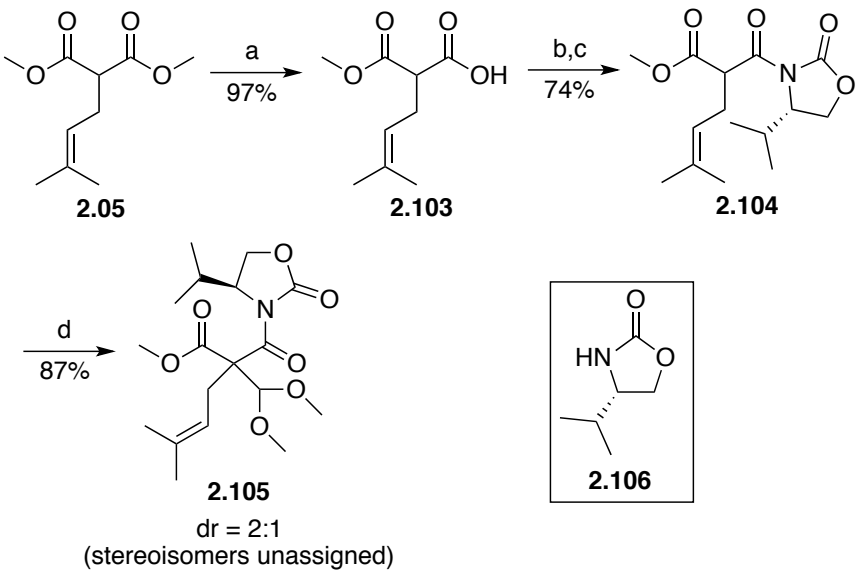
2.6.2 Auxiliary Mediated Alkylation

Finally an auxiliary mediated alkylation approach was investigated to establish diastereoselectivity in the formation of the all-carbon quaternary centre. The cyclohexyl chiral auxiliary TTC (**1.61**) was applied to the prenylation of malonates by Robert Heap (**1.5.1**). This approach is unsuitable for malonate compounds containing the dimethoxyacetal moiety however, as it is not possible to access acetal malonate **2.101** (**Scheme 2.42**). Dimethoxyacetal addition to dimethylmalonate (**1.62**) leads to the elimination of methanol and the formation of **2.102**. This compound cannot be isolated due to its instability to chromatography. The use of TTC (**1.61**) in the acetal addition to prenyl malonate **2.05** is also unsuitable due to the inherent difficulty of hydrolysing the TTC ester after alkylation as a result of its large steric bulk.



Scheme 2.41: *Reagents and conditions:* a) $TiCl_4$, $CH(OMe)_3$, NEt_3, CH_2Cl_2, 0 °C to rt.

Evans' oxazolidinone provides an alternative to TTC (**1.61**) and benefits from extensive methods for cleavage, such as conversion to a Weinreb amide or thioester, followed by reduction to an aldehyde. Amide **2.104** was prepared from prenyl malonate **2.05** by mono-hydrolysis to acid **2.103** and coupling with (*S*)-(−)-4-isopropyl-2-oxazolidinone (**2.106**) (**Scheme 2.42**). **2.104** was afforded as a 1:1 mixture of diastereoisomers, which were separated for analytical purposes. Acetal addition to one diastereoisomer of **2.104** was performed at −78 °C with no product **2.105** observed. HPLC-MS however, showed epimerisation, which suggested that the titanium enolate intermediate was being formed. Upon warming of the reaction mixture to rt, the enolate reacted with trimethylorthoformate to afford the disubstituted product **2.105** as 2:1 mixture of diastereoisomers. The major isomer was isolated but the relative stereochemistry has yet to be established.



Scheme 2.42: *Reagents and conditions:* a) KOH (1 equiv.), MeOH, 67 °C; b) oxalyl chloride, DMF, CH_2Cl_2, 0 °C to rt; c) ^{n}BuLi, (*S*)-(−)-4-isopropyl-2-oxazolidinone (**2.106**), −78 °C to rt; d) $TiCl_4$, NEt_3, $CH(OMe)_3$, CH_2Cl_2, −78 °C to rt.

The reaction provided evidence that a chiral auxiliary could be used to induce selectivity in the acetal addition reaction. In addition, it is particularly unusual to see an alkylation with Evans' auxiliary to establish a quaternary centre. Typically the deprotonation to form an enolate is not observed due to the steric clash usually observed with the auxiliary (**Figure 2.17**). It is likely that formation of the titanium enolate from **2.104** is facilitated by the adjacent ester, which leaves the auxiliary free to rotate into the lowest energy conformation. The structure of the titanium enolate is difficult to predict due to the possibility of coordination of a second titanium species to the oxazolidinone in the presence of excess $TiCl_4$. NMR or theoretical studies may be helpful in achieving a better understanding of the structure, but this is outside of the scope of the current work.

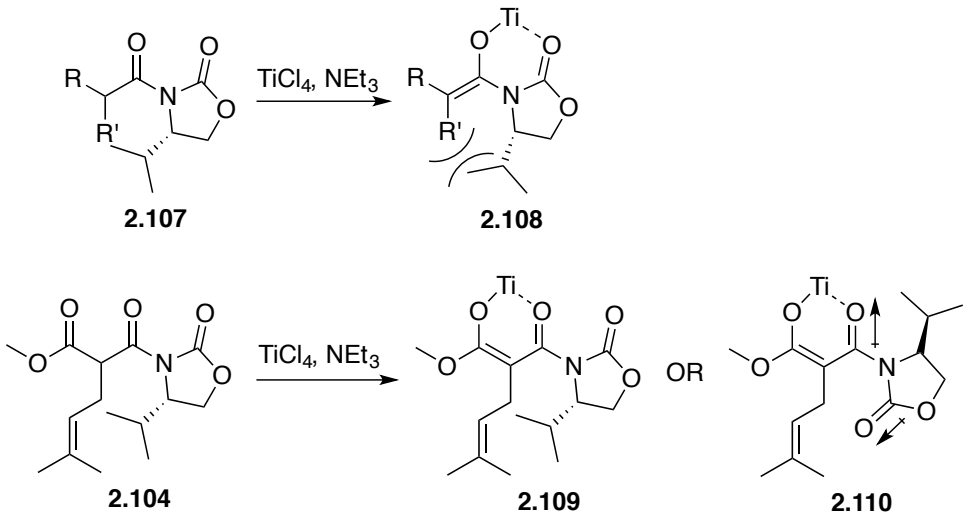
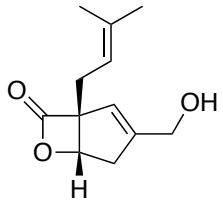


Figure 2.17: Steric clash caused by the deprotonation of **2.107** compared to **2.104**.

2.7 Conclusions

The total synthesis of (±)-vibralactone (**1.01**) has been completed in 17 and 11 steps in 4.5% and 16% yield respectively. The bicyclic system was formed via two separate routes from key intermediates **2.16** and **2.67**, which were accessed using a highly diastereoselective chelation controlled allylation. This diastereoselective reaction allows for a marked improvement over the previous total synthesis of vibralactone (**1.01**) by Snider *et al.*[2,3] with respect to control of the relative stereochemistry. The second-generation synthesis utilizes a novel deallylation/cyclisation reaction to form the β-lactone ring, which bypasses the requirement for protecting groups and shortens the synthetic route by six steps. Other novel reactions include the titanium-catalyzed addition of a dimethylacetal group to malonate derivatives and the Bn_2NH•TfOH catalyzed aldol condensation of acetal/aldehyde intermediate **2.69**.



(±)-Vibralactone (**1.01**)

1st generation = **17 steps, 4.5% yield**
2nd generation = **11 steps, 16% yield**

Figure 2.18: 1st and 2nd generation total syntheses of (±)-vibralactone (**1.01**).

Our initial investigations towards an asymmetric synthesis of (–)-vibralactone (**1.01**) show that a chiral auxiliary mediated addition of the dimethoxyacetal group may provide a means for forming the all-carbon quaternary centre of vibralactone (**1.01**) in a selective manner. More work is required to improve the selectivity of this reaction and discover a way of converging this asymmetric approach with the second-generation route to (±)-vibralactone (**1.01**).

2.8 Further Work

The most direct approach towards an enantioselective synthesis would be to explore alternative chiral auxiliaries for the selective acetal addition reaction such as Oppolzer camphorsultam (**Figure 2.19**). The disubstituted product could then be converted to enantiopure aldehyde **2.113** by a selective reduction, which would allow access to the second-generation route to vibralactone (**1.01**).

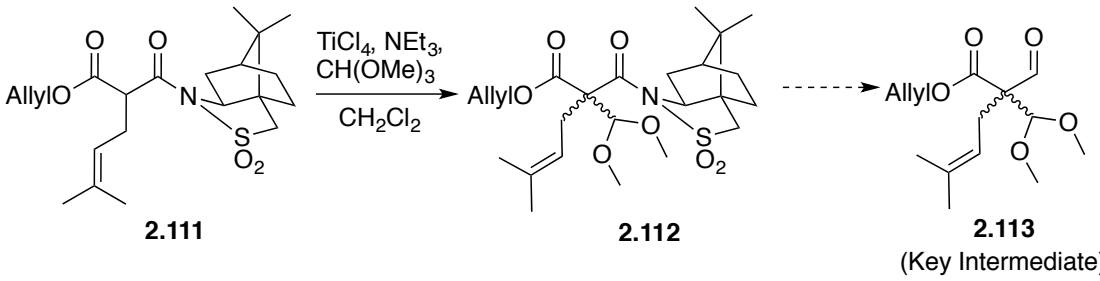
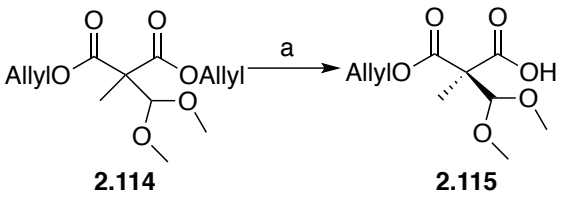


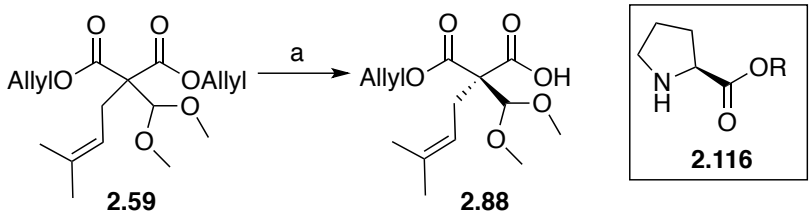
Figure 2.19: Proposed selective alkylation of **2.111**, which would ultimately lead to a pure enantiomer of aldehyde **2.113** from which the asymmetric total synthesis of vibralactone (**1.01**) could be completed.

It would be interesting to investigate the proposed enantioselective deallylation reaction further by performing the reaction on a more sterically biased system such as **2.114** (**Scheme 2.43**). This may allow for better differentiation of the two allyl esters by the chiral palladium species.



Scheme 2.43: *Reagents and conditions:* a) Pd_2dba_3 (1 mol %), chiral ligand, pyrrolidine, CH_2Cl_2, rt.

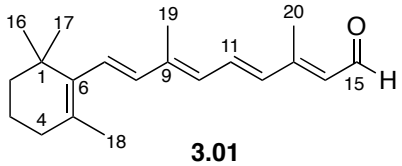
Due to the reversible nature of palladium coordination to the allyl ester double bond, the deallylation may not be under kinetic control and this could explain why no selectivity was observed with **2.59** (**Scheme 2.38** and **Figure 2.16**). Another method for inducing selectivity in the reaction may be to use a chiral amine such as proline derivative **2.116** to react with the palladium complexed intermediate (**Scheme 2.44**). To the best of our knowledge, this would be a novel use of proline in an enantioselective reaction.



Scheme 2.44: *Reagents and conditions:* a) Pd_2dba_3 (1 mol %), **2.116**, CH_2Cl_2, rt.

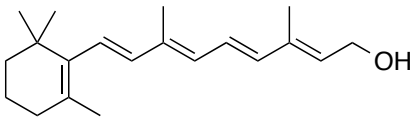
Chapter 3: Introduction to ^{13}C-Labelled Retinals

3.1 Background



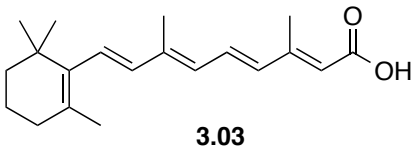
3.01

All-*trans*-Retinal (neoretinene b)



3.02

All-*trans*-Retinol (vitamin A)



3.03

All-*trans*-Retinoic Acid (Vitamin A acid)

Figure 3.1: Retinoids and numbering system

Retinoids or vitamin A derivatives are ubiquitous in nature and have been studied intensively over many decades. These compounds are mainly found within photo-reactive rhodopsin proteins, which are used for light responses such as vision in animals, ion channels in algae,[83] and proton pumps in bacteria.[84] In mammals, vitamin A also participates in gene transcription,[85] embryonic development,[86] reproduction,[87] skin and cellular health and anti-toxicity. Animals obtain retinoids through their diet, either indirectly in the retinal form (**3.01**) or by metabolism of the carotenes; α-carotene, β-carotene (**3.04**) and β-crytoxanthin (**Figure 3.2**).[88]

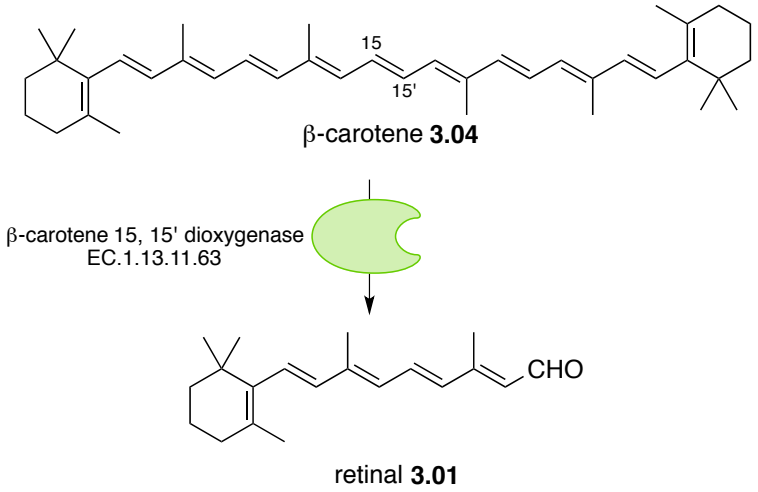


Figure 3.2: Oxidative cleavage of β-carotene to retinal by dioxygenase enzyme.[88]

Retinal is a chromophore that when covalently bound to opsin membrane proteins forms light-absorbing rhodopsin proteins, which are perhaps the most abundant prototrophic systems in nature.[89,90] These rhodopsins can be split into two homologous families that have evolved convergently to adopt similar functions: visual and archaeal (microbial) rhodospins. Visual rhodopsins are sensory pigments found in animalian eyes whereas archaeal rhodopsins are abundant in prokaryotes and some algae. Both protein families consist of a seven α-helix motif (7TM) with the retinal cofactor bound to a lysine residue in the seventh helix though a Schiff base linkage (**Figure 3.3**).[91] The retinal chromophore is utilised in the 11-*Z*/all-*E* configurations (ground state = 11-*Z*) in animal rhodopsins and the all-*E*/11-*Z* configurations (ground state = all-*E*) in archaeal rhodopsins.[92]

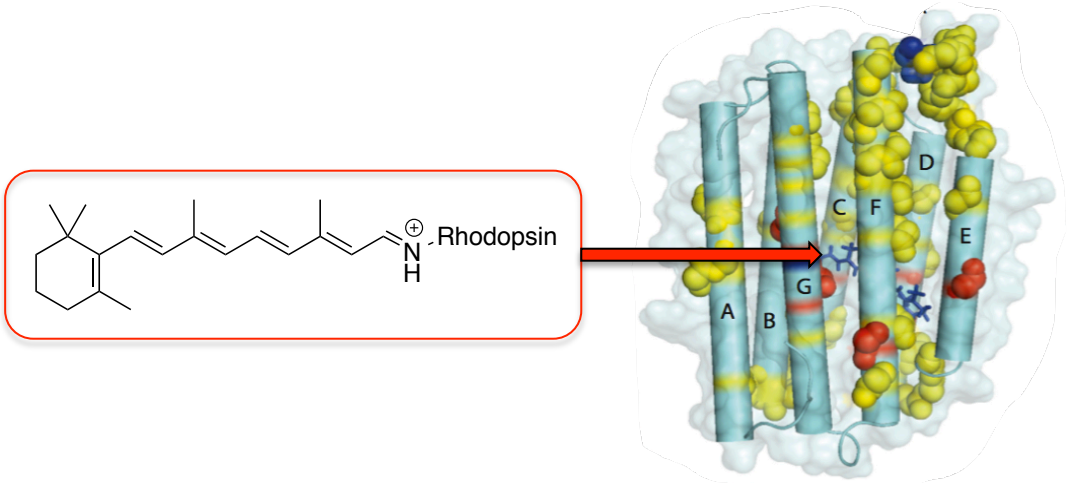


Figure 3.3: Retinoid covalently bound to the seven-helix bundle, taken from Glaubitz *et al*.[91]

3.2 Optical Characteristics

Absorption of a photon by the retinal chromophore causes photo-isomerisation of the polyene chain giving rise to a conformational change within the attached protein and, in turn, inducing a desired effect in a cell or organism. Evolutionary changes have led to these proteins becoming extremely well adapted to their environment, by alterations to the amino acid sequence both in the direct vicinity of the chromophore and in other parts of the protein.[91]

The optical absorption of the retinal chromophore is strongly influenced by the protein environment through the following factors:

- distance of the protonated Schiff base from a counter-ion complex;
- interactions of the polyene chain with the polar and polarizable amino acids;
- relative orientation of the β-ionone ring to the chain.

The absorption characteristics are also extremely important in defining the functionality of the rhodopsin protein (**section 3.3**).[91,93,94] All microbial rhodopsins include water molecules that can bind to the protein near the retinal Schiff base (SB), which are believed to stabilize the protonated SB within the hydrophobic interior of the protein.[95] Water molecules are also very important in the mechanism behind the function of these proteins. Water forms strong hydrogen bonds with protein residues and this contributes to the stability of the protein during each protointermediate.

3.3 Microbial Rhodopsins

Microbial rhodopsins, with their 7TM protein scaffold, can function as light-driven ion pumps, light gated ion channels and light senors.[95] The majority of these proteins follow a similar photocycle with a series of photointermediates that can be characterised by time-resolved optical spectroscopy. **Figure 3.4** shows the photocycle of bacteriorhodopsin.[95–100] The protonation state, isomeric configuration and planarity of the retinylidene chromophore all influence the spectroscopic properties of the photointermediates[95] as well as the position of protein residues and water molecules in the vicinity.

A photo-induced isomerisation results in the formation of the K-intermediate and a corresponding red-shift. A rapid (1 μs) blue-shift leads to the L-intermediate[101] that can be used as a proton transfer substrate in proton pump proteins such as proteorhodopsin (**Figure 3.7**). Deprotonation of the Schiff base affords the neutral M-intermediate. In proton-pump rhodopsins, re-protonation and a corresponding blue-shift leads to the N-intermediate. In chloride pumps such as halorhodopsin, the deprotonation step is by-

passed so that the L-intermediate proceeds directly to the N-intermediate[102]. The largest conformational changes within the protein are usually seen on formation of the N-intermediate. A significant tilt of the F-helix in bacteriorhodopsin at the cytoplasmic end is an important structural change in ion transport.[103] Isomerisation of the terminal alkene results in the all-*trans* O-intermediate, which undergoes a final blue-shift back to the ground state. It is important to note that the K- L- and N-intermediates have different absorption spectra due to structural changes within the protein.

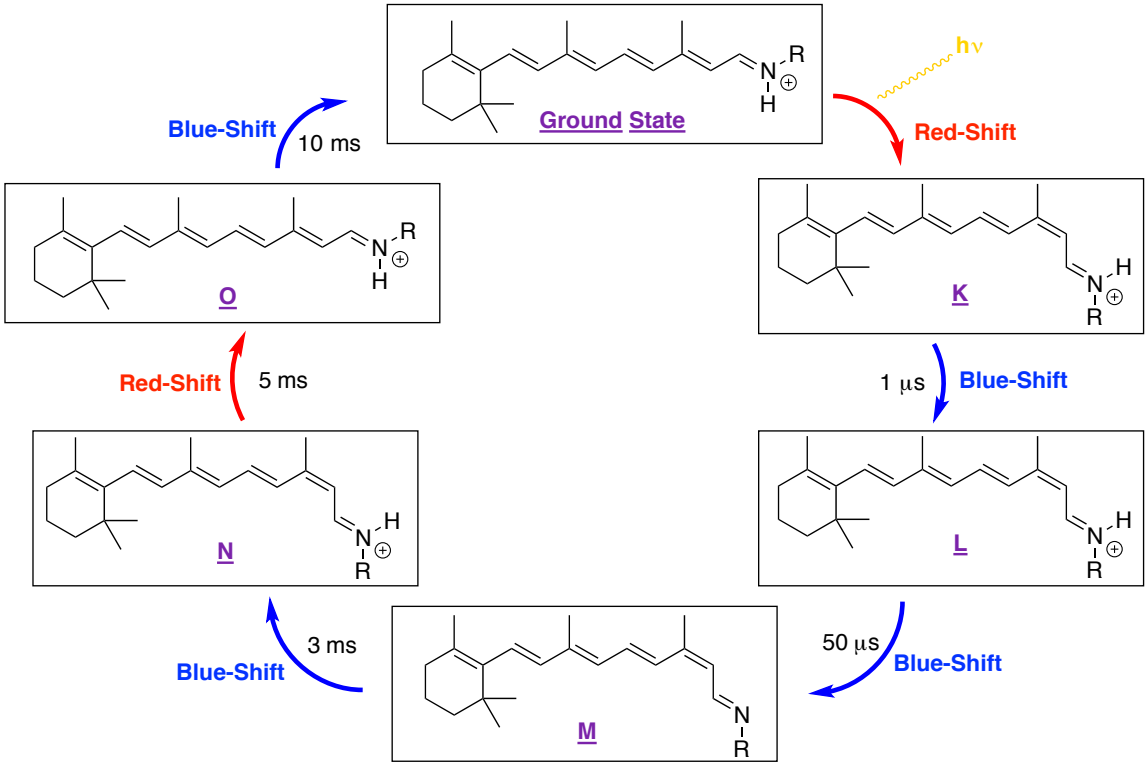


Figure 3.4: Typical microbial rhodopsin photocycle adapted from Ernst *et al.*[95]

The following sections will focus on proteorhodopsin, channelrhodopsin and KR2 microbial rhodopsins, which are currently of great interest in the field of energy harvesting and optogenetics.

3.3.1 Proteorhodopsin

Proteorhodopsin (pR) is a transmembrane retinylidene protein of marine bacterial origin that functions as a photoreactive proton pump. Its name originates from its discovery in γ-proteorbacteria, but it is not exclusive to this group of microorganisms. In fact, proteorhodopsin has been found in microorganisms including many types of bacteria, archaea, viruses and eukaryotic marine protists.[90,104,105] It is believed that due to the widespread distribution of these organisms in the oceans and lateral gene transfer of pR genes among planktonic bacteria, pR significantly contributes to global solar-energy input.[106]

There are two main families of proteorhodopsin: green absorbing (GpR, λ_{max} = 525 nm) found near to the surface of the oceans and blue absorbing (BpR, λ_{max} = 490 nm) found in deeper parts. These proteins are colour tuned so that they match the light that penetrates the water to the depth at which the bacteria inhabit.[107] The effective colour tuning property of these proteins is evident when comparing them with the protonated retinylidene Schiff base in methanol, which has an absorption maxima (λ_{max}) of 440 nm.[84] It has been shown that a single amino acid close to the chromophore is responsible for this colour tuning. The mutation GpR_{L105Q} shifts the absorption maxima of the chromophore towards blue and, conversely, the mutation BpR_{Q105L} causes a green shift.[84,90] BpR_{Q105L}, for example, is the single point mutation of glutamine-105 to leucine-105. These amino acid sequence changes affect the stabilisation of the excited state charge distribution of the chromophore, in turn causing the observed colour shift.[94]

A 20 nm red shift in the absorption spectrum of GpR has also been discovered that is due to the mutation GpR_{A178R} in the EF loop. Solid-state NMR (**section 3.4**) and time-resolved optical spectroscopy have been used to map these mutation-induced changes throughout the protein.[91] ^{13}C and ^{15}N chemical shift changes between the wild type GpR and GpR_{A178R} suggests that a distortion in the secondary structure of the EF loop propagates throughout the whole protein and changes the retinal binding pocket structure (**Figure 3.5**).[91]

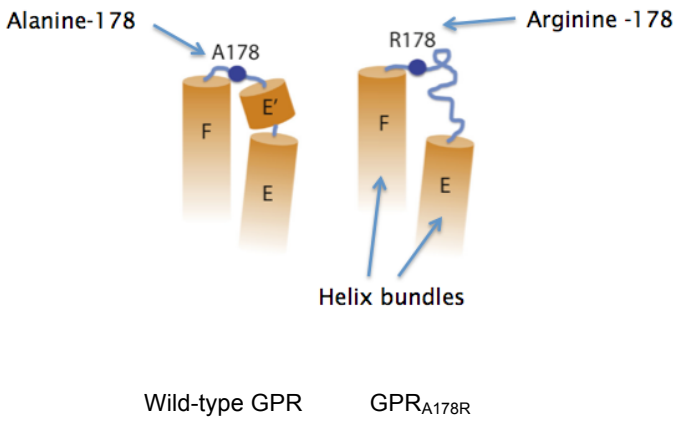


Figure 3.5: Distortion in the secondary structure of the EF loop due to GPR_{A178R} mutation.[91]

The exact mechanism of the proton pump is very complex and there have been many studies to elucidate this.[108–111] Either side of the transmembrane protein is the hydrophobic cytoplasm and the hydrophilic extracellular medium. The protein transports protons against the electrochemical gradient from the cytoplasm into the extracellular medium. The protons then pass back into the cell, where they catalyse the production of ATP by ATP synthase (**Figure 3.6**).

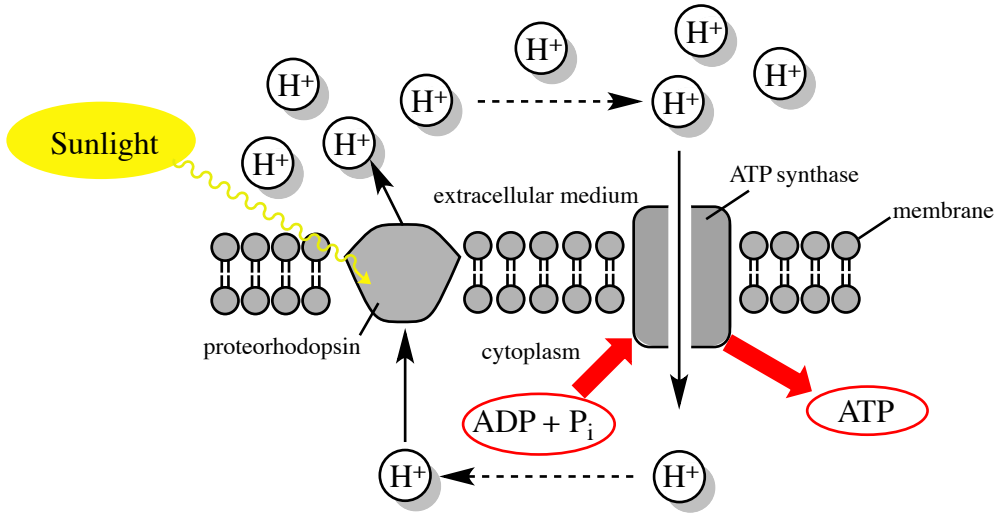


Figure 3.6: Schematic showing the action of both ATP synthase and proteorhodopsin in the photo-initiated production of ATP.

The protonated Schiff base from the retinal-lysine linkage is responsible for the proton-pump action of the protein (**Figure 3.7**). The all-*trans* structure (**3.05**) undergoes rapid photo-isomerisation to the 13-*cis*-structure/L-intermediate (**3.06**). The conformational change induced in the protein changes the dipole of the protonated Schiff base, which decreases the pK_a of the conjugate acid. This change in pK_a results in the release of the proton to the Asp97 proton acceptor. Further structural changes within the protein cause the proton to be released into the extracellular medium. The Schiff base/M-intermediate is then re-protonated by Glu108, which results in isomerisation of the chromophore back to the all-*trans* form (**3.05**).

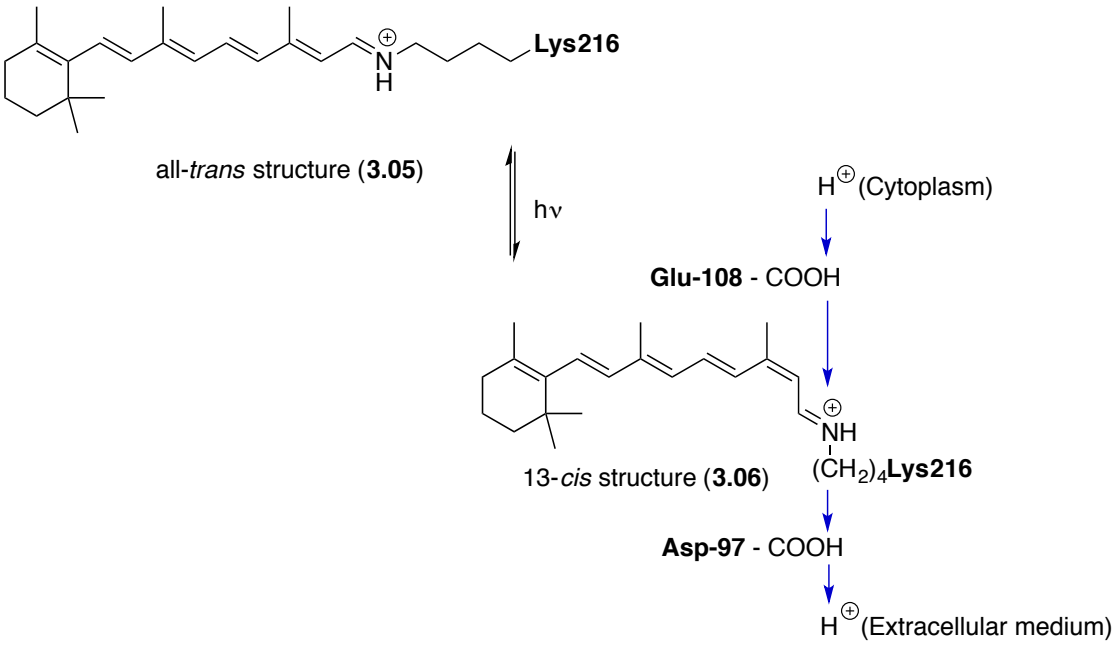


Figure 3.7: Mechanism for proton pump action of the retinal chromophore.

3.3.2 Channelrhodopsin

Ion channels are ubiquitous in living organisms and are used for signal transduction (receptor activation by an extracellular signalling molecule) and the conduction of electrical impulses. They 'gate' the movement of ions down an electrical gradient through biological membranes by rapidly opening and closing due to a specific sensory input.[112] Their water-filled pore structure allows ions to pass through whilst being shielded from the non-polar interior of the membrane, which would otherwise be impermeable.[113] These channels can either mediate many types of ions or be very selective for one particular type.

Different processes can trigger channel proteins:

- voltage-gated channels open in response to electric field changes across a membrane, such as in neurons;
- ligand-gated channels, found commonly in synapses, respond to binding with specific molecules;
- mechanosensitive channels open due to pressure changes.[114]

Channelrhodopsins (ChRs) fall into a class of their own. *Chlamydomonas reinhardtii* was the first algae from which channelrhodopsins ChR1 and ChR2 were isolated.[83,115] To date, these are the only ion channels identified that respond to light, and allow for cation gating in a fast, repetitive, reproducible and non-invasive way.[113] This property has made ChRs of great interest in the field of optogenetics; a tool created by Deisseroth in 2004 whereby the expression of light-activated proteins in neurons (inserted into the plasma membrane) allows the cells to be switched on and off with lasers by altering the cell membrane potentials.[116,117] This technique is now being used by scientists to probe how the brain functions and, in turn, potentially find methods of relieving symptoms of neurological conditions such as Parkinson's disease.[118] It is therefore clear to see why an in depth understanding of the mechanism behind these proteins is extremely valuable.

Channelrhodopsin shares the same characteristic seven transmembrane helix of proteorhodopsin and other microbial rhodopsins, with the retinal chromophore linked to a lysine residue through a protonated Schiff base. Photo-isomerisation of the C13=C14 double bond of the chromophore leads to channel opening, however the exact structural and electrostatic changes within the protein have yet to be fully understood.[113]

3.3.3 KR2 Ion Pump

Until 2013 only two groups of ions pumps had been realised: outward proton pumps such as bacteriorhodopsin (pR) and proteorhodopsin (pR), and inward chloride pumps such as halorhodopsin (hR). The absence of light promoted non-proton cation pumps was believed to be due to electrostatic repulsion that would occur with the retinylidene Schiff base proton.[119] However in 2013 *Krokinobacter eikastus* rhodopsin 2 (KR2), the first light-driven Na^+ pump, was discovered from the marine flavobacterium *Krokinobacter eikastus*.[120]

Recently Kato *et al.* published the first working model of Na^+ transport by KR2.[119] In the ground/resting state, cation transport is prevented by the protonated Schiff base in the centre of the protein cavity (**Figure 3.8**). Photoisomerisation results in the SB flipping upwards and transferring the proton to Asp116 during the K- to M-intermediate transition. Once in the M-intermediate conformation, the protonated Asp116 residue rotates to form hydrogen bonds with Ser70 and Asn112, which allows for the conduction of Na^+ by reducing the ion transport energy barrier. Finally the SB is re-protonated in the O-intermediate, which prevents backflow of Na^+.

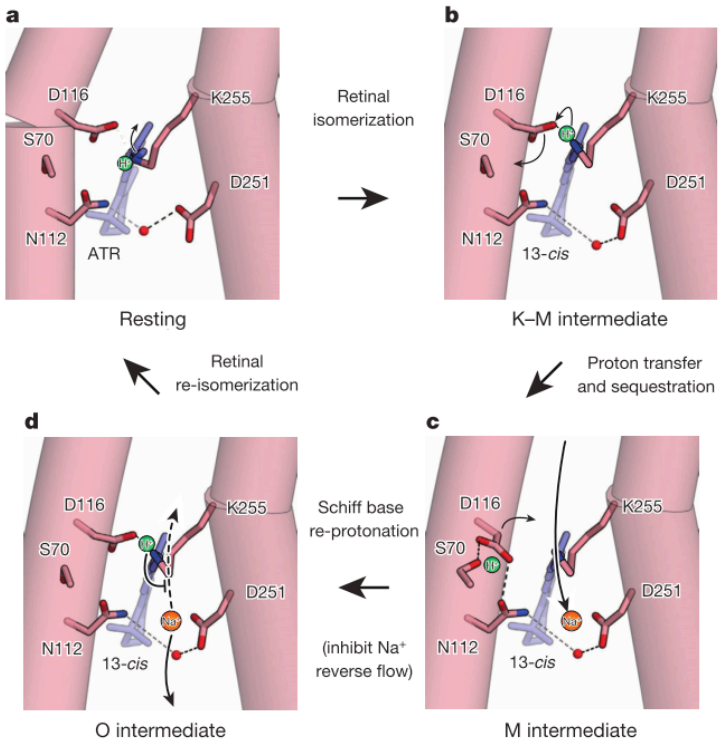


Figure 3.8: Diagram taken from Kato *et al.*[119] showing the proposed model of Na^+ transport.

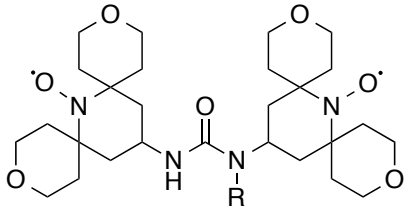
KR2 has the potential to be used as a new inhibitory optogenetics tool and further understanding into its mode of action is very important for its continued development both for its direct use and for the bioengineering of new non-proton cation pumps.

3.4 Magic-Angle Spinning (MAS) Solid-State NMR

Magic angle spinning (MAS) NMR is a widely used technique for obtaining high-resolution NMR spectra of polycrystalline and non-crystalline solids[121] as it narrows the broad peaks normally associated with solid-state spectra. Many variations of MAS NMR have been developed to both increase the sensitivity and expand upon the information that can be extracted from complex chemical systems.

3.4.1 DNP-Enhanced MAS NMR

Dynamic nuclear polarization (DNP) is a technique that can significantly enhance the sensitivity of MAS NMR and increase signal strength.[91] DNP combines electron paramagnetic resonance (EPR) with solid-state NMR by transferring the large polarization associated with unpaired electrons, from stable radical polarizing agents such as AMUPol **3.07**, to nuclei by high energy (terahertz) microwave irradiation.[122] This technique has allowed for MAS experiments on complex systems such as membrane and amyloid proteins, that are usually very challenging, and sometimes impossible, due to the low signal to noise ratio.[122] DNP is also extremely useful in the study of the retinylidene chromophore in rhodopsin proteins by enhancing the sensitivity of solid-state NMRs by orders of magnitude (up to 10^5).[91]



AMUPol

3.07

R = $(CH_2CH_2O)_4Me$

Figure 3.9: Biradical polarizing agent AMUPol (**3.07**) used in DNP-enhanced NMR.

3.4.2 Double-Quantum Filtering

Double-quantum filtering (DQF) can enhance MAS spectra by allowing for the extraction of resonances that occur between dipole-coupled spin pairs and the suppression of resonances from isolated, uncoupled spins.[121] This process can be used to simplify MAS spectra and aid assignment of complex molecules. Glaubitz *et al.* employed this technique in the study of green proteorhodopsin doped with ^{13}C-enriched retinal to suppress natural ^{13}C abundance within the protein.[91]

Single-quantum coherence is when the orientation of spin half nuclei is flipped upon irradiation in a magnetic field. When a pair of nuclei are coupled within a field and flip in opposite directions, a zero-quantum coherence is observed. Conversely, double-quantum (DQ) coherence occurs when a pair of coupled nuclei flip the same way and this results in a DQ coherence frequency roughly double that of a single-quantum coherence and outside of the normal observed range. Manipulation of the pulse sequence of MAS NMR can be used to observe signals only originating from DQ coherence.[121]

DQF can also be used to obtain CC bond lengths and HCCH torsion angles.[123] These intermolecular measurements can be obtained by inputting information from two-dimensional DQ spectra and DQF build-up curves into complex quantum mechanical formulae.[124]

3.4.3 Solid-State NMR of Retinal Proteins

As previously mentioned, ^{13}C-labelled retinals can be incorporated into rhodopsin proteins to allow for detailed structural insight by solid-state NMR. The retinal membrane proteins first require reconstitution as proteoliposomes (lipid bilayers).[91] The enriched ^{13}C atoms can then be distinguished clearly from the natural ^{13}C abundance within the protein by DQF DNP-enhanced MAS (**3.4.2**) and the small conformational changes of the retinal chromophore can be investigated by observing small variations in ^{13}C chemical shifts (**Figure 3.10**). CC bond lengths and HCCH torsion angles can also be measured. These techniques have been used extensively to determine the effect of mutations on colour tuning in proteorhodopsin (**3.3.1**).[90,91,111,125]

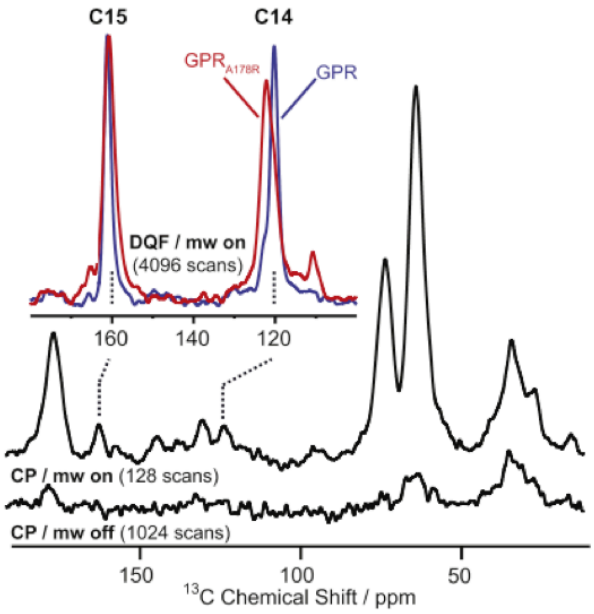
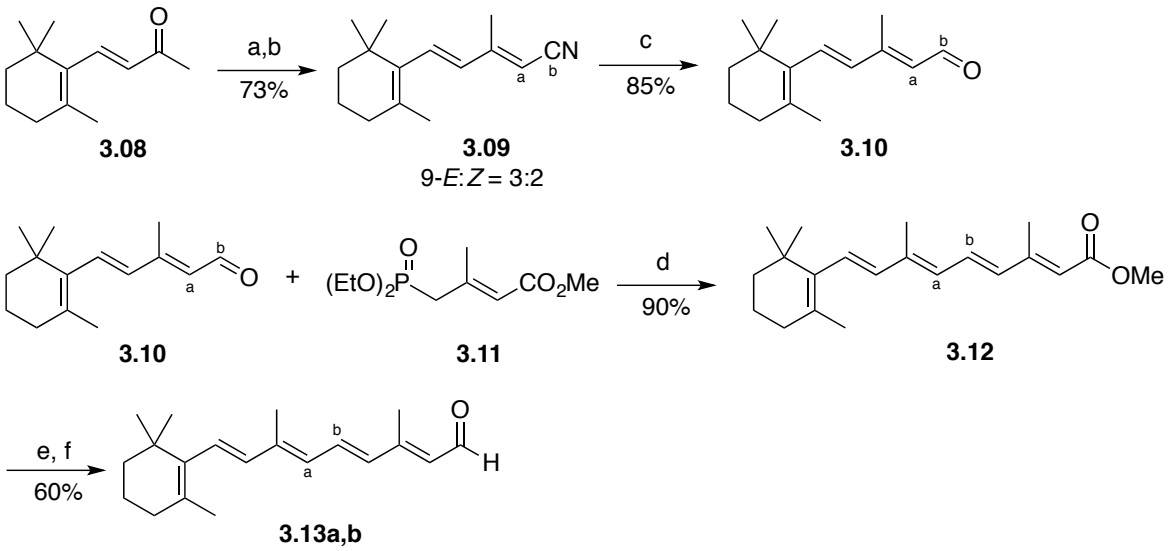


Figure 3.10: Diagram showing the signal enhancement from DNP for a ^{13}C-MAS NMR spectra of 14,15-^{13}C-labelled retinal in GPR and GPR$_{A178R}$. A double quantum filtered experiment (DQF) is used to extract the C14 and C15 peaks from which the chemical shift changes, due to the mutation, can be observed.[90,91]

3.5 ^{13}C All-*Trans* Retinal Syntheses

This section will cover a range of ^{13}C all-*trans* retinal isotopomers that have previously been synthesised using various routes to incorporate the ^{13}C labels in both the chain and the ring. Pardoen *et al*. have reported the synthesis of [10-^{13}C], [11-^{13}C], [19-^{13}C] and [20-^{13}C]-all-*trans*-retinals (**3.13a, 3.13b, 3.18 and 3.22**) using [1-^{13}C]-acetonitrile and [2-^{13}C]-acetonitrile (**Scheme 3.1**).[126]

[2-^{13}C]-Acetonitrile and [1-^{13}C]-acetonitrile were used to introduce ^{13}C enrichment in [10-^{13}C] and [11-^{13}C]-all-*trans*-retinal. A condensation reaction between the acetonitrile anion and β-ionone (**3.08**) with *N*-bromosuccinimide formed the conjugated nitrile **3.09** as a mixture of isomers. Reduction of the nitrile group with DIBAL, followed by a Horner-Emmons olefination with diethyl 3-(methoxycarbonyl)-2-methyl-2-propenylphosphonate (**3.11**), afforded methyl ester **3.12** as a mixture of isomers (ratio not reported). Subsequent reduction with $LiAlH_4$ followed by oxidation with MnO_2 provided labelled all-*trans* retinals (**3.13a** and **3.13b**) that were isolated from the isomeric mixture by column chromatography (**Scheme 3.1**).



3.08 **3.09** 9-*E*:*Z* = 3:2 **3.10**

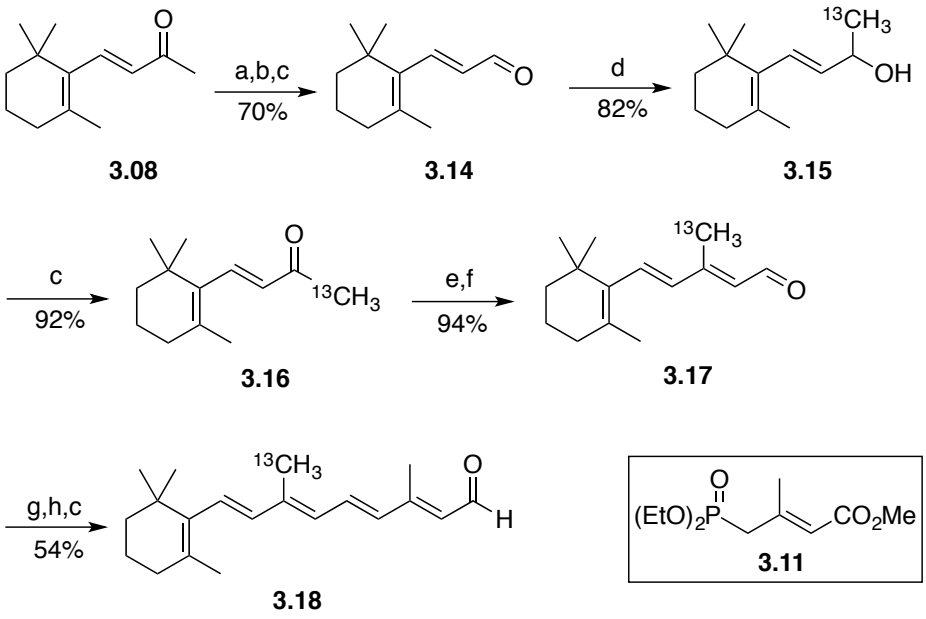
3.10 **3.11** **3.12**

3.13a,b

a = ^{13}C position for synthesis of [10-^{13}C]-all-*trans*-retinal
b = ^{13}C position for synthesis of [11-^{13}C]-all-*trans*-retinal

Scheme 3.1: *Reagents and conditions:* a) for **3.13**a: [2-^{13}C]-acetonitrile, for **3.13b**: [1-^{13}C]-acetonitrile, ^{n}BuLi, THF, −60 °C to 0 °C, b) NBS, $C_2H_4Cl_2$, 70 °C; c) i) DIBAL, pet. ether, −60 °C; ii) SiO_2, Et_2O/pet. ether/H_2O, 0 °C; d) $(EtO)_2P(O)CH_2C(CH_3)CHC(O)OMe$ (**3.11**) , NaH, THF, 0 °C to rt; e) $LiAlH_4$, Et_2O, −40 °C to 0 °C; f) MnO_2, CH_2Cl_2.

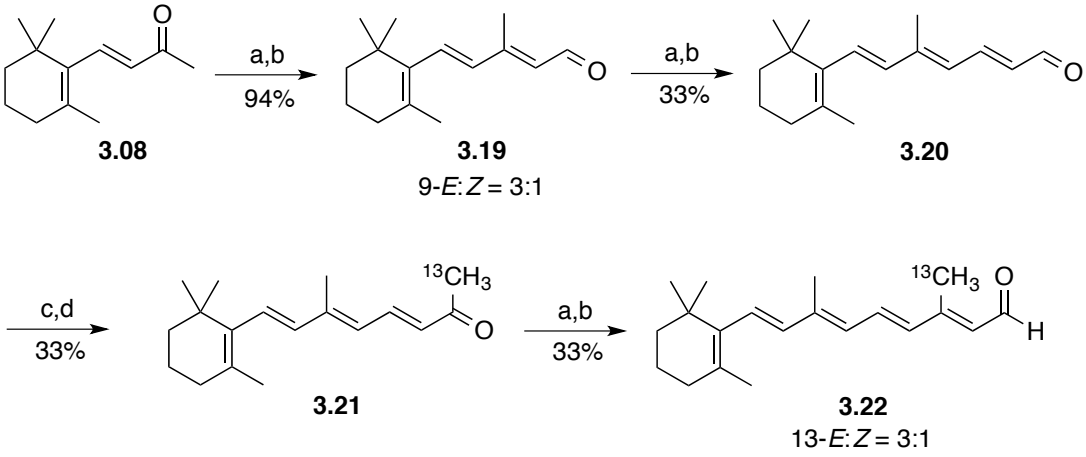
The synthesis of [19-^{13}C]-all-*trans*-retinal (**3.18**) was completed in 10 steps (27% overall yield), introducing ^{13}C enrichment with the Grignard reagent of $^{13}CH_3I$. β-Ionone (**3.08**) was oxidised to a carboxylic acid using sodium hypochlorite, reduced to an alcohol with $LiAlH_4$ and then oxidised to aldehyde **3.14** with MnO_2 in 70% over 3 steps. This manipulation allowed for the incorporation of the ^{13}C label by a Grignard reaction with ^{13}C enriched MeMgI, followed by oxidation with MnO_2 to regenerate the β-ionone structure **3.16**. Peterson olefination and acidic hydrolysis then provided aldehyde **3.17**. Finally, a Horner-Emmons olefination with diethyl 3-(methoxycarbonyl)-2-methyl-2-propenylphosphonate (**3.11**) followed reduction and oxidation afforded [19-^{13}C]-all-*trans*-retinal (**3.18**) (**Scheme 3.2**).



3.08 **3.14** **3.15** **3.16** **3.17** **3.18** **3.11**

Scheme 3.2 *Reagents and conditions:* a) NaOCl, NaOH, MeOH; b) $LiAlH_4$, Et_2O, −40 °C to 0 °C; c) MnO_2, CH_2Cl_2; d) $^{13}CH_3I$, Mg, Et_2O, rt to reflux; e) LDA, ^{t}BuNCHCH$_2$TMS, THF, −60 °C to 0 °C; f) HCOOH, THF/H_2O, 0 °C; g) **3.11**, NaH, THF, 0 °C to rt; h) $LiAlH_4$, Et_2O, −40 °C to 0 °C.

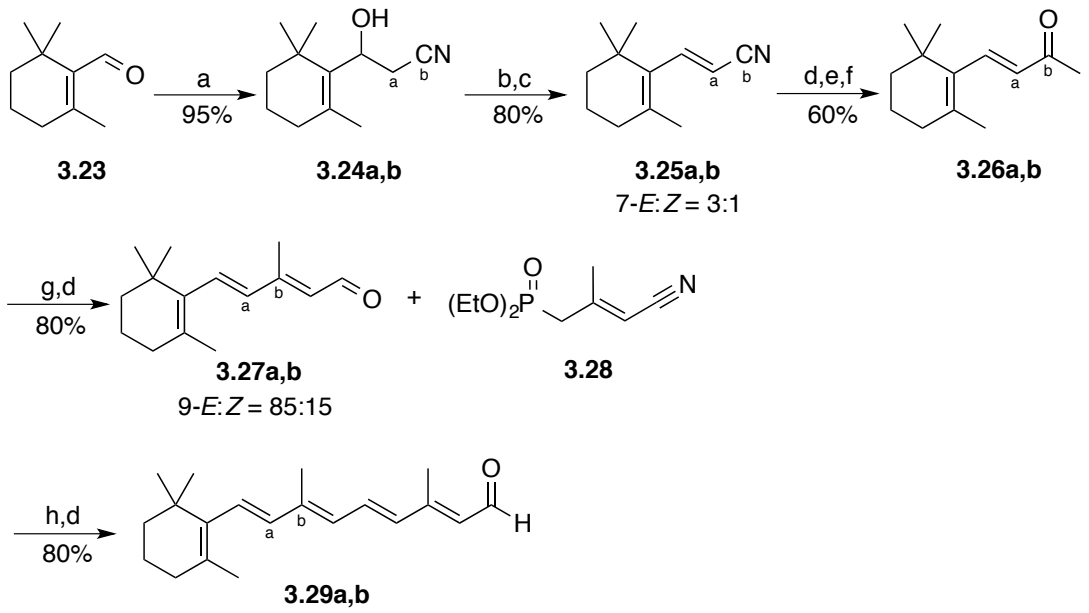
The synthesis of [20-^{13}C]-all-*trans*-retinal (**3.22**) made use of ^{13}MeMgI to incorporate the ^{13}C label (**Scheme 3.3**). Starting from β-ionone (**3.08**), two sequential Peterson olefinations provided aldehyde **3.20**, which was treated with ^{13}MeMgI and oxidised to ketone **3.21** with MnO_2. A final Peterson olefination afforded [20-^{13}C]-all-*trans*-retinal (**3.32**) as a mixture of *E*/*Z* C13 isomers, which were separated by chromatography. Overall **3.22** was obtained in 3% over 8 steps.



3.08 **3.19** 9-*E*:*Z* = 3:1 **3.20** **3.21** **3.22** 13-*E*:*Z* = 3:1

Scheme 3.3 *Reagents and conditions:* a) LDA, ^{t}BuNCHCH$_2$TMS, THF, −60 °C to 0 °C; b) HCOOH, THF, H_2O, 0 °C; c) $^{13}CH_3I$, Mg, Et_2O; d) MnO_2, CH_2Cl_2.

The synthesis of [8-^{13}C] and [9-^{13}C]-all-*trans*-retinals (**3.29a** and **3.29b**) with [2-^{13}C]-acetonitrile and [1-^{13}C]-acetonitrile respectively has also been reported by Pardeon *et al*. (**Scheme 3.4**).[127] Deprotonation of the appropriately labelled acetonitrile and reaction with aldehyde **3.23** fashioned hydroxynitriles **3.24a** and **3.24b**. Quantitative conversion to its corresponding acetate followed by base catalysed deacetylation at rt over 3 days afforded nitriles **3.25a** and **3.25b** as a mixture of isomers. Treatment of the mixture with DIBAL resulted in complete double bond isomerisation leading exclusively to the 7-*E* aldehyde. Conversion to labelled β-ionones **3.26a** and **3.26b** was then achieved by reaction with MeMgI followed by oxidation with MnO_2. A highly diastereoselective Horner-Emmons reaction with diisopropylphosphonoacetonitrile and subsequent DIBAL reduction later afforded aldehydes **3.27a** and **3.27b**. The desired [8-^{13}C] (**3.29a**) and [9-^{13}C] (**3.29b**) all-*trans*-retinals were finally realised by a Horner-Emmons olefination with diethyl-3-cyano-2-methylprop-2-enylphosphonate (**3.28**) and reduction with DIBAL (**Scheme 3.4**). The synthesis was completed in an overall yield of 29% over 10 steps.

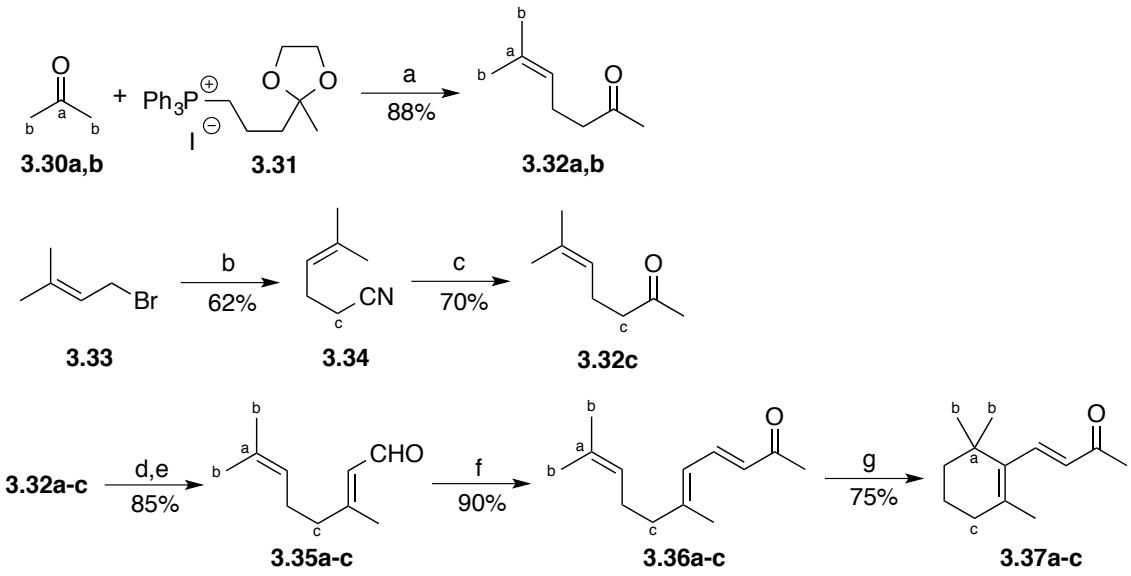


a = ^{13}C position for synthesis of [8-^{13}C]-all-*trans*-retinal
b = ^{13}C position for synthesis of [9-^{13}C]-all-*trans*-retinal

Scheme 3.4 *Reagents and conditions:* a) for **3.29**a: [2-^{13}C]-acetonitrile, for **3.29**b: [1-^{13}C]-acetonitrile, ^{n}BuLi, THF, –60 °C to 0 °C, b) Ac_2O, DMAP, pyridine, toluene; c) DBN, toluene; d) i) DIBAL, hexane/pet. ether, –60 °C to –20 °C; ii) SiO_2, H_2O/Et_2O/pet. ether; e) MeMgI, Et_2O, reflux; f) MnO_2 hexane; g) $(^{i}PrO)_2P(O)CH_2CN$, NaH, THF, 0 °C to rt; h) $(EtO)_2P(O)CH_2C(CH_3)CHCN$ (**3.28**), NaH, THF, 0 °C to rt.

The Lugtenburg group has also explored labelling of the cyclohexene ring of all-*trans*-retinals. The syntheses that enabled ^{13}C enrichment at positions 1, 4, and 16 and 17 of the β-ionone fragment are shown (**Scheme 3.5**).[128] A Wittig reaction between

phosphonium iodide **3.31** and [2-^{13}C]-acetone (**3.30a**) or [1,3-^{13}C]-acetone (**3.30b**), and subsequent acid catalysed dioxolane deprotection, afforded ketones **3.32a** and **3.32b**. Incorporation of ^{13}C at position 4 was achieved by an S_N2 reaction between prenyl bromide (**3.33**) and deprotonated [2-^{13}C]-acetonitrile to form labelled nitrile **3.34**. Treatment with excess MeLi then afforded ketone **3.32c**. A Horner-Emmons olefination of ketones **3.32a**-**c** followed by a DIBAL reduction gave access to aldehydes **3.35a**-**c** in high yield, which were converted to open-chain pseudo-ionones **3.36a**-**c** by an aldol condensation with acetone. Finally an acid-catalysed cyclisation gave the desired labelled β-ionones **3.37a**-**c** (**Scheme 3.5**).

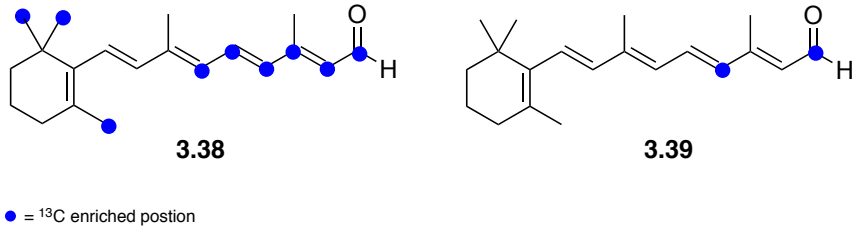


a = ^{13}C position for synthesis of [1-^{13}C]-all-*trans*-retinal
b = ^{13}C position for synthesis of [16,17-^{13}C]-all-*trans*-retinal
c = ^{13}C position for synthesis of [4-^{13}C]-all-*trans*-retinal

Scheme 3.5 *Reagents and conditions:* a) i) ^{n}BuLi, THF, −45 °C to rt.; ii) SiO_2, H_2SO_4, CH_2Cl_2; b) [2-^{13}C]-acetonitrile, ^{n}BuLi, THF, −60 °C to −50 °C; c) MeLi, Et_2O, 0 °C; d) $(EtO)_2P(O)CH_2CN$, NaH, THF; e) i) DIBAL, pet. ether, −60 °C to 10 °C; ii) SiO_2, H_2O, −30 °C to 0 °C; f) NaOH, acetone; g) H_2SO_4, $MeNO_2$, 0 °C.

3.6 Project Objectives

Together with our collaborators, the Glaubitz group (Goethe University Frankfurt), we are interested in advancing studies of retinal proteins, proteorhodopsin, channelrhodopsin and KR2 by utilizing DNP-enhanced solid state NMR. For structural insight into the channel-opening and -closing events, two all-*trans*-retinals (**3.38** and **3.39**) were designed.



3.38 **3.39**

● = ^{13}C enriched postion

Figure 3.11: [10-18-$^{13}C_9$]- all-*trans*-retinal (**3.38**) and [12,15-$^{13}C_9$]-all-*trans*-retinal (**3.39**).

We envisioned that enrichment within [10-18-$^{13}C_9$]-all-*trans*-retinal (**3.38**) would allow for new insight into the structural orientation of both the ring system and the polyene chain as well as changes of retinal-protein contacts, either by induced mutation or during photo-irradiation. Recent studies by the Glaubitz group show that only all-*trans* retinal is present in the ground state of the photocycle of channelrhodopsin. However this finding is controversial, with many academics disputing it; so further direct proof is required. [12,15-$^{13}C_2$]-all-*trans*-retinal (**3.39**) has been designed to allow for the direct measurement of the C12-C15 distance, which is different for 13-*cis* and all-*trans* retinal. The group believes that these measurements will provide the necessary evidence required to settle the dispute.

Our target was to complete the syntheses of labelled retinals **3.38** and **3.39** to facilitate the structural investigations by the Glaubitz group at Goethe University. We envisioned synthetic routes to the polyenes that make use of simple and readily available ^{13}C-enriched starting materials. Ultimately these routes would also permit access to other labelling patterns as required.

Chapter 4: Syntheses of ^{13}C-Labelled All-*Trans*-Retinals

Incorporation of all nine ^{13}C labels of [10-18-$^{13}C_9$]-all-*trans*-retinal (**3.38**) provided a difficult synthetic challenge. The use of expensive enriched starting materials that have both a low molecular mass and high volatility resulted in the need for a synthetic strategy that was efficient, concise and as optimised as possible in order to reduce the overall cost.

4.1 [10-18-$^{13}C_9$]-All-*Trans*-Retinal Initial Synthetic Strategy

Our initial synthetic strategy for [10-18-$^{13}C_9$]-all-*trans*-retinal (**3.38**) was based on the coupling of three fragments (**4.01**, **4.02** and **4.03**) by successive Horner-Emmons olefination reactions (**Figure 4.1**). It was envisioned that incorporation of the three methylene ^{13}C labels of aldehyde **4.01** would be achieved by a methylation of the commercially available β-keto ester **4.05** with $^{13}CH_3I$. $^{13}C_2$-Triethylphosphonoacetate (**4.03**) and phosphonate **4.02** can be prepared from $^{13}C_2$-ethyl bromoacetate using routes previously developed within the Brown group.[91]

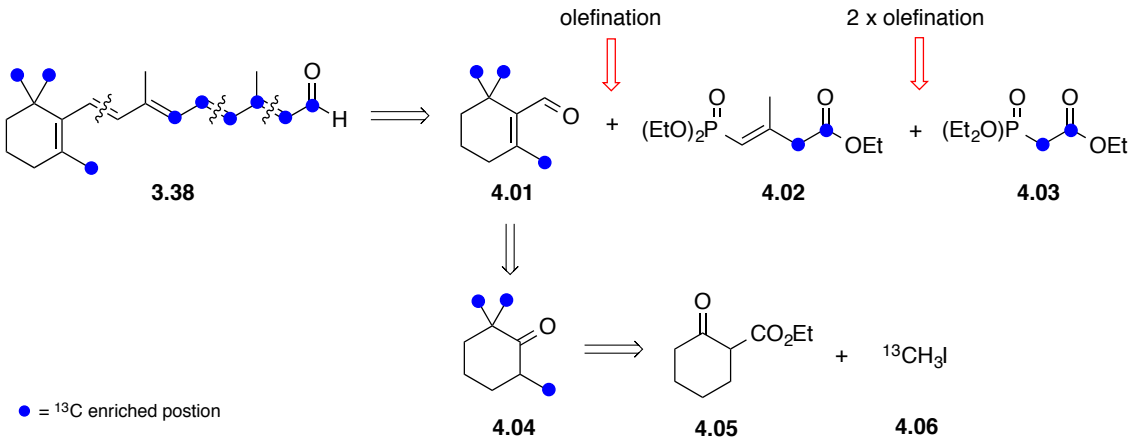


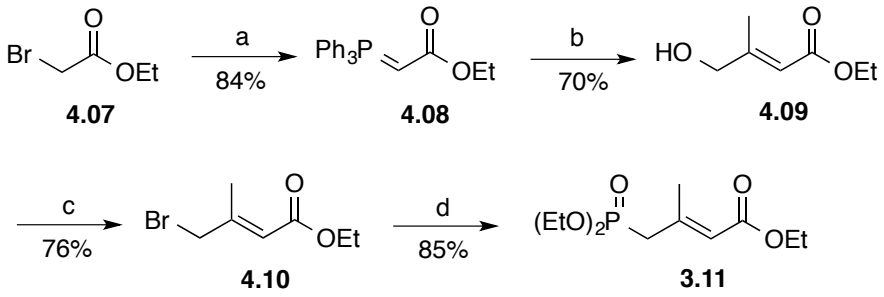
Figure 4.1: Retrosynthetic analysis of [10-18-$^{13}C_9$]-all-*trans*-retinal (**3.38**).

4.2 Unlabelled All-*Trans*-Retinal Synthesis

An initial unlabelled synthesis of all-*trans*-retinal (**3.01**) was investigated to develop and optimise an efficient route, which could later be repeated with the appropriate ^{13}C enriched starting materials to obtain the required labelling pattern.

4.2.1 Synthesis of Phosphonate Fragments 3.11 and 4.11

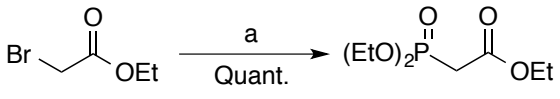
Phosphonate fragment **3.11** was prepared using a synthetic route developed previously within the Brown group and in similar overall yield.[91] Formation of ylide **4.08** and a Wittig reaction with hydroxyacetone afforded alkene **4.09** in good yield and with 100% *E*-selectivity. The stereochemistry of **4.09** was assumed based on the spectroscopic data matching those reported in the literature.[129] Conversion to the allylic bromide **4.10** proceeded cleanly and was subsequently heated with triethylphosphite in a sealed vial to complete the phosphonate fragment **3.11**.



4.07 **4.08** **4.09** **4.10** **3.11**

Scheme 4.1: *Reagents and conditions:* a) i) PPh_3, EtOAc; ii) 1 M NaOH, CH_2Cl_2; b) $HOCH_2(CO)CH_3$, MeCN, Δ; c) CBr_4, PPh_3, MeCN; d) $P(OEt)_3$, 160 °C.

Triethylphosphonoacetate (**4.11**) was produced in quantitative yield by an Arbuzov reaction of ethylbromoacetate (**4.07**) with triethylphosphite. The reaction time was reduced from 4 h in a sealed vial to 5 min in a microwave.[130]

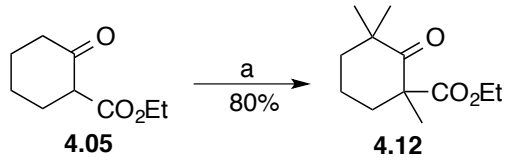


4.07 **4.11**

Scheme 4.2: *Reagents and conditions:* a) $P(OEt)_3$, μwave, 130 °C.

4.2.2 Synthesis of Aldehyde Fragment 4.17

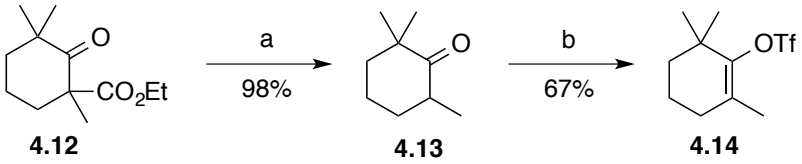
Trimethylation of β-keto ester **4.05** afforded the desired product **4.12** in high yield (**Scheme 4.3**) following a literature procedure described by Stevans *et al.*[131]



4.05 **4.12**

Scheme 4.3: *Reagents and conditions:* a) i) NaH (4.5 equiv.), THF, 0 °C; ii) MeI (3 equiv.), THF, 0 °C to rt.

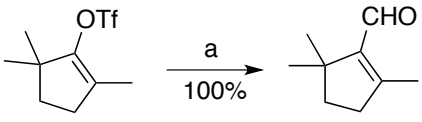
From **4.12**, acid-catalysed hydrolysis and decarboxylation afforded the relatively volatile cyclohexanone **4.13** (**Scheme 4.4**). The best yields were achieved when the reaction was heated under reflux for 3 days to ensure complete consumption of the starting material and then extracted with pentane before careful evaporation of the solvent at 400 mBar (20 °C). Triflation of ketone **4.13** by a procedure described by Breining *et al.*[132] afforded vinyl triflate **4.14** in yields ranging from 55-67% compared to the 84% yield reported. Changing the triflate source to triflic anhydride resulted in very poor conversion to the product (22%).



4.12 **4.13** **4.14**

Scheme 4.4: *Reagents and conditions:* a) conc. HCl, EtOH, 90 °C; b) LDA, $PhNTf_2$, THF, −78 °C to rt.

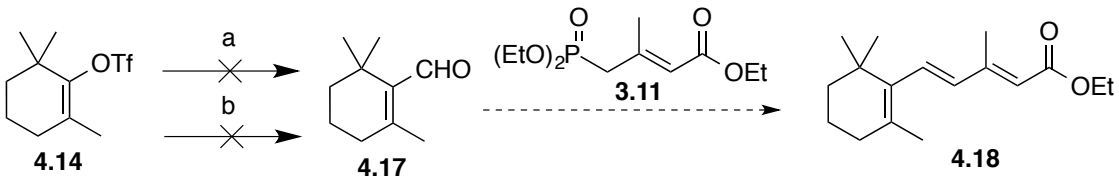
A paper by Baillargeon *et al.*[133] describes a slow but high yielding carbonylation of a 5 membered ring analogue of **4.15**, giving good precedent for the transformation to aldehyde **4.17** (**Scheme 4.5**).



4.15 **4.16**

Scheme 4.5: *Reagents and conditions:* a) CO (1 atm.), LiCl, $[Pd(PPh)_4]$ (10 mol %), Bu_3SnH, THF, 50 °C, 52 h.

Unfortunately the reaction was not applicable to vinyl triflate **4.14**, with no aldehyde product observed. Changing the hydride source to Et_3SiH was also unsuccessful.



Scheme 4.6: *Reagents and conditions:* a) CO (1 atm.), LiCl, [Pd(PPh$_3$)$_4$] (10 mol %), Bu$_3$SnH, THF, 50 °C; b) CO (1 atm.), LiCl, [Pd(PPh$_3$)$_4$] (10 mol %), Et$_3$SiH, THF, 50 °C.

4.2.3 Retinal synthesis from β-ionone

Due to difficulties accessing aldehyde **4.17**, a more efficient route to [10-18-$^{13}C_9$]-all-*trans*-retinal (**3.38**) was proposed that by-passed the carbonylation step (**Figure 4.2**). We envisioned that **3.38** could be accessed by a series of three Horner-Emmons reactions with $^{13}C_2$-triethyl phosphonoacetate (**4.03**) from labelled β-ionone **4.19**, which could be realised by a Heck coupling of vinyl triflate **4.20** with methyl vinyl ketone (**4.21**).

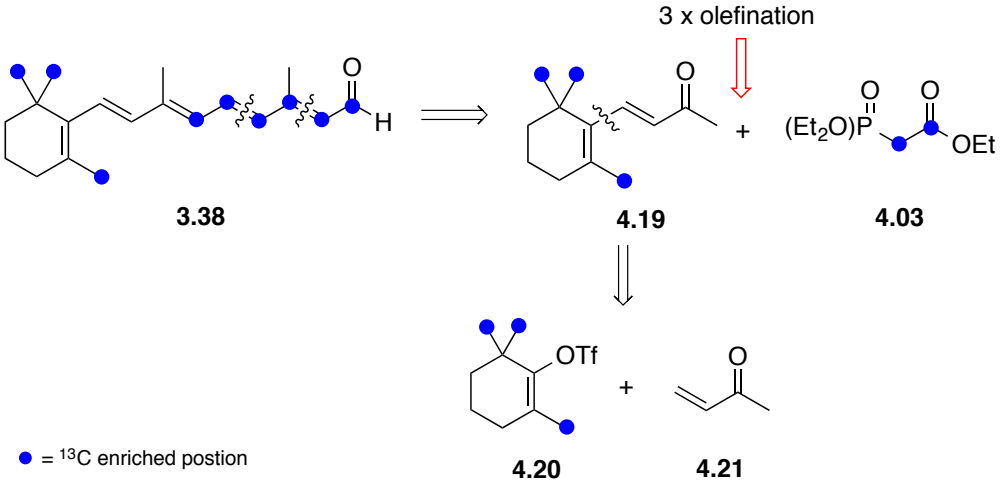
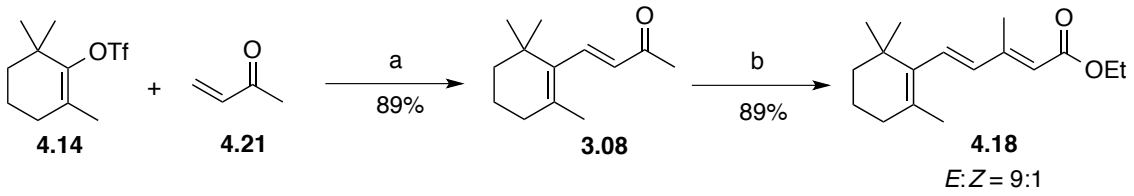


Figure 4.2: Revised retrosynthesis to [10-18-$^{13}C_9$]-all-*trans*-retinal (**3.38**).

The Heck reaction between vinyl triflate **4.14** and methyl vinyl ketone (**4.21**) proceeded cleanly to afford β-ionone (**3.08**) (**Scheme 4.7**). It is important to note that the quality of the palladium catalyst was vital for high conversion to the product. Following a procedure developed previously within the Brown Group,[134] the first Horner-Emmons reaction between β-ionone (**3.08**) and triethyl phosphonoacetate (**4.11**) provided the conjugated ester **4.18** as an inseparable mixture of isomers (*E*:*Z* = 9:1) in high yield.



Scheme 4.7: *Reagents and conditions:* a) $[Pd(PPh_3)_2Cl_2]$ (5 mol %), NEt_3, DMF, 75 °C; b) NaH, $(EtO)_2P(O)CH_2CO_2Et$ (**4.11**), Et_2O, rt.

Although a high yield was achieved for the first Horner-Emmons reaction (**Figure 4.3**), it wasn't without problems. Initial attempts produced significant side-reactions and it was discovered that the quality of the sodium hydride was responsible. When an 'old' batch of sodium hydride was used the reaction proceeded cleanly with no signs of degradation. However, with high purity NaH, high levels of polymerisation were observed. Although not complete, our best explanation is that the increased ratio of NaH to NaOH (formed from the reaction of NaH with water) in high quality sources resulted in a competing deprotonation of β-ionone and subsequent polymerisation via a 1,4-addition (**Figure 4.3**).

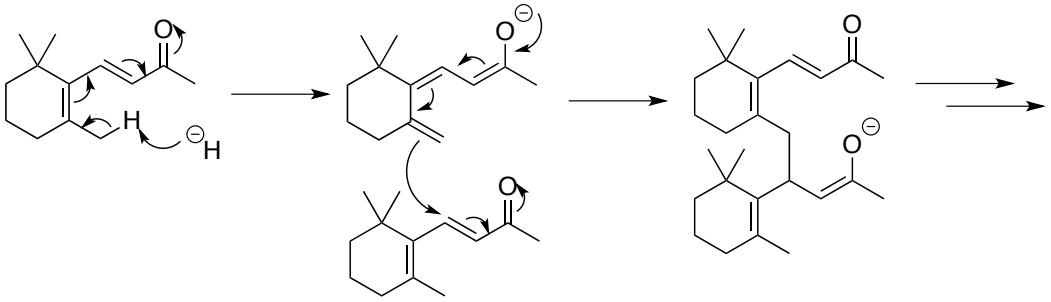
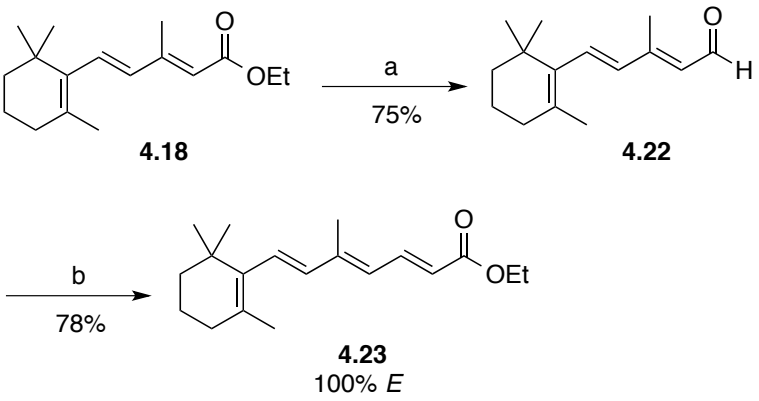


Figure 4.3: A possible anionic polymerisation mechanism initiated by the deprotonation of β-ionone.

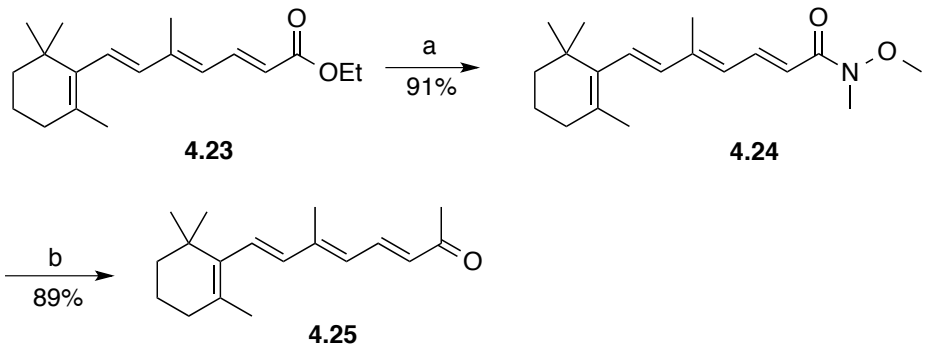
Other bases were investigated in the Horner-Emmons reaction (**Scheme 4.7**) without success. Poor conversion was achieved with NaHMDS and the mild conditions of DIPEA with LiCl[135] resulted in no reaction. In the interest of time the synthesis was continued with the same batch of NaH that did not result in polymerisation.

The conjugated ester **4.18** was converted to aldehyde **4.22** by reduction to the alcohol followed by a Ley-Griffith oxidation of the crude product.[134] DIBAL reduction of **4.18** resulted in significant over-reduction and was therefore unsuitable for this substrate. The isomers of **4.18** were separated by silica gel chromatography and the 9*E*-isomer subjected to the second Horner-Emmons olefination with triethyl phosphonoacetate (**4.11**) to afford **4.23** as a single isomer.



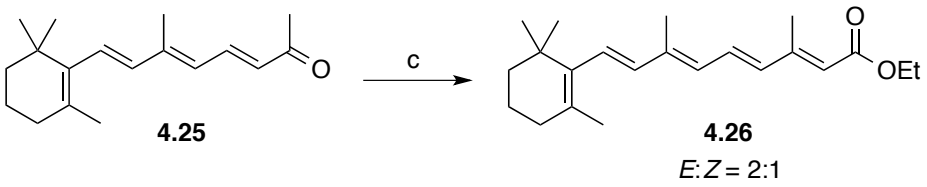
Scheme 4.8: *Reagents and conditions:* a) i) $LiAlH_4$, Et_2O, −78 °C to rt.; ii) NMO, TPAP, 4 Å sieves, CH_2Cl_2, rt.; b) NaH, $(EtO)_2P(O)CH_2CO_2Et$ (**4.11**), Et_2O, rt.

Weinreb amide intermediate **4.24** was prepared in high yield by treating ester **4.23** with the Weinreb amine anion, which was formed *in situ* by deprotonation of the Weinreb amine salt with two equivalents of ^{n}BuLi (**Scheme 4.9**). Rapid methylation at −78 °C with MeLi gave ketone **4.25** with no over-methylation observed.



Scheme 4.9: *Reagents and conditions:* a) MeONHMe.HCl, ^{n}BuLi (2 equiv.), THF, 0 °C to rt; b) MeLi, THF, −78 °C; c) NaH, $(EtO)_2P(O)CH_2CO_2Et$ Et_2O (**4.11**), rt.

Unfortunately the final Horner-Emmons reaction with triethyl phosphonoacetate (**4.11**) proceeded very slowly at room temperature and only 50% conversion to ester **4.26** was achieved after 7 days (**Scheme 4.10**). Also the product was very sensitive to silica gel and significant degradation was observed during purification. No clean material was isolated.

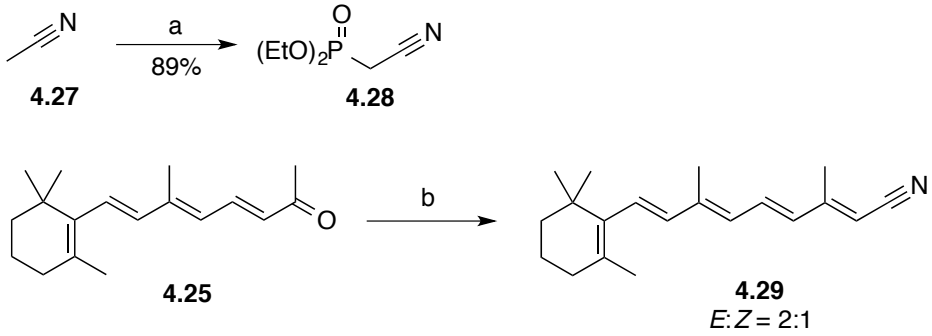


4.25 **4.26**
E:*Z* = 2:1

Scheme 4.10: *Reagents and conditions:* a) NaH, $(EtO)_2P(O)CH_2CO_2Et$ Et_2O (**4.11**), Et_2O, rt.

The Lugtenberg group have reported high yields for the olefination of **4.25** with cyanophosphonate **4.28** although the selectivity was not reported.[136] This reaction proved to be an appropriate alternative to the slow olefination with triethyl phosphonoacetate (**4.11**) (**Scheme 4.10**) and the $^{13}C_2$-acetonitrile precursor required for the labelled synthesis is widely available.

Cyanophosphonate **4.28** was prepared in high yield from acetonitrile (**4.27**) and diethyl chlorophosphate (**Scheme 4.11**). The Horner-Emmons reaction with ketone **4.25** proceeded quickly and with complete conversion to nitrile **4.29** with 2:1 *E*:*Z* selectivity. Interestingly, the nitrile product was more stable to silica than ester **4.26** however the isomers could not be separated at this stage. It is important to note that after the final Horner-Emmons all of the compounds were very light sensitive and easily underwent isomerisation. Therefore all of the highly conjugated compounds had to be handled in the dark (fume cupboard light off and samples wrapped in foil where possible).

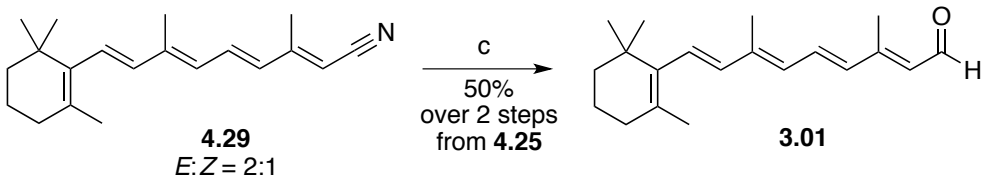


4.27 **4.28**

4.25 **4.29**
E:*Z* = 2:1

Scheme 4.11: *Reagents and conditions:* a) i) LiHMDS, THF, −78 °C; ii) $(EtO)_2P(O)Cl$, THF, −78 °C to 0 °C; b) ^{n}BuLi, **4.28**, THF, 0 °C to rt.; c) DIBAL, CH_2Cl_2, −60 °C.

The DIBAL reduction proceeded cleanly to the final retinal product **3.01** (**Scheme 4.12**). Partial separation of the two isomers was achieved on silica but preparative HPLC was required to obtain pure all-*trans*-retinal (**3.01**). It is important to note that deactivation of the silica for column chromatography with NEt_3 was essential to prevent isomerisation of the product. Overall the total synthesis of all-*trans*-retinal (**3.01**) was completed in 12 linear steps from ethyl-2-oxocyclohexanecarboxylate (**4.05**) in 15% yield. All spectroscopic data was consistent with those reported in the literature.[137]

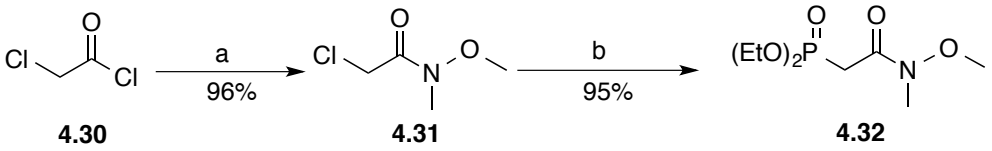


Scheme 4.12: *Reagents and conditions:* a) DIBAL, CH_2Cl_2, –60 °C.

4.2.4 Weinreb Phosphonamide Route

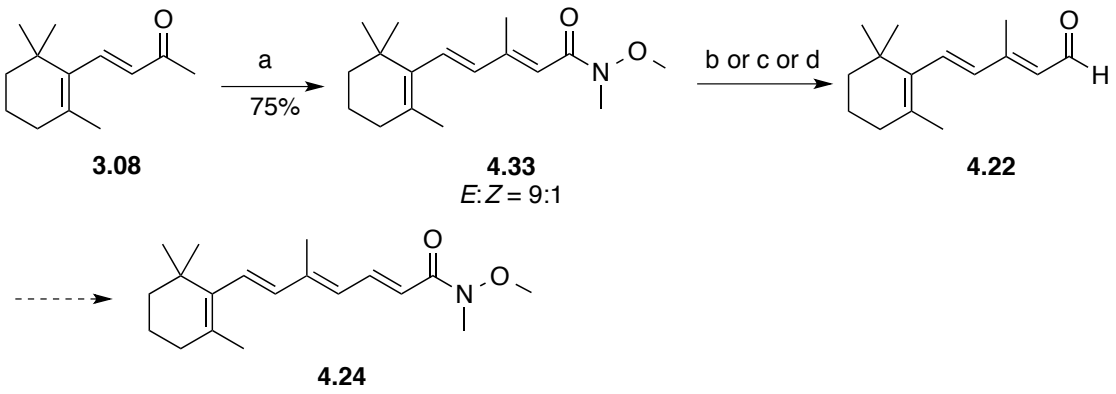
Before undertaking the synthesis of $^{13}C_9$ retinal **3.38** a more efficient route was explored using triethyl phosphonamide **4.32** (**Scheme 4.13**) in the three chain extension steps. Using a Weinreb amide analogue could allow for a single reduction step to obtain aldehyde **4.22**, and an ester to Weinreb amide functional group conversion would no longer be required.

Phosphonamide **4.32** was synthesised from chloroacetyl chloride (**4.30**) by a high yielding Schotten-Bauman reaction with the Weinreb amine salt, followed by reaction with triethyl phosphite (**Scheme 4.13**). Starting with the commercially available $^{13}C_2$-chloroacetyl chloride would allow for the desired ^{13}C enrichment.



Scheme 4.13: *Reagents and conditions:* a) MeONHMe, K_2CO_3, H_2O, ^{t}BME/H_2O –15 °C; b) $P(OEt)_3$, 120 °C.

The first Horner-Emmons coupling with β-ionone (**3.08**) proceeded efficiently, affording Weinreb amide **4.33** with an *E*:*Z* ratio of 9:1 (**Scheme 4.14**). Unfortunately, treatment of **4.33** with $LiAlH_4$, DIBAL and Schwartz reagent all showed incomplete reduction to aldehyde **4.22** and the formation of an unidentified by-product, which was inseparable from the desired product. It is likely that the Weinreb amide was more difficult to reduce than the ester equivalent (**4.18**) and as a result this allowed for competing side reactions with the polyene chain.

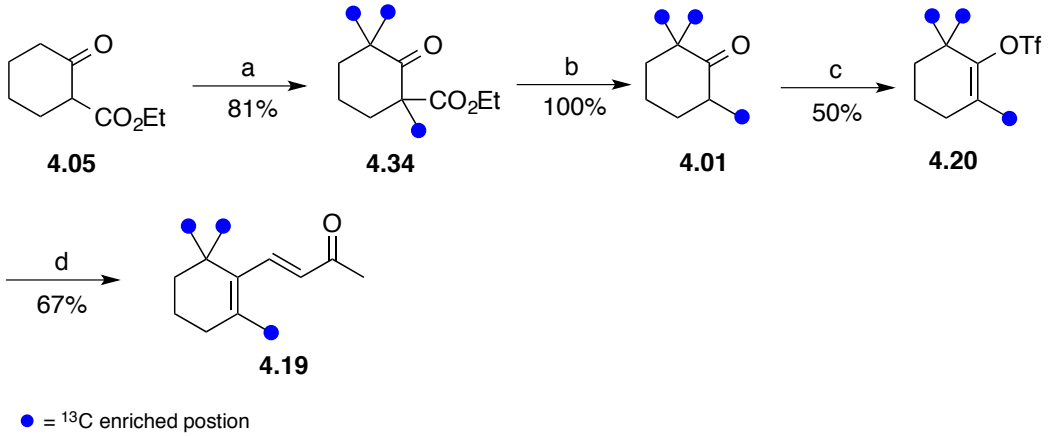


Scheme 4.14: *Reagents and conditions:* a) NaH, **4.32**, Et_2O, rt.; b) $LiAlH_4$, THF, −78 °C to rt.; c) DIBAL, THF, −78 °C; d) $ZrCp_2HCl$, THF, 0 °C.

Alternative reduction conditions that could avoid any side reactions were not obvious, and as the labelled retinal samples were required as soon as possible to begin the NMR studies, no further investigation of this route was carried out.

4.3 [10-18-$^{13}C_9$]-All-*Trans*-Retinal Synthesis

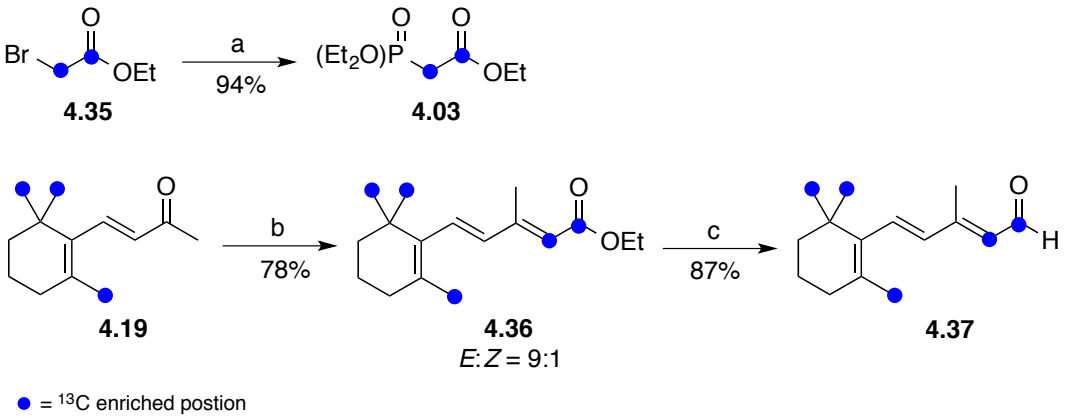
With a suitable synthetic route in hand, the synthesis of [10-18-$^{13}C_9$]-all-*trans*-retinal (**3.38**) began by trimethylation of ethyl 2-oxocyclohexanecarboxylate (**4.05**) with $^{13}CH_3I$ to afford β-keto ester **4.34** (**Scheme 4.15**). Subsequent acid catalysed hydrolysis and decarboxylation afforded the relatively volatile cyclohexanone **4.01** in quantitative yield. Difficulty separating the triflation product **4.20** from a by-product resulted in a lower than expected yield. Finally the Heck reaction with methylvinyl ketone delivered triplely labelled β-ionone **4.19**.



4.05 **4.34** **4.01** **4.20** **4.19**

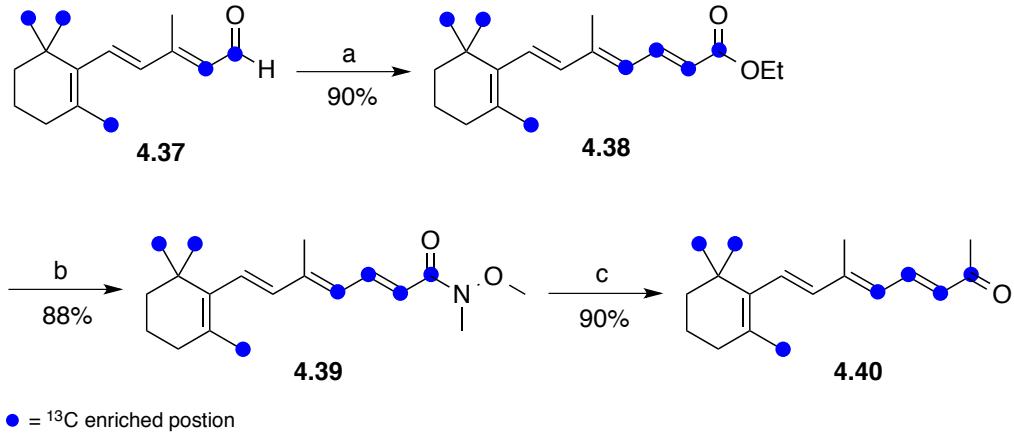
Scheme 4.15: *Reagents and conditions:* a) i) NaH (4.5 eq.), THF, 0 °C; ii) MeI (3 equiv.), THF, 0 °C to rt; b) conc. HCl, EtOH, 90 °C; c) LDA, $PhNTf_2$, THF, −78 °C to rt; d) $[Pd(PPh_3)_2Cl_2]$ (5 mol %), NEt_3, DMF, 75 °C.

The first olefination reaction using doubly ^{13}C-labelled triethyl phosphonoacetate (**4.03**) yielded trienoate **4.36** as an inseparable mixture of *E*/*Z* isomers (9:1 from ^1H NMR) (**Scheme 4.16**). Following aluminium hydride reduction and TPAP oxidation, the all-*E*-trienal **4.37** was isolated by column chromatography.



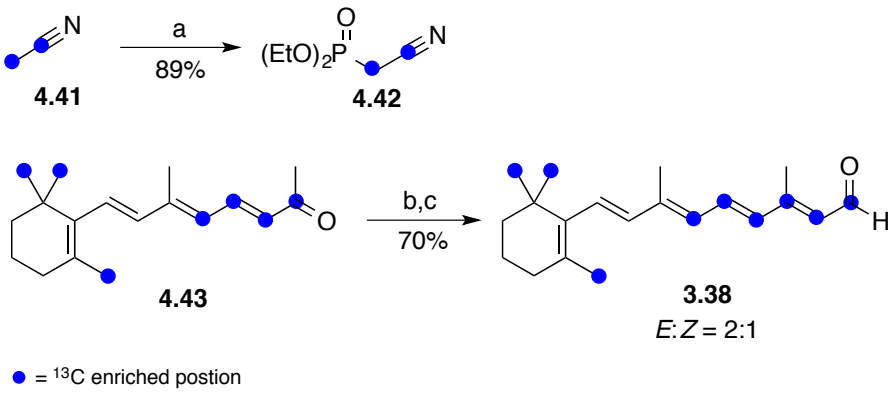
Scheme 4.16: *Reagents and conditions:* a) $P(OEt)_3$, μwave, 130 °C; b) NaH, **4.03**, Et_2O, rt; c) i) $LiAlH_4$, Et_2O, −78 °C to rt.; ii) NMO, TPAP, 4 Å sieves, CH_2Cl_2, rt.

The second olefination with $^{13}C_2$- triethyl phosphonoacetate (**4.03**) introduced the C12 and C13 labelled carbon atoms with complete selectivity for the all-*trans*-stereoisomer (**Scheme 4.17**). Formation of Weinreb amide **4.39** allowed clean and high-yielding conversion to methyl ketone **4.40**.



Scheme 4.17: *Reagents and conditions:* a) NaH, **4.03**, Et_2O, rt; c) MeONHMe.HCl (4 equiv.), ^{n}BuLi (7.8 equiv.), THF, 0 °C to rt; d) MeLi, THF, −78 °C.

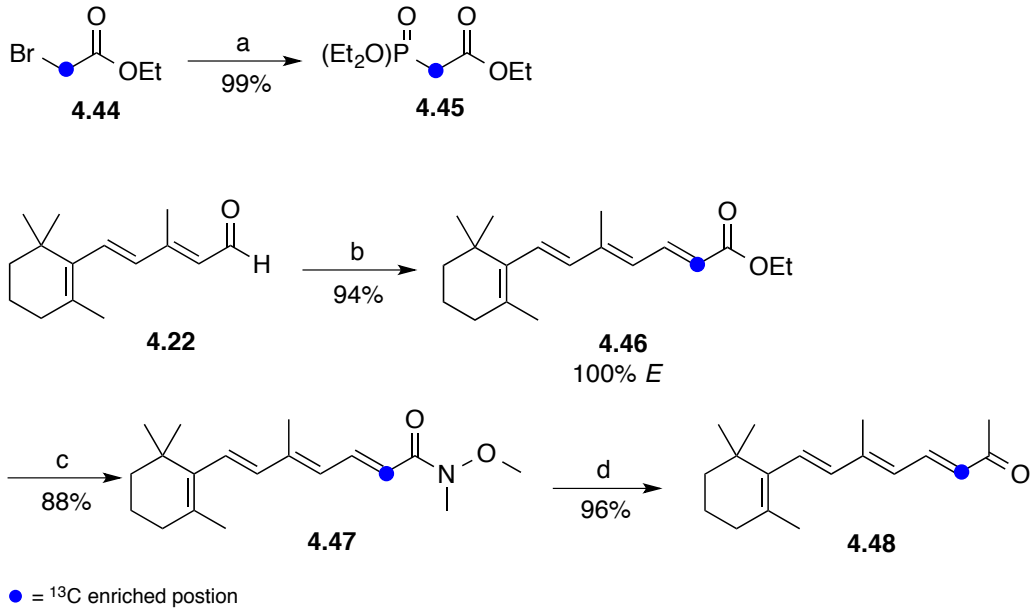
Doubly ^{13}C-labelled diethyl (cyanomethyl)phosphate (**4.42**) was prepared from labelled acetonitrile in good yield. The final olefination resulted in a mixture of 13-*E*/*Z* isomers (2:1 by ^1H NMR) which, following DIBAL reduction and purification by preparative HPLC, delivered the pure [10-18-$^{13}C_9$]-all-*trans*-retinal (**3.38**) in 9% overall yield over 11 linear steps.



Scheme 4.18: *Reagents and conditions:* a) i) LiHMDS, THF, −78 °C; ii) $(EtO)_2P(O)Cl$, THF, −78 °C to 0 °C; b) ^{n}BuLi, **4.42**, THF 0 °C to rt.; c) DIBAL, CH_2Cl_2, −60 °C.

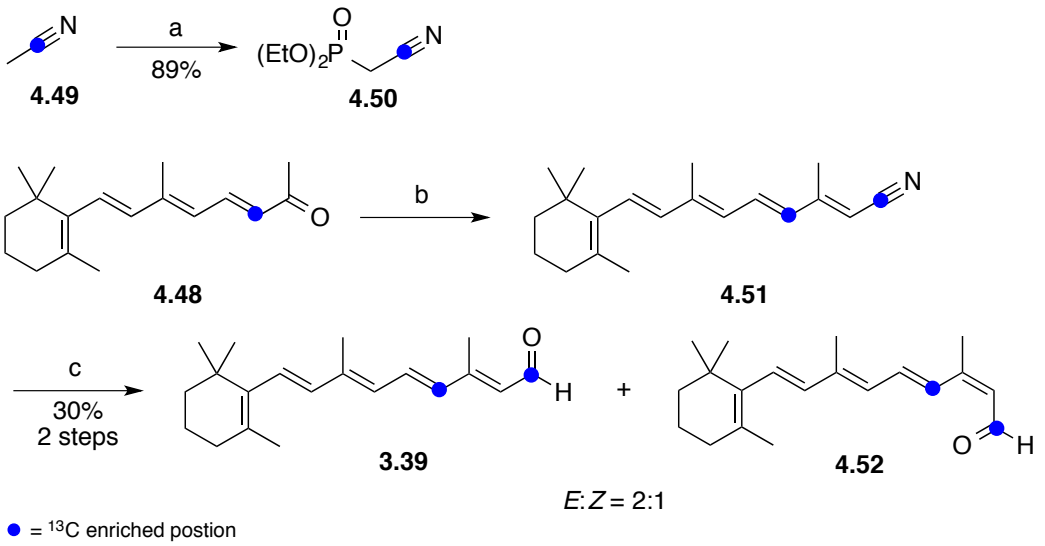
4.4 [12,15-$^{13}C_2$]-All-*Trans*-Retinal Synthesis

The synthesis of [12,15-$^{13}C_2$]-all-*trans*-retinal (**3.39**) was achieved similarly to [10-18-$^{13}C_9$]-all-*trans*-retinal (**3.38**), but starting from commercial β-ionone. The first olefination was achieved in excellent yield using singly ^{13}C-labelled phosphonate **4.45**, which was accessed from commercially available 2-^{13}C-ethyl bromoacetate (**4.44**) (**Scheme 4.19**). Conversion to Weinreb amide **4.47** followed by methylation afforded ketone **4.48** in high yield.



Scheme 4.19: *Reagents and conditions:* a) $P(OEt)_3$, μwave, 130 °C; b) NaH, **4.45**, Et_2O, rt; c) MeONHMe.HCl (4 equiv.), ^{n}BuLi (7.8 equiv.), THF, 0 °C to rt; d) MeLi, THF, −78 °C.

The second ^{13}C-label originated from 1-^{13}C-acetonitrile (**4.49**) (**Scheme 4.20**). The final Horner-Emmons coupling between singly labelled cyanophosphate **4.50** and ketone **4.48** gave access to nitrile **4.51**. The DIBAL reduction was first attempted on the crude nitrile but proved unsuccessful. After purification by column chromatography, the reduction was repeated with complete conversion to the isomeric mixture of $^{13}C_2$-retinals **3.39** and **4.52**. The low yield described was due to the difficulties with the initial reduction. Finally, separation of the retinal isomers was achieved by preparative HPLC and labelled retinals **3.39** and **4.52** were sent to the Glaubitz group for analysis.



Scheme 4.20: *Reagents and conditions:* a) i) LiHMDS, THF, −78 °C; ii) $(EtO)_2P(O)Cl$, THF, −78 °C to 0 °C; b) ^{n}BuLi, **4.50**, THF, 0 °C to rt; c) DIBAL, CH_2Cl_2, −60 °C.

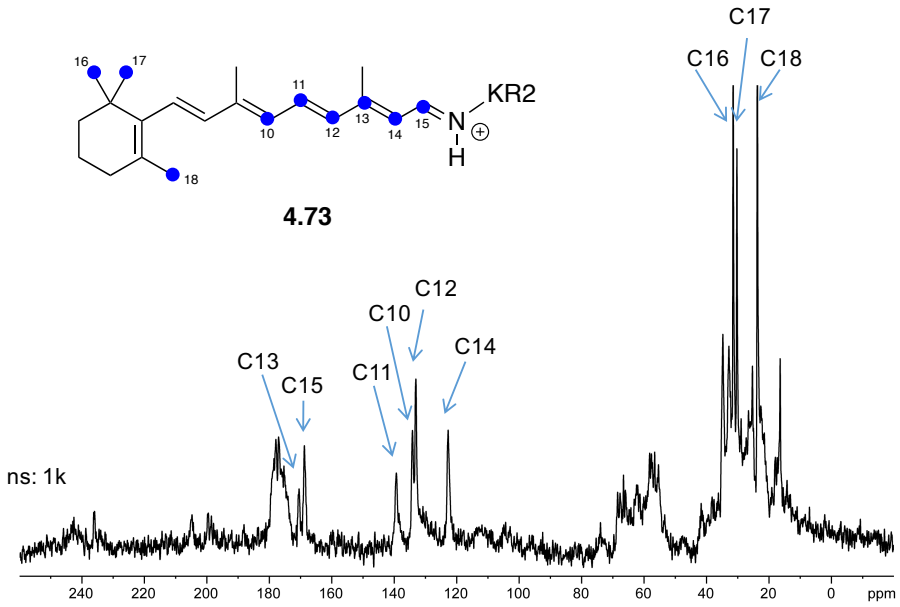
4.5 Characterisation and Assumptions

During the synthesis of retinal isotopomers **3.38** and **3.39**, all ^{13}C-enriched compounds were characterised using ^{1}H and ^{13}C NMR, IR and mass spectrometry. Signals in the ^{1}H NMR spectra showed additional coupling to the ^{13}C atoms with large coupling constants (up to 170 Hz). The remaining ^{1}H-^{1}H coupling constants for each compound matched those measured in the spectra of corresponding unlabelled compounds. For the ^{13}C NMR spectra, only the signals for the ^{13}C-enriched positions were reported. The chemical shift of these peaks matched those for the unlabelled molecules.

The IR spectra of the unlabelled and ^{13}C-enriched compounds were almost identical, with a slight shift in the wavenumber of some signals of up to 3 cm^{-1}. The mass of each isotopomer was increased by one for each ^{13}C-enriched carbon within the molecule. No kinetic isotope effect was observed during the reactions with ^{13}C-enriched material, and the stereoselectivity was assumed to be the same as for reactions with the unlabelled compounds.

4.6 Retinal Protein Structural Studies

Studies of [10-18-$^{13}C_9$]- and [12,15-$^{13}C_2$]-all-*trans*-retinals (**3.38** and **3.39**) in proteorhodopsin, channelrhodopsin and KR2 are currently ongoing and the results will be published in due course. [10-18-$^{13}C_9$]- all-*trans*-retinal (**3.38**) has so far been incorporated into the KR2 protein and a ^{13}C MAS spectra obtained (**Figure 4.4**). All nine signals from the ^{13}C-labels have been assigned using 2D-correlation spectra (not currently disclosed). The upfield shift of C15 from 190 ppm to 169 ppm shows the successful incorporation of the retinal **3.38** and formation of the protonated Schiff base **4.73**.



C	δ/ppm
C10	134.2
C11	139.3
C12	133.1
C13	170.5
C14	122.7
C15	168.8
C16	31.5
C17	30.3
C18	23.7

Figure 4.4: ^{13}C MAS of KR2-[10-18-$^{13}C_9$]-all-*trans*-retinal at 850 MHz, 14 kHz MAS, 275 K. The spectrum was recorded and samples made by Dr Jagdeep Kaur from Goethe University, Frankfurt.

4.7 Conclusions

An efficient route has been developed for the synthesis of all-*trans*-retinal (**3.01**) (**Figure 4.5**) from ethyl-2-oxocyclohexanecarboxylate (**4.05**) in 11 linear steps with a 15% overall yield. This route has been applied to the synthesis of [10-18-$^{13}C_9$]-all-*trans*-retinal (**3.38**) by employing $^{13}CH_3I$ and $^{13}C_2$-ethyl bromoacetate. In addition [12,15-$^{13}C_2$]-all-*trans*-retinal (**3.39**) has been synthesised in 6 steps from commercial β-ionone.

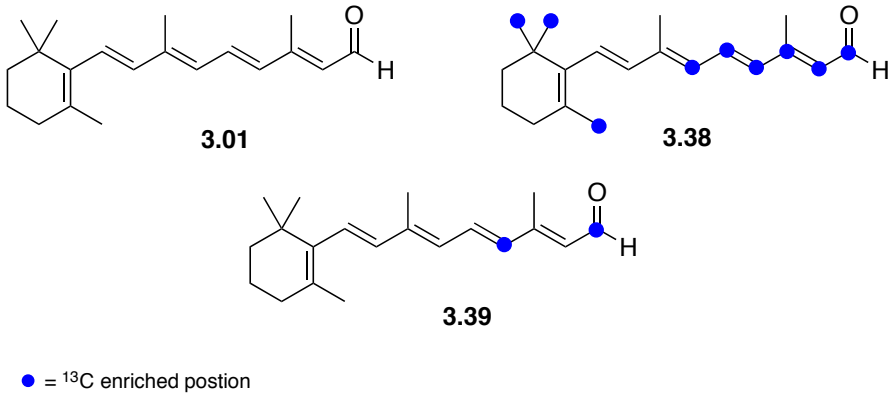
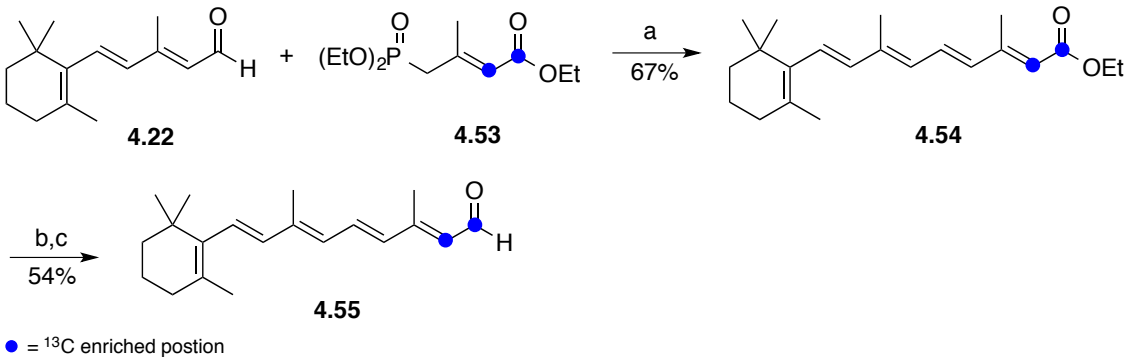


Figure 4.5: All-*trans*-retinal (**3.01**), [10-18-$^{13}C_9$]-all-*trans*-retinal (**3.38**) and [12,15-$^{13}C_2$]-all-*trans*-retinal (**3.39**).

A fully assigned ^{13}C MAS spectra of [10-18-$^{13}C_9$]- all-*trans*-retinal (**3.38**) incorporated into KR2 has been produced by the Glaubitz group and further studies are ongoing. Results from this work will be published in due course.

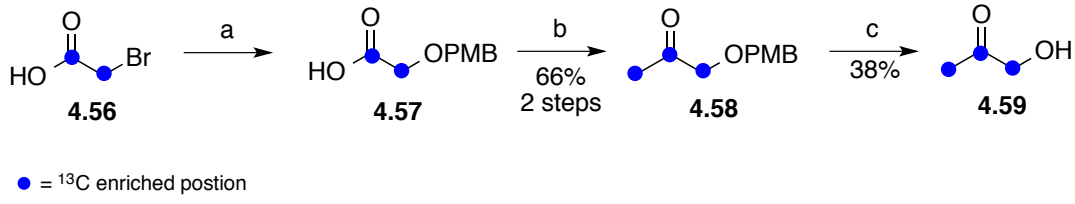
4.8 Further Work

In order to improve the fairly poor selectivity of the final olefination an adapted route could be developed. In the synthesis of [14,15-$^{13}C_2$]-all-*trans*-retinal (**4.55**) within the Brown Group,[91] the labelled phosphonate **4.53** was used in a Horner-Emmons coupling with aldehyde **4.22**. This reaction showed complete selectivity for the all-*trans*-isomer of **4.55** (**Scheme 4.21**), making the final purification of the unstable labelled retinal very straightforward.



Scheme 4.21: *Reagents and conditions:* a) DMPU, ^{n}BuLi, THF, −78 °C; b) $LiAlH_4$, Et_2O, −78 °C; c) MnO_2, CH_2Cl_2.

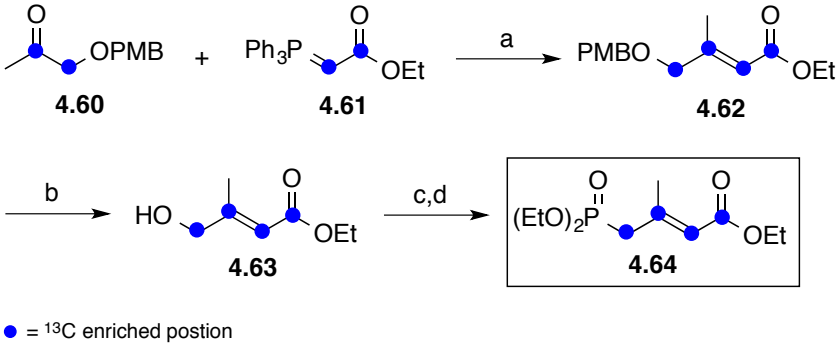
The route shown in **Scheme 4.21** was avoided for the synthesis of [10-18-$^{13}C_9$]-all-*trans*-retinal (**3.38**) due to 1,2-$^{13}C_2$-hydroxyacetone not being commercially available and the potential difficulty of synthesising such a volatile labelled compound. A paper from Dicus *et al*[138] describes the synthesis of $^{13}C_3$-hydroxyacetone (**4.59**) in moderate yield from commercially available $^{13}C_2$-2-bromoacetic acid (**4.56**) by use of a *p*-methoxybenzyl (PMB) protecting group to increase the boiling point of the substrate prior to the methylation (**Scheme 4.22**).



Scheme 4.22: *Reagents and conditions:* a) NaH, *p*-methoxybenzylalcohol, THF, 0 °C to rt; b) $^{13}CH_3Li$, Et_2O, −78 °C; c) DDQ, CH_2Cl_2/H_2O, rt.

Labelled phosphonate **4.64** could be accessed by a Wittig coupling of PMB ether **4.60** with labelled ylide **4.61** followed by PMB deprotection and conversion to the phosphonate (**Scheme 4.23**). PMB ether **4.60** could be prepared from $^{13}C_2$-2-bromoacetic acid **4.56** and unlabelled MeLi using the procedure previously discussed (**Scheme 4.22**). This method

should provide an efficient route to labelled phosphonate **4.64** that can be coupled with labelled aldehyde **4.37** (**Scheme 4.17**) with complete *E*-selectivity.



Scheme 4.23: *Reagents and conditions:* a) MeCN, Δ; b) DDQ, CH_2Cl_2/H_2O; c) CBr_4, PPh_3, MeCN; d) $P(OEt)_3$, 160 °C.

Future investigations into the ring orientation of the retinylidene chromophore within rhodopsin proteins could benefit from differentiating the methyl groups with ^{13}C labelling. Differences in the methyl chemical shift of each labelled retinal (**4.65** and **4.66**, **Figure 4.6**) within the protein could allow for precise analysis of the ring position relative to key protein residues.

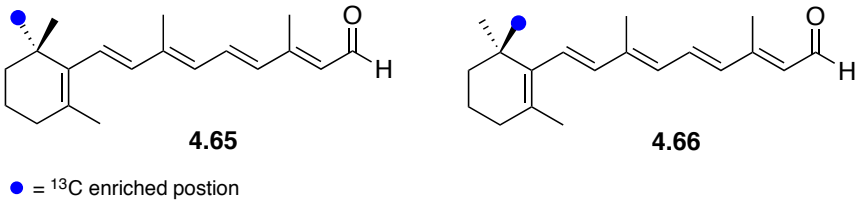


Figure 4.6: Proposed ^{13}C-labelled retinal isomers **4.65** and **4.66**.

In order to synthesise these isotopically labelled isomers, a method for introducing the ^{13}C labelled methyl group stereoselectively would have to be developed. To the best of our knowledge this type of stereoisomerism based on differentiation of methyl groups by isotopic labelling is a novel concept and would be an interesting task. The strategy proposed for this ^{13}C enrichment involves the diastereoselective methylation of β-keto ester **4.67** with $^{13}CH_3I$ using a chiral auxiliary to induce facial selectivity (**Figure 4.7**). After a second methylation, the ester group will be converted to a methyl group by reduction to alcohol **4.70** and subsequent radical deoxygenation. The resulting cyclohexanone **4.71** will then be converted to labelled β-ionone **4.72** from which the retinal synthesis could be completed.

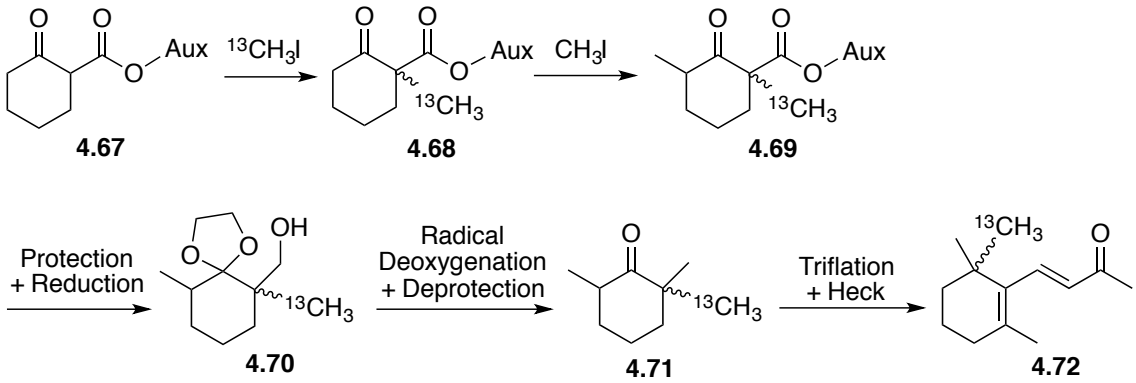


Figure 4.7: Proposed synthetic strategy for the stereoselective synthesis of ^{13}C labelled β-ionone **4.72**.

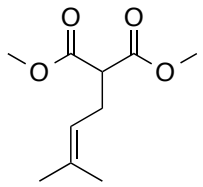
Chapter 5: Experimental

5.1 General Methods

Chemicals were purchased from Sigma-Aldrich, Fisher Scientific or Alfa Aesar. NaH was used as a 60% dispersion in oil and all ^{13}C labelled starting materials were 99% ^{13}C-enriched. All air/moisture sensitive reactions were carried out under an inert atmosphere, in oven-dried or flame-dried glassware. The solvents THF (from Na/benzophenone), CH_3CN and CH_2Cl_2 (from CaH_2) and MeOH (from $Mg(OMe)_2$) were distilled before use, and where appropriate, other reagents and solvents were purified by standard techniques.[139] TLC was performed on aluminium-precoated plates coated with silica gel 60 with an F_{254} indicator; visualised under UV light (254 nm) and/or by staining with anisaldehyde, ceric ammonium molybdate, iodine, phosphomolybdic acid, potassium permanganate or vanillin. Flash column chromatography was performed using high purity silica gel, pore size 60 Å, 230-400 mesh particle size, purchased from Merck. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$, CD_3CN, acetone-*d6* or C_6D_6 solutions (purchased from Cambridge Isotope Laboratories) at 298 K using Bruker DPX400 (400 and 101 MHz respectively) spectrometers. Chemical shifts are reported on the δ scale in ppm and were referenced to residual solvent ($CDCl_3$: 7.27 ppm for ^1H NMR spectra and 77.0 ppm for ^{13}C NMR spectra; CD_3CN: 1.94 ppm for ^1H NMR spectra and 1.4 and 118.7 ppm for ^{13}C NMR spectra; acetone-*d6*: 2.09 ppm for ^1H NMR spectra and 29.9 and 206.7 ppm for ^{13}C NMR spectra; C_6D_6: 7.16 ppm for ^1H NMR spectra and 128.06 ppm for ^{13}C NMR spectra). All spectra were reprocessed using ACD/Labs software version 2015 or ACD/Spectrus. Coupling constants (*J*) were recorded in Hz. The following abbreviations for the multiplicity of the peaks are s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), sxt (sextet), spt (septet), br (broad), and m (multiplet). Electrospray (ES) low resolution mass spectra were recorded on a Waters TQD quadrupole spectrometer. Electron impact (EI) low resolution mass spectra were recorded on a Trace 2000 Series GC-MS. High resolution mass spectra were recorded on a Bruker APEX III FT-ICR mass spectrometer. Fourier-transform infrared (FT-IR) spectra are reported in wavenumbers (cm^{-1}) and were collected as solids or neat liquids on a Nicolet 380 fitted with a Smart Orbit Goldengate attachment using OMNIC software package. The abbreviations s (strong), m (medium), w (weak) and br (broad) are used when reporting the spectra. Melting points were obtained using a Gallenkamp Electrothermal apparatus. HPLC purification was performed with a Shimadzu VP series HPLC and Phenomenex silica column, eluting with EtOAc/pet. ether.

5.2 Procedures and Characterisation Procedures

2.05 - Dimethyl 2-(3-methylbut-2-en-1-yl)malonate



$C_{10}H_{16}O_4$

200.23 $gmol^{-1}$

By adaption of a procedure by Klahn *et al.*,[140] to a suspension of oven dried (200 °C) K_2CO_3 (726 mg, 5.52 mmol) in acetone (8 mL) under N_2 was added dimethyl malonate (300 μL, 2.63 mmol) and prenyl bromide (208 μL, 1.99 mmol) and the suspension was stirred at rt for 18 h. The reaction was quenched with saturated aqueous NH_4Cl (10 mL) and the aqueous phase was extracted with EtOAc (3 x 10 mL). The combined organic solution was washed with brine, dried ($MgSO_4$) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:pet. ether = 3:97) afforded the product a colourless oil (347 mg, 1.73 mmol, 87%). Spectroscopic data were consistent with those reported.[50]

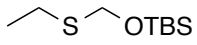
FT-IR (neat) ν_{max} 2955 (m), 1733 (s), 1148 (s) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.09-5.02 (m, 1H, -C=C**H**), 3.74 (s, 6H, -OCH_3), 3.37 (t, *J* = 7.6 Hz, 1H, -OCC**H**), 2.60 (t, *J* = 7.5 Hz, 2H, -C=CHC**H**$_2$), 1.69 (s, 3H, -CC**H**$_3$), 1.64 (s, 3H, -CC**H**$_3$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 169.60 (**C**=O), 135.10 (CH=**C**), 119.46 (**C**H=C), 52.41 (OC-C**H**), 51.91 (-O**C**H_3), 27.62 (C=CH**C**H_2), 25.73 (CH=C**C**H_3), 17.69 (CH=C**C**H_3) ppm

LRMS (ES^+) *m/z* = 201 $[M+H]^+$

2.07 - *tert*-Butyl((ethylthio)methoxy)dimethylsilane



$C_9H_{22}OSSi$

206.42 $gmol^{-1}$

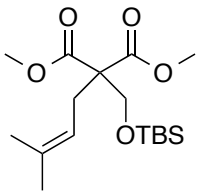
Following a procedure by Benneche *et al.*,[56] to a mixture of ethanethiol (3.00 mL, 40.5 mmol) and paraformaldehyde (1.18 g, 39.1 mmol) was added NaOMe (35 mg, 0.65 mmol). The mixture was heated to 40 °C for 30 min. After cooling to rt the residue was taken up in CH_2Cl_2 (40 mL) and TBSCl (3.36 g, 22.3 mmol) was added, followed by imidazole (2.48 g, 36.4 mmol) portion-wise. The suspension was stirred at rt for 30 min before adding saturated aqueous NH_4Cl (30 mL) and extracting with CH_2Cl_2 (3 x 30 mL). The combined organic solution was washed with brine, dried ($MgSO_4$) and the solvent removed under reduced pressure. Purification by distillation (8 mBar, 80 °C) afforded the product as a colourless oil (3.91 g, 19.0 mmol, 85%). Spectroscopic data were consistent with those reported.[56]

FT-IR (neat) ν_{max} 2929 (m), 1254 (s), 1059 (s) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.82 (s, 2H, C**H**$_2$O), 2.68 (q, *J* = 7.3 Hz, 2H, $CH_3C\mathbf{H}_2S$), 1.30 (t, *J* = 7.3 Hz, 3H, C**H**$_3CH_2S$), 0.91 (s, 9H, $C(C\mathbf{H}_3)_3$), 0.13 (s, 6H, $SiC\mathbf{H}_3$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 66.0 (**C**H_2O), 25.8 ($CH_3\mathbf{C}H_2S$), 24.5 (**C**H_3CH_2S), 18.2 ($Si\mathbf{C}(CH_3)_3$), 14.9 (**C**H_3CH_2), -5.1 ($Si\mathbf{C}H_3$) ppm

1.92 - Dimethyl 2-(((*tert*-butyldimethylsilyl)oxy)methyl)-2-(3-methylbut-2-en-1-yl)malonate



$C_{17}H_{32}O_5Si$

344.52 $gmol^{-1}$

Following a procedure by Benneche *et al.*,[56] to a stirred solution of thioether **2.07** (3.18 g, 15.5 mmol) in CH_2Cl_2 (30 mL) under Ar at 0 °C was added SO_2Cl_2 (2.14 g, 15.5 mmol) dropwise over 5 min. After 30 min at 0 °C, the solution was stirred at rt for 30 min. The solution was carefully concentrated under reduced pressure (23 °C, 100 mBar), before diluting with dry CH_2Cl_2 (20 mL) and concentrating in the same manner. This dilution and concentration was repeated twice more to afford the crude *tert*-butyl(chloromethoxy)dimethylsilane intermediate as a pale yellow oil.

To a stirred suspension of NaH (60% in mineral oil, 497 mg, 12.4 mmol) in THF (30 mL) at 0 °C under Ar was added prenyl malonate **2.05** (2.07 g, 10.4 mmol) in THF (12 mL) dropwise over 10 min. After 45 min at rt, the crude *tert*-butyl(chloromethoxy) dimethylsilane in THF (12 mL) was added dropwise, and the resulting suspension was stirred at rt for 36 h. Saturated aqueous NH_4Cl (40 mL) was added and the mixture extracted with EtOAc (3 x 40 mL). The combined organic solution was washed with brine, dried ($MgSO_4$) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 3:97) afforded the product as a colourless oil (2.87, 8.30 mmol, 74%). Spectroscopic data were consistent with those reported.[50]

FT-IR (neat) ν_{max} 2930 (m), 1736 (s), 1102 (s) cm^{-1}

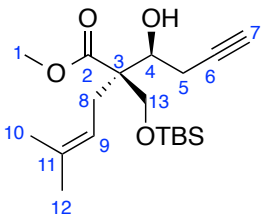
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.93 (br t, *J* = 7.7 Hz, 1H, C=C**H**), 3.95 (s, 2H, C**H**$_2$O), 3.70 (s, 6H, OC**H**$_3$), 2.73 (d, *J* = 7.7 Hz, 2H, C**H**$_2$CH), 1.70 (s, 3H, CC**H**$_3$), 1.63 (s, 3H, CC**H**$_3$), 0.86 (s, 9H, SiC(C**H**$_3$)$_3$), 0.02 (s, 6H, SiC**H**$_3$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 170.4 (**C**=O), 136.1 (**C**=CH), 117.6 (C=**C**H), 62.7 (**C**H$_2$O), 59.8 (**C**C=O), 52.2 (-O**C**H$_3$), 28.8 (**C**H$_2$CH), 26.0 (C**C**H$_3$), 25.6 (C(**C**H$_3$)$_3$), 18.0, (**C**(CH$_3$)$_3$), 17.9 (C**C**H$_3$), -5.7 (Si**C**H$_3$) ppm

LRMS (ES$^+$) *m/z* = 367 $[M+Na]^+$

HRMS (ES$^+$) For $C_{17}H_{32}NaO_5Si^+$ calculated 367.1911, found 367.1918 Da.

2.09 – Methyl (*R)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)-2-((*S**)-1-hydroxybut-3-yn-1-yl)-5-methylhex-4-enoate**



$C_{19}H_{34}O_4Si$

354.56 $gmol^{-1}$

To a stirred solution of the diester **1.92** (328 mg, 1.04 mmol) in CH_2Cl_2 (12 mL) under N_2 at −78 °C was added DIBAL (1 M in CH_2Cl_2, 1.46 mL, 1.46 mmol) dropwise over 30 min. After 5 h, saturated aqueous Rochelle's salt solution (5 mL) was added carefully at −78 °C before warming to rt and stirring the emulsion vigorously for a further 1 h. The aqueous phase was extracted with CH_2Cl_2 (3 x15 mL) and the combined organic solution was washed with brine, dried ($MgSO_4$) and the solvent removed under reduced pressure to afford the crude mono-aldehyde **1.93** (320 mg).

By adaption of a procedure by Linclau *et al.,*[51] to a stirred suspension of Mg (76 mg, 3.1 mmol) in Et_2O (2 mL) under N_2 at rt was added 1,2-dibromoethane (0.27 mL, 3.1 mmol) dropwise. After 30 min the solvent was removed under reduced pressure, and the residue re-suspended in CH_2Cl_2 (3.5 mL) under N_2 and cooled to −78 °C. To this suspension was added the crude aldehyde in CH_2Cl_2 (4 mL) dropwise over 30 min. After 45 min at −78 °C, allenyltributyltin (0.37 mL, 1.3 mmol) was added dropwise over 1 h. After a further 4 h at −78 °C, saturated aqueous $NaHCO_3$ (5 mL) was added and the aqueous phase extracted with CH_2Cl_2 (3 x 10 mL). The combined organic solution was washed with brine, dried ($MgSO_4$) and the solvent removed under reduced pressure to afford the crude alkyne **2.09** (dr = 4:1 by ^1H NMR). Purification twice by silica gel chromatography (9:1 silica:K_2CO_3 and K_2CO_3 plug, EtOAc:pet. ether = 5:95) afforded the product as a colourless oil (Major isomer - 144 mg, 0.407 mmol (92% chemical purity) and mixed isomers - 36 mg, 0.10 mmol, 47% combined yield).

FT-IR (neat) ν_{max} 3492 (br m), 1735 (s), 1096 (m), 1060 (m), 836 (s) cm^{-1}

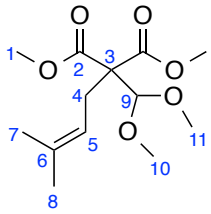
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.98 (br t, *J* = 7.4 Hz, 1H, **9**), 4.10 (td, *J* = 7.9, 3.9 Hz, 1H, **4**), 3.98 (d, *J* = 10.4 Hz, 1H, **13**), 3.78 (d, *J* = 10.4 Hz, 1H, **13**), 3.70 (s, 3H, **1**), 3.50 (d, *J* = 7.8 Hz, 1H, -O**H**), 2.61-2.34 (m, 4H, **5**+**8**), 2.04 (t, *J* = 2.6 Hz, 1H, **7**), 1.69 (s, 3H, **10**/**12**), 1.62 (s, 3H, **10**/**12**), 0.89 (s, 9H, -C(C**H**$_3$)$_3$), 0.09 (s, 3H, -SiC**H**$_3$), 0.08 (s, 3H, -SiC**H**$_3$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 174.0 (C, **2**), 135.1 (C, **11**), 118.3 (CH, **9**), 81.6 (C, **6**), 73.5 (CH, **4**), 70.0 (CH, **7**), 63.4 (CH_2, **13**), 55.0 (C, **3**), 51.7 (CH_3, **1**), 29.6 (CH_2, **8**), 26.0 (C(**C**H$_3$)$_3$), 25.8 (CH_3, **10**/**12**), 23.3 (CH_2, **5**), 18.1 (C, **C**(CH$_3$)$_3$), 17.9 (CH_3, **10**/**12**), −5.7 (CH_3, -Si**C**H$_3$), −5.8 (CH_3, -Si**C**H$_3$) ppm

LRMS (ES^+) *m/z* = 377 $[M+Na]^+$

HRMS (ES^+) For $C_{19}H_{34}NaO_4Si^+$ calculated 377.2119, found 37.2126 Da.

2.13 – Dimethyl 2-(dimethoxymethyl)-2-(3-methylbut-2-en-1-yl)malonate



$C_{13}H_{22}O_6$

274.31gmol^{-1}

By adaption of a procedure by Evans *et al.*,[141] to a solution of malonate **2.13** (742 mg, 3.37 mmol) in CH_2Cl_2 (8 mL) under N_2 at 0 °C was added NEt_3 (1.56 mL, 11.2 mmol) followed by $TiCl_4$ (1 M in CH_2Cl_2, 11.1 mL, 11.1 mmol) dropwise over 5 min. After stirring for 30 min, trimethyl orthoformate (2.24 mL, 20.5 mmol) was added dropwise over 5 min and the resulting red solution was stirred at rt for 1 h. The reaction was quenched with H_2O (15 mL) and the organic phase washed with H_2O (15 mL) and brine before drying (Na_2SO_4) and the solvent was removed under reduced pressure. Purification by silica gel chromatography (EtOAc:pet. ether = 7:93 to 4:6) afforded the product as a colourless oil (768 mg, 2.80 mmol, 83%).

FT-IR (neat) ν_{max} 2953 (w), 2839 (w), 1733 (s), 1069 (s) cm^{-1}

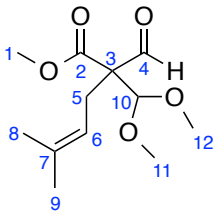
^{1}H NMR $CDCl_3$, 400 MHz): δ = 5.12 (br t, *J* = 7.3 Hz, 1H, **5**), 4.72 (s, 1H, **9**), 3.72 (s, 6H, **1**), 3.56 (s, 6H, **10**+**11**), 2.73 (br d, *J* = 7.3 Hz, 2H, **4**), 1.69 (s, 3H, **7**/**8**), 1.62 (s, 3H, **7**/**8**) ppm

^{13}C NMR ($CDCl_3$, 101MHz): δ = 169.5 (C, **2**), 134.7 (C, **6**), 118.7 (CH, **5**), 107.3 (CH, **9**), 63.2 (C, **3**), 58.6 (CH_3, **10**+**11**), 52.3 (CH_3, **1**), 29.9 (CH_2, **4**), 26.0 (CH_3, **7**/**8**), 17.7 (CH_3, **7**/**8**) ppm

LRMS (ES$^+$) *m/z* = 297 [M+H]$^+$

HRMS (ES$^+$) For $C_{13}H_{22}NaO_6^+$ calculated 297.1309, found 297.1307 Da

2.14 - Methyl 2-(dimethoxymethyl)-2-formyl-5-methylhex-4-enoate



$C_{12}H_{20}O_5$

244.29 $gmol^{-1}$

To a solution of dimethyl malonate **2.13** (343 mg, 1.25 mmol) in CH_2Cl_2 (10 mL) under N_2 atm. at −78 °C was added 1.5 equiv. of DIBAL (1 M in CH_2Cl_2, 1.88 mL, 1.88 mmol) dropwise over 90 min and the solution was stirred for a further 15 min. An additional 0.5 equiv. of DIBAL (1 M in CH_2Cl_2, 0.63 mL, 0.63 mmol) was added dropwise over 30 min and the solution was stirred for a further 45 min. Finally, another 0.5 equiv. of DIBAL (1 M in CH_2Cl_2, 0.63 mL, 0.63 mmol) was added dropwise over 30 min after which the starting material was fully consumed. The reaction was quenched by slow addition of saturated aqueous Rochelle's salt solution (6 mL) at −78 °C before allowing the mixture to warm to rt. The mixture was diluted with CH_2Cl_2 (20 mL) and saturated aqueous Rochelle's salt solution (20 mL) was added and the emulsion was stirred vigorously for 1 h until complete phase separation was observed. The aqueous phase was extracted with CH_2Cl_2 (3 x 15 mL) and the combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure to afford a colourless oil. Purification by silica gel chromatography (EtOAc:pet. ether = 7:93) afforded the product as colourless oil (189 mg, 0.774 mmol, 62%).

FT-IR (neat) ν_{max} 2955 (w), 2929 (w), 1722 (s), 1069 (s) cm^{-1}

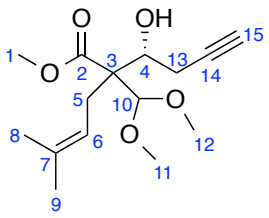
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 9.82 (s, 1H, **4**), 5.04 (br t, *J* = 7.5 Hz, 1H, **6**), 4.69 (s, 1H, **10**), 3.75 (s, 3H, **1**), 3.56 (s, 3H, **11**/**12**), 3.48 (s, 3H, **11**/**12**), 2.59 (br dd, *J* = 14.5, 6.7 Hz, 1H, **5**), 2.50 (br dd, *J* = 14.5, 8.3 Hz, 1H, **5'**), 1.68 (s, 3H, **8**/**9**), 1.61 (s, 3H, **8**/**9**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 197.8 (CH, **4**), 169.9 (C, **2**), 135.3 (C, **7**), 117.7 (CH, **6**), 108.8 (CH, **10**), 66.2 (C, **3**), 59.1 (CH_3, **11**/**12**), 58.1 (CH_3, **11**/**12**), 52.1 (CH_3, **1**), 28.1 (CH_2, **5**), 25.9 (CH_3, **8**/**9**), 17.7 (CH_3, **8**/**9**) ppm

LRMS (ES^+) *m/z* = 267 $[M+H]^+$

HRMS (ES$^+$) For $C_{12}H_{20}NaO_5^+$ calculated 267.1203, found 267.1200 Da

2.15 - Methyl (*S**)-2-(dimethoxymethyl)-2-((*S**)-1-hydroxybut-3-yn-1-yl)-5-methylhex-4-enoate



$C_{15}H_{24}O_5$

284.35 gmol^{-1}

By adaption of procedure by Linclau *et al.,*[51] to a suspension of Mg (50 mg, 2.1 mmol) in Et_2O (1.5 mL) in a 3-necked RBF fitted with a condenser was added dibromoethane (0.18 mL, 2.1 mmol) dropwise over 15 min. After 35 min the solvent was removed under reduced pressure to afford a white solid, which was taken up in CH_2Cl_2 (2 mL) under N_2 and cooled to –78 °C. A solution of the aldehyde **2.14** (169 mg, 0.692 mmol) in CH_2Cl_2 (1 mL) was added dropwise over 40 min and the mixture was stirred for a further 30 min. A solution of allenyltributyltin (0.37 mL, 1.0 mmol) in CH_2Cl_2 (1.5 mL) was added dropwise over 1 h and the mixture was stirred for a further 90 min. The reaction was allowed to warm to –41 °C (MeCN/cardice bath) and stirred for 3 h before quenching slowly with saturated aqueous $NaHCO_3$ (5 mL) and warming to rt. The aqueous phase was extracted with CH_2Cl_2 (3 x 8 mL) and the combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure to afford a colourless oil. Purification by silica gel chromatography (9:1 silica:K_2CO_3 and K_2CO_3 plug, EtOAc:pet. ether = 5:95 to 3:7) afforded the product as a colourless oil (87 mg, 0.31 mmol, 85%).

FT-IR (neat) ν_{max} 3523 (br w), 3291 (br w), 2929 (w), 1731 (s), 1062 (s) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.12-5.04 (m, 1H, **6**), 4.66 (s, 1H, **10**), 4.16 (td, *J* = 8.0, 3.8 Hz, 1H, **4**), 3.71 (s, 3H, **1**), 3.59 (s, 3H, **11**/**12**), 3.54 (d, *J* = 8.0 Hz, 1H, -O**H**), 3.50 (s, 3H, **11**/**12**), 2.70-2.56 (m, 2H, **13**), 2.51 (br dd, *J* = 14.2, 6.8 Hz, 1H, **5**), 2.43 (br dd, *J* = 14.2, 8.3 Hz, 1H, **5'**), 2.01 (t, *J* = 2.6 Hz, 1H, **15**), 1.69 (s, 3H, **8**/**9**), 1.61 (s, 3H, **8**/**9**) ppm

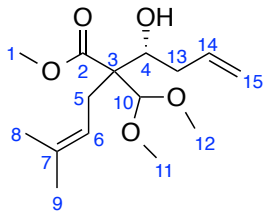
^{13}C NMR ($CDCl_3$, 101 MHz): δ = 172.8 (C, **2**), 134.8 (C, **7**), 118.4 (CH, **6**), 110.7 (CH, **10**), 83.0 (CH, **15**), 71.6 (CH, **4**), 68.9 (C, **14**), 59.8 (CH_3, **11**/**12**), 58.3 (C, **3**), 58.2 (CH_3, **11**/**12**), 51.7 (CH_3, **1**), 30.5 (CH_2, **5**), 26.0 (CH_3,

8/**9**), 24.0 (CH_2, **13**), 17.7 (CH_3, **8**/**9**) ppm

LRMS (ES^+) *m/z* = 307 $[M+H]^+$

HRMS (ES^+) For $C_{15}H_{24}NaO_5^+$ calculated 307.1516, found 307.1520 Da

2.16 - Methyl (*S)-2-(dimethoxymethyl)-2-((*S**)-1-hydroxybut-3-en-1-yl)-5-methylhex-4-enoate**



$C_{15}H_{26}O_5$

286.37 $gmol^{-1}$

By adaption of a procedure by Linclau *et al.*,[51] to a suspension of Mg (241 mg, 9.92 mmol) in Et_2O (7 mL) in a 3-necked RBF fitted with a condenser was added dibromoethane (0.860 mL, 9.92 mmol) dropwise over 15 min. After 35 min the solvent was removed under reduced pressure to afford a white solid, which was taken up in CH_2Cl_2 (9.5 mL) under N_2 and cooled to −78 °C. A solution of the aldehyde **2.14** (808 mg, 3.31 mmol) in CH_2Cl_2 (4.5 mL) was added dropwise over 40 min and the mixture was stirred for a further 30 min. A solution of allyltributylstannane (2.05 mL, 6.62 mL) in CH_2Cl_2 (7 mL) was added dropwise over 1 h and the mixture was stirred for a further 15 min. The reaction was quenched slowly with saturated aqueous $NaHCO_3$ (20 mL) and allowed to warm to rt. The aqueous phase was extracted with CH_2Cl_2 (3 x 15 mL) and the combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure to afford a colourless oil. Purification by silica gel chromatography (9:1 silica:K_2CO_3 and K_2CO_3 plug, EtOAc:pet. ether = 1:9) afforded the product as a colourless oil (801 mg, 2.80 mmol, 85%).

FT-IR (neat) ν_{max} 3528 (w), 2929 (w), 1731 (s), 1437 (m), 1063 (s) cm^{-1}

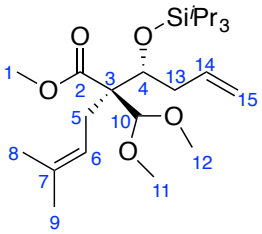
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.92 (ddt, *J* = 17.1, 10.2, 6.9 Hz, 1H, **14**), 5.15-5.02 (m, 3H, **6**+**15**), 4.69 (s, 1H, **10**), 3.97 (ddd, *J* = 10.2, 7.4, 2.5 Hz, 1H, **4**), 3.72 (s, 3H, **1**), 3.61 (s, 3H, **11**/**12**), 3.50 (s, 3H, **11**/**12**), 3.28 (d, *J* = 7.4 Hz, 1H, **-**O**H**), 2.55-2.26 (m, 4H, **5**+**14**), 1.69 (s, 3H, **8**/**9**), 1.61 (s, 3H, **8**/**9**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 173.4 (C, **2**), 136.8 (CH, **14**), 134.1 (C, **7**), 119.1 (CH, **6**), 116.1 (CH_2, **15**), 110.5 (CH, **10**), 72.6 (CH, **4**), 59.7 (CH_3, **11**/**12**), 58.7 (C, **3**), 58.1 (CH_3, **11**/**12**), 51.6 (CH_3, **1**), 37.9 (CH_2, **13**), 30.0 (CH_2, **5**), 26.0 (CH_3, **8**/**9**), 17.7 (CH_3, **8**/**9**) ppm

LRMS (ES^+) *m/z* = 309 $[M+H]^+$

HRMS (ES^+) For $C_{15}H_{26}NaO_5^+$ calculated 309.1672, found 309.1672 Da

2.18 - Methyl (*S)-2-(dimethoxymethyl)-5-methyl-2-((*R**)-1-((triisopropylsilyl)oxy)but-3-en-1-yl)hex-4-enoate**



$C_{24}H_{46}O_5Si$

442.71 $gmol^{-1}$

To a solution of the alcohol **2.16** (73 mg, 0.26 mmol), NEt_3 (0.07 mL, 0.5 mmol) and DMAP (2.0 mg, 0.016 mmol) in CH_2Cl_2 (1.5 mL) under N_2 at 0 °C was added TIPS-OTf (0.10 mL, 0.38 mmol) dropwise over 3 min and the mixture was stirred for 15 min. Further TIPS-OTf (0.10 mL, 0.38 mmol) was added dropwise over 3 min and the mixture was stirred for 15 min until the starting material was consumed. The reaction was quenched with H_2O (2 mL) and the aqueous phase extracted with CH_2Cl_2 (3 x 3 mL). The combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 1:99) afforded the product as a colourless oil (89 mg, 2.0 mmol, 77%).

FT-IR (neat) ν_{max} 2950 (m), 2866 (m), 1732 (m), 1066 (s) cm^{-1}

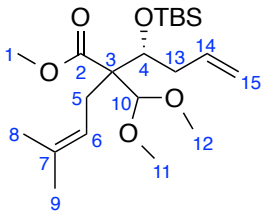
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.85 (ddt, *J* = 17.2, 10.2, 7.0 Hz, 1H, **14**), 5.44 (m, **6**), 5.13-4.96 (m, 2H, **15**), 4.57 (s, 1H, **10**), 4.37 (t, *J* = 5.4 Hz, 1H, **4**), 3.67 (s, 3H, **1**), 3.50 (s, 3H, **11**/**12**), 3.47 (s, 3H, **11**/**12**), 2.67-2.58 (m, 1H, **5**), 2.58-2.46 (m, 2H, **5'**+**13**), 2.30-2.16 (m, 1H, **13'**), 1.68 (d, *J* = 1.2 Hz, 3H, **8**), 1.62 (s, 3H, **9**), 1.11-1.04 (m, 21H, Si(C**H$_2$**C**H$_2$**C**H$_3$**)$_3$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 172.9 (C, **2**), 136.8 (CH, **14**), 131.0 (C, **7**), 121.7 (CH, **6**), 116.0 (CH_2, **15**), 109.0 (CH, **10**), 75.3 (CH, **4**), 61.0 (C, **3**), 59.0 (CH_3, **11**/**12**), 57.7 (CH_3, **11**/**12**), 51.2 (CH_3, **1**), 39.1 (CH_2, **13**), 27.3 (s, CH_2), 26.1 (CH_3, **8**/**9**), 18.4 (CH_3, **8**/**9**), 17.7 (Si(CH_2CH_2**C**H_3)$_3$), 13.5 (Si(CH_2**C**H_2CH_3)$_3$), 12.3 (Si(**C**$H_2CH_2CH_3$)$_3$) ppm

LRMS (ES^+) *m/z* = 465 $[M+Na]^+$

HRMS (ES^+) For $C_{24}H_{46}NaO_5Si^+$ calculated 465.3007, found 465.3011 Da

2.20 - Methyl (*S)-2-((*S**)-1-((*tert*-butyldimethylsilyl)oxy)but-3-en-1-yl)-2-(dimethoxymethyl)-5-methylhex-4-enoate**



$C_{21}H_{40}O_5Si$

400.63 $gmol^{-1}$

To a solution of the alkene **2.16** (100 mg, 0.349 mmol), NEt_3 (0.10 mL, 0.72 mL) and DMAP (5 mg, 0.04 mmol) in CH_2Cl_2 (1.4 mL) under N_2 at 0 °C was added TBS-OTf (0.13 mL, 5.9 mmol) dropwise over 5 min. After stirring for 5 min at 0 °C the reaction was warmed to rt and stirred for a further 30 min. The reaction was quenched with H_2O (3 mL) and the aqueous phase was extracted with CH_2Cl_2 (2 x 3 mL). The combined organic solution was dried (Na_2SO_4) and the solvent removed under reduced pressure to afford an orange oil. Purification by silica gel chromatography (EtOAc:pet. ether = 8:92) afforded the product as a colourless oil (124 mg, 0.310 mmol, 89%).

FT-IR (neat) ν_{max} 2952 (m), 2856 (m), 1732 (s), 1067 (s) cm^{-1}

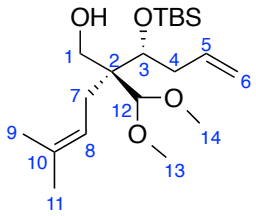
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.91-5.79 (m, 1H, **14**), 5.44-5.35 (m, 1H, **6**), 5.07-4.98 (m, 2H, **15**), 4.50 (s, 1H, **10**), 4.24 (dd, *J* = 6.4, 3.8 Hz, 1H, **4**), 3.68 (s, 3H, **1**), 3.50 (s, 3H, **11**/**12**), 3.48 (s, 3H, **11**/**12**), 2.60-2.45 (m, 3H, **5+13**), 2.23-2.11 (m, 1H, **5'**), 1.69 (s, 3H, **8**/**9**), 1.63 (s, 3H, **8**/**9**), 0.88 (s, 9H, SiC(C**H**$_3$)$_3$), 0.06 (s, 3H, SiC**H**$_3$), 0.03 (s, 3H, SiC**H**$_3$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 173.0 (C, **2**), 136.9 (CH, **14**), 131.3 (C, **7**), 121.5 (CH, **6**), 116.1 (CH_2, **15**), 108.9 (CH, **10**), 74.8 (CH, **4**), 60.8 (C, **3**), 59.0 (CH_3, **11**/**12**), 57.9 (CH_3, **11**/**12**), 51.3 (CH_3, **1**), 38.6 (CH_2, **13**), 27.3 (CH_2, **5**), 26.1 (CH_3, **8**/**9**), 26.0 (SiC(**C**H$_3$)$_3$), 18.2 (Si**C**(CH$_3$)$_3$), 17.7 (CH_3, **8**/**9**), –3.2 (Si**C**H$_3$), –4.7 (Si**C**H$_3$) ppm

LRMS (ES$^+$) *m/z* = 423 $[M+Na]^+$

HRMS (ES$^+$) For $C_{21}H_{40}NaO_5Si^+$ calculated 423.2537, found 423.2537 Da

2.21 - (*S)-2-((*S**)-1-((*tert*-Butyldimethylsilyl)oxy)but-3-en-1-yl)-2-(dimethoxymethyl)-5-methylhex-4-en-1-ol**



$C_{20}H_{40}O_4Si$

372.62 $gmol^{-1}$

To a solution of the ester **2.20** (591 mg, 1.47 mmol) in CH_2Cl_2 (12 mL) under N_2 at 0 °C was added DIBAL (1 M in CH_2Cl_2, 4.42 mL, 4.42 mmol) dropwise over 10 min before warming to rt. After 1 h the reaction was quenched by slow addition of saturated aqueous Rochelle's salt solution (20 mL). The mixture was diluted with CH_2Cl_2 (40 mL) and the emulsion was stirred vigorously for 40 min until phase separation was observed. The aqueous phase was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:pet. ether = 5:95) afforded the product as a colourless oil (386 mg, 1.04 mmol, 71%).

FT-IR (neat) ν_{max} 3527 (br w), 2955 (m), 2928 (m), 2856 (m), 1064 (s) cm^{-1}

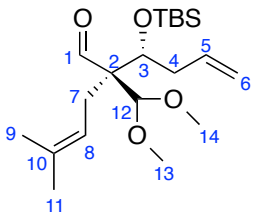
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.98-5.86 (m, 1H, **5**), 5.33-5.26 (m, 1H, **8**), 5.09-5.00 (m, 2H, **6**), 4.52 (s, 1H, **12**), 4.14 (dd, *J* = 5.8, 4.4 Hz, 1H, **3**), 3.69 (dd, *J* = 10.9, 2.6 Hz, 1H, **1**), 3.62-3.55 (m, 4H, **1'**+ **13**/**14**), 3.52 (s, 3H, **13**/**14**), 3.25-3.18 (m, 1H, **4**), 2.53-2.44 (m, 1H, **4'**), 2.33-2.24 (m, 1H, O**H**), 2.13-1.99 (m, 2H, **7**), 1.69 (d, *J* = 1.0 Hz, 3H, **9**), 1.60 (s, **11**), 0.92 (s, 9H, SiC(C**H**$_3$)$_3$), 0.11 (s, 3H, SiCC**H**$_3$), 0.09 (s, 3H, SiCC**H**$_3$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 137.0 (CH, **5**), 132.1 (C, **10**), 120.8 (CH, **8**), 116.1 (CH_2, **6**), 110.1 (CH, **12**), 74.3 (CH, **3**), 64.5 (CH_2, **1**), 59.0 (CH_3, **13**/**14**), 57.9 (CH_3, **13**/**14**), 50.4 (C, **2**), 38.1 (CH_2, **4**), 27.0 (CH_3, **9**/**11**), 26.2 (SiC(**C**H$_3$)$_3$), 26.1 (Si**C**), 18.3 (CH_2, **7**), 17.7 (CH_3, **9**/**11**), –3.6 (SiC**C**H$_3$), –4.8 (SiC**C**H$_3$) ppm

LRMS (ES^+) *m/z* = 395 $[M+Na]^+$

HRMS (ES^+) For $C_{20}H_{40}NaO_4Si^+$ calculated 395.2588, found 395.2598 Da

2.22 - (*R)-2-((*R**)-1-((*tert*-Butyldimethylsilyl)oxy)but-3-en-1-yl)-2-(dimethoxymethyl)-5-methylhex-4-enal**



$C_{20}H_{38}O_4Si$

370.61 $gmol^{-1}$

To a solution of alcohol **2.21** (270 mg, 0.717 mmol) in CH_2Cl_2 (8 mL) under N_2 at rt was added crushed 4 Å molecular sieves (200 mg) and NMO (252 mg, 2.15 mmol), followed by TPAP (25 mg, 0.072 mmol) and the suspension was stirred for 1 h. The black mixture was concentrated to dryness before purifying by silica gel chromatography (EtOAc:pet. ether = 4:96) to afford the product as a colourless oil (144 mg, 0.389 mmol, 54%).

FT-IR (neat) ν_{max} 2955 (m), 2928 (m), 2856 (m), 1727 (m), 1065 (s) cm^{-1}

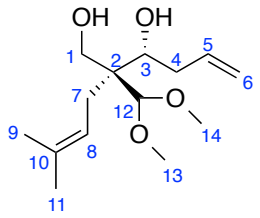
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 9.66 (s, 1H, **1**), 5.81-5.92 (m, 1H, **5**), 5.30-5.24 (m, 1H, **8**), 5.10-5.02 (m, 2H, **6**), 4.49 (s, 1H, **12**), 4.38 (dd, *J* = 6.2, 4.3 Hz, 1H, **3**), 3.50 (s, 3H, **13**/**14**), 3.48 (s, 3H, **13**/**14**), 2.56-2.41 (m, 3H, **4**+**7**), 2.32-2.22 (m, 1H, **4'**), 1.70 (d, *J* = 0.9 Hz, 3H, **9**), 1.64 (s, 3H, **11**), 0.87 (s, 9H, SiC($C\mathbf{H}_3$)$_3$), 0.07 (s, 3H, SiCC$\mathbf{H}_3$), 0.03 (s, 3H, SiCC$\mathbf{H}_3$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 204.2 (CH, **1**), 136.4 (CH, **5**), 132.7 (C, **10**), 120.2 (CH, **8**) 116.7 (CH_2, **6**), 108.5 (CH, **12**), 72.8 (CH, **3**), 62.3 (C, **2**), 58.5 (CH_3, **13**/**14**), 58.3 (CH_3, **13**/**14**), 38.6 (CH_2, **4**), 26.1 (CH_3, **9**/**11**), 26.0 (SiC($\mathbf{C}H_3$)$_3$), 25.0 (Si**C**), 18.2 (CH_3, **9**/**11**), 17.8 (CH_2, **7**), –3.30 (SiC$\mathbf{C}H_3$), -4.6 (SiC$\mathbf{C}H_3$) ppm

LRMS (ES^+) *m/z* = 393 $[M+Na]^+$

HRMS (ES^+) For $C_{20}H_{38}NaO_4Si^+$ calculated 393.2432, found 393.2429 Da

2.24 - (2*S,3*R**)-2-(Dimethoxymethyl)-2-(3-methylbut-2-en-1-yl)hex-5-ene-1,3-diol**



$C_{14}H_{26}O_4$

258.36 $gmol^{-1}$

To a solution of ester **2.16** (306 mg, 1.07 mmol) in THF (10 mL) under N_2 at 0 °C was added $LiAlH_4$ (1 M in THF, 2.14 mL, 2.14 mmol) dropwise over 5 min. After stirring for 5 min the reaction was warmed to rt and stirred for 1 h. The reaction was quenched with saturated aqueous Rochelle's salt solution at 0 °C and the resulting suspension was stirred vigorously at rt for 15 min until phase separation was observed. The aqueous phase was extracted with EtOAc (3 x 10 mL) and the combined organic solution washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 1:3) afforded the product as a colourless oil (229 mg, 0.888 mmol, 83%).

FT-IR (neat) ν_{max} 3384 (br m), 2915 (m), 1443 (m), 1063 (s) cm^{-1}

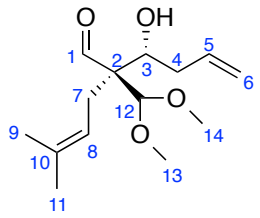
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.97-5.84 (m, 1H, **5**), 5.29-5.22 (m, 1H, **8**), 5.17-5.07 (m, 2H, **6**), 4.46 (s, 1H, **12**), 3.88 (ddd, *J* = 10.7, 4.1, 2.2 Hz, 1H, **3**), 3.76 (dd, *J* = 11.4, 5.3 Hz, 1H, **1**), 3.66 (dd, *J* = 11.4, 6.6 Hz, 1H, **1'**), 3.57 (s, 3H, **13**/**14**), 3.57 (s, 3H, **13**/**14**), 2.88-2.79 (m, 1H, CHO**H**), 2.78-2.69 (m, 1H, CH_2O**H**), 2.45-2.39 (m, 1H, **4**), 2.27-2.15 (m, 2H, **4'**+**7**), 2.07 (dd, *J* = 14.9, 7.6 Hz, 1H, **7'**), 1.72 (s, 3H, **9**/**11**), 1.64 (s, 3H, **9**/**11**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 136.6 (CH, **5**), 133.7 (C, **10**), 119.7 (CH, **8**), 117.0 (CH_2, **6**), 111.2 (CH, **12**), 73.1 (CH, **3**), 64.5 (CH_2, **1**), 58.9 (CH_3, **13**/**14**), 58.7 (CH_3, **13**/**14**), 49.2 (C, **2**), 36.8 (CH_2, **4**), 27.6 (CH_2, **7**), 26.1 (CH_3, **9**/**11**), 17.7 (CH_3, **9**/**11**) ppm

LRMS (ES^+) *m/z* = 281 $[M+Na]^+$

HRMS (ES^+) For $C_{14}H_{26}NaO_4^+$ calculated 281.1723, found 281.1720 Da

2.25 - (*R)-2-(Dimethoxymethyl)-2-((*R**)-1-hydroxybut-3-en-1-yl)-5-methylhex-4-enal**



$C_{14}H_{24}O_4$

256.34 $gmol^{-1}$

By adaption of a procedure by Yadov *et al.*,[142] to a solution of the diol **2.24** (311 mg, 1.20 mmol) in CH_2Cl_2 (12 mL) under N_2 at rt was added (diacetoxyiodo)benzene (427 mg, 1.33 mmol) followed by TEMPO (19 mg, 0.12 mmol) and stirred for 72 h. The reaction was quenched with saturated aqueous NaS_2O_3 and the mixture was stirred vigorously for 15 min. The aqueous phase was extracted with CH_2Cl_2 (3 x 10 mL) and the combined organic solution washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 8:92) afforded the product as a colourless oil (143 mg, 0.558 mmol, 46%).

FT-IR (neat) ν_{max} 3510 (br w), 2916 (m), 2837 (w), 1720 (s), 1062 (s) cm^{-1}

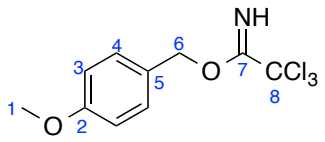
^{1}H NMR (CD_3CN, 400 MHz): δ = 9.65 (s, 1H, **1**), 5.88 (ddt, *J* = 17.1, 10.2, 6.9 Hz, 1H, **5**), 5.22-5.14 (m, 1H, **8**), 5.10-5.01 (m, 2H, **6**), 4.65 (s, 1H, **12**), 3.99 (ddd, *J* = 10.5, 5.7, 2.4 Hz, 1H, **3**), 3.53 (s, 3H, **13**/**14**), 3.50 (s, 3H, **13**/**14**), 3.18 (d, *J* = 5.7 Hz, 1H, -O**H**), 2.38-2.29 (m, 3H, **4**+**7**), 2.17-2.08 (m, 1H, **4'**), 1.68 (d, *J* = 0.9 Hz, 3H, **9**), 1.61 (s, 3H, **11**) ppm

^{13}C NMR (CD_3CN, 101 MHz): δ = 204.9 (CH, **1**), 137.6 (CH, **5**), 134.6 (C, **10**), 120.2 (CH, **8**), 117.1 (CH_2, **6**), 109.9 (CH, **12**), 72.5 (CH, **3**), 62.1 (C, **2**), 59.7 (CH_3, **13**/**14**), 59.0 (CH_3, **13**/**14**), 38.0 (CH_2, **4**), 26.7 (CH_2, **7**), 26.2 (CH_3, **9**/**11**), 18.0 (CH_3, **9**/**11**) ppm

LRMS (ES^+) *m/z* = 279 $[M+Na]^+$

HRMS (ES^+) For $C_{14}H_{24}NaO_4^+$ calculated 279.1567, found 279.1571 Da

2.29 - 4-Methoxybenzyl 2,2,2-trichloroacetimidate



$C_{10}H_{10}Cl_3NO_2$

282.55 $gmol^{-1}$

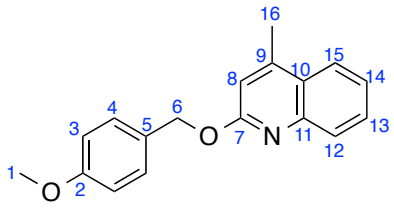
Following a procedure by Bernat *et al.*,[143] to a solution of 4-methoxybenzyl alcohol (556 mg, 4.02 mmol) in Et_2O (1.5 mL) under N_2 was added NaH (60% in mineral oil, 40 mg, 1.0 mmol) and the solution was stirred at rt for 30 min. After cooling to 0 °C, trichloroacetonitrile (0.42 mL, 4.2 mmol) was added and the solution was stirred at rt for 2 h. The reaction was quenched with saturated aqueous $NaHCO_3$ (3 mL) and the aqueous phase was extracted with Et_2O (2 x 3 mL). The combined organic solution was dried ($MgSO_4$) and the solvent removed under reduced pressure to afford the product as an orange oil (1.1 g, 3.9 mmol, 97%). Spectroscopic data were consistent with those reported.[143]

FT-IR (neat) ν_{max} 3338 (w), 2956 (w), 2836 (w), 1661 (s), 1514 (s), 1245 (w), 1072 (s) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.44-8.29 (br s, 1H, -N**H**), 7.38 (d, *J* = 8.3 Hz, 2H, **4**), 6.92 (d, *J* = 8.3 Hz, 2H, **3**), 5.29 (s, 2H, **6**), 3.83 (s, 3H, **1**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 162.6 (C, **7**), 159.7 (C, **2**), 129.7 (CH, **4**), 127.5 (C, **5**), 113.9 (CH, **3**), 91.48 (C, **8**), 70.7 (CH_2, **6**), 55.3 (CH_3, **1**) ppm

2.32 - 2-((4-Methoxybenzyl)oxy)-4-methylquinoline



$C_{18}H_{17}NO_2$

279.34 $gmol^{-1}$

Following a procedure by Dudley *et al.*,[63] a solution of 4-methoxybenzylalcohol (1.87 g, 13.5 mmol), 2-chloro-4-methylquinoline (2.40 g, 3.56 mmol), 18-crown-6 (205 mg, 0.775 mmol) and ground KOH (3.04 g, 54.4 mmol) in toluene (20 mL) was heated under reflux for 2 h with azeotropic removal of water (Dean-Stark trap). After cooling to rt, H_2O (20 mL) was added and the aqueous phase was extracted with EtOAc (3 x 20 mL). The combined organic solution was washed with brine, dried ($MgSO_4$) and the solvent removed under reduced pressure to afford an orange oil. Purification by silica gel chromatography (EtOAc:pet. ether = 5:95) afforded the product as a white solid (3.32 g, 11.9 mmol, 88%). Spectroscopic data were consistent with those reported.[63]

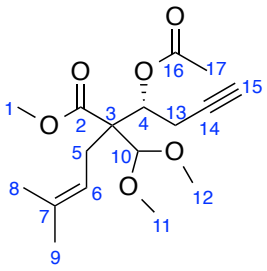
M.P 78-80°C (*solvent:* Et_2O)

FT-IR (neat) ν_{max} 2954 (w), 1611 (s), 1514 (s), 1326 (s), 1031 (s) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.93-7.83 (m, 2H, **12**+**15**), 7.64 (ddd, *J* = 8.3, 7.0, 1.3 Hz, 1H, **13**), 7.48 (d, *J* = 8.6 Hz, 2H, **4**), 7.44-7.39 (m, 1H, **14**), 6.94-6.87 (m, 2H, **3**), 6.81 (d, *J* = 0.9 Hz, 1H, **8**), 5.49 (s, 2H, **6**), 3.83 (s, 3H, **1**), 2.63 (d, *J* = 0.9 Hz, 3H, **16**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 161.8 (C, **7**), 159.4 (C, **2**), 146.8 (C, **11**), 146.5 (C, **9**), 130.0 (CH, **4**), 129.5 (C, **5**), 129.2 (CH, **13**), 127.7 (CH, **3**), 125.5 (C, **10**), 123.8 (CH, **15**), 123.6 (CH, **14**), 113.9 (CH, **3**), 113.2 (CH, **8**), 67.1 (CH_2, **6**), 55.3 (CH_3, **1**), 18.6 (CH_3, **16**) ppm

2.33 - Methyl (*S)-2-((*S**)-1-acetoxybut-3-yn-1-yl)-2-(dimethoxymethyl)-5-methylhex-4-enoate**



$C_{17}H_{26}O_6$

326.39 $gmol^{-1}$

To a solution of alcohol **2.15** (90 mg, 0.32 mmol), DMAP (11 mg, 0.094 mmol) and NEt_3 (0.35 mL, 2.5 mmol) in CH_2Cl_2 (3 mL) under N_2 at 0 °C was added Ac_2O (0.12 mL, 1.3 mmol) dropwise. After warming to rt and stirring for 10 min, the reaction was heated to 40 °C and stirred for 2 h. The solution was cooled to rt and quenched with H_2O (5 mL). The aqueous phase was extracted with CH_2Cl_2 (3 x 3 mL) and the combined organic solution washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:pet. ether = 1:9) afforded the product as a colourless oil (0.84 mg, 0.26 mmol, 81%).

FT-IR (neat) ν_{max} 3280 (w), 2929 (w), 1740 (s), 1437 (m), 1371 (m), 1222 (s), 1067 (s) cm^{-1}

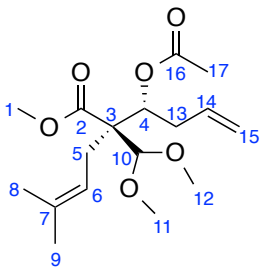
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.57 (dd, *J* = 9.5, 3.5 Hz, 1H, **4**), 5.22-5.16 (m, 1H, **6**), 4.58 (s, 1H, **10**), 3.70 (s, 3H, **1**), 3.53 (s, 3H, **11**/**12**), 3.49 (s, 3H, **11**/**12**), 2.76 (apparent dt, *J* = 17.1, 2.7 Hz, 1H, **13**), 2.60 (ddd, *J* = 17.1, 9.5, 2.7 Hz, 1H, **13'**), 2.54 (dd, *J* = 15.0, 7.5 Hz, 1H, **5**), 2.43 (dd, *J* = 15.0, 6.8 Hz, 1H, **5'**), 2.08 (s, 3H, **17**), 1.92 (t, *J* = 2.7 Hz, 1H, **15**), 1.70 (s, 3H, **8**/**9**), 1.61 (s, 3H, **8**/**9**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 171.9 (C, **2**), 169.7 (C, **16**), 133.8 (C, **7**), 119.1 (CH, **6**), 108.8 (CH, **10**), 80.8 (CH, **15**), 71.3 (CH, **4**), 69.6 (C, **14**), 59.1 (CH_3, **11**/**12**), 58.6 (C, **3**), 58.2 (CH_3, **11**/**12**), 51.8 (CH_3, **1**), 27.9 (CH_2, **5**), 26.0 (CH_3, **8**/**9**), 21.5 (CH_2, **5**), 21.0 (CH_3, **17**), 17.7 (CH_3, **8**/**9**) ppm

LRMS (ES^+) *m/z* = 349 $[M+Na]^+$

HRMS (ES^+) For $C_{17}H_{26}NaO_6^+$ calculated 349.1622, found 349.1625 Da

2.35 - Methyl (*S)-2-((*S**)-1-acetoxybut-3-en-1-yl)-2-(dimethoxymethyl)-5-methylhex-4-enoate**



$C_{17}H_{28}O_6$

328.41 $gmol^{-1}$

To a solution of alcohol **2.16** (75 mg, 0.26 mmol), NEt_3 (0.29 mL, 2.1 mmol) and DMAP (10 mg, 0.080 mmol) in CH_2Cl_2 (2.5 mL) under N_2 at 0 °C was added acetic anhydride (0.1 mL, 1 mmol) dropwise over 5 min. After 5 min the reaction was warmed to rt and stirred for a further 2 h. The reaction was quenched with H_2O (3 mL) and the aqueous phase extracted with CH_2Cl_2 (3 x 3 mL). The combined organic solution was dried (Na_2SO_4) and the solvent removed under reduced pressure to afford a yellow oil. Purification by silica gel chromatography (EtOAc:pet. ether = 1:9 to 15:85) afforded the product as a colourless oil (80 mg, 2.4 mmol, 94%).

FT-IR (neat) ν_{max} 2928 (w), 1740 (s), 1225 (s), 1070 (s) cm^{-1}

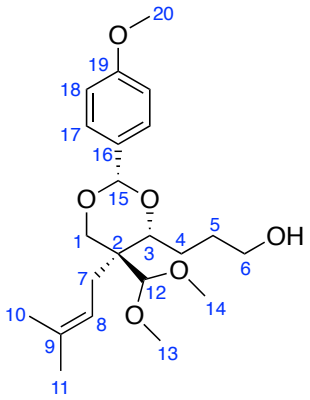
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.77-5.63 (m, 1H, **14**), 5.41 (dd, *J* = 10.5, 2.4 Hz, 1H, **4**), 5.29-5.22 (m, 1H, **5**), 5.05-4.94 (m, 2H, **15**), 4.57 (s, 1H, **10**), 3.71 (s, 3H, **1**), 3.51 (s, 3H, **11**/**12**), 3.50 (s, 3H, **11**/**12**), 2.60-2.51 (m, 2H, **5**+**13**), 2.47 (br dd, *J* = 15.0, 6.8 Hz, 1H, **5'**), 2.33-2.16 (m, 1H, **13'**), 2.00 (s, 3H, **17**), 1.70 (s, 3H, **8**/**9**), 1.63 (s, 3H, **8**/**9**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 172.3 (C, **2**), 169.9 (C, **16**), 135.0 (CH, **14**), 133.1 (C, **7**), 119.7 (CH, **6**), 117.0 (CH_2, **15**), 109.0 (CH, **10**), 73.0 (CH, **4**), 58.9 (CH_3, **11**/**12**), 58.8 (C, **3**), 58.4 (CH_3, **11**/**12**), 51.6 (CH_3, **1**), 36.2 (CH_2, **13**), 27.8 (CH_2, **6**), 26.0 (CH_3, **8**/**9**), 21.1 (CH_3, **17**), 17.7 (CH_3, **8**/**9**) ppm

LRMS (ES^+) *m/z* = 351 $[M+Na]^+$

HRMS (ES^+) For $C_{17}H_{28}NaO_6^+$ calculated 351.1778, found 351.1782 Da

2.46 - 3-((4*R,5*S**)-5-(Dimethoxymethyl)-2-(4-methoxyphenyl)-5-(3-methylbut-2-en-1-yl)-1,3-dioxan-4-yl)propan-1-ol**



$C_{22}H_{34}O_6$

394.51 $gmol^{-1}$

To a solution of diol **2.24** (3.67 g, 14.0 mmol) in CH_2Cl_2 (100 mL) under N_2 at rt, was added anisaldehyde dimethyl acetal (2.66 mL, 15.62 mmol) followed by CSA (10 mg, 0.043 mmol) and the solution was stirred for 1 h. Saturated aqueous $NaHCO_3$ (50 mL) was added and the aqueous phase was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic solution was dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 1:99 to 5:95) afforded impure alkene **2.45** as a colourless oil (5.36 g).

To a solution of impure alkene **2.45** in THF (100 mL) under N_2 at 0 °C was added 9-BBN (0.5 M in THF, 29.3 mL, 14.8 mmol) dropwise over 40 min and after 5 min the solution was warmed to rt. After 2 h additional 9-BBN (0.5 M in THF, 4.19 mL, 2.10 mmol) was added dropwise over 5 min at 0 °C and the solution was stirred for a further 2 h at rt. The solution was then cooled to 0 °C and 3 M NaOH (98.0 mL, 294 mmol) was added dropwise followed by H_2O_2 (30 wt. % in H_2O, 33.0 mL, 293 mmol) dropwise. After 5 min the reaction was warmed to rt and stirred for 1 h. The aqueous phase was extracted with EtOAc (3 x 50 mL) and the combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 3:7 to 4:6) afforded the product as a colourless oil (3.79 g, 9.60 mmol, 69%).

FT-IR (neat) ν_{max} 3423 (br m), 2930 (m), 2835 (w), 1247 (m), 1068 (s) cm^{-1}

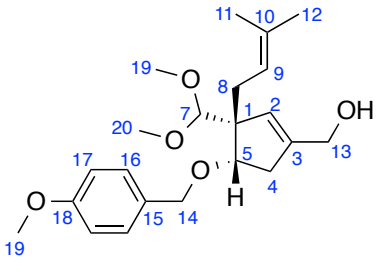
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.44-7.39 (m, 2H, **17**), 6.92-6.85 (m, 2H, **18**), 5.42 (s, 1H, **16**), 5.29-5.22 (m, 1H, **8**), 4.10 (dd, *J* = 10.1, 1.2 Hz, 1H, **3**), 4.04 (d, *J* = 11.1 Hz, 1H, **1**), 3.98 (s, 1H, **12**), 3.82-3.76 (m, 4H, **1**+**20**), 3.65 (t, *J* = 6.1 Hz, 2H, **6**), 3.52 (s, 3H, **13**/**14**), 3.51 (s, 3H, **13**/**14**), 2.67 (dd, *J* = 14.4, 8.6 Hz, 1H, **7**), 2.30 (dd, *J* = 14.4, 6.8 Hz, 1H, **7'**), 1.95 (dtd, *J* = 14.0, 7.1, 1.2 Hz, 2H, **4**), 1.76 (s, 3H, **10**/**11**), 1.71 (s, 3H, **10**/**11**), 1.81-1.63 (m, 1H, **5**), 1.62-1.51 (m, 1H, **5'**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 159.9 (C, **19**), 134.0 (C, **9**), 131.2 (C, **16**), 127.4 (CH, **17**), 119.8 (CH, **8**), 113.6 (CH, **18**), 109.9 (CH, **12**), 101.6 (CH, **15**), 80.9 (CH, **3**), 70.2 (CH_2, **1**), 62.7 (CH_2, **6**), 59.3 (CH_3, **13**/**14**), 58.5 (CH_3, **13**/**14**), 55.3 (CH_3, **20**), 44.7 (C, **2**), 29.9 (CH_2, **4**), 26.6 (CH_2, **5**), 26.2 (CH_3, **10**/**11**), 25.7 (CH_2, **7**), 17.9 (CH_3, **10**/**11**) ppm

LRMS (ES^+) *m/z* = 417 $[M+Na]^+$

HRMS (ES^+) For $C_{17}H_{28}NaO_6^+$ calculated 417.2248, found 417.2252 Da

2.48 - ((3*R,4*S**)-3-(Dimethoxymethyl)-4-((4-methoxybenzyl)oxy)-3-(3-methylbut-2-en-1-yl)cyclopent-1-en-1-yl)methanol**



$C_{22}H_{32}O_5$

376.49 $gmol^{-1}$

To a solution of *p*-methoxybenzylidene acetal **2.46** (3.79 g, 9.60 mmol) in CH_2Cl_2 (100 mL) under N_2 at 0 °C was added DIBAL (1 M in CH_2Cl_2, 33.6 mL, 33.6 mmol) dropwise over 10 min. After 5 min the reaction was warmed to rt and the solution stirred for 1 h. The reaction was then cooled to 0 °C and saturated aqueous Rochelle's salt (100 mL) was added. After 5 min the emulsion was warmed to rt and stirred vigorously for 1 h until phase separation was observed. The aqueous phase was extracted with CH_2Cl_2 (3 x 50 mL) and the combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure to afford the crude diol product.

To a solution of oxalyl chloride (2.47 mL, 28.8 mmol) in CH_2Cl_2 (36 mL) under N_2 at −78 °C was added a solution of DMSO (4.09 mL, 57.6 mmol) in CH_2Cl_2 (18 mL) dropwise over 30 min. After 20 min a solution of the crude diol in CH_2Cl_2 (35 mL) was added dropwise over 1 h. After 20 min NEt_3 (16.0 mL, 115 mmol) was added dropwise over 30 min and the reaction was allowed to slowly warm to rt over 1 h. H_2O (100 mL) was added and the phases separated. The aqueous phase was extracted with CH_2Cl_2 (2 x 50 mL) and the combined organic solution was dried (Na_2SO_4) and the solvent removed under reduced pressure (no heating) to afford the crude dialdehyde product.

The crude dialdehyde was taken up in toluene (120 mL) under N_2 and Bn_2NH•TFA (2.83 g, 9.60 mmol) was added and the resulting solution was stirred for 36 h at rt. The reaction mixture was then concentrated under reduced pressure to afford a yellow oil. Purification by silica gel chromatography (EtOAc:hexane = 8:92) afforded impure cyclopentene aldehyde **2.43** as a pale yellow oil (3.21 g).

The crude aldehyde was taken up in THF (160 mL) and H_2O (10 drops) and cooled to 0 °C before adding $NaBH_4$ (726 mg, 19.2 mmol). The reaction was warmed to rt and after 10 min concentrated under reduced pressure. The colourless residue was taken up in EtOAc (150 mL) and washed sequentially with H_2O and brine before drying (Na_2SO_4) and removing the solvent under reduced pressure. Purification by silica gel chromatography

(EtOAc:hexane = 1:4) afforded the product as a colourless oil (1.91 g, 5.06 mmol, 52% over 4 steps).

FT-IR (neat) ν_{max} 3423 (br), 2911 (m), 2836 (m), 1514 (s), 1247 (s), 1097 (s), 1069 (s) cm^{-1}

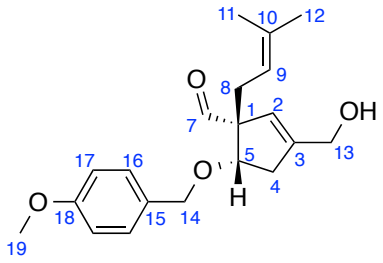
^{1}H NMR (Acetone-*d6*, 400 MHz): δ = 7.30 (d, *J* = 8.7 Hz, 2H, **16**), 6.90 (d, *J* = 8.7 Hz, 2H, **17**), 5.32-5.28 (m, 1H, **2**), 5.15 (dddd, *J* = 7.5, 6.1, 2.9, 1.3 Hz, 1H, **9**), 4.51 (d, *J* = 11.3 Hz, 1H, **14**), 4.39 (d, *J* = 11.3 Hz, 1H, **14'**), 4.36 (s, 1H, **7**), 4.05 (d, *J* = 5.7 Hz, 2H, **13**), 3.96 (t, *J* = 7.0 Hz, 1H, **5**), 3.79 (s, 3H, **19**), 3.69 (t, *J* = 5.7 Hz, 1H, CH_2O**H**), 3.43 (s, 3H, **19**/**20**), 3.36 (s, 3H, **19**/**20**), 2.52-2.32 (m, 3H, **4**+**8**), 2.13-2.06 (m, 1H, **8**'), 1.66 (s, 3H, **11**/**12**), 1.58 (s, 3H, **11**/**12**) ppm

^{13}C NMR (Acetone-*d6*, 101 MHz): δ = 160.2 (C, **18)**, 144.5 (C, **15**), 132.8 (C, **10**), 132.2 (C, **3**), 130.1 (CH, **16**), 126.9 (CH, **2**), 122.2 (CH, **9**), 114.5 (CH, **17**), 110.3 (CH, **7**), 85.1 (CH, **5**), 72.3 (CH_2, **14**), 62.1 (C, **1**), 60.2 (CH_2, **13**), 58.8 (CH_3, **19**/**20**), 55.6 (CH_3, **19**/**20**), 39.2 (CH_2, **4**), 33.5 (CH_2, **8**), 26.3 (CH_3, **11**/**12**), 18.0 (CH_3, **11**/**12**) ppm

LRMS (ES^+) *m/z* = 399 $[M+Na]^+$

HRMS (ES^+) For $C_{22}H_{32}NaO_5^+$ calculated 399.2151, found 399.2142 Da

2.49 - (1*R,5*S**)-3-(Hydroxymethyl)-5-((4-methoxybenzyl)oxy)-1-(3-methylbut-2-en-1-yl)cyclopent-2-ene-1-carbaldehyde**



$C_{20}H_{26}O_4$

330.42 $gmol^{-1}$

A solution of acetal **2.48** (520 mg, 1.38 mmol) in THF (16 mL), H_2O (16 mL) and acetic acid (10 mL) was heated at 60 °C for 2 h before cooling to rt. The mixture was diluted with H_2O and extracted with EtOAc (3 x 30 mL). The combined organic solution was stirred with 3 M NaOH (50 mL) for 15 min before separating the phases and extracting the aqueous phase with EtOAc (3 x 30 mL). The combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 3:7) afforded the product as a colourless oil (444 mg, 1.34 mmol, 97%).

FT-IR (neat) ν_{max} 3423 (br), 2912 (w), 2856 (w), 1717 (s), 1513 (s), 1245 (s), 1086 (m), 1031 (s) cm^{-1}

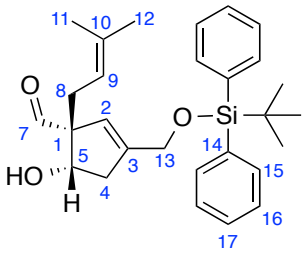
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 9.71 (s, 1H, **7**), 7.21 (d, *J* = 8.7 Hz, 2H, **16**), 6.87 (d, *J* = 8.7 Hz, 2H, **17**), 5.40 (t, *J* = 1.7 Hz, 1H, **2**), 5.08-4.96 (m, 1H, **9**), 4.49 (d, *J* = 11.5 Hz, 1H, **14**), 4.37 (d, *J* = 11.5 Hz, 1H, **14'**), 4.25-4.19 (m, 3H, **5**, **13**), 3.81 (s, 3H, **19**), 2.72-2.62 (m, 1H, **4**), 2.59-2.49 (m, 1H, **4'**), 2.44 (dd, *J* = 14.9, 6.7 Hz, 1H, **8**), 2.36 (dd, *J* = 14.9, 8.3 Hz, 1H, **8'**), 1.74 (br s, 1H, -O**H**), 1.68 (d, *J* = 0.7 Hz, 3H, **11**), 1.58 (s, 3H, **12**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 202.4 (CH, **7**), 159.2 (C, **18**), 146.7 (C, **3**), 134.6 (C, **10**), 129.9 (C, **15**), 129.2 (CH, **16**), 123.9 (CH, **2**), 118.9 (CH, **9**), 113.8 (CH, **17**), 84.8 (CH, **5**), 71.7 (CH_2, **14**), 66.7 (C, **1**), 62.0 (CH_2, **13**), 55.3 (CH_3, **19**), 38.3 (CH_2, **4**), 30.7 (s, CH_2), 25.9 (CH_3, **11**/**12**), 17.9 (CH_3, **11**/**12**) ppm

LRMS (ES^+) *m/z* = 353 $[M+Na]^+$

HRMS (ES$^+$) For $C_{20}H_{26}NaO_4^+$ calculated 353.1723, found 353.1723 Da

2.52 - (1*R,5*S**)-3-(((*tert*-Butyldiphenylsilyl)oxy)methyl)-5-hydroxy-1-(3-methylbut-2-en-1-yl)cyclopent-2-ene-1-carbaldehyde**



$C_{28}H_{36}O_3Si$

448.68 gmol^{-1}

To a solution of **2.49** (424 mg, 1.28 mmol), NEt_3 (0.33 mL, 2.3 mmol) and DMAP (10 mg, 0.082 mmol) in CH_2Cl_2 (16 mL) under N_2 was added TBDPS-Cl (0.35 mL, 1.3 mmol) at rt and the resulting solution was stirred for 3 h. The reaction was quenched with H_2O (10 mL) and the aqueous phase extracted with CH_2Cl_2 (3 x 10 mL). The combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure. Filtration through a silica plug, eluting with EtOAc:hexane = 4:96, afforded the silyl protected alcohol as an impure colourless oil (742 mg).

To a vigorously stirred solution of the impure silyl protected alcohol in CH_2Cl_2 (11 mL) and H_2O (0.7 mL) at rt was added DDQ (365 mg, 1.61 mmol) and the resulting suspension was stirred for 1 h. The dark orange mixture was concentrated under reduced pressure and the resulting orange paste was purified by silica gel chromatography (EtOAc:hexane = 5:95) to afford hydroxyaldehyde product **2.52** as a colourless oil (418 mg, 0.932 mmol, 73%).

FT-IR (neat) ν_{max} 3434 (br), 2912 (w), 2929 (w), 2856 (w), 1720 (m), 1513 (s), 1427 (s), 1110 (s), 1056 (s) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 9.65 (d, *J* = 0.6 Hz, 1H, **7**), 7.70-7.66 (m, 4H, **15**), 7.45-7.37 (m, 6H, **16**+**17**), 5.50 (t, *J* = 1.7 Hz, 1H, **2**), 5.19-5.11 (m, 1H, **9**), 4.38 (t, *J* = 7.4 Hz, 1H, **5**), 4.25 (d, *J* = 1.1 Hz, 2H, **13**), 2.67 (ddd, *J* = 16.4, 7.4, 1.1 Hz, 1H, **4**), 2.45-2.35 (m, 3H, **4'**+**8**), 1.73 (d, *J* = 0.7 Hz, 3H, **11**), 1.66 (s, 3H, **12**), 1.08 (s, 9H, SiC(C**H**$_3$)$_3$) ppm

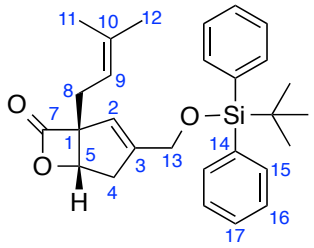
^{13}C NMR ($CDCl_3$, 101 MHz): δ = 203.3 (CH, **7**), 147.0 (C, **3**), 135.5 (CH, **3**), 135.2 (C, **10**), 133.4 (C, **14**), 129.8 (CH, **17**), 127.7 (CH, **16**), 122.6 (CH, **2**),

118.5 (CH, **9**), 78.5 (CH, **5**), 66.3 (C, **1**), 63.0 (CH_2, **13**), 40.8 (CH_2, **4**), 31.0 (CH_2, **8**), 26.8 ($C(\mathbf{C}H_3)_3$, 26.0 (CH_3, **11**/**12**), 19.3 ($C(\mathbf{C}H_3)_3$), 18.0 (CH_3, **11**/**12**) ppm

LRMS (ES^+) *m/z* = 449 $[M+H]^+$, 466 $[M+NH_4]^+$, 471 $[M+Na]^+$

HRMS (ES^+) For $C_{28}H_{36}NaO_3Si^+$ calculated 471.2326, found 471.2335 Da

2.53 - (1*R,5*S**)-3-(((*tert*-Butyldiphenylsilyl)oxy)methyl)-1-(3-methylbut-2-en-1-yl)-6-oxabicyclo[3.2.0]hept-2-en-7-one**



$C_{28}H_{34}O_3Si$

446.66 $gmol^{-1}$

To a solution of aldehyde **2.52** (189 mg, 0.421 mmol) in MeCN (5 mL) at −5 °C was added a solution of NaH_2PO_4 (76 mg, 0.64 mmol) in H_2O (4 mL) followed by H_2O_2 (0.62 mL, 5.5 mmol). After 10 min $NaClO_2$ (190 mg, 2.11 mmol) in H_2O (3 mL) was added dropwise over 40 min. After stirring at 0 °C for 3 h 30 min, the reaction mixture was extracted with EtOAc (4 x 5 mL). The combined organic solution was washed with $NaHCO_3$, dried (Na_2SO_4) and the solvent removed under reduced pressure to afford the crude hydroxyacid.

To a solution of the crude hydroxyacid in pyridine (6 mL) under N_2 at −5 °C was added *p*TsCl (200 mg, 1.05 mmol) and placed in a 5 °C fridge for 60 h. Additional *p*TsCl (200 mg, 1.05 mmol) was then added and the solution was stirred at 0 °C for 5 h. The reaction was quenched with H_2O (5 mL) and the aqueous phase was extracted with EtOAc (3 x 10 mL). The combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 2.5:97.5) afforded β-lactone **2.53** as a colourless oil (116 mg, 0.260 mmol, 62%).

FT-IR (neat) ν_{max} 2930 (w), 2856 (w), 1817 (s), 1106 (s), 1074 (m) cm^{-1}

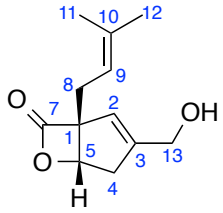
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.69-7.64 (m, 4H, **15**), 7.48-7.38 (m, 6H, **16**+**17**), 5.63-5.59 (m, 1H, **2**), 5.17-5.11 (m, 1H, **9**), 4.78 (dd, *J* = 4.5, 1.5 Hz, 1H, **5**), 4.24 (d, *J* = 1.5 Hz, 2H, **13**), 2.70-2.66 (m, 2H, **4**), 2.63 (dd, *J* = 15.0, 7.5 Hz, 1H, **8**), 2.47-2.38 (m, 1H, **8'**), 1.74 (d, *J* = 0.9 Hz, 3H, **11**), 1.66 (s, 3H, **12**), 1.08 (s, 9H, $SiC(C\mathbf{H}_3)_3$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 173.0 (C, **7**), 146.4 (C, **3**), 135.8 (C, **14**), 135.5 (CH, **15**), 133.2 (C, **10**), 129.8 (CH, **17**), 127.8 (CH, **16**), 122.0 (CH, **2**), 117.5 (CH, **9**), 78.5 (CH, **5**), 75.1 (C, **1**), 62.5 (CH_2, **13**), 37.3 (CH_2, **4**), 27.7 (CH_2, **8**), 26.8 ($C(\mathbf{C}H_3)_3$), 25.8 (CH_3, **11**/**12**), 19.2 ($C(\mathbf{C}H_3)_3$), 18.0 (CH_3, **11**/**12**) ppm

LRMS (ES^+) *m/z* = 464 $[M+NH_4]^+$, 469 $[M+Na]^+$

HRMS (ES^+) For $C_{28}H_{34}NaO_3Si^+$ calculated 469.2169, found 469.2176 Da

1.01 - (±)-Vibralactone



$C_{12}H_{16}O_3$

208.26 $gmol^{-1}$

First Generation Synthesis from 2.53

To a solution of silyl ether **2.53** (34 mg, 0.076 mmol) in THF (1 mL) at rt was added TBAF (1 M in THF, 0.15 mL, 0.15 mmol) dropwise over 30 s. After 90 min the reaction mixture was partitioned between H_2O and Et_2O and the aqueous phase extracted with Et_2O (3 x 5 mL). The combined organic solution was washed with brine, dried ($MgSO_4$) and the solvent removed under reduced pressure to afford a yellow oil. Purification by silica gel chromatography (EtOAc:hexane = 2:3) afforded the product as a colourless oil (14 mg, 0.067 mmol, 88%). Spectrascopic data were consistent with those reported.[1–3]

Second Generation Synthesis from 2.69

To a solution of dialdehyde **2.69** (aldehyde **2.69**:ketone **2.71** = 9:1, 6 mg, 0.02 mmol) in toluene (0.3 mL) under N_2 was added Bn_2NH•TfOH (35 mg, 0.10 mmol) and the solution was stirred at rt for 20 h. The solvent was removed under reduced pressure and purified by silica gel chromatography (EtOAc:hexane = 1:4) to afford the impure aldehyde product **1.14**.

Following a procedure by Snider *et al.*,[2] the impure aldehyde **1.14** was taken up in DME (0.3 mL) with 2 drops of H_2O and cooled to rt. $NaBH_4$ (2 mg, 0.05 mmol) was added and the solution was stirred for 10 min. The solvent was removed under reduced pressure and the resulting oil purified by silica gel chromatography (EtOAc:hexane = 1:2) to afford the product as a colourless oil (3.0 mg, 0.015 mmol, 75%). Spectrascopic data were consistent with those reported.[1–3]

FT-IR (neat) ν_{max} 3415 (br w), 2914 (w), 1809 (s), 1109 (m), 821 (s) cm^{-1}

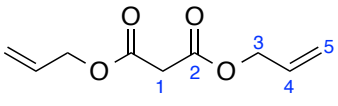
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.65-5.60 (m, 1H, **2**), 5.18-5.07 (m, 1H, **9**), 4.81 (dd, *J* = 4.5, 1.8 Hz, 1H, **5**), 4.25 (d, *J* = 1.1 Hz, 2H, **13**), 2.78-2.73 (s, 2H, **4**), 2.62 (dd, *J* = 15.1, 7.3 Hz, 1H, **8**), 2.44 (dd, *J* = 15.1, 7.3 Hz, 1H, **8'**), 1.73 (d, *J* = 0.9 Hz, 3H, **11**), 1.70 (br s, 1H, -O**H**), 1.64 (s, 3H, **12**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 172.9 (C, **7**), 146.5 (C, **3**), 136.0 (C, **10**), 122.6 (CH, **2**), 117.3 (CH, **9**), 78.5 (CH, **5**), 75.2 (C, **1**), 61.4 (CH_2, **13**), 37.3 (CH_2, **4**), 27.6 (CH_2, **8**), 25.8 (CH_3, **11**/**12**), 18.0 (CH_3, **11**/**12**) ppm

LRMS (EI) *m/z* = 208 $[M]^{+\cdot}$

HRMS (EI) For $C_{12}H_{16}O_3^{+\cdot}$ calculated 208.10940, found 208.11039 Da

2.57 - Diallyl malonate



$C_9H_{12}O_4$

184.19 $gmol^{-1}$

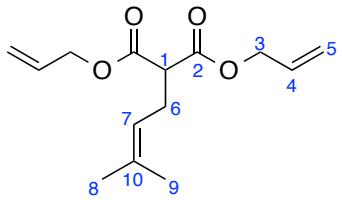
By adaption of a procedure by Shelkov *et al.*,[65] to a solution of malonic acid (1.00 g, 9.61 mmol) and allyl alcohol (1.31 mL, 19.2 mmol) in MeCN (30 mL) at 0 °C was added a solution of DCC (3.97 g, 19.2 mmol) in MeCN (25 mL) over 10 min before warming the suspension to rt. After 30 min the white precipitate was filtered off and washed with EtOAc (2 x 40 mL) before concentrating the filtrate. Purification by Kugelrohr distillation ($5.7x10^{-2}$ mbar, 135 °C) afforded the product as a colourless oil (1.60 g, 8.69 mmol, 91%). Spectroscopic data were consistent with those reported.[65]

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.92 (ddt, *J* = 17.2, 10.5, 5.7 Hz, 2H, **4**), 5.38-5.24 (m, 4H, **5**), 4.66 (dt, *J* = 5.7, 1.3 Hz, 4H, **3**), 3.44 (s, 2H, **1**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 166.1 (C, **2**), 131.5 (CH, **4**), 118.8 (CH_2, **5**), 66.1 (CH_2, **3**), 41.4 (CH_2, **1**) ppm

LRMS (ES^+) *m/z* = 185 $[M+H]^+$

2.58 - Diallyl 2-(3-methylbut-2-en-1-yl)malonate



$C_{14}H_{20}O_4$

252.31 $gmol^{-1}$

By adaption of a procedure by Klahn *et al*.,[140] to a solution of diallyl malonate (**2.57**, 4.603 g, 25.00 mmol) in acetone (30 mL) under N_2 at rt was added oven-dried K_2CO_3 (6.91 g, 50.0 mmol) followed by prenyl bromide (1.93 mL, 16.7 mmol) dropwise and the solution was stirred for 24 h. Saturated aqueous NH_4Cl (40 mL) was added and the aqueous phase was then extracted with EtOAc (3 x 70 mL). The combined organic solution was dried ($MgSO_4$) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 3:95) afforded the product as a colourless oil (3.48 g, 13.8 mmol, 83%).

FT-IR (neat) ν_{max} 2931 (w), 1732 (s), 1143 (m), 1030 (s) cm^{-1}

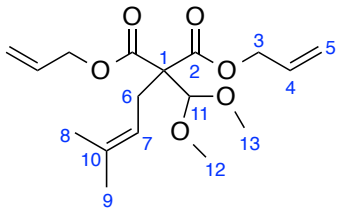
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.89 (ddt, *J* = 17.2, 10.5, 5.7 Hz, 2H, **4**), 5.32 (dq, *J* = 17.2, 1.5 Hz, 2H, **5**), 5.24 (dq, *J* = 10.5, 1.5 Hz, 2H, **5'**), 5.11-5.04 (m, 1H, **7**), 4.63 (dt, *J* = 5.7, 1.4 Hz, 4H, **3**), 3.42 (t, *J* = 7.7 Hz, 1H, **1**), 2.62 (t, *J* = 7.5 Hz, 2H, **6**), 1.68 (s, 3H, **8**/**9**), 1.63 (s, 3H, **8**/**9**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 168.8 (C, **2**), 135.1 (C, **10**), 131.6 (CH, **4**), 119.5 (CH, **7**), 118.4 (CH_2, **5**), 65.8 (CH_2, **3**), 52.1 (CH, **1**), 27.6 (CH_2, **6**), 25.7 (CH_3, **8**/**9**), 17.8 (CH_3, **8**/**9**) ppm

LRMS (ES^+) *m/z* = 253 $[M+H]^+$, 275 $[M+Na]^+$

HRMS (ES^+) For $C_{14}H_{20}NaO_4^+$ calculated 275.1254, found 275.1250 Da

2.59 - Diallyl 2-(dimethoxymethyl)-2-(3-methylbut-2-en-1-yl)malonate



$C_{17}H_{26}O_6$

326.39 $gmol^{-1}$

By adaption of a procedure by Evans *et al.*,[141] to a solution of malonate **2.58** (371 mg, 1.47 mmol) in CH_2Cl_2 (7 mL) under N_2 at 0 °C was added NEt_3 (0.41 mL, 2.9 mmol) followed by $TiCl_4$ (1 M in CH_2Cl_2, 2.94 mL, 2.94 mmol) dropwise over 5 min. After stirring for 45 min, trimethyl orthoformate (0.80 mL, 7.4 mmol) was added dropwise over 5 min and the orange solution was stirred at rt for 30 min. The reaction was cooled to 0 °C and quenched by slow addition of H_2O (15 mL). After warming to rt, the organic phase was washed with H_2O (15 mL) and brine before drying (Na_2SO_4) and removing the solvent under reduced pressure. Purification by silica gel chromatography (EtOAc:pet. ether = 7:93) afforded the product as a colourless oil (364 mg, 1.12 mmol, 76%).

FT-IR (neat) ν_{max} 2930 (w), 1733 (s), 1183 (m), 1071 (s) cm^{-1}

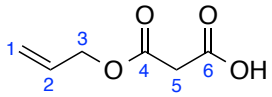
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.87 (ddt, *J* = 17.2, 10.5, 5.7 Hz, 2H, **4**), 5.32 (dq, *J* = 17.2, 1.5 Hz, 2H, **5**), 5.22 (dq, *J* = 10.5, 1.5 Hz, 2H, **5'**), 5.20-5.14 (m, 1H, **7**), 4.77 (s, 1H, **11**), 4.70-4.56 (m, 4H, **3**), 3.56 (s, 6H, **12**+**13**), 2.76 (d, *J* = 7.5 Hz, 2H, **6**), 1.67 (s, 3H, **8**/**9**), 1.61 (s, 3H, **8**/**9**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 168.7 (C, **2**), 134.4 (C, **10**), 131.7 (CH, **4**), 118.9 (CH, **7**), 118.2 (CH_2, **5**), 107.2 (CH, **11**), 65.7 (CH_2, **3**), 63.3 (C, **1**), 58.6 (CH_3, **12**+**13**), 29.4 (CH_2, **6**), 26.0 (CH_3, **8**/**9**), 17.8 (CH_3, **8**/**9**) ppm

LRMS (ES^+) *m/z* = 349 $[M+Na]^+$

HRMS (ES^+) For $C_{17}H_{26}NaO_6^+$ calculated 349.1622, found 349.1618 Da

2.63 - 3-(Allyloxy)-3-oxopropanoic acid



$C_6H_8O_4$

144.13 $gmol^{-1}$

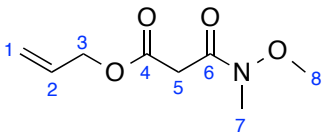
By adaption of a procedure by Shelkov *et al.*,[65] to a solution of malonic acid (10.4 g, 99.9 mmol) and allyl alcohol (13.6 mL, 200 mmol) in MeCN (200 mL) under N_2 at 0 °C was added a solution of DCC (22.7 g, 110 mmol) in MeCN (75 mL) and the suspension was stirred at rt for 2 h. The white solid was removed by filtration, washing with EtOAc. The filtrate was then concentrated under reduced pressure. The colourless residue was taken up in Et_2O (500 mL) and extracted with saturated aqueous $NaHCO_3$ (3 x 200 mL) The aqueous phase was then acidified to pH 1 with 2 M HCl (750 mL) before extracting with EtOAc (3 x 300 mL). The combined organic solution was dried ($MgSO_4$) and the solvent removed to afford the product as a colourless oil (10.5 g, 72.9 mmol, 73%). Spectroscopic data were consistent with those reported.[144]

FT-IR (neat) ν_{max} 2948 (br w), 1713 (s), 1149 (s), 990 (m), 736 (m) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.71 (br s, O**H**), 5.92 (ddt, *J* = 17.1, 10.5, 5.7 Hz, 1H, **2**), 5.39-5.32 (m, 1H, **1**), 5.28 (dd, *J* = 10.5, 1.0 Hz, 1H, **1'**), 4.68 (d, *J* = 5.7 Hz, 2H, **3**), 3.47 (s, 2H, **5**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 171.2 (C, **6**), 166.4 (C, **4**), 131.2 (CH, **2**), 119.1 (CH_2, **1**), 66.4 (CH_2, **3**), 40.8 (CH_2, **5**) ppm

2.64 - Allyl 3-(methoxy(methyl)amino)-3-oxopropanoate



$C_8H_{13}NO_4$

187.20 $gmol^{-1}$

To a solution of acid **2.63** (8.59 g, 59.6 mmol) and 5 drops of DMF in CH_2Cl_2 (250 mL) under N_2 at 0 °C was added oxalyl chloride (6.05 mL, 71.5 mmol) dropwise over 10 min. After 5 min the reaction was warmed to rt and stirred for 2.5 h. The solvent was removed under reduced pressure to afford the crude acid chloride product.

To a vigorously stirred solution of K_2CO_3 (16.6 g, 120 mmol) in H_2O (100 mL) at 0 °C was added *N*,*O*-dimethylhydroxyamide (6.440 g, 66.02 mmol) portion-wise. A solution of the acid chloride in Et_2O (100 mL) was added dropwise over 15 min. After 30 min the phases were separated and the aqueous phase extracted with Et_2O (3 x 100 mL). The combined organic solution was washed with brine, dried ($MgSO_4$) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 2:5) afforded the product as an orange oil (9.12 g, 48.7 mmol, 81%).

FT-IR (neat) ν_{max} 2944 (w), 1738 (s), 1665 (s), 1151 (s), 989 (s), 933 (m) cm^{-1}

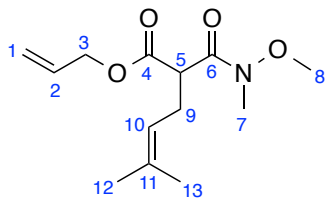
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.91 (ddt, *J* = 17.2, 10.5, 5.7 Hz, 1H, **2**), 5.34 (dq, *J* = 17.2, 1.5 Hz, 1H, **1**), 5.24 (dq, *J* = 10.4, 1.5 Hz, 1H, **1'**), 4.64 (dt, *J* = 5.7, 1.3 Hz, 2H, **3**), 3.70 (s, 3H, **8**), 3.52 (s, 2H, **5**), 3.21 (s, 3H, **7**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 167.1 (C, **6**), 167.0 (C, **4**), 131.7 (CH, **2**), 118.6 (CH_2, **1**), 65.8 (CH_2, **3**), 61.3 (CH_3, **8**), 40.1 (CH_2, **5**), 32.2 (CH_3, **7**) ppm

LRMS (ES^+) *m/z* = 188 $[M+H]^+$, 210 $[M+Na]^+$

HRMS (ES^+) For $C_8H_{14}NO_4^+$ calculated 188.0917, found 188.0923 Da

2.65 - Allyl 2-(methoxy(methyl)carbamoyl)-5-methylhex-4-enoate



$C_{13}H_{21}NO_4$

255.31 $gmol^{-1}$

To a solution of allyl 3-(methoxy(methyl)amino)-3-oxopropanoate (**2.65**, 8.75 g, 46.7 mmol) in THF (230 mL) under N_2 at 0 °C was added NaHMDS (1 M in THF, 46.7 mL, 46.7 mmol) dropwise over 15 min and stirred for 5 min before warming to rt. After 90 min the reaction was cooled to −78 °C and prenyl bromide (4.88 mL, 42.1 mmol) was added dropwise over 10 min. The reaction was allowed to warm slowly to rt in the cardice/acetone bath over 19 h. The reaction was quenched with H_2O (100 mL) and the aqueous phase extracted with EtOAc (3 x 150 mL). The combined organic solution was washed with brine, dried ($MgSO_4$) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 3:1) afforded the product as an orange oil (9.34 g, 26.4 mmol, 87%).

FT-IR (neat) ν_{max} 2968 (w), 2937 (w), 1736 (s), 1666 (s), 1378 (m), 1191 (m), 1149 (s), 986 (s) cm^{-1}

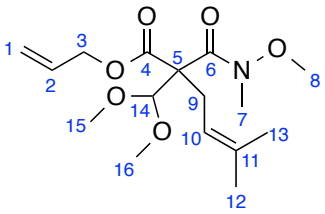
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.89 (ddt, *J* = 17.2, 10.5, 5.6 Hz, 1H, **2**), 5.31 (dq, *J* = 17.2, 1.5 Hz, 1H, **1**), 5.22 (dq, *J* = 10.5, 1.5 Hz, 1H, **1'**), 5.15-5.09 (m, 1H, **10**), 4.66-4.55 (m, 2H, **3**), 3.69 (s, 3H, **8**), 3.21 (s, 3H, **7**), 2.61 (t, *J* = 7.4 Hz, 2H, **9**), 1.68 (d, *J* = 1.0 Hz, 3H, **13**), 1.64 (s, 3H, **12**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 170.0 (C, **6**), 169.4 (C, **4**), 134.4 (C, **11**), 131.9 (CH, **2**), 120.3 (CH, **10**), 118.2 (CH_2, **1**), 65.6 (CH_2, **3**), 61.3 (CH_3, **8**), 48.8 (CH, **5**), 32.5 (CH_3, **7**), 27.3 (CH_2, **9**), 25.7 (CH_3, **12**/**13**), 17.8 (CH_3, **12**/**13**) ppm

LRMS (ES^+) *m/z* = 256 $[M+H]^+$, 278 $[M+Na]^+$

HRMS (ES^+) For $C_{13}H_{22}NO_4^+$ calculated 256.1543, found 256.1546 Da

2.66 - Allyl 2-(dimethoxymethyl)-2-(methoxy(methyl)carbamoyl)-5-methylhex-4-enoate



$C_{16}H_{27}NO_6$

329.39 $gmol^{-1}$

By adaption of a procedure by Evans *et al.*,[141] to a solution of malonate **2.65** (1.78 g, 6.78 mmol) and NEt_3 (2.09 mL, 15.0 mmol) in CH_2Cl_2 (37 mL) under N_2 at 0 °C was added $TiCl_4$ (1 M in CH_2Cl_2, 7.50 mL, 7.50 mmol) dropwise over 10 min. The resulting orange solution was warmed to rt and stirred for 30 min. Trimethyl orthoformate (1.62 mL, 15.0 mmol) was added dropwise over 5 min and the resulting solution was stirred for 1 h. Additional $TiCl_4$ (1 M in CH_2Cl_2, 7.50 mL, 7.50 mmol) was added dropwise over 5 min and the resulting solution was stirred for a further 1 h. The reaction was cooled to 0 °C and quenched by slow addition of H_2O (15 mL). After warming to rt, the organic phase was washed with H_2O (15 mL) and brine before drying (Na_2SO_4) and removing the solvent under reduced pressure. Purification by silica gel column chromatography (EtOAc:hexane = 1:4) afforded the product as a yellow oil (1.85 g, 5.63 mmol, 83%).

FT-IR (neat) ν_{max} 2936 (m), 1736 (s), 1664 (s),1378 (m), 1150 (s) cm^{-1}

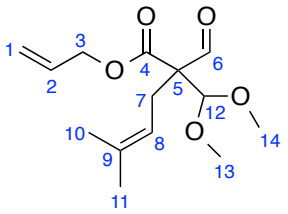
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.90 (ddt, *J* = 17.2, 10.4, 5.6 Hz, 1H, **2**), 5.33 (dq, *J* = 17.2, 1.3 Hz, 1H, **1**), 5.21 (dq, *J* = 10.5, 1.3 Hz, 1H, **1'**), 5.14-5.07 (m, 1H, **10**), 4.80 (s, 1H, **14**), 4.65 (ddt, *J* = 13.3, 5.6, 1.4 Hz, 1H, **3**), 4.56 (ddt, *J* = 13.3, 5.6, 1.4 Hz, 1H, **3'**), 3.56 (s, 3H, **8**/**15**/**16**), 3.56 (s, 3H, **8**/**15**/**16**), 3.56 (s, 3H, **8**/**15**/**16**), 3.16 (s, 3H, **7**), 2.80 (d, *J* = 7.2 Hz, 2H, **9**), 1.68 (d, *J* = 1.0 Hz, 3H, **13**), 1.62 (s, 3H, **12**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 170.5 (C, **6**), 168.9 (C, **4**), 133.9 (C, **11**), 132.1 (CH, **2**), 119.3 (CH, **10**), 118.0 (CH_2, **1**), 107.0 (CH, **14**), 65.4 (CH_2, **3**), 61.8 (CH_3, **8**), 60.2 (C, **5**), 58.5 (CH_3, **15**/**16**), 58.0 (CH_3, **15**/**16**), 33.2 (CH_3, **7**), 28.9 (CH_2, **9**), 26.0 (CH_3, **12**/**13**), 17.8 (CH_3, **12**/**13**) ppm

LRMS (ES^+) *m/z* = 352 $[M+Na]^+$

HRMS (ES$^+$) For $C_{16}H_{27}NNaO_6^+$ calculated 352.1731, found 352.1730 Da

2.61 - Allyl 2-(dimethoxymethyl)-2-formyl-5-methylhex-4-enoate



$C_{14}H_{22}O_5$

270.33 gmol^{-1}

To a solution of Weinreb amide **2.66** (1.43 g, 4.36 mmol) in CH_2Cl_2 (25 mL) under N_2 at −78 °C was added DIBAL (1 M in CH_2Cl_2, 13.0 mL, 13.0 mmol) dropwise over 1 h. The reaction was quenched by slow addition of MeOH (20 mL) and the solution allowed to warm to rt. Saturated aqueous Rochelle's salt solution (100 mL) was added and stirred vigorously for 2 h until complete phase separation was observed. The aqueous phase was extracted with CH_2Cl_2 (3 x 40 mL) and the combined organic solution was washed with brine, dried ($MgSO_4$) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 1:9) afforded the product as a colourless oil (977 mg, 3.61 mmol, 83%).

FT-IR (neat) ν_{max} 2931 (w), 2837 (w), 1721 (s), 1069 (s) cm^{-1}

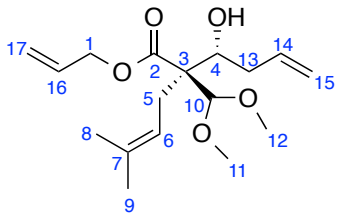
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 9.84 (s, 1H, **6**), 5.88 (ddt, *J* = 17.2, 10.4, 5.6 Hz, 1H, **2**), 5.33 (apparent dd, *J* = 17.2, 1.4 Hz, 1H, **1**), 5.25 (apparent dd, *J* = 10.4, 1.1 Hz, 1H, **1'**), 5.10-5.01 (m, 1H, **8**), 4.70 (s, 1H, **12**), 4.69-4.59 (m, 2H, **3**), 3.56 (s, 3H, **13**/**14**), 3.48 (s, 3H, **13**/**14**), 2.60 (dd, *J* = 14.4, 6.5 Hz, 1H, **7**), 2.50 (dd, *J* = 14.4, 8.4 Hz, 1H, **7'**), 1.67 (s, 3H, **10**/**11**), 1.61 (s, 3H, **10**/**11**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 197.7 (CH, **6**), 169.1 (C, **4**), 135.2 (C, **9**), 131.5 (CH, **2**), 118.6 (CH_2, **1**), 117.7 (CH, **8**), 108.9 (CH, **12**), 66.2 (C, **5**), 65.7 (CH_2, **3**), 59.1 (CH_3, **13**/**14**), 58.1 (CH_3, **13**/**14**), 28.0 (CH_2, **7**), 25.9 (CH_3, **10**/**11**), 17.7 (CH_3, **10**/**11**) ppm

LRMS (ES$^+$) *m/z* = 293 [M+Na]$^+$

HRMS (ES$^+$) For $C_{14}H_{22}NaO_5^+$ calculated 293.1359, found 293.1359 Da

2.67 - Allyl 2-(dimethoxymethyl)-2-((*R**)-1-hydroxybut-3-en-1-yl)-5-methylhex-4-enoate



$C_{17}H_{28}O_5$

312.41 gmol^{-1}

By adaption of a procedure by Linclau *et al*.,[51] to a suspension of Mg (228 mg, 9.38 mmol) in Et_2O (7 mL) in a 3-necked RBF fitted with a condenser was added dibromoethane (0.81 mL, 9.4 mmol) dropwise over 10 min. After 35 min the solvent was removed under reduced pressure to afford a white solid, which was taken up in CH_2Cl_2 (9.5 mL) under N_2 and cooled to −78 °C. A solution of aldehyde **2.61** (845 mg, 3.13 mmol) in CH_2Cl_2 (4.5 mL) was added dropwise over 40 min and the solution was stirred for a further 30 min. A solution of allyltributylstannane (1.94 mL, 6.26 mmol) in CH_2Cl_2 (7 mL) was added dropwise over 1 h. After 3 h the reaction was quenched by slow addition of saturated aqueous $NaHCO_3$ (20 mL) before warming to rt. The aqueous phase was extracted with CH_2Cl_2 (3 x 15 mL) and the combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure to afford a colourless oil. Purification by silica gel chromatography (9:1 silica:K_2CO_3 and K_2CO_3 plug, EtOAc:hexane = 1:9) afforded the product as a colourless oil (874 mg, 2.80 mmol, 89%).

FT-IR (neat) ν_{max} 3518 (br w), 2928 (w), 1728 (s), 1187 (m), 1063 (s) cm^{-1}

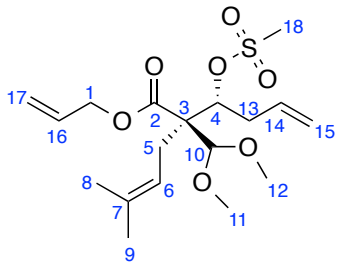
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.00-5.87 (m, 2H, **14**+**16**), 5.40-5.23 (m, 2H, **17**), 5.18-5.12, (m, 1H, **6**), 5.12-5.02 (m, 2H, **15**), 4.70 (s, 1H, **10**), 4.63 (dt, *J* = 5.7, 1.4 Hz, 2H, **1**), 3.98 (ddd, *J* = 10.2, 7.5, 2.6 Hz, 1H, **4**), 3.61 (s, 3H, **11**/**12**), 3.51 (s, 3H, **11**/**12**), 3.28 (d, *J* = 7.5 Hz, 1H, -O**H**), 2.54 (br dd, *J* = 14.4, 6.7 Hz, 1H, **5**), 2.48-2.39 (m, 2H, **5'**+**13**), 2.39-2.28 (m, 1H, **13'**), 1.69 (d, *J* = 0.7 Hz, 3H, **8**), 1.61 (s, 3H, **9**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 172.5 (C, **2**), 136.9 (CH, **14**), 134.1 (C, **7**), 132.0 (CH, **16**), 119.0 (CH_2, **17**), 118.4 (CH, **6**), 116.1 (CH_2, **15**), 110.6 (CH, **10**), 72.7 (CH, **4**), 65.3 (CH_2, **1**), 59.7 (CH_3, **11**/**12**), 58.7 (C, **3**), 58.2 (CH_3, **11**/**12**), 37.9 (CH_2, **13**), 30.1 (CH, **6**), 26.0 (CH_3, **8**/**9**), 17.8 (CH_3, **8**/**9**) ppm

LRMS (ES^+) *m/z* = 335 $[M+Na]^+$

HRMS (ES^+) For $C_{17}H_{28}NaO_5^+$ calculated 335.1829, found 335.1827 Da

2.54 - Allyl (*S)-2-(dimethoxymethyl)-5-methyl-2-((*R**)-1-((methylsulfonyl)oxy)but-3-en-1-yl)hex-4-enoate**



$C_{18}H_{30}O_7S$

390.49 $gmol^{-1}$

To a solution of alcohol **2.67** (864 mg, 2.77 mmol), pyridine (0.9 mL, 11.1 mmol) and DMAP (20 mg, 0.16 mmol) in CH_2Cl_2 (14 mL) under N_2 at rt was added Ms_2O (1.45 g, 8.31 mmol) and stirred for 90 min. The reaction was quenched with H_2O (10 mL) and the aqueous phase extracted with CH_2Cl_2 (3 x 15 mL). The combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 8:92) afforded the product as a colourless oil (1.01 g, 2.59 mmol, 94%).

FT-IR (neat) ν_{max} 2934 (w), 1731 (s), 1340 (s), 1172 (s), 1069 (s), 863 (s) cm^{-1}

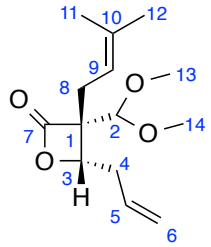
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.00-5.90 (m, 1H, **16**), 5.90-5.81 (m, 1H, **14**), 5.39 (dq, *J* = 17.2, 1.5 Hz, 1H, **17**), 5.36-5.30 (m, 1H, **6**), 5.27 (dq, *J* = 10.5, 1.5 Hz, 1H, **17'**), 5.23 (dd, *J* = 9.5, 2.6 Hz, 1H, **4**), 5.17-5.10 (m, 2H, **15**), 4.72 (s, 1H, **10**), 4.65 (ddt, *J* = 5.5, 3.9, 1.5 Hz, 2H, **1**), 3.54 (s, 3H, **11**/**12**), 3.54 (s, 3H, **11**/**12**), 3.01 (s, 3H, **16**), 2.70-2.53 (m, 3H, **5**+**13**), 2.48-2.36 (m, 1H, **13'**), 1.70 (d, *J* = 0.7 Hz, 3H, **8**), 1.63 (s, 3H, **9**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 170.9 (C, **2**), 134.9 (CH, **14**), 133.6 (C, **7**), 131.9 (CH, **16**), 119.4 (CH, **6**), 118.4 (CH_2, **17**), 118.2 (CH_2, **15**), 108.4 (CH, **10**), 83.3 (CH, **4**), 65.6 (CH_2, **1**), 59.6 (CH_3, **11**/**12**), 58.8 (C, **3**), 58.1 (CH_3, **11**/**12**), 39.4 (CH_3, **18**), 36.7 (CH_2, **13**), 28.1 (CH_2, **5**), 26.1 (CH_3, **8**/**9**), 17.8 (CH_3, **8**/**9**) ppm

LRMS (ES^+) *m/z* = 413 $[M+Na]^+$

HRMS (ES^+) For $C_{18}H_{30}NaO_7S^+$ calculated 413.1604, found 413.1606 Da

2.18 - (3*S,4*S**)-4-Allyl-3-(dimethoxymethyl)-3-(3-methylbut-2-en-1-yl)oxetan-2-one**



$C_{14}H_{22}O_4$

254.33 $gmol^{-1}$

To a solution of allyl ester **2.54** (62.0 mg, 0.16 mmol) and $[Pd(PPh_3)_4]$ (2 mg, 0.002 mmol) in CH_2Cl_2 (3 mL) under N_2 was added pyrrolidine (0.040 mL, 0.48 mmol) and the orange solution was stirred for 2 min. The solvent was removed under reduced pressure to afford a yellow oil/solid. Purification by silica gel chromatography (EtOAc:hexane = 5:95) afforded the product as a colourless oil (38.4 mg, 0.152 mmol, 95%).

FT-IR (neat) ν_{max} 2932 (w), 1819 (s), 1445 (w), 1068 (s) cm^{-1}

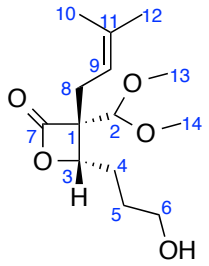
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.83 (ddt, *J* = 17.1, 10.3, 6.7 Hz, 1H, **5**), 5.21-5.10 (m, 3H, **9**+**6**), 4.50 (s, 1H, **2**), 4.29 (dd, *J* = 9.2, 4.7 Hz, 1H, **3**), 3.51 (s, 3H, **13**/**14**), 3.50 (s, 3H, **13**/**14**), 2.88-2.78 (m, 1H, **4**), 2.71-2.61 (m, 1H, **4'**), 2.51 (dd, *J* = 14.8, 7.1 Hz, 1H, **8**), 2.45 (dd, *J* = 14.8, 7.9 Hz, 1H, **8'**), 1.75 (d, *J* = 0.7 Hz, 3H, **11**), 1.65 (s, 3H, **12**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 170.4 (C, **7**), 136.9 (C, **10**), 133.2 (CH, **5**), 117.9 (CH_2, **6**), 117.0 (CH, **9**), 104.3 (CH, **2**), 78.6 (CH, **3**), 66.3 (C, **1**), 57.2 (CH_3, **11**/**12**), 56.3 (CH_3, **11**/**12**), 34.1 (CH_2, **4**), 28.7 (CH_3, **13**/**14**), 25.9 (CH_2, **8**), 18.0 (CH_3, **13**/**14**) ppm

LRMS (ES^+) *m/z* = 277 $[M+Na]^+$

HRMS (ES^+) For $C_{14}H_{22}NaO_4^+$ calculated 277.1410, found 277.1404 Da

2.70 - (3*S,4**S*)-3-(Dimethoxymethyl)-4-(3-hydroxypropyl)-3-(3-methylbut-2-en-1-yl)oxetan-2-one**



$C_{14}H_{24}O_5$

272.34 $gmol^{-1}$

To a solution of alkene **2.18** (42 mg, 0.17 mmol) in THF (0.85 mL) under N_2 at rt was added 9-BBN (0.5 M in THF, 0.68 mL, 0.34 mmol) dropwise over 2 min. The reaction was heated at 60 °C for 16 h before cooling to 0 °C. H_2O (2 mL) was added followed by $NaBO_3.4H_2O$ (8 mg 0.5 mmol), and the resulting suspension was warmed to rt and stirred for 1 h. The aqueous phase was extracted with EtOAc (3 x 2 mL) and the combined organics washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 8:92 to 3:7) afforded the product as a colourless oil (16 mg, 0.059 mmol, 35%) along with the recovered starting material (39 mg, 0.15 mmol).

FT-IR (neat) ν_{max} 3423 (br m), 2931 (m), 1816 (s), 1100 (m), 1068 (s) cm^{-1}

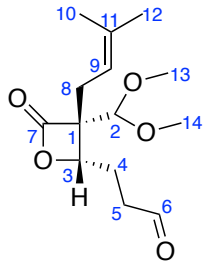
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.21-5.13 (m, 1H, **9**), 4.47 (s, 1H, **2**), 4.27 (dd, *J* = 9.7, 3.9 Hz, 1H, **3**), 3.77-3.64 (m, 2H, **6**), 3.50 (s, 3H, **13**/**14**), 3.50 (s, 3H, **13**/**14**), 2.50 (dd, *J* = 14.7, 7.1 Hz, 1H, **8**), 2.45 (dd, *J* = 14.7, 7.9 Hz, 1H, **8'**), 2.18-2.06 (m, 1H, **4**), 2.06-1.95 (m, 1H, **4'**), 1.75 (s, 3H, **13**/**14**), 1.65 (s, 3H, **13**/**14**), 1.81-1.61 (m, 2H, **5**), 1.53 (br s, 1H, O**H**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 170.6 (C, **7**), 136.8 (C, **11**), 117.0 (CH, **9**), 104.7 (CH, **2**), 79.9 (CH, **3**), 66.1 (C, **1**), 62.2 (CH_2, **6**), 57.5 (CH_3, **13**/**14**), 56.6 (CH_3, **13**/**14**), 29.4 (CH_2, **5**), 28.6 (CH_2, **4**), 26.1 (CH_2, **8**), 25.9 (CH_3, **10**/**12**), 18.0 (CH_3, **10**/**12**) ppm

LRMS (ES^+) *m/z* = 295 $[M+Na]^+$

HRMS (ES^+) For $C_{14}H_{24}NaO_5^+$ calculated 295.1513, found 295.1518 Da

2.69 - 3-((2*S,3*S**)-3-(Dimethoxymethyl)-3-(3-methylbut-2-en-1-yl)-4-oxooxetan-2-yl)propanal**



$C_{14}H_{22}O_5$

270.33 $gmol^{-1}$

By adaption of a procedure by Grubbs *et al.*,[67] a mixture of $PdCl_2(PhCN)_2$ (4 mg, 0.01 mmol), $CuCl_2.H_2O$ (2.0 mg, 0.012 mmol) and $AgNO_2$ (2.0 mg, 0.015 mmol) was sparged for 1 min with O_2 (1 atm, balloon). Separately, a solution of alkene **2.18** (22 mg, 0.087 mmol) in ^tBuOH (0.9 mL) and $MeNO_2$ (0.05 mmol) was sparged with O_2 (1 atm, balloon) for 1 min and the solution was then added dropwise to the first reaction vessel. The resulting solution was sparged for a further 2 min with O_2 (1 atm, balloon) and stirred for 1 h under a positive pressure of O_2. H_2O (1 mL) was added and the aqueous phase extracted with CH_2Cl_2 (3 x 3 mL). The combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent was removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 1:9) afforded the aldehyde product **2.69** as a 9:1 mixture with the corresponding ketone (21 mg, 0.077 mmol, 78%).

FT-IR (neat) ν_{max} 2932 (w), 1816 (s), 1721 (m), 1097 (m), 1067 (s) cm^{-1}

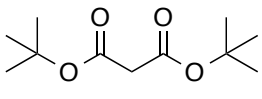
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 9.81 (t, *J* = 1.0 Hz, 1H, **6**), 5.19-5.13 (m, 1H, **9**), 4.46 (s, 1H, **2**), 4.24 (dd, *J* = 10.0, 4.0 Hz, 1H, **3**), 3.51 (s, 3H, **13**/**14**), 3.49 (s, 3H, **13**/**14**), 3.32 (dd, *J* = 18.1, 7.1 Hz, 1H, **8**), 3.18 (dd, *J* = 18.1, 7.1 Hz, 1H, **8'**), 2.72-2.55 (m, 2H, **4**), 2.53-2.43 (m, 2H, **4'**+**5**), 2.40-2.31 (m, 1H, **5'**), 1.74 (d, *J* = 1.0 Hz, 3H, **10**), 1.64 (s, 3H, **12**) ppm (Only aldehyde **2.69** peaks reported)

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 200.9 (CH, **9**), 170.2 (C, **7**), 137.1 (C, **11**), 116.8 (CH, **9**), 105.0 (CH, **2**), 78.6 (CH, **3**), 66.2 (C, **1**), 57.5 (CH_3, **13**/**14**), 57.1 (CH_3, **13**/**14**), 40.1 (CH_2, **5**), 28.8 (CH_3, **10**/**12**), 25.9 (CH_2, **8**), 22.2 (CH_2, **4**), 17.9 (CH_3, **10**/**12**) ppm

LRMS (ES^+) *m/z* = 293 $[M+Na]^+$

HRMS (ES^+) For $C_{14}H_{22}NaO_5^+$ calculated 293.1359, found 293.1361 Da

2.56 - Di-*tert*-butyl malonate



$C_{11}H_{20}O_4$

216.28 $gmol^{-1}$

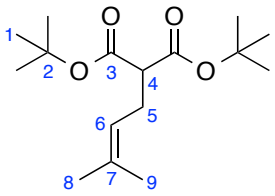
By adaption of a procedure by Shelkov *et al.*,[65] to a solution of malonic acid (3.937 g, 29.19 mmol) and ^{t}BuOH (4.319 g, 58.27 mmol) in MeCN (40 mL) under N_2 at 0 °C was added a solution of DCC (12.04 g, 58.37 mmol) in MeCN (60 mL) over 10 min. After 15 min the reaction was warmed to rt and stirred for 1 h. The white precipitate was removed by filtration and washed with EtOAc before concentrating the filtrate under reduced pressure. Purification by Kugelrohr distillation (160 °C at 30 mbar) afforded the product as a colourless oil (5.800 g, 26.92 mmol, 92%). The spectroscopic data were consistent with a commercial sample.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 3.18 (s, 2H, C**H$_2$**), 1.47 (s, 18H, C**H**$_3$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 166.2 (**C**=O), 81.6 (**C**CH_3), 44.3 (**C**H_2), 27.9 (**C**H_3) ppm

LRMS (ES^+) *m/z* = 239 $[M+Na]^+$

2.83 - Di-*tert*-butyl 2-(3-methylbut-2-en-1-yl)malonate



$C_{16}H_{28}O_4$

284.40 $gmol^{-1}$

By adaption of a procedure by Klahn *et al*.,[140] to a mixture of di-*tert*-butyl malonate (311 mg, 1.09 mmol) and K_2CO_3 (276 mg, 1.78 mmol) in acetone (9 mL) under N_2 at rt was added prenyl bromide (0.08 mL, 0.7 mmol) dropwise over 5 min and the solution was stirred for 42 h. The reaction was quenched with H_2O (10 mL) and the aqueous phase extracted with EtOAc (3 x 10 mL). The combined organic solution was washed with brine, dried ($MgSO_4$) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 1:99) afforded the product as a colourless oil (37 mg, 0.13 mmol, 12%).

FT-IR (neat) ν_{max} 2931 (w), 1732 (s), 1143 (s), 930 (m) cm^{-1}

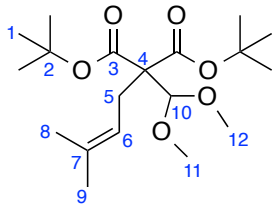
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.10-5.04 (m, 1H, **6**), 3.14 (t, *J* = 7.8 Hz, 1H, **4**), 2.50 (t, *J* = 7.5 Hz, 2H, **5**), 1.68 (d, *J* = 0.9 Hz, 3H, **9**), 1.64 (s, 3H, **8**), 1.45 (s, 18H, **1**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 168.7 (C, **3**), 134.1 (C, **7**), 120.2 (CH, **6**), 81.2 (C, **2**), 54.1 (CH, **4**), 27.9 (CH_3, **1**), 27.5 (CH_3, **8**/**9**), 25.7 (CH_2, **5**), 17.8 (CH_3, **8**/**9**) ppm

LRMS (ES$^+$) *m/z* = 307 $[M+Na]^+$

HRMS (ES$^+$) For $C_{16}H_{28}NaO_4^+$ calculated 307.1880, found 307.1882 Da

2.84 - Di-*tert*-butyl 2-(dimethoxymethyl)-2-(3-methylbut-2-en-1-yl)malonate



$C_{19}H_{34}O_6$

358.48 $gmol^{-1}$

By adaption of a procedure by Evans *et al*.,[141] to a solution of malonate **2.83** (190 mg, 0.668 mmol) and NEt_3 (0.47 mL, 3.3 mmol) in CH_2Cl_2 (4 mL) under N_2 at 0 °C was added $TiCl_4$ (0.47 mL, 0.47 mmol) dropwise over 10 min and the resulting red solution was stirred for a further 40 min at 0 °C. Trimethyl orthoformate (0.36 mL, 0.35 mmol) was added dropwise over 2 min and then warmed to rt. After 18 h the reaction was quenched with H_2O (10 mL). The organic phase was washed with H_2O (2 x 10 mL), dried (Na_2SO_4) and the solvent removed under reduced pressure to afford an orange oil. Purification by silica gel chromatography (EtOAc:hexane = 5:95) afforded the product as a white solid (149 mg, 0.416 mmol, 62%).

M.P 102-103.1 °C (solvent: Et_2O)

FT-IR (neat) ν_{max} 2954 (w), 1733 (s), 1445 (s), 1211 (m), 1071 (s) cm^{-1}

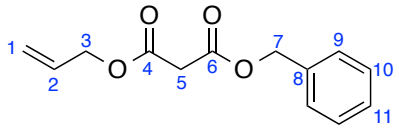
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.32-5.25 (m, 1H, **6**), 4.74 (s, 1H, **10**), 3.57 (s, 6H, **11**+**12**), 2.67 (d, *J* = 7.1 Hz, 2H, **5**), 1.67 (d, *J* = 1.0 Hz, 3H, **8**), 1.63 (s, 3H, **9**), 1.44 (s, 18H, **1**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 168.3 (C, **3**), 132.8 (C, **7**), 120.5 (CH, **6**), 107.7 (CH, **10**), 81.0 (C, **2**), 63.7 (C, **4**), 58.4 (CH_3, **11**+**12**), 27.9 (CH_3, **1**), 27.8 (CH_3, **5**), 26.0 (CH_3, **8**/**9**), 17.8 (CH_3, **8**/**9**) ppm

LRMS (ES^+) *m/z* = 381 $[M+Na]^+$

HRMS (ES^+) For $C_{19}H_{34}NaO_6^+$ calculated 381.2248, found 381.2257 Da

2.93 - Allyl benzyl malonate



$C_{13}H_{14}O_4$

234.25 $gmol^{-1}$

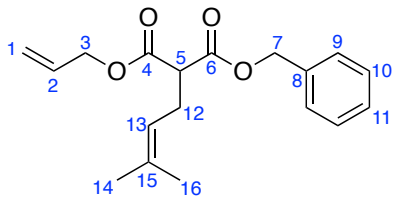
To a solution of acid **2.63** (1.50 g, 10.4 mmol) and benzyl alcohol (1.08 mL, 10.4 mmol) in MeCN (40 mL) under N_2 at rt was added a solution of DCC (2.15 g, 10.4 mmol) in MeCN (10 mL) dropwise over 5 min. After 30 min the white solid was filtered and washed with EtOAc (100 mL). The filtrate was concentrated under reduced pressure to afford an orange oil. Purification by kugelrohr distillation (220 °C, 0.1 mbar) followed by silica gel chromatography (EtOAc:hexane = 4:96) afforded the product as a colourless oil (2.27 g, 9.70 mmol, 93%). Spectroscopic data were consistent with those reported.[145]

FT-IR (neat) ν_{max} 2930 (w), 1732 (s), 1210 (m), 1143 (s) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.41-7.31 (m, 5H, **9**+**10**+**11**), 5.89 (ddt, *J* = 17.2, 10.4, 5.6 Hz, 1H, **2**), 5.33 (dq, *J* = 17.2, 1.3 Hz, 1H, **1**), 5.25 (dq, *J* = 10.4, 1.3 Hz, 1H, **1'**), 5.20 (s, 2H, **7**), 4.65 (dt, *J* = 5.6, 1.3 Hz, 2H, **3**), 3.47 (s, 2H, **5**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 166.2 (C, **4**/**6**), 166.0 (C, **4**/**6**), 135.2 (C, **8**), 131.4 (CH, **2**), 128.5 (CH, **9**/**10**/**11**), 128.4 (CH, **9**/**10**/**11**), 128.3 (CH, **9**/**10**/**11**), 118.8 (CH_2, **1**), 67.2 (CH_2, **7**), 66.1 (CH_2, **3**), 41.5 (CH_2, **5**) ppm

2.94 - 1-Allyl 3-benzyl 2-(3-methylbut-2-en-1-yl)malonate



$C_{18}H_{22}O_4$

302.37 $gmol^{-1}$

By adaption of a procedure by Klahn *et al.*,[140] to a suspension of malonate **2.93** (1.76 g, 7.52 mmol) and K_2CO_3 (2.08 g, 15.0 mmol) in acetone (40 mL) under N_2 at rt was added prenyl bromide (0.65 mL, 5.6 mmol) dropwise over 2 min and the resulting mixture was stirred for 19 h. H_2O (50 mL) was added and the aqueous phase was extracted with EtOAc (3 x 20 mL). The combined organic solution was washed with brine, dried ($MgSO_4$) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 3.5:96.5) afforded the product as a colourless oil (1.40 g, 4.65 mmol, 82%).

FT-IR (neat) ν_{max} 2950 (w), 1731 (s), 1269 (m), 1141 (s) cm^{-1}

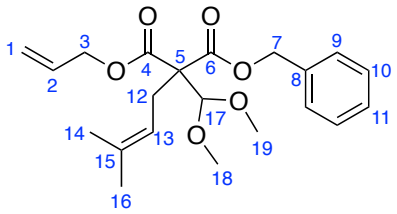
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.39-7.31 (m, 5H, **9**+**10**+**11**), 5.85 (ddt, *J* = 17.2, 10.5, 5.7 Hz, 1H, **2**), 5.29 (dq, *J* = 17.2, 1.5 Hz, 1H, **1**), 5.21 (dq, *J* = 10.5, 1.5 Hz, 1H, **1'**), 5.18 (s, 2H, **7**), 5.09-5.03 (m, 1H, **13**), 4.61 (dt, *J* = 5.7, 1.4 Hz, 2H, **3**), 3.44 (t, *J* = 7.7 Hz, 1H, **5**), 2.63 (t, *J* = 7.5 Hz, 2H, **12**), 1.67 (d, *J* = 1.0 Hz, 3H, **16**), 1.61 (s, 3H, **14**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 169.0 (C, **4**/**6**), 168.7 (C, **4**/**6**), 135.5 (C, **8**), 135.1 (C, **15**), 131.6 (CH, **2**), 128.5 (CH, **10**), 128.3 (CH, **11**), 128.2 (CH, **9**), 119.5 (CH, **13**), 118.5 (CH_2, **1**), 67.0 (CH_2, **7**), 65.9 (CH_2, **3**), 52.2 (CH, **5**), 27.6 (CH_2, **12**), 25.7 (CH_3, **14**/**16**), 17.8 (CH_3, **14**/**16**) ppm

LRMS (ES^+) *m/z* = 325 $[M+Na]^+$

HRMS (ES^+) For $C_{18}H_{22}NaO_4^+$ calculated 325.1410, found 325.1410 Da

2.89 - 1-Allyl 3-benzyl 2-(dimethoxymethyl)-2-(3-methylbut-2-en-1-yl)malonate



$C_{21}H_{28}O_6$

376.45 $gmol^{-1}$

By adaption of a procedure by Evans *et al.*,[141] to a solution of malonate **2.94** (800 mg, 2.65 mmol) and NEt_3 (1.85 mL, 13.3 mmol) in CH_2Cl_2 (13 mL) under N_2 at 0 °C was added $TiCl_4$ (1 M in CH_2Cl_2, 5.30 mL, 5.30 mmol) dropwise over 5 min and the resulting solution stirred for 25 min. Trimethyl orthoformate (1.44 mL, 13.3 mmol) was added dropwise over 2 min and after 5 min the solution was warmed to rt. After 40 min the reaction was quenched with H_2O (10 mL). The organic phase was washed with H_2O (2 x 20 mL), dried (Na_2SO_4) and the solvent removed under reduced pressure to afford an orange oil. Purification by silica gel chromatography (EtOAc:hexane = 9:91) afforded the product as a colourless oil (861 mg, 2.29 mmol, 86%).

FT-IR (neat) ν_{max} 2930 (w), 1733 (s), 1183 (m), 1070 (s) cm^{-1}

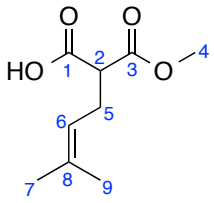
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.33 (s, 5H, **9**+**10**+**11**), 5.80 (ddt, *J* = 17.2, 10.1, 5.8 Hz, 1H, **2**), 5.28 (dd, *J* = 17.2, 1.6 Hz, 1H, **1**), 5.19-13 (m, **1'** + **13**), 4.78 (s, 1H, **17**), 4.60 (ddt, *J* = 13.3, 5.8, 1.2 Hz, 1H, **3**), 4.56 (ddt, *J* = 13.3, 5.8, 1.5 Hz, 1H, **3'**), 3.55 (s, 3H, **18**/**19**), 3.52 (s, 3H, **18**/**19**), 2.78 (d, *J* = 7.3 Hz, 2H, **12**), 1.65 (d, *J* = 1.0 Hz, 3H, **14**), 1.58 (s, 3H, **16**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 168.8 (C, **4**/**6**), 168.6 (C, **4**/**6**), 135.6 (C, **8**), 134.4 (C, **15**), 131.7 (CH, **2**), 128.4 (CH, **9**), 128.1 (CH, **10**), 128.1 (C, **11**), 118.9 (CH, **13**), 118.2 (CH_2, **1**), 107.2 (CH, **17**), 66.8 (CH_2, **7**), 65.7 (CH_2, **3**), 63.3 (C, **5**), 58.6 (CH_3, **18**/**19**), 58.5 (CH_3, **18**/**19**), 29.3 (CH_2, **12**), 26.0 (CH_3, **14**/**16**), 17.7 (CH_3, **14**/**16**) ppm

LRMS (ES^+) *m/z* = 399 $[M+Na]^+$

HRMS (ES^+) For $C_{21}H_{28}NaO_6^+$ calculated 399.1778, found 399.1782 Da

2.103 - 2-(Methoxycarbonyl)-5-methylhex-4-enoic acid



$C_9H_{14}O_4$

186.21 $gmol^{-1}$

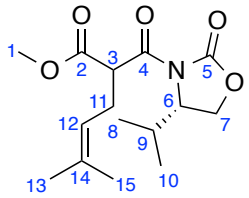
To a solution of the prenyl malonate **2.05** (9.33 g, 42.4 mmol) in MeOH (150 mL) was added KOH (>85%, 2.70 g, 42.40 mmol) and heated at 67 °C for 48 h. The reaction was then cooled to rt and concentrated under reduced pressure. The residue was taken up in H_2O (50 mL) and basified (pH 10) with 1 M NaOH before extracting with EtOAc (3 x 50 mL) to remove trace starting material. The aqueous phase was then acidified (pH 2) with 2 M HCl and extracted with EtOAc (4 x 150 mL). The combined organic solution was then dried ($MgSO_4$) and the solvent removed under reduced pressure to afford the pure product as a colourless oil (7.65 g, 41.1 mmol, 97%). Spectroscopic data were consistent with those reported.[140]

FT-IR (neat) ν_{max} 2956 (m), 2918 (m), 1712 (s), 1437 (m) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.12-5.05 (m, 1H, **6**), 3.77 (s, 3H, **4**), 3.42 (t, *J* = 7.5 Hz, 1H, **2**), 2.64 (apparent t, *J* = 7.3 Hz, 2H, **5**), 1.71 (s, 3H, **7**/**9**), 1.64 (s, 3H, **7**/**9**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 174.1 (C, **1**), 169.8 (C, **3**), 135.7 (C, **8**), 118.9 (CH, **6**), 52.7 (CH, **2**), 51.5 (CH_3, **4**), 27.7 (CH_2, **5**), 25.8 (CH_3, **7**/**9**), 17.7 (CH_3, **7**/**9**) ppm

2.104 - Methyl 2-((*S*)-4-isopropyl-2-oxooxazolidine-3-carbonyl)-5-methylhex-4-enoate



$C_{15}H_{23}NO_5$

297.35 $gmol^{-1}$

To a solution of acid **2.103** (252 mg, 1.19 mmol) and DMF (2 drops) in CH_2Cl_2 (6 mL) under N_2 at 0 °C was added oxalyl chloride (0.12 mL, 1.4 mmol) dropwise and the solution was warmed to rt after 5 min. After 75 min the reaction mixture was concentrated under reduced pressure to afford the crude acid chloride as an orange oil.

To a solution of (*S*)-(−)-4-isopropyl-2-oxazolidinone (136 g, 1.08 mmol) in THF (5 mL) under N_2 at −78 °C was added ^{n}BuLi (2.5 M in hexanes, 0.45 mL, 1.13 mmol) dropwise over 5 min. After 1 h a solution of the crude acid chloride in THF (5 mL) was added dropwise over 10 min. After 30 min the reaction was quenched with saturated aqueous NH_4Cl and warmed to rt. The aqueous phase was extracted with EtOAc (3 x 10 mL) and the combined organic solution was washed with brine, dried ($MgSO_4$) and the solvent removed under reduced pressure to afford the crude product (dr = 1:1 by ^{1}H NMR). Purification by silica gel chromatography (EtOAc:hexane = 1:9) afforded the two diastereoisomers of **2.104** as colourless oils (isomer 1 = 103 mg, isomer 2 = 15 mg, isomeric mixture = 118 mg, total = 236 mg, 0.794 mmol, 74%).

Diastereoisomer 1

FT-IR (neat) ν_{max} 2965 (w), 1775 (s), 1734 (m), 1702 (s), 1371 (s), 1203 (s) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.15-5.08 (m, 1H, **12**), 4.51 (dd, *J* = 9.2, 5.0 Hz, 1H, **3**), 4.46 (dt, *J* = 8.0, 3.5 Hz, 1H, **6**), 4.29 (dd, *J* = 9.1, 8.0 Hz, 1H, **7**), 4.24 (dd, *J* = 9.1, 3.5 Hz, 1H, **7'**), 3.72 (s, 3H, **1**), 2.71 (d, *J* = 8.3 Hz, 1H, **11**), 2.63-2.54 (m, 1H, **11'**), 2.48 (sptd, *J* = 6.8, 3.5 Hz, 1H, **9**), 1.68 (d, *J* = 0.6 Hz, 3H, **15**), 1.65 (s, 3H, **13**), 0.94 (d, *J* = 3.5 Hz, 3H, **8**/**10**), 0.92 (d, *J* = 3.5 Hz, 3H, **8**/**10**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 169.9 (C, **2**), 168.4 (C, **4**), 153.9 (C, **5**), 134.7 (C, **14**), 120.2 (CH, **12**), 63.5 (CH_2, **7**), 58.7 (CH, **6**), 52.3 (CH_3, **1**), 51.1 (CH, **3**), 28.1 (CH, **9**), 27.0 (CH_2, **11**), 25.7 (CH_3, **13**/**15**), 17.9 (CH_3, **13**/**15**), 17.7 (CH_3, **8**/**10**), 14.4 (CH_3, **8**/**10**) ppm

LRMS (ES^+) *m/z* = 298 $[M+H]^+$

HRMS (ES^+) For $C_{15}H_{24}NO_5^+$ calculated 298.1649, found 298.1653 Da

Diastereoisomer 2

FT-IR (neat) ν_{max} 2964 (w), 1776 (s), 1739 (m), 1670 (s), 1372 (s), 1201 (s) cm^{-1}

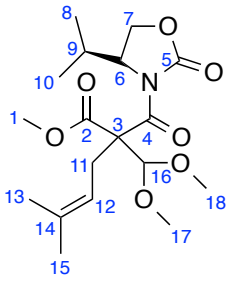
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.12 (ddt, *J* = 8.0, 6.6, 1.4 Hz, 1H, **12**), 4.76 (dd, *J* = 9.1, 5.4 Hz, 1H, **3**), 4.51 (ddd, *J* = 8.4, 3.9, 3.2 Hz, 1H, **6**), 4.31 (t, *J* = 8.8 Hz, 1H, **7**), 4.21 (dd, *J* = 9.2, 3.2 Hz, 1H, **7'**), 3.71 (s, 3H, **1**), 2.79-2.68 (m, 1H, **11**), 2.68-2.57 (m, 1H, **11'**), 2.33 (sptd, *J* = 6.8, 4.0 Hz, 1H, **9**), 1.66 (s, 3H, **13**/**15**), 1.64 (s, 3H, **13**/**15**), 0.92 (d, *J* = 6.8 Hz, 3H, **8**/**10**), 0.86 (d, *J* = 6.8 Hz, 3H, **8**/**10**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 169.7 (C, **2**), 169.0 (C, **4**), 153.9 (C, **5**), 135.0 (C, **14**), 119.8 (CH, **12**), 63.4 (CH_2, **7**), 58.6 (CH, **6**), 52.4 (CH, **1**), 50.4 (CH, **3**), 28.4 (CH, **9**), 27.7 (CH_2, **11**), 25.8 (CH_3, **13**/**15**), 17.8 (CH_3, **13**/**15**), 17.7 (CH_3, **8**/**10**), 14.5 (CH_3, **8**/**10**) ppm

LRMS (ES^+) *m/z* = 298 $[M+H]^+$

HRMS (ES^+) For $C_{15}H_{24}NO_5^+$ calculated 298.1649, found 298.1655 Da

2.105 - Methyl 2-(dimethoxymethyl)-2-((*S*)-4-isopropyl-2-oxooxazolidine-3-carbonyl)-5-methylhex-4-enoate



$C_{18}H_{29}NO_7$

371.43 $gmol^{-1}$

To a solution of malonate **2.104** (84 mg, 0.28 mmol) and NEt_3 (0.12 mL, 0.84 mmol) in CH_2Cl_2 (1.5 mL) under N_2 at −78 °C was added $TiCl_4$ (1 M in CH_2Cl_2, 0.31 mL, 0.31 mmol) dropwise over 5 min. After 30 min trimethyl orthoformate (0.46 mL, 4.2 mmol) was added dropwise over 10 min and the resulting red solution was allowed to warm to rt over 45 min. The reaction was cooled to 0 °C and additional $TiCl_4$ (1 M in CH_2Cl_2, 0.62 mL, 0.62 mmol) was added dropwise before warming to rt. After 40 min the reaction was quenched with H_2O (5 mL). The organic phase was washed with H_2O (2 x 10 mL) before drying ($MgSO_4$) and removing the solvent under reduced pressure to afford the crude product (dr = 2:1 by ^1H NMR). Purification by silica gel chromatography (EtOAc:hexane = 7:93 to 9:91) afforded the product as a thick colourless oil (major isomer = 55 mg, isomeric mixture = 36 mg, total = 91 mg, 0.24 mmol, 87%).

FT-IR (neat) ν_{max} 2962 (w), 1776 (s), 1692 (m), 1204 (m), 1068 (s) cm^{-1}

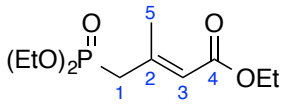
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.06-5.00 (m, 1H, **12**), 4.96 (s, 1H, **16**), 4.52 (ddd, *J* = 8.2, 3.6, 2.8 Hz, 1H, **6**), 4.28 (dd, *J* = 9.1, 8.3 Hz, 1H, **7**), 4.21 (dd, *J* = 8.8, 2.7 Hz, 1H, **7'**), 3.69 (s, 3H, **17**/**18**), 3.62 (s, 3H, **17**/**18**), 3.48 (s, 3H, **1**), 3.05 (dd, *J* = 15.5, 7.7 Hz, 1H, **11**), 2.91 (dd, *J* = 15.4, 6.8 Hz, 1H, **11'**), 2.37 (sptd, *J* = 7.1, 3.7 Hz, 1H, **9**), 1.68 (d, *J* = 1.1 Hz, 3H, **13**), 1.63 (s, 3H, **15**), 0.93 (d, *J* = 7.1 Hz, 3H, **8**/**10**), 0.89 (d, *J* = 6.8 Hz, 3H, **8**/**10**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 169.1 (C, **2**), 168.2 (C, **4**), 153.4 (C, **5**), 134.8 ((C, **14**), 118.4 (CH, **12**), 106.1 (CH, **16**), 64.3 (C, **3**), 63.7 (CH, **7**), 59.6 (CH, **6**), 59.1 (CH_3, **17**/**18**), 57.8 (CH_3, **17**/**18**), 52.1 (CH_3, **1**), 29.5 (CH, **9**), 28.5 (CH_2, **11**), 26.0 (CH_3, **13**/**15**), 18.0 (CH_3, **13**/**15**), 17.9 (CH_3, **8**/**10**), 14.6 (CH_3, **8**/**10**) ppm

LRMS (ES$^+$) *m/z* = 394 $[M+Na]^+$

HRMS (ES$^+$) For $C_{18}H_{29}NNaO_7$ calculated 394.1836, found 394.1839 Da

3.11 - Ethyl (*E*)-4-(diethoxyphosphanyl)-3-methylbut-2-enoate



$C_{11}H_{21}O_4P$

264.11 $gmol^{-1}$

By adaptation of a procedure by Kiddle *et al.*,[146] a solution of ethyl (*E*)-4-bromo-3-methylbut-2-enoate (**4.10**, 1.88 g, 9.05 mmol) and $P(OEt)_3$ (1.69 mL, 9.98 mL) was heated at 160 °C in a pressure vial for 4.5 h. Purification by silica gel chromatography (EtOAc:hexane = 3:1) afforded the product as a colourless oil (2.03 g, 7.69 mmol, 85%). Spectroscopic and physical data were consistent with those reported.[129]

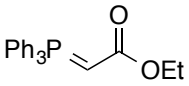
FT-IR (neat) ν_{max} 2982 (w), 1713 (s), 1646 (m), 1292 (s), 1020 (s) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.77 (br d, *J* = 4.6 Hz, **3**), 4.18-4.03 (m, 6H, -OC**H**$_2$CH$_3$), 2.67 (d, J_{HP} = 23.5 Hz, 2H, **1**), 2.29 (d, *J* = 2.0 Hz, 3H, **5**), 1.31 (t, *J* = 7.0 Hz, 6H, -POCH$_2$C**H**$_3$), 1.26 (t, *J* = 7.0 Hz, 3H, -OCH$_2$C**H**$_3$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 166.0 (d, J_{CP} = 3.7 Hz, C, **4**), 149.43 (d, J_{CP} = 11.0, C, **2**), 120.0 (d, J_{CP} = 11.7 Hz, CH, **3**), 62.2 (d, J_{CP} = 6.7 Hz, P-O-**C**H$_2$CH$_3$), 59.7 (-O**C**H$_2$CH$_3$), 38.5 (d, J_{CP} = 135.0 Hz, CH$_2$, **1**), 20.0 (d, J_{CP} = 2.9 Hz, CH$_3$, **5**), 16.3 (d, J_{CP} = 5.9 Hz, P-O-CH$_2$**C**H$_3$), 14.2 (-OCH$_2$**C**H$_3$) ppm

LRMS (EI) m/z = 264 $[M]^+\cdot$ (13%), 218.1 $[M-OEt]^+$ (79%), 190.06 $[M-CO_2Et]^+$ (100%)

4.08 - Ethyl 2-(triphenylphosphaneylidene)acetate



$C_{22}H_{21}O_2P$

348.38 $gmol^{-1}$

By adaption of a procedure by Mondal *et al.*,[147] to a solution of PPh_3 (15.9 g, 60.5 mmol) in EtOAc (50 mL) under N_2 at rt was added ethyl bromoacetate (10.1 g, 59.9 mmol) in EtOAc (50 mL) dropwise over 10 min. After stirring for 18 h the white precipitate was filtered and washed with Et_2O (5x10 mL). The filtrate was concentrated under reduced pressure to afford the crude product as a white solid, which was taken up in CH_2Cl_2 (200 mL). A solution of NaOH (1 M, 200 mL) was added and the mixture stirred vigorously for 45 min. The aqueous phase was extracted with CH_2Cl_2 (3 x 15 mL) and the combined organic solution washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure to afford the product as a white solid (17.1 g, 49.7 mmol, 87%). Spectroscopic and physical data were consistent with those reported.[148]

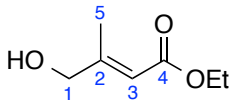
MP 125-127°C [literature 125-127.5 °C (CH_2Cl_2)[148]]

FT-IR (neat) ν_{max} 3056 (w), 1604 (s), 1328 (m) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz) δ = 7.77-7.37 (m, 15H, C**H**$_{Ar}$) 3.40 (q, *J* = 7.1 Hz 2H, -OC**H**$_2$$CH_3$) 2.90 (br s, 1 H, P=C**H**) 1.24 (t, *J* = 7.1 Hz, 3H, OCH_2C**H**$_3$) ppm

LRMS (ES^+) *m/z* = 349 $[M+H]^+$

4.09 - Ethyl (*E*)-4-hydroxy-3-methylbut-2-enoate



$C_7H_{12}O_3$

144.17 $gmol^{-1}$

By adapation of a procedure by Radha Krishna *et al.*,[149] a suspension of ethyl 2-(triphenylphosphanylidene)acetate (**4.08**, 8.065 g, 23.15 mmol) and hydroxyacetone (1.92 mL, 25.3 mmol) in MeCN (50 mL) was heated under reflux for 17 h. After cooling to rt the solvent was removed under reduced pressure and the resulting white solid triturated with Et_2O (100 mL) before cooling in a freezer (–20 °C) for 1 h. The resulting white solid was filtered and discarded and the filtrate concentrated under reduced pressure to afford an orange oil. Purification by silica gel chromatography (EtOAc:hexane = 1:3) afforded the product as a colourless oil (2.33 g, 16.2 mmol, 70%). Spectroscopic and physical data were consistent with those reported.[149]

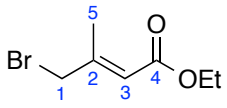
FT-IR (neat) ν_{max} 3410 (s, br), 2982 (w), 1691 (s), 1658 (s), 1284 (s) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.57 (s, 1H, **3**), 4.15 (q, 2H, *J* = 7.1 Hz, -OC**H**$_2$CH$_3$), 4.13-4.09 (m, 2H, **1**), 2.41 (unresolved t, 1H, O**H**), 2.07 (s, 3H, **5**), 1.27 (t, *J* = 7.1 Hz, 3H, O-CH$_2$C**H**$_3$) ppm

^{13}C NMR ($CDCl_3$, 400 MHz): δ = 166.9 (C, **4**), 157.3 (C, **2**), 113.6 (CH, **3**), 66.9 (CH_2, **1**), 59.7 (-O-**C**H$_2$CH$_3$), 15.5 (CH_3, **5**), 14.2 (-OCH$_2$**C**H$_3$) ppm

LRMS (EI) m/z = 98.07 $[M\text{-}H\text{-}OEt]^+$, 144.06 $[M]^{+\cdot}$

4.10 - Ethyl (*E*)-4-bromo-3-methylbut-2-enoate



$C_7H_{11}BrO_2$

207.07 $gmol^{-1}$

Following a procedure by Magoulas *et al.*,[129] to an ice-cold solution of ethyl (*E*)-4-hydroxy-3-methylbut-2-enoate (**4.09**, 2.26 g, 15.7 mmol) and PPh_3 (4.14 g, 15.8 mmol) in MeCN (10 mL) under N_2 was added CBr_4 (5.23 g, 15.8 mmol) and stirred at rt for 4 h. The solvent was removed under reduced pressure and purified by silica gel chromatography (hexane:CH_2Cl_2 = 1:0 to 1:1, to 0:1). The product was afforded as a colourless oil (2.47 g, 11.9 mmol, 76%). Spectroscopic and physical data were consistent with those reported.[150]

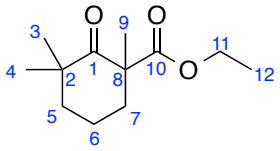
FT-IR (neat) ν_{max} 2981 (w), 1712 (s), 1648 (m), 1281 (s), 620 (m) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.97-5.94 (m, 1H, **3**), 4.17 (q, *J* = 7.1 Hz, 2H, -OC**H**$_2$CH$_3$), 3.94 (d, *J* = 0.7 Hz, 2H, **1**), 2.27 (d, *J* = 1.5 Hz, 3H, **5**), 1.28 (t, *J* = 7.1 Hz, 3H, O-CH$_2$C**H**$_3$) ppm

^{13}C NMR ($CDCl_3$, 400 MHz): δ = 165.9 (C, **4**), 152.3 (C, **2**), 119.5 (CH, **3**), 60.1 (-O-**C**H$_2$-CH$_3$), 38.2 (CH$_2$, **1**), 17.2 (CH$_3$, **5**), 14.2 (-OCH$_2$**C**H$_3$) ppm

LRMS (EI) m/z = 208 [M (^{81}Br)]$^+$· (26%), 206 [M (^{79}Br)]$^+$· (29%), 162.93 [M-OEt (^{81}Br)]$^+$ (94%), 160.91 [M-OEt (^{79}Br)]$^+$ (100%)

4.12 - Ethyl 1,3,3-trimethyl-2-oxocyclohexane-1-carboxylate



$C_{12}H_{20}O_3$

212.29 $gmol^{-1}$

By adaptation of a procedure by Stevans *et al.,*[131] to a stirred, ice-cold suspension of NaH (60% in mineral oil, 7.41 g, 185 mmol) in THF (100 mL) under N_2 was added a solution of ethyl 2-oxocyclohexane-1-carboxylate (6.58 mL, 41.1 mmol) in THF (100 mL) dropwise over 1 h. The mixture was stirred on ice for 1 h before adding iodomethane (10.3 mL, 165 mmol) dropwise over 10 minutes. The mixture was stirred at rt for 18 h with the formation of a white precipitate after 1 h. The mixture was diluted with Et_2O (100 mL) and cooled to 0 °C before quenching with ice-cold H_2O (25 mL). The aqueous phase was extracted with Et_2O (3 x 40 mL) and the combined organic solution washed with brine (30 mL), dried ($MgSO_4$) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 3:97) afforded the product as a colourless oil (6.99 g, 32.9 mmol, 80%). Previous literature syntheses reported no spectroscopic data.

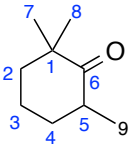
FT-IR (neat) ν_{max} 3011 (m), 1733 (s), 1243 (m) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.23-4.04 (m, 2H, **11**), 2.54 (ddd, *J* = 13.7, 6.2, 3.5 Hz, 1H, $\mathbf{7_{eq}}$), 2.06-1.91 (m, 1H, $\mathbf{5_{ax}}$), 1.77-1.68 (m, 1H, $\mathbf{5_{eq}}$), 1.66-1.53 (m, 2H, **6**), 1.46-1.36 (ddd, *J* = 13.7, 12.3, 4.5 Hz, 1H, $\mathbf{7_{ax}}$), 1.29 (s, 3H, **9**), 1.23 (t, *J* = 7.1 Hz, 3H, **12**), 1.09 (s, 3H, **3**/**4**), 1.07 (s, 3H, **3**/**4**) ppm.

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 211.6 (C, **1**), 172.7 (C, **10**), 61.2 (CH_2, **11**), 55.1 (C, **8**), 46.2 (C, **2**), 40.7 (CH_2, **5**), 36.8 (CH_2, **7**), 26.8 (CH_3, **3/4**), 25.6 (CH_3, **3/4**), 23.6 (CH_3, **9**), 18.5 (CH_2, **6**), 14.0 (CH_3, **12**) ppm

LRMS (EI): *m/z* = 212 $[M]^{+}\cdot$, 167 $[M\text{-}OEt]^{+}$, 139 $[M\text{-}CO_2Et]^{+}$

4.13 - 2,2,6-Trimethylcyclohexan-1-one



$C_9H_{16}O$

140.13 $gmol^{-1}$

A solution of ethyl 1,3,3-trimethyl-2-oxocyclohexane-1-carboxylate (**4.12**, 6.99 g, 32.9 mmol) and conc. HCl (45 mL) in EtOH (30 mL) heated under reflux for 3 days. The mixture was then cooled and diluted with H_2O (20 mL) before adding 1 M NaOH (50 mL). The aqueous layer was extracted with pentane (4 x 15 mL) and the combined organic solution was washed with brine and dried ($MgSO_4$). The solvent was removed under reduced pressure (400 mBar, 20 °C) to afford the pure product as a colourless oil (4.50 g, 32.1 mmol, 98%). Spectroscopic data were consistent with those reported.[151]

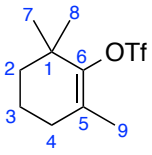
FT-IR (neat) ν_{max} 2967 (w), 2929 (m), 2868 (w), 1704 (s), 1454 (m) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.65 (dquin, *J* = 12.6, 6.4 Hz, 1H, **5$_{ax}$**), 2.05 (dddt, *J* = 13.2, 6.6, 3.7, 2.2 Hz, 1H, **4$_{eq}$**), 1.88 (qd, *J* = 13.4, 3.7 Hz, 1H, **3$_{ax}$**), 1.76 (ddd, *J* = 13.4, 6.6, 2.7, 1H, **2$_{eq}$**), 1.64 (dtt, *J* = 13.7, 3.9, 2.9 Hz, 1H, **3$_{eq}$**), 1.54 (td, *J* = 13.4, 3.9 Hz, 1H, **2$_{ax}$**), 1.31 (qd, *J* = 13.1, 4.0 Hz, 1H, **4$_{ax}$**), 1.18 (s, 3H, **7**/**8**), 1.04 (s, 3H, **7**/**8**), 0.99 (d, *J* = 6.6 Hz, 3H, **9**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 217.3 (C, C**6**), 45.2 (C, C**1**), 41.8 (CH, C**5**), 40.7 (CH_2, C**2**), 36.8 (CH_2, C**4**), 25.6 (CH_3, C**7**/C**8**), 25.3 (CH_3, C**7**/C**8**), 21.5 (CH_2, C**3**), 15.0 (CH_3, C**9**) ppm

LRMS (EI) *m/z* = 140 $[M]^{+\cdot}$ (61%), 82 (100%)

4.14 - 2,6,6-Trimethylcyclohex-1-en-1-yl trifluoromethanesulfonate



$C_{10}H_{15}F_3O_3S$

272.28 $gmol^{-1}$

By adaptation of a procedure by Breining *et al*.,[146] to a solution of LDA (1.91 M, 4.51 mL, 8.57 mmol) in THF (4 mL) under N_2 at −78 °C was added 2,2,6-trimethylcyclohexan-1-one (**4.13**, 1.00 g, 7.14 mmol) in THF (4 mL). The mixture was stirred at −78 °C for 2 h before adding a solution of *N*-phenyl-bis(trifluoromethanesulfonimide) (3.06 g, 8.57 mmol) in THF (4 mL) dropwise. The mixture was allowed to warm naturally to rt over 24 h. The solvent was removed under reduced pressure and the resulting oil poured into vigorously stirred hexane (20 mL). The brown precipitate formed was filtered and the filtrate concentrated. This was repeated again to afford a yellow oil, which was purified by silica gel chromatography (hexane) to afford the product as a colourless oil (1.29 g, 4.72 mmol, 67%). Spectroscopic data were consistent with those reported.[132]

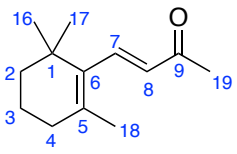
FT-IR (neat) ν_{max} 2940 (w), 1445 (w), 1400 (s), 1242 (s), 1201 (s), 1139 (s), 999 (m), 894 (s), 858 (s), 605 (s) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.16 (t, *J* = 5.9 Hz, 2H, **4**), 1.76 (s, 3H, **9**), 1.58-1.70 (m, 4H, **2**+**3**), 1.17-1.14 (m, 6H, **7**+**8**) ppm

^{19}F NMR ($CDCl_3$, 400 MHz): δ = −73.45 (s, C**F$_3$**) ppm

LRMS (EI) *m/z* = 272.06 $[M]^{+\cdot}$ (12%), 257.01 $[M\text{-}CH_3]^+$ (22%), 69.02 $[CF_3]^{+\cdot}$ (100%)

3.08 - β-Ionone



$C_{13}H_{20}O$

192.30 $gmol^{-1}$

By adaptation of a procedure by Breining *et al.*,[146] a solution of 2,6,6-trimethylcyclohex-1-en-1-yl trifluoromethanesulfonate (**4.14**, 640 mg, 2.39 mmol), NEt_3 (1.0 mL, 7.16 mmol), methyl vinyl ketone (0.39 mL, 4.8 mmol) and $[Pd(PPh_3)_2Cl_2]$ (84 mg, 0.12 mmol) in DMF (8 mL) under N_2 was heated at 75 °C for 18 h. The reaction was quenched with H_2O (20 mL) and the aqueous phase extracted with pentane (5 x 16 mL). The combined organic solution was washed with H_2O (2 x 20 mL) and brine and then dried (Na_2SO_4) before removing the solvent under reduced pressure. Purification by silica gel chromatography (Et_2O:hexane = 3:97) afforded the product as a pale yellow oil (408 mg, 2.12 mmol, 89%). Spectroscopic data were consistent with those reported.[132]

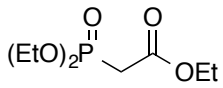
FT-IR (neat) ν_{max} 2929 (m), 2866 (m), 1691 (w), 1668 (s) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.27 (d, *J* = 16.4 Hz, 1H, **7**), 6.11 (d, *J* = 16.4 Hz, 1H, **8**), 2.29 (s, 3H, **19**), 2.07 (t, *J* = 6.3 Hz, 2H, **4**), 1.76 (s, 3H, **18**), 1.59-1.67 (m, 2H, **3**), 1.51-1.46 (m, 2H, **2**), 1.07 (s, 6H, **16**+**17**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 198.7 (C, C**9**), 143.1 (CH, C**7**), 135.9 (C, C**6**), 131.6 (CH, C**8**), 39.7 (CH_2, C**2**), 34.0 (C, C**1**), 33.5 (CH_2, C**4**), 28.8 (CH_3, C**16**+C**17**), 27.1 (CH_3, C**19**), 21.7 (CH_2, C**3**), 18.9 (CH_3, C**18**) ppm

LRMS (ES^+) *m/z* = 193 $[M+H]^+$

4.11 - Triethyl phosphonoacetate



$C_8H_{17}O_5P$

224.19 $gmol^{-1}$

By adaptation of a procedure by Kiddle *et al.*,[146] a solution of ethyl bromoacetate (1.99 mL, 18.0 mmol) and $P(OEt)_3$ (1.98 mL, 18.0 mmol) under N_2 was heated at 130 °C in a microwave for 5 min. The product was afforded as a colourless oil (3.97 g, 1.77 mmol, 98%). Spectroscopic data were consistent with those reported.[152]

FT-IR (neat) ν_{max} 2983 (m), 1773 (s), 1256 (s), 1018 (s), 963 (s) cm^{-1}

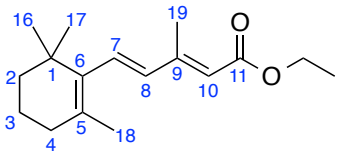
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.17 (dq, J_{HP} = 14.5, J_{HH} = 7.1 Hz, 6H, -OC**H**$_2$C**H**$_3$), 2.95 (d, J = 21.5 Hz, 2H, -PC**H**$_2$), 1.34 (t, J = 7.1 Hz, 6H, -P(O)OCH$_2$C**H**$_3$), 1.28 (t, J = 7.1 Hz, 3H, -C(O)OCH$_2$C**H**$_3$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 165.7 (O=**C**), 62.6 (d, J_{CP} = 6.6 Hz, P-O**C**H$_2$CH$_3$), 61.5 ((O)CO**C**H$_2$CH$_3$), 34.3 (d, J_{CP} = 133.5 Hz, -P**C**H$_2$), 16.3 (d, J_{CP} = 6.4 Hz, P-OCH$_2$**C**H$_3$), 14.1 ((O)COCH$_2$**C**H$_3$) ppm

^{31}P NMR ($CDCl_3$, 162 MHz): δ = 19.82 ppm

LRMS (ES^+) *m/z* = 225 $[M+H]^+$, 247 $[M+Na]^+$

4.18 - Ethyl (2*E*,4*E*)-3-methyl-5-(2,6,6-trimethylcyclohex-1-en-1-yl)penta-2,4-dienoate



$C_{17}H_{26}O_2$

262.39 gmol^{-1}

Following a procedure by Mclean *et al.*,[134] to a suspension of NaH (60% in mineral oil, 456 mg, 11.6 mmol) in Et_2O (7 mL) under N_2 was added a solution of triethyl phosphonoacetate (2.20 mL, 10.1 mmol) in Et_2O (3 mL) dropwise and stirred for 1 h at rt. A solution of β-ionone (**3.08**, 1.00 g, 5.20 mmol) in Et_2O (3 mL) was added dropwise and stirred for 60 h. The reaction was then quenched with H_2O (20 mL) and the aqueous phase was extracted with Et_2O (3 x 10 mL). The combined organic solution was washed with brine, dried ($MgSO_4$) and the solvent removed under reduced pressure. Purification by silica gel chromatography (Et_2O:hexane = 1:99) afforded the product as a pale yellow oil (408 mg, 2.12 mmol, 89%, 9*E*:9*Z* = 9:1). Spectroscopic data were consistent with those reported.[134]

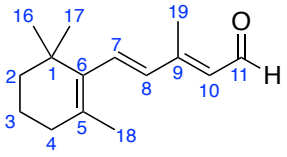
FT-IR (neat) ν_{max} 2957 (m), 2928 (m), 2865 (m), 1709 (s), 1606 (m), 1443 (m), 1233 (m) 1155 (s), 1047 (m) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.56 (d, *J* = 16.1 Hz, 1H, **7**), 6.09 (d, *J* = 16.1 Hz, 1H, **8**), 5.74 (s, 1H, **10**), 4.18 (q, *J* = 7.1 Hz, 2H, -OC**H**$_2$CH$_3$), 2.34 (d, *J* = 1.1 Hz, 3H, **19**), 2.03 (t, *J* = 6.2 Hz, 2H, **4**), 1.70 (s, 3H, **18**), 1.59-1.66 (m, 2H, **3**), 1.51-1.44 (m, 2H, **2**), 1.29 (t, *J* = 7.1 Hz, 3H, -OCH$_2$C**H**$_3$), 1.02 (s, 6H, **16**+**17**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 167.2 (C, C**11**), 152.8 (C, C**9**), 137.1 (C, C**5**), 136.2 (CH, C**8**), 133.6 (CH, C**7**), 131.0 (C, C**6**), 118.0 (CH, C**10**), 59.6 (O**C**H$_2$CH$_3$), 39.5 (CH$_2$, C**2**), 34.2 (C, C**1**), 33.0 (CH$_2$, C**4**), 28.0 (CH$_3$, C**16**+C**17**), 21.6 (CH$_3$, C**18**), 19.1 (CH$_2$, C**3**), 14.4 (CH$_3$, -OCH$_2$**C**H$_3$), 13.6 (CH$_3$, C**19**) ppm

LRMS (ES$^+$) *m/z* = 263 [M+H]$^+$

4.22 - (2*E*,4*E*)-3-Methyl-5-(2,6,6-trimethylcyclohex-1-en-1-yl)penta-2,4-dienal



$C_{15}H_{22}O$

218.34 $gmol^{-1}$

Following a procedure by Mclean *et al.*,[134] to a slurry of $LiAlH_4$ (185 mg, 4.87 mmol) in Et_2O (38 mL) under N_2 was added a solution of ethyl (2*E*,4*E*)-3-methyl-5-(2,6,6-trimethylcyclohex-1-en-1-yl)penta-2,4-dienoate (**4.18**, 1.16 g, 4.43 mmol) in Et_2O (12 mL) dropwise at −78 °C and stirred at −78 °C for 30 min. The mixture was warmed to rt and stirred for a further 2 h. The reaction was quenched with H_2O (3 mL), 1 M NaOH (3 mL) and H_2O (2 mL) sequentially and the white solid was filtered through celite. The filtrate was dried ($MgSO_4$) and concentrated under reduced pressure to furnish a colourless oil, which was re-dissolved in CH_2Cl_2 (40 mL). Crushed molecular sieves (2.00 g), NMO (1.04 g, 8.85 mmol) and TPAP (105 mg, 0.299 mmol) were added and stirred at rt for 30 min. The black mixture was concentrated under reduced pressure and the resulting black oil purified by silica gel chromatography (4:96 = EtOAc:hexane) to afford the product as a yellow oil (725 mg, 3.32 mmol, 75%). Spectroscopic data were consistent were those reported.[134]

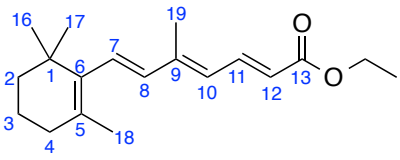
FT-IR (neat) ν_{max} 2931 (m), 2927 (m), 2863 (m), 1661 (s), 1604 (m), 1444 (m) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 10.14 (d, *J* = 8.1 Hz, 1H, **11**), 6.75 (d, *J* = 16.1 Hz, 1H, **7**), 6.22 (d, *J* = 16.1 Hz, 1H **8**), 5.94 (d, *J* = 8.1 Hz, 1H, **10**), 2.32 (s, 3H, **19**), 2.05 (t, *J* = 6.3 Hz, 2H, **4**), 1.73 (s, 3H, **18**), 1.69-1.58 (m, 2H **3**), 1.53-1.43 (m, 2H **2**), 1.05 (s, 6H, **16**+**17**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 191.7 (CH, **11**), 155.4 (C, **9**), 137.4 (C, **6**), 136.0 (CH, **8**), 135.9 (C, **5**), 133.1 (CH, **7**), 129.1 (CH, **10**), 39.8 (CH_2, **2**), 34.6 (C, **1**), 33.6 (CH, **4**), 29.3 (CH_3, **16**+**17**), 22.1 (CH_3, **18**), 19.36 (CH_2, **3**), 13.27 (CH_2, **19**) ppm

LRMS (ES^+) *m/z* = 219 $[M+H]^+$

4.23 - Ethyl (2*E*,4*E*,6*E*)-5-methyl-7-(2,6,6-trimethylcyclohex-1-en-1-yl)hepta-2,4,6-trienoate



$C_{19}H_{28}O_2$

288.43 $gmol^{-1}$

By adaptation of a procedure by Mclean *et al.*,[134] to a suspension of NaH (60% in mineral oil, 49 mg, 1.2 mmol) in Et_2O (2 mL) under N_2 was added a solution of triethyl phosphonoacetate (0.21 mL, 1.0 mmol) in Et_2O (1 mL) dropwise and stirred for 2 h at rt. A solution of (2*E*,4*E*)-3-methyl-5-(2,6,6-trimethylcyclohex-1-en-1-yl)penta-2,4-dienal (**4.22**, 150 mg, 0.690 mmol) in Et_2O (1 mL) was added dropwise and stirred for a further 2 h. The reaction was then quenched with H_2O (3 mL) and the aqueous phase was extracted with Et_2O (3 x 3 mL). The combined organic solution was washed with brine, dried ($MgSO_4$) and the solvent was removed under reduced pressure. Purification by silica gel chromatography (Et_2O:hexane = 3:97) afforded the product as a yellow/green oil (152 mg, 0.527 mmol, 78%). Spectroscopic data were consistent with those reported.[136]

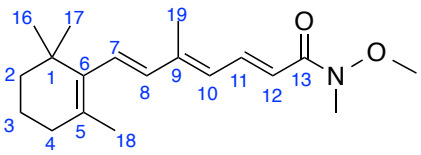
FT-IR (neat) ν_{max} 2927 (m), 2864 (m), 1707 (s), 1619 (m), 1597 (m) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.64 (dd, *J* = 15.0, 12.0 Hz, 1H, **11**), 6.32 (d, *J* = 16.0 Hz, 1H, **7**), 6.12-6.04 (m, 2H, **8**+**10**), 5.80 (d, *J* = 15.0 Hz, 1H, **12**), 4.15 (q, *J* = 7.1 Hz, 2H, -OC**H**$_2$CH$_3$), 1.97 (d, *J* = 0.6 Hz, 3H, **19**), 1.95 (t, *J* = 6.2 Hz, 2H, **4**), 1.64 (d, *J* = 0.6 Hz, 3H, **18**), 1.58-1.51 (m, 2H, **3**), 1.42-1.38 (m, 2H, **2**), 1.24 (t, *J* = 7.1 Hz, 3H, -OCH$_2$C**H**$_3$), 0.96 (s, 6H, **16**+**17**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 167.5 (C, **13**), 144.3 (C, **9**), 140.6 (CH, **10**), 137.5 (C, **6**), 136.7 (CH, **8**), 130.8 (CH, **7**), 130.7 (C, **5**), 127.2 (CH, **10**), 120.0 (CH, **12**), 60.2 (-O**C**H$_2$CH$_3$), 39.6 (CH$_2$, **2**), 34.2 (C, **1**), 33.1 (CH$_2$, **4**), 28.9 (CH$_3$, **16**+**17**), 21.7 (CH, **18**), 19.2 (CH$_2$, **3**), 14.3 (-OCH$_2$**C**H$_3$), 13.0 (CH$_3$, **19**) ppm

LRMS (ES$^+$): *m/z* = 289 [M+H]$^+$

4.24 - (2*E*,4*E*,6*E*)-*N*-Methoxy-*N*,5-dimethyl-7-(2,6,6-trimethylcyclohex-1-en-1-yl)hepta-2,4,6-trienamide



$C_{19}H_{29}NO_2$

303.45 $gmol^{-1}$

By adaptation of a procedure by Grosbeek *et al.*,[136] to a solution of *N*,*O*-dimethylhydroxylamine hydrochloride (302 mg, 3.09 mmol) in THF (7.5 mL) under N_2 at −15 °C was added ^{n}BuLi (2.3 M in hexanes, 2.62 mL, 6.03 mmol) dropwise over 5 min. After stirring the solution at −15 °C for 45 min, a solution of ethyl (2*E*,4*E*,6*E*)-5-methyl-7-(2,6,6-trimethylcyclohexen-1-yl)hepta-2,4,6-trienoate (**4.23**, 223 mg, 0.770 mmol) in THF (5 mL) was added dropwise over 5 min at −15 °C. After 30 min at −15 °C, the reaction was quenched with saturated aqueous NH_4Cl and the aqueous phase was extracted with Et_2O (3 x 8 mL). The combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by silica gel chromatography (gradient from 7-60% EtOAc in hexane) afforded the product as a yellow/green oil (213 mg, 0.702 mmol, 91%). Spectroscopic data were consistent was those reported.[136]

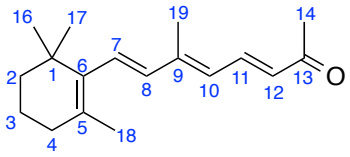
FT-IR (neat) ν_{max} 2956 (w), 2928 (m), 2864 (w), 1649 (s), 1594 (m), 1409 (m), 1375 (s), 1002 (m), 979 (m) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.78 (dd, *J* = 14.9, 11.9 Hz, 1H, **11**), 6.48 (d, *J* = 14.9 Hz, 1H, **12**), 6.37 (d, *J* = 16.1 Hz, 1H, **7**), 6.23 (d, *J* = 11.9 Hz, 1H, **10**), 6.16 (d, *J* = 16.1 Hz, 1H, **8**), 3.72 (s, 3H, -OC**H**$_3$), 3.28 (s, 3H, -NC**H**$_3$), 2.06 (s, 3H, **19**), 2.04 (t, *J* = 7.1 Hz, 2H, **4**), 1.72 (s, 3H, **18**), 1.67-1.59 (m, 2H, **H3**), 1.51-1.45 (m, 2H, **2**), 1.04 (s, 6H, **16**+**17**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 167.7 (C, **13**), 143.7 (C, **9**), 139.5 (CH, **11**), 137.6 (C, **6**), 136.9 (CH, **8**), 130.4 (C, **5**), 130.3 (CH, **7**), 127.9 (CH, **10**), 117.9 (CH, **12**), 61.7 (-O**C**H$_3$), 39.6 (CH_2, **2**), 34.2 (C, **1**), 33.1 (CH_2, **4**), 32.5 (-N**C**H$_3$), 28.9 (CH_3, **16**+**17**), 21.7 (CH_3, **18**), 19.2 (CH_2, **3**), 13.0 (CH_3, **19**) ppm

LRMS (ES^+): *m/z* = 304 $[M+H]^+$

4.25 - (3*E*,5*E*,7*E*)-6-Methyl-8-(2,6,6-trimethylcyclohex-1-en-1-yl)octa-3,5,7-trien-2-one



$C_{18}H_{26}O$

258.40 $gmol^{-1}$

By adaptation of a procedure by Grosbeek *et al.*,[136] to a solution of (2*E*,4*E*,6*E*)-*N*-methoxy-N,5-dimethyl-7-(2,6,6-trimethylcyclohexen-1-yl)hepta-2,4,6-trienamide (**4.24**, 200 mg, 0.660 mmol) in THF (6 mL) under N_2 at −78 °C was added methyl lithium (1.51 M in Et_2O, 0.52 mL, 0.79 mmol) dropwise over 2 min. After 10 min at −78 °C the reaction was quenched by addition of a slurry of silica (700 mg) and H_2O (2 mL) and the suspension was stirred for 15 min. The mixture was dried ($MgSO_4$), the solids filtered and the filtrate concentrated under reduced pressure to afford the product as viscous green oil (150 mg, 0.581 mmol, 89%). Spectroscopic data were consistent with those reported.[136]

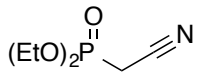
FT-IR (neat) ν_{max} 2927 (m), 2864 (w), 1715 (w), 1659 (s), 1586 (s), 1445 (w), 1359 (m), 1252 (s), 1020 (s) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.58 (dd, *J* = 16.2, 11.8 Hz, 1H, **11**), 6.43 (d, *J* = 16.2 Hz, 1H, **12**), 6.18 (d, *J* = 16.2 Hz, 1H, **7**), 6.17 (d, *J* = 11.8 Hz, 1H, **10**), 6.17 (d, *J* = 16.2 Hz, 1H, **8**), 2.30 (s, 3H, **14**), 2.07 (s, 3H, **19**), 2.04 (br t, *J* = 5.9 Hz, 2H, **4**), 1.72 (s, 3H, **18**), 1.67-1.59 (m, 2H, **3**), 1.50-1.45 (m, 2H, **2**), 1.04 (s, 6H, **16**+**17**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 198.7 (C, **13**), 143.7 (C, **9**), 139.3 (CH, **11**), 137.6 (C, **6**), 136.7 (CH_2, **8**), 131.3 (C, **5**), 131.0 (CH, **7**), 129.3 (CH, **10**), 127.7 (CH, **12**), 39.6 (CH_2, **2**), 34.3 (C, **1**), 33.2 (CH_2, **4**), 28.9 (CH_3, **16**+**17**), 27.7 (CH_3, **14**), 21.7 (CH_3, **18**), 19.2 (CH_2, **3**), 13.1 (CH_3, **19**) ppm

LRMS (ES^+) *m/z* = 259 $[M+H]^+$

4.28 - Diethyl (cyanomethyl)phosphonate



$C_6H_{12}NO_3P$

177.14 $gmol^{-1}$

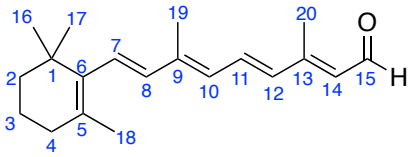
By adaptation of a procedure by Grosbeek *et al.*,[136] to a solution of ^{n}BuLi (2.30 M in hexanes, 5.39 mL, 12.4 mmol) in THF (8 mL) under N_2 at −78 °C was added a solution of HMDS (2.65 mL, 12.6 mmol) in THF (6 mL) dropwise over 20 min. After stirring for 20 min, a solution of MeCN (0.33 mL, 6.3 mmol) in THF (6 mL) was added dropwise over 10 min and the solution was then stirred for a further 30 min. A solution diethyl chlorophosphate (1.00 mL, 6.92 mmol) in THF (6 mL) was added dropwise over 10 min and the resulting mixture stirred for a further 40 min. The mixture was allowed to warm to rt and stirred for 30 min before pouring into a stirred mixture of 2 M HCl (20 mL) and CH_2Cl_2 (30 mL). The aqueous phase was extracted with CH_2Cl_2 (3 x 10 mL) and the combined organic solution washed with H_2O before drying ($MgSO_4$) and removing the solvent under reduced pressure to afford an orange oil. Purification by silica gel chromatography (EtOAc:hexane = 7:3 to 100:0) afforded the product as an orange oil (987 mg, 5.57 mmol, 89%). Spectroscopic data were consistent with those reported.[153]

FT-IR (neat) ν_{max} 2986 (w), 2910 (w), 2256 (w), 1260 (m), 1012 (s) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.30-4.20 (m, 4H, -OC**H$_2$**CH_3), 2.87 (d, J_{HP} = 20.9 Hz, 2H, -C**H$_2$**CN), 1.40 (t, J = 7.0 Hz, 6H, -OCH_2C**H$_3$**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 112.6 (d, J_{CP} = 11.0 Hz, -**C**N), 63.9 (d, J_{CP} = 6.6 Hz, -O**C**H_2CH_3), 16.5 (d, J_{CP} = 5.9 Hz, -OCH_2**C**H_3), 16.3 (d, J_{CP} = 143.8 Hz, -**C**H_2CN) ppm

3.01 - All-*trans*-retinal



$C_{20}H_{28}O$

284.44 $gmol^{-1}$

By adaptation of a procedure by Grosbeek *et al.*,[136] to a solution of diethyl (cyanomethyl)phosphonate (200 mg, 1.13 mmol) in THF (1.5 mL) under N_2 at 0 °C was added ^{n}BuLi (2.31 M in hexanes, 0.44 mL, 1.0 mmol) dropwise over 3 min and then stirred at rt for 2 h. A solution of (3*E*,5*E*,7*E*)-6-methyl-8-(2,6,6-trimethylcyclohex-1-en-1-yl)octa-3,5,7-trien-2-one (**4.25**, 140 mg, 0.542 mmol) in THF (4 mL) was added dropwise and stirred at rt for 19 h. The reaction was quenched with saturated aqueous NH_4Cl and the aqueous phase extracted with EtOAc (3 x 8 mL). The combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure to afford the crude nitrile product as an orange oil.

The orange oil was taken up in CH_2Cl_2 (4 mL) under N_2 and DIBAL (1 M in CH_2Cl_2, 1.90 mL, 1.90 mmol) was added dropwise at −60 °C. After stirring at −60 °C for 1 h, a slurry of silica and H_2O was added and the suspension was stirred for 30 min at rt. The solids were filtered and the filtrate dried twice (Na_2SO_4) before concentrating under reduced pressure to afford an orange oil. Purification by silica gel chromatography (EtOAc:hexane = 4:96, SiO_2 deactivated with NEt_3) afforded the product as an orange oil (77 mg, 0.271 mmol, 50%, 13*E*:13*Z* = 2:1). The isomers were separated by preparative HPLC (EtOAc:pet. ether = 3.5:96.5). Spectroscopic data were consistent with those reported.[153]

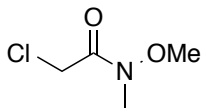
FT-IR (neat) ν_{max} 2928 (w), 1716 (w), 1662 (w), 971 (m) cm^{-1}

^{1}H NMR (C_6D_6, 400 MHz): δ = 10.01 (d, *J* = 7.8 Hz, 1H, **15**), 6.83 (dd, *J* = 15.2, 11.3 Hz, 1H, **11**), 6.37 (d, *J* = 16.1 Hz, 1H, **7**), 6.26 (d, *J* = 16.1 Hz, 1H, **8**), 6.02 (apparent d, *J* = 14.3 Hz, 2H, **10**+**12**), 5.96 (d, *J* = 7.8 Hz, 1H, **14**), 1.96 (t, *J* = 6.1 Hz, 2H, **4**), 1.78 (s, 3H, **18**/**19**), 1.77 (s, 3H, **18**/**19**), 1.73 (s, 3H, **20**), 1.62-1.55 (m, 2H, **2**), 1.49-1.45 (m, 2H, **3**), 1.12 (s, 6H, **16**+**17**) ppm

^{13}C NMR (C_6D_6, 101 MHz): δ = 190.2 (CH, **15**), 153.5 (C, **13**), 140.5 (C, **9**), 138.4 (C, **6**), 138.3 (CH, **8**), 135.9 (CH, **12**), 131.9 (CH, **11**), 130.7 (C, **5**), 130.7 (CH, **10**), 130.1 (CH, **7**), 129.7 (CH, **14**), 40.2 (CH_2, **2**), 34.9 (C, **1**), 33.7 (CH_2, **4**), 29.5 (CH_3, **16**+**17**), 22.3 (CH_3, **18**), 20.0 (CH_2, **3**), 13.1 (CH_3, **19**), 12.8 (CH_3, **20**) ppm

LRMS (ES$^+$) *m/z* = 285 $[M+H]^+$

4.31 - 2-Chloro-*N*-methoxy-*N*-methylacetamide



$C_4H_8ClNO_2$

137.56 gmol^{-1}

Following a procedure by Pace *et al.*,[154] to a solution of K_2CO_3 (6.97 g, 50.5 mmol) in H_2O (35 mL) at 0 °C was added *N*,*O*-dimethylhydroxylamine hydrochloride (2.71 g, 27.8 mmol) and *t*-BME (35 mL) sequentially. The biphasic mixture was then cooled to −15 °C and 2-chloroacetyl chloride (2.01 mL, 25.2 mmol) was added dropwise. The mixture was stirred at 0 °C for 40 min. The aqueous phase was extracted with Et_2O (3 x 30 mL) and the combined organic solution washed with brine and dried (Na_2SO_4). The solvent was removed under reduced pressure to afford the product as a white solid (3.33 g, 24.2 mmol, 96%). Spectroscopic data was consistent with those reported.[154]

M.P. 49-50 °C [literature 49-51 °C (Et_2O)[154]]

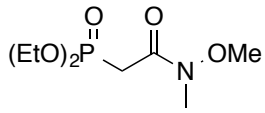
FT-IR (neat) ν_{max} 1669 (s), 1468 (m), 1444 (m), 1405 (m), 1182 (m) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.24 (s, 2H, Cl-C**H**$_2$), 3.75 (s, 3H, -OC**H**$_3$), 3.23 (s, 3H, -NC**H**$_3$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 167.5 (O=**C**), 61.6 (-OC**H**$_3$), 40.8 (Cl-**C**H$_2$), 32.6 (N**C**H$_3$) ppm

LRMS (ES$^+$) *m/z* = 138 and 140 $[M+H]^+$

4.32 - Diethyl (2-(methoxy(methyl)amino)-2-oxoethyl)phosphonate



$C_8H_{18}NO_5P$

239.21 $gmol^{-1}$

A solution of 2-chloro-*N*-methoxy-*N*-methyl-acetamide (2.21 g, 16.1 mmol) and triethylphosphite (2.80 mL, 16.8 mmol) under N_2 was heated at 120 °C for 20 h in a sealed vial. Purification by silica gel chromatography (MeOH:EtOAc = 1:9) afforded the product as a brown oil (3.65 g, 15.3 mmol, 95%). Spectroscopic data were consistent with those reported.[155]

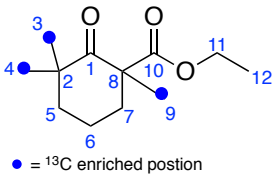
FT-IR (neat) ν_{max} 2982 (w), 1657 (s), 1380 (m), 1252 (s), 1019 (s) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.24-4.12 (m, 4H, P-OC**H**$_2$), 3.77 (s, 3H, -OC**H**$_3$), 3.21 (s, 3H, -NC**H**$_3$), 3.16 (d, *J* = 22.1 Hz, 2H, -PC**H**$_2$), 1.34 (t, *J* = 7.1 Hz, 6H, -OCH$_2$C**H**$_3$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 166.1 (O=**C**), 62.5 (d, J_{CP} = 6.6 Hz, -P-O**C**H$_2$), 61.5 (-O**C**H$_3$), 32.1 (-N**C**H$_3$), 30.7 (d, J_{CP} = 135.7 Hz, -P**C**H$_2$), 16.3 (d, J_{CP} = 6.6 Hz, -OCH$_2$**C**H$_3$) ppm

LRMS (ES$^+$) *m/z* = 240 $[M+H]^+$, 262 $[M+Na]^+$

4.34 - Ethyl 1,3,3-tri(methyl-^{13}C)-2-oxocyclohexane-1-carboxylate



$C_9{}^{13}C_3H_{20}O_3$

215.27 gmol^{-1}

To a suspension of NaH (60% in mineral oil, 5.60 g, 140 mmol) in THF (80 mL) under N_2 at 0 °C was added a solution of ethyl 2-oxocyclohexane-1-carboxylate (5.91 mL, 34.5 mmol) in THF (80 mL) dropwise over 90 min. The mixture was stirred on ice for 30 min before addition of $^{13}CH_3I$ (6.47 mL, 104 mmol) dropwise over 20 min. The mixture was stirred at rt for 36 h with the formation of a white precipitate after 1 h. The mixture was diluted with Et_2O (100 mL) and cooled to 0 °C before quenching with ice-cold H_2O (25 mL). The aqueous layer was extracted with Et_2O (3 x 30 mL) and the combined organic solution was washed with brine (30 mL), dried ($MgSO_4$) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 2:98) afforded the product as a colourless oil (6.01 g, 27.9 mmol, 81%).

FT-IR (neat) ν_{max} 2939 (m), 1732 (m), 1703 (s), 1423 (m), 1074 (m) cm^{-1}

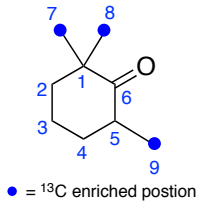
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.23-4.04 (m, 2H, **11**), 2.58-2.50 (m, 1H, **7$_{eq}$**), 2.06-1.91 (m, 1H, **7$_{ax}$**), 1.76-1.68 (m, 1H, **5$_{eq}$**), 1.65-1.49 (m, 2H, **5$_{ax}$**), 1.45-1.35 (m, 2H, **6**), 1.29 (d, J_{CH} = 131.0 Hz, 3H, **9**), 1.23 (t, J_{HH} = 7.1 Hz, 3H, **12**), 1.09 (dd, J_{CH} = 128.0, J_{CH} = 5.0 Hz, 3H, **3**/**4**), 1.07 (dd, J_{CH} = 127.7, J_{CH} = 5.0 Hz, 3H, **3**/**4**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 26.8 ($^{13}CH_3$, **3/4**), 25.6 ($^{13}CH_3$, **3/4**), 23.6 ($^{13}CH_3$, **9**) ppm (Only peaks for ^{13}C labelled carbons reported)

LRMS (ES$^+$) *m/z* = 216 [M+H]$^+$, 238 [M+Na]$^+$

HRMS (ES$^+$) For $C_9{}^{13}C_3H_{20}O_3Na^+$ calculated 238.1405, found 238.1402 Da

4.01 - 2,2,6-Tri(methyl-^{13}C)cyclohexan-1-one



$C_6{}^{13}C_3H_{16}O$

143.20 gmol^{-1}

A solution of ethyl 1,3,3-tri(methyl-^{13}C)-2-oxocyclohexane-1-carboxylate (**4.01**, 5.48 g, 25.5 mmol) and conc. HCl (40 mL) in EtOH (80 mL) was refluxed for 3 days. The mixture was then cooled and diluted with H_2O (20 mL) and neutralised with 1 M NaOH (170 mL). The aqueous layer was extracted with pentane (4 x 50 mL) and the combined organic solution washed with brine and dried ($MgSO_4$). The solvent was removed under reduced pressure (400 mBar, 20 °C) to afford the product as a colourless oil (3.64 g, 32.1 mmol, 100%).

FT-IR (neat) ν_{max} 2967 (w), 2929 (m), 2868 (w), 1704 (s), 1454 (m) cm^{-1}

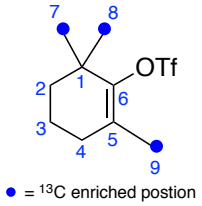
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.72-2.59 (m, 1H, **5**), 2.10-2.00 (m, 1H, $\mathbf{4_{eq}}$), 1.88 (qt, J_{HH} = 13.4, J_{HH} = 3.8 Hz, 1H, $\mathbf{3_{ax}}$), 1.81-1.72 (m, 1H, $\mathbf{2_{eq}}$), 1.69-1.61 (m, 1H, $\mathbf{3_{eq}}$), 1.60-1.49 (m, 1H, $\mathbf{2_{ax}}$), 1.38-1.22 (m, 1H, $\mathbf{4_{ax}}$), 1.18 (dd, J_{CH} = 127.0, J_{CH} = 4.9 Hz, 3H, **7/8**), 1.04 (dd, J_{CH} = 126.4, J_{CH} = 4.9 Hz, 3H, **7/8**), 0.99 (dd, J_{CH} = 126.8, J_{HH} = 6.4 Hz, 3H, **9**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 25.6 ($^{13}CH_3$, **7/8**), 25.3 ($^{13}CH_3$, **7/8**), 15.0 ($^{13}CH_3$, **9**) ppm (Only peaks for ^{13}C labelled carbons reported)

LRMS (EI) *m/z* = 143 $[M]^{+\cdot}$ (33%), 84 (100%)

HRMS (EI) For $C_6{}^{13}C_3H_{16}O^{+\cdot}$ calculated 143.13018, found 143.12999 Da

4.20 - 2,6,6-Tri(methyl-^{13}C)cyclohex-1-en-1-yl trifluoromethanesulfonate



$C_7{}^{13}C_3H_{15}F_3O_3S$

275.26 gmol^{-1}

To a solution of LDA (21.3 mL, 40.50 mmol) in THF (35 mL) under N_2 at −78 °C was added 2,2,6-tri-(methyl-^{13}C)cyclohexan-1-one (**4.01**, 3.62 g, 25.3 mmol) in THF (35 mL) dropwise over 45 min. The mixture was stirred at −78 °C for 2 h before adding a solution of *N*-phenyl-bis(trifluoromethanesulfonimide) (9.02 g, 25.3 mmol) in THF (40 mL) dropwise. The mixture was allowed to warm to rt over 24 h. The reaction was quenched with NH_4Cl (80 mL) and the aqueous phase extracted with pentane (3 x 40 mL). The combined organic solution was washed with brine, dried ($MgSO_4$) and the solvent removed under reduced pressure (200 mbar, 23 °C) to furnish an orange oil. Purification by silica gel chromatography (pentane) afforded the product as a colourless oil (3.47 g, 12.6 mmol, 50%).

FT-IR (neat) ν_{max} 2940 (w), 1458 (w), 1400 (s), 1243 (s), 1201 (s), 1139 (s), cm^{-1}

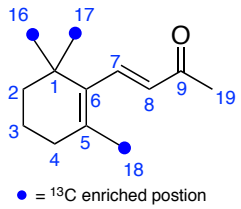
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.16 (t, J_{HH} = 5.9 Hz, 2H, **4**), 1.71-1.58 (m, 4H, **2+3**), 1.76 (d, J_{CH} = 128.0 Hz, 3H, **9**), 1.16 (dd, J_{CH} = 126.9, J_{CH} = 4.7 Hz, 6H, **7+8**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 26.4 ($^{13}CH_3$, **7+8**), 17.6 ($^{13}CH_3$, **9**) ppm (Only peaks for ^{13}C labelled carbons reported)

LRMS (EI) *m/z* = 275 $[M]^{+\cdot}$ (27%), 259 $[M\text{-}CH_3]^+$ (33%), 69 $[CF_3]^{+\cdot}$ (100%)

HRMS (EI) For $C_7{}^{13}C_3H_{15}F_3O_3S^{+\cdot}$ calculated 275.07947, found 275.07902 Da

4.19 - (*E*)-4-(2,6,6-Tri(methyl-^{13}C)cyclohex-1-en-1-yl)but-3-en-2-one



$C_{10}{}^{13}C_3H_{20}O$

195.28 gmol^{-1}

A solution of 2,6,6-tri(methyl-^{13}C)cyclohex-1-en-1-yl trifluoromethanesulfonate (**4.20**, 3.33 g, 12.1 mmol), NEt_3 (6.75 mL, 48.4 mmol), methyl vinyl ketone (1.97 mL, 48.4 mmol) and $[Pd(PPh_3)_2Cl_2]$ (425 mg, 0.610 mmol) in DMF (60 mL) under N_2 was heated at 75 °C for 18 h. The reaction was quenched with H_2O (50 mL) and the aqueous phase extracted with pentane (4 x 50 mL). The combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by silica gel chromatography (Et_2O:hexane = 3:97) afforded the product as a pale yellow oil (1.58 mg, 8.08 mmol, 67%).

FT-IR (neat) ν_{max} 2930 (m), 2863 (m), 1692 (w), 1670 (s) cm^{-1}

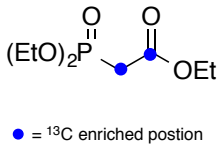
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.28 (br d, J_{HH} = 16.5 Hz, 1H, **7**), 6.12 (d, J_{HH} = 16.5 Hz, 1H, **8**), 2.30 (s, 3H, **19**), 2.12-2.05 (unresolved t, 2H, **4**), 1.77 (d, J_{CH} = 126.1 Hz, 3H, **18**), 1.67-1.62 (m, 2H, **3**), 1.53-1.46 (m, 2H, **2**), 1.08 (dd, J_{CH} = 125.4, J_{CH} = 4.9 Hz, 6H, **16+17**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): 28.7 ($^{13}CH_3$, **16**+**17**), 21.7 ($^{13}CH_3$, **18**) ppm
(Only peaks for ^{13}C labelled carbons reported)

LRMS (ES$^+$) *m/z* = 196 $[M+H]^+$

HRMS (ES$^+$) For $C_{10}{}^{13}C_3H_{21}O^+$ calculated 196.1688, found 196.1692 Da

4.03 - Triethylphosphonoacetate-$^{13}C_2$



$C_6{}^{13}C_2H_{17}O_5P$

226.18 $gmol^{-1}$

A solution of 2-^{13}C-ethyl bromoacetate (2.15 g, 12.7 mmol) and $P(OEt)_3$ (2.05 g, 12.4 mmol) under N_2 was heated at 130 °C in a microwave for 10 min. Purification by silica gel chromatography (EtOAc:hexane = 85:15) afforded the product as a colourless oil (2.62g, 11.60 mmol, 94%). ^{1}H and ^{13}C NMR spectroscopic data were consistent with those reported.[156]

FT-IR (neat) ν_{max} 2980 (m), 1692 (s), 1252 (s), 1020 (s), 963 (s) cm^{-1}

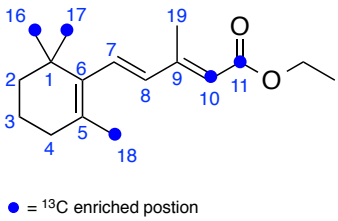
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.26-4.09 (m, 6H, -OC**H$_2$**CH_3), 2.96 (ddd, J_{CH} = 129.9, J_{HP} = 21.5, J_{HH} = 7.3 Hz, 2H, P-^{13}C**H$_2$**), 1.36 (t, J_{HH} = 7.0 Hz, 6H, -$POCH_2$C**H$_3$**), 1.29 (t, J_{HH} = 7.2 Hz, 3H, (O)^{13}$COCH_2$C**H$_3$**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 165.7 (d, J_{CC} = 58.7, J_{CP} = 5.9 Hz, ^{13}C=O), 34.3 (d, J_{CP} = 134.3, J_{CC} = 59.4 Hz, P-^{13}**C**H_2) ppm (Only peaks for ^{13}C labelled carbons reported)

^{31}P NMR ($CDCl_3$, 162 MHz): δ = 19.83 ppm (dd, J_{CP} = 133.1, J_{CP} = 6.0 Hz) ppm

LRMS (ES^+) *m/z* = 227 $[M+H]^+$

4.36 - Ethyl (2*E*,4*E*)-3-methyl-5-(2,6,6-tri(methyl-^{13}C)cyclohex-1-en-1-yl)penta-2,4-dienoate-1,2-$^{13}C_2$



● = ^{13}C enriched postion

$C_{12}{}^{13}C_5H_{26}O_2$

267.35gmol^{-1}

To a suspension of NaH (60% in mineral oil, 630 mg, 15.7 mmol) in Et_2O (12 mL) under N_2 at 0 °C was added a solution of triethylphosphonoacetate-$^{13}C_2$ (**4.03**, 3.51 mL, 15.5 mmol) in Et_2O (8 mL) dropwise and stirred for 2 h at rt. A solution of (*E*)-4-(2,6,6-tri(methyl-^{13}C)cyclohex-1-en-1-yl)but-3-en-2-one (**4.19**, 1.54 g, 7.87 mmol) in Et_2O (8 mL) was added dropwise and stirred for 96 h. The reaction was quenched with H_2O (10 mL) and the aqueous phase was extracted with Et_2O (3 x 10 mL). The combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by silica gel chromatography (Et_2O:hexane = 0.5:99.5) afforded the product as a pale yellow oil and a mixture of inseparable isomers (1.65 g, 6.15 mmol, 78%, 9*E*:9*Z* = 9:1).

FT-IR (neat) ν_{max} 2957 (m), 2928 (m), 2865 (m), 1709 (s), 1606 (m), 1443 (m), 1233 (m) 1155 (s), 1047 (m), 967 (m), 875 (w) cm^{-1}

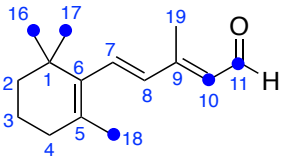
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.64 (dd, J_{HH} = 16.4, J_{HH} = 2.3 Hz, 1H, **8*Z***), 6.56 (d, J_{HH} = 16.1 Hz, 1H, **7*E***), 6.10 (dd, J_{HH} = 16.1, J_{CH} = 5.3 Hz, 1H, **8*E***), 5.74 (d, J_{CH} = 159.3 Hz, 1H, **10*E***), 5.65 (d, J_{CH} = 160.3 Hz, 1H, **10*Z***), 4.18 (qd, J_{HH} = 7.1, J_{CH} = 3.1 Hz, 2H, -O-C**H**$_2$), 2.34 (d, J_{CH} = 4.6 Hz, 3H, **19**), 2.07-2.00 (m, 2H, **4**), 1.70 (d, J_{CH} = 125.8 Hz, 3H, **18**), 1.66-1.58 (m, 2H, **3**), 1.52-1.43 (m, 2H, **2**), 1.29 (t, J_{HH} = 7.1 Hz, 3H, -OCH$_2$C**H**$_3$), 1.07 (dd, J_{CH} = 125.3, J_{CH} = 4.8 Hz, 6H, **17*Z*+18*Z***), 1.02 (dd, J_{CH} = 125.3, J_{CH} = 4.8 Hz, 6H, **17*E*+18*E***) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 167.3 (d, J_{CC} = 77.0 Hz, ^{13}C, **11*E***), 166.5 (d, J_{CC} = 77.0 Hz, ^{13}C, **11*Z***), 118.0 (d, J_{CC} = 77.0 Hz, ^{13}CH, **10*E***), 116.2 (d, J_{CC} = 77.0, ^{13}CH, **10*Z***), 28.9 ($^{13}CH_3$, **16*Z*+17*Z***), 28.9 ($^{13}CH_3$, **16*E*+17*E***), 21.75 ($^{13}CH_3$, **18*Z***), 21.6 ($^{13}CH_3$, **18*E***) ppm (Only peaks for ^{13}C labelled carbons reported)

LRMS (ES$^+$) *m/z* = 262 [M+H]$^+$

HRMS (ES$^+$) For $C_{12}{}^{13}C_5H_{26}NaO_2^+$ calculated 290.1993, found 290.2000

4.37 - (2*E*,4*E*)-3-Methyl-5-(2,6,6-tri(methyl-^{13}C)cyclohex-1-en-1-yl)penta-2,4-dienal-1,2-$^{13}C_2$



$C_{10}{}^{13}C_5H_{22}O$

223.30 gmol^{-1}

To a $LiAlH_4$ (1 M in THF, 7.15 mL, 7.15 mmol) in Et_2O (15 mL) under N_2 at −78 °C was added a solution of ethyl (2*E*,4*E*)-3-methyl-5-(2,6,6-tri(methyl-^{13}C)cyclohex-1-en-1-yl)penta-2,4-dienoate-1,2-$^{13}C_2$ (**4.36**, 1.59 g, 5.95 mmol) in Et_2O (30 mL) dropwise and stirred at −78 °C for 30 min. The mixture was then warmed to rt and stirred for 20 min. The reaction was quenched with H_2O (6 mL), 1 M NaOH (2 mL) and H_2O (2 mL) sequentially and the white solid was filtered through celite. The filtrate was dried ($MgSO_4$) and the solvent removed under reduced pressure to afford a colourless oil which was re-dissolved in CH_2Cl_2 (40 mL). Crushed molecular sieves (2.00 g), NMO (1.40 g, 11.9 mmol) and TPAP (105 mg, 2.98 mmol) was added and stirred at rt for 30 min. The black mixture was concentrated and the resulting black oil purified by silica gel chromatography (EtOAc:hexane = 4:96) to afford the product as a yellow oil (1.16 g, 5.17 mmol, 87%).

FT-IR (neat) ν_{max} 2928 (m), 2861 (m), 1627 (s), 1601 (s), 1442 (m) cm^{-1}

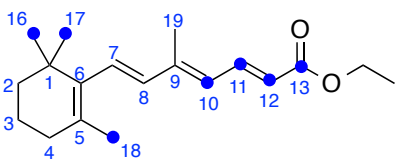
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 10.13 (ddd, J_{CH} = 169.7, J_{CH} = 24.6, J_{HH} = 8.1 Hz, 1H, **11**), 6.74 (d, J_{HH} = 16.1 Hz, 1H, **7**), 6.22 (dd, J_{HH} = 16.1, J_{CH} = 4.9 Hz, 1H, **8**), 5.94 (dd, J_{CH} = 157.5, J_{HH} = 8.1 Hz, 1H, **10**), 2.32 (d, J_{CH} = 4.0 Hz, 3H, **19**), 2.09-2.02 (m, 2H, **4**), 1.73 (d, J_{CH} = 125.8 Hz, 3H, **18**), 1.68-1.59 (m, 2H, **3**), 1.53-1.45 (m, 2H, **2**), 1.05 (dd, J_{CH} = 125.3, J_{CH} = 4.9 Hz, 6H, **16+17**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 191.3 (d, *J* = 77.0 Hz, ^{13}C, **11**), 128.7 (d, *J* = 77.0 Hz, ^{13}CH, **10**), 28.9 ($^{13}CH_3$, **16+17**), 21.7 ($^{13}CH_3$, **18**) ppm (Only peaks for ^{13}C labelled carbons reported)

LRMS (ES^+) *m/z* = 224 $[M+H]^+$

HRMS (ES^+) For $C_{10}{}^{13}C_5H_{22}NaO^+$ calculated 246.1731, found 246.1739 Da

4.38 – Ethyl (2*E*,4*E*,6*E*)-5-methyl-7-(2,6,6-tri(methyl-^{13}C)cyclohex-1-en-1-yl)hepta-2,4,6-trienoate-1,2,3,4-$^{13}C_4$



● = ^{13}C enriched postion

$C_{12}{}^{13}C_7H_{28}O_2$

295.38 $gmol^{-1}$

To a suspension of NaH (60% in mineral oil, 354 mg, 8.86 mmol) in Et_2O (5 mL) under N_2 at 0 °C was added a solution of $^{13}C_2$-triethylphosphonoacetate (2.01 g, 8.86 mmol) in Et_2O (5 mL) dropwise and the suspension was stirred for 1 h at rt. A solution of (2*E*,4*E*)-3-methyl-5-(2,6,6-tri(methyl-^{13}C)cyclohex-1-en-1-yl)penta-2,4-dienal-1,2-$^{13}C_2$ (**4.37**, 1.03 g, 4.65 mmol) in Et_2O (5 mL) was added dropwise and stirred at rt for 18 h. The reaction was then quenched with H_2O (6 mL) and the aqueous phase was extracted with Et_2O (3 x 6 mL). The combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 2:97) afforded the product as a yellow/green oil (1.24 g, 4.19 mmol, 90%).

FT-IR (neat) ν_{max} 2927 (m), 2864 (m), 2361 (m), 1707 (s), 1619 (m), 1597 (m) cm^{-1}

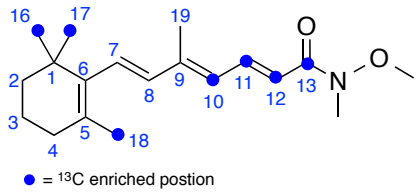
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.92-7.52 (m, 1H, **11**), 6.40 (d, J_{HH} = 16.2 Hz, 1H, **7**), 6.16 (br dd, J_{HH} = 16.2, J_{CH} = 5.3 Hz, 1H, **8**), 6.15 (dd, J_{CH} = 153.9, J_{HH} = 12.5 Hz, 1H, **10**), 5.87 (dddd, J_{CH} = 162.3, J_{HH} = 15.0, J_{CH} = 7.0, J_{CH} = 2.9 Hz, 1H, **12**), 4.22 (qd, J_{HH} = 7.1, J_{CH} = 3.1 Hz, 2H, -OC**H**$_2$), 2.05 (d, J_{CH} = 4.9 Hz, 3H, **19**), 2.03 (t, J_{HH} = 6.2 Hz, 2H, **4**), 1.72 (d, J_{CH} = 125.7 Hz, 3H, **18**), 1.66-1.57 (m, 2H, **3**), 1.50-1.45 (m, 2H, **2**), 1.31 (t, J_{HH} = 7.1 Hz, 3H, -OCH_2C**H**$_3$), 1.03 (dd, J_{CH} = 125.3, J_{CH} = 4.8 Hz, 6H, **16+17**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 167.5 (ddd, J_{CC} = 76.5, J_{CC} = 7.9, J_{CC} = 1.5 Hz, ^{13}C, **13**), 140.6 (ddd, J_{CC} = 70.0, J_{CC} = 57.6, J_{CC} = 1.5 Hz, ^{13}CH, **11**), 127.2 (ddd, J_{CC} = 57.6, J_{CC} = 8.1, J_{CC} = 1.0 Hz, ^{13}CH, **10**), 120.0 (ddd, J_{CC} = 76.5, J_{CC} = 70.0, J_{CC} = 1.5 Hz, ^{13}CH, **12**), 28.9 ($^{13}CH_3$, **16+17**), 21.7 ($^{13}CH_3$, **18**) ppm (Only peaks for ^{13}C labelled carbons reported)

LRMS (ES^+): *m/z* = 296 $[M+H]^+$

HRMS (ES^+) For $C_{12}{}^{13}C_7H_{28}O_2Na^+$ calculated 318.2216, found 318.2218 Da

4.39 - (2*E*,4*E*,6*E*)-*N*-Methoxy-*N*,5-dimethyl-7-(2,6,6-tri(methyl-^{13}C)cyclohex-1-en-1-yl)hepta-2,4,6-trienamide-1,2,3,4-$^{13}C_4$



$C_{12}{}^{13}C_7H_{29}NO_2$

310.39 gmol^{-1}

To a solution of *N*,*O*-dimethylhydroxylamine hydrochloride (1.83 g, 18.7 mmol) in THF (30 mL) under N_2 at −15 °C was added ^{n}BuLi (2.27 M in hexanes, 16.1 mL, 36.5 mmol) dropwise over 15 min. After stirring at −15 °C for 1 h a solution of ethyl (2*E*,4*E*,6*E*)-5-methyl-7-(2,6,6-trimethylcyclohex-1-en-1-yl)hepta-2,4,6-trienoate-2-^{13}C (**4.38**, 1.23 g, 4.25 mmol) in THF (20 mL) was added dropwise over 15 min at −15 °C. After 30 min at −15 °C, the reaction was quenched with saturated aqueous NH_4Cl and the aqueous phase was extracted with Et_2O (3 x 10 mL). The combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 15:85) afforded the product as a yellow/green oil (1.13 g, 3.72 mmol, 88%).

FT-IR (neat) ν_{max} 2932 (m), 2861 (w), 1603 (s), 1537 (s), 1364 (m) cm^{-1}

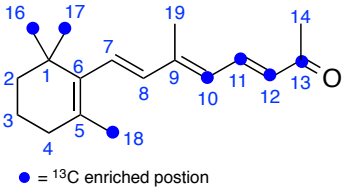
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.02-7.52 (m, 1H, **11**), 6.47 (dddd, J_{CH} = 161.6, J_{HH} = 14.9, J_{CH} = 7.0, J_{CH} = 3.7 Hz, 1H, **12**), 6.37 (br d, J_{HH} = 16.5 Hz, 1H, **7**), 6.22 (br dd, J_{CH} = 152.7, J_{HH} = 12.0 Hz, 1H, **10**), 6.16 (dd, J_{HH} = 16.5, J_{CH} = 5.4 Hz, 1H, **8**), 3.72 (s, 3H, -OC**H**$_3$), 3.27 (d, J_{CH} = 2.0 Hz, 3H, -NC**H**$_3$), 2.08-2.00 (m, 5H, **4**+**19**), 1.72 (d, J_{CH} = 125.6 Hz, 3H, **18**), 1.66-1.58 (m, 1H, **3**), 1.53-1.42 (m, 2H. **2**), 1.03 (dd, J_{CH} = 125.3, J_{CH} = 5.1 Hz, 6H, **16+17**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 167.6 (dd, J_{CC} = 71.2, J_{CC} = 7.0 Hz, ^{13}C, **13**), 139.5 (ddd, J_{CC} = 71.2, J_{CC} = 58.0, J_{CC} = 1.5 Hz, ^{13}CH, **11**), 127.9 (ddd, J_{CC} = 71.2, J_{CC} = 69.0, J_{CC} = 2.2 Hz, ^{13}CH, **10**), 117.8 (ddd, J_{CC} = 71.2, J_{CC} = 69.0, J_{CC} = 2.2 Hz, ^{13}CH, **12**), 28.9 ($^{13}CH_3$, **16**+**17**), 21.7 ($^{13}CH_3$, **18**) ppm (Only peaks for ^{13}C labelled carbons reported)

LRMS (ES$^+$): *m/z* = 311 [M+H]$^+$

HRMS (ES$^+$) For $C_{12}{}^{13}C_7H_{30}NO_2^+$ calculated 311.2506, found 311.2513 Da

4.40 - (3*E*,5*E*,7*E*)-6-Methyl-8-(2,6,6-tri(methyl-^{13}C)cyclohex-1-en-1-yl)octa-3,5,7-trien-2-one-2,3,4,5-$^{13}C_4$



$C_{11}{}^{13}C_7H_{26}O$

265.35 gmol^{-1}

To a solution of (2*E*,4*E*,6*E*)-*N*-methoxy-*N*,5-dimethyl-7-(2,6,6-tri(methyl-^{13}C)cyclohex-1-en-1-yl)hepta-2,4,6-trienamide-1,2,3,4-$^{13}C_4$ (**4.39**, 289 mg, 0.931 mmol) in THF (15 mL) at −78 °C was added methyl lithium (1.48 M in Et_2O, 0.750 mL, 1.12 mmol) dropwise over 10 min. After 10 min at −78 °C the reaction was quenched by addition of a slurry of silica (1 g) and H_2O (2 mL) and the resulting suspension was stirred for 15 min. The mixture was dried (Na_2SO_4), the solids filtered and the filtrate concentrated under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 1:9) afforded the product as a viscous green oil (222 mg, 8.37 mmol, 90%).

FT-IR (neat) ν_{max} 2927 (m), 2864 (w), 1715 (w), 1659 (s), 1586 (s), 1445 (w), 1359 (m), 1252 (s), 1020 (s) cm^{-1}

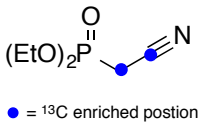
^{1}H NMR (C_6D_6, 400 MHz): δ = 7.70-7.29 (m, 1H, **11**), 6.34 (d, J_{HH} = 16.0 Hz, 1H, **7**), 6.18 (dd, J_{HH} = 16.0, J_{CH} = 5.4 Hz, 1H, **8**), 6.05 (dddd, J_{CH} = 156.7, J_{HH} = 15.0, J_{CH} = 7.2, J_{CH} = 3.3 Hz, 1H, **12**), 6.01-5.53 (m, 1H, **10**), 1.93 (m, 2H, **4**), 1.79 (d, J_{CH} = 125.3 Hz, 3H, **18**), 1.71 (d, J_{CH} = 5.0 Hz, 3H, **14**), 1.60-1.52 (m, 2H, **3**), 1.47-1.41 (m, 2H, **2**), 1.06 (dd, J_{CH} = 125.3, J_{CH} = 4.8 Hz, 6H, **16**+**17**) ppm

^{13}C NMR (C_6D_6, 101 MHz): δ = 196.55 (ddd, J_{CC} = 54.3, J_{CC} = 6.6, J_{CC} = 1.5 Hz, ^{13}C, **13**), 138.4 (dd, J_{CC} = 68.0, J_{CC} = 57.2 Hz, ^{13}CH, **11**), 130.4 (dd, J_{CC} = 68.0, J_{CC} = 54.3 Hz, ^{13}CH, **12**), 129.0 (dd, J_{CC} = 57.2, J_{CC} = 6.6 Hz, ^{13}CH, **10**), 29.4 ($^{13}CH_3$, **18**), 22.2 ($^{13}CH_3$, **16**+**17**) ppm (Only peaks for ^{13}C labelled carbons reported)

LRMS (ES$^+$) *m/z* = 266 [M+H]$^+$

HRMS (ES$^+$) For $C_{11}{}^{13}C_7H_{26}NaO^+$ calculated 288.2111, found 288.2115 Da

4.42 - Diethyl ((cyano-^{13}C)methyl-^{13}*C*)phosphonate



$C_4{}^{13}C_2H_{12}NO_3P$

179.12 gmol^{-1}

To a solution of ^{n}BuLi (2.32 M in hexanes, 4.76 mL, 11.0 mmol) in THF (8 mL) under N_2 at −78 °C was added a solution of HMDS (2.45 mL, 11.6 mmol) in THF (6 mL) dropwise over 15 min. After stirring the solution for 15 min a solution of $^{13}CH_3{}^{13}CN$ (250 mg, 5.81 mmol) in THF (6 mL) was added dropwise over 15 min and the solution was stirred for a further 40 min. A solution diethylchlorophosphate (0.92 mL, 6.4 mmol) in THF (6 mL) was added dropwise over 10 min and the solution was stirred for a further 40 min. The mixture was allowed to warm to rt and stirred for 40 min before pouring into a stirred mixture of 2 M HCl (10 mL) and CH_2Cl_2 (15 mL). The aqueous phase was extracted with CH_2Cl_2 (3 x 10 mL) and the combined organic solution was washed with H_2O before drying ($MgSO_4$) and removing the solvent under reduced pressure to afford an orange oil. Purification by silica gel chromatography (EtOAc:hexane = 7:3 to 100:0) afforded the product as an orange oil (926 mg, 5.17 mmol, 89%). Spectroscopic data were consistent with those reported.[157]

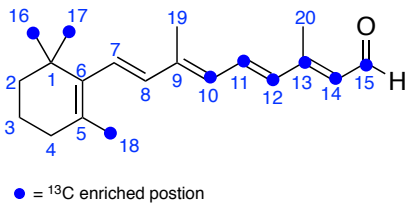
FT-IR (neat) ν_{max} 2984 (w), 2909 (w), 2255 (w), 1260 (m), 1012 (s) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.30-4.20 (m, 4H, -C**H$_2$**CH$_3$), 2.87 (ddd, J_{CH} = 135.0, J_{HP} = 21.0, J_{CH} = 10.5 Hz, 2H, -C**H$_2$**CN), 1.39 (t, J_{HH} = 7.1 Hz, 6H, -CH$_2$C**H$_3$**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 112.6 (d, J = 57.9, 11.4 Hz, -CH$_2$**C**N), 16.5 (d, J_{CC} = 143.8, J_{CP} = 60.2, -**C**H$_2$CN) ppm (Only peaks for ^{13}C labelled carbons reported)

LRMS (ES$^+$) *m/z* = 180 [M+H]$^+$

3.38 - [10,11,12,13,14,15,16,17,18-$^{13}C_9$]-All-*trans*-retinal



$C_{11}{}^{13}C_9H_{28}O$

293.37 gmol^{-1}

To a solution of diethyl ((cyano-^{13}C)methyl)phosphonate (**4.42**, 205 mg, 1.14 mmol) in THF (1.5 mL) under N_2 at 0 °C was added ^{n}BuLi (2.32 M in hexanes, 0.45 mL, 1.0 mmol) dropwise over 5 min and the solution was then stirred at rt for 90 min. A solution of (3*E*,5*E*,7*E*)-6-methyl-8-(2,6,6-tri(methyl-^{13}C)cyclohex-1-en-1-yl)octa-3,5,7-trien-2-one-2,3,4,5-$^{13}C_4$ (**4.43**, 138 mg, 0.520 mmol) in THF (5 mL) was added dropwise and stirred at rt for 18 h. The reaction was quenched with saturated aqueous NH_4Cl and the aqueous phase extracted with EtOAc (3 x 8 mL). The combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent reduced pressure to afford an orange oil. Purification by silica gel chromatography (EtOAc:hexane = 4:96, silica deactivated with NEt_3) afforded the impure nitrile intermediate as an orange oil (145 mg).

The orange oil was taken up in CH_2Cl_2 (3.5 mL) under N_2 and DIBAL (1 M in CH_2Cl_2, 0.76 mL, 0.76 mmol) was added dropwise at −60 °C. After stirring at −60 °C for 15 min, a slurry of SiO_2 and H_2O was added and the resulting suspension was stirred for 15 min at rt. The solids were filtered and the filtrate dried twice (Na_2SO_4) before removing the solvent under reduced pressure to afford an orange oil. Purification by silica gel chromatography (EtOAc:hexane = 4:96 to 1:9, silica deactivated with NEt_3) afforded the product as an orange oil (106 mg, 0.364 mmol, 70%, 13*E*:13*Z* = 2:1). The all-*trans* isomer was isolated by preparative HPLC (EtOAc:pet. ether = 3.5:96.5).

FT-IR (neat) ν_{max} 2928 (w), 1716 (w), 1662 (w), 971 (m) cm^{-1}

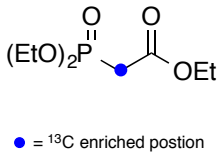
^{1}H NMR (C_6D_6, 500 MHz): δ = 10.01 (ddd, J_{CH} = 169.1, J_{CH} = 25.4, J_{HH} = 7.8 Hz, 1H, **15**), 7.03-6.62 (m, 1H, **11**), 6.37 (br d, J_{HH} = 16.1 Hz, 1H, **7**), 6.26 (dd, J_{HH} = 16.1, J_{CH} = 4.9 Hz, 1H, **8**), 6.21-5.83 (m, 1H, **10**+**12**), 5.96 (dt, J_{CH} = 157.1, J_{HH} = 7.8 Hz, 1H, **14**), 1.99-1.93 (m, 2H, **4**), 1.78 (d, J_{CH} = 4.4 Hz, 3H, **19**), 1.75-1.70 (m, 3H, **20**), 1.76 (d, J_{CH} = 125.6 Hz, 3H, **18**), 1.62-1.55 (m, 2H, **2**), 1.50-1.44 (m, 2H, **3**), 1.12 (dd, J_{CH} = 125.2, J_{CH} = 4.7 Hz, 6H, **16**+**17**) ppm

^{13}C NMR (C_6D_6, 126 MHz): δ = 189.4 (ddd, J_{CC} = 56.7, J_{CC} = 6.8, J_{CC} = 2.9 Hz, ^{13}C, **15**), 152.6 (dddd, J_{CC} = 67.1, J_{CC} = 54.2, J_{CC} = 6.8, J_{CC} = 2.9 Hz, ^{13}C, **13**), 135.2 (ddddd, J_{CC} = 67.1, J_{CC} = 54.2, J_{CC} = 6.8, J_{CC} = 3.3, J_{CC} = 1.7 Hz, ^{13}CH, **12**), 131.2 (ddd, J_{CC} = 67.1, J_{CC} = 58.4, J_{CC} = 7.6 Hz, ^{13}CH, **11**), 130.3-129.6 (m, ^{13}CH, **10**), 130.0-128.8 (m, ^{13}CH, **14**), 28.8 ($^{13}CH_3$, **16**+**17**), 21.6 ($^{13}CH_3$, **18**) ppm (Only peaks for ^{13}C labelled carbons reported)

LRMS (ES^+) *m/z* = 294 $[M+H]^+$

HRMS (ES^+) For $C_{11}{}^{13}C_9H_{29}O^+$ calculated 294.2515, found 294.2519 Da

4.45 - Triethylphosphonoacetate-2-^{13}C



$C_7{}^{13}CH_{17}O_5P$

225.19 gmol^{-1}

A solution of 2-^{13}C-ethyl bromoacetate (1.77 g, 10.5 mmol) and $P(OEt)_3$ (1.70 g, 10.3 mmol) under N_2 was heated at 130 °C in a microwave for 10 min. Purification by silica gel chromatography (EtOAc:hexane = 85:15) afforded the product was afforded as a colourless oil (2.28 g, 10.1 mmol, 99%). ^{1}H and ^{13}C NMR spectroscopic data were consistent with those reported.[158]

FT-IR (neat) ν_{max} 2983 (m), 1773 (s), 1253 (s), 1017 (s), 963 (s) cm^{-1}

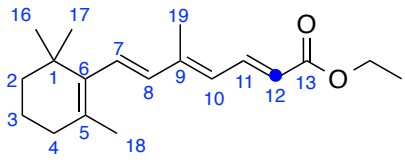
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.18 (dq, J_{HP} = 14.6, J_{HH} = 7.2 Hz, 6H, -OC**H$_2$**CH$_3$), 2.95 (dd, J_{CH} = 130.0, J_{HP} = 21.5 Hz, 2H, P-^{13}C**H$_2$**), 1.34 (t, J_{HH} = 7.2 Hz, 6H, -POCH$_2$C**H$_3$**), 1.28 (t, J_{HH} = 7.2 Hz, 3H, (O)COCH$_2$C**H$_3$**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 34.3 (d, J_{CP} = 134.3 Hz, P-^{13}**C**H$_2$) ppm (Only peak for ^{13}C labelled carbon reported)

^{31}P NMR ($CDCl_3$, 162 MHz): δ = 19.8 (d, J_{CP} = 134.6 Hz) ppm

LRMS (ES$^+$) *m/z* = 226 [M+H]$^+$

4.46 - Ethyl (2*E*,4*E*,6*E*)-5-methyl-7-(2,6,6-trimethylcyclohex-1-en-1-yl)hepta-2,4,6-trienoate-2-^{13}C



● = ^{13}C enriched postion

$C_{18}{}^{13}CH_{28}O_2$

289.42 gmol^{-1}

To a suspension of NaH (60% in mineral oil, 427 mg, 10.7 mmol) in Et_2O (8 mL) under N_2 at 0 °C was added a solution of triethylphosphonoacetate-2-^{13}C (**4.45**, 2.25 g, 10.0 mmol) in Et_2O (4 mL) dropwise and the solution was stirred for 90 min at rt. A solution of (2*E*,4*E*)-3-methyl-5-(2,6,6-trimethylcyclohex-1-en-1-yl)penta-2,4-dienal (**4.22**, 1.10 g, 5.04 mmol) in Et_2O (4 mL) was added dropwise and the solution was stirred at rt for 2 h. The reaction was then quenched with H_2O (10 mL) and the aqueous phase was extracted with Et_2O (3 x 8 mL). The combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 2:97) afforded the product as a yellow/green oil (1.37 g, 4.73 mmol, 94%).

FT-IR (neat) ν_{max} 2929 (m), 2861 (m), 1663 (s), 1565 (m) cm^{-1}

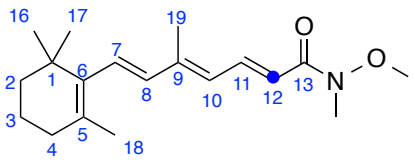
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.72 (ddd, J_{HH} = 15.0, J_{HH} = 12.1, J_{CH} = 2.5 Hz, 1H, **11**), 6.40 (br d, J_{HH} = 16.0 Hz, 1H, **7**), 6.20-6.12 (m, 2H, **8+10**), 5.88 (dd, J_{CH} = 162.4, J_{HH} = 15.0 Hz, 1H, **12**), 4.22 (d, J_{HH} = 7.1 Hz, 2H, -OC**H**$_2$), 2.05 (s, 3H, **19**), 2.03 (t, J_{HH} = 5.9 Hz, 2H, **4**), 1.72 (s, 3H, **18**), 1.67-1.59 (m, 2H, **3**), 1.52-1.45 (m, 2H, **2**), 1.32 (t, J_{HH} = 7.1 Hz, 3H, -OCH$_2$C**H**$_3$), 1.04 (s, 6H, **16+17**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 120.1 (^{13}CH, **12**) ppm (Only peak for ^{13}C labelled carbon reported)

LRMS (ES$^+$): *m/z* = 290 [M+H]$^+$

HRMS (ES$^+$) $C_{18}{}^{13}CH_{28}NaO_2^+$ calculated 312.2015, found 312.2018 Da

4.47 - (2*E*,4*E*,6*E*)-*N*-Methoxy-*N*,5-dimethyl-7-(2,6,6-trimethylcyclohex-1-en-1-yl)hepta-2,4,6-trienamide-2-^{13}C



● = ^{13}C enriched postion

$C_{18}{}^{13}CH_{29}NO_2$

304.44 gmol^{-1}

To a solution of *N*,*O*-dimethylhydroxylamine hydrochloride (1.83 g, 18.7 mmol) in THF (30 mL) under N_2 at −15 °C was added ^{n}BuLi (2.27 M in hexanes, 16.1 mL, 36.5 mmol) dropwise over 15 min. After stirring the solution at −15 °C for 1 h, a solution of ethyl (2*E*,4*E*,6*E*)-5-methyl-7-(2,6,6-trimethylcyclohex-1-en-1-yl)hepta-2,4,6-trienoate-2-^{13}C (**4.46**, 1.23 g, 4.25 mmol) in THF (20 mL) was added dropwise over 15 min at −15 °C. After 30 min at −15 °C, the reaction was quenched with saturated aqueous NH_4Cl and the aqueous phase was extracted with Et_2O (3 x 10 mL). The combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by silica gel chromatography (EtOAc:hexane = 15:85) afforded the product as a yellow/green oil (1.13 g, 3.72 mmol, 88%).

FT-IR (neat) ν_{max} 2928 (w), 2963 (m), 1645 (s), 1589 (m), 1408 (m), 1371 (s) cm^{-1}

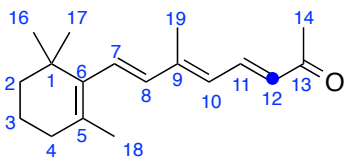
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.77 (ddd, J_{HH} = 14.9, J_{HH} = 12.0, J_{CH} = 2.9 Hz, 1H, **11**), 6.47 (br dd, J_{HH} = 161.9, J_{HH} = 14.9 Hz, 1H, **12**), 6.37 (br d, J_{HH} = 16.1 Hz, 1H, **7**), 6.22 (dd, J_{HH} = 12.0, J_{HH} = 4.4 Hz, 1H, **10**), 6.15 (d, J_{HH} = 16.1 Hz, 1H, **8**), 3.71 (s, 3H, -OC**H**$_3$), 3.27 (s, 3H, -NC**H**$_3$), 2.06 (s, 3H, **19**), 2.03 (t, J_{HH} = 6.0 Hz, 2H, **4**), 1.71 (s, 3 H, **18**), 1.67-1.58 (m, 2H, **3**), 1.51-1.45 (m, 2H, **2**), 1.03 (s, 6H, **16+17**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 117.9 (^{13}CH, **12**) ppm (Only peak for ^{13}C labelled carbon reported)

LRMS (ES$^+$): *m/z* = 305 [M+H]$^+$

HRMS (ES$^+$) For $C_{18}{}^{13}CH_{30}NO_2{}^+$ calculated 305.2305, found 305.2303 Da

4.48 - (3*E*,5*E*,7*E*)-6-Methyl-8-(2,6,6-trimethylcyclohex-1-en-1-yl)octa-3,5,7-trien-2-one-3-^{13}C



● = ^{13}C enriched postion

$C_{17}{}^{13}CH_{26}O$

259.40 gmol^{-1}

To a solution of (2*E*,4*E*,6*E*)-*N*-methoxy-*N*,5-dimethyl-7-(2,6,6-trimethylcyclohex-1-en-1-yl)hepta-2,4,6-trienamide-2-^{13}C (**4.47**, 964 mg, 3.18 mmol) in THF (50 mL) at −78 °C was added methyl lithium (1.45 M in Et_2O, 2.62 mL, 3.80 mmol) dropwise over 10 min. After 10 min at −78 °C the reaction was quenched by addition of a slurry of silica (2 g) and H_2O (10 mL) and the resulting suspension was stirred for 15 min at rt. The mixture was dried ($MgSO_4$), the solids filtered and the filtrate concentrated under reduced pressure to afford the product as viscous green oil (788 mg, 3.04 mmol, 96%).

FT-IR (neat) ν_{max} 2927 (m), 2864 (w), 1657 (s), 1585 (s), 1445 (w), 1359 (m), 1020 (s) cm^{-1}

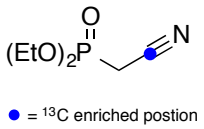
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.58 (ddd, J_{HH} = 15.1, J_{HH} = 11.9, J_{CH} = 1.2 Hz, 1H, **11**), 6.43 (br d, J_{HH} = 16.0 Hz, 1H, **7**), 6.18 (br d, J_{HH} = 16.0 Hz, 1H, **8**), 6.17 (br dd, J_{HH} = 11.9, J_{CH} = 4.5 Hz, 1H, **10**), 6.17 (dd, J_{CH} = 157.5, J_{HH} = 15.1 Hz, 1H, **12**), 2.30 (d, J_{CH} = 1.0 Hz, 3H, **14**), 2.07 (s, 3H, **19**), 2.04 (br t, J_{HH} = 6.2 Hz, 2H, **4**), 1.72 (s, 3H, **18**), 1.67-1.59 (m, 2H, **3**), 1.51-1.45 (m, 2H, **2**), 1.04 (s, 6H, **16+17**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 129.3 (^{13}CH, **12**) ppm (Only peak for ^{13}C labelled carbon reported)

LRMS (ES$^+$) *m/z* = 260 [M+H]$^+$

HRMS (ES$^+$) For $C_{17}{}^{13}CH_{26}NaO^+$ calculated 282.1909, found 282.1918 Da

4.50 - Diethyl ((cyano-^{13}C)methyl)phosphonate



$C_5{}^{13}CH_{12}NO_3P$

178.13 gmol^{-1}

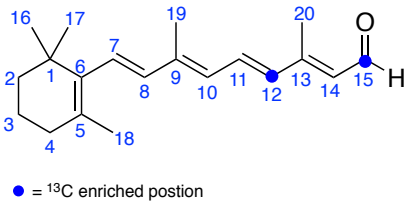
To a solution of ^{n}BuLi (2.17 M in hexanes, 10.8 mL, 23.4 mmol) in THF (16 mL) under N_2 at −78 °C was added a solution of HMDS (5.02 mL, 23.8 mmol) in THF (12 mL) dropwise over 15 min. After stirring for 15 min a solution of $^{13}CH_3CN$ (500 mg, 11.9 mmol) in THF (12 mL) was added dropwise over 15 min and the solution was then stirred for a further 40 min. A solution diethylchlorophosphate (1.89mL, 13.1 mmol) in THF (12 mL) was added dropwise over 10 min and the solution was stirred for a further 40 min. The mixture was allowed to warm to rt and stirred for 40 min before pouring into a stirred mixture of 2 M HCl (20 mL) and CH_2Cl_2 (30 mL). The aqueous phase was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic solution was washed with H_2O before drying ($MgSO_4$) and removing the solvent under reduced pressure to afford an orange oil. Purification by silica gel chromatography (EtOAc:hexane = 7:3 to 100:0) afforded the product as an orange oil (1.89 g, 10.6 mmol, 89%). ^{1}H and ^{13}C NMR spectroscopic data were consistent with those reported.[157]

FT-IR (neat) ν_{max} 2984 (w), 2909 (w), 2256 (w), 1260 (m), 1012 (s) cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.31-4.19 (m, 4H, -C**H$_2$**CH$_3$), 2.87 (dd, J_{HP} = 21.1, J_{CH} = 10.6 Hz, 2H, -C**H$_2$**CN), 1.39 (t, J_{CH} = 7.1 Hz, 6H, -CH$_2$C**H$_3$**) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 112.6 (J_{CP} = 11.0 Hz, -CH$_2$**C**N) ppm (Only peak for ^{13}C labelled carbon reported)

3.39 - [12-15-$^{13}C_2$]-All-*trans*-retinal



$C_{18}{}^{13}C_2H_{28}O$

286.43 gmol^{-1}

To a solution of diethyl ((cyano-^{13}C)methyl)-phosphonate (250 mg, 1.41 mmol) in THF (1.5 mL) under N_2 at 0 °C was added ^{n}BuLi (2.31 M in hexanes, 0.55 mL, 1.3 mmol) dropwise over 5 min and the solution was then stirred at rt for 75 min. A solution of (3*E*,5*E*,7*E*)-6-methyl-8-(2,6,6-trimethylcyclohex-1-en-1-yl)octa-3,5,7-trien-2-one-3-^{13}C (**4.48**, 165 mg, 0.639 mmol) in THF (5 mL) was added dropwise and the solution was stirred at rt for 18 h. The reaction was quenched with saturated aqueous NH_4Cl and the aqueous phase extracted with EtOAc (3 x 8 mL). The combined organic solution was washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure to afford an orange oil. Purification by silica gel chromatography (EtOAc:hexane = 3:97, deactivated with NEt_3) afforded the impure nitrile intermediate as an orange oil (121 mg).

The orange oil was taken up in CH_2Cl_2 (3 mL) under N_2 and DIBAL (1 M in CH_2Cl_2, 0.64 mL, 0.64 mmol) was added dropwise at −60 °C. After stirring at −60 °C for 1 h, a slurry of silica and H_2O was added and the resulting suspension was stirred for 15 min at rt. The solids were filtered and the filtrate dried twice (Na_2SO_4) before removing the solvent under reduced pressure to afford an orange oil. Purification by silica gel chromatography (EtOAc:hexane = 4:96 to 1:9, SiO_2 deactivated with NEt_3) afforded the product as an orange oil (120 mg, 0.419 mmol, 30%, 13*E*:13*Z* = 2:1). The isomers were separated by preparative HPLC (EtOAc:pet. ether = 3.5:96.5).

FT-IR (neat) ν_{max} 2928 (w), 1716 (w), 1662 (w), 971 (m) cm^{-1}

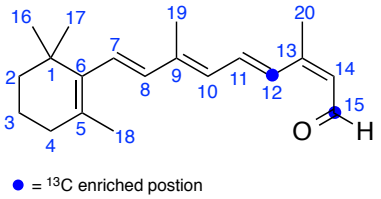
^{1}H NMR (C_6D_6, 400 MHz): δ = 10.01 (dd, J_{CH} = 169.2, J_{HH} = 8.1 Hz, 1H, **15**), 6.83 (dd, J_{HH} = 15.2, J_{HH} = 11.5 Hz, 1H, **11**), 6.37 (d, J_{HH} = 16.1 Hz, 1H, **7**), 6.26 (d, J_{HH} = 16.1 Hz, 1H, **8**), 6.02 (dd, J_{HH} = 11.5, J_{CH} = 4.8 Hz, 1H, **10**), 6.02 (dd, J_{CH} = 154.4, J_{HH} = 15.2 Hz, 1H, **12**), 5.96 (apparent t, $J_{HH/CH}$ = 7.9 Hz, 1H, **14**), 1.96 (t, J_{HH} = 6.0 Hz, 2H, **4**), 1.78 (s, 3H, **18**+**19**), 1.77 (s, 3H, **18**+**19**), 1.73 (d, J_{CH} = 4.0 Hz, 3H, **20**), 1.62-1.55 (m, 2H, **2**), 1.51-1.44 (m, 2H, **3**), 1.12 (s, 6H, **16**+**17**) ppm

^{13}C NMR (C_6D_6, 101 MHz): δ = 190.1 (d, J_{CC} = 7.0 Hz, ^{13}C, **15**), 135.9 (d, J_{CC} = 7.0 Hz, ^{13}CH, **20**) ppm (Only peaks for ^{13}C labelled carbons reported)

LRMS (ES^+) *m/z* = 287 $[M+H]^+$

HRMS (ES^+) For $C_{18}{}^{13}C_2H_{28}NaO^+$ calculated 309.2099, found 309.2098

4.52 - [12-15-$^{13}C_2$]-13-*cis*-retinal



$C_{18}{}^{13}C_2H_{28}O$

286.43 $gmol^{-1}$

^{1}H NMR (C_6D_6, 400 MHz): δ = 10.15 (dd, J_{CH} = 169.7, J_{HH} = 7.2 Hz, 1H, **15**), 7.07 (dd, J_{CH} = 153.8, J_{HH} = 15.0 Hz, 1H, **12**), 6.74 (dd, J_{HH} = 15.0, J_{HH} = 11.4 Hz, 1H, **11**), 6.37 (d, J_{HH} = 16.1 Hz, 1H, **7**), 6.28 (d, J_{HH} = 16.1 Hz, 1H, **8**), 6.05 (dd, J_{HH} = 11.4, J_{CH} = 4.9 Hz, 1H, **10**), 5.76 (apparent t, $J_{CH/HH}$ = 8.3 Hz, 1H, **14**), 1.96 (br t, J_{HH} = 6.1 Hz, 2H, **4**), 1.82-1.75 (m, 6H, **18**+**19**), 1.59 (br d, J_{CH} = 4.0 Hz, 3H, **20**), 1.62-1.55 (m, 2H, **2**), 1.50-1.45 (m, 2H, **3**), 1.13 (s, 6H, **16**+**17**) ppm

^{13}C NMR (C_6D_6, 101 MHz): δ = 189.0 (d, J_{CC} = 5.1 Hz, ^{13}C, **15**), 127.9 (d, J_{CC} = 5.1 Hz, ^{13}CH, **12**) ppm (Only peaks for ^{13}C labelled carbons reported)

LRMS (ES^+) *m/z* = 287 $[M+H]^+$

HRMS (ES^+) For $C_{18}{}^{13}C_2H_{28}NaO^+$ calculated 309.2099, found 309.20102

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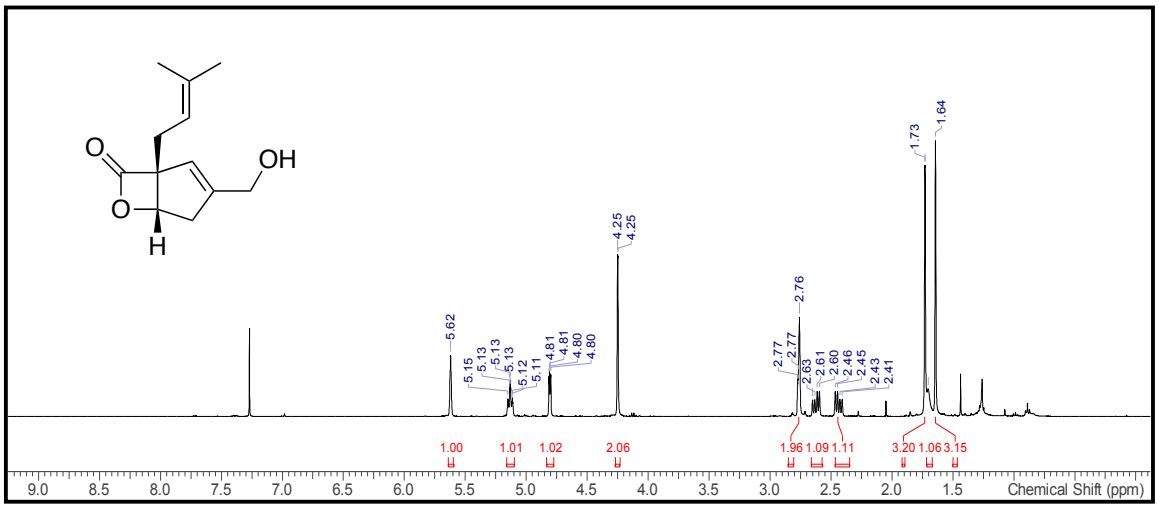
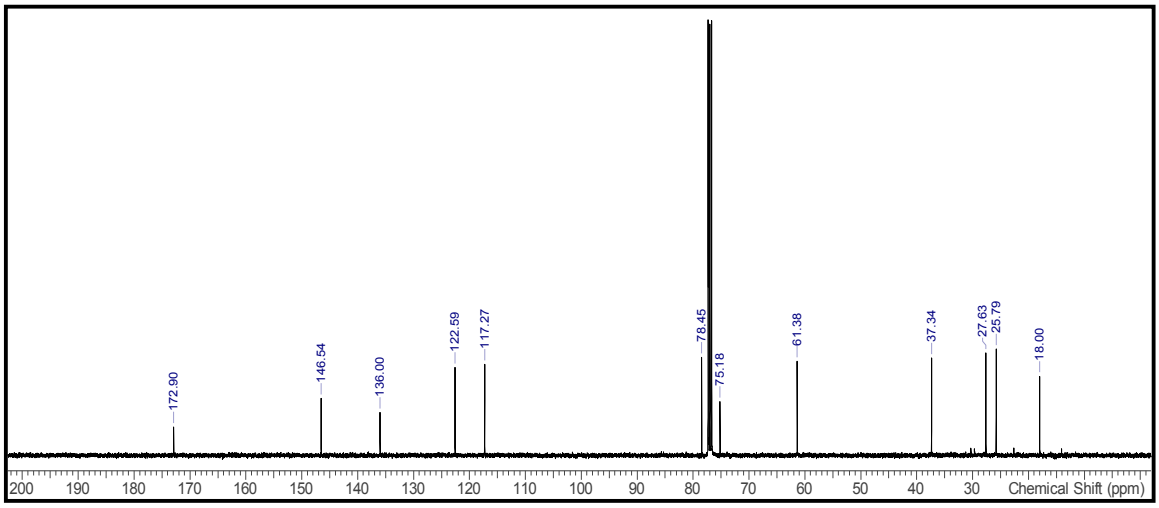
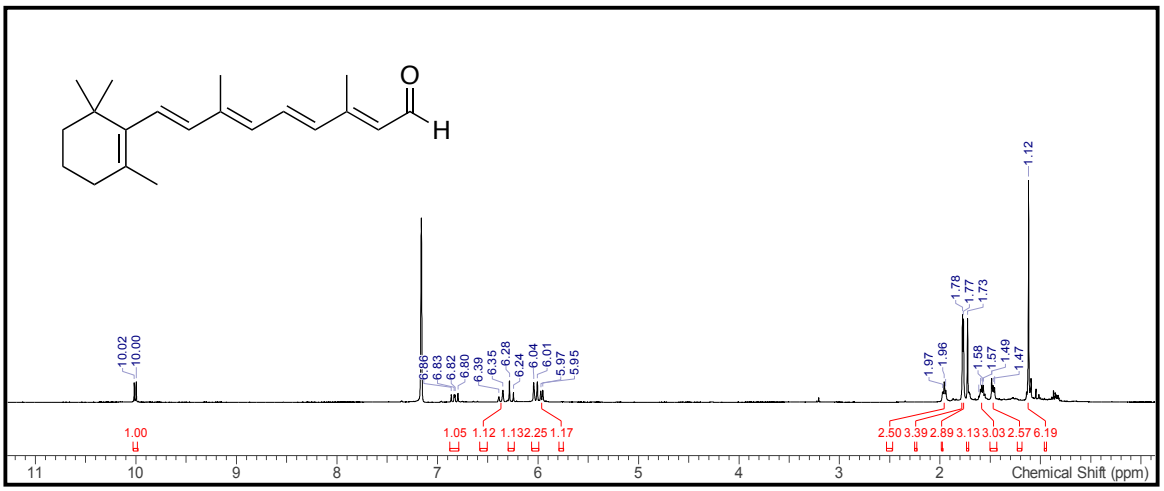
Appendix A

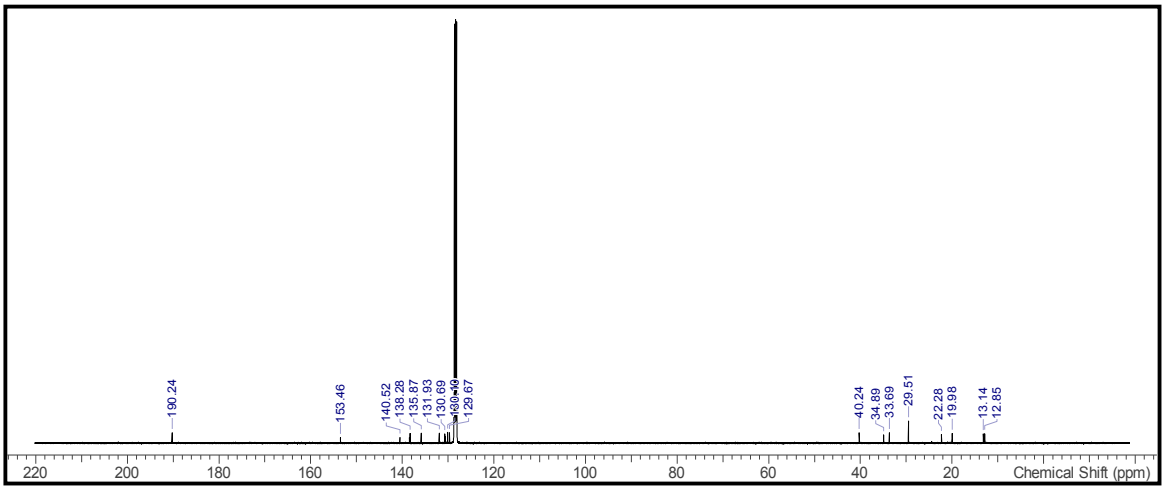
^{1}H (400 MHz; $CDCl_3$) and ^{13}C (100 MHz; $CDCl_3$) NMR spectra for (±)-Vibralactone (1.01)



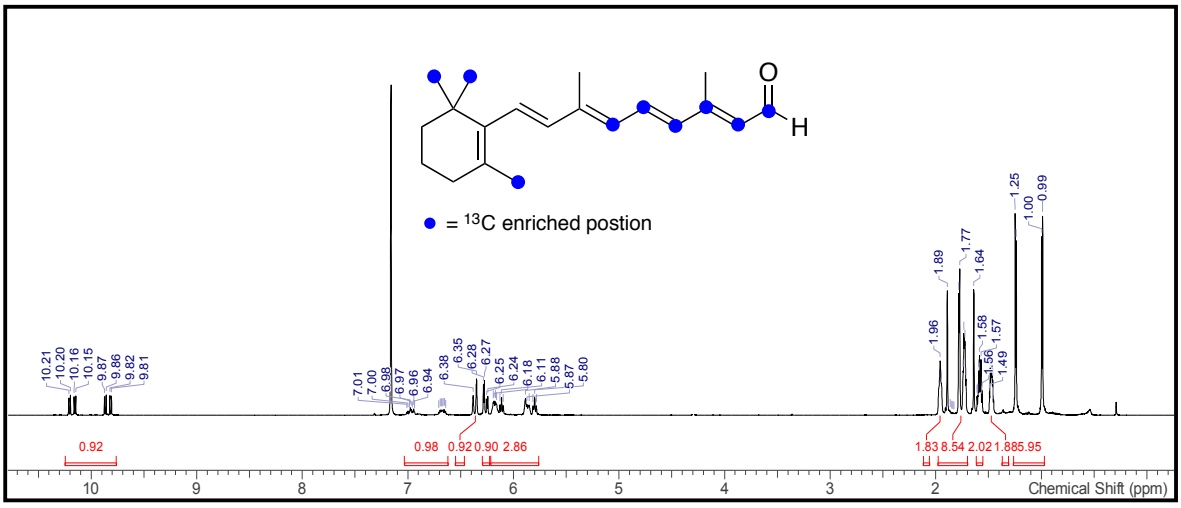


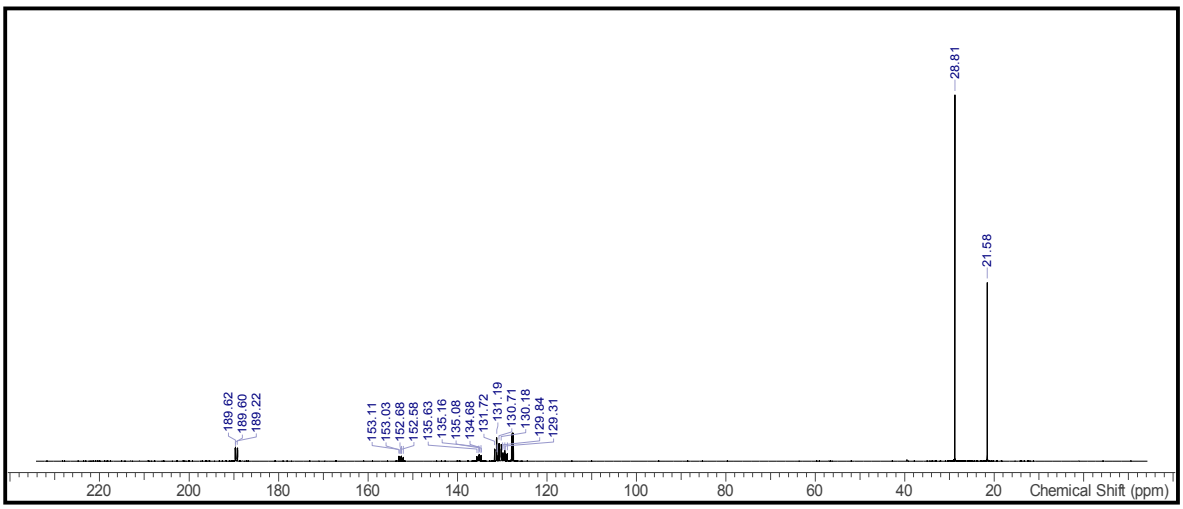
^{1}H (400 MHz; $CDCl_3$) and ^{13}C (100 MHz; $CDCl_3$) NMR spectra for all-*trans*-retinal (3.01)



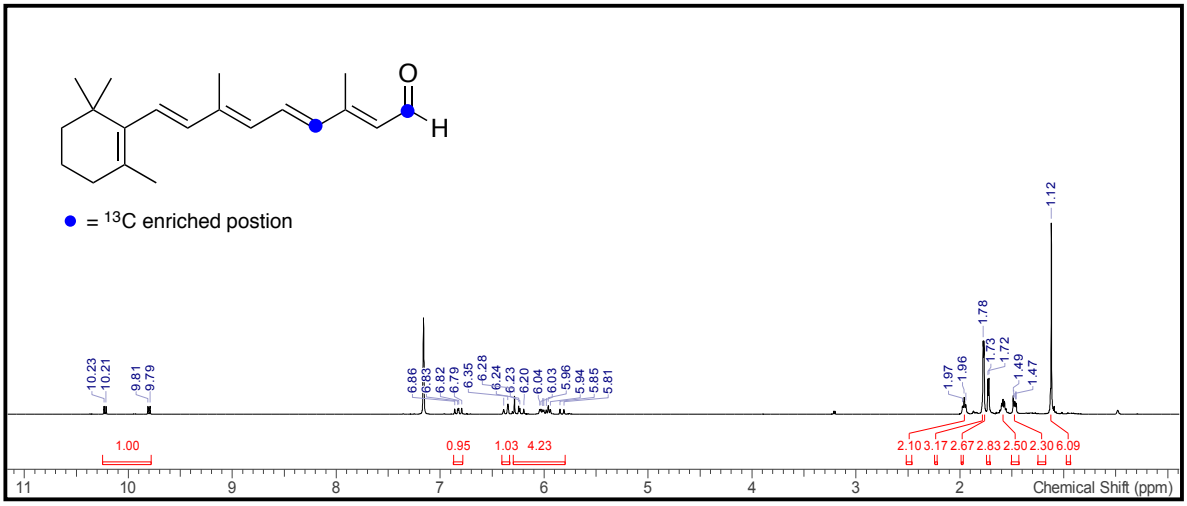


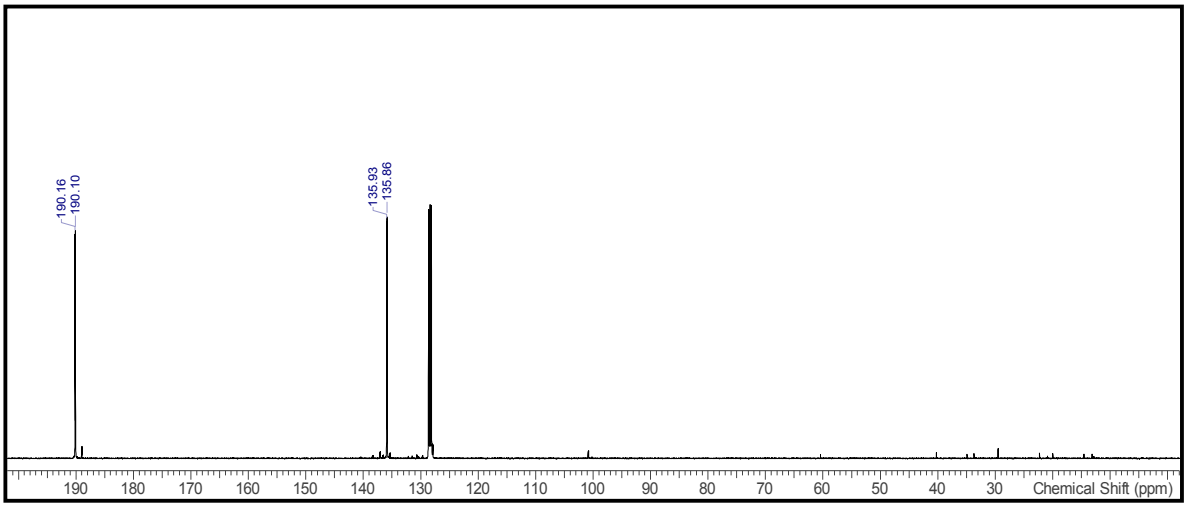
^{1}H (400 MHz; $CDCl_3$) and ^{13}C (100 MHz; $CDCl_3$) NMR spectra for [10,11,12,13,14,15,16,17,18-$^{13}C_9$]-all-*trans*-retinal (3.38)





^{1}H (400 MHz; $CDCl_3$) and ^{13}C (100 MHz; $CDCl_3$) NMR spectra for [12-15-$^{13}C_2$]-all-*trans*-retinal (3.39)





^{1}H (400 MHz; $CDCl_3$) and ^{13}C (100 MHz; $CDCl_3$) NMR spectra for [12-15-$^{13}C_2$]-13-*cis*-retinal (4.52)