UNIVERSITY OF SOUTHAMPTON

Faculty of Engineering, Science and Mathematics

The Stereoselective Synthesis of $^{13}C_2$-Labelled 11*Z*-Retinals and the Total Synthesis of (−)-Galanthamine

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ABSTRACT

FACULTY OF ENGINEERING, SCIENCE AND MATHEMATICS

SCHOOL OF CHEMISTRY

Doctor of Philosophy

PART 1: THE STEREOSELECTIVE SYNTHESIS OF $^{13}C_2$-LABELLED 11*Z*-RETINALS
PART 2: TOTAL SYNTHESIS OF (–)-GALANTHAMINE

By Neville James McLean

Rhodopsin a G-protein coupled receptor (GPCR), consists of the apoprotein opsin and a 11*Z*-retinylidene chromophore and is responsible for dim light vision. On absorption of a photon, the 11*Z*-retinylidene chromophore specifically photoisomerises to all-*E*-retinylidene in approximately 200 fs. To allow investigation by solid state NMR into how the protein environment accelerates and controls this specific isomerisation and of the photointermediates, synthetic strategies to a series of $^{13}C_2$ labelled 11*Z*-retinals were devised. [9,10-$^{13}C_2$]-11*Z*-Retinal (**2.45**), [10,11-$^{13}C_2$]-11*Z*-retinal (**2.53**) and [11,12-$^{13}C_2$]-11*Z*-retinal (**2.81**) were successfully synthesised, employing a novel Weinreb amide analogue and a labelled ethoxy analogue of the Bestmann-Ohira reagent (**2.78**) as key reagents.

(–)-Galanthamine (**3.1**) is a naturally occurring tertiary alkaloid, with it's hydrobromide salt used in the treatment of mild to moderate Alzheimer's disease. Two synthetic routes to (–)-galanthamine (**3.1**) were investigated. The first synthetic route used a Heck cross-coupling and an enyne metathesis to form rings B and C respectively. An azepine ring closure completed the synthesis giving (–)-galanthamine (**3.1**). Investigation of the second route was commenced. Efforts to synthesis a 1,3-*anti*-diol fragment were explored, using the Sharpless asymmetric epoxidation to induce asymmetry.

Contents

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Abbreviations

Ac	acetyl
APC	allyl palladium chloride dimer
BBN	9-borabicyclo[3.3.1]nonane
Boc	1,1-dimethylethoxycarbonyl
br	broad (NMR and IR)
Bu	butyl
Bn	benzyl
CI	chemical ionisation
d	doublet (NMR)
Da	Dalton
DBAD	di-*tert*-butyl azodicarboxylate
DET	diethyl tartrate
DIBAL-H	di*iso*butylaluminium hydride
DIAD	di*iso*propyl diazodicarboxylate
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
Cp	cyclopentadiene
dppp	1,3-bis(diphenylphosphino)propane
dr	diastereomeric ratio
EDCI	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
ee	enantiomeric excess
EI	electron ionisation
Et	ethyl
FT	fourier transform
GC	gas chromatography
h	hour(s)
HMDS	hexamethyldisilazane
HMPA	hexamethylphosphoric triamide
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
Hz	hertz

i	*iso*
IR	infrared
J	coupling constant (NMR)
lbpe	low boiling petroleum ether
LDA	lithium diisopropylamide
LRMS	low resolution mass spectrometry
m	multiplet (NMR) or medium (IR)
M	$moldm^{-3}$
MAS	magic angle spinning
Me	methyl
min	minute
MPM	*p*-methoxybenzyl
Mpt	melting point
Ms	methanesulfonyl (mesyl)
m/z	mass/charge ratio
NMO	*N*-methylmorpholine *N*-oxide
NMR	nuclear magnetic resonance
PCC	pyridinium chlorochromate
Ph	phenyl
ppm	parts per million
Pr	propyl
q	quartet (NMR)
qn	qunitet (NMR)
RCM	Ring closing metathesis
rt	room temperature
s	singlet (NMR) or strong (IR)
t	tertiary
t	triplet (NMR)
TASF	tris(dimethylamino)sulfonium difluorotrimethylsilicate
TBAF	tetrabutylammonium fluoride
TBDMS	*t*-butyldimethylsilyl
Tf	trifluoromethanesulfonyl (triflyl)
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride

THF	tetrahydrofuran
TMS	trimethylsilyl
TPAP	Tetra-*n*-propylammonium perruthenate
Ts	*p*-toluenesulfonyl (tosyl)
w	weak (IR)

Chapter 1 Rhodopsin and 11*Z*-Retinal

1.1 Background

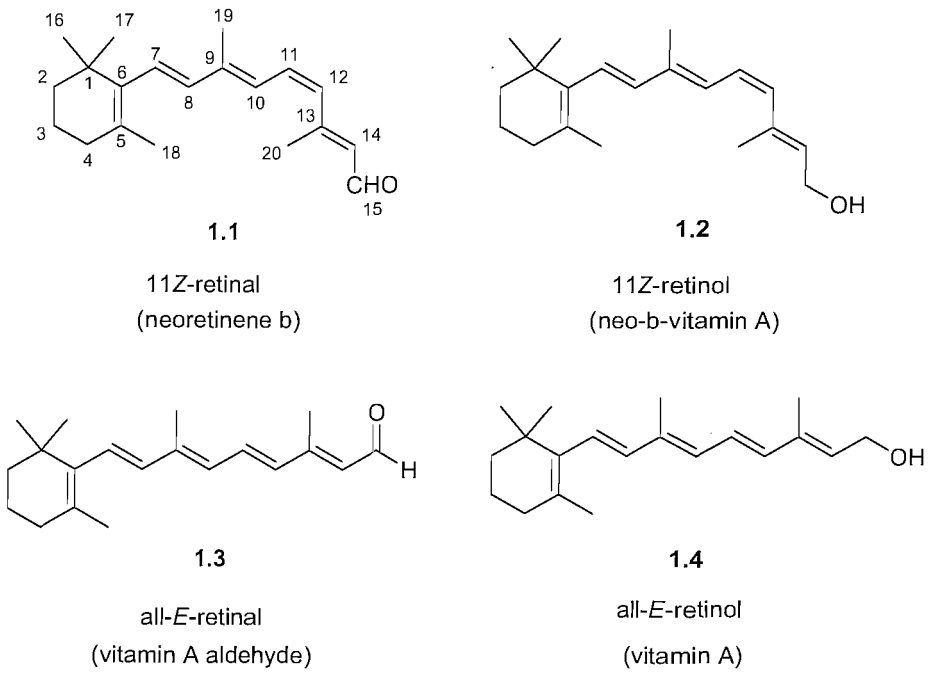


Figure 1.1 Retinoids and IUPAC numbering system for 11*Z*-retinal (**1.1**)

Rhodopsin is responsible for dim light vision in vertebrates and is located in the rod cells of the retina. Rhodopsin is a 40-kd 7-transmembrane G-protein coupled receptor (GPCR), consisting of the apoprotein opsin and a 11*Z*-retinylidene chromophore. The retinylidene chromophore is bound to opsin by a protonated Schiff base (PSB) linkage to the ε-amino group of lysine 296.[1] On absorption of a photon, the bound 11*Z*-retinal (**1.1**) specifically photoisomerises to all-*E*-retinal (**1.3**) in approximately 200 fs. The strained all-*E* chromophore gives rise to conformational changes within the protein, leading to the active form of rhodopsin as metarhodopsin II *via* transient photointermediates (Figure 1.2).[2] GPCRs are a superfamily of receptors, which are frequently used as pharmaceutical targets, making insight into how the protein environment accelerates the isomerisation of the chromophore of great interest.

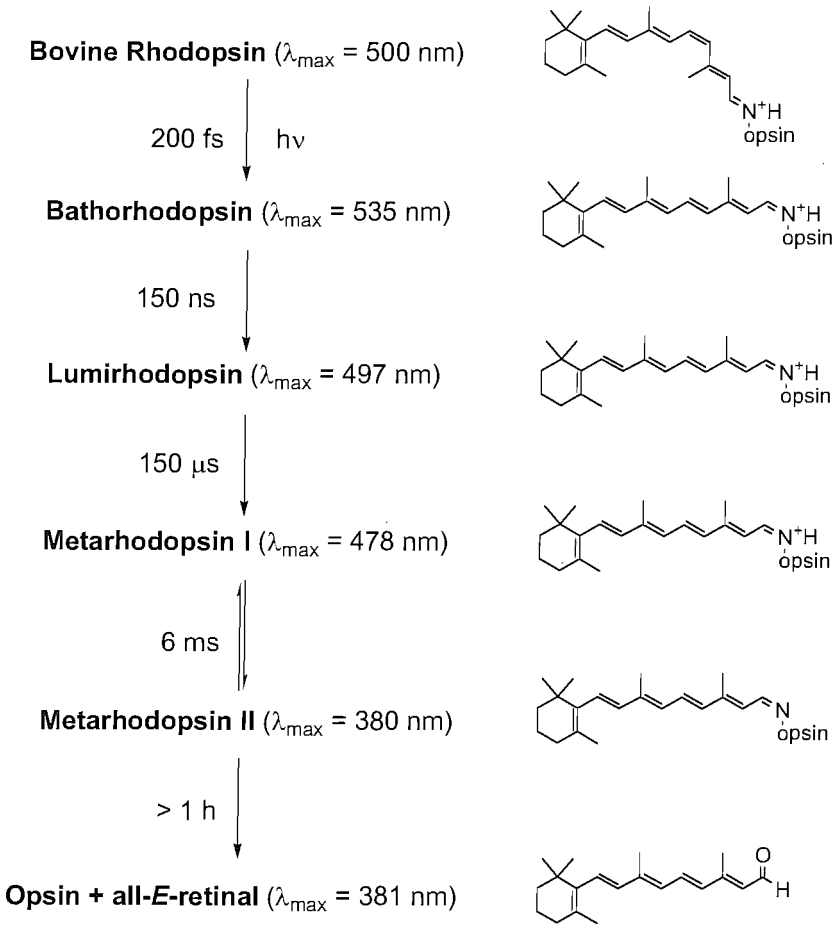


Figure 1.2 - The photointermediates of rhodopsin

It was George Wald in 1934 who discovered the importance of retinal in the visual process.[3] The precursor of 11*Z*-retinal (**1.1**) is all-*E*-retinol (**1.4**) (vitamin A), this is a necessary dietary requirement for mammals as it cannot be synthesised *de novo* in mammals. A deficiency of vitamin A leads to night blindness and can progress to the deterioration of the outer segments of the rod cells and eventually leads to blindness.[1]

Rhodopsin is extremely sensitive as the absorption of a single photon produces a measurable response.[4] In the photocycle, conversion of metarhodopsin I to metarhodopsin II (photoexcited rhodopsin) by the deprotonation of the protonated Schiff base, causes an enzymatic cascade resulting in the hydrolysis of cGMP. The unprotonated Schiff base is then hydrolysed to give opsin and all-*E*-retinal (1.**3**). The enzymatic cascade involves the binding of metarhodopsin II to transducin (T), a heterotrimeric G-protein containing T_α, T_β and T_γ subunits. GDP is bound to the alpha subunit and is converted to GTP once transducin is bound to metarhodopsin II. Once

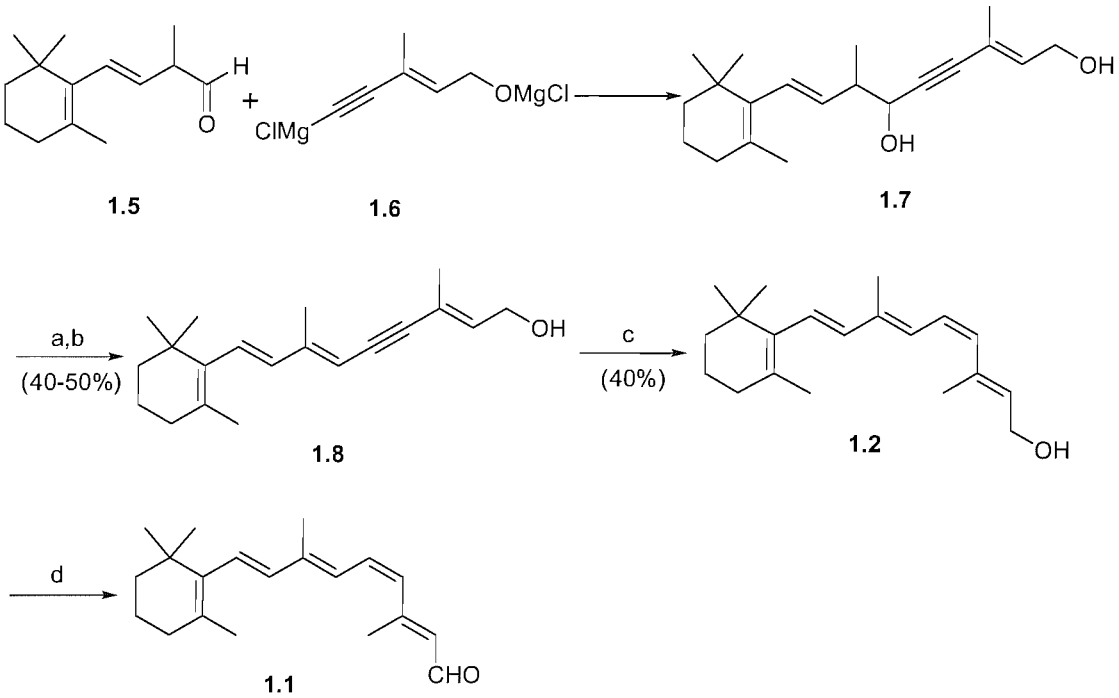
this has occurred the active form of transducin is produced, leading to the release of the metarhodopsin II, which allows the conversion of another molecule of transducin. A single metarhodopsin II can catalyse the activation of 500 transducin molecules before being deactivated. The binding of GTP also causes the release of the $T_{\beta,\gamma}$ subunits from T_{α}-GTP. The released T_{α}-GTP then activates a phosphodiesterase, which in turn catalyses the hydrolysis of cGMP to GMP. cGMP within the cell opens Na^{+}/K^{+} channels in the plasma membrane keeping the cell depolarised. The drop in cGMP from the hydrolysis causes the closure of the ion channels. The decrease of Na^{+} influx gives an increased potential across the membrane and the plasma membrane becomes hyperpolarized. This causes a reduced production of neurotransmitter in the synaptical terminus of the cell, which causes a pulse to be sent *via* the bipolar second order retina neurones through the optical nerve to the brain. The system returns to the dark state by the hydrolysis of T_{α} bound GTP to GDP, therefore deactivating the phosphodiesterase. This occurs approximately a second after the T_{α}-GTP initially binds to the phosphodiesterase. Metarhodopsin II is deactivated by the multiple phosphorylation in the carboxyl-terminus region by rhodopsin kinase, which allows the binding of arrestin. Arrestin's role is to act as inhibitory protein to stop the binding of transducin to metarhodopsin II. The return to the dark state also requires guanylate cyclase to convert GTP to cGMP, a process stimulated by a drop in Ca^{2+} which is induced by illumination.[1]

The free all-*E*-retinal (**1.3**) is converted to vitamin A (**1.4**) by specific nicotinamide linked retinol dehydrogenases prRDH, setSDR and RDH12.[5] Vitamin A (**1.4**) is then transported from the outer rod segment to the pigment epithelium where it is esterified then enzymatically converted to the 11*Z* isomer and oxidised to 11*Z*-retinal (**1.1**). The newly formed 11*Z*-retinal then binds to opsin to regenerate the light sensitive rhodopsin.[1]

1.2 Past Syntheses of 11*Z*-Retinal (1.1)

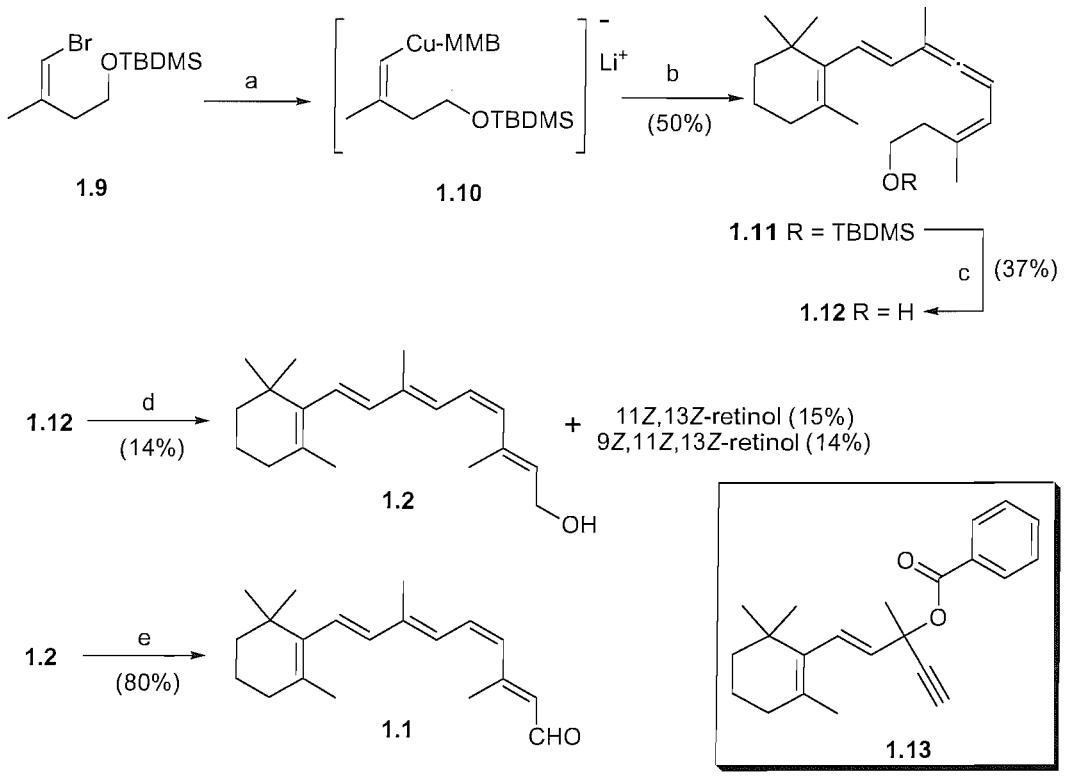
There are only a handful of successful reports on the stereoselective synthesis of 11*Z*-retinal (**1.1**).[6-9] With many reports resulting in low yields or producing complex

mixtures of stereoisomers.[10-15] In 1956, Oroshnik reported the first synthesis of 11*Z*-retinal (**1.1**) (Scheme 1.1).[15] Reaction between aldehyde **1.5** and Grignard reagent **1.6** gave the desired 13*E*-glycol **1.7**. Monoacetylation and subsequent dehydration produced dehydroretinol **1.8** in moderate yield. A catalytic semihydrogenation was utilised to install the 11*Z* double bond. Following this, a MnO_2 oxidation of retinol **1.2** gave 11Z-retinal (**1.1**), which was identified by condensation with opsin, yielding rhodopsin.



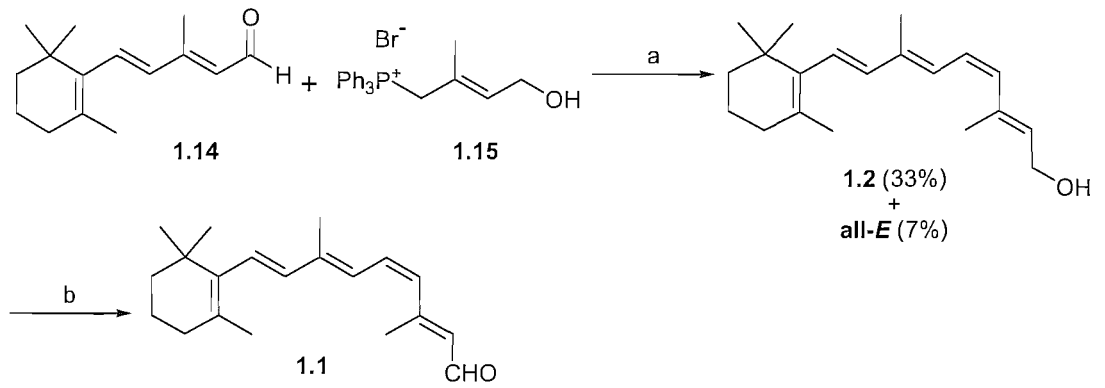
Scheme 1.1 *Reagents and conditions:* a) Monoacetylation; b) tosylic acid, benzene; c) catalytic semihydrogentation; d) MnO_2.

Knudsen *et al.* utilised a thermal sigmatropic rearrangement to acquire the desired 11*Z* stereochemistry (Scheme 1.2).[10] Formation of allene silyl ether **1.11** was accomplished with the coupling of mixed cuprate **1.10** and propargyl benzoate **1.13** in a modest yield of 50%. The silyl ether **1.11** was then deprotected with TBAF in 37% yield. Knudsen and co-workers found that the deprotection of crude allene silyl ether **1.11** gave an improved yield of 25% over two steps. With allene **1.12** prepared, the [1,5]-sigmatropic reaction was attempted in refluxing hexane. The reaction resulted in the formation of three isomers, these being, 11*Z*,13*Z*-retinol (15%), 9*Z*,11*Z*,13*Z*-retinol (14%) and the desired 11*Z*-retinol (**1.2**) (14%). The oxidation of 11*Z*-retinol (**1.2**) with activated MnO_2 gave 11*Z*-retinal (**1.1**) in a high yield of 80%.



Scheme 1.2 *Reagents and conditions:* a) *t*-BuLi, Et_2O, –78 °C, then copper 3-methoxy-3-methylbutyne; b) **1.13**, Et_2O, –78 °C to rt; c) TBAF, THF; d) hexane, reflux; e) MnO_2, lbpe, 4 °C.

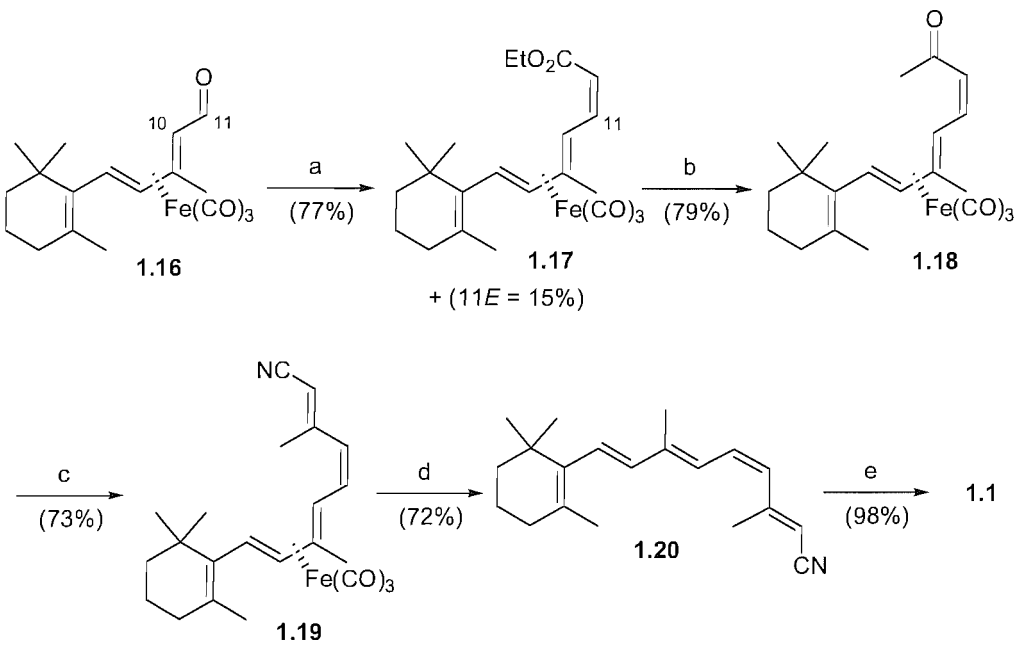
In 1987 Hosoda *et al.* described a synthesis of 11*Z*-retinal (**1.1**) (Scheme 1.3).[11] The concise synthesis utilised a *Z*-selective Wittig reaction between aldehyde **1.14** and phosphonium salt **1.15** to introduce the 11*Z* stereochemistry. Initial model compound optimisation of the Wittig reaction found that using $KN(TMS)_2$ as the base afforded the highest *Z*-selectivity. To obtain 11*Z*-retinal (**1.1**) the mixture of 11*Z* and all-*E*-retinols were oxidised with MnO_2. Pure 11*Z*-retinal (**1.1**) was obtained by preparative HPLC.



Scheme 1.3 *Reagents and conditions:* a) $KN(TMS)_2$, THF, −78 °C to rt; b) MnO_2.

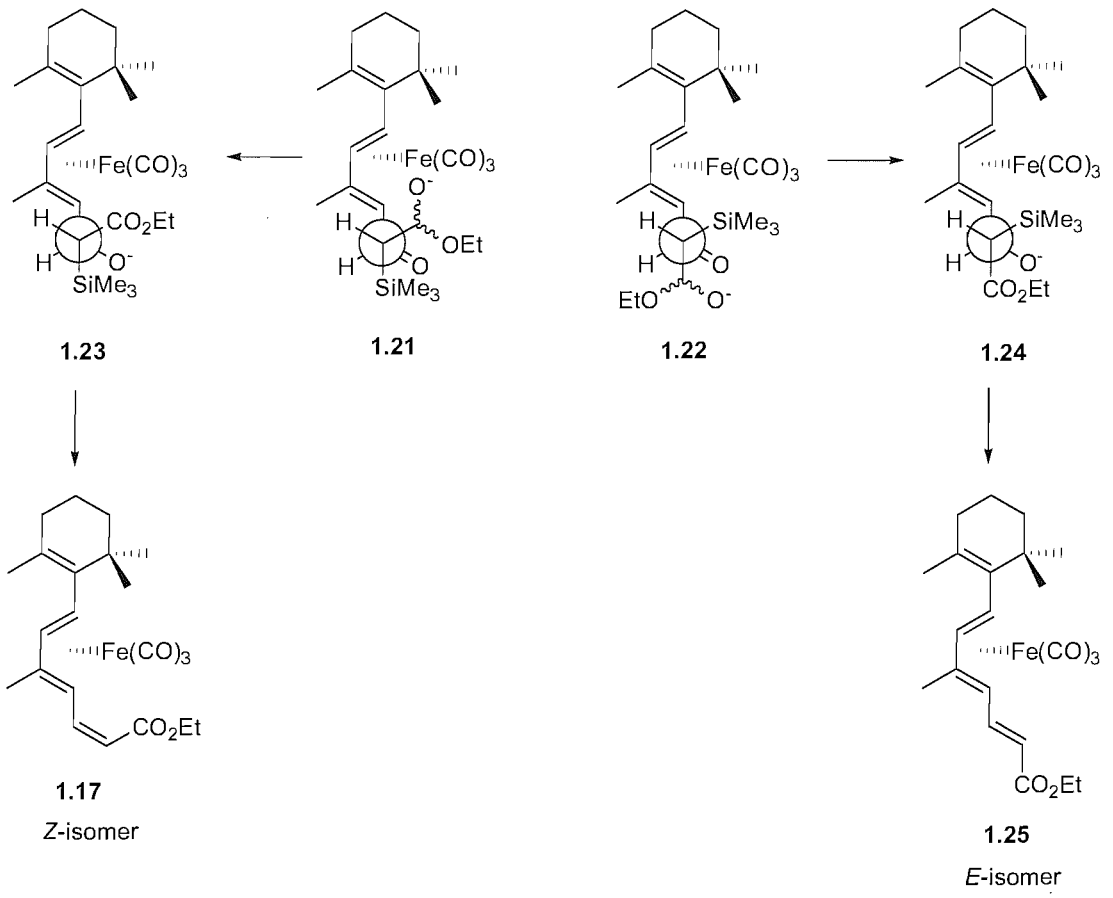
The above routes all suffered from poor yields and low 11*Z* selectivity, three novel approaches to the synthesis of 11*Z*-retinal (**1.1**) are described below exhibiting high yields and high 11*Z* selectivity.

Wada *et al.* reported the first stereoselective synthesis of 11*Z*-retinal (**1.1**) in 1996, with elaboration in 2000 (Scheme 1.4).[8,9] Wada's synthesis used a tricarbonyl iron complex in a stereoselective Peterson reaction to obtain the 11*Z* geometry. *β*-Ionylideneacetaldehyde-tricarbonyl complex **1.16** was derived from the reaction between *β*-ionone tricarbonyl complex and lithioacetonitrile followed by DIBAL-H reduction. Wada and co-workers attempted to synthesise ester **1.17** from aldehyde **1.16** using Wittig and Horner-Emmons reactions, however in these reactions the *E*-isomer was exclusively observed. They found that a Peterson reaction with aldehyde **1.16** using lithium ethyl trimethylsilylacetonoate as the olefinating agent achieved good stereoselectivity for the desired *Z*-isomer, giving ester **1.17**.



Scheme 1.4 *Reagents and conditions:* a) $EtOC(O)CH_2TMS$, LDA, THF, –78 °C; b) Ph_3SnCH_2I, *n*-BuLi, THF; c) $(^iPrO)_2P(O)CH_2CN$, NaH, THF; d) $CuCl_2$, EtOH; e) DIBAL-H, toluene.

The selectivity of the Peterson reaction is thought to derive from the steric hindrance between the C19-methyl group and carbonyl group in aldehyde **1.16**. This steric hindrance leads to the s-*trans* conformation across the C_{10}-C_{11} single bond in **1.16**. Wada *et al.* observed that the Peterson reaction with a 9-demethyl analogue of aldehyde **1.16** gave a 1:1 mixture of C11 stereoisomers, corroborating the importance of substitution at C9 for the stereoselectivity. Wada *et al.* proposed that conformer **1.21** is preferred relative to **1.22**, this is due to the undesired steric interaction between the trimethylsilyl group and the diene-tricarbonyl complex (Scheme 1.5). Therefore, reactive conformation **1.23** produces the desired *Z*-isomer *via* syn elimination.[16]

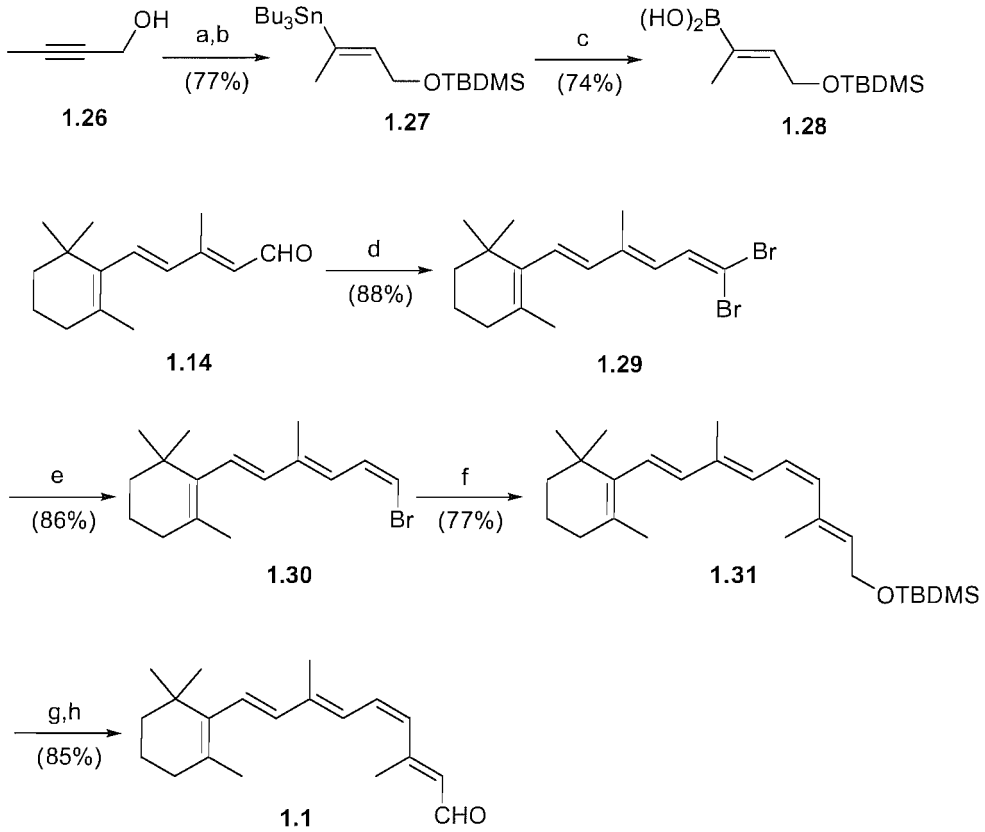


Scheme 1.5 - Peterson reaction reactive conformations

With the essential 11*Z* double bond introduced, the synthesis was completed with no isomerisation at C11. Ester **1.17** was converted smoothly to ketone **1.18** with triphenylstannylmethyllithium (Scheme 1.4). Horner-Emmons reaction with di*iso*propyl cyanomethylphosphonate and subsequent decomplexation with $CuCl_2$ gave nitrile **1.20** in good yield. 11*Z*-Retinal (**1.1**) was given by DIBAL-H reduction of the nitrile in excellent yield.

Uenishi *et al.* reported an efficient and concise synthesis of geometrically pure 11*Z*-retinal (**1.1**) in 1998 (Scheme 1.6).[7] The synthesis started with the dibromomethylation of known aldehyde **1.14** with carbon tetrabromide and triphenylphosphine giving dibromide **1.29** in high yield. Using tributyltin hydride and a catalytic quantity of $Pd(PPh_3)_4$ gave the stereoselective reduction of dibromide **1.29** which proceeded cleanly in high yield to afford vinyl bromide **1.30**. It was noted that vinyl bromide **1.30** was unstable to light or acid, but could be stored for one month in frozen benzene with a small amount of PPh_3. With vinyl bromide **1.30** prepared, boronic acid **1.28** was

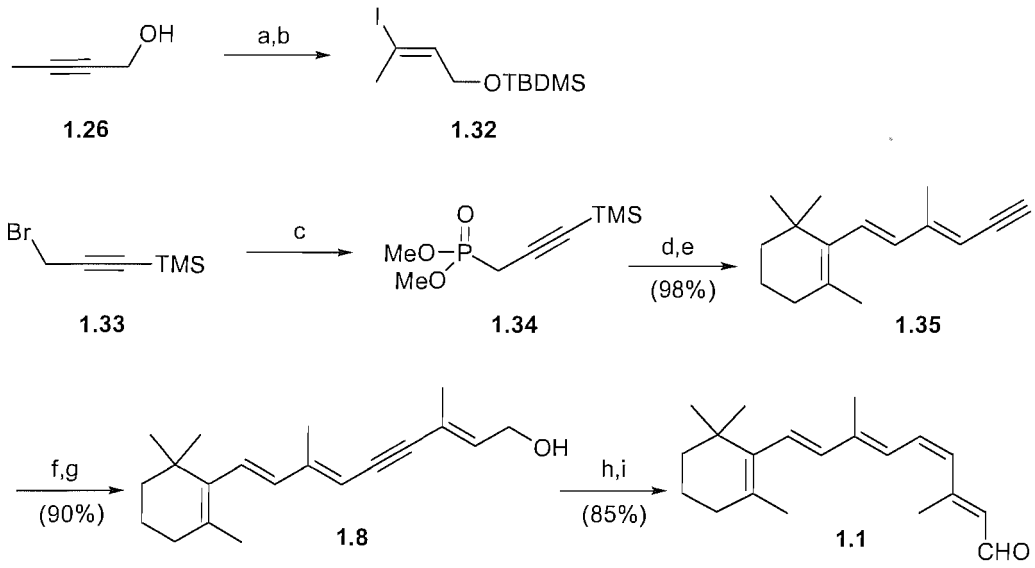
prepared to complete the retinal framework. Propargyl alcohol (**1.26**) was hydrostannylated regio and stereoselectively with tributylstannyl cuprate in 83% yield. Subsequent protection as the TBDMS silyl ether in an excellent yield of 93% gave stannane **1.27**. Transmetallation of tributylstannane **1.27** with *n*-BuLi followed by quenching with tri*iso*propyl boronate and hydrolysis with HCl, yielded boronic acid **1.28** in good yield. Suzuki coupling of fragments **1.28** and **1.30** gave isomerically pure TBDMS protected 11*Z*-retinol **1.31** in good yield. 11*Z*-Retinal (**1.1**) was obtained from TBDMS ether **1.31** by TBAF deprotection and $BaMnO_4$ oxidation, proceeding in excellent yield.



Scheme 1.6 *Reagents and conditions:* a) $(Bu_3Sn)_2CuCNLi_2$, MeOH/THF, –10 °C; b) TBDMSCl, KH, THF; c) i) *n*-BuLi, THF, –78 °C; ii) $B(O^iPr)_3$; iii) HCl; d) CBr_4, PPh_3, CH_2Cl_2, 0 °C; e) $Pd(PPh_3)_4$, Bu_3SnH, benzene; f) **1.28**, $Pd(PPh_3)_4$, KOH, Ag_2CO_3, THF; g) TBAF, THF; h) $BaMnO_4$, CH_2Cl_2.

In 1999 Borhan *et al.* reported an efficient synthesis of 11*Z*-retinal (**1.1**) utilising a zinc-mediated semi-hydrogenation to introduce the 11*Z* double bond (Scheme 1.7).[6] Borhan and co-workers attempted the synthesis of 11*Z*-retinal (**1.1**) *via* four

approaches, these being Horner-Emmons olefination, Stille coupling, Michael type high order cuprate addition and a semi-hydrogenation. The first three approaches were found to be fruitless giving predominantly 11-*E* products. The semi-hydrogenation route required the synthesis of fragments **1.32** and **1.35**. The right-hand fragment **1.32** was prepared by a method described by Sato *et al.* from propargyl alcohol (**1.26**).[17] The synthesis of the left-hand fragment **1.35** was achieved from propargyl bromide **1.33**, by conversion to phosphonate **1.34** with dimethyl phosphite as reported by Gibson and co-workers.[18] Olefination of *β*-ionone with phosphonate **1.34** proceeded in excellent yield but an undisclosed C9 *E*/*Z* ratio. Subsequent TBAF deprotection afforded terminal alkyne **1.35** in excellent yield. The Sonogashira coupling of fragments **1.32** and **1.35** with $Pd(PPh_3)_4$ and CuI in $^{i}PrNH_2$ with subsequent TBAF deprotection gave dehydroretinol **1.8** in excellent yield with no reported isomerisation. The semi-hydrogenation was attempted using various reducing systems, such as Lindlar catalyst with a number of poisons, nickel boride bound to borohydride exchange resin. These did not accomplish the selective reduction of polyene **1.8**. Borhan found a zinc-mediated procedure described by Boland *et al.* proved to be successful.[19] The procedure gave 11*Z*-retinol (**1.2**) in a high yield of 85% and an 11-*Z*/*E* ratio of 13:1. The 11*Z*-retinol (**1.2**) was then oxidised with TPAP and NMO in quantitative yield giving 11*Z*-retinal (**1.1**). The group found that using MnO_2 gave the desired 11*Z*-retinal (**1.1**) but in lower yield.



Scheme 1.7 *Reagents and conditions:* a) i) ^{i}BuMgCl, Cp_2TiCl_2, Et_2O; ii) I_2, Et_2O, −78 °C; b) TBDMSCl, Et_3N, CH_2Cl_2; c) $(MeO)_2P(O)H$, NaHMDS, THF; d) *n*-BuLi, then *β*-ionone, THF, 0 °C to rt; e) TBAF, THF; f) **1.32**, $Pd(PPh_3)_4$, CuI, $^{i}PrNH_2$; g) TBAF, THF; h) Zn, $Cu(OAc)_2$, $AgNO_3$, $^{i}PrOH/H_2O$; i) TPAP, NMO, CH_2Cl_2.

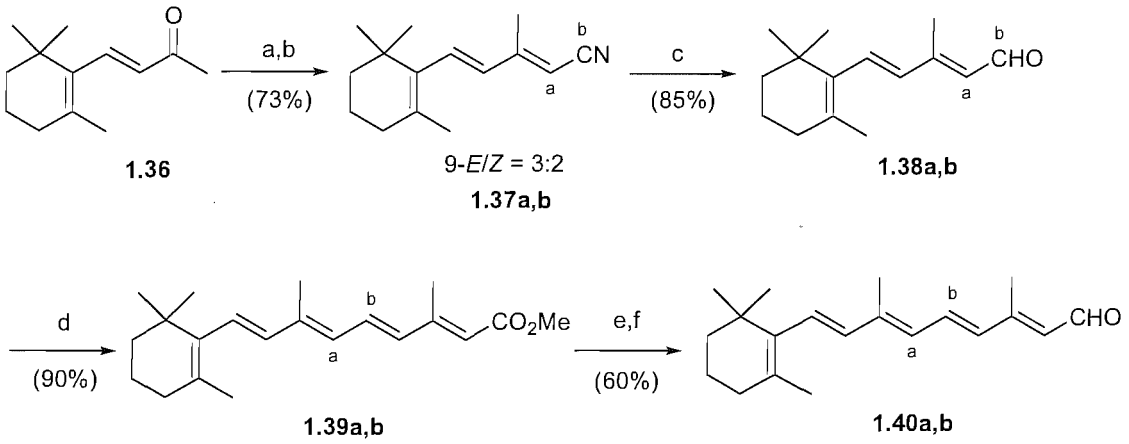
1.3 Synthesis of ^{13}C Enriched Retinals

The past syntheses of ^{13}C labelled retinals have all been of all-*E*-retinal (**1.3**). If the 11*Z* isomer was required, it was achieved by photoisomerisation of an acetonitrile solution of all-*E*-retinal (**1.3**), isolating pure 11*Z*-retinal (**1.1**) by preparative HPLC of the complex mixture of isomers. Previous syntheses of ^{13}C enriched all-*E*-retinals have predominantly been by the Lugtenburg group.[20-30]

In 1983 Pardoen *et al.* reported the synthesis of [10-^{13}C], [11-^{13}C], [19-^{13}C] and [20-^{13}C]-all-*E*-retinals (Schemes 1.8-1.10).[20] The series of mono ^{13}C labelled all-*E*-retinals were synthesised for the incorporation into bacteriorhodopsin for analysis by MAS ^{13}C NMR.

In the synthesis of [10-^{13}C] and [11-^{13}C]-all-*E*-retinals, [2-^{13}C]-acetonitrile and [1-^{13}C]-acetonitrile were utilised for ^{13}C incorporation at positions C10 and C11 respectively (Scheme 1.8). *β*-Ionylideneacetonitrile **1.37** was prepared by the condensation between labelled lithioacetonitrile and *β*-ionone (**1.36**) with subsequent dehydration with *N*-

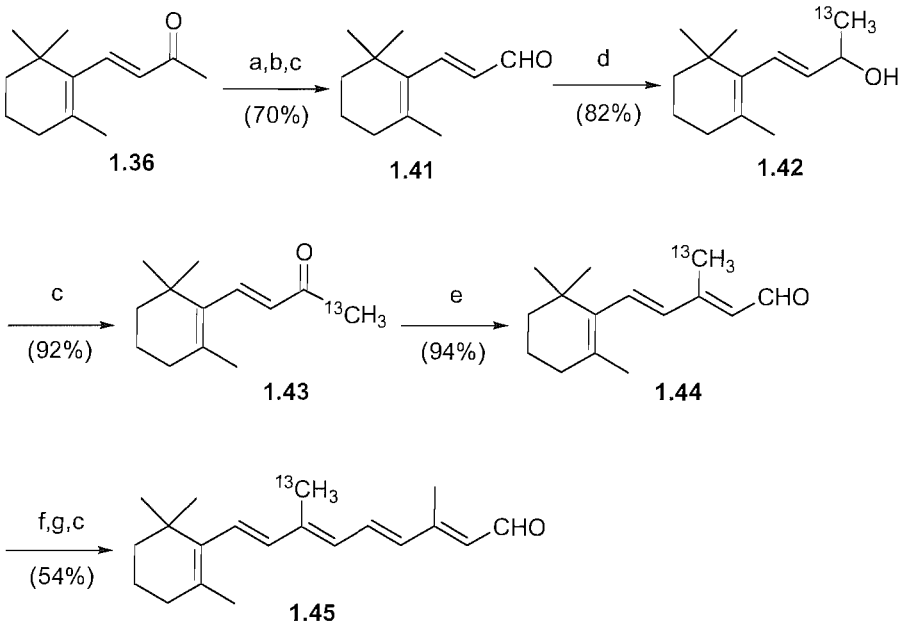
bromosuccinimide. Reduction of nitrile **1.37** with DIBAL-H and Horner-Emmons reaction with diethyl 3-(methoxycarbonyl)-2-methyl-2-propenylphosphonate gave methyl ester **1.39** as a mixture of isomers in high yield. Reduction of the mixture of methyl ester isomers **1.39** with $LiAlH_4$ and subsequent oxidation with MnO_2 afforded the desired [10-^{13}C] (**1.40a**) and [11-^{13}C]-all-*E*-retinals (**1.40b**).



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

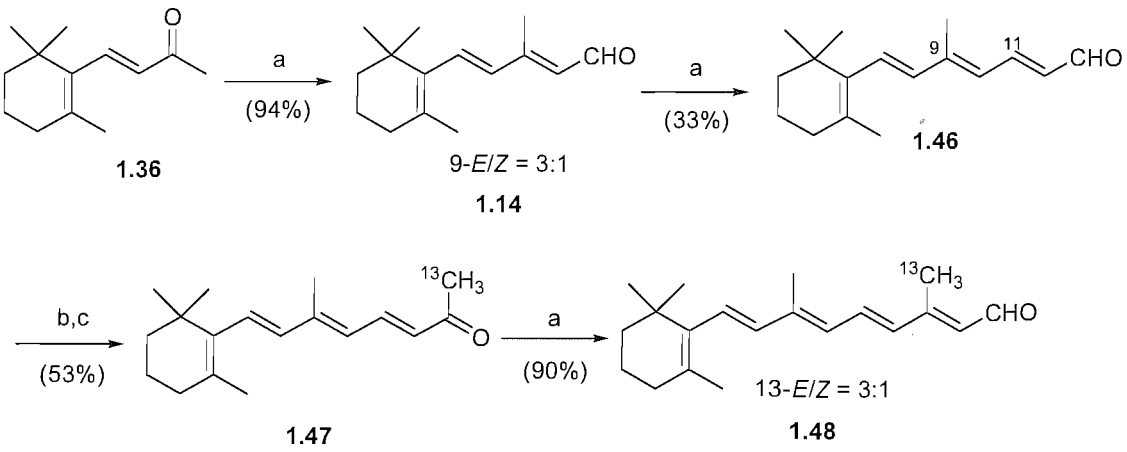
Scheme 1.8 *Reagents and conditions:* a) for **1.37a**: [2-$^{13}C_2$]-acetonitrile, for **1.37b**: [1-$^{13}C_2$]-acetonitrile, *n*-BuLi, THF, –60 °C to 0 °C; b) NBS, $C_2H_4Cl_2$, 70 °C; c) i) DIBAL-H, petroleum ether, –60 °C; ii) SiO_2, Et_2O/petroleum ether/H_2O, 0 °C; d) $(EtO)_2P(O)CH_2C(CH_3)CHC(O)OMe$, NaH, THF, 0 °C to rt; e) $LiAlH_4$, Et_2O, –40 °C to 0 °C; f) MnO_2, CH_2Cl_2.

In their synthesis of [19-^{13}C]-all-*E*-retinal (1.45) the ^{13}C label at C19 was introduced with ^{13}MeMgI which was derived from ^{13}MeI (Scheme 1.9).[20] *β*-Ionone (**1.36**) was oxidised with sodium hypochlorite, reduced with $LiAlH_4$ then oxidised to aldehyde **1.41** with MnO_2 in an overall high yield. With aldehyde **1.41** in place, the key ^{13}C introduction at C19 with ^{13}MeMgI was accomplished in high yield. *β*-Ionol **1.42** was oxidised with MnO_2, affording [19-^{13}C]-*β*-ionone (**1.43**) in excellent yield. Subsequent Peterson olefination and acidic hydrolysis gave rise to aldehyde **1.44** in excellent yield. Aldehyde **1.44** was converted to the desired [19-^{13}C]-all-*E*-retinal (**1.45**) by Horner-Emmons olefination with diethyl 3-(methoxycarbonyl)-2-methyl-2-propenylphosphonate, reduction and oxidation in good yield.



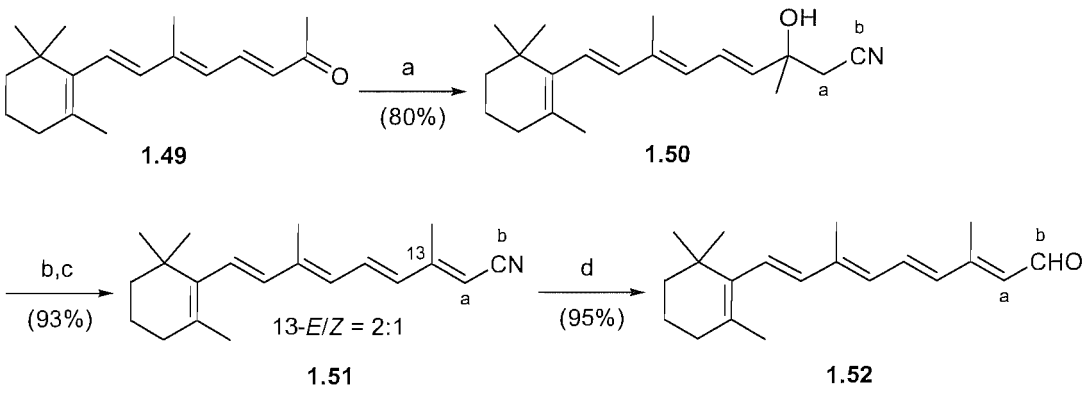
Scheme 1.9 *Reagents and conditions:* a) NaOCl, NaOH, MeOH; b) $LiAlH_4$, Et_2O, –40 °C to 0 °C; c) MnO_2, CH_2Cl_2; d) $^{13}CH_3I$, Mg, Et_2O, rt to reflux; e) i) LDA, ^{t}BuNCHCH$_2$TMS, THF, –60 °C to 0 °C; ii) HCOOH, pH 3, THF/H_2O, 0 °C; f) $(EtO)_2P(O)CH_2C(CH_3)CHC(O)OMe$, NaH, THF, 0 °C to rt; g) $LiAlH_4$, Et_2O, –40 °C to 0 °C.

Pardoen *et al.* also synthesised [20-^{13}C]-all-*E*-retinal (**1.48**), again using ^{13}MeMgI to incorporate the ^{13}C label (Scheme 1.10).[20] The synthesis started with *β*-ionone (**1.36**) using two sequential Peterson olefinations obtaining aldehyde **1.46** as a mixture of 9*E*, 11*Z*, 9,11-*Z* and all-*E* (33%) isomers. Addition of ^{13}MeMgI and oxidation with MnO_2 furnished ketone **1.47**, a further Peterson olefination produced the desired [20-^{13}C]-all-*E*-retinal (**1.48**) as a mixture of C13 *E*/*Z* isomers.



Scheme 1.10 *Reagents and conditions:* a) i) LDA, ^{t}BuNCHCH$_2$TMS, THF, –60 °C to 0 °C; ii) HCOOH, pH 3, THF/H$_2$O, 0 °C; b) ^{13}CH$_3$I, Mg, Et$_2$O; c) MnO$_2$, CH$_2$Cl$_2$.

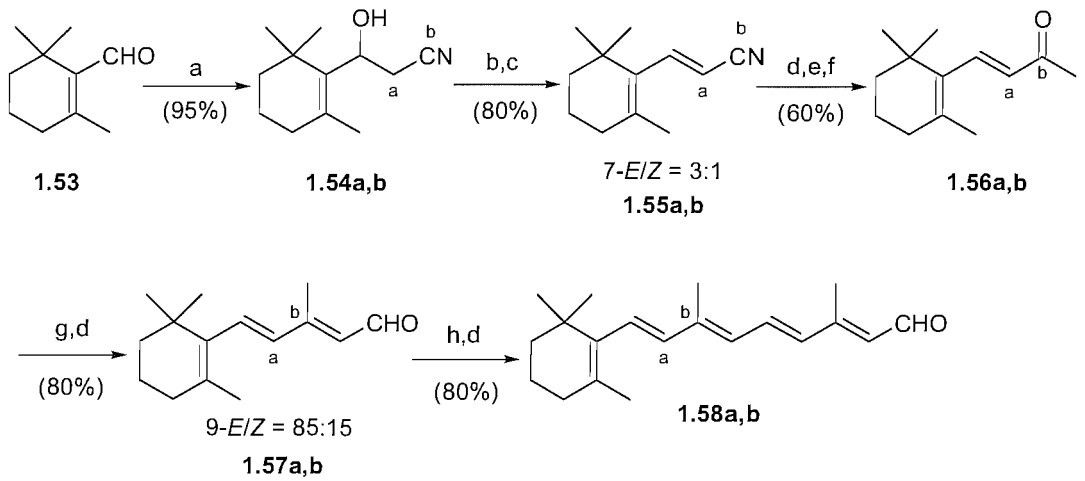
Later work by Pardoen *et al.* in 1984 described the synthesis of [14-^{13}C] (**1.52a**) and [15-^{13}C]-all-*E*-retinal (**1.52b**) using labelled acetonitrile for the enrichment (Scheme 1.11).[21] Reacting known ketone **1.49**[31] with [2-^{13}C]-lithioacetonitrile or [1-^{13}C]-lithioacetonitrile gave hydroxynitriles **1.50a** and **1.50b** respectively in good yield. Acetylation and dehydration with DBN gave nitriles **1.51a** and **1.51b** as a 2:1 mixture of 13-*E*/*Z* isomers. DIBAL-H reduction of nitriles **1.51a** and **1.51b** produced the required retinals **1.52a** and **1.52b** in excellent yield.



a = ^{13}C position for [14-^{13}C]-all-*E*-retinal synthesis
b = ^{13}C position for [15-^{13}C]-all-*E*-retinal synthesis

Scheme 1.11 *Reagents and conditions:* a) for **1.50a**: [2-^{13}C$_2$]-acetonitrile, for **1.50b**: [1-^{13}C$_2$]-acetonitrile, *n*-BuLi, THF, –60 °C to 0 °C; b) Ac$_2$O, DMAP, toluene; c) DBN, toluene, reflux; d) i) DIBAL-H, petroleum ether, –60 °C to –20 °C; ii) SiO$_2$, H$_2$O/Et$_2$O/petroleum ether, 0 °C.

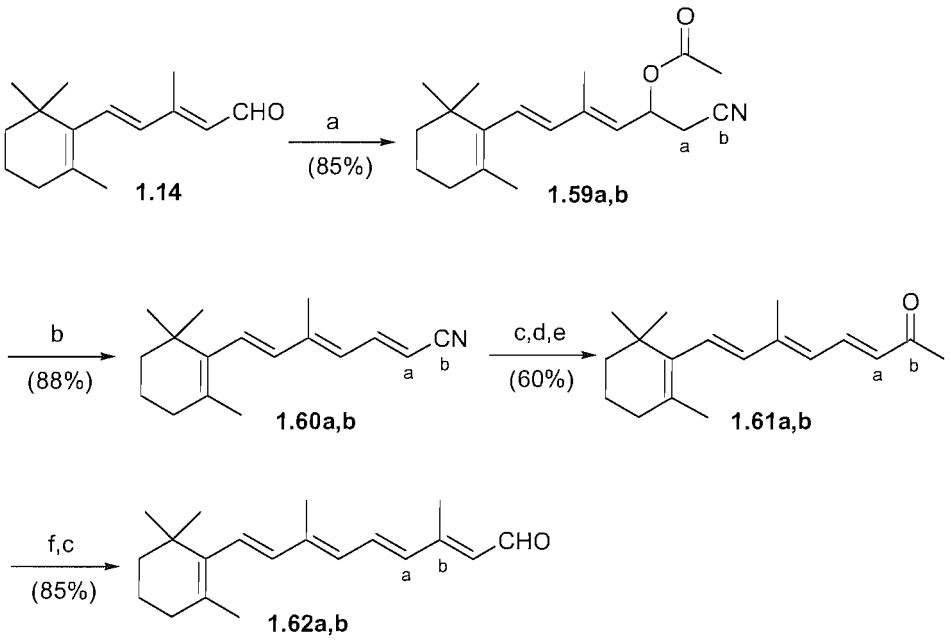
In 1985 Pardoen *et al.* synthesised [8-^{13}C] (**1.57a**), [9-^{13}C] (**1.57b**), [12-^{13}C] (**1.57c**) and [13-^{13}C]-all-*E*-retinal (**1.57d**) (Schemes 1.12 and 1.13).[23] Addition of [2-^{13}C]-lithioacetonitrile or [1-^{13}C]-lithioacetonitrile to *β*-cyclocitral **1.53** gave the formation of hydroxynitriles **1.54a** and **1.54b** respectively in excellent yield Scheme (1.12). Acetylation and dehydration with DBN afforded nitriles **1.55a** and **1.55b** as a mixture of C7 *E/Z* isomers in good yield. Nitriles **1.55a** and **1.55b** were converted in three steps to [8-^{13}C]-*β*-ionone (**1.56a**) and [9-^{13}C]-*β*-ionone (**1.56b**) respectively as pure 7*E* isomers. Horner-Emmons olefination with di*iso*propylphosphonoacetonitrile and subsequent reduction with DIBAL-H gave aldehydes **1.57a** and **1.57b** with high C9 stereoselectivity. Horner-Emmons olefination with diethyl-3-cyano-2-methylprop-2-enylphosphonate and subsequent reduction with DIBAL-H of aldehydes **1.57a** and **1.57b** afforded the desired [8-^{13}C] (**1.58a**) and [9-^{13}C] (**1.58b**) all-*E*-retinals in high yield.



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

Scheme 1.12 *Reagents and conditions:* a) for **1.54a**: [2-$^{13}C_2$]-acetonitrile, for **1.54b**: [1-$^{13}C_2$]-acetonitrile, *n*-BuLi, THF, –60 °C; b) Ac_2O, DMAP, pyridine, toluene; c) DBN, toluene; d) i) DIBAL-H, hexane/petroleum ether, –60 °C to –20 °C; ii) SiO_2, H_2O/Et_2O/petroleum ether; e) MeMgI, Et_2O, reflux; f) MnO_2, hexane; g) $(^iPrO)_2P(O)CH_2CN$, NaH, THF, 0 °C to rt; h) $(EtO)_2P(O)CH_2C(CH_3)CHCN$, NaH, THF, 0 °C to rt.

Using a similar strategy to their synthesis of [8-^{13}C] (**1.58a**) and [9-^{13}C]-all-*E*-retinals (**1.58b**) as described above (Scheme 1.12), Lugtenburg's group were able to synthesise [12-^{13}C] (**1.62a**) and [13-^{13}C]-all-*E*-retinal (**1.62b**) (Scheme 1.13).[26] Once again, [2-^{13}C]-lithioacetonitrile and [1-^{13}C]-lithioacetonitrile were utilised to incorporate the ^{13}C labels in [12-^{13}C] (**1.62a**) and [13-^{13}C]-all-*E*-retinal (**1.62a**) respectively. The intermediate hydroxynitriles were acetylated and a DBN induced acetic acid elimination gave nitriles **1.60a** and **1.60b** in high yield. The synthesis was completed in a similar manner to that in the above synthesis of [8-^{13}C] (**1.58a**) and [9-^{13}C]-all-*E*-retinal (**1.58b**), therefore achieving the synthesis of [12-^{13}C] (**1.62a**) and [13-^{13}C]-all-*E*-retinal (**1.62b**) in a good overall yield.



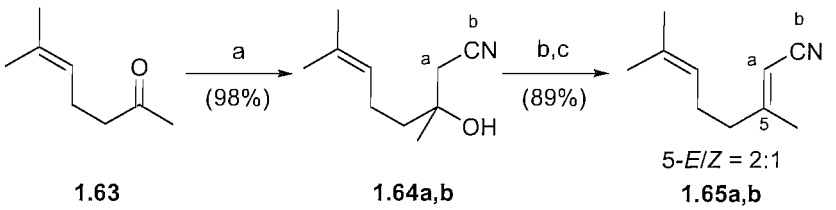
a = ^{13}C position for [12-^{13}C]-all-*E*-retinal synthesis
b = ^{13}C position for [13-^{13}C]-all-*E*-retinal synthesis

Scheme 1.13 *Reagents and conditions:* a) i) for **1.59a**: [2-$^{13}C_2$]-acetonitrile, for **1.59b**: [1-$^{13}C_2$]-acetonitrile, *n*-BuLi, THF, –60 °C to –20 °C; ii) AcCl, THF, –90 °C to 10 °C; b) DBN, toluene, reflux; c) i) DIBAL-H, hexane/petroleum ether, –60 °C to –20 °C; ii) SiO_2, H_2O/Et_2O/petroleum ether; d) MeMgI, Et_2O, reflux; e) MnO_2, hexane; f) $(^iPrO)_2P(O)CH_2CN$, NaH, THF, 0 °C to rt.

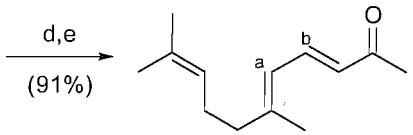
The Lugtenburg group carried out the synthesis of [5-^{13}C] (**1.76a**), [6-^{13}C] (**1.76b**), [7-^{13}C] (**1.76c**) and [18-^{13}C]-all-*E*-retinal (**1.76d**) (Schemes 1.14-1.16).[32] Introducing ^{13}C

labels into the cyclohexane ring and at C7 of *β*-ionone (**1.36**) made synthetic access to this series of labelled retinals more challenging. To produce ^{13}C enriched *β*-ionones **1.74a-d**, precursor ketones **1.66a,b** and **1.73c,d** had firstly to be prepared (Schemes 1.14 and 1.15).

For the construction of ketones **1.66a,b**, [2-^{13}C]-lithioacetonitrile and [1-^{13}C]-lithioacetonitrile were utilised for ^{13}C incorporation at C6 and C7 (Scheme 1.14). Using the group's standard methods, nitriles **1.65a** and **1.65b** were assembled from enone **1.63** by addition of labelled lithioacetonitrile, acetylation and elimination. This gave the desired nitriles **1.65a** and **1.65b** in excellent yield but poor *E/Z* stereoselectivity at C5. This lack of stereocontrol did not pose a problem, as in the acid catalysed cyclisation of enones **1.66a,b** led to an isomerisation at C5 giving the correct stereochemistry (Scheme 1.16). Nitriles **1.65a,b** were reduced with DIBAL-H in excellent yield, followed by aldol condensation of the resulting aldehydes with acetone to furnish *β*-ionone precursors **1.66a,b** also in excellent yields (Scheme 1.14).



1.63 **1.64a,b** **1.65a,b**



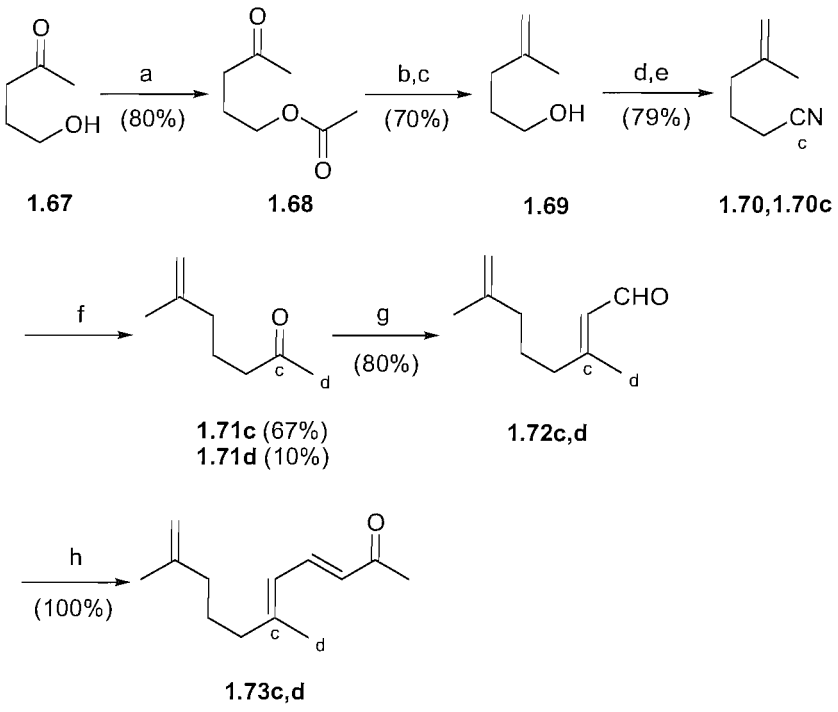
5-*E*/*Z* = 2:1

1.66a,b

a = ^{13}C position for [6-^{13}C]-all-*E*-retinal synthesis
b = ^{13}C position for [7-^{13}C]-all-*E*-retinal synthesis

Scheme 1.14 *Reagents and conditions:* a) for **1.64a**: [2-$^{13}C_2$]-acetonitrile, *n*-BuLi, THF, –60 °C to 0 °C; for **1.64b**: [1-$^{13}C_2$]-acetonitrile, *n*-BuLi, THF, –60 °C to 0 °C; b) Ac_2O, DMAP, toluene; c) DBN, toluene, reflux; d) i) DIBAL-H, petroleum ether, –60 °C to –20 °C; ii) SiO_2, H_2O/petroleum ether, 0 °C; e) NaOH, acetone.

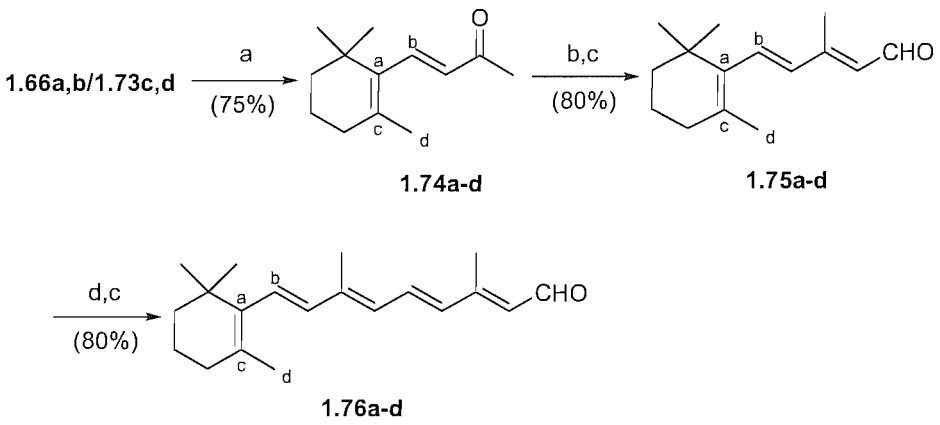
For the ^{13}C incorporation at C5 and C18, β-ionone precursor ketones **1.73c,d** had to be prepared (Scheme 1.15). Commercially available 5-hydroxy-2-pentanone (**1.67**) was protected with acetic anhydride prior to Wittig olefination with triphenylmethylphosphonium iodide and subsequently deprotected with KOH giving alcohol **1.69** in good yield. Post tosylation, the resulting tosylate was treated with KCN or $K^{13}CN$ yielding nitriles **1.70** and **1.70c** respectively. For the ^{13}C incorporation at C18, nitrile **1.70** was treated with an excess of $^{13}MeMgI$. This proved to be a convenient route for ^{13}C incorporation at C18, however the reaction was poor yielding. Labelled nitrile **1.70c** was treated with an excess of MeLi leading to ketone **1.71c** in a respectable yield. Peterson olefination of ketones **1.71c** and **1.71d,** followed by aldol condensation with acetone gave *iso*-pseudo-ionones **1.73c** and **1.73d** in high yields.



c = ^{13}C position for [5-^{13}C]-all-*E*-retinal synthesis
d = ^{13}C position for [18-^{13}C]-all-*E*-retinal synthesis

Scheme 1.15 *Reagents and conditions:* a) Ac_2O, pyridine, toluene; b) $(Ph)_3P^+CH_3I^-$, *n*-BuLi, THF, –30 °C to rt; c) KOH, MeOH; d) TsCl, pyridine, < 20 °C; e) for **1.70**: KCN; for **1.70c**: $K^{13}CN$, NaI, DMSO; f) for **1.71c**: MeLi, Et_2O, 0 °C to rt; for **1.71d**: $^{13}CH_3I$, Mg, Et_2O, 0 °C to 35 °C; g) i) LDA, ^{t}BuNCHCH$_2$TMS, THF, –60 °C to 0 °C; ii) HCOOH, pH 3, THF/H_2O, 0 °C; h) NaOH, acetone.

With pseudo-ionones **1.66a**, **1.66b** and *iso*-pseudo-ionones **1.73c, 1.73d** in hand, their conversion to labelled *β*-ionones **1.74a-d** was accomplished by treatment with H_2SO_4 in good yield (Scheme 1.16). As in their earlier work, completion of the synthesis was achieved by two consecutive sets of sequential Horner-Emmons olefinations and DIBAL-H reductions.



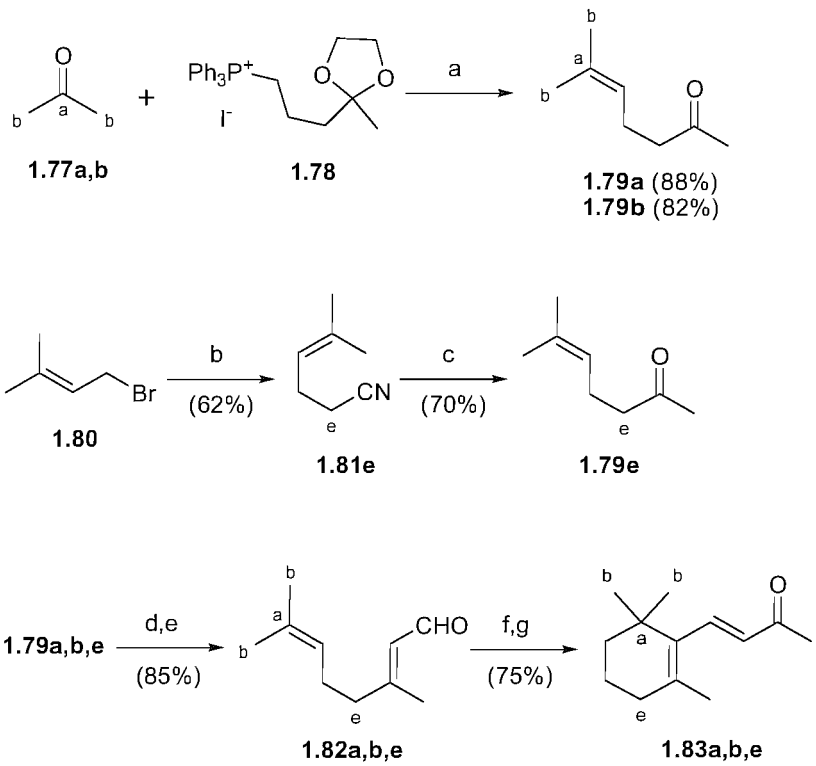
a = ^{13}C position for [6-^{13}C]-all-*E*-retinal synthesis
b = ^{13}C position for [7-^{13}C]-all-*E*-retinal synthesis
c = ^{13}C position for [5-^{13}C]-all-*E*-retinal synthesis
d = ^{13}C position for [18-^{13}C]-all-*E*-retinal synthesis

Scheme 1.16 *Reagents and conditions:* a) H_2SO_4, $MeNO_2$, 0 °C; b) $(^iPrO)_2P(O)CH_2CN$, NaH, THF, 0 °C to rt; c) i) DIBAL-H, hexane/petroleum ether, –60 °C to –20 °C; ii) SiO_2, H_2O/Et_2O/petroleum ether; d) $(EtO)_2P(O)CH_2C(CH_3)CHCN$, NaH, THF, 0 °C to rt.

In 1989 the Lugtenburg group reported the synthesis of further all-*E*-retinals with ^{13}C enrichment in the cyclohexene ring at positions 1, 2, 3, 4, 16 and 17 (Schemes 1.17-1.19).[24] These isomers were synthesised to act as tools for the investigation of rhodopsin and bacteriorhodopsin by MAS ^{13}C NMR.

The first part of the synthesis was to prepare ^{13}C labelled *β*-ionones **1.83a-e** as key intermediates in the synthesis of this series of retinal isotopomers (Scheme 1.17). With respect to the synthesis of *β*-ionones **1.83a,b,e**, ketones **1.79a,b,e** were initially formed. The Wittig reaction between phosphonium iodide **1.78** and [2-^{13}C]-acetone (**1.77a**) or [1,3-$^{13}C_2$]-acetone (**1.77b**), followed by dioxolane deprotection afforded ketones **1.79a** and **1.79b** respectively in high yield. For the ^{13}C incorporation at C4, ketone **1.79e** was

prepared by the S_N2 reaction of bromide **1.80** and [2-^{13}C]-lithioacetonitrile in good yield. The formed nitrile **1.81e** was then converted to ketone **1.79e** with an excess of MeLi. With ketones **1.79a,b,e** in place they were subjected to a Horner-Emmons olefination before the intermediate nitriles were reduced with DIBAL-H affording citrals **1.82a,b,e** in excellent yield. Aldol condensation with acetone and treatment with concentrated H_2SO_4 gave the key β-ionones **1.83a,b,e** in high yield.

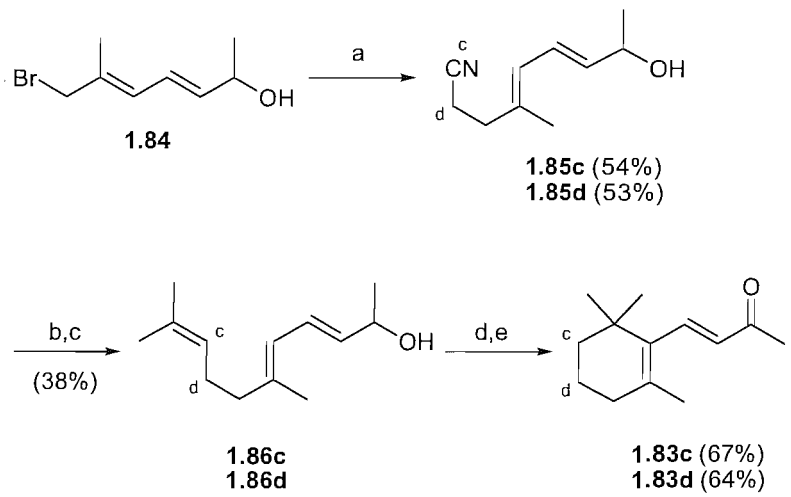


a = ^{13}C position for [1-^{13}C]-all-*E*-retinal synthesis
b = ^{13}C position for [16,17-$^{13}C_2$]-all-*E*-retinal synthesis
e = ^{13}C position for [4-^{13}C]-all-*E*-retinal synthesis

Scheme 1.17 *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, 0 °C to rt; e) i) DIBAL-H, petroleum 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.

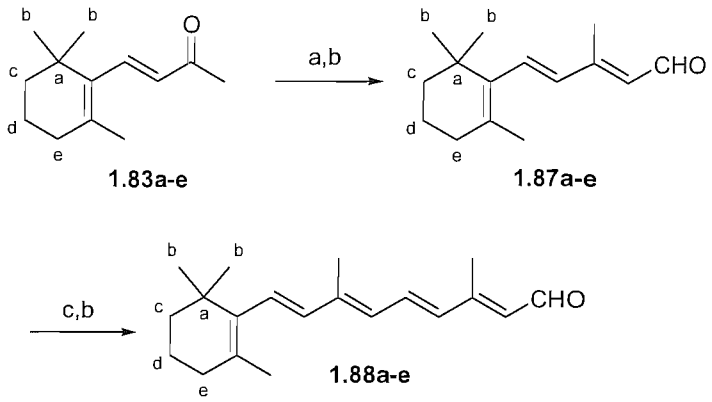
Incorporation at C2 and C3 position required an alternative route to reach β-ionones **1.83c,d** (Scheme 1.18). Alcohol **1.84** was deprotonated with LDA prior to the S_N2 reaction with [1-^{13}C]-lithioacetonitrile or [2-^{13}C]-lithioacetonitrile furnishing nitriles

1.85c and **1.85d** respectively. Reduction of the nitrile group afforded an intermediate aldehyde, which subsequently was olefinated with *iso*propyltriphenylphosphonium iodide giving access to trienols **1.86c** and **1.86d**. MnO_2 oxidation and treatment with concentrated H_2SO_4 afforded [2-^{13}C]-β-ionone (**1.83c**) and [3-^{13}C]-β-ionone (**1.83d**).



Scheme 1.18 *Reagents and conditions:* a) i) **1.84**, LDA, THF, –85 °C; ii) for **1.85c**: [1-^{13}C]-acetonitrile, for **1.85d**: [2-^{13}C]-acetonitrile, *n*-BuLi, THF, –85 °C to < –60 °C; b) i) DIBAL-H, Et_2O/light petroleum ether, –70 °C to –30 °C; ii) AcOH, –70 °C to rt; c) $(Ph)_3P^+CH(CH_3)_2I^-$, *n*-BuLi, THF, 0 °C to rt; d) MnO_2, hexane; e) H_2SO_4, $MeNO_2$, 0 °C.

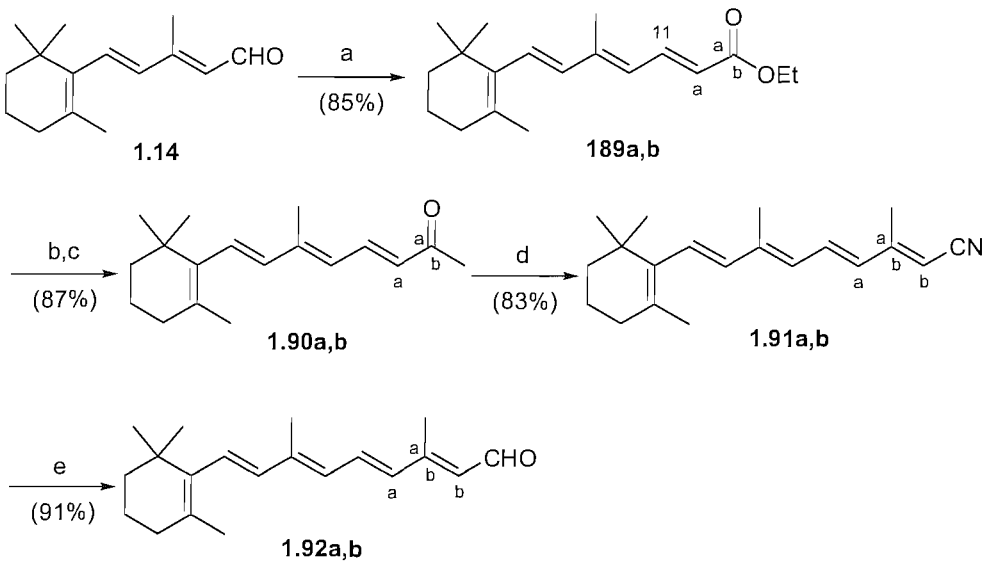
The β-ionone isotopomers **1.83a-e** were converted to [1-^{13}C], [16,17-^{13}C], [2-^{13}C], [3-^{13}C] and [4-^{13}C]-all-*E*-retinals (**1.88a-e**) respectively as previously discussed (Scheme 1.19).



a = ^{13}C position for [1-^{13}C]-all-*E*-retinal synthesis
b = ^{13}C position for [16,17-$^{13}C_2$]-all-*E*-retinal synthesis
c = ^{13}C position for [2-^{13}C]-all-*E*-retinal synthesis
d = ^{13}C position for [3-^{13}C]-all-*E*-retinal synthesis
e = ^{13}C position for [4-^{13}C]-all-*E*-retinal synthesis

Scheme 1.19 *Reagents and conditions:* a) $(EtO)_2P(O)CH_2CN$, NaH, THF, 0 °C to rt; b) i) DIBAL-H, petroleum ether, –60 °C to –20 °C; ii) SiO_2, H_2O/Et_2O/petroleum ether; c) $(EtO)_2P(O)CHC(CH_3)CHCN$, NaH, THF, 0 °C to rt.

Groesbeek *et al.* synthesised [12,13-$^{13}C_2$] (**1.92a**) and [13,14-$^{13}C_2$]-all-*E*-retinals (**1.92b**) for the measurement of internuclear distances in bacteriorhodopsin and rhodopsin by MAS ^{13}C NMR (Scheme 1.20).[26] The synthesis started from the known aldehyde **1.14**. Horner-Emmons olefination of **1.14** with [1,2-$^{13}C_2$]-diethyl phosphonoacetate and [1-^{13}C]-diethyl phosphonoacetate gave ester **1.89a** and **1.89b** respectively as a mixture of isomers at C11. Conversion of the esters to Weinreb amides and reaction with MeLi afforded ketones **1.90a** and **1.90b** in high yield. Ketones **1.90a** and **1.90b** were olefinated with diethyl phosphonoacetonitrile and [2-^{13}C]-diethyl phosphonoacetonitrile respectively. These reactions gave nitriles **1.91a** and **1.91b** in high yield. The [2-^{13}C]-diethyl phosphonoacetonitrile was prepared from [2-^{13}C]-acetonitrile and diethyl chlorophosphate. DIBAL-H reduction of the nitriles gave the desired [12,13-$^{13}C_2$] (**1.92a**) and [13,14-$^{13}C_2$]-all-*E*-retinal (**1.92b**) in excellent yields.

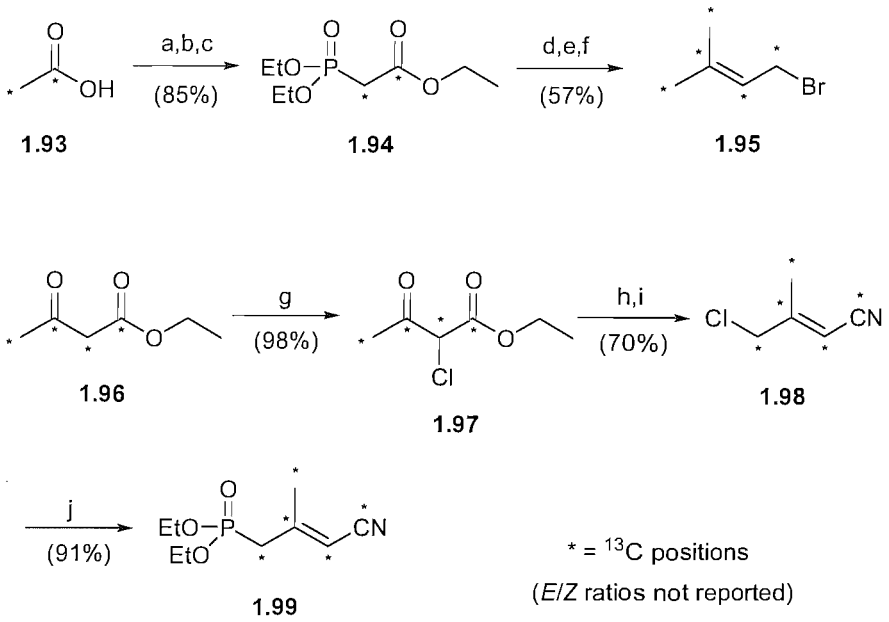


a = ^{13}C position for [12,13-$^{13}C_2$]-all-*E*-retinal synthesis
b = ^{13}C position for [13,14-$^{13}C_2$]-all-*E*-retinal synthesis

(*E*/*Z* ratios not reported)

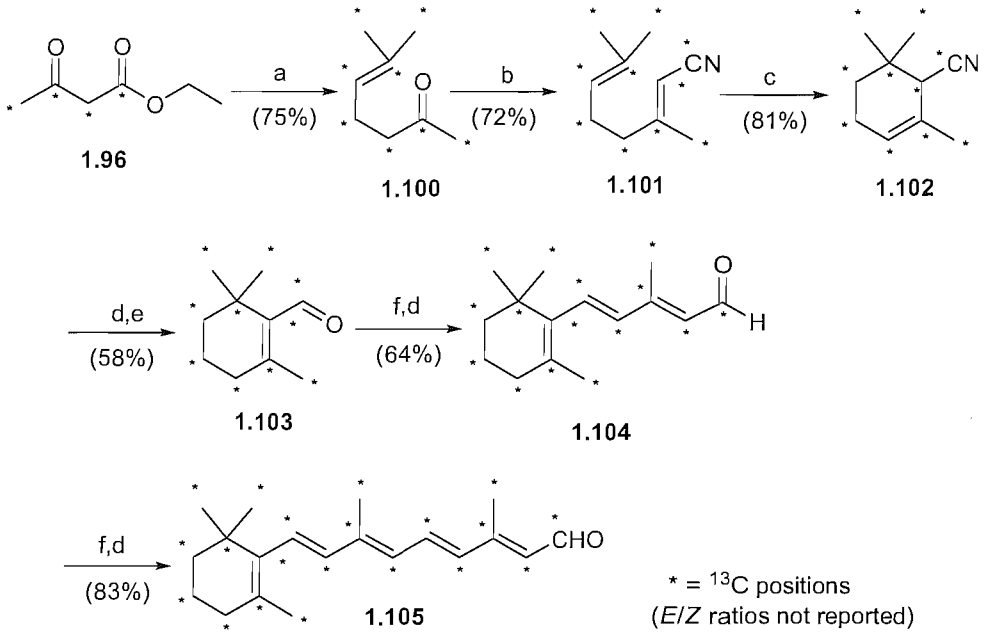
Scheme 1.20 *Reagents and conditions:* a) for **1.89a**: [1,2-$^{13}C_2$]-diethyl phosphonoacetate; for **1.89b**: [1-^{13}C]-diethyl phosphonoacetate, *n*-BuLi, THF, 0 °C to rt; b) MeONHMe, *n*-BuLi, THF, 0 °C to rt; c) MeLi, THF, –20 °C to 0 °C; d) for **1.91a**: $(EtO)_2P(O)CH_2CN$, *n*-BuLi, THF, 0 °C to rt; for **1.91b**: LDA, [2-^{13}C]-acetonitrile, $(EtO)_2P(O)Cl$, THF, –60 °C to 0 °C; e) i) DIBAL-H, petroleum ether, –60 °C; ii) SiO_2, H_2O/petroleum ether, 0 °C.

In 2002 the Lugtenburg group published an impressive synthesis of [U-$^{13}C_{20}$]-all-*E*-retinal (**1.105**) (Scheme 1.22).[30] For the synthesis, two labelled reagents **1.95** and **1.99** firstly had to be constructed (Scheme 1.21). [1,2-$^{13}C_2$]-Acetic acid was converted to [1,2-$^{13}C_2$]-triethyl phosphonoacetate (**1.94**) in three steps in an overall yield of 85%. The phosphonate **1.94** was reacted with universally labelled acetone, reduced and converted to bromide **1.95**. The synthesis of phosphonate **1.99** was also required. Its synthesis was achieved by the chlorination of [1,2,3,4-$^{13}C_4$]-ethyl acetoacetate (**1.96**) followed by acid hydrolysis and decarboxylation to [1,2,3-$^{13}C_3$]-chloroacetone in high yield. Horner-Emmons reaction between the formed [1,2,3-$^{13}C_3$]-chloroacetone and [1,2-$^{13}C_2$]-diethyl cyanomethylphosphonate gave chloride **1.98** in high yield. Arbuzov reaction with triethyl phosphite afforded the required phosphonate **1.99** in excellent yield.



Scheme 1.21 *Reagents and conditions:* a) Br_2, TFAA; b) $(COCl)_2$ then EtOH, 0 °C to rt; c) $P(OEt)_3$, 180 °C; d) [1,2,3-$^{13}C_3$]-acetone, *n*-BuLi, THF, 0 °C to rt; e) i) DIBAL-H, light petroleum ether, −60 °C; ii) SiO_2, H_2O/light petroleum ether, 0 °C; f) HBr, CH_2Cl_2, 0 °C; g) SO_2Cl_2, 0 °C; h) H_2SO_4, THF/H_2O, reflux; i) LDA, [$^{13}C_2$]-acetonitrile, $(EtO)_2P(O)Cl$, THF, −60 °C to rt; j) $P(OEt)_3$, 180 °C.

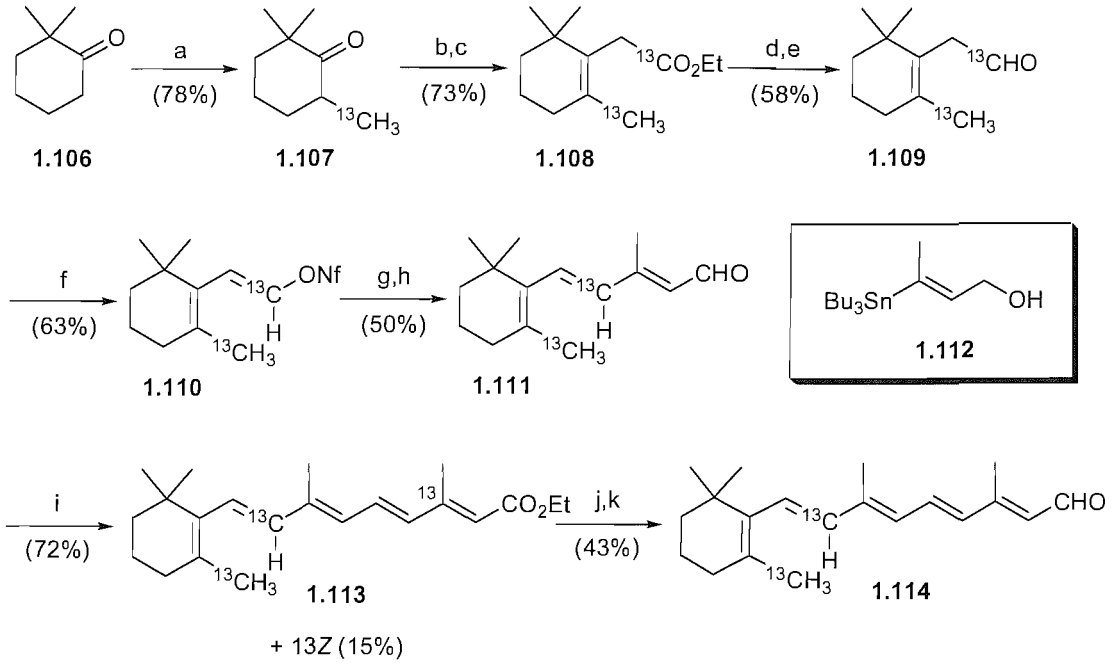
With labelled reagents **1.95** and **1.99** in place the synthesis of [U-$^{13}C_{20}$]-all-*E*-retinal (**1.105**) could proceed (Scheme 1.22). The synthesis was carried out predominately as in their previous syntheses of ^{13}C labelled retinals, discussed above. This is with the exception of the construction of labelled enone **1.100**. This was carried out from [1,2,3,4-$^{13}C_4$]-ethyl acetoacetate, which was deprotonated and reacted with bromide **1.95**. The resulting *β*-keto ester was saponified and subsequently subjected to acid catalysed decarboxylation, giving enone **1.100** in high yield.



Scheme 1.22 *Reagents and conditions:* a) i) **1.95**, NaOEt, EtOH, 0 °C to reflux; ii) 10% NaOH, rt to 60 °C; iii) H^+ b) LDA, [$^{13}C_2$]-acetonitrile, $(EtO)_2P(O)Cl$, THF, –60 °C to rt; c) H_2SO_4, $MeNO_2$, 0 °C; d) i) DIBAL-H, light petroleum ether, –60 °C to –40 °C; ii) SiO_2, H_2O/light petroleum ether, 0 °C; e) 5% KOH, MeOH, 0 °C; f) **1.99**, *n*-BuLi, THF, 0 °C to rt.

In 2005 Wada *et al.* reported their highly stereoselective synthesis of [8,18-$^{13}C_2$]-all-*E*-retinal (**1.114**), to be used as a investigative tool to determine the conformation of the cyclohexene ring (6-s-*cis* or 6-s-*trans*) in phoborhodopsin (Scheme 1.23).[33]

The introduction of the ^{13}C label at position C18 was achieved by the ^{13}C methylation of dimethyl cyclohexanone **1.106**. For the introduction of the ^{13}C at C8, [18-^{13}C]-2,6,6-trimethyl cyclohexanone (**1.107**) was reacted with the enolate of [1-^{13}C]-ethyl acetate and then dehydrated with thionyl chloride producing ester **1.108**. $LiAlH_4$ reduction, oxidation and conversion to the nonaflate **1.110** gave access to the key Stille reaction with stannane **1.112** affording aldehyde **1.111**. With aldehyde **1.111** in hand, olefination with triethyl phosphonocrotonate lead to ester **1.113** as an 83:17 mixture of 13-*E*/*Z* isomers. The simple reduction and oxidation of ester **1.113** as a mixture of isomers furnished [8,18-$^{13}C_2$]-retinal (**1.114**) in a modest yield of 43%, with the all-*E* isomer isolated by HPLC.



Scheme 1.23 *Reagents and conditions:* a) LDA, $^{13}CH_3I$, THF; b) LDA, [1-^{13}C]-EtOAc, THF, −78 °C; c) $SOCl_2$, pyridine, 0 °C; d) $LiAlH_4$, Et_2O, 0 °C; e) ^{i}PrMgBr, azocarbonyldipiperidine, Et_2O; f) ^{t}BuOK, nonafluorobutanesulfonyl fluoride, THF, reflux; g) **1.112**, $Pd_2(dba)_3.CHCl_3$, Ph_3As, DMF; h) MnO_2, CH_2Cl_2; i) $(EtO)_2P(O)CH_2CHCHCOOEt$, *n*-BuLi, THF, −78 °C; j) $LiAlH_4$, Et_2O, 0 °C; k) MnO_2, CH_2Cl_2.

1.4 Determination of Structure

Various methods have been used for the structural determination of bovine rhodopsin, including electron cyromicroscopy, X-ray crystallography and ^{13}C solid state NMR.

1.4.1 11*Z*-Retinal Crystal Structure

In 1954 Dieterle *et al.* reported their isolation of 11*Z*-retinal (**1.1**) and all-*E*-retinal (**1.3**) crystals.[34] Their procedure began with the photoisomerisation of all-*E*-retinal (**1.3**) into a photostationary isomeric mixture. The isomeric mixture was concentrated and purified by column chromatography on sodium silico-aluminate, achieving a yellow filtrate. This filtrate was concentrated then dissolved in petroleum ether, on cooling to

−18 °C a mixture of 11*Z*-retinal (**1.1**) as orange prisms and all-*E*-retinal (**1.3**) as yellow needles were obtained. The crystals were separated manually giving pure crystalline 11*Z*-retinal (**1.1**).

Gilardi *et al.* determined the crystal structure of 11*Z*-retinal (**1.1**) in 1972.[35] The crystal structure shows the configuration of 11*Z*-retinal (**1.1**) as being C6-C7 s-*cis* and C12-C13 s-*cis*. The configuration was originally thought to be C6-C7 s-*cis* and C12-C13 s-*trans*. The geometry of the C6-C7 bond has also been established and confirmed by Fujimoto *et al.* as 6-s-*cis* by incubation of 6-s-locked retinoids with opsin.[36]

1.4.2 Rhodopsin Structure

Rhodopsin is currently the best model for the GPCR family, as it is the only GPCR to have been crystallised.[37] Therefore, there has been great interest into investigating the protein structure and the retinylidene chromophore of rhodopsin. This is to gain a greater understanding of how the protein environment accelerates and directs the specific isomerisation.

Since 2002 there have been a number of reviews on rhodopsin that have been published by Stenkamp *et al.*, Filipek *et al.* and most recently in 2006 by Palczewski.[5,38,39] The reviews discuss the discovery, function and predominantly the elucidation of the structure of rhodopsin.

1.4.2.1 Rhodopsin X-Ray Crystal Structure

The three-dimensional structure of bovine rhodopsin was determined in 2000 by Palczewski *et al.*, this is the first detailed high-resolution crystal structure of a GPCR.[37] The original data was obtained at a resolution of 2.8 Å, which was later refined in 2001, indicating more amino acid residues than in the original work.[40] In 2002 a 2.6 Å resolution structure was reported by Okada *et al.* showing seven water molecules within the structure, with two being located close to the chromophore binding site. The

above data sets were of tetragonal $P4_1$ crystals, in 2004 new purification techniques allowed Li *et al.* to determine the structure of bovine rhodopsin in a trigonal $P3_1$ crystal form at a resolution of 2.65 Å.[41] With this data they were able to compare the two crystal forms. The main structural differences between the crystals are the orientation of the C2 loop, conformation of the C3 loop, the lengths of H5 and H6 and the C-terminal fragment. It is thought that the trigonal crystal represents the native receptor more closely than the tetragonal crystal structure.

The highest resolution crystal structure to date was reported by Okada *et al.* in 2004 at a resolution of 2.2 Å (Figure 1.3).[42] With a new crystallisation technique it allowed the improved resolution, which resulted in the full resolution of the complete polypeptide chain. The model also gave further information on the C6-C7 single bond conformation of the chromophore.

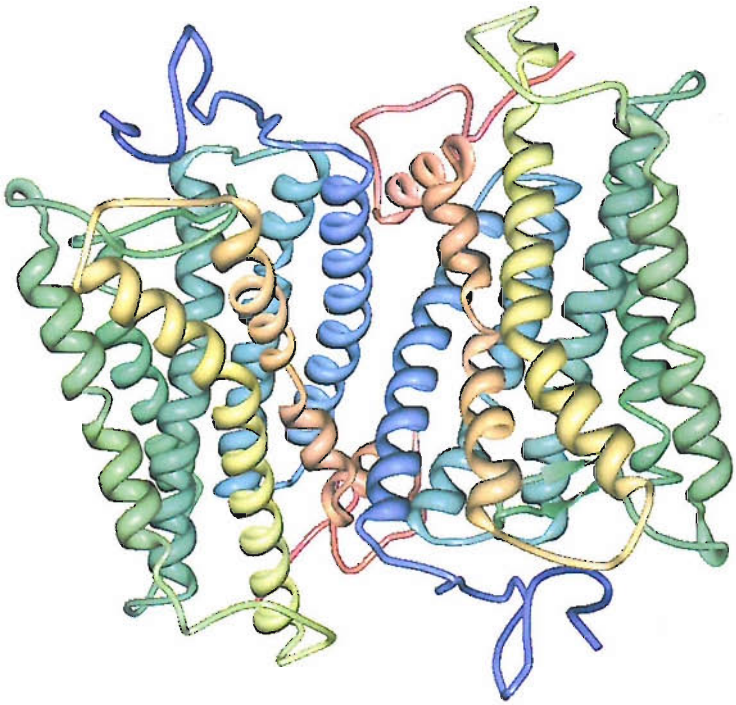


Figure 1.3 - Crystal structure of rhodopsin dimmer: H1 dark blue; H2 light blue; H3 green blue; H4 green; H5 yellow green; H6 yellow; H7 orange (created with The Molecular Biology Toolkit).[43]

The key features of the bovine rhodopsin crystal structure are that its overall shape is an elliptical cylinder with the seven transmembrane helices varying in length between 20

to 33 residues with the exhibition of kinking. The intra and extracellular regions are evenly distributed with 65% of the amino acids located within the transmembrane region.

The 11*Z*-retinylidene chromophore is located between the seven transmembrane helices, offset towards the extracellular region of the membrane. The chromophore is non-planar and is twisted due to packing and steric interactions within the protein. Hydrophobic phenylalanine residues are located around the β-ionone ring, with the side chain of tryptophan 265 located between the Schiff base and the β-ionone ring causing the chromophore to bend. The retinal binding pocket consists of the alpha helices of the transmembrane region and of a plug section formed by an anti-parallel β-sheet from extracellular loop II. In the protonated form of the chromophore, glutamic acid 113 acts as the counterion for the protonated Schiff base, this interaction stabilises the Schiff base inhibiting its spontaneous hydrolysis.[44] Okada and co-worker's 2.2 Å crystal structure determined the configuration of the chromophore within bovine rhodopsin to be C6-C7 s-*cis* with a substantial negative twist and C12-C13 as s-*trans*.[45]

1.4.2.2 Rhodopsin Electron Cyromicroscopy

The most recent rhodopsin structure determined by electron cyromicroscopy was reported by Krebs *et al.* in 2003.[46] The group achieved a three-dimensional density map from two-dimensional crystals with $p22_12_1$ symmetry. With refinement they were able to obtain the most accurate density map to date, at a resolution of 5.5 Å in the membrane plane and ~13 Å perpendicular to it. Electron cryomicroscopy has the advantage over three-dimensional crystals of gaining information about the centre of the membrane plane and the orientation of the molecule relative to the bilayer.

1.4.2.3 Rhodopsin Solid State ^{13}C NMR

^{13}C NMR of rhodopsin has been carried out with single, double and uniformly labelled retinylidene chromophores to investigate configurations, conformations, internuclear distances, torsional angles and chromophore-protein interactions. The use of solution state NMR was found not to give much success. As an example, Millet *et al.* were only able to detect membrane lipids due to low rotational correlation time giving broadened protein resonances beyond detection.[47] Using [14-^{13}C]-all-*E*-retinal Shriver *et al.* carried out solution NMR where they detected mostly released [14-^{13}C]-all-*E*-retinal.[48] With the use of magic angle spinning (MAS) solid state NMR these issues were resolved giving clearer signals and spectra. To increase the resolution of the spectra further, a double-quantum (DQ) filter was used (Figure 1.4).[49] The DQ filtering works by suppressing ^{13}C signals which are uncoupled, therefore enhancing the signals from the $^{13}C_2$-retinylidene ligand.

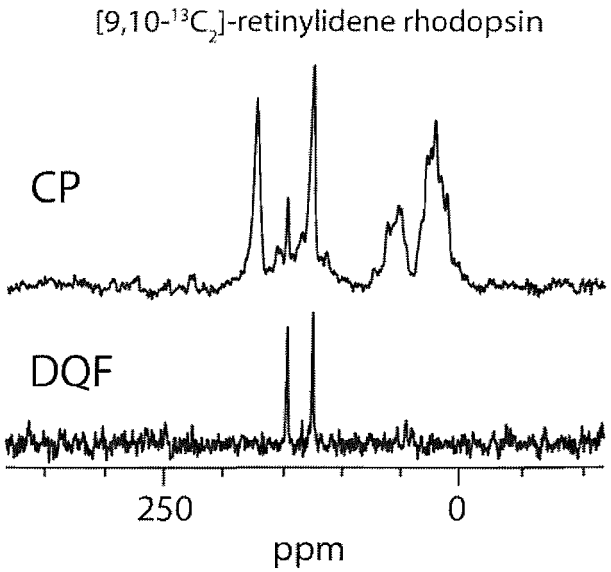


Figure 1.4 - MAS ^{13}C NMR spectra of [9,10-$^{13}C_2$]-retinylidene rhodopsin, at a spinning frequency of 10.00 kHz, a field of 9.4 T, and a temperature of 170 K. (CP) Cross-polarization spectrum. (DQF) Double-quantum-filtered spectrum showing the peaks from the ^{13}C labels.

Past MAS solid state NMR experiments of singly and doubly ^{13}C labelled retinylidene chromophores have focused on the conformation of the C6-C7 single bond. Low-temperature experiments measuring the isotropic chemical shifts and chemical shift

tensor values of a [5-^{13}C]-11*Z*-retinylidene in ground state rhodopsin reported the bond to be 6-s-*cis* by comparison with retinal derivatives in 6-s-*trans* and 6-s-*cis* conformations.[50,51] Mollevanger *et al.* carried out similar work to the work described above, reaching the same result.[52]

Spooner *et al.* used [8,18-$^{13}C_2$]-retinylidene and [8,16/17-$^{13}C_2$]-retinylidenes to measure the internuclear distances in the chromophore. From their results they were able to deduce that the chromophore has a twisted 6-s-*cis* conformation. The distance between C8 and C18 as 2.95 Å with a torsional angle between C5-C6-C7-C8 of –28 ± 7°, confirming previous reports (Figure 1.5).[53]

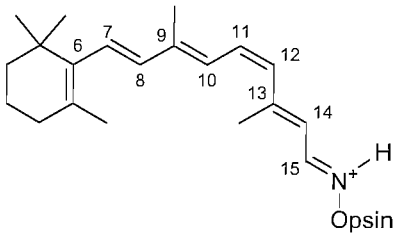


Figure 1.5 - Retinylidene chromophore.

Gröbner *et al.* reported conflicting results from their ^{2}H NMR experiments.[54,55] They reported the 6-s bond to be in the 6-s-*trans* conformation as in bacteriorhodopsin (Figure 1.6). However this data has been met by general scepticism from other groups who have reported the 6-s-*cis* configuration.

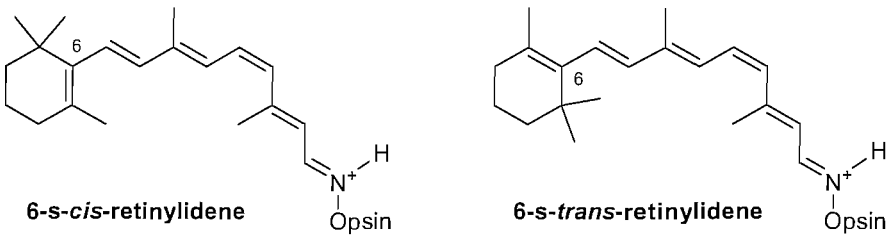


Figure 1.6 - 6-s-*cis* and 6-s-*trans* 11*Z*-retinylidene conformations.

Smith *et al.* also identified the protonated Schiff base to be in the *anti* configuration by comparing the isotropic chemical shifts and chemical shift tensor results of rhodopsin with all-*E* and 11-*Z* retinal PSB models (*anti*) and a bacteriorhodopsin sample (*syn*).

The values fitted with the *anti* configuration values, agreeing with Raman and Fourier transform infrared spectroscopy.[56,57]

Creemers *et al.* published the complete ^{1}H and ^{13}C assignments of the 11*Z*-retinylidene chromophore.[58] In doing so, they established that the normally equivalent carbons C16 and C17 in the protein environment become non-equivalent, with the C16 methyl positioned equatorially and C17 methyl group axially. Their data also suggested a non-planar 12-s bond in the chromophore chain.

In 2001 Carravetta *et al.* investigated a series of $^{13}C_2$ labelled chromophores in rhodopsin to measure adjacent bond lengths between C8-C15.[59] They also measured the H-C10-C11-H torsional angle to be 160 ± 10° indicating a significant deviation form the planar 10-11-s-*trans* conformation.

As well as conformations and configuration analysis, protein interactions were examined by a number of groups.[52,58,60,61] Reports have confirmed the localisation of a complex counterion for the PSB of the chromophore, this has been identified as residue Glu-113 by X-ray crystallography.[51,60]

Creemers *et al.* using results from rhodopsin with an uniformly labelled retinylidene chromophore described non-bonding interactions between positions C16,17,18 of the chromophore and residues Phe-208 and Phe-212 and Trp-265 of the protein environment.[58]

In 2005, use of uniformly labelled retinal and the use of MAS SIDY (selective interface detection spectroscopy) technique achieved a ^{1}H-^{13}C heteronuclear correlation spectra of Rhodopsin.[62] Kiihne and co-workers method allowed them to remove the ligand-ligand interactions so that they were able to observe only $^{1}H_{GPCR}$-$^{13}C_{lig}$ interactions. This allowed them to produce a model of the closest amino acid contacts in the chromophore binding pocket.

1.4.3 Photointermediate Analysis

1.4.3.1 Bathorhodopsin

Bathorhodopsin is the first of the photointermediates from the illumination of rhodopsin (Figure 1.2). MAS NMR[63] and X-ray crystallography[64] have been used to examine the structure of bathorhodopsin and the chromophore. In 1991 Smith *et al.* reported their MAS NMR studies of bathorhodopsin at <135 K, utilising mono labelled retinals to measure the isotropic chemical shifts at positions 8, 10-14 and 15.[63] To gain insight into bathorhodopsin they compared the isotropic chemical shifts of bathorhodopsin, all-*trans*-retinyl-*n*-butylimmonium chloride, rhodopsin and isorhodopsin. From the data they determined that the C=N continued to be in the *anti* configuration as in rhodopsin, positions 12-15 were observed to be barely effected by the isomerisation, their overall comment was that the electrostatic protein-retinal interactions stay essentially the same as in rhodopsin in the areas examined.

In 2006 Okada *et al.* published the first structural view of bathorhodopsin by X-ray crystallography.[64] Their data was collected from frozen crystals with or without illumination at 95 K to allow them to compare rhodopsin and bathorhodopsin. They found that the β-ionone ring undergoes some movement and the major changes are unsurprisingly around the C11-C12 double bond. They also highlighted the methyl group at C19 as a key group in the stabilisation of 11*Z*-retinylidene and the isomerisation process due to the presence of the residues Thr118, Ile189, Tyr191 and Tyr268 being within 4.5 Å of C19.

1.4.3.2 Lumirhodopsin

Okada *et al.* in 2006 reported a X-ray crystallographic model of lumirhodopsin at a resolution of 2.8 Å.[64] The data shows the movement of the β-ionone ring towards helices III and IV. With regards to the retinylidene chain, it is observed to take a nearly complete all-*trans* conformation. Even though the ring has flattened to some extent, the C9-C10 and C11-C12 double bonds are still twisted.

1.4.3.3 Metarhodopsin I

Metarhodopsin I has been investigated by electron microscopy[65] and MAS NMR[66,67]. Schertler in 2005 published his electron microscopy map of two-dimensional crystals of metarhodopsin I.[65] The three publications all report that the β-ionone ring of the chromophore is still held in a position similar to the ground state[65-67] and the chain of the retinylidene is reported to have achieved an overall relaxed all-*E* structure.[66]

1.4.3.4 Metarhodopsin II

MAS NMR and, most recently, X-ray crystallography have investigated the active state of rhodopsin, metarhodopsin II.[68-70] These investigations have elucidated that the major changes in the protein are in the H5, H6, H7 regions of the transmembrane helices. Cross-polarised MAS NMR experiments by Spooner *et al.* used [16,17-$^{13}C_2$]-retinal to probe the β-ionone ring interactions in metarhodopsin II.[68] Their work reported that the steric interactions that differentiate C16 and C17 became even stronger in the active form, indicating a possible hydrophobic activation switch of the aromatic cluster around the ring of helix 6. In 2004 Patel *et al.* published their high-resolution NMR measurements between C12, C14, C15, C19 and C20 ^{13}C mono labelled retinals and specific ^{13}C labelling on tyrosine, glycine, serine and threonine in the retinal binding site.[69] Their results, as with Spooner and co-workers suggest motion in H5, H6 and H7. They observed a large rotation at C20 and translation towards H5, disrupting the interactions of H5, H6 and H7 with the H1-H4 core leading to receptor activation. They proposed that interactions between the β-ionone ring and His-211 in H5, move the H5 helix into an active orientation. H6 is thought to undergo an outward rotation due to the translation of the chromophore towards H5 by interaction between Trp-265 and, Lys-296 or Ala-295 side chains. The large rotation of C20 towards extracellular loop 2 coupled with the translation of the retinal towards H5 is consistent with the proposed counterion switch from Glu-113 on H3 to Glu-181 on extracellular loop 2 in metarhodopsin I.[71] In 2006 Salom published the first crystal structure of metarhodopsin II at a resolution of 4.15 Å, overcoming previous post illumination difficulties of the loss of diffraction of the crystals.[70] From their work they proposed that upon

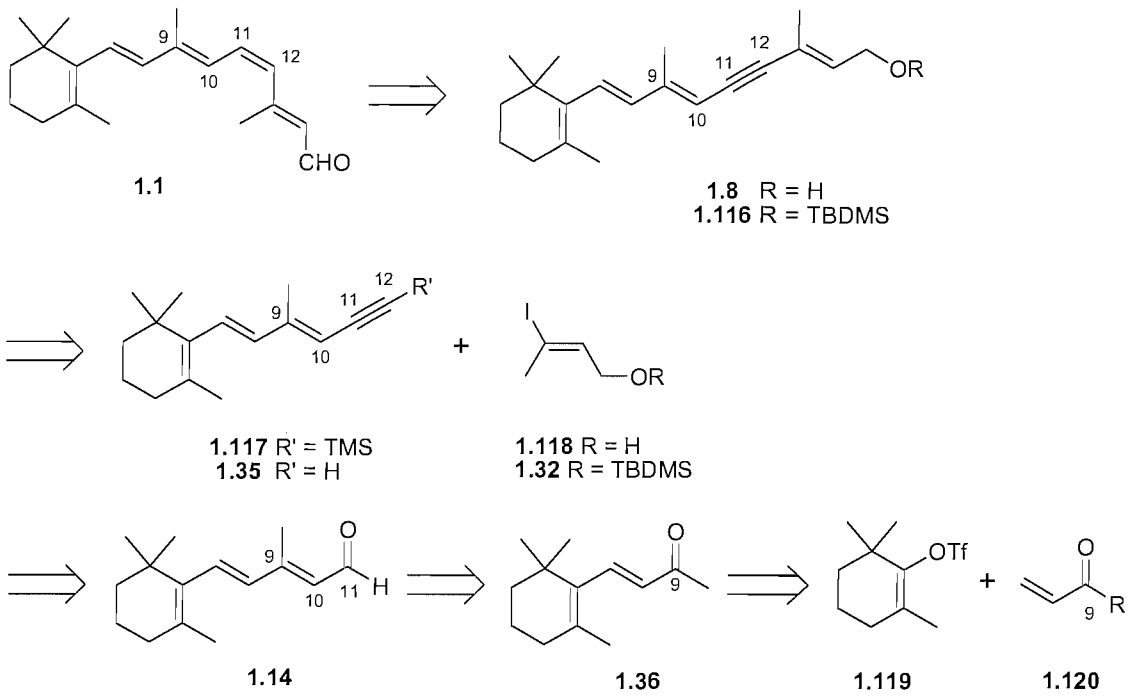
photoactivation the structural changes are less than previously suggested by calculations.[72] With photoactivation, rhodopsin relaxes and loses its counterion and the cytoplasmic loops become flexible allowing the binding to transducin.

1.5 Project Objectives

To allow the Levitt group to continue their investigations into the 11*Z*-retinylidene chromophore of rhodopsin and later the photointermediates of rhodopsin, a series of $^{13}C_2$-11*Z*-retinals were to be synthesised. The series of $^{13}C_2$-11*Z*-retinals once condensed with opsin, would allow the measurement of internuclear distances, torsional angles, *J* couplings and dipolar couplings using methodology developed in their group. With these measurements, further understanding of the conformation chromophore and of the isomerisation mechanism will be gained.[59,73,74]

1.6 11*Z*-Retinal (1.1) Synthetic Strategy

Our planned route to 11*Z*-retinal (**1.1**) is based on the coupling of key fragments **1.35/1.117** and **1.118/1.32** to afford dehydroretinols **1.8/1.116** (Scheme 1.24). Once the dehydroretinol (**1.8**) is prepared, the 11*Z*-double bond will be introduced with a zinc mediated selective semi-hydrogenation.[6] Subsequent oxidation will afford 11*Z*-retinal (**1.1**). Alkyne fragments **1.35/1.117** were to be derived from aldehyde **1.14** by homologation. To incorporate a ^{13}C label at C12, a labelled homologating reagent will be required. Aldehyde **1.14** will be construction from *β*-ionone (**1.36**) by Horner-Emmons olefination, therefore labelling at positions C10 and C11 will require labelled phosphonates in the synthesis. The introduction of a ^{13}C label at C9 will require a *β*-ionone (**1.36**) synthesis, this will be achieved by Heck coupling of triflate **1.119** and an acrylate equivalent **1.120**.



Scheme 1.24 - Retrosynthetic analysis of 11*Z*-retinal (**1.1**).

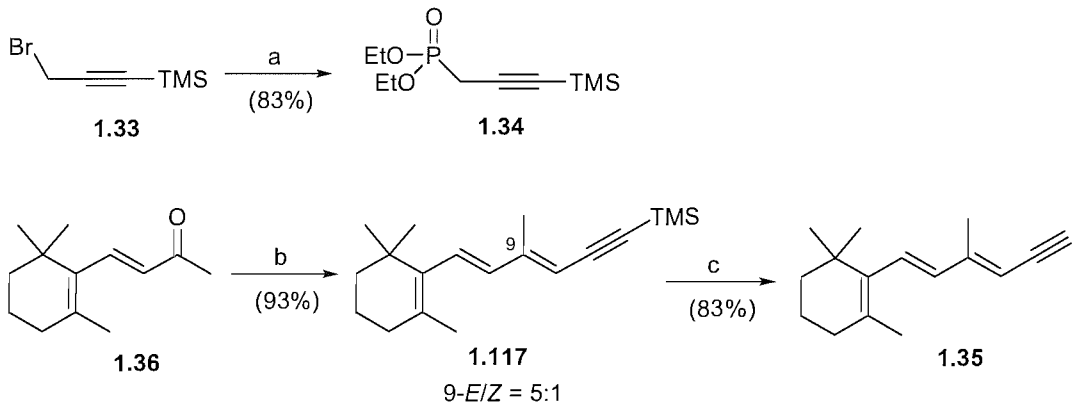
The following chapter will describe implementation of the routes discussed above to the synthesis of [9,10-$^{13}C_2$], [10,11-$^{13}C_2$] and [11,12-$^{13}C_2$]-11*Z*-retinals.

Chapter 2 Synthesis of 11*Z*-Retinal and $^{13}C_2$-11*Z*-Retinals

2.1 Unlabelled 11*Z*-Retinal Synthesis

An initial unlabelled synthesis of 11*Z*-retinal (**1.1**) was conducted to investigate various pathways to key intermediates **1.117**, **1.35**, **1.118** and **1.32**. Firstly, the synthesis of the alkyne fragments **1.117** and **1.35** was investigated. These fragments were to be used in a silyl cross-coupling or a Sonogashira cross-coupling respectively (Scheme 2.1).

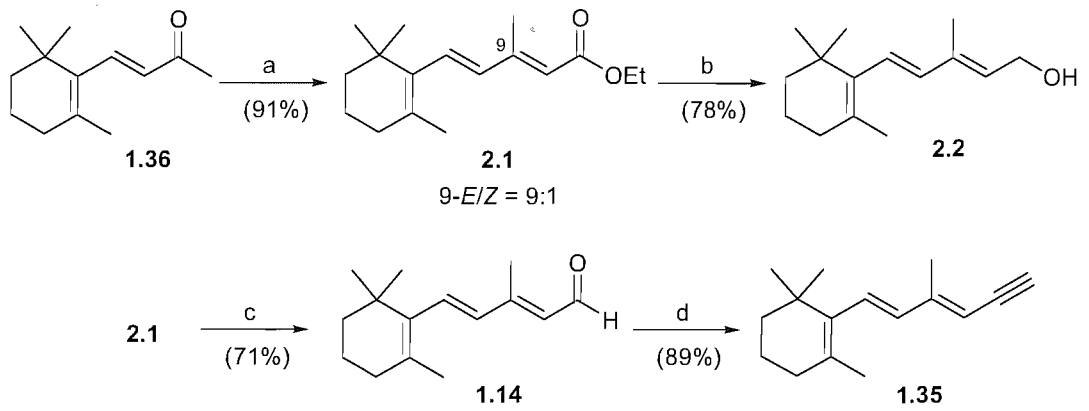
2.1.1 Synthesis of Alkyne Fragment



Scheme 2.1 *Reagents and conditions:* a) NaHMDS, $(EtO)_2P(O)H$, THF, –10 °C to rt; b) **1.34**, *n*-BuLi, THF, 0 °C to rt; c) TBAF, THF.

Phosphonate **1.34** was prepared by a method reported by Gibson *et al.* in a high and reproducible yield (Scheme 2.1).[18] Subsequent reaction between phosphonate **1.34** and *β*-ionone (**1.36**) gave desired TMS alkyne **1.117** in excellent yield and an *E*/*Z* ratio at the 9-10 double bond identical to that reported in the literature.[6] Using more of Borhan's methodology TMS alkyne **1.117** was smoothly desilylated with TBAF giving a second alkyne fragment **1.35** in good yield.[6] The synthetic route was simple and gave high to excellent yields, however the moderate *E*/*Z* ratio (5:1) produced in the Horner-Emmons reaction was considered too low for the synthesis of the ^{13}C labelled isotopomers. In addition, the separation of the stereoisomers was found to be difficult due to the low polarity of alkynes **1.117** and **1.35**. However, the use of preparative

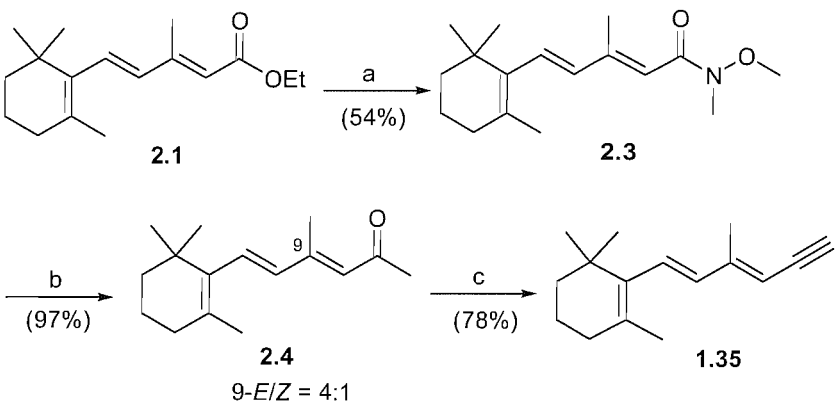
HPLC allowed separation of the isomers at the TMS stage. Preparative HPLC on a 1 g scale was considered undesirable. Owing to these findings, new routes to alkyne **1.35** were investigated in the hope to find a more effective synthesis that did not require preparative HPLC purification.



Scheme 2.2 *Reagents and conditions:* a) NaH, $(EtO)_2P(O)CH_2C(O)OEt$, Et_2O; b) $LiAlH_4$, THF, –78 °C to rt; c) i) $LiAlH_4$, Et_2O, –78 °C to rt; ii) TPAP, NMO, MS (4 Å), CH_2Cl_2; d) $TMSCHN_2$, LDA, THF, –78 °C to rt.

A second pathway was adopted, based on methodology reported by Bergen *et al.* and later by Tanumihardjo, permitting the efficient production of aldehyde **1.14** (Scheme 2.2).[75,76] Formation of ester **2.1** using the method described by Bergen and co-workers gave an excellent yield and improved *E/Z* selectivity.[75] In addition, separation of the stereoisomers was achievable by flash column chromatography. The solvent and base were modified in an effort to discover whether the *E/Z* selectivity could be improved further. Changing the solvent to THF was found to decrease the selectivity and yield, as did changing the base to NaHMDS or LiHMDS. After this limited search it was decided that the original conditions were acceptable for the synthesis of $^{13}C_2$ labelled retinals. Reduction of ester **2.1** to alcohol **2.2** was found to proceed in good yield.[75] It also was observed that alcohol **2.2** could undergo decomposition on purification by column chromatography. Due to this discovery, the oxidation to aldehyde **1.14** was carried out on crude alcohol **2.2**. The oxidation of crude alcohol **2.2** was then examined using three methods. The first procedure used $BaMnO_4$ as the oxidising agent, the reaction was found to be sluggish, requiring 65 hours at room temperature, affording aldehyde **1.14** in moderate yield (43%). The second method used activated MnO_2 as the oxidising agent.[76] This procedure required 24 hours at room temperature, furnishing

aldehyde **1.14** in a 62% yield. The final procedure used TPAP and NMO as the oxidising agents, requiring only 30 minutes at room temperature for complete oxidation in an improved yield of 71%. The direct transformation of ester **2.1** to aldehyde **1.14** was also attempted, using DIBAL-H in toluene and quenching at –78 °C. This led only to complete reduction, giving alcohol **2.2** with no observed aldehyde **1.14**.



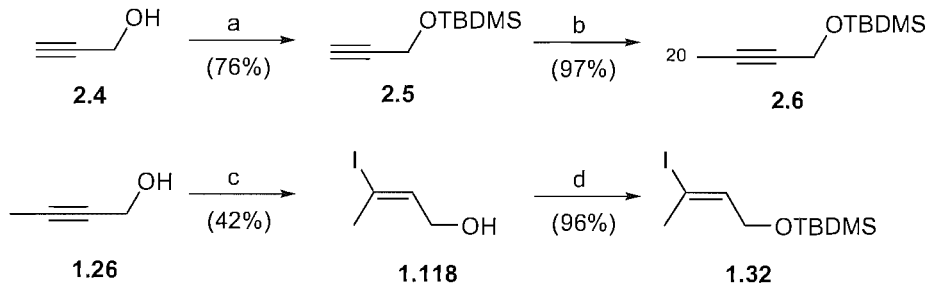
Scheme 2.3 *Reagents and conditions:* a) MeNHOMe.HCl, *n*-BuLi, THF, –25 °C to rt; b) MeLi, THF, –78 °C to –20 °C; c) i) LDA, THF, –78 °C; ii) $(EtO)_2P(O)Cl$, –78 °C to rt; iii) LDA, –78 °C to rt.

A modified sequence for the conversion of **2.1** to **1.35** was investigated (Scheme 2.3). The first step in the route involved the substitution of the ethoxy group with *N,O*-dimethylhydroxylamine, providing Weinreb amide **2.3** in moderate yield. The unoptimised reaction was found to produce a number of unidentifiable by-products, giving the disappointing yield. Methylation with MeLi advanced rapidly and in excellent yield, although isomerisation of the 9-10 double bond occurred under the reactions work-up conditions, leading to a 4:1 *E/Z* ratio. The isomerisation is thought to have occurred on acidic work up. The protonation of the ketone allowed a 1,4-reversible conjugated addition of a nucleophile, leading to the isomerisation. The mixture of ketone isomers **2.4** were converted into the desired alkyne **1.35** *via* an intermediate vinyl phosphate. The reaction gave alkyne **1.35** in good yield and did not produce any further isomerisation of the 9-10 double bond. The above route was deemed inappropriate due to the low yielding Weinreb amide formation and the extensive isomerisation during methylation.

Of the three routes described, the second route (Scheme 2.2) proved to be the most appropriate synthetic path to alkyne **1.35** for the application to the synthesis of $^{13}C_2$ labelled 11*Z*-retinals.

2.1.2 Synthesis of C_{13}-C_{15} Iodide Fragment

Attention then turned to the synthesis of iodide fragments **1.118** and **1.32,** which would be suitable for cross-coupling with alkyne fragments **1.117** or **1.35** respectively.



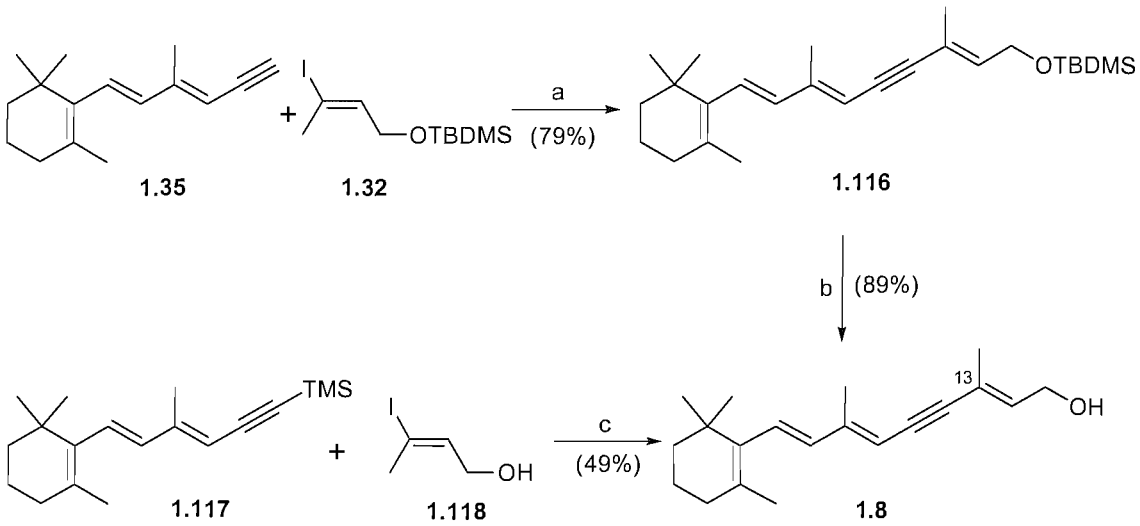
Scheme 2.4 *Reagents and conditions:* a) TBDMSCl, DMAP, Et_3N, CH_2Cl_2, 0 °C to rt; b) i) *n*-BuLi, THF, –70 °C; ii) MeI, THF, –70 °C to rt; c) i) ^{i}BuMgCl, Cp_2TiCl_2, Et_2O, –5 °C to rt; ii) I_2, Et_2O/THF, –70 °C to rt; d) TBDMSCl, DMAP, Et_3N, CH_2Cl_2, 0 °C to rt.

TBDMS protection of propargyl alcohol (**2.4**) proceeded in good yield (Scheme 2.4). After protection, methylation with MeI proceeded smoothly in excellent yield. This reaction could allow efficient access to ^{13}C incorporation at C20 with ^{13}MeI.

Two methods for the regioselective iodination of 2-butyn-1-ol (**1.26**) were investigated. Treatment of 2-butyn-1-ol (**1.26**) with $LiAlH_4$ and NaOMe in refluxing THF followed by addition of I_2 in THF at –70 °C gave an overall yield of 59%. Unfortunately, the product was obtained as an 8:1 mixture of unseparable regioisomers. The method was deemed unsuitable due to its lack of regioselectivity. The second iodination method proceeded *via* a hydromagnesiation reaction, which was initially carried out by a method described by Sato *et al.* giving vinyl iodide **1.118** in poor yields ranging between 9% and 26%.[77] Exchanging Et_2O for THF in the I_2 addition led to an improved yield of 42% (Scheme 2.4).[78] This alteration gave a suitable route for the construction

of iodide **1.118**. TBDMS protection of vinyl iodide **1.118** gave the required iodide fragment **1.32**, in excellent yield.

2.1.3 Coupling of the Fragments and Synthesis of 11*Z*-Retinal



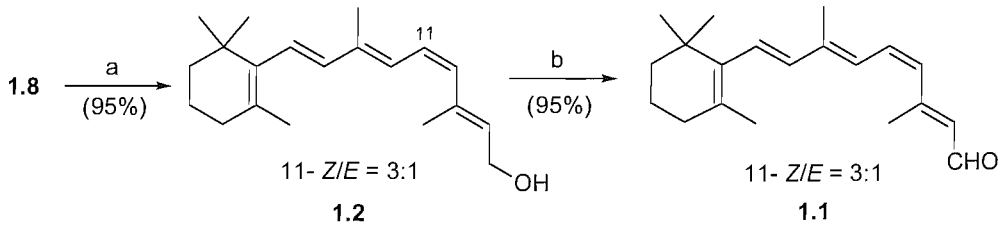
Scheme 2.5 *Reagents and conditions:* a) $Pd(PPh_3)_4$, CuI, $^iPrNH_2$; b) TBAF, THF, 0 °C to rt; c) TASF, APC, THF, 0 °C to rt.

Two routes to dehydroretinol (**1.8**) were attempted *via* palladium-catalysed cross-coupling reactions between alkynes **1.35** and **1.117** with iodides **1.32** and **1.118** respectively (Scheme 2.5).

Firstly the Sonogashira route will be discussed. The reaction utilised methodology reported by Borhan *et al.* in their synthesis of 11*Z*-retinal.[6] The reaction gave the TBDMS protected dehydroretinol **1.116** in high yield. Purification by column chromatography allowed separation of excess iodide **1.32** from the desired product **1.116**. The TBDMS protection was cleanly removed with TBAF giving dehydroretinol (**1.8**).

A second route to dehydroretinol (**1.8**) was achieved following methodology described by Denmark and co-workers.[79] On paper this route was more appealing, firstly because fewer reaction steps were required and TBDMS protection of C15 alcohol was not required. Also having the TMS group on alkyne **1.117** improved its stability in

comparison with terminal alkyne **1.35**. Application of the silyl cross-coupling methodology to alkyne **1.117** and iodide **1.118**, gave dehydroretinol (**1.8**) in a moderate yield of 49% (Scheme 2.5). The poor yield is thought to, at least in part, due to the hydroscopic nature of the TASF reagent, making it difficult to handle. Also the absorbed water is considered to impede the moisture sensitive reaction.



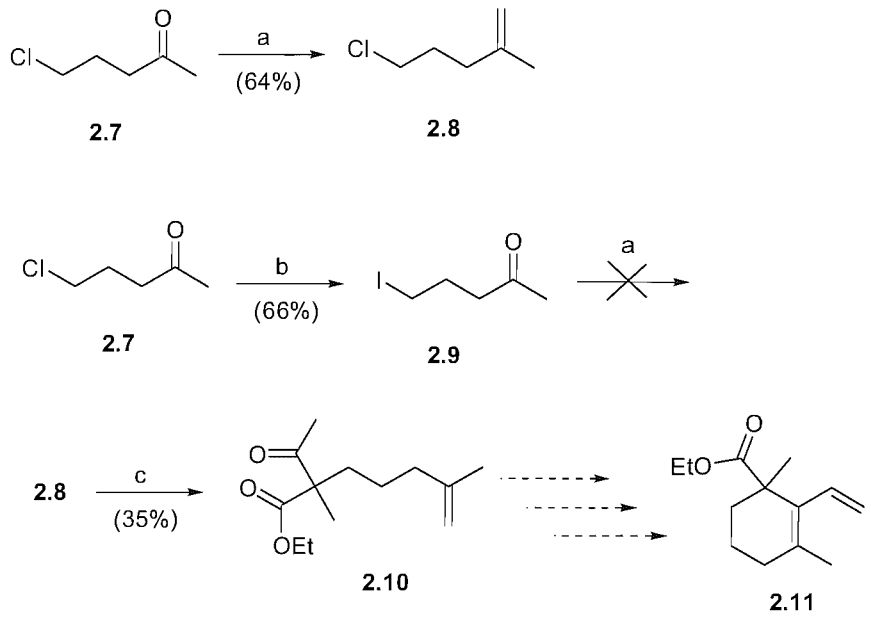
Scheme 2.6 *Reagents and conditions:* a) Zn, $Cu(OAc)_2$, $AgNO_3$, H_2O/MeOH, 40 °C; b) TPAP, NMO, MS (4 Å), CH_2Cl_2.

Utilising the last stages of the work described by Borhan *et al.*, the conversion of dehydroretinol **1.8** to 11*Z*-retinal (**1.1**) was achieved (Scheme 2.6).[6] S*yn* hydrogenation of dehydroretinol (**1.8**) with activated zinc only accomplished 13% completion after 21 hours at room temperature using the procedure by Borhan and co-workers.[6] After work up, the mixture was redissolved and treated with a fresh batch of activated zinc and stirred for a further 21 hours at room temperature, affording a 1:1 mixture of starting material (**1.8**) and 11*Z*-retinol (**1.2**). The *syn* selective reduction was attempted again at a higher temperature (40 °C).[19] Gratifyingly, after 21 hours, complete hydrogenation was achieved with a *Z*/*E* ratio at C11 of 3:1, which was less than the 13:1 ratio reported by Borhan and co-workers.[6] Oxidation of the unseparated retinols with TPAP and NMO was swift and in excellent yield (95%) with no isomerisation. Preparative HPLC was used to obtain the pure 11*Z*-retinal (**1.1**) and all-*E*-retinal (**1.3**).

2.1.4 Development of Methodology to Label the Cyclohexene Ring

We were interested in developing a method to allow introduction of ^{13}C labels into the cyclohexene ring, therefore a strategy to form the cyclohexene ring was proposed. The strategy was centred on the use of an enyne RCM that would lead to diene **2.11**. A route was proposed from *β*-keto ester **2.10**, which required the conversion of the ketone

functionality to a terminal alkyne with LDA and chloro diethylphosphonate as discussed earlier (Scheme 2.3). With the enyne in place the RCM would then be implemented. To reach *β*-ionone (**1.36**) from diene **2.11**, it would require the reduction and deoxygenation of the ester, followed by cross metathesis with methyl acryolein.



Scheme 2.7 *Reagents and conditions:* a) $Ph_3P^+CH_2Cl^-$, *n*-BuLi, THF, –30 °C to 0 °C; b) NaI, acetone, reflux; c) i) NaI, acetone, reflux; ii) $CH_3C(O)CH(CH_3)CO_2Et$, NaOEt, EtOH, rt to 50 °C.

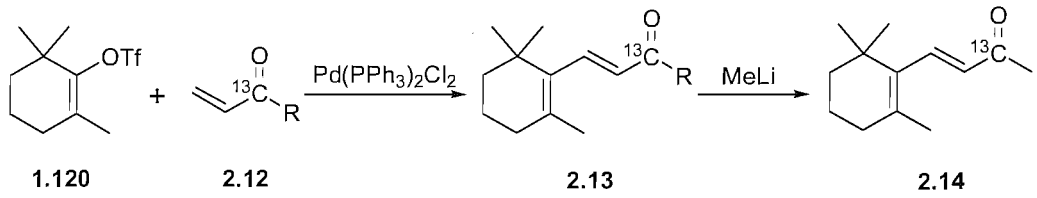
Wittig reaction of ketone **2.7** afforded alkene **2.8** in good yield according to a procedure described by Spijker-Assink *et al.*[80] Due to the product having a boiling point of 104 °C the complete removal of hexane was deemed responsible for the lower yield than that published (84%). The chloride **2.8** underwent halogen exchange with NaI to the desired iodide **2.9** and was used crude.[81] Reversing the order of halogen-halogen exchange and the Wittig reaction steps gave inferior results due to the lability of the iodo group. Deprotonation of ethyl 2-methyl acetoacetate with NaOEt and subsequent treatment with crude iodide gave the alkene **2.10** in poor yield. The reaction was very slow due to the steric bulk around the enolate, requiring the reaction to be warmed to 50 °C. Unfortunately, the increased reaction temperature also led to increased decomposition of the formed iodide and a disappointing yield. With more time, the alkylation would have be carried out using ethyl acetoacetate introducing the methyl group in a second alkylation step. Once the alkylation with the intermediate

iodide was complete, the methyl group could be introduced after further deprotonation and treatment with MeI in the hope to achieve alkene **2.10**. Construction of the cyclohexene ring was not investigated further due to ^{13}C labelling in the ring was of a relatively low priority in the overall project.

2.2 Synthesis of [9,10-$^{13}C_2$]-11*Z*-Retinal

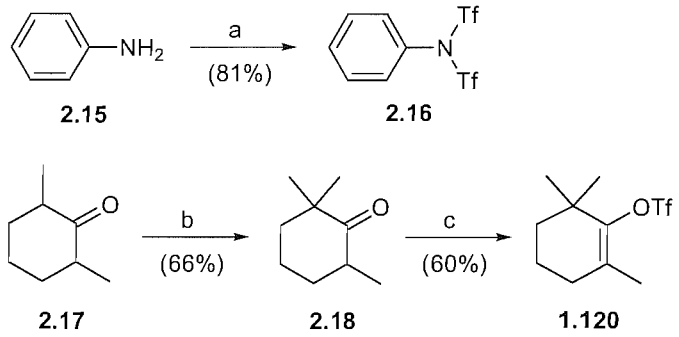
2.2.1 Unlabelled Investigation into the Incorporation of ^{13}C at C9

The first consideration in the synthesis of [9,10-$^{13}C_2$]-11*Z*-retinal (**2.45**) was how to incorporate the ^{13}C label at the C9 position in [9-^{13}C]-*β*-ionone (**2.14**). In the literature Breining *et al.* reported a synthesis of unlabelled *β*-ionone (**1.36**) by the Heck coupling of triflate **1.120** and methyl vinyl ketone in a 88% yield.[82] A similar strategy was utilised in the synthesis of [9-^{13}C]-*β*-ionone (**2.14**), however labelled methyl vinyl ketone was deemed unsuitable as a synthetic building block. This is because it has a low boiling point, leading to a potential loss of labelled material. Therefore a strategy involving the use of a labelled high boiling acrylate equivalent was proposed (Scheme 2.8).



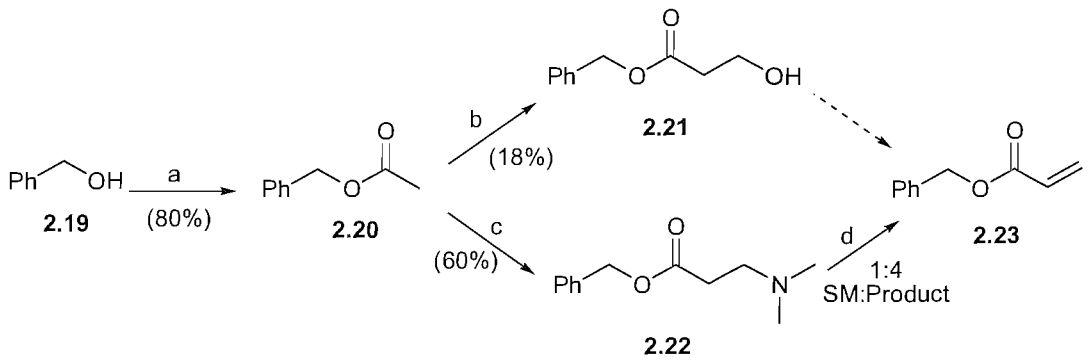
Scheme 2.8 Strategy for [9-^{13}C]-*β*-ionone synthesis

Triflate **1.12** was prepared using procedures reported in the literature and was straightforward (Scheme 2.9).[82] For the triflation of trimethyl ketone **2.18**, the triflating reagent **2.16** had to be firstly prepared. This was accomplished using a method described by Hendrickson *et al.*, ditriflating aniline (**2.15**) with triflic anhydride in high yield.[83] The methylation of dimethyl cyclohexanone (**2.17**) proceeded well giving ketone **2.18**.[84] Triflation of ketone **2.18** by a procedure reported by Breining *et al.* gave varying results ranging between 50% to 60% compared to the 84% yield reported.[85]



Scheme 2.9 *Reagents and conditions:* a) Tf_2O, Et_3N, CH_2Cl_2, –78 °C to rt; b) i) LDA, THF, –10 °C to 0 °C; ii) MeI, THF, 0 °C to rt; c) LDA, **2.16**, THF, –78 °C to rt.

With the cyclohexene fragment **1.120** in place, the acrylate fragment was to be synthesised next. Two acrylate equivalents were examined, firstly benzyl acrylate (**2.23**) and secondly a modified Weinreb amide **2.32** (Schemes 2.10 to 2.14).



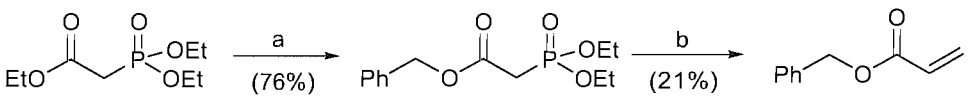
Scheme 2.10 *Reagents and conditions:* a) AcCl, DMAP, Et_3N, 0 °C to rt; b) i) LDA, THF, –78 °C to –30 °C; ii) $CH_2O_{(g)}$, THF, –30 °C; c) i) LDA, THF, –78 °C; ii) $Me_2(CH_2)N^+I^-$, THF, –78 °C to rt; d) i) HCl, Et_2O; ii) MeCN, μW, 180 °C.

Three methods to prepare benzyl acrylate (**2.23**) were attempted; aldol condensation with gaseous formaldehyde, Mannich reaction and a Horner-Emmons olefination (Schemes 2.10 and 2.11).

Benzyl esters **2.21** and **2.22** were prepared from benzyl acetate (**2.20**) in poor and moderate yields respectively (Scheme 2.10). Deprotonation of benzyl acetate (**2.20**) with LDA and subsequent treatment with bubbling gaseous formaldehyde afforded *β*-hydroxyl ester **2.21** in poor yield. The poor yield was caused by the technical challenge

of introducing gaseous formaldehyde into a cold reaction, and the elimination of benzyl alcohol from the pre-formed enolate. At low temperature the gaseous formaldehyde solidified on contact with the reaction mixture, increasing the reaction temperature from –78 °C to –30 °C helped to some extent. Unfortunately the increase in reaction temperature allowed the elimination of benzyl alcohol from the enolate to occur faster than aldol reaction. An optimum temperature was not discovered and this route to benzyl acrylate (**2.23**) was not pursued.

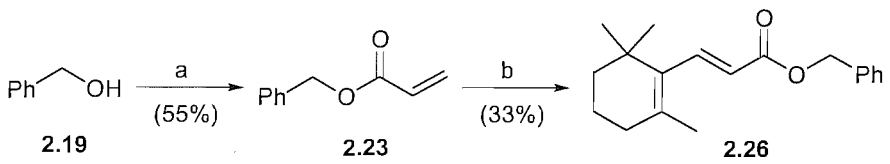
An alternative elimination route to benzyl acrylate (**2.23**) *via* tertiary amine **2.22** was then investigated (Scheme 2.10). Benzyl acetate (**2.20**) was once again deprotonated with LDA, this time it was treated with *N*,*N*-dimethylmethyleneammonium iodide affording the desired amine **2.22** in a respectable yield of 60%. The E_2 elimination to achieve the desired benzyl acrylate (**2.23**) was accomplished by forming the hydrochloride salt followed by microwave irradiation at 180 °C. This led to a 80% conversion determined by ^{1}H NMR to acrylate **2.23**. This route was also determined not to meet the requirements for use in the $^{13}C_2$ labelled synthesis due to poor conversion to the acrylate **2.23** from **2.22**, therefore the above route was not investigated further.



2.24 **2.25** **2.23**

Scheme 2.11 *Reagents and conditions:* a) BuOH, DMAP, toluene, reflux; b) $CH_2O_{(aq)}$, K_2CO_3, H_2O.

A third route to benzyl acrylate (**2.23**) was attempted (Scheme 2.11). Trans-esterification of triethyl phosphonoacetate with BuOH gave the desired phosphonate **2.25** in good yield. Attempts to increase the yield further by extending the reaction period were not successful, suggesting that an equilibrium had been reached. Horner-Emmons reaction of **2.25** with aqueous formaldehyde led to a messy reaction and an unsatisfactory yield of **2.23**. The reaction was also investigated with paraformaldehyde and K_2CO_3 in dioxane. Under these conditions the phosphonate **2.25** was consumed but none of the desired product was observed.

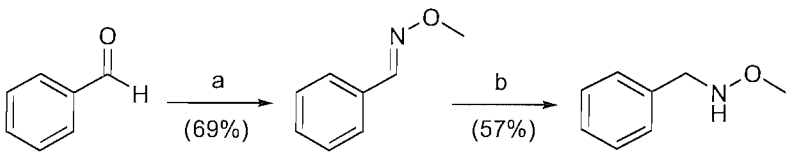


2.19 2.23 2.26

Scheme 2.12 *Reagents and conditions:* a) $H_2C{=}CC(O)Cl$, DMAP, Et_3N, CH_2Cl_2; b) **1.120**, $Pd(PPh_3)_2Cl_2$, Et_3N, DMF, 75 °C.

In parallel with the investigations of the routes to benzyl acrylate (**2.23**) described above, the Heck cross-coupling between triflate **1.120** and benzyl acrylate (**2.23**) was studied (Scheme 2.12). Unlabelled benzyl acrylate (**2.23**) was prepared from benzyl alcohol (**2.19**) and acryloyl chloride, in moderate yield. The moderate yield was not of an issue as the reaction would not be used in the labelled synthesis. The Heck reaction was carried out using the conditions reported by Breining *et al.* in their synthesis of *β*-ionone (**1.36**).[82] Heck reaction between benzyl acrylate (**2.23**) and the triflate **1.120** gave the desired product **2.26** in moderate yield (33%) along with recovered starting material and some unidentified by-products. In view of the inefficiency of the overall approach, our attention turned at this point to the synthesis of Weinreb amide fragment **2.32** (Scheme 2.14).

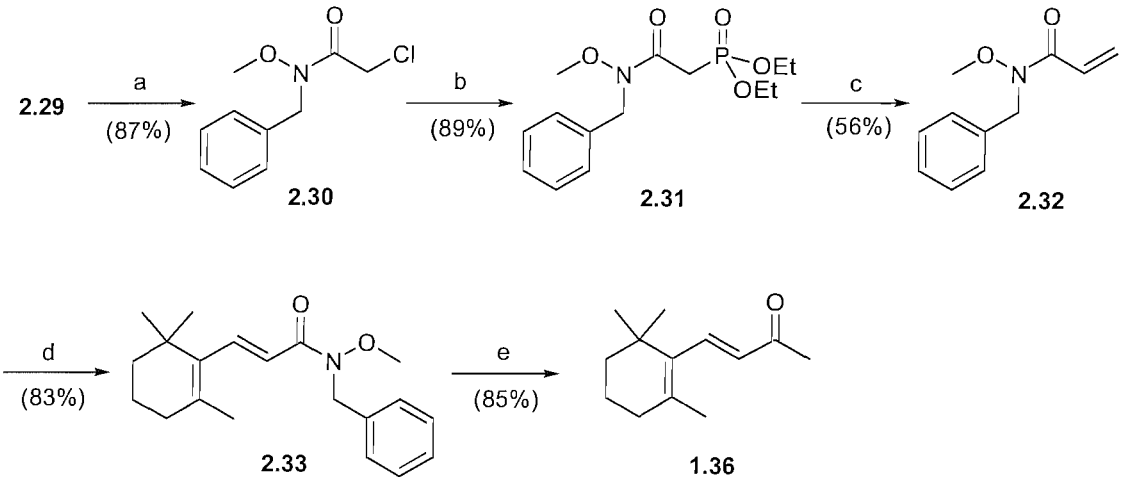
We were concerned about the potential volatility of the acrylate fragment, so a heavier Weinreb amide analogue was developed (Scheme 2.14). For the production of the modified Weinreb amide **2.30**, *N*-benzyl-*O*-methylhydroxylamine (**2.29**) had to be synthesised (Scheme 2.13). This was achieved by the formation of methoxime **2.28**, followed by reduction with $NaBH_3CN$ as reported by Keck *et al.* giving methoxyamine **2.29** in good yield.[86] A direct approach to the hydrochloride salt of methoxyamine **2.29** was attempted by alkylation of methoxyamine with benzyl bromide, this failed to give an adequate yield. Bhattacharyya's one-pot reductive amination with $Ti(^iOPr)_4$ and $NaBH_4$ was also unsuccessful.[87]



2.27 2.28 2.29

Scheme 2.13 *Reagents and conditions:* a) $MeONH_2.HCl$, NaOAc, EtOH, reflux; b) $NaCNBH_3$, 2 M HCl in MeOH, CH_2Cl_2.

With the synthesis of *N*-benzyl-*O*-methylhydroxylamine (**2.29**) validated, the synthetic route to *β*-ionone (**1.36**) was then investigated (Scheme 2.14).

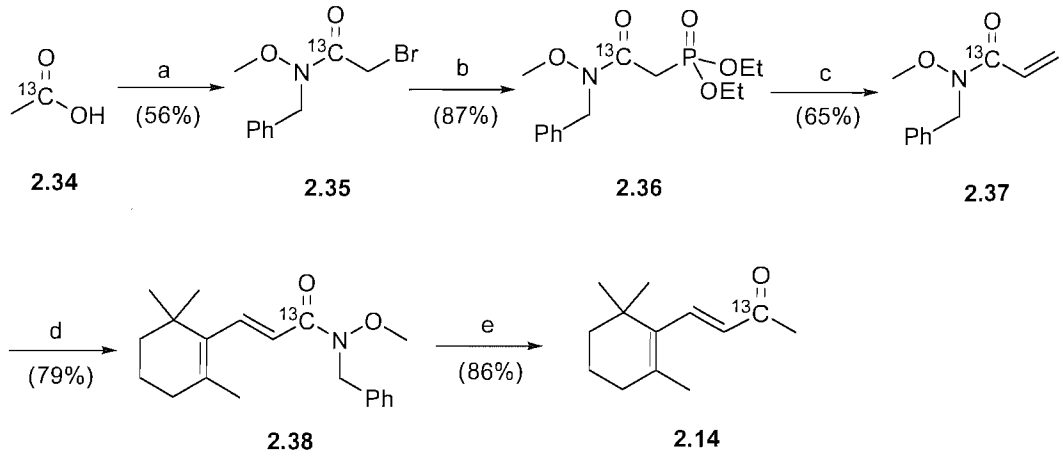


Scheme 2.14 *Reagents and conditions:* a) $ClCOCH_2Cl$, Et_3N, CH_2Cl_2, 0 °C to rt; b) $P(OEt)_3$, 180 °C; c) $CH_2O_{(aq)}$, K_2CO_3, 40 °C; d) **1.120**, $Pd(PPh_3)_2Cl_2$, Et_3N, DMF, 75 °C; e) MeLi, THF, −78 °C to −20 °C.

Reaction between **2.29** and chloro acetylchloride formed amide **2.30** in good yield (Scheme 2.14). The chloroacetate derivative **2.30** underwent a rapid and efficient Arbuzov reaction with triethyl phosphite to give phosphonate **2.31**. Horner-Emmons reaction between phosphonate **2.31** and aqueous formaldehyde gave acrylamide **2.32** in moderate yield. In the hope of increasing the yield a range of conditions for the Horner-Emmons were attempted, such as Et_3N and LiBr in MeCN, these conditions however did not give an increase in yield. Heck coupling between triflate **1.120** and acrylamide **2.32** afforded benzyl Weinreb amide **2.33** in a reproducible high yield. MeLi addition to Weinreb amide **2.33**, gave *β*-ionone (**1.36**) cleanly and in high yield. The above synthetic route gave an efficient and high yielding pathway to *β*-ionone (**1.36**) and was

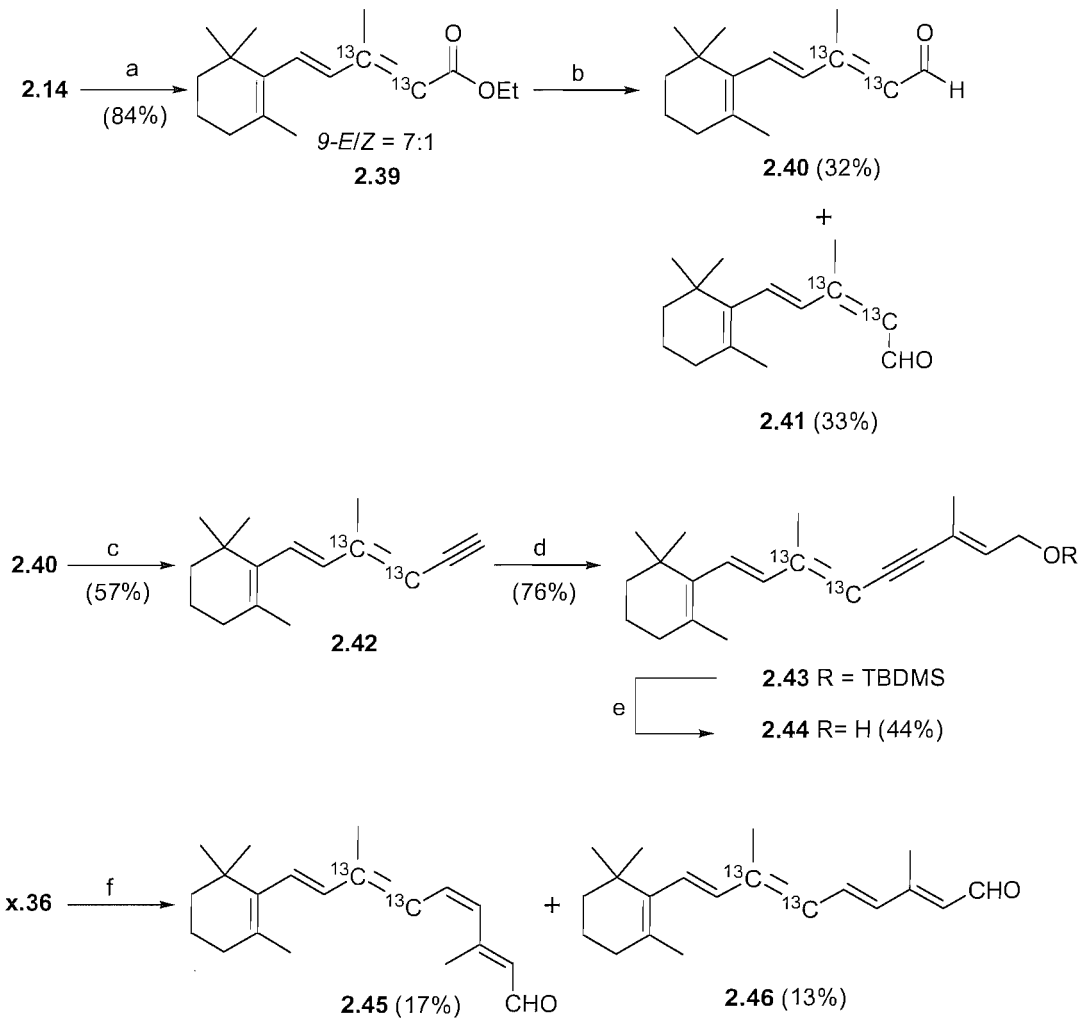
deemed suitable for use in the labelled synthesis. Combining the above route to β-ionone (**1.36**) with the 11*Z*-retinal (**1.1**) synthesis methodology described earlier (Schemes 2.2 and 2.5) allowed the efficient production of [9,10-$^{13}C_2$]-11*Z*-retinal (**2.45**) (Schemes 2.15 and 2.16).

2.2.2 Synthesis of [9,10-$^{13}C_2$]-11*Z*-Retinal



Scheme 2.15 *Reagents and conditions:* a) i) [1-^{13}C]-acetic acid, PBr_3, Br_2, reflux; ii) **2.29**, Et_3N, CH_2Cl_2, 0 °C; b) $P(OEt)_3$, 180 °C; c) $CH_2O_{(aq)}$, K_2CO_3, H_2O, 40 °C; d) **1.120**, $Pd(PPh_3)_2Cl_2$, Et_3N, DMF, 75 °C; e) MeLi, THF, –78 °C to –20 °C.

Unfortunately, commercial stocks of [1-^{13}C]-chloro acetylchloride were no longer available. This meant [1-^{13}C]-acetic acid was used as a precursor to [1-^{13}C]-bromo acetylbromide. Bromination of [1-^{13}C]-acetic acid with PBr_3 and Br_2 gave good conversion to the desired [1-^{13}C]-bromo acetylbromide (Scheme 2.15). Subsequent treatment with methoxyamine **2.29** returned bromide **2.35** in a good yield of 56% over two steps. Conversion to [9-^{13}C]-β-ionone **2.14** from bromide **2.35** proceeded smoothly as in the unlabelled synthesis.



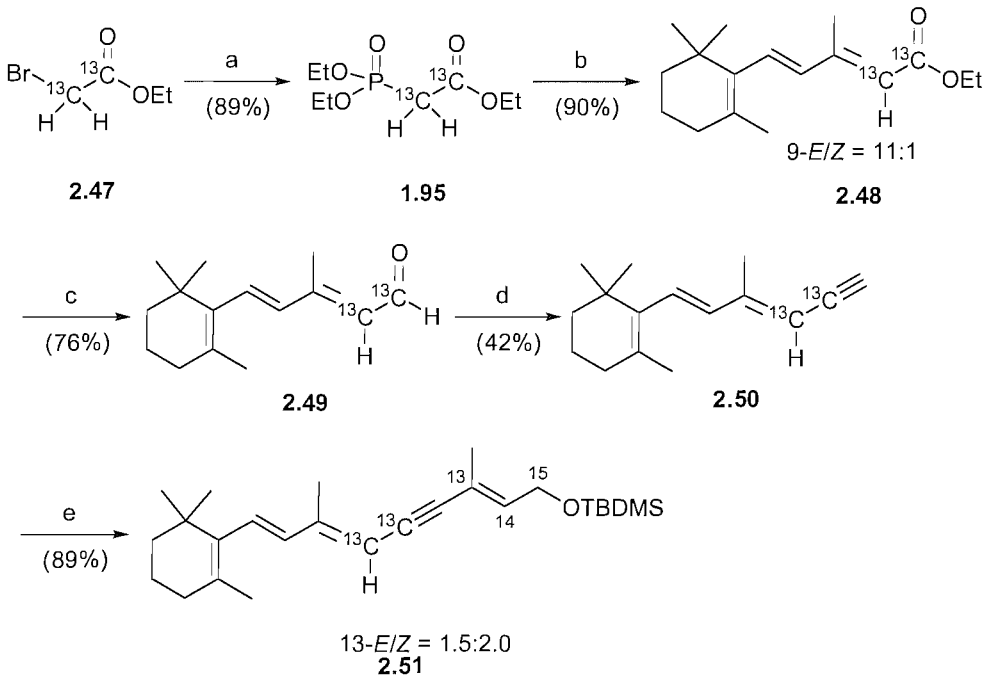
Scheme 2.16 *Reagents and conditions:* a) [2-^{13}C]-triethyl phosphonoacetate, NaH, Et_2O; b) i) $LiAlH_4$, Et_2O, –78 °C to rt; ii) TPAP, NMO, MS (4 Å), CH_2Cl_2; c) $TMSCHN_2$, LDA, THF, –78 °C; d) **1.32**, $Pd(PPh_3)_4$, CuI, $^{i}PrNH_2$; e) TBAF, THF, 0 °C to rt; f) i) Zn, $Cu(OAc)_2$, $AgNO_3$, H_2O/MeOH, 40 °C; ii) TPAP, NMO, MS (4 Å), CH_2Cl_2.

The next step in the synthesis was the Horner-Emmons reaction between [9-^{13}C]-*β*-ionone (**2.14**) and [2-^{13}C]-triethyl phosphonoacetate (Scheme 2.16). The reaction proceeded well and gave a yield and *E*/*Z* ratio similar to previous unlabelled reactions. The two step reduction-oxidation sequence to prepare aldehyde **2.40** from ester **2.39** gave an unexpected poor yield. The poor yield originated from acid catalysed isomerisation at C9 of aldehyde **2.40** on purification by column chromatography giving 33% of the incorrect stereoisomer. The reactions converting aldehyde **2.40** to [9,10-$^{13}C_2$]-11*Z*-retinal (**2.45**) all suffered reduced yields in comparison to the unlabelled

optimised reactions. These reduced yields were subsequently attributed to a poor batch of silica gel, unfortunately it was not identified before the end of the synthesis.

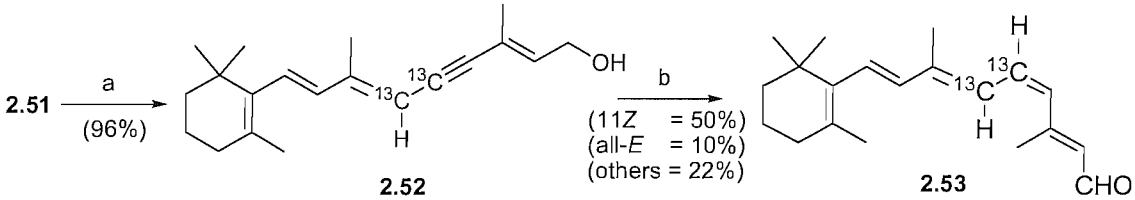
2.3 Synthesis of [10,11-$^{13}C_2$]-11*Z*-Retinal

[10,11-$^{13}C_2$]-11*Z*-Retinal (**2.53**) was the most straightforward of the three isotopomers to prepare because no special reagents or intermediates were required. Both labels to be introduced originated from commercial [1,2-$^{13}C_2$]-bromoethyl acetate (**2.47**). The Arbuzov reaction between [1,2-$^{13}C_2$]-bromoethyl acetate (**2.47**) and triethyl phosphite proceeded in excellent yield (Scheme 2.17).[30] Sequential Horner-Emmons reaction with *β*-ionone (**1.36**), reduction and oxidation gave aldehyde **2.49** in a superb yield and *E*/*Z* ratio. A further advance was the reduction in stoichiometry of phosphonoacetate **1.95** required in the Horner-Emmons reaction from 2.2 equivalents to 1.5 equivalents, making the reaction more ^{13}C label efficient. Attempts to further reduce the amount of phosphonoacetate **1.95** led to a dramatic drop in conversion. Previously the alkyne formation has worked in high yield, unfortunately when aldehyde **2.49** was treated with $TMSCHN_2$ it resulted in a disappointing yield of 42% with 7% starting material. The crude NMR showed a relatively clean spectrum with negligible amounts of starting material. Therefore it is thought that the low yield is due to decomposition on purification by column chromatography. Later synthetic experience of the alkyne found that the use of a non-polar solvent system for chromatography causes some decomposition on silica. If a more polar solvent system was used, this drop in yield may be avoided. The Sonogashira reaction with iodide **1.32** worked efficiently giving the desired protected dehydroretinol **2.51** in high yield. Distressingly on purification isomerisation at C13 (*E*/*Z* = 1.5:2.0) occurred, which had not been observed in previous syntheses. The crude material was dry loaded onto silica due to solubility problems in the eluent system, this and the formation of a palladium-allyl complex may have caused the isomerisation.



Scheme 2.17 *Reagents and conditions:* a) $P(OEt)_3$, 180 °C; b) *β*-ionone, NaH, Et_2O; c) i) $LiAlH_4$, Et_2O, −78 °C to rt; ii) TPAP, NMO, MS (4 Å), CH_2Cl_2; d) $TMSCHN_2$, LDA, THF, −78 °C; e) **1.32**, $Pd(PPh_3)_4$, CuI, $^{i}PrNH_2$.

The mixture of protected dehydroretinol isomers **2.51** was deprotected with TBAF, in a superb yield (Scheme 2.18). At this point it was possible to isolate the isomers with no further conversion to the unwanted 13*Z*-dehydroretinol. The selective hydrogenation proceeded extremely well with no observable all-*E*-retinol, which is a remarkable improvement from 3:1 previously achieved and superior than reported by Borhan *et al.* who attained a ratio of 13:1.[6] The use of HPLC grade solvents (including water) and exclusion of light in the reaction is thought to give pure 11*Z*-retinol. The oxidation also proceeded smoothly giving crude isomerically pure [10,11-$^{13}C_2$]-11*Z*-retinal (**2.53**). Purification by preparative HPLC was utilised to obtain pure [10,11-$^{13}C_2$]-11*Z*-retinal (**2.53**).

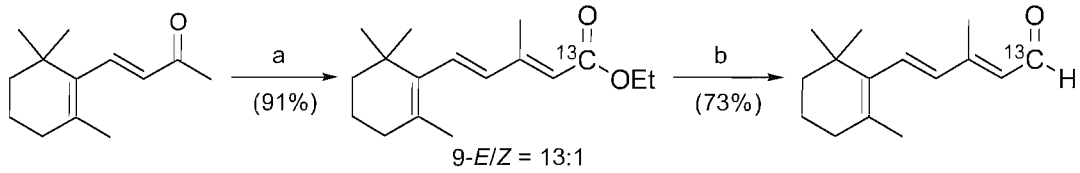


Scheme 2.18 *Reagents and conditions:* a) TBAF, THF, 0 °C to rt; b) i) Zn, $Cu(OAc)_2$, $AgNO_3$, $H_2O/^{i}PrOH$, 40 °C; ii) TPAP, NMO, MS (4 Å), CH_2Cl_2.

2.4 Synthesis of [11,12-$^{13}C_2$]-11*Z*-Retinal

2.4.1 Unlabelled Investigation into the Incorporation of ^{13}C at C11

As in the synthesis of [10,11-$^{13}C_2$]-11*Z*-retinal (**2.53**) the ^{13}C label at C11 was incorporated from ^{13}C labelled triethyl phosphonoacetate in a Horner-Emmons reaction with *β*-ionone (**1.36**) (Scheme 2.19). The Horner-Emmons reaction afforded the ester **2.54** with the best yield and *E*/*Z* ratio observed thus far. The reduction and immediate oxidation to aldehyde **2.55** proceeded efficiently.

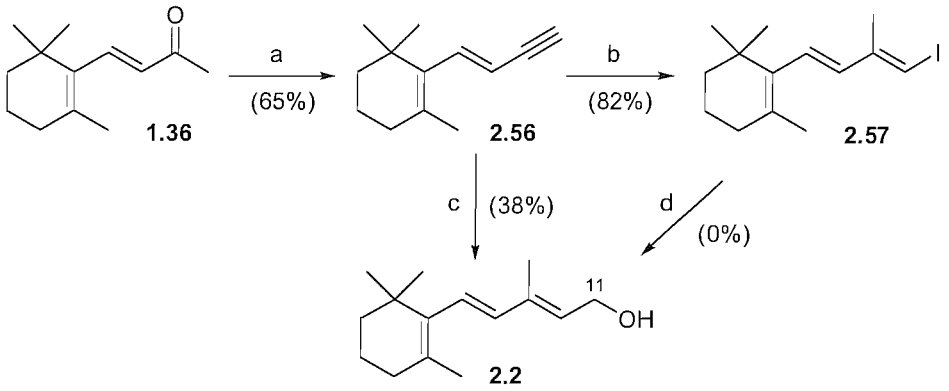


1.36 **2.54** **2.55**

Scheme 2.19 *Reagents and conditions:* a) [1-^{13}C]-triethyl phosphonoacetate, NaH, Et_2O; b) i) $LiAlH_4$, Et_2O, −78 °C to rt; ii) TPAP, NMO, MS (4 Å), CH_2Cl_2.

An alternative route to ^{13}C incorporation at C11 was also attempted (Scheme 2.20). The route would have utilised [1-^{13}C]-paraformaldehyde as the ^{13}C labelled starting material, which is very cheap and readily available. The aim of the approach was to reach alcohol **2.2** *via* methyl aluminium addition across alkyne **2.56** followed by the addition of ^{13}C labelled paraformaldehyde. Once this was achieved, alcohol **2.2** would be oxidised to aldehyde **1.14** and the synthesis to [11,12-$^{13}C_2$]-11*Z*-retinal (**2.81**) continued. The formation of alkyne **2.56** from *β*-ionone (**1.36**) was found to be respectable (unoptimised). Treatment with $AlMe_3$ and quenching with I_2 gave iodide **2.57**. It was found that iodide **2.57** exhibited poor stability making it an unwise choice for the use in the labelled synthesis. Attempts to obtain alcohol **2.2** from iodide **2.57** by halogen-metal exchange and reaction with paraformaldehyde failed, giving an unidentified decomposed material. Successful halogen-metal exchange was inferred by G.C. analysis of the quenched products but the subsequent reaction with paraformaldehyde did not occur. To avoid the unstable iodide **2.57** an *in situ* approach to alcohol **2.2** from alkyne **2.56** was investigated. This method produced the desired

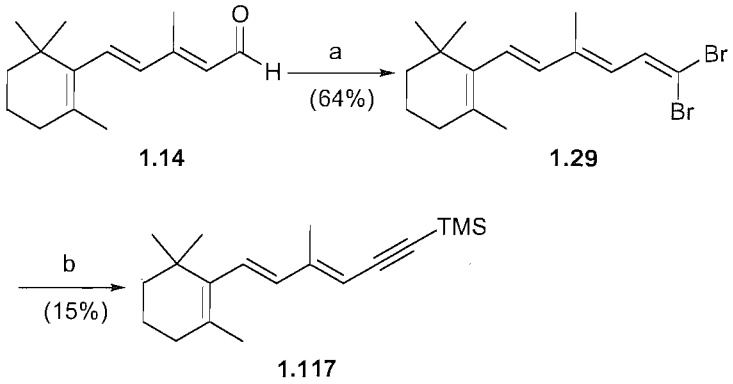
alcohol **2.2** but only in a poor yield of 38%, this poor yield is again attributed to the poor solubility of paraformaldehyde.



Scheme 2.20 *Reagents and conditions:* a) i) LDA, THF, –78 °C; ii) $(EtO)_2P(O)Cl$, –78 °C to rt; iii) LDA, THF, –78 °C to rt; b) i) $AlMe_3$, Cp_2ZrCl_2, CH_2Cl_2, 0 °C to rt; ii) I_2, THF, –50 °C; c) i) $AlMe_3$, Cp_2ZrCl_2, CH_2Cl_2, 0 °C to rt; ii) *n*-BuLi, $(CH_2O)_n$, THF; d) i) *n*-BuLi, THF, –78 °C; ii) $(CH_2O)_n$, THF, –78 °C to rt.

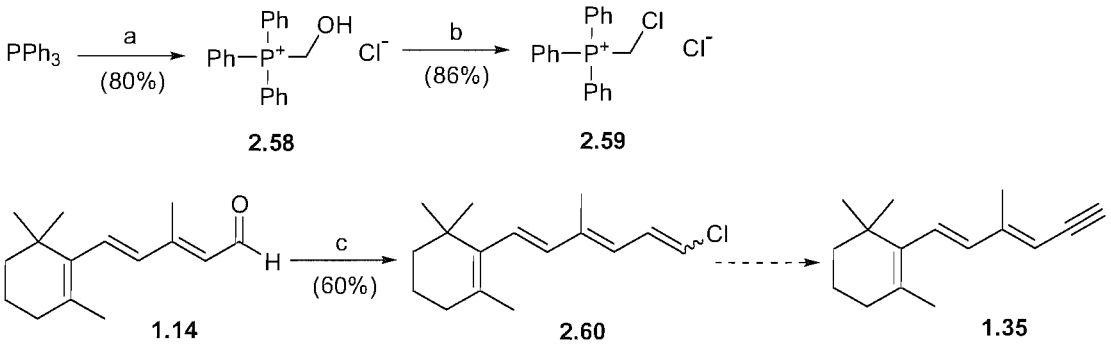
2.4.2 Unlabelled Investigation into the Incorporation of ^{13}C at C12

In the previous syntheses of labelled retinals the C12 carbon has been introduced with trimethylsilyldiazomethane. Unfortunately the preparation of ^{13}C labelled $TMSCHN_2$ was not readily achievable from available ^{13}C starting materials. The preparation would require the synthesis of ^{13}C labelled chloromethyltrimethylsilane as the starting material of the synthesis.[88] Therefore three approaches to incorporate the ^{13}C label at C12 are described below.



Scheme 2.21 *Reagents and conditions:* a) CBr_4, PPh_3, CH_2Cl_2, 0 °C; b) i) *n*-BuLi, THF, –78 °C; ii) TMSCl, THF, –78 °C to rt.

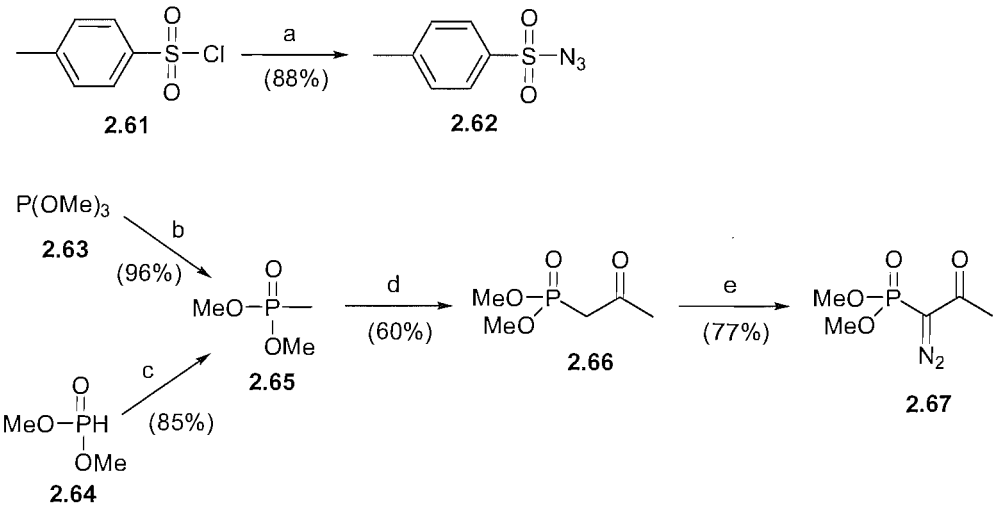
The first approach to incorporate a ^{13}C label at C12 utilised the Corey-Fuchs[89] procedure from $^{13}CBr_4$.[7] The dibromomethylenation of aldehyde **1.14** with CBr_4 and PPh_3 was extremely facile, going to completion within 5 minutes (Scheme 2.21). Uenshi *et al.* reported a 98% yield for the transformation, the yield actually achieved was slightly less. It was observed that the dibromide **1.29** once isolated had a very short shelf life (approximately 2 hours at room temperature). This decomposition was retarded by storage at –15 °C, but not completely prevented. Attempts to treat dibromide **1.29** with *n*-BuLi and subsequently trap the anion with TMSCl gave low yields. In view of the above results, this line of investigation was ceased.



Scheme 2.22 *Reagents and conditions:* a) $(CH_2O)_n$, PPh_3, HCl, Et_2O; b) $SOCl_2$, CH_2Cl_2, reflux; c) **2.59**, piperidine, *n*-BuLi, Et_2O.

The second approach used a Wittig reaction between aldehyde **1.14** and phosphonium salt **2.59**, giving vinyl chloride **2.60** as the precursor to alkyne **1.35** (Scheme 2.22). Preparation of phosphonium chloride **2.59** was relatively straightforward and

proceeded in good overall yield.[90,91] The Wittig reaction between aldehyde **1.14** and phosphonium chloride **2.59** was attempted with various conditions. The most effective conditions were found to be piperidine and *n*-BuLi in Et_2O. Even so, the yield was never raised above 60%. This approach did not meet the practical requirements for the labelled synthesis. This meant the conversion of the unstable chloride **2.60** to alkyne **1.35** was not attempted.



Scheme 2.23 *Reagents and conditions:* a) NaN_3, acetone/H_2O; b) MeI, 70 °C, μW; c) K_2CO_3, MeI; d) i) *n*-BuLi, CuI, THF, –60 °C to –30 °C; ii) AcCl, THF, –40 °C to rt; e) **2.62**, NaH, THF, 0 °C.

The final and successful approach to alkyne **1.35** utilised the Bestmann-Ohira reagent (**2.67**) to introduce the ^{13}C label (Scheme 2.23). In the synthesis of the Bestmann-Ohira reagent (**2.67**), tosyl azide (**2.62**) was used as the diazo transfer reagent. Tosyl azide (**2.62**) was prepared by a method described by Ghosh *et al.* from tosyl chloride and sodium azide in good yield.[92] An Arbuzov reaction and a Michaelis-Becker reaction were used to construct methyl phosphonate **2.65**.[93,94] The Arbuzov reaction was extremely fast and very exothermic [CAUTION], the reaction was heated to 70 °C with microwave irradiation.[93] On a 1mL scale of MeI, once the reaction reached 70 °C the reaction exothermed to 155 °C and the reaction was halted with the emergency stop button! When the reaction was carried out on a 0.5 mL scale, the reaction did not give a sufficient exotherm to complete the reaction. This meant that the reaction had to be irradiated for a longer period at 80 °C to reach completion. This method allowed the quick and efficient synthesis of methyl phosphonate **2.65** for the purposes of later test

reactions, but consideration of the mechanism indicated a mixture of $^{13}C/^{12}C$ isotopomers would be produced (Figure 2.1). Therefore the simplified Michaelis-Becker reaction was applied to avoid the $^{13}C/^{12}C$ scrambling.[94] The reaction was found to give methyl phosphonate **2.65** in good yield.

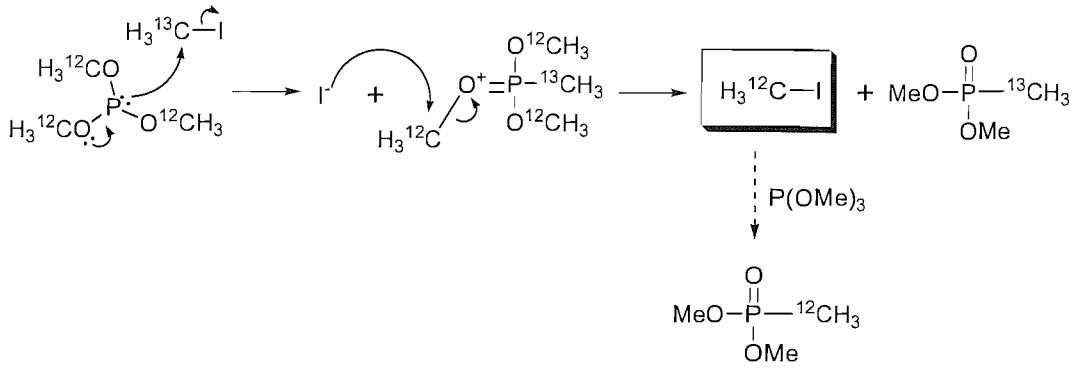


Figure 2.1 The Arbuzov Mechanism

Acetylation of **2.65** proceeded in good yield, with recovered starting material (Scheme 2.23).[95] It was later found that the purity of the copper iodide has a dramatic effect on the yield and efficiency of the lithium-copper exchange. Formation of Bestmann-Ohira (**2.67**) with tosyl azide (**2.62**) was accomplished by diazo transfer in high yield.[92,96]

Reaction of the Bestmann-Ohira reagent (**2.67**) with aldehyde **1.14** using the standard conditions of K_2CO_3 in methanol gave alkyne **1.35** in a yield of 74%. However the presence of methoxide led to the isomerisation of the aldehyde before reaction occurred, giving the alkyne as a 6:1 *E/Z* mixture at C9. The isomerisation occurred by the Michael addition-elimination of methoxide. In the literature unsaturated systems when reacted with the Bestmann-Ohira reagent (**2.67**) gave an undesired homopropargylic methyl ether from the Michael addition of methoxide, protonation and subsequent reaction with the Bestmann-Ohira reagent (Figure 2.2).[97] However in the instance of polyene aldehyde **1.14** the homopropargylic methyl ether product was not observed, only the desired terminal alkyne **1.35**. Presumably, this is due to the increased thermodynamic stability of the conjugated polyene system.

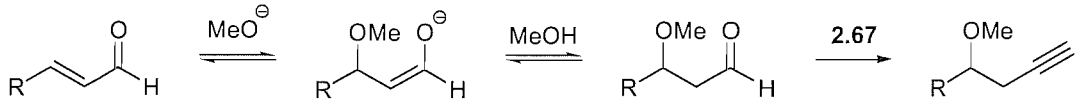


Figure 2.2 Bestmann-Ohira reagent with an enone

It was hoped that pre-treatment of the Bestmann-Ohira reagent with a controlled quantity of methoxide would lead to initial deacylation of the Bestmann-Ohira reagent. It was anticipated that the deacetylation and consequent comsumption of methoxide would supress the addition-elimination pathway for isomerisation of aldehyde **1.14** (Figure 2.3). Therefore, the Bestmann-Ohira reaction was carried out using NaOMe in THF at –78 °C.[98]

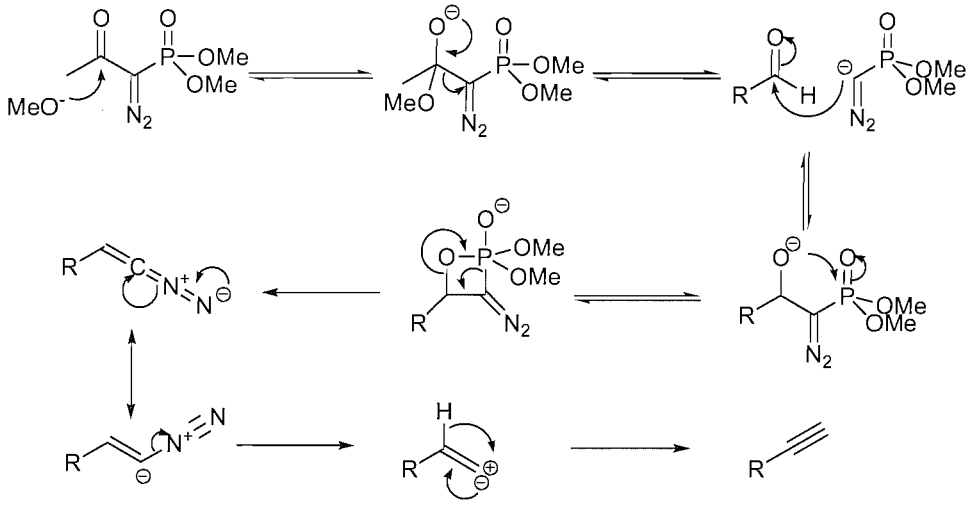
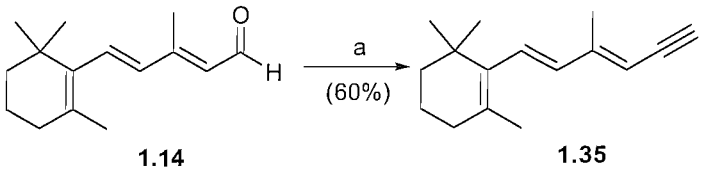


Figure 2.3 Bestmann-Ohira Mechanism

These new conditions were tested, allowing an hour for the deacylation to occur before addition of aldehyde **1.14**. The reaction proceeded slowly with no isomerisation, giving the desired alkyne **1.35** in a respectable yield (Scheme 2.24). It was decided that a labelled Bestmann-Ohira reagent derived from ^{13}MeI would be used in the labelled synthesis.



Scheme 2.24 *Reagents and conditions:* a) **2.67**, NaOMe, MeOH, THF, –78 °C to rt.

2.4.3 Synthesis of [11,12-$^{13}C_2$]-11*Z*-Retinal

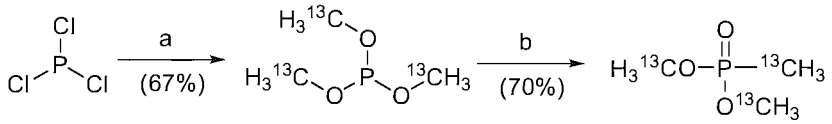
With aldehyde **2.55** in hand (Scheme 2.19), the next stage of the synthesis was the preparation of ^{13}C labelled Bestmann-Ohira reagent by the simplified Michaelis-Becker reaction (Scheme 2.25).[94] To our great surprise the reaction gave the product as a 7:1 mixture of ^{13}C and ^{12}C at the methyl position. The isotope dilution was due to the formation of ^{12}MeI in the reaction.

2.64

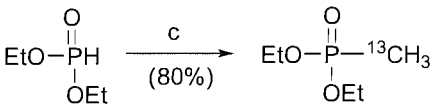
2.68 R = $^{13}CH_3$ (88%)
2.69 R = $^{12}CH_3$ (12%)

Scheme 2.25 *Reagents and conditions:* a) $^{13}CH_3I$, K_2CO_3.

Due to the unexpected isotope mixing, two ideas were considered to secure methyl phosphonate **2.68** with no isotope dilution. The first method was to synthesise fully ^{13}C labelled trimethyl phosphite **2.71**, then react it with ^{13}MeI (Scheme 2.26). The second approach was to synthesise an ethoxy analogue of the methyl phosphonate to stop the isotope dilution.



2.70 **2.71** **2.72**

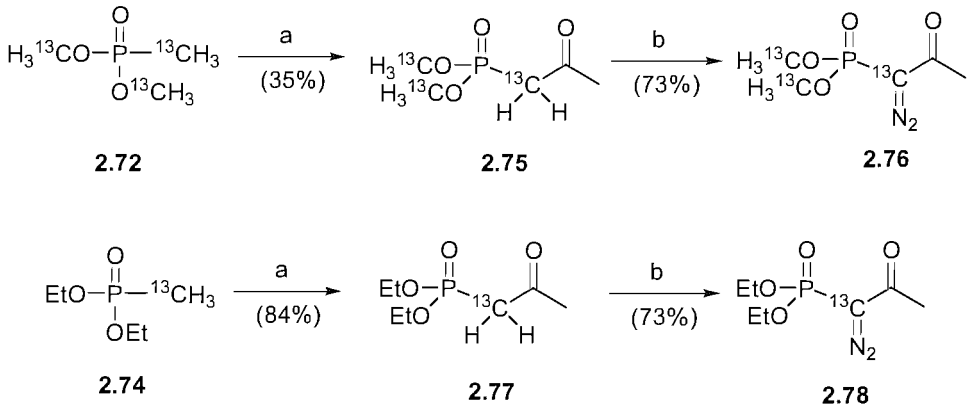


2.73 **2.74**

Scheme 2.26 *Reagents and conditions:* a) $^{13}CH_3OH$, *n*-Bu_3N, tetralin, 0 °C to rt; b) $^{13}CH_3I$, μW, 120 °C; c) $^{13}CH_3I$, K_2CO_3, 35 °C then μW, 110 °C.

The production of [1-$^{13}C_3$]-trimethyl phosphite (**2.71**) was carried out by a method reported by Ohlendorf *et al.* giving the desired product **2.71** in a respectable yield.[99] The method utilises a high boiling solvent and tertiary amine, so that the desired phosphite can be distilled away from them. However purification by Kugelröhr

distillation led to an unseparable mixture of tetralin and the desired phosphite **2.71**. This meant the Arbuzov reaction with ^{13}MeI was carried out on the unseparated mixture. This resulted in the reaction proceeding less effectively than when neat reagents were used, requiring a higher temperature and longer reaction period to reach completion. Even with these complications the reaction still gave $^{13}C_3$-trimethyl phosphonate **2.72** in good yield. Removal of the tetralin was still not possible at this stage, as the boiling points of tetralin and [1,2-$^{13}C_3$]-trimethyl phosphonate (**2.72**) are almost identical. Fortunately the ethoxy analogue **2.74** was prepared in high yield using the simplified Michaelis-Becker reaction, with no isotope mixing at the methyl position.

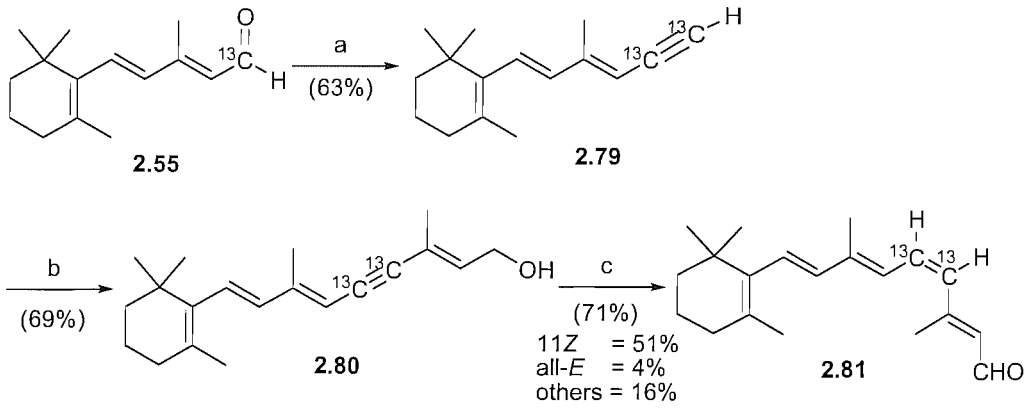


2.72 **2.75** **2.76**

2.74 **2.77** **2.78**

Scheme 2.27 *Reagents and conditions:* a) i) *n*-BuLi, CuI, THF, –60 °C to –30 °C; ii) AcCl, THF, –40 °C to rt; e) **2.62**, NaH, benzene/THF, 0 °C to rt.

With [1,2-$^{13}C_3$]-trimethyl phosphonate (**2.72**) and [1-^{13}C]-diethyl methylphosphonate (**2.74**) in hand, they were then converted to ^{13}C labelled Bestmann-Ohira reagent **2.76** and ethoxy analogue of Bestmann-Ohira reagent **2.78** respectively (Scheme 2.27). The acetylation of the phosphonates varied dramatically. The contrast in yields was due to the presence of tetralin in the acetylation of $^{13}C_3$ labelled phosphonate **2.72**. The tetralin was removed from *β*-keto phosphonate **2.75** as it could be purified by column chromatography. The diazo transfer reaction gave identically good results furnishing [1,2-$^{13}C_3$]-Bestmann-Ohira reagent **2.76** and ethoxy analogue **2.78**.



Scheme 2.28 *Reagents and conditions:* a) **2.78**, NaOMe, MeOH, THF, –78 °C to rt; b) i) **1.32**, $Pd(PPh_3)_4$, CuI, $^{i}PrNH_2$; ii) TBAF, THF; c) i) Zn, $Cu(OAc)_2$, $AgNO_3$, $H_2O/^{i}PrOH$, 40 °C; ii) TPAP, NMO, MS (4 Å), CH_2Cl_2.

[1,2-$^{13}C_3$]-Bestmann-Ohira reagent **2.76** and ethoxy analogue **2.78** were both used to produce alkyne **2.79** (Scheme 2.28). It was found that the ethoxy analogue **2.78** gave a superior result of 63% with 15% starting material, compared to 55% with 8% starting material for the [1,2-$^{13}C_3$]-Bestmann-Ohira reagent **2.76**. Both reactions were quenched before completion in fear of decomposition of alkyne **2.79** over extended periods at room temperature. Sonogashira reaction between alkyne **2.79** and iodide **1.32** proceeded cleanly. The TBDMS protected dehydroretinol was not purified before TBAF deprotection for concerns of isomerisation at C13 as in the synthesis of [10,11-$^{13}C_2$]-11*Z*-retinal (**2.81**). After the efficient removal of the TBDMS group a test purification was attempted and it was observed that the isomerisation still occurred at C13. Therefore the isomerisation was attributed to the presence of the remaining palladium catalyst. Purification by flash column chromatography on neutral alumina allowed the removal of the catalyst with very minor isomerisation, further purification on silica gel gave pure dehydroretinol **2.80**. Reduction with activated zinc gave isomerically pure crude 11*Z*-retinol. Subsequent oxidation with TPAP and NMO gave isomerically pure crude [11,12-$^{13}C_2$]-11*Z*-retinal (**2.81**). Purification by preparative HPLC yielded pure [11,12-$^{13}C_2$]-11*Z*-retinal (**2.81**) and a range of other isomers.

2.5 Conclusions and Future Work

The stereoselective syntheses of the three isotopomers, [9,10-$^{13}C_2$]-11*Z*-retinal (**2.45**), [10,11-$^{13}C_2$]-11*Z*-retinal (**2.53**) and [11,12-$^{13}C_2$]-11*Z*-retinal (**2.81**) have been successfully accomplished (Scheme 2.29). The syntheses utilised cheap ^{13}C enriched building blocks, therefore meeting the original project objectives. The synthetic routes investigated can allow ^{13}C enrichment at positions 9-12, 19, 20 and unselectively at 16 and 17.

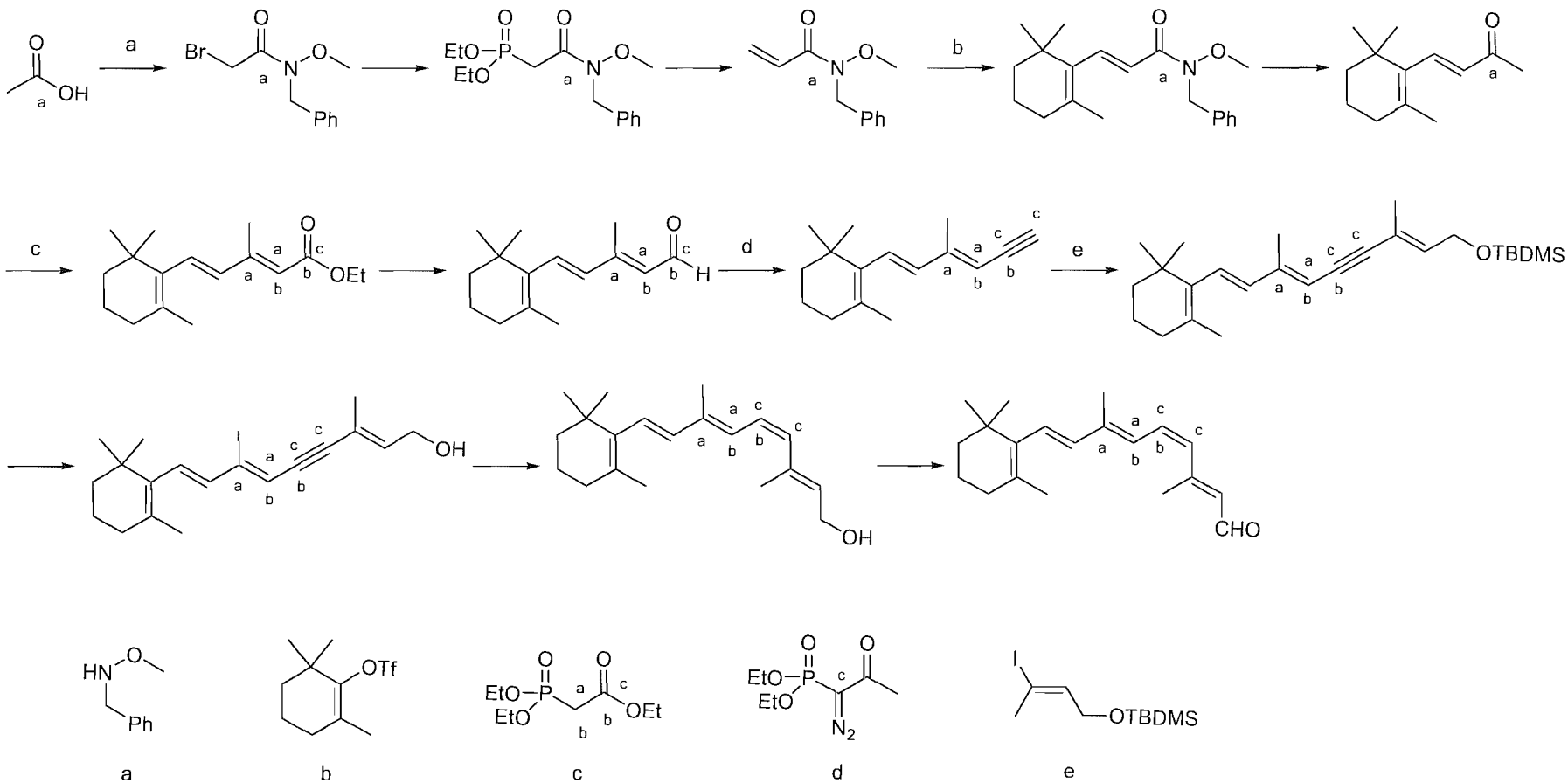
In the synthesis of [9,10-$^{13}C_2$]-11*Z*-retinal (**2.45**), due to concerns about the potential volatility of the acrylate fragment **2.12,** a novel Weinreb amide analogue **2.32** was developed. The synthesis suffered reduced yields, which were subsequently attributed to a poor batch of silica gel employed in some of the purification steps. Even in these adverse circumstances [9,10-$^{13}C_2$]-11*Z*-retinal (**2.45**) was isolated and condensed with opsin giving rhodopsin. This allowed the Levitt group to measure the intramolecular distances between the C9-C10 of the retinylidene chromophore in ground state rhodopsin. The results of these experiments were published in 2006.[49] The isolated [9,10-$^{13}C_2$]-all-*E*-retinal (**2.45**) was also constructively used by the Levitt group as a model for their analytical theory of γ-encoded double-quantum recoupling sequences in solid -state NMR.[100]

The synthesis of [10,11-$^{13}C_2$]-11*Z*-retinal (**2.53**) was successful. Importantly, isomerically pure 11*Z*-retinol was obtained from the activated zinc triple bond reduction step.

For the synthesis of [11,12-$^{13}C_2$]-11*Z*-retinal (**2.81**) a labelled ethoxy analogue of the Bestmann-Ohira reagent was prepared and successfully applied. This is the first example of the use of the Bestmann-Ohira reagent on an unsaturated substrate, using pre-deacetylation of the reagent to stop isomerisation of aldehyde **2.55**.

[10,11-$^{13}C_2$]-11*Z*-Retinal (**2.53**) and [11,12-$^{13}C_2$]-11*Z*-retinal (**2.81**) are due to be condensed with opsin and subsequently used in solid state NMR experiments by the

Levitt group. The Levitt group is currently attempting to trap doubly labelled rhodopsin photointermediates at low temperature and probe the chromophore with MAS NMR.

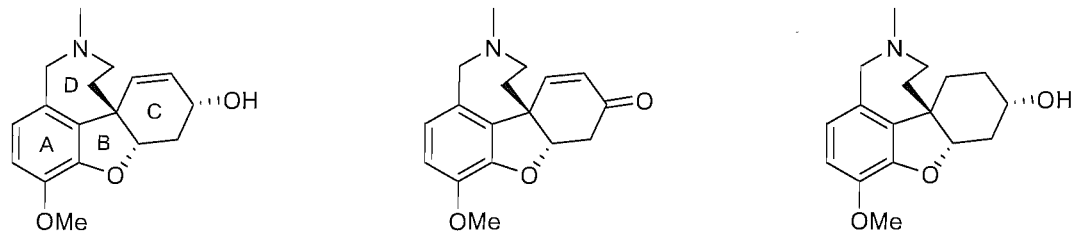


Scheme 2.29 - $^{13}C_2$-11*Z*-Retinal Synthetic Routes (a = [9,10-$^{13}C_2$], b = [10,11-$^{13}C_2$] and c = [11,12-$^{13}C_2$]).

Chapter 3 (–)-Galanthamine

3.1 Background

(–)-Galanthamine (**3.1**) is a tertiary alkaloid isolated from the bulbs and flowers of Caucasian snowdrop (*Galanthus woronowii*). Since then galanthamine has been isolated from the bulbs of various species of the *Amaryllidaceace* family.[101,102] Galanthamine is the parent member of the *amaryllidaceace* alkaloids (Figure 3.1). It acts as a reversible, competitive inhibitor of acetylcholinesterase (AChE) and as an allosteric potentiator of neuronal nicotinic acetylcholine receptors (nAChR).[103] Owing to these interactions, (–)-galanthamine hydrobromide is used for the treatment of mild to moderate Alzheimer's disease under the trade names of Reminyl® (Europe) and Razadyne® (USA).



(–)-galanthamine (3.1) (–)-narwedine (3.2) (–)-lycoramine (3.3)

Figure 3.1 The *amaryllidaceace* alkaloids

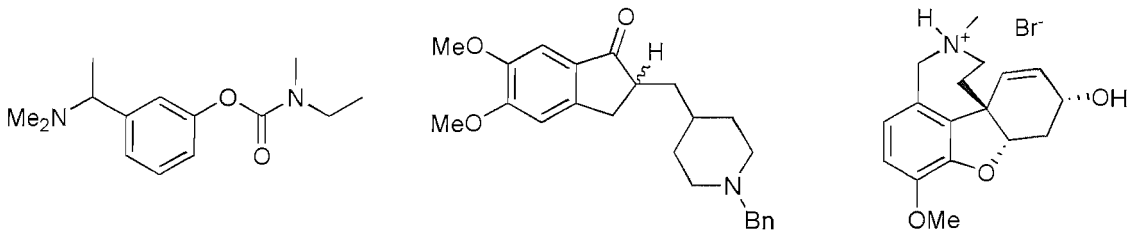
3.2 Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative age dependent disorder, leading to the deterioration of memory, judgement and comprehension. These symptoms are due to the formation of β-amyloid plaques, neurofibrillary tangles and the loss of cholinergic neurons in the hippocampus, the entorhinal cortex and the amygdala.[104-106] The amorphous amyloid plaques are found extracellularly and consist of the amyloid proteins Aβ40 and Aβ42. The proteins are produced by the proteolytic cleavage of amyloid precursor protein (APP), a membrane protein expressed in many cells including in the central nervous system (CNS). It is thought that the β-plaques cause

neuronal apoptosis leading to neurodegeneration. The associated interneuronal neurofibrillary tangles are produced by the abnormal phosphorylation of the protein Tau naturally found in cells. The phosphorylated Tau proteins form paired helical filaments, which are deposited intracelluarly. After cell death the filaments aggregate as neurofillary tangles. Loss of cholinergic neurones in the cortex and hippocampus and subsequent reduction in ACh release is thought to induce the symptoms of AD. Therefore the therapeutic approach of inhibition of acetylcholinesterase to increase levels of ACh led to the development of various AChE inhibitors.[107]

3.3 The Symptomatic Treatment of Alzheimer's Disease by AChE Inhibition

The first generation of AChE inhibitors included tacrine and physostigamine, these inhibited acetyl and butylcholinesterase with similar potencies.[108,109] In addition to their lack of selectivity they were also found to be hepatotoxic and subsequently their use was discontinued.[110] The second generation of AChE inhibitors includes rivastigamine (Exelon®), donepezil (Aricept®) and finally galanthamine (Reminyl®) (Figure 3.2). These drugs are all licensed for the treatment of mild to moderate Alzheimer's disease, with donepezil and galanthamine acting as selective AChE inhibitors. Galanthamine binds competitively and reversibly to the active site of AChE, slowing the catabolism of ACh at the synapse, this leads to an increased synaptic concentration of ACh. This counteracts the loss of ACh caused by the depletion of the cholinerginic neurones.[103] Galanthamine also acts as an allosteric nicotinic acetylcholine receptor potentiator, binding to the allosteric site produces a conformational change of the receptor increasing the effect of acetylcholine binding. This once again increases the concentration of acetylcholine in the brain. Unfortunately these drugs are less effective as the disease progresses with further loss of cholinergic neurones. Memantine (Ebixa®) is a *N*-methyl-*D*-aspartate (NMDA) antagonist and is used in the treatment of moderate to severe AD. It acts by blocking the native agonist glutamate, which is an excitotoxic neurotransmitter. With excess levels of glutamate the cell excites itself to death, which can lead to a chain reaction of cell death.



Exelon Aricept Reminyl

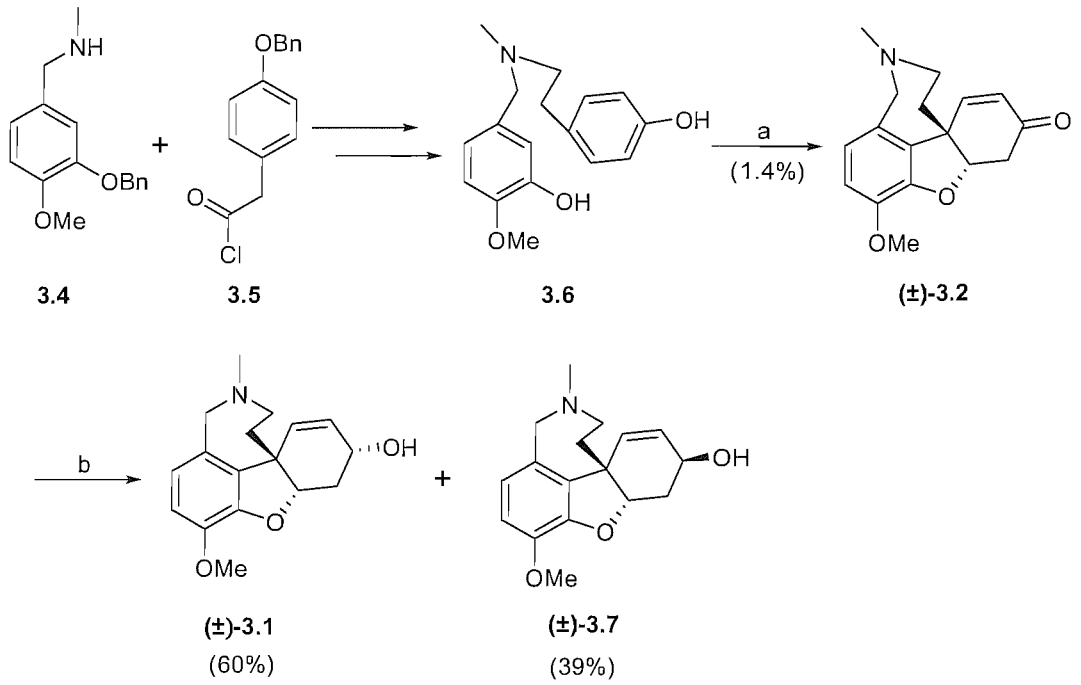
Figure 3.2 Rivastigamine, Donepezil and Galanthamine

3.4 Past Syntheses of Galanthamine

The isolation of galanthamine from natural sources is limited and costly, making synthetic strategies to galanthamine of great interest.[111,112] The past syntheses can be categorised into two broad approaches, the biomimetic oxidative phenolic coupling approach and non-biomimetic approaches. Commercially, (−)-galanthamine is prepared by oxidative coupling and subsequently chemically resolved to gain the desired (−)-enantiomer.[111,113,114] The pharmacology and synthesis of galanthamine has been discussed in a recent review by Marco-Contelles *et al.*, also recently by Stephen Kemp and Vachiporn Satcharoen within the Brown group.[115-117] Below is a brief outline of the key syntheses of galanthamine.

3.4.1 Biomimetic Syntheses

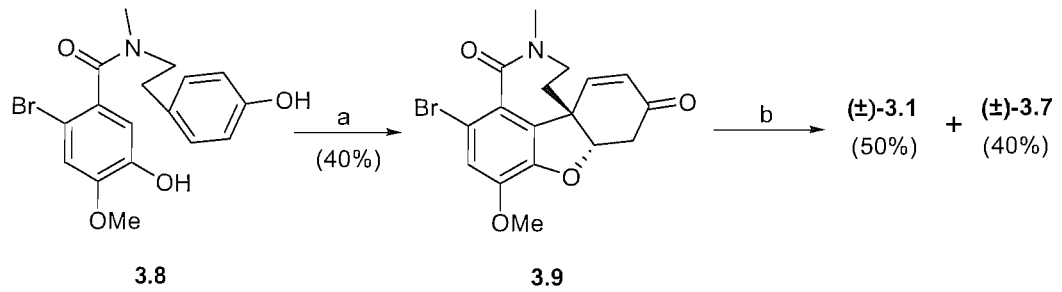
In 1962 Barton and Kirby reported the first synthesis of (±)-galanthamine using the biomimetic phenolic oxidative coupling approach (Scheme 3.1).[118] The synthesis proved to be a quick and simple route to racemic galanthamine, however the key oxidative phenolic coupling of diol **3.6** with $K_3Fe(CN)_6$ proved to give a very poor yield. The poor yield is thought to be partly due to a lack of regioselectivity in the coupling reaction, leading to reaction at the desired ortho position and at the undesired para position relative to the hydroxyl of the methoxy phenol moiety in **3.6**. Subsequent reduction of (±)-narwedine (**3.2**) with $LiAlH_4$ gave a mixture of (±)-galanthamine (**3.1**) and (±)-*epi*-galanthamine (**3.7**).



Scheme 3.1 *Reagents and conditions:* a) $K_3Fe(CN)_6$, $H_2O/NaHCO_3$; b) $LiAlH_4$, Et_2O.

Various approaches to raise the yield of the oxidative phenolic coupling have been reported, using bromine[119-125] and TMS[126] groups to block the para position with varying success.

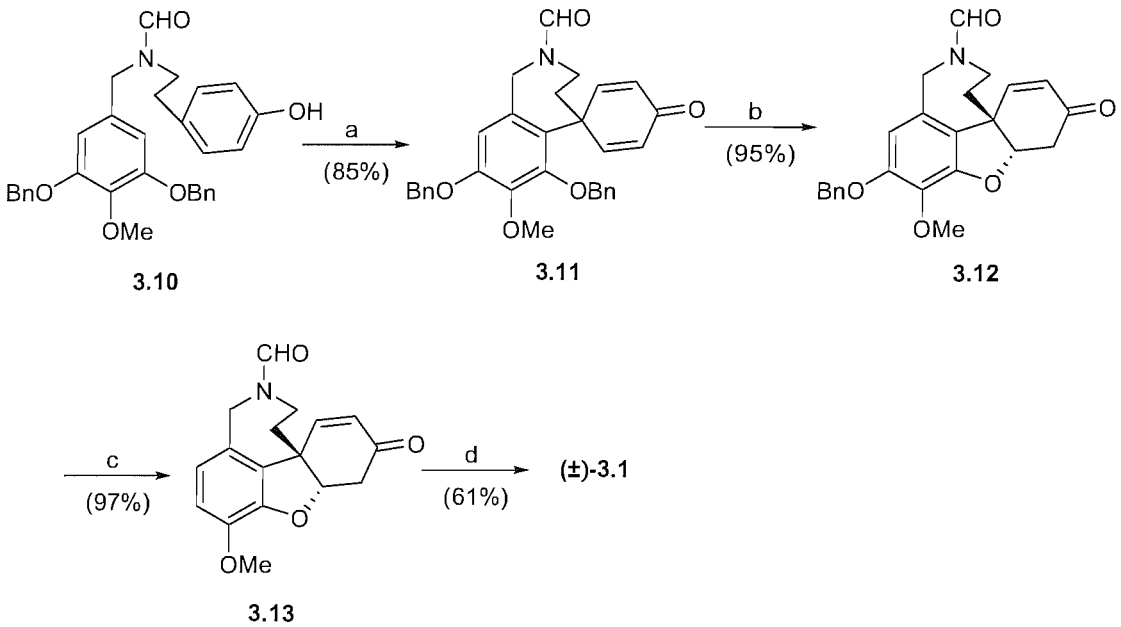
The first attempt was reported by Kametani *et al.* utilising bromine as the blocking group. This gave a vastly improved yield using $K_3Fe(CN)_6$ as the oxidant (Scheme 3.2).[119,120,127]



Scheme 3.2 *Reagents and conditions:* a) $K_3Fe(CN)_6$, $NaHCO_3$, $H_2O/CHCl_3$, 60 °C; b) $LiAlH_4$, THF, rt to reflux.

In conjunction with the use of blocking groups, phenyliodide (III) *bis*(trifluoroacetate) (PIFA) was used as the oxidant in place of $K_3Fe(CN)_6$ by a number of research

groups.[125,126,128] The most successful route was reported by Node *et al.* using substrate **3.10** in the coupling reaction with PIFA (Scheme 3.3).[128] This route used a slightly different strategy, rather than blocking the para position with a bromide atom, two benzoxy groups were used so that the A-ring became symmetrical, removing the regioselectivity issue. After the coupling, removal of a single benzyl group with BCl_3 allowed spontaneous cyclisation giving **3.12**. Subsequent functional group interconversion and removal of the superfluous benzoxy group returned compound **3.13**. In 1998, Kita *et al.* found that in their synthesis of (±)-galanthamine (**3.1**) that reduction of the α,β-unsaturated ketone with L-selectride rather than $LiAlH_4$ led to a diastereoselective reduction giving only the desired (±)-galanthamine (**3.1**).[126]

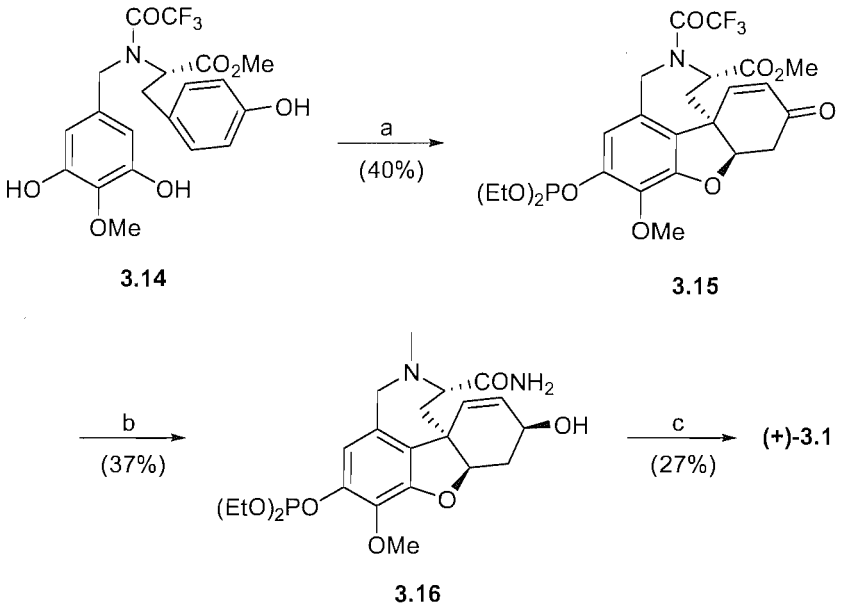


Scheme 3.3 *Reagents and conditions:* a) PIFA; b) BCl_3, CH_2Cl_2, −78 °C; c) i) Tf_2O, pyridine; ii) $Pd(OAc)_2$ PPh_3, Et_3N, HCO_2H; d) i) L-Selectride, THF, −78 °C; ii) $LiAlH_4$, THF.

3.4.1.1 First Asymmetric Synthesis Using the Biomimetic Approach

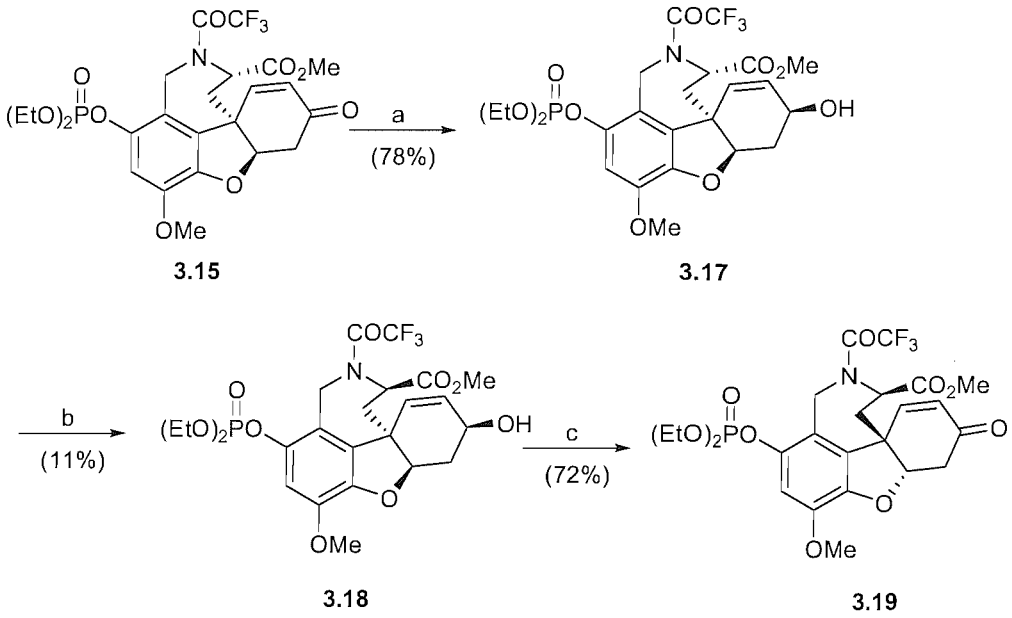
Koga *et al.* reported the first asymmetric biomimetic synthesis of galanthamine using $Mn(acac)_3$ as the oxidant (Scheme 3.4).[129-131] *L*-Tyrosine methyl ester was used to incorporate the desired chirality in ester **3.14**, the ester group was used to direct the configuration of the phenolic coupling. The coupling only produced trace amounts of

the unwanted diastereoisomer. On completion of the synthesis it was elucidated that the (+)-galanthamine (**3.1**) had actually been synthesised rather than the natural (−)-enantiomer.



Scheme 3.4 *Reagents and conditions:* a) i) $Mn(acac)_3$; ii) $(C_2H_5O)_2POCl$, Et_3N; b) i) $NaBH_4$; ii) 35% $CH_2O_{(aq)}$, 85% $HCOOH_{(aq)}$,; ii) NH_3; c) i) Ac_2O, pyridine; ii) $POCl_3$, pyridine then $LiAlH_4$; iii) Na, NH_3.

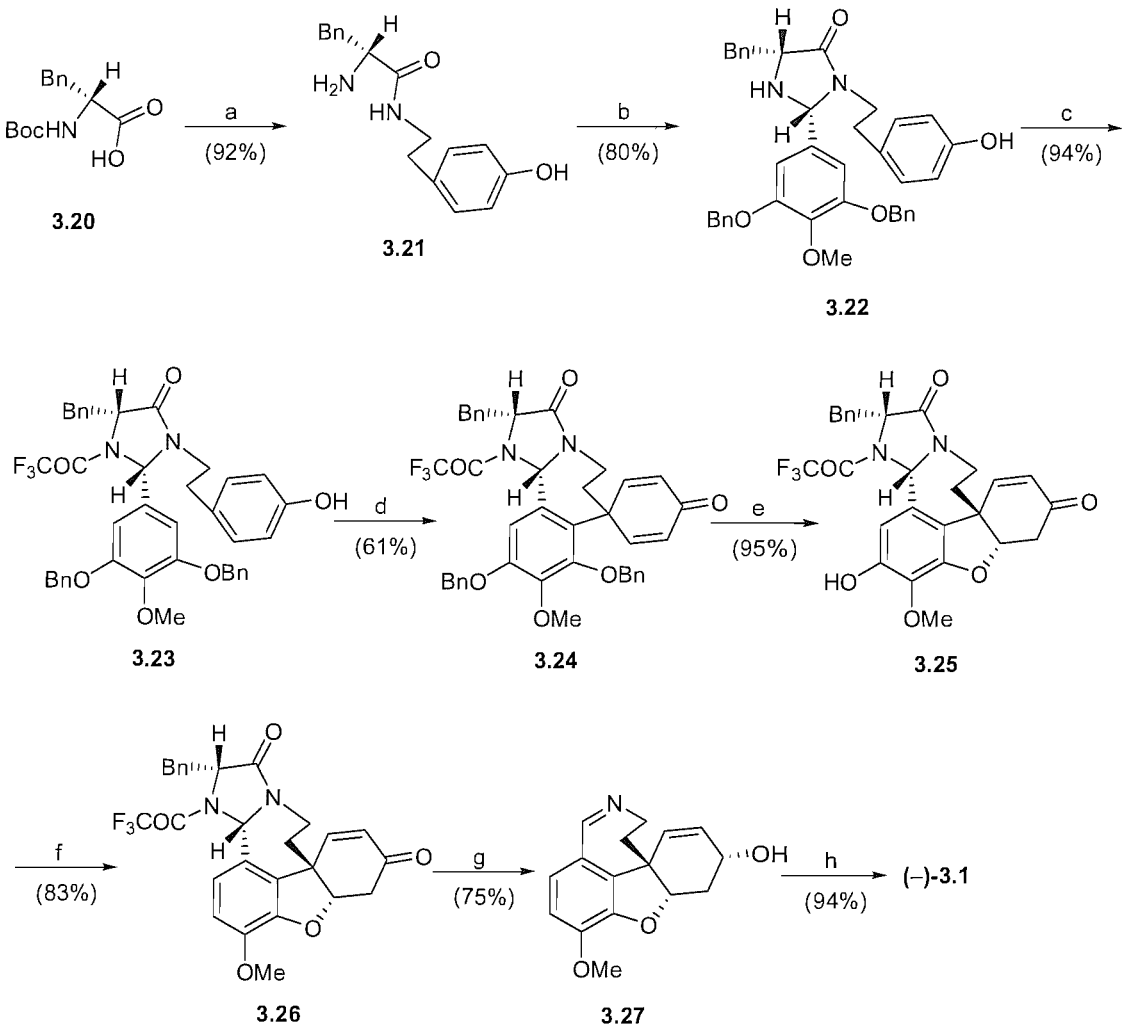
The use of *D*-tyrosine in place of natural tyrosine would allow the synthesis of (−)-galanthamine (**3.1**). Koga *et al.* were able to carry out the formal synthesis of (−)-galanthamine (**3.1**) from intermediate **3.15** (Scheme 3.5). This was carried out by the reduction of enone **3.15** followed by treatment with triflic anhydride then $KHCO_3$ in methanol, giving allylic alcohol **3.17**. Treatment of **3.17** with LDA gave the desired ester group epimerisation, subsequent oxidation to ketone **3.19** allowed the carbon skeleton to slowly epimerise to the correct configuration completing the formal synthesis.



Scheme 3.5 *Reagents and conditions:* a) i) $NaBH_4$; ii) Tf_2O, pyridine; iii) 5% $KHCO_{3(aq)}$, MeOH; b) LDA, HMPT; c) PCC.

3.4.1.2 Second Asymmetric Synthesis Using the Biomimetic Approach

In 2004, Node *et al.* reported an asymmetric biomimetic approach using remote induction (Scheme 3.6).[132] Formation of **3.23** was found to proceed in high yield giving a diastereoisomerically pure product. With this in hand the oxidative phenolic coupling could proceed with PIFA producing the desired stereochemistry. The remaining steps followed closely to the groups past work, except for the removal of the imidazolidinone functionality.[128]

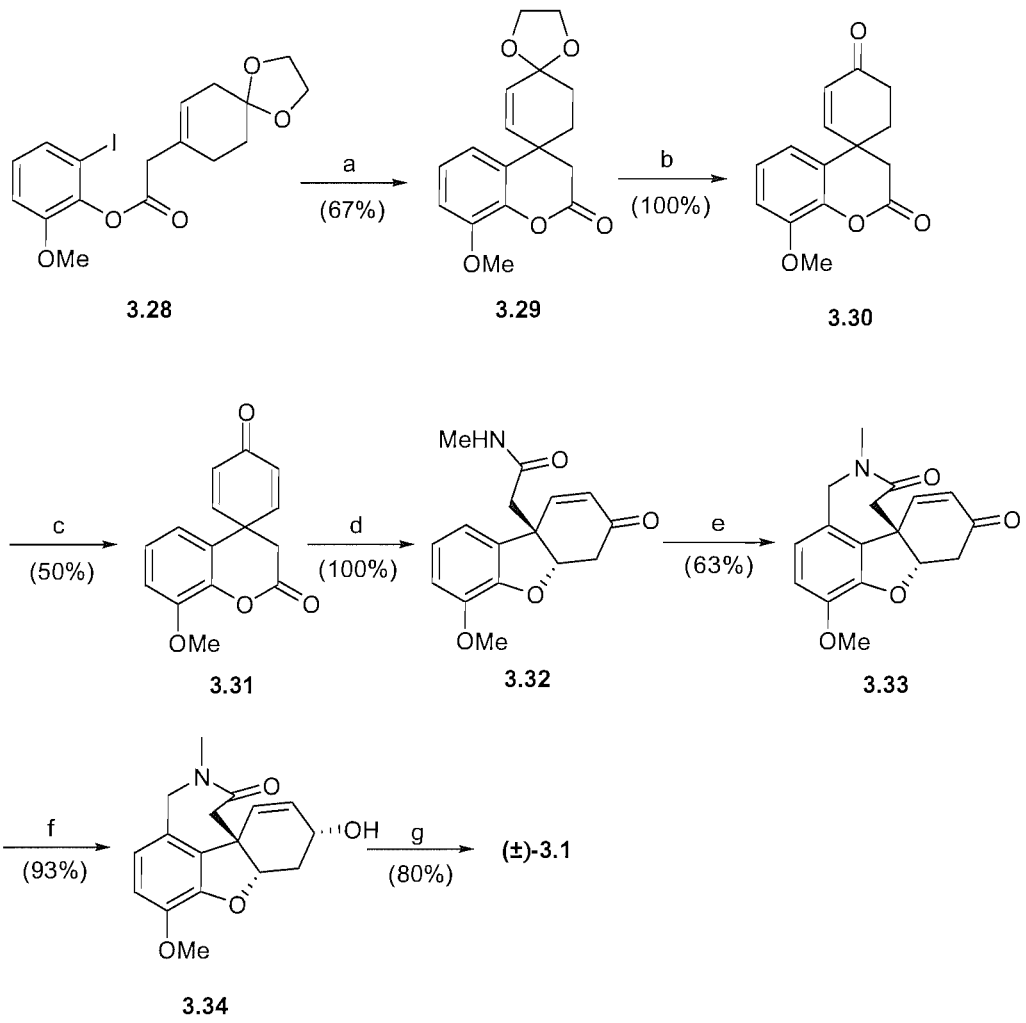


Scheme 3.6 *Reagents and conditions:* a) i) tyramine, EDC.HCl, HOBt, THF; ii) MsOH, MeOH, 40 °C; b) i) 3,5-dibenzyloxy-4-methoxybenzaldehyde, dioxane; ii) HCl, dioxane; c) TFAA, pyridine, 0 °C; d) PIFA, CF_3CH_2OH, –40 °C; e) BCl_3, –78 °C; f) i) Tf_2O, pyridine; ii) $Pd(OAc)_2$, PPh_3, HCO_2H, DMF; g) i) L-Selectride, THF, –78 °C; ii) KOH, EtOH; h) i) $NaBH_4$, MeOH, then HCO_2Et; ii) $LiAlH_4$, THF.

3.4.2 Non-Biomimetic Approaches

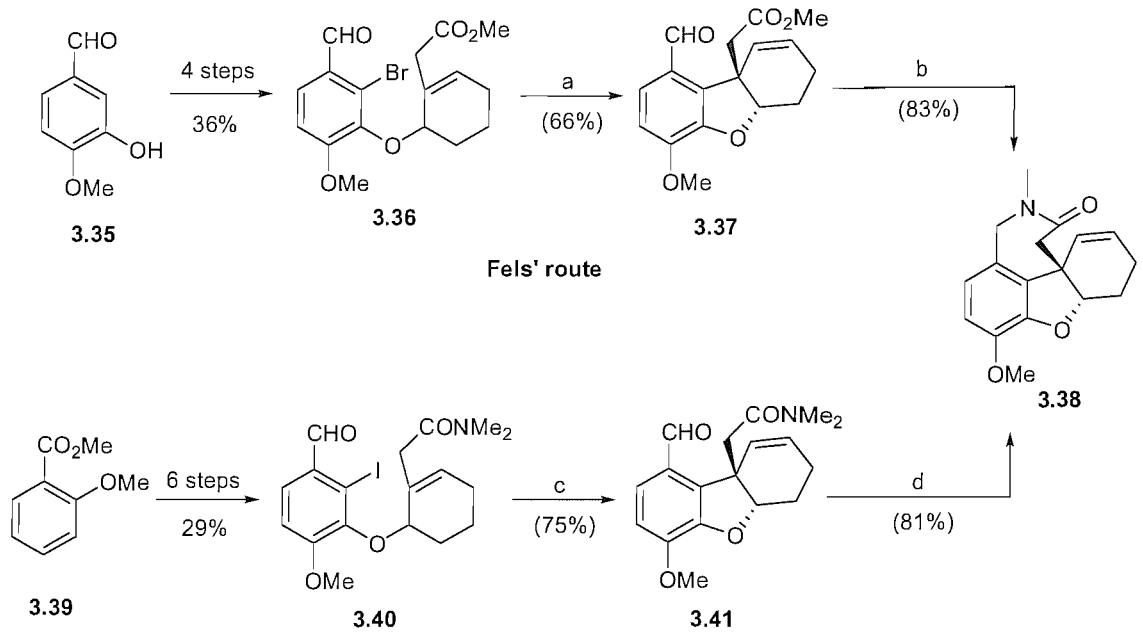
In 2001 Guillou *et al.* reported a non-biomimetic route to (±)-galanthamine (**3.1**) (Scheme 3.7).[133] Their work developed Hoshino and co-workers synthesis of (±)-lycoramine (**3.3**), substituting the radical formation of the spiro quaternary carbon with a Heck cross-coupling.[134] Guillou *et al.* originally used their simplified route for the formal synthesis of (±)-lycoramine (**3.3**) in 1999.[135] Where Hoshino *et al.* failed to

oxidise enone **3.30**, Guillou and co-workers were able to achieve the transformation with $(PhSeO)_2O$ by the addition of molecular sieves, allowing them to accomplish in their total synthesis of (±)-galanthamine (**3.1**).



Scheme 3.7 *Reagents and conditions:* a) $Pd_2(dba)_3$, dppe, TlOAc, MeCN; b) Ph_3CBF_4, CH_2Cl_2; c) $(PhSeO)_2O$, MS (4Å), CH_2Cl_2, reflux; d) 40% $MeNH_{2(aq)}$, THF; e) $(CH_2O)_n$, TFA, DCE, 60 °C; f) L-Selectride, THF, –78 °C; g) $LiAlH_4$, DME, 50 °C.

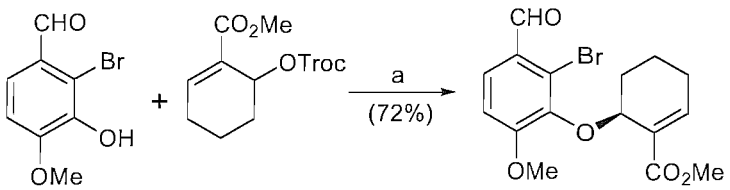
On noting Guillou and co-workers' successful implementation of a Heck cross-coupling in their formal synthesis of (±)-lycoramine (**3.3**), Fels *et al.* devised a route to racemic amide **3.38** as a precursor to (±)-galanthamine (**3.1**) (Scheme 3.8).[136] Fels and co-workers publication then prompted Parsons and co-workers to report their research into their approach to same galanthamine precursor **3.38** (Scheme 3.8).[137] Parsons *et al.* reported that attempts to achieve the allylic oxidation of **3.38** were unsuccessful.[137]

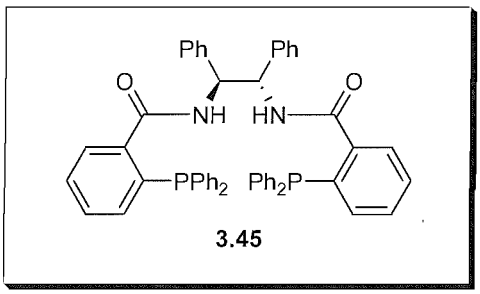


Scheme 3.8 *Reagents and conditions:* a) $Pd(PPh)_4$, K_2CO_3, toluene, 107 °C; b) i) $MeNH_2$, MeOH; ii) $NaBH_4$, 0 °C; c) $Pd(OAc)_2$, dppe, Ag_2CO_3, DMF, reflux; d) i) $MeNH_2$, EtOH then $NaBH_4$, MeOH; ii) HCl, MeOH.

3.4.2.1 Trost's Asymmetric Synthesis of (–)-Galanthamine

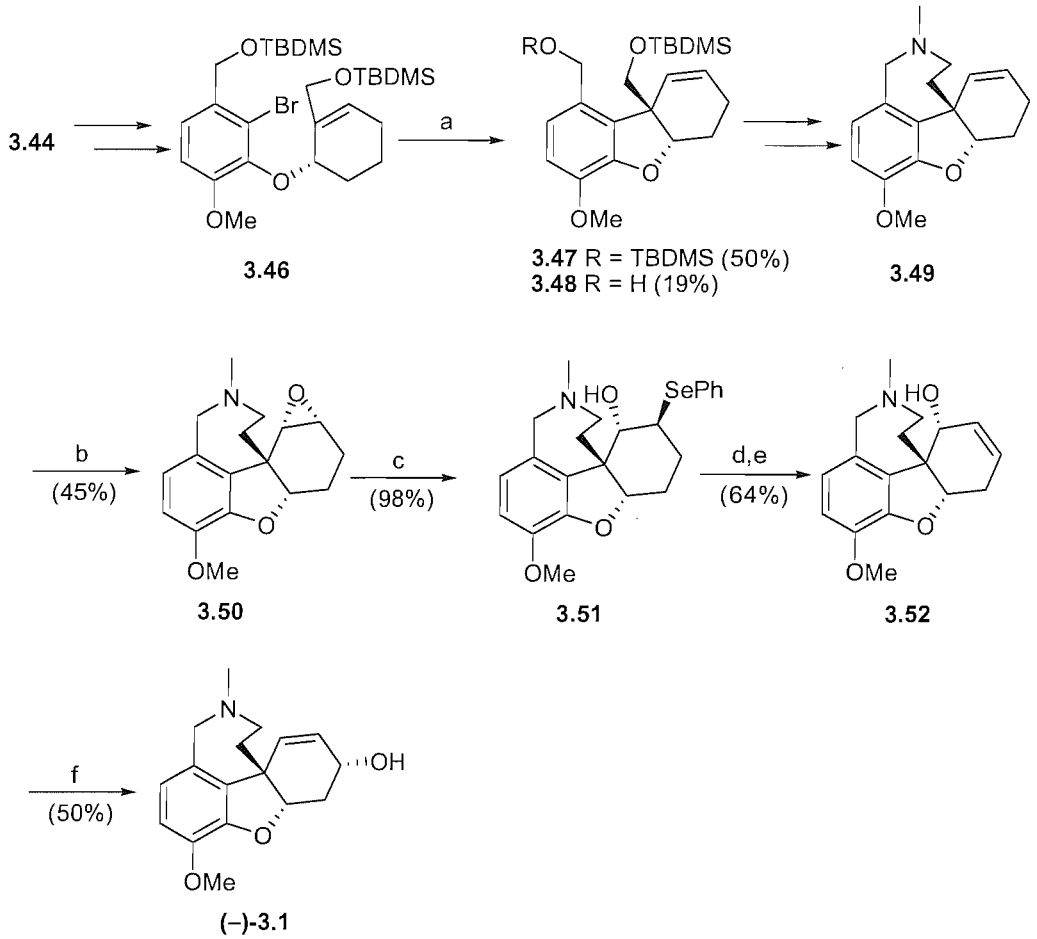
In 2001 Trost *et al.* accomplished the first non-biomimetic asymmetric synthesis of (–)-galanthamine (**3.1**) using an intramolecular Heck reaction to create the quaternary spiro carbon.[138] The first stage of their synthesis required the asymmetric formation of ether **3.44** (Scheme 3.9). Trost implemented their palladium-catalysed asymmetric allylic alkylation (AAA) methodology to afford ether **3.44** in a high yield and an *ee* of 88%, with the enantioselectivity arising from ligand **3.45**.





Scheme 3.9 *Reagents and conditions:* a) **3.45**, $[(\eta^3\text{-}C_3H_5)PdCl]_2$, Et_3N, CH_2Cl_2.

Their attempts of a direct Heck cross-coupling with ether **3.44** did not yield the desired cyclised product but resulted in the ionisation of the phenoxy moiety giving phenol **3.42**. Conversion of the ether **3.44** to *bis*TMS compound **3.46** and subsequent Heck cross-coupling using $Pd(OAc)_2$ with a range of bases gave the desired tricyclic compound **3.47** in low yields (Scheme 3.9). These low yields were due to catalyst decomposition, therefore a range of ligands and conditions were screened in an attempt to increase the yield. It was found that dpce, DMA and a proton sponge were the optimum conditions. The reaction gave no ionisation but did lead to desilylation at the benzylic oxygen. The desilylation was not of a concern as the following step was a deprotection with TBAF. Closure of the D-ring was achieved in six steps giving (–)-deoxygalanthamine **3.49**. The next key step in the synthesis was the allylic oxidation of (–)-deoxygalanthamine **3.49**, achieving (–)-galanthamine (**3.1**). They decided on the direct approach of allylic oxidation initally with SeO_2 as reported by Muxfeldt *et al.* in their synthesis of crinine.[139] This however gave a complex mixture of products, as did other oxidising agents. A three step approach was successfully implemented, firstly deoxygalanthamine (**3.49**) was selectively epoxidised. Epoxide **3.50** was then opened with phenylselenide, proceeding in excellent yield. Selenide **3.51** was oxidised with sodium periodate and with subsequent elimination at 80 °C gave (–)-isogalanthamine (**3.52**). Isomerisation to (–)-galanthamine (**3.1**) with *p*-toluenesulfonic acid then $Ph_3SiOReO_3$ proceeded in a moderate yield with 20% recovered starting material.

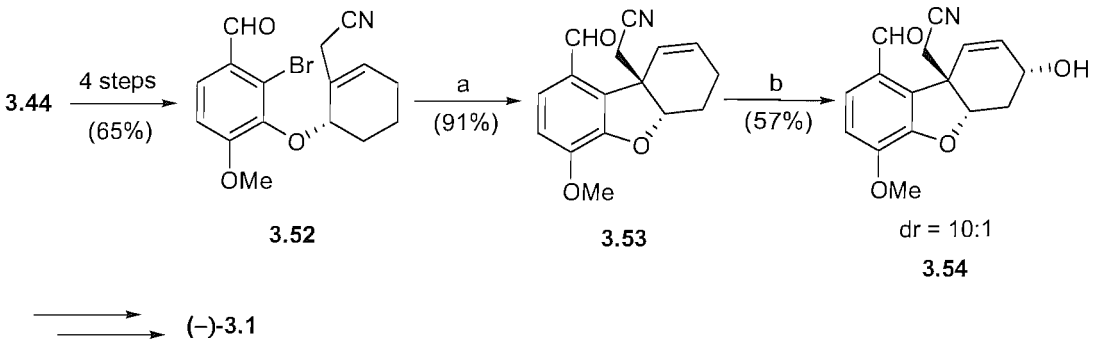


Scheme 3.10 *Reagents and conditions:* a) $Pd(OAc)_2$, dcpe, proton sponge, DMA, 80 °C; b) i) TsOH, CH_2Cl_2 then dimethyldioxirane, acetone; ii) DBU, CH_2Cl_2, 40 °C; c) PhSeSePh, $NaBH_4$, EtOH, 80 °C; d) $NaIO_4$, THF/H_2O; e) $CHCl_3$, 80 °C; f) $Ph_3SiOReO_3$, TsOH, benzene, 60 °C.

3.4.2.2 Trost's Second and Third Asymmetric Synthesis of (–)-Galanthamine

Trost *et al.* reported a rather inefficient second generation synthesis centred on an iodolactonisation to introduce the allylic alcohol, reaching (–)-galanthamine (**3.1**) in 12 steps and an 1.2% overall yield.[140] This lack of success prompted a third strategy to introduce the allylic alcohol simply and efficiently. The third strategy once again used a palladium catalysed asymmetric allylic alkylation (AAA) to into give enantioenriched ether **3.44** in high yield and good *ee* (Scheme 3.11).[140] Several functional group interconversions followed by recrystallisation raised the *ee* from 88% to 96% as ether **3.52**. With nitrile **3.52** in place, the Heck cross-coupling proceeded in excellent yield

after optimisation. The allylic oxidation with SeO_2 gave a dr of 10:1 as the SeO_2 reacted on the less hindered concave face through an ene mechanism. With the allylic oxidation complete, the closure of the D-ring was completed in three steps affording (–)-galanthamine (**3.1**) in a yield of 62%.

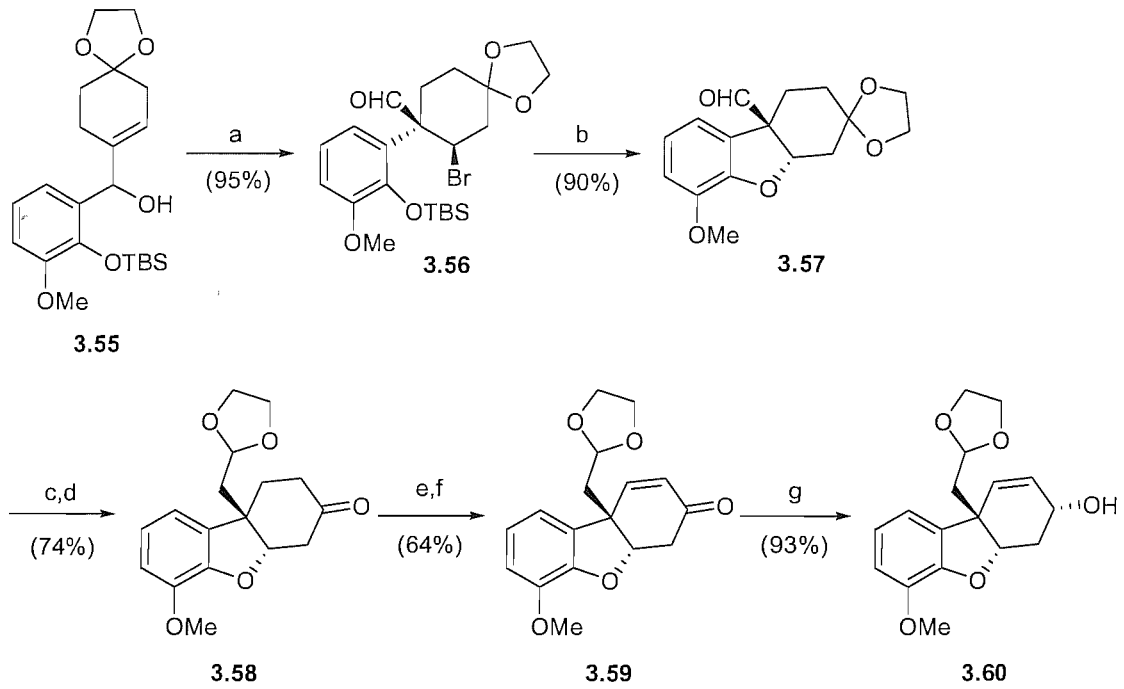


Scheme 3.11 *Reagents and conditions:* a) $Pd(OAc)_2$, dppp, $AgCO_3$, toluene, 107 °C; b) SeO_2, NaH_2PO_4, dioxane, 150 °C.

3.4.2.3 Synthesis by Tu *et al.*

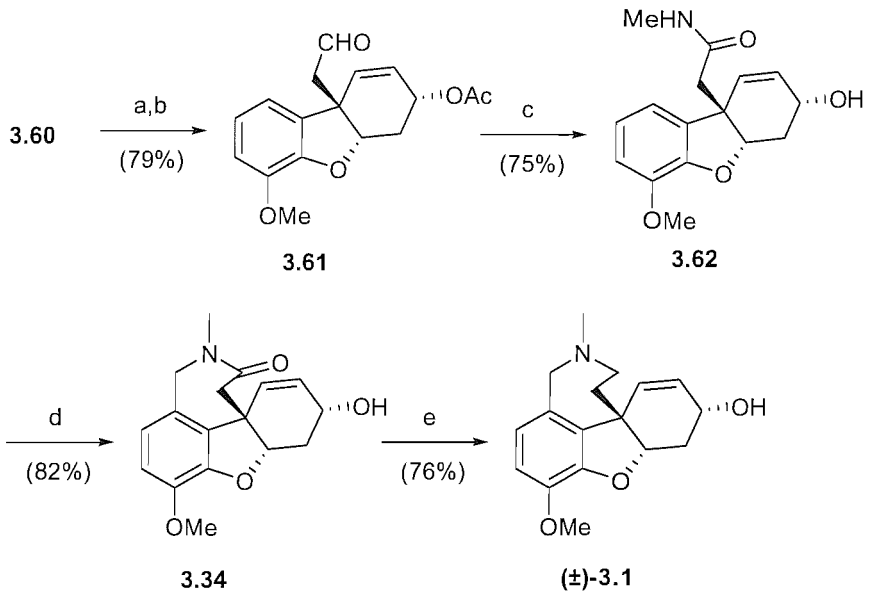
The latest synthesis of galanthamine has been reported by Tu *et al.* in 2006.[141] Their novel strategy used a semipinacol rearrangement and subsequent desilyation to create the core structure. This was followed by a modified Saegusa-Ito oxidation to establish the allylic alcohol moiety (Scheme 3.12).

Allylic alcohol **3.55** was prepared in two steps from commercially available starting materials. Treatment of allylic alcohol **3.55** with NBS gave rise to the semipinacol rearrangement giving aldehyde **3.56** in excellent yield. Desilyation with DBN in DMSO led to a spontaneous cyclisation to afford tetracycle **3.57**. Wittig reaction and subsequent dioxolane deprotection gave the unexpected product **3.58**. The group discovered that under standard acidic conditions the reaction produced ketone **3.58** in a poor yield, with the addition of glycol the yield improved dramatically. With ketone **3.58** in hand, the modified Saegusa-Ito oxidation was implemented giving the desired enone **3.59** in good yield. As in previous syntheses, enone **3.60** was reduced in an diastereoselective fashion with L-selectride in excellent yield.



Scheme 3.12 *Reagents and conditions:* a) NBS, CH_2Cl_2, 0 °C; b) DBU, DMSO, 95 °C; c) $MeOCH{=}PPh_3$, ^tBuOK, THF; d) PTS, acetone, glycol; e) LDA, TMSCl, THF, −78 °C; f) $Pd(OAc)_2$, Na_2CO_3, MeCN; g) L-selectride, THF, −78 °C.

The synthesis was completed by exploiting methodology developed during the group's synthesis of (±)-lycoramine (**3.3**) (Scheme 3.13).[142] Deprotection of the dioxolane **3.60** with HCl followed by protection of the allylic alcohol gave aldehyde **3.61**. Radical formation of acid bromide and reaction with gaseous methylamine produced amide **3.62**. The D-ring was closed with a modified Pictet-Spengler reaction with paraformaldehyde and TFA, before lactam **3.36** was reduced with $LiAlH_4$ giving the target racemic galanthamine (**3.1**).

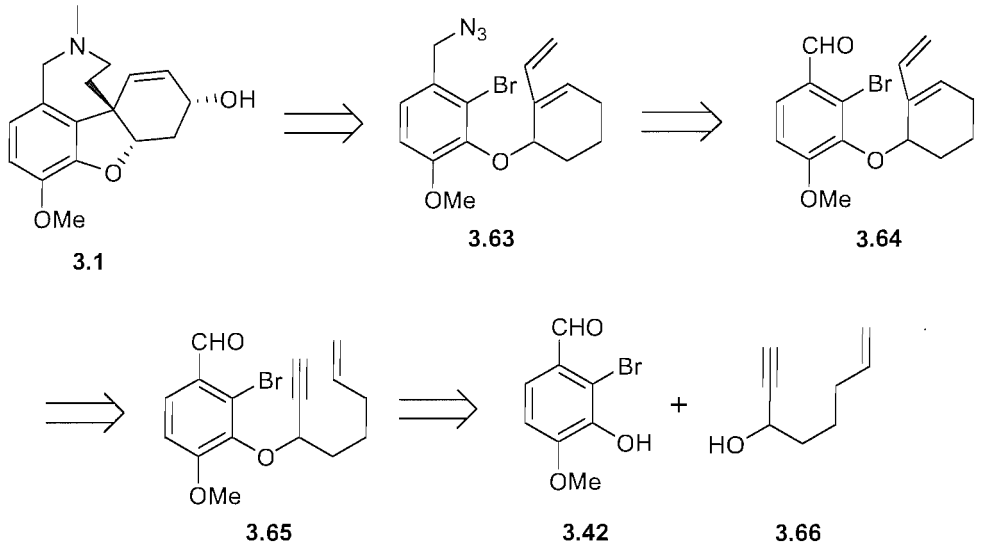


Scheme 3.13 *Reagents and conditions:* a) HCl, THF, 40 °C; b) Ac_2O, DMAP, pyridine, CH_2Cl_2; c) i) NBS, AIBN, CCl_4, 95 °C; ii) $MeNH_{2(g)}$, CCl_4; d) $(CH_2O)_n$, TFA, DCE; e) $LiAlH_4$, DME.

3.5 Previous Work in the Group

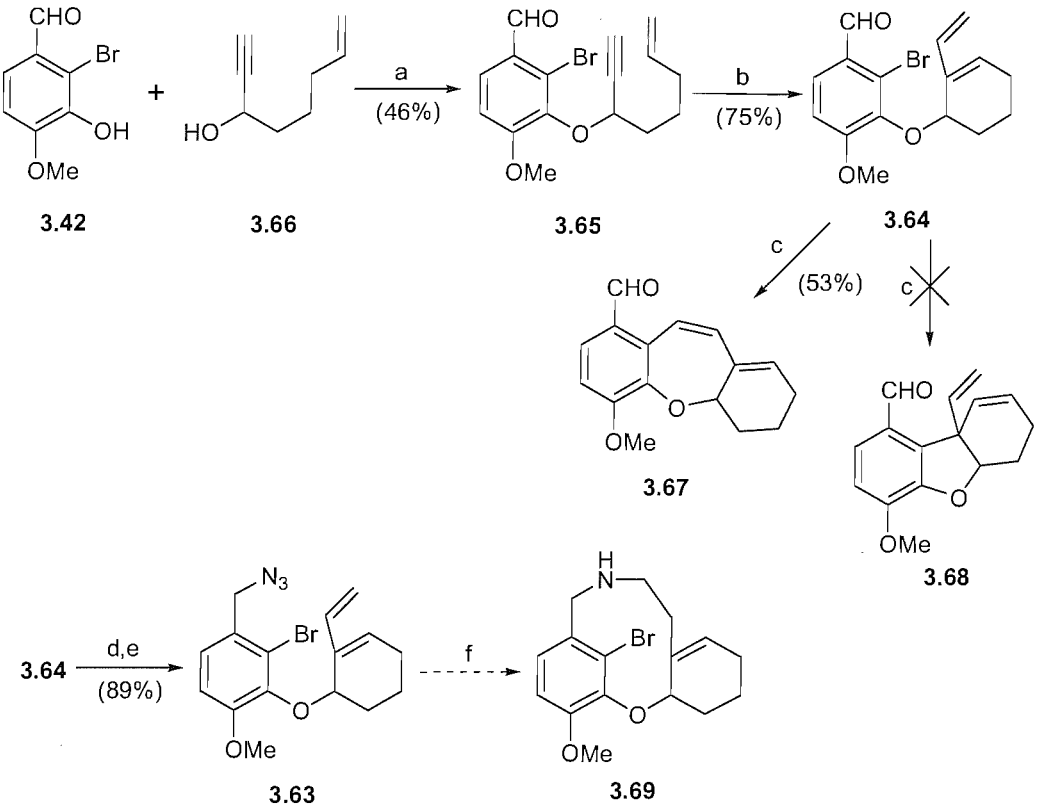
3.5.1 Kemp's Work

Kemp's proposed route aimed to use azide **3.63** to create the galanthamine skeleton using a reaction developed by H. C. Brown (Scheme 3.14).[143] Hydroboration of azide **3.63**, would lead to nucleophilic attack of the azide onto the boron group. With alkyl migration and loss of nitrogen would afford the azepine ring, with *N*-methylation and allylic oxidation to complete the synthesis.



Scheme 3.14 - Kemp's proposed route.

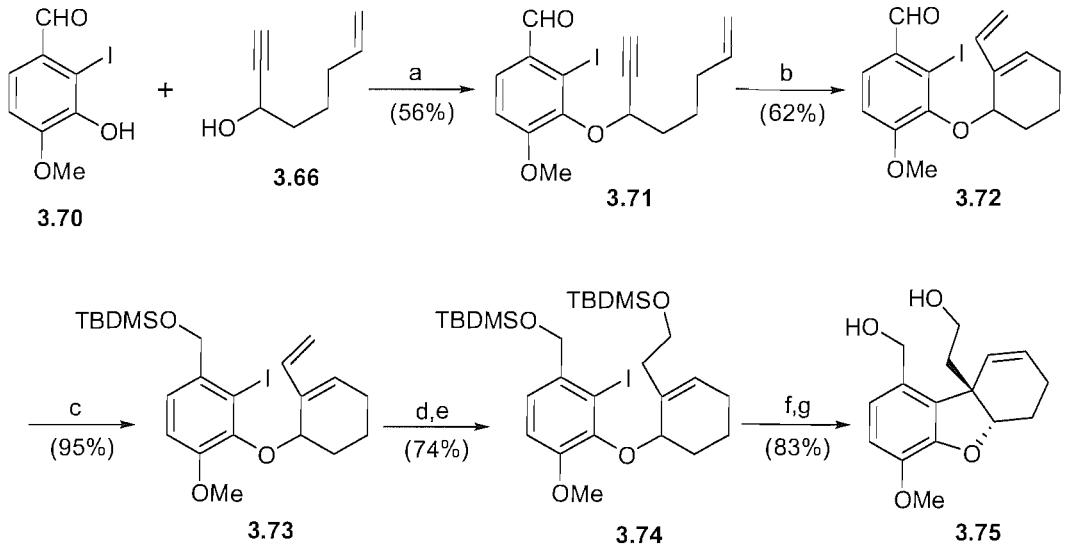
Kemp's work began with the formation of ether **3.65** by Mitsunobu reaction between phenol **3.42** and alcohol **3.66** with DIAD in a moderate yield. After enyne metathesis with Grubbs' 1st generation catalyst gave diene **3.64**, it was envisaged that the formation of the B-ring would be possible *via* an intramolecular Heck reaction. This however failed as the external alkene reacted in preference to the desired internal alkene. To avoid this unwanted reaction, azide **3.63** was constructed in the hope to hydroborate the external alkene selectively. Once the hydroboration had been effected it was hoped that the azide, would spontaneously react closing a 10-member ring. This would eliminate the possibility of the Heck reacting with the external alkene allowing the formation of the B-ring smoothly. Due to a lack of time Kemp was unable to continue his investigations further than compound **3.63**. However, initial efforts to hydroborate the diene **3.64** met without success.



Scheme 3.15 *Reagents and conditions:* a) DIAD, PPh_3, THF, reflux; b) Grubbs' 1st Generation catalyst, CH_2Cl_2, reflux; c) $Pd(OAc)_2$, dppp, Ag_2CO_3, toluene, reflux; d) $NaBH_4$, MeOH; e) $(PhO)_2P(O)N_3$, DBU, toluene; f) HBR_2.

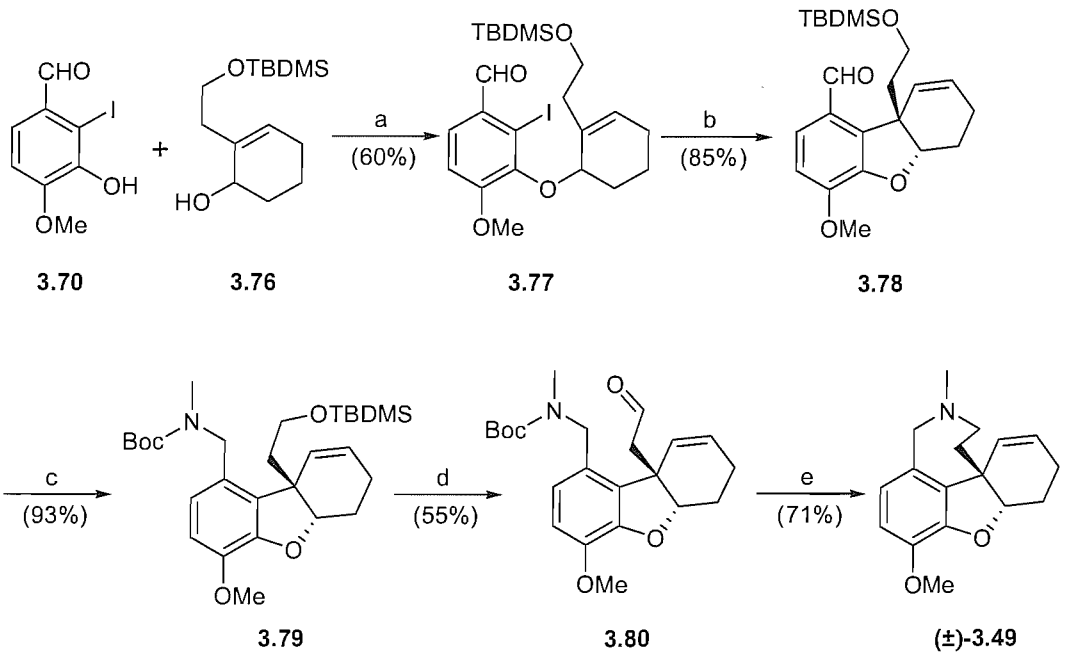
3.5.2 Satcharoen's Synthesis of (±)-Deoxygalanthamine

Satcharoen accomplished the total synthesis of (±)-deoxygalanthamine (**3.49**).[117] Building on the discoveries of Kemp, Satcharoen completed a number of alterations to the synthetic scheme to optimise the pathway. Bromide **3.42** was replaced with iodide **3.70**, leading to improved results in the Heck reaction (Scheme 3.16). As with Kemp, reactions towards diene **3.73** proceeded smoothly in good yields. Satcharoen was able to successfully hydroborate diene **3.73** with 9-BBN. This could be due to having an silyl ether in place of the azide group in Kemp's intermediate **3.63**. The primary alcohol was protected as silyl ether **3.74** before the Heck reaction was attempted. Gratifyingly, the Heck proceeded as hoped followed by removal of the silyl protection with TBAF. At this point a *bis*-tosylation was attempted, in the hope to close the D-ring with methylamine. This however, could not be achieved.



Scheme 3.16 *Reagents and conditions:* a) DBAD, *n*-Bu_3P, THF; b) Grubbs' 1st generation catalyst, CH_2Cl_2; c) i) $NaBH_4$, MeOH; ii) TBDMSCl, DMAP, imidazole, Et_3N, CH_2Cl_2; d) 9-BBN, THF, NaOH, H_2O_2; e) TBDMSCl, DMAP, imidazole, Et_3N, CH_2Cl_2; f) $Pd(OAc)_2$, Ag_2CO_3, dppp, toluene; g) TBAF, THF.

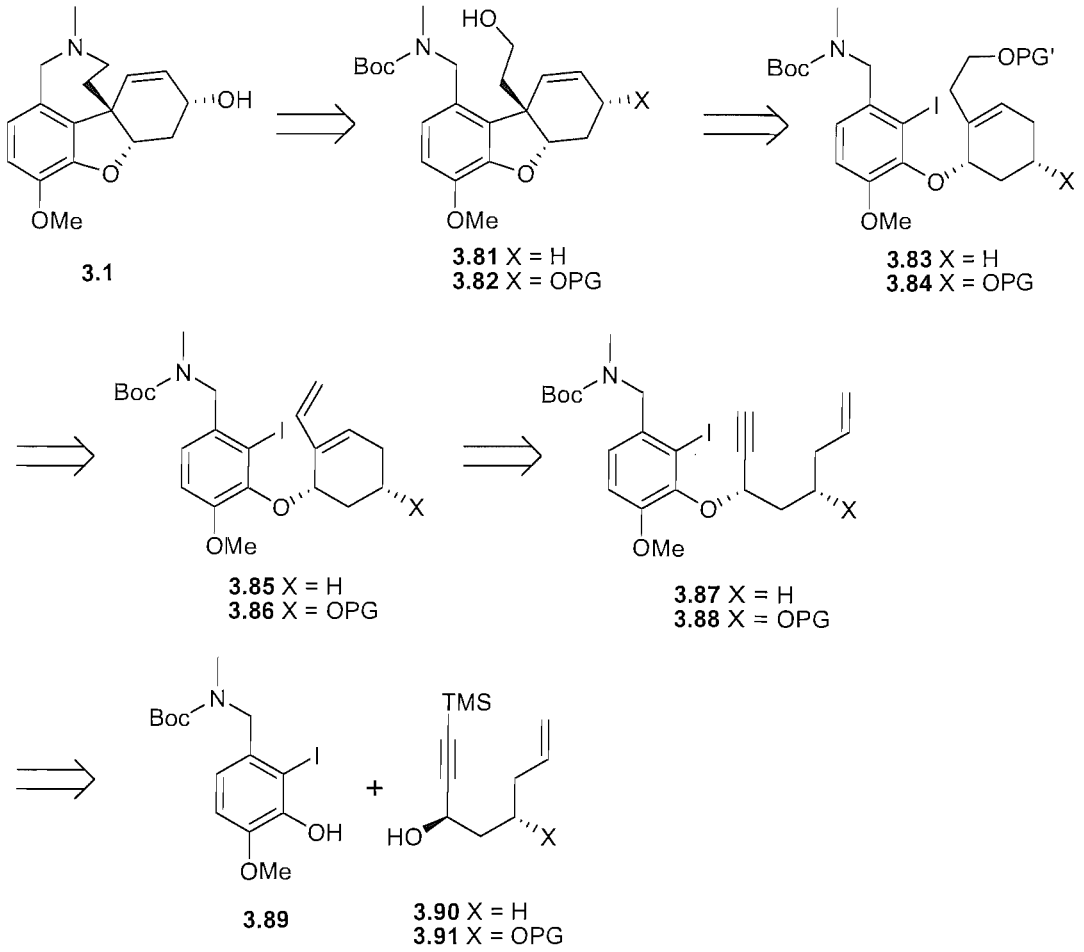
An alternative route to (±)-deoxygalanthamine (**3.49**) was devised and investigated by Satcharoen (Scheme 3.17).[117] In this synthesis the cyclohexane moiety **3.76** was preformed prior to the Mitsunobu reaction with iodide **3.70**. Post Mitsunobu reaction the Heck cross-coupling was attempted and resulted in the desired tricycle **3.78** in high yield. Reductive amination of tricycle **3.78** and carbamate formation gave **3.79**. Once this was achieved removal of the silyl protection and oxidation with Dess-Martin periodinane afforded **3.80**. Deprotection with TFA and subsequent reductive amination led to the closure of the D-ring, yielding (±)-deoxygalanthamine (**3.49**) in good yield.



Scheme 3.17 *Reagents and conditions:* a) DBAD, *n*-Bu_3P, THF; b) $Pd(OAc)_2$, Ag_2CO_3, dppp, $PhCH_3$; c) i) $MeNH_2.HCl$, EtOH, $Ti(O^iPr)_4$, ii) $NaBH_4$; iii) Boc_2O, NEt_3, CH_2Cl_2; d) i) TBAF, THF; ii) Dess-Martin periodinane, CH_2Cl_2; e) TFA, MS (4 Å), CH_2Cl_2, then $NaCNBH_3$, MeOH.

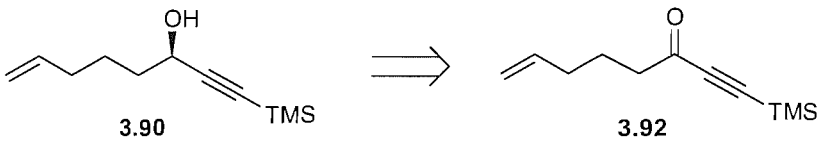
3.6 Proposed Routes to (–)-Galanthamine

Following from Kemp's and Satcharoen's previous work and successes, two routes to (–)-galanthamine (**3.1**) were proposed. The latter stages of the two routes would proceed in the same way, the major variation being how the allylic alcohol moiety in (–)-galanthamine (**3.1**) was to be introduced. In route A the allylic alcohol was to be introduced by the allylic oxidation of **3.81** with SeO_2, which has been previously shown possible by Trost *et al.*[144] The second approach (Route B) would incorporate the hydroxyl functionality early in the synthesis prior to the Mitsunobu reaction between iodide **3.89** and alcohol **3.91**. It was ultimately hoped that this second generation approach would allow the potentially low yielding allylic oxidation to be avoided.



Scheme 3.18 Retrosynthetic analysis of (–)-galanthamine.

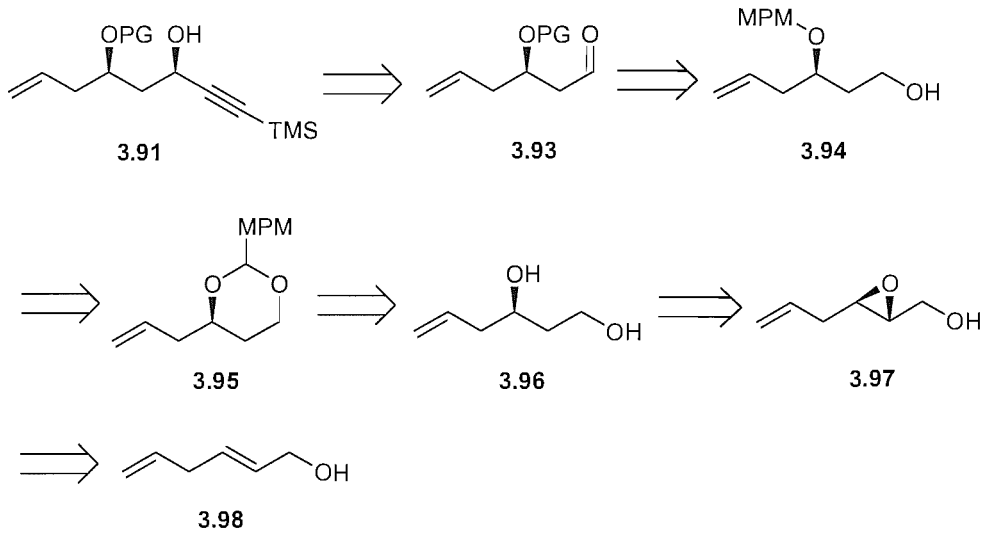
For route A, production of alcohol **3.90** would require the asymmetric reduction of ketone **3.92** (Scheme 3.19).



Scheme 3.19 Retrosynthetic analysis of propargylic alcohol **3.90**.

The synthesis of alcohol **3.91** would be more involved. The required stereochemistry in **3.93** was planned to be introduced by the Sharpless asymmetric epoxidation of diene **3.98** (Scheme 3.20).[145] With the epoxide **3.97** in place, regioselective opening with Red-Al® and conversion to the protected 1,3-diol **3.95** would be investigated. It was then hoped that regioselective DIBAL-H reduction of acetal **3.95** would afford primary

alcohol **3.94,** which was to be oxidised and subjected to an asymmetric alkynation to achieve the desired propargylic alcohol **3.91**.



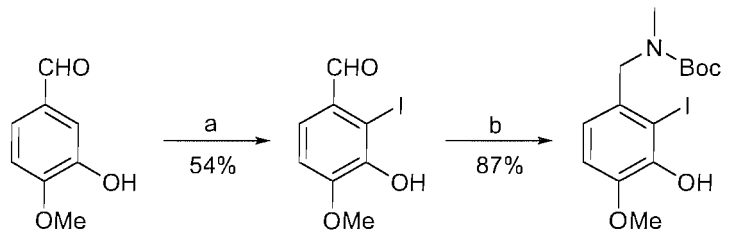
Scheme 3.20 Retrosynthetic analysis of diol fragment **3.91**.

The following chapter will describe implementation of the routes discussed above to the total synthesis of (–)-galanthamine (**3.1**).

Two approaches to (–)-galanthamine (**3.1**) were investigated, namely route A and route B (Scheme 3.18). Route A was proposed to start from an enantiomerically enriched alcohol, with the introduction of the allylic alcohol to be faced later in the synthesis by an allylic oxidation with SeO_2.[140] Whereas route B was designed to have both alkoxy moietys in place prior to the Mitsunobu coupling with aryl fragment **3.89**.

4.1 Route A

For route A, the formation the two key fragments, TMS propargylic alcohol **3.90** and phenol **3.89** was required. Once prepared, they were to be coupled by a Mitsunobu reaction giving the carbon skeleton of (–)-galanthamine (**3.1**) (Scheme 4.5).



Scheme 4.1 *Reagents and conditions:* a) ICl, pyridine, dioxane; b) i) $MeNH_2$, MeOH; ii) $NaBH_4$, MS (4 Å), MeOH; iii) $(Boc)_2O$, dioxane/H_2O.

The first fragment synthesised was phenol **3.89** (Scheme 4.1). Isovanillin (**3.35**) was iodinated regioselectively using ICl as reported by Markovich *et al.*, giving iodide **3.70** in a 54% yield.[146] The reaction was found to progress slowly, after stirring for 6 days the reaction was quenched and found that it had not reached completion (product:starting material = 3:2 estimated by ^1H NMR).

Reductive amination of aldehyde **3.70** was originally attempted by an elegant one-pot method that has been successfully used in the group previously.[116,117] The methodology was shown to be an efficient method for the reductive amination of various benzaldehydes with methylamine hydrochloride.[147] The method utilises $Ti(O^iPr)_4$

which is thought to form an intermediate methylaminoalcoholatotitanium complex (Figure 4.1). The complex is then reduced *in situ* with $NaBH_4$ giving the desired secondary amine.

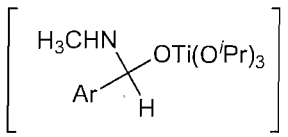


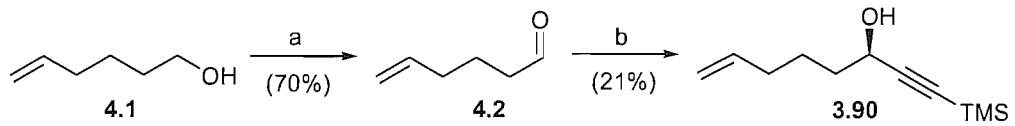
Figure 4.1 Intermediate methylaminoalcoholatotitanium complex

In the case of our substrate **3.70**, the reaction unfortunately did not give the desired secondary amine. It was discovered that the reaction gave an incredibly poor mass recovery after removal of a precipitate. This is thought to be due to phenol **3.70** reacting with $Ti(^{i}OPr)_4$ to form an insoluble titanium complex. To deduce that the insoluble precipitate was not the desired secondary amine, an *in situ* Boc protection was attempted in the hope to obtain an isolable product. This attempt however did not lead to dissolution of the complex. In an attempt to resolve this complex formation, phenol **3.70** was protected with an acetyl group in good yield using acetic anhydride in pyridine. The reductive amination was attempted again with Bhattacharyya's conditions. This led to the rapid removal of the acetate by methylamine and subsequent precipitation of the presumed complex. The reductive amination was found to proceed well with a two step reaction using a methanolic methylamine solution followed by reduction with $NaBH_4$ as described by Hart *et al.* (Scheme 4.1).[148]

Boc protection of the secondary amine was firstly attempted with $(Boc)_2O$ in $^{t}BuOH$. This lead to complete consumption of the amine but gave a 4:1 mixture of the desired product **3.89** with an undesired carbonate by-product. The additional phenolic Boc group was easily removed in excellent yield using 7 N NH_3 in MeOH as described by Nakamura *et al.*[149] The additional step to remove the phenolic Boc protection was undesired therefore alternative conditions reported by Ueno *et al.*, using $(Boc)_2O$ in dioxane and H_2O were examined.[150] These conditions gave the desired product **3.89** in excellent yield with none of the carbonate by-product formed (Scheme 4.1).

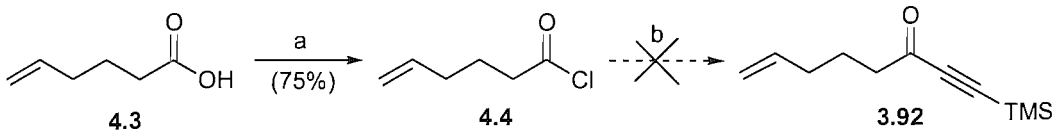
The conversion of the aldehyde **3.70** to carbamate **3.89** was initially decided to be carried out stepwise. It was found that the intermediate unprotected secondary amine

was relatively insoluble in most solvents, making purification awkward. Therefore, the reactions were carried out sequentially on the crude intermediates giving the methyl carbamate **3.89** in excellent yield (87%) over three steps.



Scheme 4.2 *Reagents and conditions:* a) PCC, CH_2Cl_2; b) i) $Zn(OTf)_2$, (+)-methylephedrine, Et_3N, toluene; ii) TMSC≡CH, toluene, 60 °C.

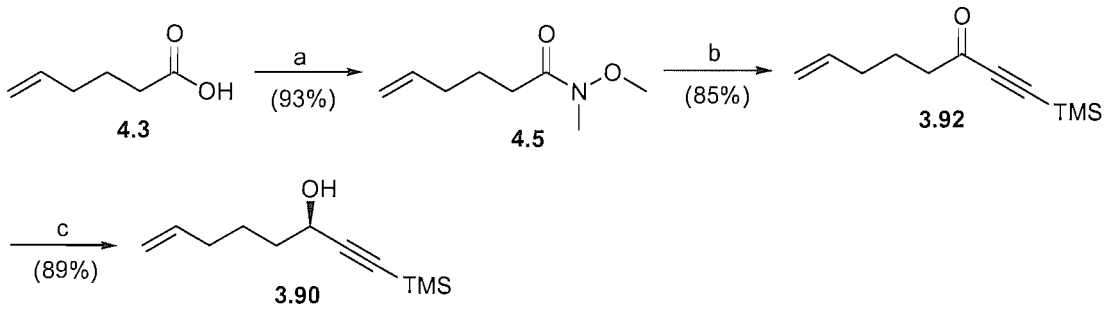
The next stage of the synthesis was to develop an enantioselective synthesis of alcohol **3.90** (Scheme 4.2). Oxidation of 5-hexen-1-ol (**4.1**) with PCC as reported by Meyer *et al.* gave aldehyde **4.2** in good yield.[151] The oxidation was also attempted with TPAP to avoid the use of PCC, unfortunately this did not produce any desired aldehyde **4.2**. Using a mild method described by Frantz *et al.*, propargyl alcohol **3.90** was formed from the enantioselective addition of TMS acetylene using $Zn(OTf)_2$ and (+)-methylephedrine.[152] Initial attempts produced very small quantities of the desired propargyl alcohol **3.90** at room temperature. Strand *et al.* reported that their substrate required the reaction to be carried out at 60 °C, which gave their desired product in good yield and diastereoselectivity.[153] With the increase in reaction temperature it was found to improve the yield to 21% and give an *ee* of 85%, which was still determined to be unsatisfactory. The poor yield was thought to result from self aldol condensation of the aldehyde, which could be reduced but not avoided.



Scheme 4.3 *Reagents and conditions:* a) DMF, $(COCl)_2$; b) $AlCl_3$, TMSC≡CTMS, CH_2Cl_2, −78 °C to 0 °C.

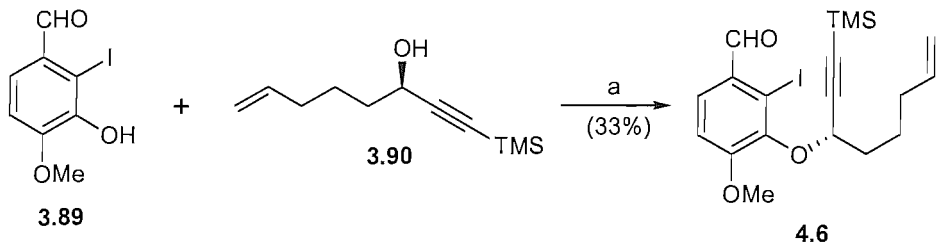
With the disappointing results from the asymmetric addition of TMS acetylene to aldehyde **4.2**, it was decided that propargyl alcohol **3.90** would be synthesised by asymmetric reduction of propargylic ketone **3.92**. The synthesis of ketone **3.92** was initially attempted from 5-hexenoyl chloride (**4.4**) (Scheme 4.3). Conversion of 5-

hexenoic acid (**4.3**) to acid chloride **4.4** proceeded rapidly using a procedure reported by Ahrendt *et al.*, however addition of TMS acetylene with *bis* TMS acetylene and $AlCl_3$ did not proceed as hoped.[154,155] The method was found to be intolerant of the terminal alkene causing cyclisation of the acid chloride **4.4**, giving cyclohex-2-enone.



Scheme 4.4 *Reagents and conditions:* a) EDCI, DMAP, *N,O*-dimethylhydroxyamine hydrochloride, CH_2Cl_2; b) TMSC≡CH, *n*-BuLi, THF, –40 °C to –10 °C; c) *R*-alpine borane, THF, 0 °C to rt.

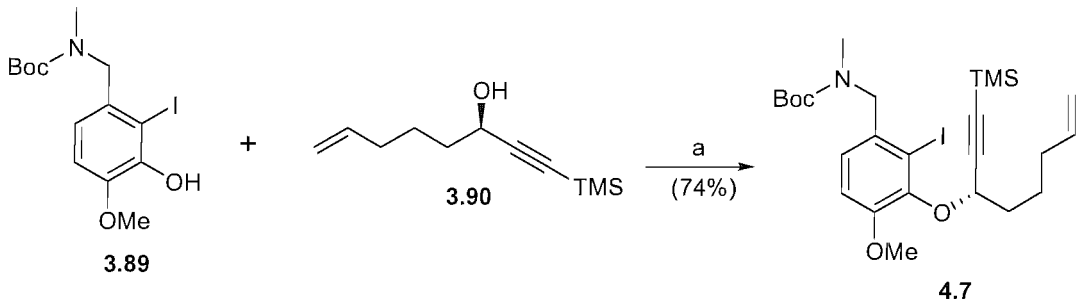
A new route to ketone **3.92** was then developed (Scheme 4.4). Formation of Weinreb amide **4.5** proceeded smoothly in excellent yield using EDCI as the coupling agent. Carbonyl diimidazole was also used but produced a slightly lower yield of 80%. The addition of TMS acetylene to Weinreb amide **4.5** also gave consistently high yields. Following the TMS acetylene addition, the asymmetric reduction with *R*-Alpine Borane™ gave the desired propargyl alcohol **3.90** in high yield with an enantiomeric excess of 92%. The *ee* was determined with analytical chiral HPLC (OD-H column) after derivation by Mitsunobu coupling between propargyl alcohol **3.90** and phenol **3.89** (Scheme 4.5).



Scheme 4.5 *Reagents and conditions:* a) DIAD, PPh_3, THF, reflux.

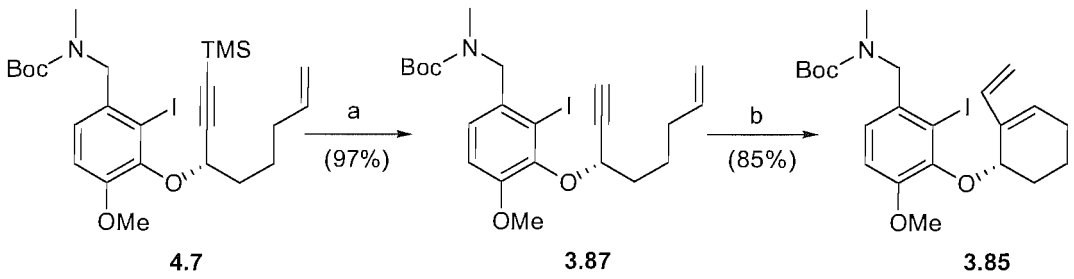
With the synthetic routes to key fragments **3.89** and **3.90** established, the fragments were coupled using Mitsunobu conditions (Scheme 4.6). Previous work carried out by

Satcharoen coupled phenol **3.70** and alcohol **3.66** (Scheme 3.16). It was found that the Mitsunobu reaction carried out by Satcharoen, DBAD was the azodicarboxylate of choice, giving a yield of 56%. DIAD was also used by Satcharoen in the coupling but was found to be less effective giving a yield of 48%.[117] However in the case of **3.90** and **3.89**, the opposite was observed with DBAD affording almost no reaction and DIAD giving a good yield of 74% (Scheme 4.6). The result in the case of DBAD was possibly due to poor reagents rather than substrate. Because the use of DIAD gave good results, the use of DBAD was not further investigated.



Scheme 4.6 *Reagents and conditions:* a) DIAD, PPh_3, THF, reflux.

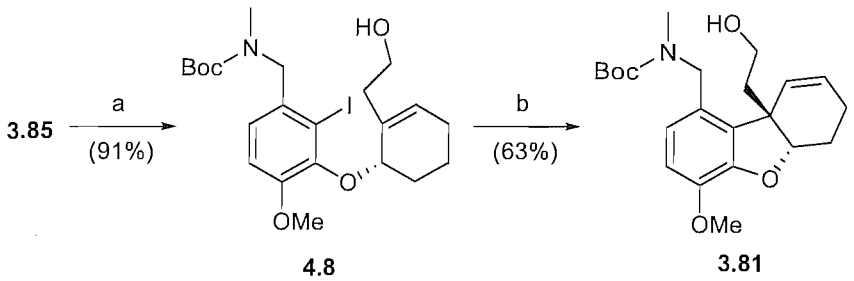
With the carbon skeleton in place, the closure of rings B, C, D were investigated to give (−)-galanthamine (**3.1**).



Scheme 4.7 *Reagents and conditions:* a) K_2CO_3, MeOH; b) Grubbs' 1st generation catalyst, CH_2Cl_2.

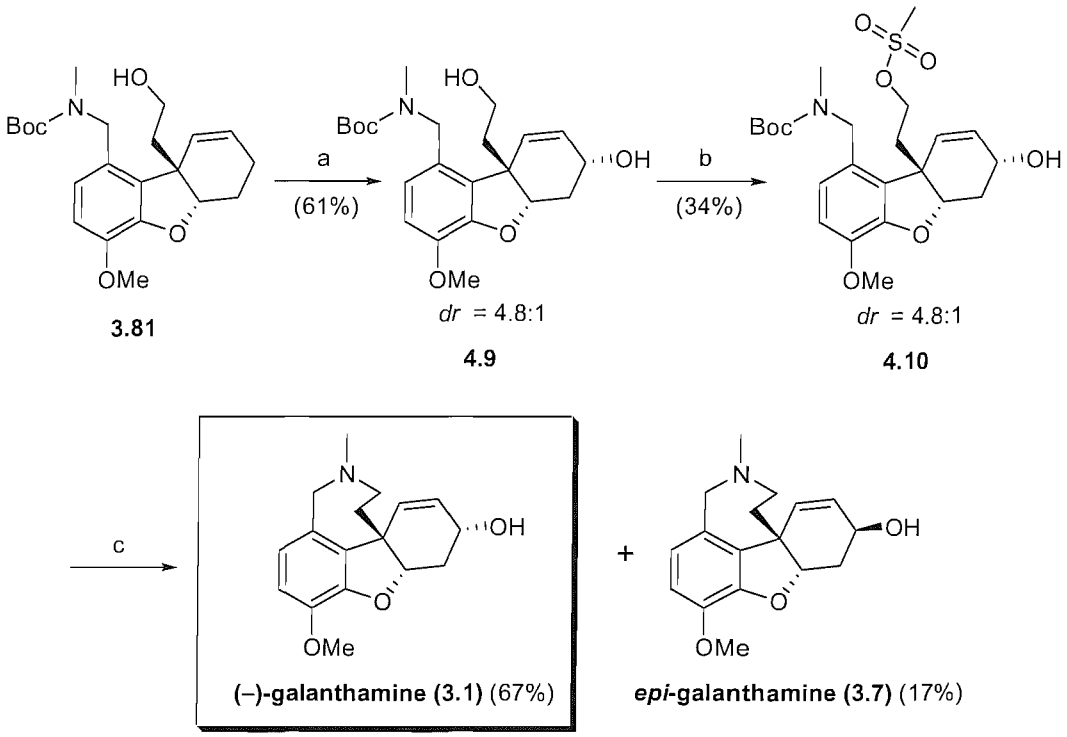
Removal of the TMS protection was extremely straightforward, affording enyne **3.87**, which was used without further purification (Scheme 4.7). The formation of the C ring was carried out with Grubbs' 1st generation catalyst in high yield. The reaction was optimised from Satcharoen's original reaction conditions.[117] The reaction was carried

out at rt for a shorter reaction period, also the catalyst loading was reduced from 10 mol% to 5 mol%, without an adverse effect on the yield or reaction rate.



Scheme 4.8 *Reagents and conditions:* a) i) 9-BBN, THF, 17 h; ii) 3 M NaOH followed by H_2O_2, THF; b) $AgCO_3$, $Pd(OAc)_2$, dppp, toluene, reflux.

The next ring to be closed was the B ring *via* a Heck cross-coupling which has been proved effective in previous work in our group by Satcharoen, and the groups discussed earlier.[117,133,136,137,140] It was found that if the Heck was carried out on diene **3.85** it would close with the external double bond to form an unwanted seven-membered ring.[116] Therefore the strategy of hydroborating and oxidising the external double bond prior to the Heck cyclisation was carried out.[117] The hydroboration and oxidation of diene **3.85** advanced smoothly in excellent yield, giving alkene **4.8** without complications (Scheme 4.8). The Heck reaction was investigated without the protection of the free hydroxyl and was found to work efficiently affording the desired tricyclic compound **3.81** in good yield considering the avoidance of protecting groups.



Scheme 4.9 *Reagents and conditions:* a) SeO_2, NaH_2PO_4, quartz sand, dioxane, 150 °C, sealed tube; b) MsCl, Et_3N, THF then CH_2Cl_2, 0 °C to rt; c) i) TFA, CH_2Cl_2, 0 °C to rt; ii) $NaHCO_3$, $CHCl_3$, 0 °C to rt.

In Trost's synthesis of (–)-galanthamine (**3.1**) a diastereoselective allylic oxidation with SeO_2 was implemented to incorporate the secondary alcohol in a modest yield of 57% and a good dr of 10:1.[140] This procedure was utilised on alkene **3.81** with a comparable yield but an unfortunately reduced diastereoselectivity (dr = 4.8:1). The moderate yield in both cases is due to decomposition of the starting material over the long reaction time. To accelerate the reaction, the use of microwave irradiation was investigated, but unfortunately this led to rapid decomposition of starting material, making microwave acceleration an ineffective method. In the reaction the diastereoselectivity is controlled by the orientation of the axial proton which is required for π-overlap with the double bond in the ene mechanism (Figure 4.2).[140]

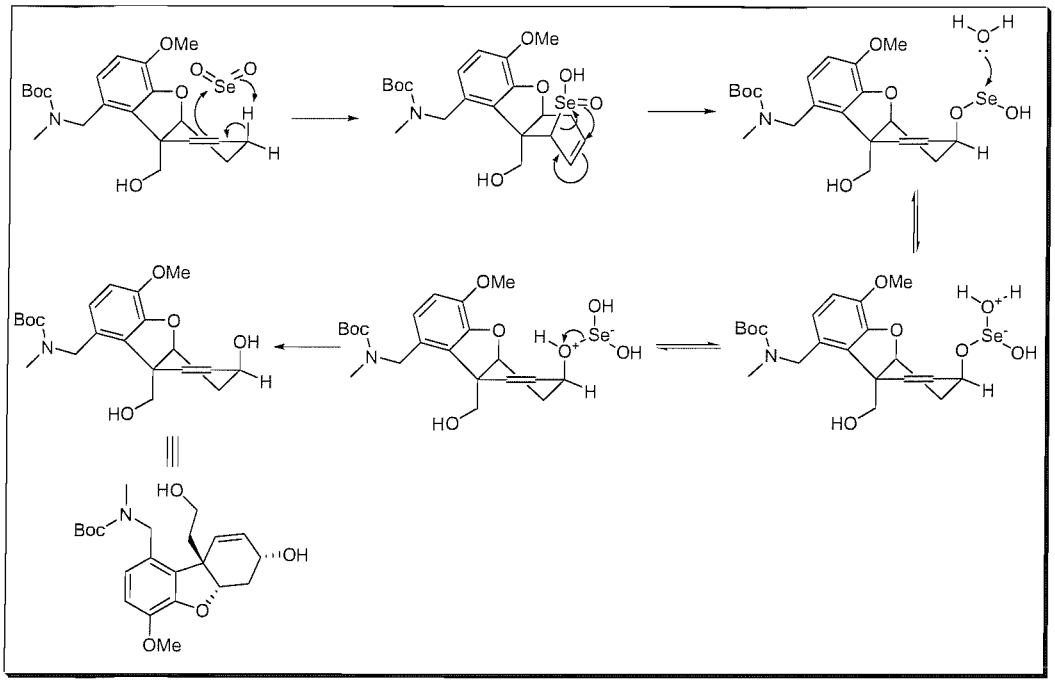


Figure 4.2 Allylic oxidation with SeO_2

The selective primary mesylation of diastereomeric diols **4.9** (dr = 5:1) was hoped to be high yielding and straightforward (Scheme 4.9). Taber *et al.* reported a very effective mesylation on a similar motif in their synthesis of (–)-mesembrine.[156] Their method gave the desired mesylate a high yield of 84% with no reported dimesylation. This method was implemented to diol **4.9** (dr = 4.8:1), it was apparent that Et_2O was incompatible with our diol due to solubility, therefore it was exchanged with THF. Unfortunately the reaction was found to proceed extremely slowly, giving to a second change of solvent system to CH_2Cl_2. With the use of CH_2Cl_2 the reaction started to progress but at a slow rate, even with further equivalents of MsCl the reaction was slow. After a total of 91.5 hours at room temperature the reaction was quenched. The reaction was found to consist of a mixture of starting material (10%), desired product (34%) and dimesylated material (17%). With further time and material, a selective tosylation would have been attempted, with hopefully improved results.

The final steps required to convert mesylate **4.10** to (–)-galanthamine (**3.1**) were a Boc deprotection and a ring closure (Scheme 4.9). Mesylate **4.10** (dr = 4.8:1) was treated with TFA in CH_2Cl_2 which led to the clean and efficient cleavage of the Boc protecting group. The acidic mixture was then diluted with $CHCl_3$ and basified with $NaHCO_3$, leading to smooth azepine ring closure giving a mixture of (–)-galanthamine (3.1) and

epi-galanthamine (**3.7**). The epimers where easily separated by column chromatography, affording pure isolated (–)-galanthamine (67%) and *epi*-galanthamine (17%). For a ^{1}H NMR spectra of (–)-galanthamine (**3.1**) see Figure 4.3.

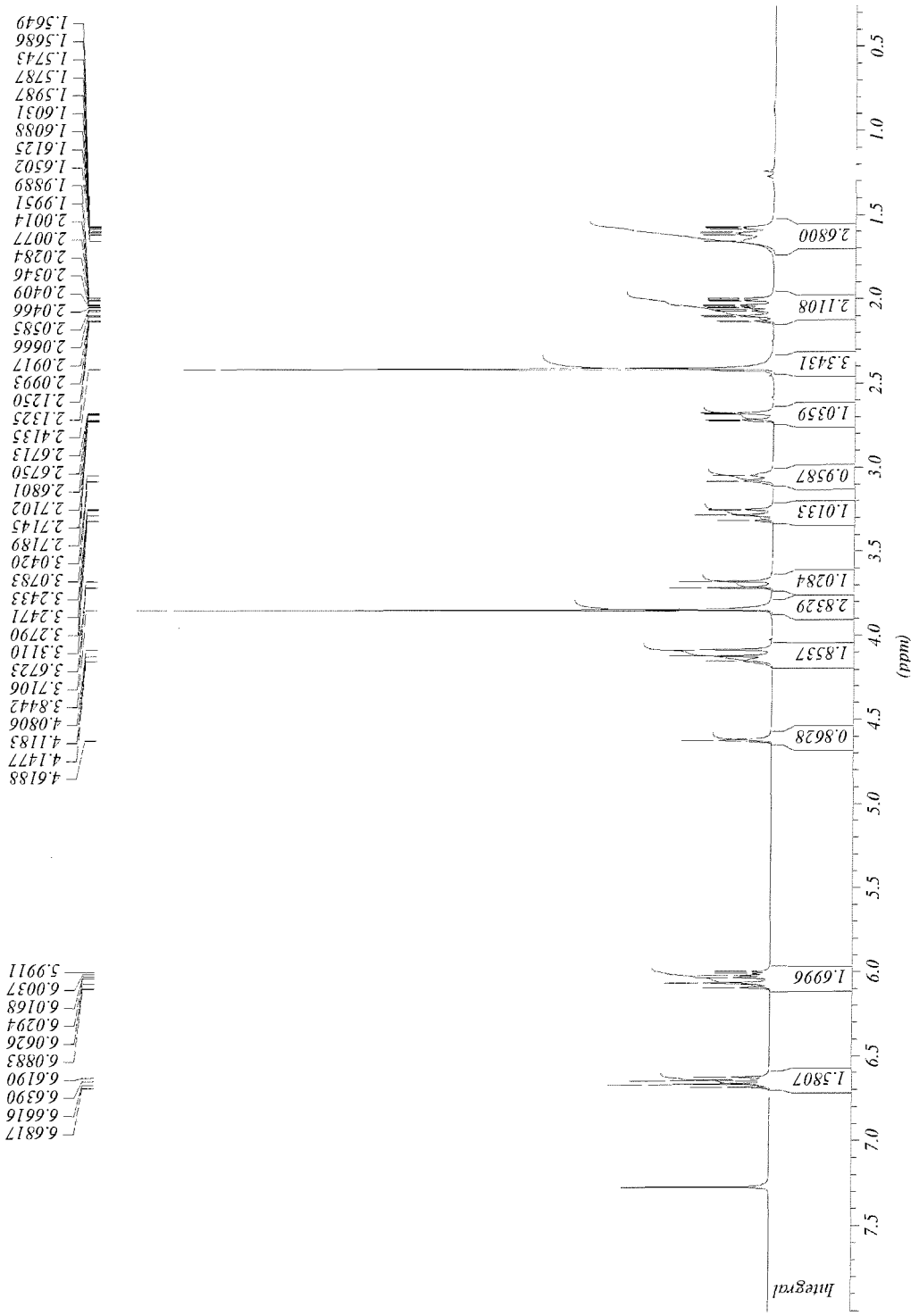
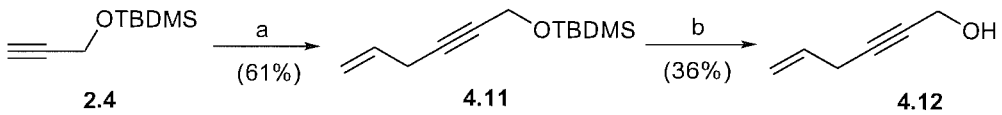


Figure 4.3 ^{1}H NMR of (–)-galanthamine (**3.1**)

4.2 Route B

For route B, diene **3.98** was proposed as the substrate for the key Sharpless asymmetric epoxidation, which will introduce the enantioselectivity at the ether junction of ring B. Diene **3.98** was to be synthesised from propargyl alcohol **4.12** by $LiAlH_4$ reduction. Therefore the route B synthesis commenced with the investigation into two routes to intermediate propargyl alcohol **4.12** (Schemes 4.10 and 4.11).

The first route to propargylic alcohol **4.12** commenced by allylation of TBDMS ether **2.4** followed by TBAF deprotection (Scheme 4.10). This route was found to give the desired alcohol **4.12** in a poor overall yield of 19%. The poor yield was partly due to the allylation reaction being sluggish and producing several by-products. Also the seemingly straightforward deprotection with TBAF was found to give a poor yield, this is thought to be caused by losses during multiple purifications in efforts to acquire pure material.



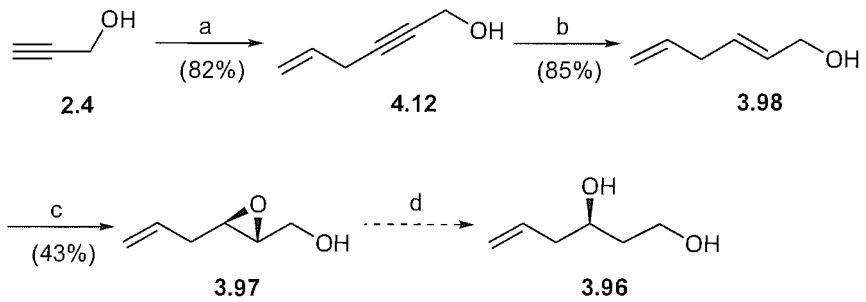
Scheme 4.10 *Reagents and conditions:* a) i) *n*-BuLi, THF, –78 °C; ii) $H_2C{=}CHCH_2Br$, HMPA, –78 °C to rt; b) TBAF, THF.

The second route to the desired propargylic alcohol **4.12** was effected by direct allylation of the dianion of propargyl alcohol (**2.4**), formed using two equivalents of *n*-BuLi and HMPA. This however did not produce the desired product or a viable route to diene **3.98**.

4.3 Ongoing Work

With further consideration and searching of the literature a new and shorter pathway to alcohol **4.12** was investigated (Scheme 4.11). The pathway started with the efficient allylation of propargyl alcohol **2.4** as described by Taber *et al.* leading directly to alcohol **4.12** in high yield.[157] Alcohol **4.12** was then cleanly reduced to diene **3.98** with

$LiAlH_4$ in high yield.[158] With diene **3.98** in hand, the Sharpless asymmetric epoxidation was attempted a number of times, giving epoxide **3.97** in disappointing yields (36-43%).[145] Analysis of the crude material showed that the reaction had gone to completion leading to the conclusion that the epoxide was decomposing on purification. This was surprising as Ma *et al.* also purified the same epoxide **3.97** by column chromatography, achieving a yield of 86%.[159]



Scheme 4.11 *Reagents and conditions:* a) i) EtMgBr, THF/Et_2O, 0 °C, 2 h; ii) H_2C=$CHCH_2Br$, CuBr.Me_2S, THF/Et_2O, 0 °C to rt; b) $LiAlH_4$, THF, reflux; c) ^{t}BuOOH, MS (4 Å), *D*-DET, $Ti(O^iPr)_4$, CH_2Cl_2, –20 °C; d) Red-Al®, THF.

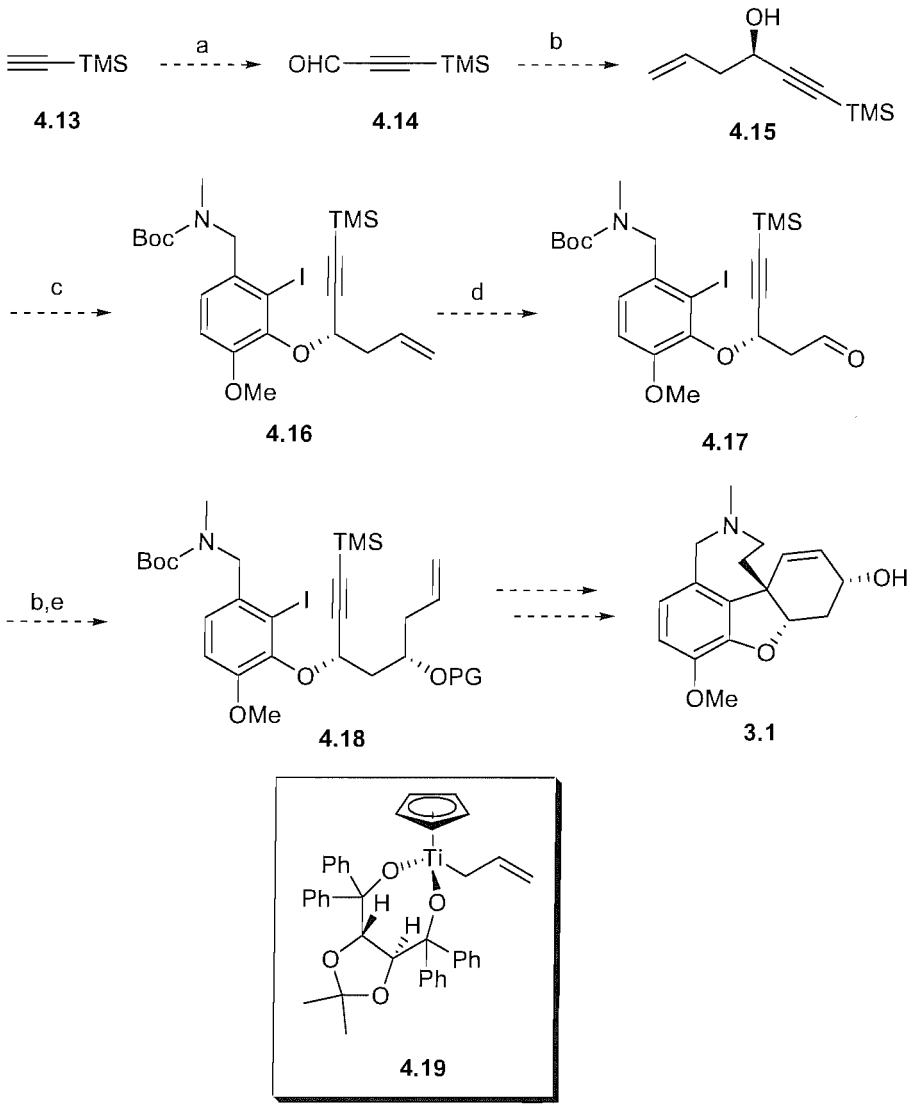
Further investigation into the above route to (–)-galanthamine (**3.1**) was not continued due to a lack of time.

4.4 Conclusions and Further Work

Two routes towards (–)-galanthamine (3.1) were investigated. Route A, led to the successful total synthesis of (–)-galanthamine (**3.1**) in 11 steps with an overall yield of 4%. The route utilised an asymmetric reduction (*ee* = 92%) and allylic oxidation (dr = 4.8:1) to introduce two of the three required stereocentres of the tetracyclic core. The other tertiary stereogenic centre was introduced with complete diastereoselectivity using a Heck cyclisation. The synthesis proved to be concise and included many high yielding steps, with the unfortunate exception of the selective mesylation of diol (**4.8**). With further time and material, selective tosylation would have been investigated. Effecting a successful tosylation, the overall yield of the synthesis could be raised to 11%, based on a conservative 90% yield for the tosylation. Work on route B, towards

(−)-galanthamine (**3.1**) was commenced. The epoxide intermediate **3.97**, required for the synthesis of fragment **3.96** was successfully prepared. However, insufficient time was available to pursue the route further.

Future work will introduce a new route to implement the required stereochemistry in the allylic alcohol and B-ring ether (Scheme 4.12). The new synthesis will utilise asymmetric allylation to introduce both of the required stereocentres. The titanium allylating reagent is derived from *D*-tartaric acid and will introduce the desired *R*-configuration for both stereocentres in **4.14** and **4.17**.[160-162] Once **4.17** is obtained, the synthesis will continue along similar lines to route A.



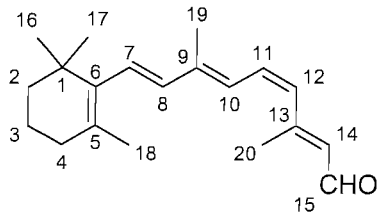
Scheme 4.12 *Reagents and conditions:* a) *n*-BuLi, DMF; b) **4.18**, NH_4F, H_2O; c) **3.89**, DIAD, PPh_3, THF, reflux; d) $NaIO_4$, OsO_4; e) Protection.

Chapter 5 Experimental

General Experimental

All reactions were carried out under an inert atmosphere in oven dried glassware. THF and Et_2O were distilled from sodium and benzophenone prior to use. Triethylamine and dichloromethane were dried by distillation from CaH_2. All other solvents and reagents were purified, if required, by standard methods.[163] Column chromatography was carried out using Merck Kieselgel 60 and column dimensions are quoted in cm (width x depth). Thin layer chromatography was carried out on Merck silica gel 60 F_{254} and visualised under UV illumination (254 nm) and stained with potassium permanganate. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ or C_6D_6 solutions using a Bruker AC300 and AV300 (300 and 75 MHz respectively) or on a Bruker DPX400 (400 and 100 MHz respectively). ^{19}F and ^{31}P NMR spectra were recorded in solution on a Bruker AV300 (282 and 121 MHz respectively). Chemical shifts are reported in δ units using $CHCl_3$ or C_6H_6 as an internal standard (δ 7.27 ppm ^1H, δ 77.36 ppm ^{13}C, δ 7.15 ppm ^1H, δ 128.62 ppm ^{13}C respectively). 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), qn (quintet), br (broad) and m (multiplet). Infrared spectra were recorded on a Nicolet 380 fitted with a Diamond platform, as solids or neat liquids. The IR spectra are reported in wavenumbers (cm^{-1}). The abbreviations, (s) strong, (m) medium, (w) weak and (br) broad were used when reporting the spectra. Melting points were obtained using a Gallenkamp Electrothermal apparatus and are uncorrected. Electron impact and chemical ionisation mass spectra were obtained using a Fisons VG platform single quadropole mass spectrometer. Electrospray mass spectra were obtained using a Micromass platform mass analyser with an electrospray ion source. Reactions introducing and containing the 11*Z* double bond were carried out in dim red light conditions and base washed glassware. HPLC purification of retinal was performed with a Shimadzu VP series HPLC and Phenomenex silica column, eluting with Et_2O/hexane. The numbering system for retinal and it's precursors are according to the IUPAC approved numbering of 11*Z*-retinal (**1.1**).[164] Enantiomeric excesses were determined with chiral HPLC analysis, performed on a Hewlett-Packard 1090 series II HPLC and Daicell Chemical Industries Chiralcel OD-H column, eluting with IPA/hexane.

11*Z*-Retinal (1.1)



$C_{20}H_{28}O$

m.w. = 284.44 g/mol

Yellow oil

To a solution of 11*Z*-retinol (**1.2**) (38 mg, 0.133 mmol) in CH_2Cl_2 (3 mL) was added crushed 4 Å molecular sieves (100 mg), NMO (26 mg, 0.226 mmol) and TPAP (15 mg, 44 μmol). This mixture was stirred for 1 hour before filtration through a pad of celite and neutral alumina, flushing with Et_2O. The solution was concentrated *in vacuo* and evaporated to dryness under high vacuum to give the title compound as a yellow oil (36 mg, 0.127 mmol, 95%, 11*Z*/all-*E* = 1.6:1). Pure 11*Z*-retinal (**1.1**) was isolated with HPLC eluting with Et_2O (2.00 mL/minute) and hexane (7.99 mL/minute), U.V. detector = 360 nm. Spectrocopic data was consistent with literature.[25]

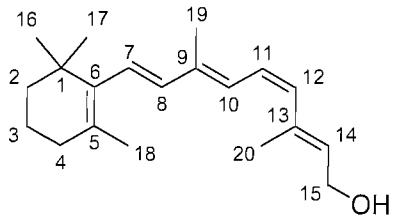
FT-IR (neat) ν_{max} 2955 (m), 2927 (m), 2863 (m), 1661 (s), 1573 (m) cm^{-1}.

^{1}H NMR (400 MHz, C_6D_6) δ 9.91 (1H, d, *J* = 7.7 Hz, **15**), 6.58 (1H, d, *J* = 12.1 Hz, **10**), 6.38 (1H, t, *J* = 12.1 Hz, **11**), 6.34 (1H, d, *J* = 16.1 Hz, **7**), 6.21 (1H, d, *J* = 16.1 Hz, **8**), 6.09 (1H, d, *J* = 7.7 Hz, **14**), 5.59 (1H, d, *J* = 11.8 Hz, **12**), 1.92 (2H, t, *J* = 6.0 Hz, **4**), 1.77 (3H, s, **20**), 1.74 (3H, s, **19**), 1.68 (3H, s, **18**), 1.60-1.50 (2H, m, **3**), 1.46-1.40 (2H, m, **2**), 1.11 (6H, s, **16**+**17**) ppm.

^{13}C NMR (100 MHz, C_6D_6) δ 190.34 (CH, **15**), 154.63 (C, **13**), 141.42 (C, **9**), 138.81 (CH, **8**), 138.58 (C, **6**), 131.69 (CH, **12**), 131.30 (CH, **11**), 131.09 (CH, **14**), 130.66 (C, **5**), 130.08 (CH, **7**), 127.07 (CH, **10**), 40.41 (CH_2, **2**), 35.10 (C, **1**), 33.82 (CH_2, **4**), 29.68 (CH_3, **16**+**17**), 22.42 (CH_3, **18**), 20.22 (CH_2, **3**), 18.00 (CH_3, **20**), 12.86 (CH_3, **19**) ppm.

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

11*Z*-Retinol (1.2)



$C_{20}H_{30}O$

m.w. = 286.45 g/mol

Orange oil

11-*Z*-retinol (**1.2**) was prepared by a modification of a method described by Borhan *et al.*[6] Argon was bubbled through a suspension of zinc dust (1.00 g, 15.29 mmol) in H_2O (6 mL) and stirred for 15 minutes. After this time $Cu(OAc)_2$ (100 mg, 0.55 mmol) was added and the mixture was stirred for a further 15 minutes. $AgNO_3$ (100 mg, 0.59 mmol) was added and the reaction mixture was stirred for a further 30 minutes. The resulting black suspension was filtered and washed with H_2O, MeOH and Et_2O, allowing the black solid to stay moist with Et_2O. The activated zinc catalyst was immediately transferred to a mixture of H_2O (2 mL) and MeOH (2 mL), before a solution of allylic alcohol **1.8** (39 mg, 0.14 mmol) in MeOH (2 mL) was added dropwise. The reaction mixture was warmed to 40 °C and stirred for 21 hours before the mixture was filtered through a pad of celite, washing with H_2O and Et_2O. The phases were separated and the organic was washed with brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo*. Evaporation to dryness under high vacuum furnished the title compound (**1.2**) as an orange oil (38 mg, 0.133 mmol, 95%, 11-*Z*/*E* = 3:1). The spectroscopic data was consistent with literature values.[6]

FT-IR (neat) ν_{max} 3342 (br), 2924 (m), 2861 (w), 1448 (w) cm^{-1}.

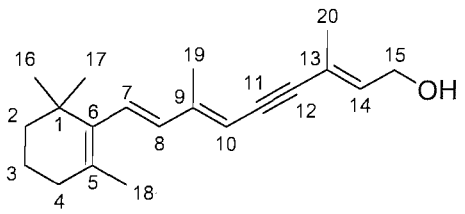
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.81 (1H, d, *J* = 12.0 Hz, **10**), 6.37 (1H, t, *J* = 11.8 Hz, **11**), 6.34 (1H, d, *J* = 16.3 Hz, **7**), 6.28 (1H, d, *J* = 16.3 Hz, **8**), 5.87 (1H, d, *J* = 11.8 Hz, **12**), 5.70 (1H, t, *J* = 6.6 Hz, **14**), 3.96 (2H, d, *J* = 6.6 Hz, **15**), 2.00-1.88 (2H, m, **4**), 1.86 (3H, s, **19**), 1.72 (3H, s, **20**), 1.67 (3H,

s, **18**), 1.63-1.53 (2H, m, **3**), 1.51-1.41 (2H, m, **2**), 1.09 (6H, s, **16**+**17**) ppm. No OH peak observed.

^{13}C NMR ($CDCl_3$) δ 139.17 (CH, **11**), 137.61 (C, **6**), 136.16 (C, **13**), 135.31 (C, **9**), 133.98 (CH, **12**), 132.48 (CH, **14**), 129.79 (C, **5**), 127.92 (CH, **10**), 127.84 (CH, **8**), 125.90 (CH, **7**), 59.99 (CH_2, **15**), 40.47 (CH_2, **2**), 35.13 (C, **1**), 33.81 (CH_2, **4**), 29.73 (CH_3, **16**+**17**), 22.49 (CH_3, **20**), 20.31 (CH_3, **3**), 17.67 (CH_3, **18**), 12.91 (CH_3, **19**) ppm.

LRMS (CI, NH_3) *m/z* 287 [$M+H^+$].

3,7-Dimethyl-9-(2,6,6-trimethyl-cyclohex-1-enyl)-nona-2,6,8-trien-4-yn-1-ol (1.8)



$C_{20}H_{28}O$

m.w. = 284.44 g/mol

Amber oil

Alcohol **1.8** was prepared by a method described by Borhan *et al.*[6] To a solution of alkyne **1.35** (100 mg, 0.25 mmol) in THF (1 mL) at 0 °C was added TBAF (1.0 M in THF, 0.43 mL, 0.43 mmol) dropwise. The reaction was warmed to rt and stirred for 1.5 hours before the reaction was quenched with a saturated solution of NH_4Cl (3 mL). The mixture was extracted with Et_2O (4 x 15 mL) and the combined organic phases were washed with brine (5 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving a yellow oil. Purification by flash column chromatography on silica gel (3.5 cm x 15 cm) eluting with 15% EtOAc/hexane gave the desired product **1.8** as an amber oil (63 mg, 0.22 mmol, 89%).

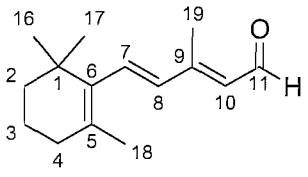
FT-IR (neat) ν_{max} 3350 (br), 2926 (m), 2864 (m), 1629 (w), 1441 (m), 1376 (m), 1360 (m), 1263 (w), 1003 (s), 965 (s), 732 (m), 526 (m) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.27 (1H, d, J = 16.1 Hz, **7**), 6.12 (1H, d, J = 16.1 Hz, **8**), 6.01 (1H, t, J = 6.8 Hz, **14**), 5.53 (1H, s, **10**), 4.27 (2H, d, J = 6.8 Hz, **15**), 2.07 (3H, s, **19**), 2.02 (2H, t, J = 6.0 Hz, **4**), 1.90 (3H, s, **20**), 1.70 (3H, s, **18**), 1.66-1.57 (2H, m, **3**), 1.51-1.42 (2H, m, **2**), 1.03 (6H, s, **16**+**17**) ppm. No OH observed.

^{13}C NMR (100 MHz, $CDCl_3$) δ 147.81 (C, **9**), 137.81 (C, **6**), 135.81 (CH, **8**), 134.69 (CH, **14**), 130.44 (C, **5**), 129.91 (CH, **7**), 121.97 (C, **13**), 108.76 (CH, **10**), 98.76 (C, **11**), 87.32 (C, **12**), 59.62 (CH_2, **15**), 39.94 (CH_2, **2**), 34.59 (C, **1**), 33.41 (CH_2, **4**), 29.26 (CH_3, **16**+**17**), 22.01 (CH_3, **18**), 19.57 (CH_2, **3**), 18.02 (CH_3, **20**), 15.41 (CH_3, **19**) ppm.

LRMS (CI, NH_3) *m/z* 285 [$M+H^+$].

(2*E*,4*E*)-3-Methyl-5-(2,6,6-trimethylcyclohex-1-enyl)penta-2,4-dienal (1.14)



$C_{15}H_{22}O$

m.w. = 218.33 g/mol

Colourless oil

To a slurry of $LiAlH_4$ (231 mg, 6.10 mmol) in Et_2O (10 mL) at –78 °C was added a solution of ester **2.1** (1.00 g, 3.81 mmol) in Et_2O (40 mL) dropwise and the reaction was stirred at –78 °C for 1 hour. The reaction was allowed to warmed to rt and stirred for a further 1.5 hours before the reaction was quenched with H_2O (0.3 mL), 15% NaOH (0.3 mL) and H_2O (0.9 mL) sequentially resulting in a white precipitate. The precipitate was dried ($MgSO_4$) then removed by filtration through a pad of celite, flushing with Et_2O. The solution was concentrated *in vacuo* giving a colourless oil. This oil was dissolved in CH_2Cl_2 (40 mL) and to this solution was added, ground 4 Å molecular sieves (2.27 g), NMO (893 mg, 7.62 mmol) and TPAP (134 mg, 0.38 mmol). The mixture was stirred at rt for 30 minutes before the black solution was concentrated *in vacuo* to give a black oil. Purification by flash column chromatography on silica gel

(4.5 cm x 15 cm) eluting with 5% EtOAc/hexane afforded the title compound **1.14** as a colourless oil (592 mg, 2.71 mmol, 71%). Spectroscopic details were consistent with those observed in the literature.[76]

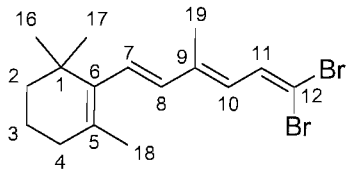
FT-IR (neat) ν_{max} 2931 (m), 2867 (m), 1712 (m), 1685 (s), 1446 (m), 1139 (m) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 10.14 (1H, d, *J* = 8.1 Hz, **11**), 6.75 (1H, d, *J* = 16.1 Hz, **7**), 6.22 (1H, d, *J* = 16.1 Hz, **8**), 5.94 (1H, d, *J* = 8.1 Hz, **10**), 2.32 (3H, s, **19**), 2.05 (2H, t, *J* = 6.3 Hz, **4**), 1.73 (3H, s, **18**), 1.69-1.58 (2H, m, **3**), 1.53-1.43 (2H, m, **2**), 1.05 (6H, s, **16**+**17**) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 191.74 (CH, **11**), 155.42 (C, **9**), 137.36 (C, **6**), 136.02 (CH, **8**), 135.90 (C, **5**), 133.10 (CH, **7**), 129.11 (CH, **10**), 39.82 (CH_2, **2**), 34.58 (C, **1**), 33.55 (CH_2, **4**), 29.26 (CH_3, **16**+**17**), 22.10 (CH_3, **18**), 19.36 (CH_2, **3**), 13.27 (CH_2, **19**) ppm.

LRMS (CI, NH_3) *m/z* 219 [$M+H^+$].

2-(6,6-Dibromo-3-methylhexa-1,3,5-trienyl)-1,3,3-trimethylcyclohexene (1.29)



$C_{16}H_{22}Br$

m.w. = 374.15 g/mol

Colourless oil

Dibromomethylenylation of aldehyde **1.14** was achieved using a method described by Uenishi *et al.*[7] To a solution of *β*-ionylideneethyl aldehyde (**1.14**) (46 mg, 0.21 mmol) in CH_2Cl_2 (1 mL) at 0 °C was added CBr_4 (126 mg, 0.38 mmol) followed by PPh_3 (220 mg, 0.84 mmol). The reaction mixture was stirred for 10 minutes before concentration *in vacuo* giving a brown oil. Purification by flash column chromatography on silica gel

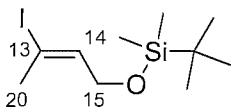
(2.0 cm x 15 cm) eluting with hexane afforded the title compound **1.29** as a colourless oil (50 mg, 0.13 mmol, 64%). Spectroscopic details were consistent with the literature.[7]

FT-IR (neat) ν_{max} 3120 (m), 3032 (m), 2961 (m), 2862 (m), 1715 (w), 1443 (w), 1392 (s), 963 (m) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (1H, d, J = 10.8 Hz, **11**), 6.34 (1H, d, J = 16.1 Hz, **8**), 6.14 (1H, d, J = 16.1 Hz, **7**), 6.02 (1H, d, J = 10.8 Hz, **10**), 2.02 (2H, t, J = 6.2 Hz, **4**), 1.92 (3H, s, **19**), 1.72 (3H, s, **18**) 1.67-1.58 (2H, m, **3**), 1.51-1.45 (2H, m, **2**), 1.03 (6H, s, **16**+**17**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 139.90 (C, **9**), 137.96 (C, **6**), 137.14 (CH, **8**), 134.04 (CH, **11**), 130.45 (C, **5**), 129.94 (CH, **7**), 126.05 (CH, **10**), 91.01 (C, **12**), 39.93 (CH_2, **2**), 34.55 (C, **1**), 33.43 (CH_2, **4**), 29.29 (CH_3, **16**+**17**), 22.09 (CH_3, **18**), 19.56 (CH_2, **3**), 13.86 (CH_3, **19**) ppm.

((*E*)-3-Iodobut-2-enyloxy)(*tert*-butyl)dimethylsilane (1.32)



$C_{10}H_{21}IOSi$

m.w. = 312.26 g/mol

Pale yellow oil

To a solution of TBDMSCl (1.00 g, 6.64 mmol) in CH_2Cl_2 (6 mL) at 0 °C was added Et_3N (1.01 mL, 7.25 mmol), DMAP (3 mg, 0.02 mmol) and a solution of (*E*)-3-iodobut-2-en-1-ol **(1.118)** (1.195 g, 6.04 mmol) in CH_2Cl_2 (3 mL) dropwise. The reaction mixture was warmed to rt and stirred for 18.5 hours. The solution was washed with H_2O (10 mL) before the aqueous phase was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic phases were washed with H_2O (10 mL) and brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by distillation under reduced pressure (10 mbar, 110-120 °C) gave the title compound **1.32** as a pale yellow oil (1.81 g, 5.79

mmol, 96%). Spectroscopic details were consistent with those reported in the literature.[165]

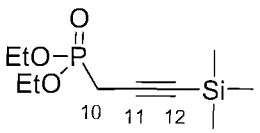
FT-IR (neat) ν_{max} 2954 (w), 2929 (w), 2852 (w), 1634 (w), 1467 (w), 1364 (w), 1250 (m), 1082 (s) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.31 (1H, tq, J = 6.5, 1.5 Hz, **14**), 4.13 (2H, dq, J = 6.5, 0.9 Hz, **15**), 2.42 (3H, dt, J = 1.5, 0.9 Hz, **20**), 0.91 (9H, s, -SiC($C\mathbf{H}_3$)$_3$), 0.08 (6H, s, -OSi($C\mathbf{H}_3$)$_2$) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 141.04 (CH, **14**), 96.30 (C, **13**), 61.04 (CH_2, **15**), 28.44 (CH_3, **20**), 26.24 (CH_3, -SiC($\mathbf{C}H_3$)$_3$), 18.68 (C, -Si$\mathbf{C}$(CH_3)$_3$), −4.86 (CH_3, -Si($\mathbf{C}H_3$)$_2$) ppm.

LRMS (CI, NH_3) m/z 313 [M+H$^+$].

Diethyl 3-(trimethylsilyl)prop-2-ynylphosphonate (1.34)



$C_{10}H_{21}O_3PSi$

m.w. = 248.34 g/mol

Colourless oil

Diethyl-(3-trimethylsilyl-2-propynl)-phosphonate (**1.34**) was prepared by a method described by Gibson *et al.*[18] To NaHMDS (1.0 M in THF, 5.23 mL, 5.23 mmol) at –10 °C was added a solution of diethyl phosphite (0.67 mL, 5.23 mmol) in THF (1.5 mL) dropwise. After stirring for 15 minutes, 3-bromo-1-(trimethylsilyl)-1-propyne (0.82 mL, 5.23 mmol) in THF (1.5 mL) was added and the reaction was stirred for 2 hours at rt. After which, the mixture was quenched with H_2O (10 mL) and extracted with Et_2O (3 x 10 mL). The combined organic phases were washed with 2 M HCl (10 mL), H_2O (10 mL) and brine (10 mL) before the organic was dried ($MgSO_4$) and concentrated *in vacuo* giving a pale yellow oil. Purification by distillation under reduced pressure (0.4 mbar, 100-120 °C) followed by flash column chromatography on silica gel (3.5 cm x 20 cm) eluting with 60% EtOAc/hexane gave the desired phosphonate **1.34** as a

colourless oil (1.08 g, 4.34 mmol, 83%). Spectroscopic details are consistent with those reported in the literature.[18]

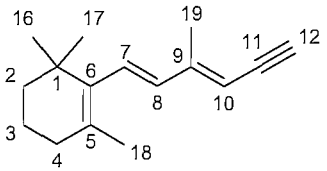
FT-IR (neat) ν_{max} 2983 (w), 2962 (w), 2901 (w), 2179 (m), 1392 (w), 1250 (s), 1023 (s) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.20 (2H, qd, J = 7.1, 1.3 Hz, -POC**H**$_2$CH$_3$), 4.18 (2H, dq, J = 7.1, 1.3 Hz, -POC**H**$_2$CH$_3$), 2.80 (2H, d, J = 22.3 Hz, **10**), 1.35 (6H, t, J = 7.0 Hz, -P(OCH$_2$C**H**$_3$)$_2$), 0.15 (9H, s, -Si(C**H**$_3$)$_3$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 96.07 (C, d, J = 13.5 Hz, **11**), 88.22 (C, d, J = 8.7 Hz, **12**), 63.35 (CH$_2$, d, J = 6.8 Hz, -PO**C**H$_2$CH$_3$), 19.66 (CH$_2$, d, J = 143.9 Hz, **10**), 16.70 (CH$_3$, d, J = 6.8 Hz, -POCH$_2$**C**H$_3$), 0.14 (CH$_3$, -Si(**C**H$_3$)$_3$) ppm.

LRMS (CI, NH_3) *m/z* 249 $[M+H]^+$.

1,3,3-Trimethyl-2-(3-methyl-hexa-1,3-dien-5-ynyl)-cyclohexene (1.35)



$C_{16}H_{22}$

m.w. = 214.35 g/mol

Brown red oil

Terminal alkyne **1.35** was prepared by the method described by Borhan *et al.*[6] TMS alkyne **1.117** (90 mg, 0.31 mmol) was stirred in THF (1 mL) and treated with TBAF (1.0 M in THF, 0.31 mL, 0.31 mmol). The reaction was stirred for 2 hours before quenching with a saturated solution of NH_4Cl (3 mL). The mixture was extracted with Et_2O (2 x 10 mL) and the combined organic phases were washed with brine (3 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give a brown oil. Purification by flash column chromatography on silica gel (3.5 cm x 15 cm) eluting 10% Et_2O/hexane yielded the title compound **1.35** as a brown red oil (55 mg, 0.26 mmol, 83%).

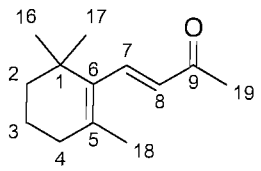
FT-IR (neat) ν_{max} 3309 (w), 2828 (m), 2867 (m), 1716 (m), 1582 (w), 1447 (m), 1378 (m), 1362 (m), 971 (m) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.29 (1H, d, J = 16.1 Hz, **7**), 6.10 (1H, 16.1 Hz, **8**), 5.41 (1H, s, **10**), 3.29 (1H, s, **12**), 2.09 (3H, s, **19**), 2.02 (2H, t, J = 6.3 Hz, **4**), 1.70 (3H, s, **18**), 1.67-1.57 (2H, m, **3**), 1.51-1.43 (2H, m, **2**), 1.03 (6H, s, **16**+**17**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 149.62 (C, **9**), 137.70 (C, **6**), 135.44 (CH, **8**), 130.58 (CH, **7**), 130.54 (C, **5**), 107.61 (CH, **10**), 83.80 (CH, **12**), 82.07 (C, **11**), 39.91 (CH_2, **2**), 34.56 (C, **1**), 33.37 (CH_2, **4**), 29.24 (CH_3, **16**+**17**), 21.99 (CH_3, **18**), 19.49 (CH_2, **3**), 15.40 (CH_3, **19**) ppm.

LRMS (EI) *m/z* (relative intensity) 214 (60%) [$M^{\cdot+}$].

β-Ionone (1.36)



$C_{13}H_{20}O$
m.w. = 192.30 g/mol
Pale yellow oil

To a solution of acrylamide **2.33** (175 mg, 0.56 mmol) in THF (9 mL) at –78 °C was added MeLi (0.66 mL, 1.06 mmol) dropwise. The resulting reaction mixture was stirred at –78 °C for 45 minutes then warmed to –20 °C and stirred for a further 20 minutes. The reaction was quenched with a saturated solution of NH_4Cl (5 mL) and the mixture was extracted with Et_2O (4 x 20 mL) before the combined organic phases were washed with a saturated solution of NH_4Cl (5 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving a pale yellow oil. Purification by flash column chromatography on silica gel (2.5 cm x 15 cm) eluting with 5% Et_2O/hexane gave the title compound **1.36** as a pale yellow oil (91 mg, 0.47 mmol, 85%). Spectroscopic details were consistent with those reported in the literature.[24]

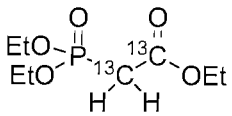
FT-IR (neat) ν_{max} 2957 (m), 2929 (m), 2866 (m), 2826 (w), 1692 (m), 1667 (s) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (1H, d, J = 16.5 Hz, **7**), 6.12 (1H, d, J = 16.5 Hz, **8**), 2.30 (3H, s, **19**), 2.08 (2H, t, J = 6.0 Hz, **4**), 1.77 (3H, s, **18**), 1.70-1.60 (2H, m, **3**), 1.55-1.40 (2H, m, **2**), 1.08 (6H, s, **16**+**17**) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 198.95 (C, **9**), 143.45 (CH, **7**), 136.35 (C, **5**), 136.29 (C, **6**), 131.97 (CH, **8**), 40.14 (CH_2, **2**), 34.44 (C, **1**), 33.90 (CH_2, **4**), 29.15 (CH_3, **16**+**17**), 27.49 (CH_3, **19**), 22.04 (CH_3, **18**), 19.26 (CH_2, **3**).

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

[1,2-$^{13}C_2$]-Triethyl phosphonoacetate (1.95)



$C_6{}^{13}C_2H_{15}O_5P$

m.w. = 226.18 g/mol

Colourless oil

Triethyl phosphonoacetate **1.95** was prepared by a method described by Creemers *et al.*[30] A mixture of 1,2-^{13}C-bromoethylacetate (1.00 g, 5.92 mmol) and triethyl phosphite (1.12 mL, 6.51 mmol) was heated to 180 °C and stirred for 4 hours. The reaction was cooled giving a colourless oil. Purification by distillation under reduced pressure (10 mbar, 120-140 °C) yielded the desired phosphonoacetate **1.95** as a colourless oil (1.19 g, 5.27 mmol, 89%).

FT-IR (neat) ν_{max} 3466 (br), 2984 (m), 2936 (w), 2911 (w), 1690 (s), 1478 (w), 1446 (w), 1393 (m) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.25-4.05 (2H, m, -C(O)OC**H$_2$**CH_3), 4.16 (4H, q, J = 7.0 Hz, -P(OC**H$_2$**CH_3)$_2$), 4.57 (2H, ddd, J = 129.9,

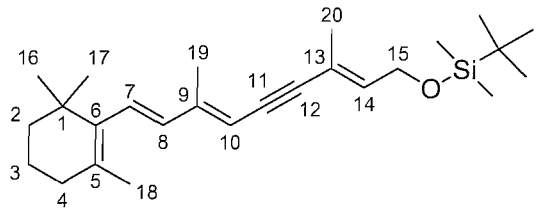
21.6, 7.3 Hz, -PC**H**$_2$-), 1.35 (6H, t, J = 7.0 Hz, -P(OCH$_2$C**H**$_3$)$_2$), 1.29 (3H, t, J = 7.1 Hz, -C(O)OCH$_2$C**H**$_3$) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 166.14 (C, dd, J = 58.6, 6.1, -**C**=O), 62.99 (CH_2, d, J = 6.1 Hz, -P(O**C**H$_2$CH$_3$)$_2$), 61.86 (CH_2, d, J = 1.7 Hz, -C(O)**C**H$_2$CH$_3$), 34.75 (CH_2, dd, J = 134.3, 58.6 Hz, -P**C**H$_2$-), 16.66 (CH_3, d, J = 6.1 Hz, -P(OCH$_2$**C**H$_3$)$_2$), 14.42 (CH_3, d, J = 1.7 Hz, -C(O)CH$_2$**C**H$_3$) ppm.

^{31}P NMR (121 MHz, $CDCl_3$) δ 20.39 (dd, J = 134.3, 6.2 Hz) ppm.

LRMS (ES^+) *m/z* 227 [$M+H^+$], 149 [$M+Na^+$], 475 [$2M+Na^+$].

((2*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,6,8-trien-4-ynyloxy)(*tert*-butyl)dimethylsilane (1.116)



$C_{26}H_{42}OSi$
m.w. = 398.70 g/mol
Amber oil

TBDMS protected dehydro retinol **1.116** was prepared by a method described by Borhan *et al.*[6] A solution of iodide **1.32** (189 mg, 605 μmol) and $Pd(PPh_3)_4$ (5 mg, 5 μmol) in $^{i}PrNH_2$ (1.8 mL) was stirred for 5 minutes before the addition of CuI (1 mg, 5 μmol) to the mixture and the reaction was stirred for 5 minutes. To the resulting mixture was added alkyne **1.35** (100 mg, 0.47 mmol) in $^{i}PrNH_2$ (1.8 mL) dropwise. After 5 hours the reaction was concentrated *in vacuo* and dissolved in Et_2O (20 mL) and a saturated solution of NH_4Cl (5 mL). The organic phase was separated and washed with brine (5 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving an amber oil. Purification by flash column chromatography on silica gel (2.5 cm x 15 cm) eluting with hexane gave the desired product **1.116** as an amber oil (148 mg, 0.37 mmol, 79%). Spectroscopic details were consistent with those reported in the literature.[166]

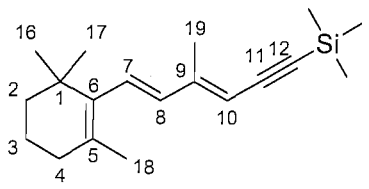
FT-IR (neat) ν_{max} 2929 (m), 2857 (m), 1723 (m), 1462 (m), 1362 (m), 1257 (m), 1092 (m), 835 (s) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.25 (1H, d, J = 16.1 Hz, **7**), 6.11 (1H, d, J = 16.1 Hz, **8**), 5.91 (1H, t, J = 6.4 Hz, **14**), 5.53 (1H, s, **10**), 4.29 (2H, d, J = 6.4 Hz, **15**), 2.06 (3H, s, **19**), 2.02 (2H, t, J = 6.2 Hz, **4**), 1.85 (3H, s, **20**), 1.70 (3H, s, **18**), 1.67-1.56 (2H, m, **3**), 1.51-1.42 (2H, m, **2**), 1.02 (6H, s, **16**+**17**), 0.92 (9H, s, -SiC(CH_3)$_3$), 0.09 (6H, s, -Si(CH_3)$_2$) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 147.43 (CH, **9**), 137.84 (C, **6**), 136.35 (CH, **8**), 135.91 (CH, **14**), 130.32 (C, **5**), 129.63 (CH, **7**), 119.71 (C, **13**), 108.97 (CH, **10**), 99.17 (C, **12**), 86.50 (C, **11**), 60.47 (CH_2, **15**), 39.93 (CH_2, **2**), 34.58 (C, **1**), 33.40 (CH_2, **4**), 29.27 (CH_3, **16**+**17**), 26.29 (CH_3, -SiC(CH_3)$_3$), 22.01 (CH_3, **18**), 19.57 (CH_2, **3**), 18.71 (C, -Si**C**(CH_3)$_3$), 18.08 (CH_3, **20**), 15.39 (CH_3, **19**), –4.80 (CH_3, -Si(CH_3)$_2$) ppm.

LRMS (CI, NH_3) *m/z* 400 [M+H^+].

Trimethyl-[4-methyl-6-(2,6,-trimethyl-cyclohex-1-enyl)-hexa-3,5-dien-1-ynyl]-silane (1.117)



$C_{19}H_{30}Si$
m.w. = 286.53 g/mol
Pale yellow oil

The TMS alkyne **1.117** was prepared by a method described by Borhan *et al.*[6] Dimethyl-(3-trimethylsilyl-2-propynl)-phosphonate (**1.34**) (1.376 g, 5.54 mmol) in THF (26 mL) was cooled to 0 °C before *n*-BuLi (2.35 M in hexane, 2.36 mL, 5.54

mmol) was added dropwise. The resultant red solution was allowed to warm to rt and stirred for 10 minutes before the addition of a solution of β-ionone (**1.36**) (0.533 g, 2.77 mmol) in THF (3 mL) dropwise. After 3.5 hours the reaction was quenched with a saturated solution of NH_4Cl (20 mL) and extracted with Et_2O (3 x 30 mL). The combined organic phases were washed with brine (20 mL) and dried ($MgSO_4$) before concentration *in vacuo* giving an orange yellow oil. Purification by flash column chromatography on silica gel (3.5 cm x 15 cm) eluting with 10% Et_2O/hexane gave the title compound **1.117** as a pale yellow oil (0.734 g, 2.56 mmol, 93 %, C9-*E*/*Z* = 5:1). The isomers were separated by HPLC, eluting with Et_2O (0.01 mL/minute) and hexane (9.98 mL/minute), U.V detector = 302 nm. The spectroscopic data was in good accordance to the literature.[6]

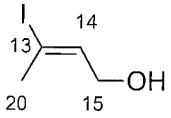
FT-IR (neat) ν_{max} 2958 (m), 2928 (m), 2127 (m), 1738 (w), 1446 (w), 1360 (w), 1249 (m), 1090 (w), 965 (w), 840 (s), 759 (m) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.27 (1H, d, J = 16.1 Hz, **7**), 6.08 (1H, d, J = 16.1 Hz, **8**), 5.44 (1H, s, **10**), 2.07 (3H, s, **19**), 2.02 (2H, t, J = 6.0 Hz, **4**), 1.69 (3H, s, **18**), 1.66-1.57 (2H, m, **3**), 1.50-1.42 (2H, m, **2**), 1.02 (6H, s, **16**+**17**), 0.22 (9H, s, -Si(C**H**$_3$)$_3$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 149.18 (C, **9**), 137.77 (C, **6**), 135.73 (CH, **8**), 130.44 (C, **5**), 130.37 (CH, **7**), 108.74 (CH, **10**), 104.39 (C, **11**), 101.54 (C, **12**), 39.91 (CH_2, **2**), 34.57 (C, **1**), 33.38 (CH_2, **4**), 29.24 (CH_3, **16**+**17**), 21.97 (CH_3, **18**), 19.57 (CH_2, **3**), 15.48 (CH_3, **19**), 0.44 (CH_3, -Si(**C**H$_3$)$_3$) ppm.

LRMS (CI, NH_3) *m/z* 287 [M+H$^+$], 73 [Si(Me)$_3^+$].

(*E*)-3-Iodo-but-2-en-1-ol (1.118)



C_4H_7IO

m.w. = 198.00 g/mol

Colourless oil

To a solution of ^{i}BuMgCl (2.0 M in Et_2O, 17.12 mL, 34.24 mmol) in Et_2O (7 mL) at –5 °C was added Cp_2TiCl_2 (355 mg, 34.24 mmol), this mixture was stirred at –5 °C for 15 minutes. To the brown reaction mixture was added a solution of 2-butyn-1-ol (**1.26**) (1.07 mL, 14.27 mmol) in Et_2O (22 mL) dropwise. The brown black solution was allowed to warm to rt and stir for 72 hours giving a brown suspension. The suspension was cooled to –70 °C before a solution of iodine (6.88 g, 27.10 mmol) in THF (22 mL) was added dropwise. After which, the reaction was warmed to –60 °C and stirred for 15 minutes before warming to rt and stirring for 1 hour. The reaction was quenched with 2 M HCl (20 mL) and the aqueous was extracted with Et_2O (5 x 50 mL). The combined organic phases were washed with Na_2CO_3 (15 mL), brine (15 mL) and $Na_2S_2O_3$ (15 mL) sequentially. The organic was dried ($MgSO_4$) and concentrated *in vacuo* giving a green oil. Purification by flash column chromatography on silica gel (5.0 cm x 9 cm) eluting with 5% Et_2O/CH_2Cl_2 afforded the desired iodide **1.118** as a colourless oil (1.195 g, 6.04 mmol, 42%). Spectroscopic details are consistent with those reported in the literature.[165]

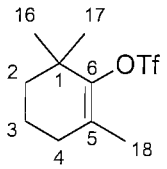
FT-IR (neat) ν_{max} 3297 (br), 2916 (m), 2871 (m), 1637 (m), 1424 (m), 1376 (m), 1218 (m), 1096 (m), 999 (s) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.41 (1H, tq, J = 7.0, 1.5 Hz, **14**), 4.09 (2H, dq, J = 7.0, 0.8 Hz, **15**), 2.46 (3H, br s, **20**), 1.62 (1H, br s, -O**H**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 140.06 (CH, **14**), 98.81 (C, **13**), 60.26 (CH_2, **15**), 28.35 (CH_3, **20**) ppm.

LRMS (CI, NH_3) *m/z* 198 [M-H_2O+NH_4^+], 72 [M-I+NH_4^+].

2,6,6-Trimethylcyclohex-1-enyl trifluoromethanesulfonate (1.119)



$C_{10}H_{15}F_3O_3S$

m.w. = 272.28 g/mol

Colourless oil

Triflate **1.119** was prepared by a method described by Breining *et al.*[82] To a solution of LDA (1.8 M, THF/heptane/ethylbenzene, 0.87 mL, 1.57 mmol) in THF (2 mL) at −78 °C was added a solution of 2,2,6-trimethyl cyclohexanone (**2.18**) (200 mg, 1.43 mmol) in THF (2 mL) dropwise. The solution was stirred at −78 °C for 2 hours at which point *N*-phenyl-trifluoromethane sulfonimide (545 mg, 1.53 mmol) in THF (2 mL) was added dropwise. The reaction was stirred for 24 hours whilst naturally warming to rt. The reaction mixture was concentrated *in vacuo* then dissolved in 2 M HCl (10 mL) and Et_2O (10 mL). After separation the aqueous phase was extracted with Et_2O (3 x 10 mL) before the combined organic phases were washed with 2 M HCl (5 mL), 2 M NaOH (2 x 5 mL) and brine (5 mL). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* giving an amber oil and solid. Purification by flash column chromatography on silica gel (3.0 cm x 13 cm) eluting with 1% Et_2O/hexane furnished the desired product **1.119** as a colourless oil (235 mg, 0.86 mmol, 60%).

FT-IR (neat) ν_{max} 2968 (w), 2940 (w), 2875 (w), 2838 (w), 1686 (w), 1458 (w), 1399 (s), 1243 (m), 1204 (s), 1142 (s), 1058 (w), 1001 (m), 895 (s), 860 (s), 609 (s) cm^{-1}.

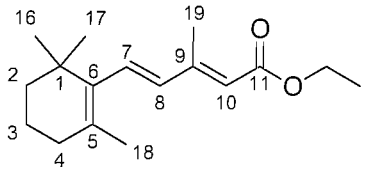
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.16 (2H, t, J = 6.2 Hz, **4**), 1.76 (3H, s, **18**), 1.69-1.59 (4H, m, **2**+**3**), 1.16 (6H, s, **16**+**17**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 150.58 (C, **6**), 126.43 (C, **5**), 119.14 (CF_3, d, J = 319.8 Hz, -**C**F_3), 40.92 (CH_2, **2**), 35.97 (C, **1**), 32.85 (CH_2, **4**), 26.74 (CH_3, **16**+**17**), 18.96 (CH_2, **3**), 17.94 (CH_3, **18**) ppm.

^{19}F NMR (282 MHz, $CDCl_3$) δ −73.59 (F, -OS(O)$_2$C**F**$_3$) ppm.

LRMS (EI) *m/z* (relative intensity) 272 (17 %) [$M^{\cdot +}$], 69 (100 %) [$CF_3^{\cdot +}$].

(2*E*,4*E*)-Ethyl 3-methyl-5-(2,6,6-trimethylcyclohex-1-enyl)penta-2,4-dienoate (2.1)



$C_{17}H_{26}O_2$

m.w. = 262.39 g/mol

Pale yellow oil

To a suspension of NaH (2.63 g, 65.75 mmol) in Et_2O (40 mL), triethyl phosphonoacetate (11.30 mL, 57.20 mmol) in Et_2O (30 mL) was added dropwise before the reaction mixture was stirred for 2 hours at rt. To the reaction mixture was added a solution of *β*-ionone (**1.36**) (5.00 g, 26.00 mmol) in Et_2O (20 mL) dropwise before stirring for 60 hours. The reaction was then quenched with H_2O (20 mL) and the mixture was diluted with hexane (40 mL). The organic phase was separated and washed with H_2O (2 x 20 mL) and brine (2 x 10 mL). The aqueous phase was extracted with hexane (2 x 15 mL) before the combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo* to yield a yellow oil. Purification by flash chromatography on silica gel (4.0 cm x 17 cm) eluting with 3% EtOAc/hexane yielded the desired product (**2.1**) as a pale yellow oil (6.20 g, 23.63 mmol, 91 %, C9-*E*/*Z* = 9:1). Spectroscopic details were consistent with those observed in the literature.[76]

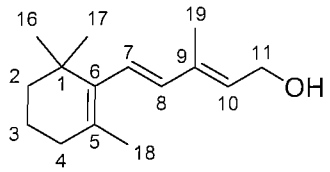
FT-IR (neat) ν_{max} 2957 (m), 2928 (m), 2866 (m), 2828 (w), 1710 (s), 1607 (m), 1445 (m), 1365 (m), 1233 (m), 1155 (s), 1047 (m), 968 (m), 876 (w) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.56 (1H, d, *J* = 16.1 Hz, **7**), 6.09 (1H, d, *J* = 16.1 Hz, **8**), 5.74 (1H, s, **10**), 4.18 (2H, q, *J* = 7.2 Hz, -OC**H$_2$**CH_3), 2.34 (3H, s, **19**), 2.03 (2H, t, *J* = 6.0 Hz, **4**), 1.70 (3H, s, **18**), 1.66-1.55 (2H, m, **3**), 1.49-1.44 (2H, m, **3**), 1.29 (3H, t, *J* = 7.2, -OCH_2C**H$_3$**), 1.02 (6H, s, **16**+**17**) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 167.63 (C, **11**), 153.14 (C, **9**), 137.48 (C, **5**), 136.56 (CH, **8**), 133.96 (CH, **7**), 131.40 (C, **6**), 118.36 (CH, **10**), 59.94 (CH_2, -O**C**H_2CH$_3$), 39.79 (CH_2, **2**), 34.53 (C, **1**), 33.37 (CH_2, **4**), 29.20 (CH_3, **16**+**17**), 21.98 (CH_3, **18**), 19.45 (CH_2, **3**), 14.70 (CH_3, -OCH$_2$**C**H$_3$), 13.95 (CH_3, **19**) ppm.

LRMS (CI, NH_3) *m/z* 263 [M+H$^+$].

(2*E*,4*E*)-3-Methyl-5-(2,6,6-trimethylcyclohex-1-enyl)penta-2,4-dien-1-ol (2.2)



$C_{15}H_{24}O$

m.w. = 220.35 g/mol

Colourless oil

β-ionylideneethyl alcohol (**2.2**) was prepared according to the method described by Tanumihardjo.[76] To a suspension of $LiAlH_4$ (232 mg, 6.11 mmol) in THF (10 mL) at –78 °C was added a solution of ethyl-*β*-ionylidene acetate (**2.1**) (1.00 g, 3.82 mmol, C9-*E*/*Z* = 10:1) in THF (40 mL) dropwise. After 1 hour the suspension was allowed to warm to rt before stirring for a further 1.5 hours. After this period the reaction was quenched with ice chips (~30 mL) cautiously. After the effervescence had ceased, the white suspension was filtered through a pad of celite washing with H_2O and Et_2O. The organic phase was separated before the aqueous phase was extracted with Et_2O (2 x 20 mL). The combined organic phases were sequentially washed with H_2O (2 x 30 mL) and brine (20 mL). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* yielding a yellow oil. Purification by flash column chromatography on silica gel (3.0 cm x 17 cm) eluting with 1% Et_3N/10% EtOAc/hexane gave the desired product (**2.2**) as a colourless oil (472 mg, 2.14 mmol, 56%) and 9-*Z* alcohol as a colourless oil (188 mg, 0.85 mmol, 22%). Spectroscopic details were consistent with those observed in the literature.[76]

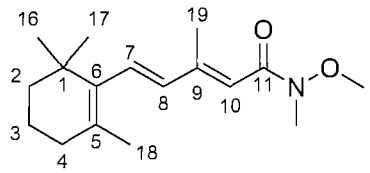
FT-IR (neat) ν_{max} 3384 (br), 2934 (m), 1713 (s), 1454 (m), 1377 (m), 1147 (s) cm^{-1}.

^{1}H NMR (400 MHz, C_6D_6) δ 6.17 (1H, d, J = 16.0 Hz, **7**), 6.14 (1H, d, J = 16.0 Hz, **8**), 5.57 (1H, t, J = 6.8 Hz, **10**), 4.04 (2H, d, J = 6.8 Hz, **11**), 1.94 (2H, t, J = 6.3 Hz, **4**), 1.73 (3H, s, **18**), 1.65 (3H, s, **19**), 1.62-1.51 (2H, m, **3**), 1.49-1.43 (2H, m, **2**), 1.09 (6H, s, **16**+**17**) ppm. No OH observed.

^{13}C NMR (100 MHz, C_6D_6) δ 138.64 (CH, **8**), 136.41 (C, **9**), 130.97 (CH, **10**), 129.46 (C, **5**), 128.92 (C, **6**), 127.22 (CH, **7**), 59.97 (CH_2, **11**), 40.44 (CH_2, **2**), 35.07 (C, **1**), 33.71 (CH_2, **4**), 29.67 (CH_3, **16**+**17**), 22.38 (CH_3, **18**), 20.31 (CH_2, **3**), 13.00 (CH_3, **19**) ppm.

LRMS (CI, NH_3) *m/z* 203 [M–H_2O+H^+].

(2*E*,4*E*)-*N*-Methoxy-*N*-3-dimethyl-5-(2,6,6-trimethylcyclohex-1-enyl)penta-2,4-dienamide (2.3)



$C_{17}H_{27}NO_2$

m.w. = 277.40 g/mol

Colourless oil

To a solution of *N*,*O*-dimethylhydroxyamine hydrochloride (1.115 g, 11.43 mmol) in THF (100 mL) at –25 °C was added *n*-BuLi (2.31 M in hexane, 4.79 mL, 11.06 mmol) dropwise. The reaction mixture was stirred for 40 minutes at –25 °C before the addition of a solution of ester (**2.1**) (1.000 g, 3.81 mmol) in THF (15 mL) dropwise. The reaction mixture was warmed to rt slowly and stirred for 20 hours. The reaction was quenched by concentration *in vacuo* and the residue was dissolved in EtOAc (30 mL) and H_2O (10 mL) before the phases were separated. The aqueous phase was extracted with EtOAc (3 x 25 mL) and the combined organic phases were washed with brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving an amber oil. Purification by flash column chromatography on silica gel (4.0 cm x 17 cm) eluting with 15%

EtOAc/hexane afforded the desired Weinreb amide (**2.3**) as a colourless oil (576 mg, 2.08 mmol, 54%).

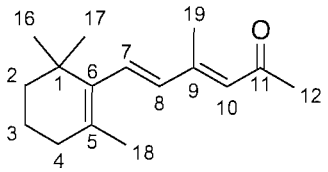
FT-IR (neat) ν_{max} 2928 (m), 2864 (m), 1646 (s), 1599 (s), 1440 (m), 1407 (m), 1363 (s) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.52 (1H, d, J = 16.1 Hz, **7**), 6.20 (1H, s, **10**), 6.14 (1H, d, J = 16.1 Hz, **8**), 3.70 (3H, s, -NOC**H$_3$**), 3.24 (3H, s, -NC**H$_3$**), 2.31 (3H, d, J = 1.3 Hz, **19**), 2.03 (2H, t, J = 6.3 Hz, **4**), 1.72 (3H, s, **18**), 1.68-1.57 (2H, m, **3**), 1.52-1.44 (2H, m, **2**), 1.03 (6H, s, **16**+**17**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 168.81 (C, **11**), 150.68 (C, **9**), 137.67 (C, **6**), 137.33 (CH, **8**), 132.84 (CH, **7**), 130.87 (C, **5**), 117.04 (CH, **10**), 61.86 (CH_3, -NO**C**H$_3$), 39.88 (CH_2, **2**), 34.59 (C, **1**), 33.38 (CH_2, **4**), 32.76 (CH_3, -N**C**H$_3$), 29.24 (CH_3, **16**+**17**), 22.01 (CH_3, **18**), 19.53 (CH_2, **3**), 14.14 (CH_3, **19**) ppm.

LRMS (ES^+) *m/z* 278 [$M+H^+$], 578 [$2M+Na^+$].

(3*E*,5*E*)-4-Methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-3,5-dien-2-one (2.4)



$C_{16}H_{24}O$

m.w. = 232.36 g/mol

Colourless oil

To a solution of Weinreb amide **2.3** (400 mg, 1.44 mmol) in THF (24 mL) at –78 °C was added MeLi (1.8 M in THF, 1.52 mL, 2.74 mmol) dropwise and the resultant mixture was stirred at –78 °C for 45 minutes. The reaction was warmed to –20 °C and stirred for 20 minutes before the reaction was quenched with a saturated solution of NH_4Cl (5 mL). The phases were separated and the aqueous phase was extracted with

Et_2O (3 x 20 mL). The combined organic phases were washed with brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving a yellow oil. Purification by flash column chromatography on silica gel (3.5 cm x 14 cm) eluting with 10% EtOAc/hexane gave the ketone **2.4** as a colourless oil (325 mg, 1.40 mmol, 97%, 9-*E*/*Z* = 4:1).

9-*E* Isomer

FT-IR (neat) ν_{max} 2957 (m), 2927 (m), 2865 (w), 1713 (w), 1677 (s), 1577 (s) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.64 (1H, d, *J* = 16.1 Hz, **7**), 6.12 (1H, s, 10), 6.07 (1H, d, *J* = 16.1 Hz, **8**), 2.31 (3H, s, **19**), 2.23 (3H, s, **12**), 2.04 (2H, t, *J* = 6.0 Hz, **4**), 1.71 (3H, s, **18**), 1.67-1.57 (2H, m, **3**), 1.52-1.44 (2H, m, **2**), 1.04 (6H, s, **16**+**17**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 199.49 (C, **11**), 151.44 (C, **9**), 137.67 (C, **6**), 136.84 (CH, **8**), 135.49 (CH, **7**), 131.72 (C, **5**), 126.01 (CH, **10**), 39.91 (CH_2, **2**), 34.60 (C, **1**), 33.47 (CH_2, **4**), 32.41 (CH_3, **12**), 29.24 (CH_3, **16**+**17**), 22.01 (CH_3, **18**), 19.48 (CH_2, **3**), 14.26 (CH_3, **19**) ppm.

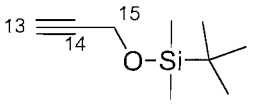
LRMS (ES^+) *m/z* 233 [$M+H^+$], 255 [$M+Na^+$].

9-*Z* Isomer

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (1H, d, *J* = 16.6 Hz, **8**), 6.64 (1H, d, *J* = 16.6 Hz, **7**), 6.02 (1H, s, **10**), 2.31 (3H, s, **19**), 2.20 (3H, s, **12**), 2.04 (2H, t, *J* = 6.0 Hz, **4**), 1.79 (3H, s, **18**), 1.67-1.57 (2H, m, **3**), 1.52-1.44 (2H, m, **2**), 1.08 (6H, s, **16**+**17**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 198.74 (C, **11**), 15018 (C, **9**), 137.60 (C, **6**), 136.23 (CH, **8**), 133.08 (C, **5**), 130.88 (CH, **7**), 124.32 (CH, **10**), 40.27 (CH_2, **2**), 34.51 (C, **1**), 33.81 (CH_2, **4**), 32.29 (CH_3, **12**), 29.30 (CH_3, **16**+**17**), 21.11 (CH_3, **18**), 19.48 (CH_2, **3**), 14.26 (CH_3, **19**) ppm.

***Tert*-butyldimethyl(prop-2-ynyloxy)silane (2.5)**



$C_9H_{18}OSi$

m.w. = 170.33 g/mol

Colourless oil

To a solution of TBDMSCl (2.95 g, 19.57 mmol), Et_3N (2.98 mL, 21.44 mmol) and DMAP (9 mg, 0.07 mmol) in CH_2Cl_2 (6 mL) at 0 °C, was added a solution of propargyl alcohol (**2.4**) (1.00 g, 17.83 mmol) in CH_2Cl_2 (2 mL) dropwise. After complete addition, the white suspension was allowed to warm to rt and stirred for 21 hours. The reaction mixture was washed with H_2O (18 mL) before the aqueous phase was extracted with CH_2Cl_2 (2 x 10 mL). The combined organic phases were washed with H_2O (15 mL) and brine (15 mL) sequentially, dried ($MgSO_4$) and concentrated *in vacuo* to yield a mobile peach oil. Purification by distillation under reduced pressure (0.4 mbar, 20-30 °C) gave the title compound **2.5** as a colourless oil (2.29 g, 13.45 mmol, 76%). Spectroscopic details were consistent with the literature.[167]

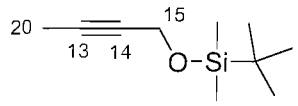
FT-IR (neat) ν_{max} 3312 (w), 2956 (w), 2931 (w), 2887 (w), 2859 (w), 1255 (m), 1095 (m), 835 (m) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.32 (2H, d, J = 2.4 Hz, **15**), 2.40 (1H, t, J = 2.3 Hz, **13**), 0.92 (9H, s, $(CH_3)_3CSi$-), 0.13 (6H, s, $(CH_3)_2Si$) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 82.74 (C, **14**), 73.18 (CH, **13**), 51.85 (CH_2, **15**), 26.12 (CH_3, -SiC(CH_3)$_3$), 18.62 (C, -Si**C**(CH_3)$_3$), -4.87 (CH_3, -Si(CH_3)$_2$) ppm.

LRMS (CI, NH_3) *m/z* 83 [$OCH_2CCH+NH_4^+$].

***Tert*-butyl(but-2-ynyloxy)dimethylsilane (2.6)**



$C_{10}H_{20}OSi$
m.w. = 184.33 g/mol
Colourless oil

To a solution of alkyne **2.5** (1.00 g, 5.87 mmol) in THF (23 mL) at –70 °C was added *n*-BuLi (2.50 M, 2.82 mL, 7.05 mmol) dropwise. The solution was stirred for 15 minutes before the dropwise addition of a solution of MeI (1.83 mL, 29.35 mmol) in THF (2 mL). Once this was achieved, the reaction mixture was warmed to rt and stirred for 22 hours. The reaction was quenched with H_2O (45 mL) and extracted with Et_2O (3 x 45 mL). The combined organic phases were washed with brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo* to yield a pale yellow oil. Purification by distillation under reduced pressure (0.4 mbar, 48-52 °C) gave the title compound **2.6** as a colourless oil (1.05 g, 5.70 mmol, 97%).

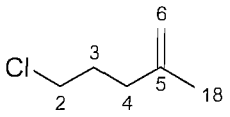
FT-IR (neat) ν_{max} 2955 (w), 2930 (w), 2896 (w), 2858 (w), 1254 (m), 1144 (m), 1078 (s), 835 (s) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.27 (2H, q, *J* = 2.2 Hz, **15**), 1.84 (3H, t, *J* = 2.2 Hz, **20**), 0.91 (9H, s, $(CH_3)_3CSi$-), 0.11 (6H, s, $(CH_3)_2Si$-) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 81.21 (C, **14**), 78.06 (C, **13**), 52.30 (CH_2, **15**), 26.21 (CH_3, $(CH_3)_3CSi$-), 18.70 (C, $(CH_3)_3CSi$-), 3.96 (CH_3, **20**), 4.85 (CH_3, -$Si(CH_3)_2$) ppm.

LRMS (CI, NH_3) *m/z* 185 [$M+H^+$].

5-Chloro-2-methyl-pent-1-ene (2.8)



$C_6H_{11}Cl$

m.w. = 118.60 g/mol

Colourless oil

To a solution of methyltriphenylphosphonium chloride (13.08 g, 41.82 mmol) in THF (40 mL) at –30 °C was treated with *n*-BuLi (2.31 M in hexane, 12.93 mL, 29.87 mmol). The reaction was warmed to 0 °C and stirred for 1.3 hours. The solution was cooled to –20 °C before the addition of a solution of 5-chloropenta-2-one (**2.7**) (3.00 g, 24.89 mmol) in THF (10 mL). After complete addition the reaction was warmed to 0 °C and stirred for 1 hour. After which the reaction was quenched with a saturated solution of NH_4Cl (30 mL) and diluted with hexane (15 mL). This mixture was filtered through a plug of silica gel before the phases were separated. The aqueous phase was extracted with hexane (3 x 10 mL) before the combined organic phases were washed with brine (10 mL) and dried ($MgSO_4$). The solvent was removed by distillation at atmospheric pressure. Purification by distillation at atmospheric pressure (102-104 °C) furnished the title compound (**2.8**) as a colourless oil (1.88 g, 15.86 mmol, 64%). Spectroscopic details were consistent with the literature.[80]

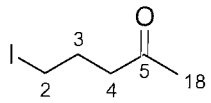
FT-IR (neat) ν_{max} 2959 (m), 1650 (m), 1443 (m), 889 (s) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.77 (1H, s, **6**), 4.73 (1H, s, **6**), 3.55 (2H, t, J = 6.8 Hz, **2**), 2.17 (2H, t, J = 7.4 Hz, **4**), 1.92 (2H, tt, J = 7.4, 6.8 Hz, **3**), 1.74 (3H, s, **18**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 144.46 (C, **5**), 111.23 (CH_2, **6**), 44.85 (CH_2, **2**), 35.17 (CH_2, **4**), 30.84 (CH_2, **3**), 22.65 (CH_3, **18**) ppm.

LRMS (CI, NH_3) *m/z* 56 [M–C_2H_4Cl+H^+].

5-Iodopentan-2-one (2.9)



C_5H_9IO

m.w. = 212.03 g/mol

Colourless oil

The iodide **2.9** was prepared by a method described by Chiarello *et al.*[81] To a solution of 5-chloropentanone (1.000 g, 8.29 mmol) in acetone (50 mL) was added NaI (6.221 g, 41.47 mmol) in one portion. The reaction mixture was heated to reflux and stirred for 17 hours. The reaction mixture was diluted Et_2O (50 mL), $NaHCO_3$ (15 mL) and $Na_2S_2O_3$ (15 mL) then separated. The aqueous phase was extracted with Et_2O (3 x 20 mL) and the combined organic phase was washed with brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving an amber oil. Purification by flash column chromatography (3.5 cm x 18 cm) eluting with 25% Et_2O/hexane furnished the title compound **2.9** as an amber oil (1.155 g, 5.44 mmol, 66%). Spectroscopic details were consistent with the literature.[81]

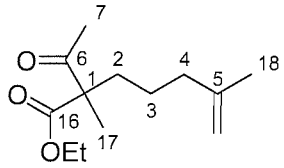
FT-IR (neat) ν_{max} 2960 (w), 2904 (w), 1711 (s), 1424 (m), 1364 (m), 1286 (w), 1220 (m), 1174 (s), 904 (w), 727 (w) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.23 (2H, t, J = 6.8 Hz, **2**), 2.59 (2H, t, J = 6.8 Hz, **4**), 2.17 (3H, s, **18**), 2.07 (2H, qn, J = 6.8 Hz, **3**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 207.43 (C, **5**), 44.16 (CH_2, **4**), 30.44 (CH_3, **18**), 27.40 (CH_2, **3**), 6.69 (CH_2, **2**) ppm.

LRMS (EI) *m/z* (relative intensity) 43 (100%) [$Ac^{\cdot+}$], 85 (60%) [$M^{\cdot+}$–I].

Ethyl 2-acetyl-2,6-dimethylhept-6-enoate (2.10)



$C_{13}H_{22}O_3$

m.w. = 226.31 g/mol

Colourless oil

To a solution of ethyl-2-methyl acetoacetate (245 mg, 1.70 mmol) in EtOH (7 mL) was added NaOEt (98 mg, 1.44 mmol) and the reaction was stirred for 1 hour. After this time a solution of iodide **2.9** (275 mg, 1.31 mmol) in EtOH (2 mL) was added dropwise. The resultant reaction mixture was stirred for 42 hours at rt then warmed to 50 °C and stirred for 26.5 hours. The reaction was concentrated *in vacuo* and the amber solid dissolved in H_2O (10 mL) and extracted with Et_2O (5 x 20 mL). The combined organic phases were washed with brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving a colourless oil. Purification by flash column chromatography on silica gel (4.0 cm x 14 cm) eluting with 15% Et_2O/hexane gave the title compound **2.10** as a colourless oil (85 mg, 0.46 mmol, 35%).

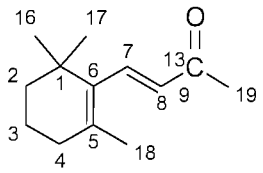
FT-IR (neat) ν_{max} 2980 (w), 2937 (w), 1709 (s), 1649 (w), 1447 (w) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.72 (1H, br s, -C=C**H**$_2$), 4.67 (1H, br s, -C=C**H**$_2$), 4.20 (2H, q, J = 7.1 Hz, -OC**H**$_2$CH$_3$), 2.15 (3H, s, **7**), 2.03 (2H, t, J = 7.4 Hz, **4**), 1.96-1.82 (2H, m, **2**), 1.80-1.68 (2H, m, **3**), 1.70 (3H, s, **18**), 1.34 (3H, s, **17**), 1.27 (3H, t, J = 7.1 Hz, -OCH$_2$C**H**$_3$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 206.01 (C, **6**), 173.41 (C, **16**), 145.38 (C, **5**), 110.73 (CH_2, -C=**C**H$_2$), 61.57 (CH_2, -O**C**H$_2$CH$_3$), 59.96 (C, **1**), 38.28 (CH_2, **4**), 34.71 (CH_2, **2**), 26.45 (CH_3, **7**), 22.57 (CH_3, **18**), 22.45 (CH_2, **3**), 19.22 (CH_3, **17**), 14.41 (CH_3, -OCH$_2$**C**H$_3$) ppm.

LRMS (CI, NH_3) *m/z* 227 [M+H$^+$].

[9-^{13}C]-β-Ionone (2.14)



$C_{12}{}^{13}CH_{20}O$

m.w. = 193.29 g/mol

Pale yellow oil

To a solution of acrylamide **2.38** (702 mg, 2.23 mmol) in THF (40 mL) at –78 °C was added MeLi (1.6 M in Et_2O, 2.23 mL, 3.57 mmol) slowly dropwise. The resulting reaction mixture was stirred at –78 °C for 45 minutes then warmed to –20 °C and stirred for 20 minutes. The reaction was quenched with a saturated solution of NH_4Cl (10 mL), diluted with Et_2O (40 mL) and separated. The organic phase was washed with a saturated solution of NH_4Cl (10 mL) before the aqueous phase was extracted with Et_2O (3 x 30 mL). The combined organic phases were washed with brine (10 mL), dried ($MgSO_4$) and concentrated to give a pale yellow oil. Purification by flash column chromatography on silica gel (4.0 cm x 13 cm) eluting with 10% Et_2O/hexane gave the title compound **2.14** as a pale yellow oil (370 mg, 1.91 mmol, 86%).

FT-IR (neat) ν_{max} 2960 (m), 2935 (m), 1617 (s), 1454 (m), 1359 (s), 1224 (s), 983 (m) cm^{-1}.

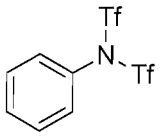
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (1H, dd, J = 16.5, 6.8 Hz, **8**), 6.12 (1H, dd, J = 16.5, 3.1 Hz, **7**), 2.30 (3H, d, J = 5.7 Hz, **19**), 2.08 (2H, t, J = 6.1 Hz, **4**), 1.77 (3H, s, **18**), 1.70-1.55 (2H, m, **3**), 1.53-1.40 (2H, m, **2**), 1.08 (6H, s, **16+17**) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 199.10 (C, **9**), 143.54 (CH, d, J = 1.7 Hz, **7**), 136.41 (2 x C, **5+6**), 131.94 (CH, d, J = 52.7 Hz, **8**), 40.10 (CH_2, **2**), 34.42 (C, **1**), 33.91 (CH_2, **4**), 29.15 (2 x CH_3, **16+17**), 27.50 (CH_3, d, J = 42.0 Hz, **19**), 22.08 (CH_3, **18**), 19.24 (CH_2, **3**) ppm.

LRMS (CI, NH_3) *m/z* 194 [$M+H^+$].

HRMS (EI) for $C_{12}{}^{13}CH_{20}O$, calculated 193.1548, found 193.1549 Da.

Phenyl triflimide (2.16)



$C_8H_5F_6NO_4S_2$

m.w. = 357.25 g/mol

White solid

Triflimide **2.16** was prepared by a method described by Hendrickson *et al.*[83] To a solution of aniline (1.96 mL, 21.48 mmol) in CH_2Cl_2 (16 mL) at –78 °C was added Et_3N (5.99 mL, 42.95 mmol) dropwise then stirred for 10 minutes at –78 °C. After which, triflic anhydride (3.61 mL, 21.48 mmol) was added dropwise over a period of 15 minutes. The mixture was warmed to rt and stirred for 15 hours. After this time, the reaction was diluted with CH_2Cl_2 (20 mL) and 2 M HCl (15 mL). The phases were separated before the organic phase was washed with 2 M HCl (2 x 15 mL), water (2 x 15 mL) and brine (15 mL). The solution was dried ($MgSO_4$) and concentrated *in vacuo* to give a brown grey solid. Purification by recrystallisation with CH_2Cl_2 gave the title compound **2.16** as a white solid (6.25 g, 17.48 mmol, 81%). Spectroscopic details were consistent with the literature.[83]

MPt 98-101 °C.

FT-IR (neat) ν_{max} 2970 (w), 2935 (w), 1439 (s), 1417 (s), 1206 (s), 1117 (s) cm^{-1}.

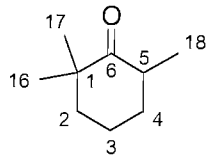
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (1H, t, J = 7.8 Hz, -NCCHCHC**H**-), 7.53 (2H, t, J = 7.8 Hz, -NCCHC**H**CH-), 7.42 (2H, d, J = 7.8 Hz, -NCC**H**CHCH-) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 132.45 (CH, -NCCHCH**C**H-), 132.34 (C, -N**C**CHCHCH-), 131.34 (CH, -NC**C**HCHCH-), 130.31 (CH, -NCCH**C**HCH-) ppm.

^{19}F NMR (282 MHz, $CDCl_3$) δ –70.87 (F, -OS(O)$_2$C**F**$_3$) ppm.

LRMS (EI) *m/z* 91 (100%) [$M^{\cdot+}$–2 x Tf], 357 (16%) [$M^{\cdot+}$].

2,2,6-Trimethyl cyclohexanone (2.18)



$C_9H_{16}O$

m.w. = 140.22 g/mol

Colourless oil

Cyclohexanone **2.18** was prepared by a method described by Liotta *et al.*[84] To a solution of LDA (1.8 M in THF/heptane/ethylbenzene, 9.68 mL, 17.43 mmol) in THF (24 mL) at −10 °C was added a solution of 2,6-dimethyl cyclohexanone (2.16 mL, 15.85 mmol) dropwise. The reaction was then stirred for 1.5 hours at 0 °C before the addition of MeI (1.48 mL, 23.77 mmol) dropwise. The solution was warmed to rt and stirred for 15 hours then quenched with a saturated solution of NH_4Cl (15 mL) and the reaction mixture was extracted with Et_2O (4 x 20 mL). The combined organic was washed with 2 M HCl (10 mL), $NaHCO_3$ (10 mL), water (10 mL) and brine (10 mL) sequentially. The organic was dried ($MgSO_4$) and concentrated *in vacuo* to give a yellow oil. Purification by flash column chromatography on silica gel (4.5 cm x 19 cm) eluting with 5% Et_2O/hexane afforded the desired product **2.18** as a colourless oil (1.47 g, 10.48 mmol, 66%). Spectroscopic details were consistent with the literature.[84]

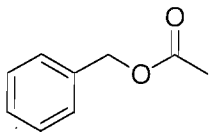
FT-IR (neat) ν_{max} 2967 (w), 2929 (m), 2869 (w), 1704 (s), 1454 (m) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.66 (1H, ddq, J = 13.0, 6.4, 5.7 Hz, **5**), 2.06 (1H, dddt, J = 13.0, 5.7, 3.8, 2.8 Hz, $\mathbf{4_{eq}}$), 1.89 (1H, qt, J = 13.3, 3.8 Hz, $\mathbf{3_{ax}}$), 1.77 (1H, ddq, J = 13.3, 2.8, 0.8 Hz, $\mathbf{2_{eq}}$), 1.66 (1H, dtt, J = 13.3, 4.0, 2.8 Hz, $\mathbf{3_{eq}}$), 1.55 (1H, td, J = 13.3, 4.3 Hz, $\mathbf{2_{ax}}$), 1.32 (1H, qd, J = 13.0, 4.0 Hz, $\mathbf{4_{ax}}$), 1.18 (3H, s, **16/17**), 1.12 (3H, s, **16/17**), 1.00 (3H, d, J = 6.5 Hz, **18**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 217.62 (C, **6**), 45.57 (C, **1**), 42.19 (CH_2, **2**), 41.12 (CH, **5**), 37.12 (CH_2, **4**), 25.99 (CH_3, **16/17**), 25.66 (CH_3, **16/17**), 21.90 (CH_2, **3**), 15.32 (CH_3, **18**) ppm.

LRMS (EI) *m/z* (relative intensity) 140 (25%) [$M^{\cdot+}$].

Benzyl acetate (2.20)



$C_9H_{10}O_2$

m.w. = 150.17 g/mol

Colourless oil

To a solution of benzyl alcohol (**2.19**) (1.46 mL, 14.69 mmol) in CH_2Cl_2 (60 mL) at 0 °C was added Et_3N (3.93 mL, 28.18 mmol), DMAP (157 mg, 1.28 mmol) and AcCl (0.91 mL, 12.81 mmol). The solution was warmed to rt and stirred for 16 hours before the reaction was quenched with 2 M HCl (20 mL). The phases were separated before the aqueous phase was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic phases were washed with brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give a cloudy colourless oil. Purification by flash column chromatography on silica gel (4.0 cm x 15 cm) eluting with 10% Et_2O/hexane afforded the title compound **2.20** as a colourless oil (1.55 g, 10.31 mmol, 80%). Spectroscopic details were consistent with the literature.[168]

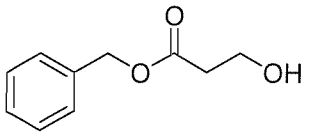
FT-IR (neat) ν_{max} 3035 (w), 2958 (w), 1737 (s), 1498 (w), 1455 (w), 1380 (m), 1382 (m), 1223 (s), 1025 (s), 747 (m), 697 (s) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.33 (5H, m, Ar**H**), 5.13 (2H, s, ArC**H$_2$**-), 2.12 (3H, s, -C(O)C**H$_3$**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.18 (C, -**C**=O), 136.30 (C, -**C**CH_2O-), 128.90 (CH, -OCH_2CCH**C**HCH-), 128.58 (2 x CH, -OCH_2**C**$CHCH$**C**H-), 66.64 (CH_2, -**C**H_2O-), 21.33 (CH_3, -C(O)**C**H_3) ppm.

LRMS (CI, NH_3) *m/z* 168 [$M+NH_4^+$], 108 [$M-Ac+H^+$].

3-Hydroxy-propionic acid benzyl ester (2.21)



$C_{10}H_{12}O_3$

m.w. = 180.20 g/mol

Colourless oil

To a solution of LDA (1.8 M in THF/heptane/ethylbenzene, 0.41 mL, 0.73 mmol) in THF (5 mL) at –78 °C was added a solution of benzyl acetate (**2.20**) (110 mg, 0.73 mmol) in THF (1 mL) dropwise. The mixture was stirred at –78 °C for 1.5 hours before being warmed to –30 °C. Paraformaldehyde (133 mg, 4.41 mmol) and phosphorus pentoxide (50 mg) were heated, liberating gaseous formaldehyde. This gas was then bubbled through the enolate solution. The solution was stirred at –30 °C for 1 hour before the reaction was quenched with a saturated solution of NH_4Cl (1 mL) and diluted with Et_2O (3 x 15 mL). The combined organic phases were washed with brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving an amber oil. Purification by column chromatography on silica gel (3.0 cm x 18 cm) eluting with 30% EtOAc/hexane gave the title compound **2.21** as a colourless oil (24 mg, 0.13 mmol, 18%).

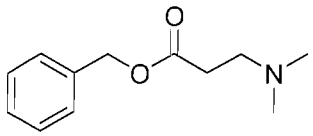
FT-IR (neat) ν_{max} 3444 (br), 2960 (w), 2919 (w), 2891 (w), 1729 (s), 1497 (w), 1162 (s), 1041 (s) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45-7.25 (5H, m, Ar**H**), 5.17 (2H, s, PhC**H$_2$**O-), 3.90 (2H, t, J = 5.5 Hz, -C**H$_2$**OH), 2.64 (2H, t, J = 5.5 Hz, -C(O)C**H$_2$**CH_2OH), 2.45 (1H, br s, -O**H**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 173.00 (C, -**C**=O), 135.96 (C, -OCH_2**C**CHCHCH-), 128.95 (CH, -OCH_2CCH**C**HCH-), 128.70 (CH, -OCH_2CCHCH**C**H-), 128.58 (CH, -OCH_2**C**CHCHCH-), 66.85 (CH_2, Ph**C**H_2O-), 58.57 (CH_2, -**C**H_2OH), 37.18 (CH_2, -C(O)**C**H_2CH_2OH) ppm.

LRMS (ES^+) *m/z* 203 [$M+Na^+$].

3-Dimethylamino-propionic acid benzyl ester (2.22)



$C_{12}H_{17}NO_2$

m.w. = 207.27 g/mol

Colourless oil

To a solution of LDA (1.8 M in THF/heptane/ethylbenzene, 0.74 mL, 1.33 mmol) in THF (10 mL) at –78 °C was added a solution of benzyl acetate **2.20** (200 mg, 1.33 mmol) in THF (1 mL) dropwise. This solution was stirred at –78 °C for 2 hours, after which *N,N*-dimethylmethyleneammonium iodide (370 mg, 2.00 mmol) in THF (15 mL) was added dropwise over 40 minutes. The reaction was stirred at –78 °C for 2.5 hours before slowly warming to rt and stirring for 13 hours. The reaction was quenched with water (10 mL) and then extracted with Et_2O (4 x 15 mL). The combined organic phases were washed with brine (15 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving an amber oil. Purification by flash column chromatography on silica gel (2.5 cm x 17 cm) eluting with 1% Et_3N/10% MeOH/DCM gave the title compound **2.22** as a colourless oil (166 mg, 0.80 mmol, 60%).

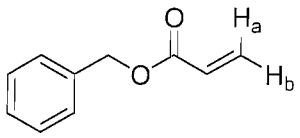
FT-IR (neat) ν_{max} 2967 (m), 2819 (m), 2768 (m), 1734 (s), 1456 (m) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.30 (5H, m, Ar**H**), 5.14 (2H, s, -OC**H$_2$**-), 2.66 (2H, t, J = 7.0 Hz, -C**H$_2$**N(CH$_3$)$_2$), 2.55 (2H, t, J = 7.0 Hz, -C(O)C**H$_2$**-), 2.26 (6H, s, -N(C**H$_3$**)$_3$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.60 (C, -**C**=O), 136.31 (C, -OCH$_2$**C**CHCHCH-), 128.87 (CH, -OCH$_2$CCH**C**HCH-), 128.52 (2 x CH -OCH$_2$C**C**HCH**C**H-), 66.61 (CH_2, -O**C**H$_2$CCHCHCH-), 55.04 (CH_2, -**C**H$_2$N(CH$_3$)$_2$), 45.54 (CH_3, -N(**C**H$_3$)$_3$), 33.21 (CH_2, -C(O)**C**H$_2$-) ppm.

LRMS (ES^+) *m/z* 208 [$M+H^+$].

Benzyl acrylate (2.23)



$C_{10}H_{10}O_2$

m.w. = 162.19 g/mol

Colourless oil

To a solution of benzyl alcohol (**2.19**) (1.46 mL, 14.69 mmol) in CH_2Cl_2 (60 mL) at 0 °C was added Et_3N (5.36 mL, 38.43 mmol), DMAP (157 mg, 1.28 mmol) and acryloyl chloride (1.04 mL, 12.81 mmol) sequentially. The reaction mixture was warmed to rt and stirred for 23 hours. The reaction was quenched with 2 M HCl (15 mL) and the phases separated, the aqueous was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic phases were washed with brine (15 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving an orange oil and solid. Purification by flash column chromatography on silica gel (4.0 cm x 12 cm) eluting with 5% Et_2O/hexane gave the desired product **2.23** as a colourless oil (1.15 g, 7.12 mmol, 55%). Spectroscopic details are consistent with those reported in the literature.[169]

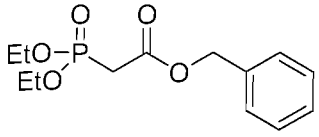
FT-IR (neat) ν_{max} 3034 (w), 2956 (w), 1722 (s), 1634 (w), 1498 (w), 1455 (w), 1405 (m), 1295 (m), 1267 (m), 1173 (s), 1048 (m) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44-7.34 (5H, m, Ar**H**), 6.47 (1H, dd, J = 17.3, 1.5 Hz, $\mathbf{H_a}$), 6.19 (1H, dd, J = 17.3, 10.5 Hz, -C**H**=CH_2), 5.86 (1H, dd, J = 10.5, 1.5 Hz, $\mathbf{H_b}$), 5.22 (2H, s, ArC$\mathbf{H_2}$-) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 166.36 (C, -**C**=O), 136.26 (C, -OCH_2**C**$CHCHCH$-), 131.38 (CH_2, -CH=**C**H_2), 128.92 (CH, -$OCH_2CCHCHCH$-), 128.70 (CH, -**C**H=CH_2), 128.61 (CH, -$OCH_2CCHCHCH$-), 128.58 (CH, -$OCH_2CCHCHCH$-), 66.67 (CH_2, -O**C**H_2-) ppm.

LRMS (EI) *m/z* (relative intensity) 162 (46 %) [$M^{\cdot+}$], 91 (100 %) [$PhCH_2^{\cdot+}$].

Phenylmethyl 2-[di(ethoxy)phosphoryl]ethanoate (2.25)



$C_{13}H_{19}O_5P$

m.w. = 286.26 g/mol

Colourless oil

To a solution of benzyl alcohol (**2.19**) (0.14 mL, 1.34 mmol) and DMAP (49 mg, 0.40 mmol) in toluene (2 mL) was added triethyl phosphonoacetate (0.27 mL, 1.34 mmol) dropwise. This solution was then heated to reflux and stirred for 32 hours before being concentrated *in vacuo* giving an amber oil. Purification by flash column chromatography (4.0 cm x 14 cm) eluting with 90% EtOAc/hexane gave the desired product **2.25** as a colourless oil (290 mg, 1.01 mmol, 76%). Spectroscopic details are consistent with those reported in the literature.[170]

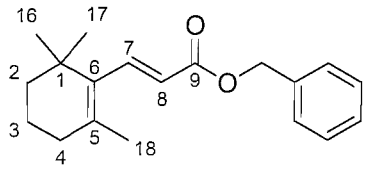
FT-IR (neat) ν_{max} 2984 (w), 2936 (w), 1734 (s), 1455 (w), 1373 (w), 1258 (s), 1112 (m), 1018 (s), 965 (m) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45-7.29 (5H, m, Ar**H**), 5.18 (2H, s, -OC**H$_2$**Ar), 4.13 (4H, dq, *J* = 8.3, 7.0 Hz, ($CH_3C\mathbf{H_2}O)_2$P-), 3.01 (2H, d, *J* = 21.6 Hz, -PC**H$_2$**-), 1.30 (6H, t, *J* = 7.0 Hz, ($C\mathbf{H_3}CH_2O)_2$P-) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 165.97 (C, d, *J* = 5.8 Hz, -**C**=O), 135.70 (C, -OCH_2**C**CHCHCH-), 128.87 (CH, , -OCH_2CCH**C**HCH-), 128.71 (CH, -OCH_2CCHCH**C**H-), 128.67 (CH, -OCH_2**C**CHCHCH-), 67.57 (CH_2, -O**C**$H_2CCHCHCH$-), 63.02 (CH_2, d, *J* = 5.8 Hz, (CH_3**C**$H_2O)_2$P-), 34.72 (CH_2, d, *J* = 134.1 Hz, -P**C**H_2-), 16.59 (CH_3, d, *J* = 6.8 Hz, (**C**$H_3CH_2O)_2$P-) ppm.

LRMS (EI) *m/z* (relative intensity) 286 (4%) [$M^{\cdot +}$], 91 (100%) [$PhCH_2^{\cdot +}$].

(2*E*)-Benzyl 3-(2,6,6-trimethylcyclohex-1-enyl)acrylate (2.26)



$C_{19}H_{24}O_2$

m.w. = 284.39 g/mol

Colourless oil

To a slurry of $Pd(PPh_3)Cl_2$ (6 mg, 0.01 mmol) in DMF (0.8 mL) was added a solution of triflate **1.120** (100 mg, 0.37 mmol), benzyl acrylate (**2.23**) (131 mg, 0.81 mmol) and Et_3N (0.18 mL, 1.29 mmol) in DMF (0.8 mL) dropwise. This mixture was heated to 75 °C and stirred for 23 hours before the reaction was quenched with H_2O (5 mL). The mixture was extracted with Et_2O (5 x 15 mL) and the combined organic phases were washed with H_2O (2 x 5 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving an amber oil. Purification by flash column chromatography (2.5 cm x 18 cm) eluting with 5% Et_2O/hexane furnished the desired product **2.26** as a colourless oil (35 mg, 0.12 mmol, 33%).

FT-IR (neat) ν_{max} 3031 (w), 2953 (w), 2928 (m), 2864 (w), 1711 (s), 1611 (m), 1454 (m), 1374 (m), 1291 (m), 1258 (m), 1154 (s), 1133 (m), 980 (m), 909 (m), 730 (s), 694 (s) cm^{-1}.

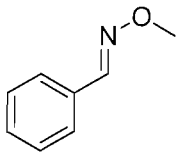
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (1H, d, J = 16.3 Hz, **7**), 7.45-7.30 (5H, m, Ar**H**), 5.89 (1H, d, J = 16.3 Hz, **8**), 5.22 (2H, s, -OC**H$_2$**Ar), 2.07 (2H, t, J = 5.9 Hz, **4**), 1.78 (3H, s, **18**), 1.67-1.58 (2H, m, **3**), 1.51-1.43 (2H, m, **2**), 1.08 (6H, s, **16**+**17**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 167.49 (C, **9**), 145.17 (CH, **7**), 136.68 (C, -OCH_2**C**CHCHCH-), 136.28 (C, **6**), 136.22 (C, **5**), 128.89 (CH, -OCH_2CCH**C**HCH-), 128.54 (CH, -OCH_2**C**CHCHCH-), 128.46 (CH, -OCH_2CCHCH**C**H-), 121.62 (CH, **8**), 66.40 (CH_2, -O**C**H_2Ar), 40.17 (CH_2, **2**), 34.38 (C, **1**), 33.89 (CH_2, **4**), 29.09 (CH_3, **16**+**17**), 22.01 (CH_3, **18**), 19.26 (CH_2, **3**) ppm.

LRMS (ES^+) *m/z* 307 [$M+Na^+$], 348 [$M+K^+$].

HRMS (ES^+) for $C_{19}H_{25}O_2$, calculated 285.1849, found 285.1853 Da.

Benzaldehyde *O*-methyloxime (2.28)



C_8H_9NO

m.w. = 135.16 g/mol

Colourless oil

To a solution of methoxyamine hydrochloride (1.00 g, 11.97 mmol) in EtOH (20 mL) was added NaOAc (893 mg, 10.88 mmol) and benzaldehyde (1.11 mL, 10.88 mmol). The reaction mixture was heated to reflux and stirred for 15 hours. After cooling, the reaction was neutralised with $CaCO_3$ and filtered. The residue was dissolved in Et_2O (50 mL) and H_2O (15 mL) and the phases were separated. The aqueous phase was extracted with Et_2O (3 x 30 mL) before the combined organic phases were washed with brine (15 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving a colourless oil. Purification by flash column chromatography on silica gel (4.0 cm x 14 cm) eluting with 1%→4% Et_2O/hexane afforded the desired methyloxime **2.28** as a colourless oil (1.02 g, 7.55 mmol, 69%). Spectroscopic details were consistent with the literature.[171]

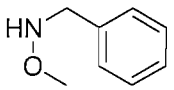
FT-IR (neat) ν_{max} 2935 (w), 2896 (w), 2816 (w), 1446 (m), 1211 (m), 1048 (s), 945 (m), 914 (s) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (1H, s, CH_3ON=C**H**-), 7.63-7.55 (2H, m, -N=CHCC**H**CHCH-), 7.41-7.34 (3H, m, -N=CHCCHC**H**C**H**-), 4.00 (3H, s, C**H**$_3$O-) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 148.90 (CH, CH_3ON=**C**H-), 132.59 (C, -N=CH**C**CHCHCH-), 130.13 (CH, -N=CHCCHCH**C**H-), 129.02 (CH, -N=CHC**C**HCHCH-), 127.36 (CH, -N=CHCCH**C**HCH-), 62.34 (**C**H_3, **C**H_3ON=CH-) ppm.

LRMS (EI) *m/z* (relative intensity) 135 (100 %) [$M^{\cdot+}$].

***N*-Benzyl-*O*-methylhydroxylamine (2.29)**



$C_8H_{11}NO$

m.w. = 137.18 g/mol

Colourless oil

N-benzyl-*O*-methylhydroxylamine (**2.29**) was prepared by a method described by Keck *et al.*[86] To a solution of benzyaldehyde *O*-methyloxime (**2.28**) (1.00 g, 7.40 mmol) in CH_2Cl_2 (9 mL) was added $NaCNBH_3$ (744 mg, 11.84 mmol) in one portion. Then a solution of HCl (2 M in methanol, 7 mL, 14.00 mmol) was added slowly over 10 minutes to the suspension. The brown solution was stirred for 20 hours before the addition of 2 M NaOH until pH 9 was achieved. The mixture was then extracted with CH_2Cl_2 (4 x 25 mL). The combined organic phases were washed with brine (10 mL), dried ($MgSO_4$) then filtered through a pad of celite and $MgSO_4$. The filtered solution was concentrated *in vacuo* giving a colourless oil. Purification by flash column chromatography on silica gel (4.0 cm x 9 cm) eluting with 10% Et_2O/hexane furnished the title compound **2.29** as a colourless oil (582 mg, 4.24 mmol, 57%). Spectroscopic details were consistent with the literature.[86]

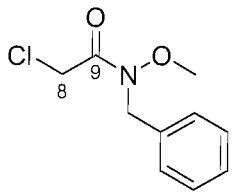
FT-IR (neat) ν_{max} 3243 (w), 3028 (w), 2934 (m), 2892 (m), 2806 (w), 1494 (m), 1452 (s), 1057 (s), 991 (s) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.28 (5H, m, Ar**H**), 5.73 (1H, br s, N**H**), 4.07 (2H, s, -NHC**H$_2$**Ar), 3.52 (3H, s, C**H$_3$**O-) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 137.97 (C, -NHCH$_2$**C**CHCHCH-), 129.18 (CH, -NHCH$_2$CCH**C**HCH-), 128.79 (CH, -NHCH$_2$C**C**HCHCH-), 127.78 (CH, -NHCH$_2$CCHCH**C**H-), 62.16 (CH_3, **C**H$_3$O-), 56.58 (CH_2, -NH**C**H$_2$CCHCHCH-) ppm.

LRMS (EI) *m/z* (relative intensity) 137 (12 %) [$M^{\cdot+}$], 91 (100 %) [$PhCH_2^{+}$].

***N*-Benzyl-2-chloro-*N*-methoxyacetamide (2.30)**



$C_{10}H_{12}ClNO_2$
m.w. = 213.66 g/mol
Colourless oil

To a solution of *N*-benzyl-*O*-methylhydroxylamine (**2.29**) (750 mg, 5.47 mmol) in CH_2Cl_2 (15 mL) at 0 °C was added chloroacetyl chloride (0.44 mL, 5.47 mmol) and Et_3N (0.76 mL, 5.47 mmol) dropwise. The mixture was allowed to warm to rt and stirred for 2 hours before being quenched with H_2O (10 mL) and the phases separated. The aqueous phase was extracted with CH_2Cl_2 (4 x 15 mL) before the combined organic phases were washed with brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving a colourless oil. Purification by flash column chromatography on silica gel (4.0 cm x 14 cm) eluting with 20% EtOAc/hexane gave the title compound **2.30** as a colourless oil (1.02 g, 4.76 mmol, 87%).

FT-IR (neat) ν_{max} 3064 (w), 3023 (w), 2954 (w), 1671 (s), 1426 (s), 1397 (s), 1356 (s), 1230 (s) cm^{-1}.

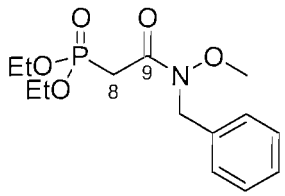
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43-7.28 (5H, m, Ar**H**), 4.83 (2H, s, **8**), 4.29 (2H, s, Ar**CH$_2$**-), 3.70 (3H, s, **CH$_3$**O-) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 168.01 (C, **9**), 135.95 (C, -NHCH$_2$**C**CHCHCH-), 129.02 (CH, -NHCH$_2$CCH**C**HCH-), 128.87 (CH, -NHCH$_2$C**C**HCHCH-), 128.34 (CH, -NHCH$_2$CCHCH**C**H-), 62.92 (CH$_3$, **C**H$_3$O-), 50.03 (CH$_2$, -NH**C**H$_2$CCHCHCH-), 41.47 (CH$_2$, **8**) ppm.

LRMS (CI, NH_3) *m/z* 214 [M+H$^+$].

HRMS (ES^+) for $C_{10}H_{13}NO_2Cl$, calculated 214.0629, found 214.0629 Da.

Diethyl (*N*-benzyl-*N*-methoxycarbamoyl)methylphosphonate (2.31)



$C_{14}H_{22}NO_5P$

m.w. = 315.30 g/mol

Colourless oil

A mixture of amide **2.30** (1.00 g, 4.68 mmol) and triethyl phosphite (0.80 mL, 4.68 mmol) was heated to 180 °C and stirred for 1 hour. Purification by flash column chromatography on silica gel (4.5 cm x 11 cm) eluting with EtOAc→10% MeOH/CH_2Cl_2 gave the desired product **2.31** as a colourless oil (1.31 g, 4.15 mmol, 89%).

FT-IR (neat) ν_{max} 2978 (w), 2938 (w), 2901 (w), 1654 (s), 1438 (m), 1390 (m), 1251 (s), 1164 (m), 1050 (m), 1019 (s), 960 (s) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.20 (5H, m, Ar**H**), 4.83 (2H, s, -NC**H$_2$**-), 4.47 (4H, dq, J = 8.1, 7.1 Hz, -P(OC**H$_2$**CH_3)$_2$), 3.72 (3H, s, -NOC**H$_3$**), 3.21 (2H, d, J = 22.0 Hz, -PC**H$_2$**C=O), 1.33 (6H, dt, J = 7.1, 0.5 Hz, P(OCH$_2$C**H$_3$**)$_2$) ppm.

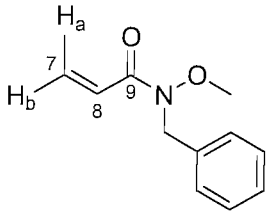
^{13}C NMR (100 MHz, $CDCl_3$) δ 166.51 (C, **9**), 136.30 (C, -NCH$_2$**C**CHCHCH-), 128.87 (CH, -NCH$_2$CCH**C**HCH-), 128.76 (CH, -NCH$_2$C**C**HCHCH-), 128.08 (CH, -NCH$_2$CCHCH**C**H-), 62.92 (CH_2, d, J = 6.0 Hz, P(O**C**H$_2$CH$_3$)$_2$), 62.61 (CH_3, -NO**C**H$_3$), 49.05 (CH_2, -N**C**H$_2$Ar), 31.96 (CH_2, d, J = 133.0 Hz, -P**C**H$_2$C=O), 16.65 (CH_3, d, J = 6.6 Hz, -POCH$_2$**C**H$_3$).

^{31}P NMR (121 MHz, $CDCl_3$) δ 21.59 (s) ppm.

LRMS (ES^+) *m/z* 338 [$M+Na^+$].

HRMS (ES^+) for $C_{14}H_{23}NO_5P$, calculated 316.1309, found 316.1307 Da.

***N*-Benzyl-*N*-methoxyacrylamide (2.32)**



$C_{11}H_{13}NO_2$
m.w. = 191.23 g/mol
Colourless oil

A mixture of phosphonate **2.31** (200 mg, 0.63 mmol), K_2CO_3 (263 mg, 1.90 mmol) and $CH_2O_{(aq)}$ (0.32 mL, 3.94 mmol) were heated at 40 °C for 20 minutes. The reaction was diluted with H_2O (5 mL) and the mixture was extracted with Et_2O (4 x 10 mL). The combined organic phases were washed with brine (5 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving a colourless oil. Purification by flash column chromatography on silica gel (3.5 cm x 17 cm) eluting with 1% Et_2O/CH_2Cl_2→10% Et_2O/CH_2Cl_2 gave the desired product **2.32** as a colourless oil (68 mg, 0.36 mmol, 56%).

FT-IR (neat) ν_{max} 3029 (w), 2970 (w), 2935 (w), 1649 (s), 1620 (m), 1491 (m), 1416 (s), 1342 (m), 1227 (m), 982 (s) cm^{-1}.

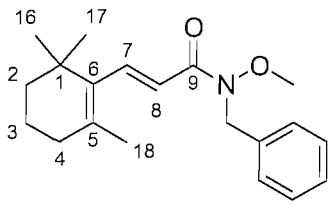
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.20 (5H, m, Ar**H**), 6.77 (1H, dd, *J* = 17.1, 10.3 Hz, **8**), 6.50 (1H, dd, *J* = 17.1, 2.0 Hz, **7a**), 5.80 (1H, dd, *J* = 10.3, 2.0 Hz, **7b**), 4.87 (2H, s, -NC**H$_2$**Ar), 3.65 (3H, s, -NOC**H$_3$**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 166.81 (C, **9**), 136.70 (C, -NCH_2**C**CHCHCH-), 129.92 (CH_2, **7**), 128.91 (CH, -NCH_2CCH**C**HCH-), 128.75 (CH, -NCH_2**C**CHCHCH-), 128.05 (CH, -NCH_2CCHCH**C**H-), 126.43 (CH, **8**), 63.11 (CH_3, -NO**C**H_3), 49.68 (CH_2, -N**C**H_2Ar) ppm.

LRMS (CI, NH_3) *m/z* 214 [$M+Na^+$].

HRMS (ES^+) for $C_{11}H_{20}NO_2Na$, calculated 214.0838, found 214.0835 Da.

(2*E*)-*N*-Benzyl-*N*-methoxy-3-(2,6,6-trimethylcyclohex-1-enyl)acrylamide (2.33)



$C_{20}H_{27}NO_2$

m.w. = 313.43 g/mol

Pale yellow oil

To a suspension of $Pd(PPh_3)_2Cl_2$ (8 mg, 0.01 mmol) in DMF (1 mL) was added a solution of amide **2.32** (100 mg, 0.52 mmol), triflate **1.120** (214 mg, 0.78 mmol) and Et_3N (0.26 mL, 1.83 mmol) in DMF (1 mL) dropwise. The mixture was heated to 75 °C and stirred for 16 hours. The reaction was quenched with H_2O (10 mL) and extracted with Et_2O (3 x 20 mL) before the combined organic phases were washed with brine (3 x 5 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving an amber oil. Purification by flash column chromatography on silica gel (2.5 cm x 12 cm) eluting with 15% EtOAc/hexane gave the title compound **2.33** as an amber oil (135 mg, 0.43 mmol, 83%).

FT-IR (neat) ν_{max} 2958 (m), 2929 (m), 2868 (m), 2819 (w), 1650 (s), 1601 (s), 1495 (m), 1426 (m), 1389 (s), 988 (s) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (1H, d, *J* = 16.1 Hz, **7**), 7.40-7.20 (5H, m, -NCH_2ArH), 6.43 (1H, d, *J* = 16.1 Hz, **8**), 4.89 (2H, s, -NCH_2Ar), 3.64 (3H, s, -$NOCH_3$), 2.07 (2H, t, *J* = 5.9 Hz, **4**), 1.79 (3H, s, **18**), 1.70-1.56 (2H, m, **3**), 1.55-1.40 (2H, m, **2**), 1.09 (6H, s, **16**+**17**) ppm.

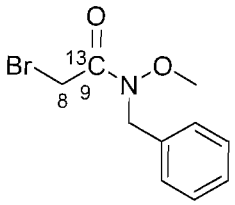
^{13}C NMR (75 MHz, $CDCl_3$) δ 167.77 (C, **9**), 143.86 (CH, **7**), 137.09 (C, **6**), 136.94 (C, -$NCH_2CCHCHCH$-), 134.97 (C, **5**), 128.89 (CH, -$NCH_2CCHCHCH$-), 128.82 (CH, -$NCH_2CCHCHCH$-

), 127.94 (CH, -NCH$_2$CCHCHCH-), 119.95 (CH, **8**), 63.18 (CH_3, -NOCH_3), 49.86 (CH_2, -NCH_2Ar), 40.17 (CH_2, **2**), 34.46 (C, **1**), 33.82 (CH_2, **4**), 29.16 (CH_3, **16**+**17**), 22.05 (CH_3, **18**), 19.34 (CH_2, **3**) ppm.

LRMS (CI, NH_3) *m/z* 336 [M+Na^+].

HRMS (ES^+) for $C_{20}H_{28}NO_2$, calculated 314.2115, found 314.2122 Da.

[9-^{13}C]-*N*-Benzyl-2-bromo-*N*-methoxyacetamide (2.35)



$C_9{}^{13}CH_{12}BrNO_2$

m.w. = 259.10 g/mol

Colourless oil

To a mixture of [1-^{13}C]-acetic acid (1.00 g, 16.38 mmol) and PBr_3 (1.95 mL, 16.38 mmol) was added bromine (2.10 mL, 40.96 mmol) slowly dropwise. The mixture was heated to 75 °C and stirred for 3 hours. Excess bromine was removed by distillation at atmospheric pressure before [1-^{13}C]-bromoacetyl bromide was distilled at atmospheric pressure (155-158 °C). To a solution of [1-^{13}C]-bromoacetyl bromide in CH_2Cl_2 (45 mL) at 0 °C was added *N*-benzyl-*O*-methylhydroxylamine (2.25 g, 16.38 mmol) in CH_2Cl_2 (5 mL) dropwise slowly and stirred for 10 minutes. After which, Et_3N (2.28 mL, 16.38 mmol) was added dropwise to the solution and stirred at 0 °C. After 1 hour the reaction was quenched with H_2O (10 mL) and the mixture separated. The aqueous phase was extracted with CH_2Cl_2 (4 x 20 mL) before the combined organic phases were washed with brine (15 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give a cloudy orange oil. Purification by flash column chromatography on silica gel (5.0 cm x 17 cm) eluting with 1% Et_2O/CH_2Cl_2 gave the desired compound **2.35** as a colourless oil (2.39 g, 9.21 mmol, 56%).

FT-IR (neat) ν_{max} 3086 (w), 3063 (w), 3031 (w), 2974 (w), 2939 (w), 2820 (w), 1620 (s), 1496 (m), 1425 (m), 1391 (m),

1347 (m), 1209 (w), 1140 (w), 988 (m), 715 (s), 697 (s) cm^{-1}.

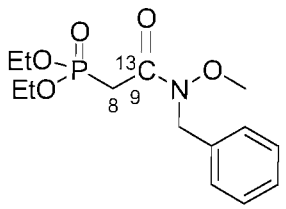
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45-7.30 (5H, m, Ar**H**), 4.83 (2H, d, J = 1.8 Hz, -CH_2Ph), 4.05 (2H, d, J = 3.8 Hz, **8**), 3.74 (3H, s, -OCH_3) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 168.11 (C, **9**), 135.94 (C, -CH_2**C**CHCHCH-), 128.98 (CH, -CH_2CCH**C**HCH-), 128.75 (CH, -CH_2C**C**HCHCH-), 128.28 (CH, -CH_2CCHCH**C**H-), 62.82 (CH_3, -O**C**H_3), 49.73 (CH_2, -**C**H_2Ph), 25.83 (CH_2, d, J = 57.7 Hz, **8**) ppm.

LRMS (CI, NH_3) *m/z* 259 [M(^{79}Br)+H^+], 261 [M(^{81}Br)+H^+].

HRMS (ES^+) for $C_9{}^{13}CH_{13}{}^{79}BrNO_2$, calculated 259.0159, found 259.0160 Da.

[9-^{13}C]-Diethyl (*N*-benzyl-*N*-methoxycarbamoyl)methylphosphonate (2.36)



$C_{13}{}^{13}CH_{22}NO_5P$

m.w. = 316.29 g/mol

Colourless oil

A mixture of amide **2.35** (2.37 g, 9.16 mmol) and triethyl phosphite (1.57 mL, 9.16 mmol) was heated to 180 °C and stirred for 1 hour giving a colourless oil. Purification by column chromatography on silica gel (4.5 cm x 11 cm) eluting with EtOAc→10% MeOH/CH_2Cl_2 gave the desired product **2.36** as a colourless oil (2.51 g, 7.95 mmol, 87%).

FT-IR (neat) ν_{max} 3488 (br), 2982 (w), 2938 (w), 2909 (w), 1615 (s), 1390 (m), 1250 (s), 1159 (m), 1052 (m), 1018 (s), 960 (s) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40-7.20 (5H, m, Ar**H**-), 4.83 (2H, d, *J* = 2.2 Hz, Ar**CH$_2$**-), 4.17 (4H, dq, *J* = 8.2, 7.1 Hz, -P(O**CH$_2$**CH$_3$)$_2$), 3.72 (3H, s, -NOC**H$_3$**), 3.20 (2H, dd, *J* = 22.0, 6.8 Hz, **8**), 1.33 (6H, dt, *J* = 7.0, 0.6 Hz, -P(OCH$_2$C**H$_3$**)$_2$) ppm.

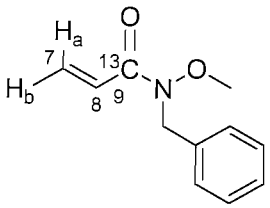
^{13}C NMR (75 MHz, $CDCl_3$) δ 166.51 (C, **9**), 136.32 (C, -NCH$_2$**C**CHCHCH-), 128.87 (CH, -NCH$_2$CCH**C**HCH-), 128.76 (CH, -NCH$_2$**C**CHCHCH-), 128.08 (CH, -NCH$_2$CCHCH**C**H-), 62.93 (CH_2, d, *J* = 6.1 Hz, -P(O**C**H$_2$CH$_3$)$_2$), 62.61 (CH_3, -NO**C**H$_3$), 49.10 (CH_2, -N**C**H$_2$CCHCHCH-), 31.98 (CH_2, dd, *J* = 135.5, 53.1 Hz, **8**), 16.65 (CH_3, d, *J* = 6.0 Hz, -P(OCH$_2$**C**H$_3$)$_2$) ppm.

^{31}P NMR (121 MHz, $CDCl_3$) δ 21.59 (s) ppm.

LRMS (ES^+) *m/z* 339 [M+Na^+].

HRMS (ES^+) for $C_{13}{}^{13}CH_{23}NO_5P$, calculated 317.1342, found 317.1340 Da.

[9-^{13}C]-*N*-Benzyl-*N*-methoxyacrylamide (2.37)



$C_{10}{}^{13}CH_{13}NO_2$
m.w. = 192.22 g/mol
Colourless oil

Phosphonate **2.36** (2.45 g, 7.73 mmol) and K_2CO_3 (3.207 g, 23.20 mmol) were suspended in H_2O (5 mL) and stirred for 15 minutes. To the solution was added $CH_2O_{(aq)}$ (1.15 mL, 15.47 mmol) slowly dropwise before the reaction was warmed to 40 °C and stirred for 30 minutes. To the reaction 6 aliquots of $CH_2O_{(aq)}$ (0.58 mL, 7.73 mmol) were made in 30 minute intervals. The reaction was diluted with Et_2O (40 mL) and H_2O (15 mL) then separated before the aqueous phase was extracted with Et_2O (3 x

20 mL). The combined organic phases were washed with brine (15 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give a pale yellow oil. Purification by flash column chromatography on silica gel (5.0 cm x 9 cm) eluting with 30% EtOAc/hexane gave the desired compound **2.37** as a colourless oil (965 mg, 5.02 mmol, 65%).

FT-IR (neat) ν_{max} 3088 (w), 3065 (w), 3032 (w), 2970 (w), 2937 (w), 1634 (s), 1595 (s), 1495 (m), 1404 (s), 1346 (m), 1223 (m), 988 (s), 769 (m), 733 (m), 699 (s) cm^{-1}.

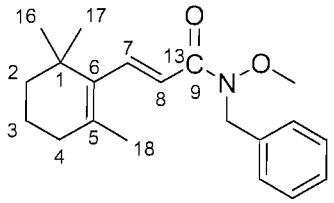
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45-7.22 (5H, m, Ar**H**), 6.77 (1H, ddd, J = 17.0, 10.2, 4.4 Hz, **8**), 6.50 (1H, ddd, J = 17.0, 6.8, 2.0 Hz, **7a**), 5.81 (1H, ddd, J = 12.4, 10.2, 2.0 Hz, **7b**), 4.87 (2H, d, J = 2.0 Hz, -NC**H$_2$**Ar), 3.65 (3H, s, -NOC**H$_3$**) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 166.82 (C, **9**), 136.71 (C, -NCH_2**C**CHCHCH-), 129.97 (CH_2, **7**), 128.93 (CH, -NCH_2CCH**C**HCH-), 128.76 (CH, -NCH_2**C**CHCHCH-), 128.06 (CH, -NCH_2CCHCH**C**H-), 126.42 (CH, J = 65.8 Hz, **8**), 63.13 (CH_3, -NO**C**H_3), 49.68 (CH_2, -N**C**H_2Ar) ppm.

LRMS (ES^+) *m/z* 215 [$M+Na^+$].

HRMS (ES^+) for $C_{10}{}^{13}CH_{14}NO_2$, calculated 193.1053, found 193.1050 Da.

[9-^{13}C]-(2*E*)-*N*-Benzyl-*N*-methoxy-3-(2,6,6-trimethylcyclohex-1-enyl)acrylamide (2.38)



$C_{19}{}^{13}CH_{27}NO_2$

m.w. = 314.43 g/mol

Colourless oil

$Pd(PPh_3)_2Cl_2$ (56 mg, 0.08 mmol) was suspended in DMF (5 mL), to this was added a solution of amide **2.37** (548 mg, 2.85 mmol), triflate **1.120** (981 mg, 3.60 mmol) and Et_3N (1.76 mL, 12.61 mmol) in DMF (5 mL) before the reaction was warmed to 75 °C and stirred for 22 hours. The reaction was diluted with Et_2O (40 mL) and H_2O (20 mL) then separated. The aqueous phase was extracted with Et_2O (4 x 20 mL) before the combined organic phases were washed with brine (3 x 10 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving a brown oil. Purification by flash column chromatography on silica gel (4.0 cm x 14 cm) eluting with 15% EtOAc/hexane gave the title compound **2.38** as an amber oil (708 mg, 2.25 mmol, 79%).

FT-IR (neat) ν_{max} 2960 (m), 2935 (m), 2869 (w), 1631 (m), 1596 (s), 1496 (m), 1456 (m), 1384 (s), 1259 (m), 1215 (m), 1031 (m), 990 (s), 698 (s) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (1H, dd, *J* = 16.1, 6.5 Hz, **7**), 7.40-7.25 (5H, m, Ar**H**), 6.43 (1H, dd, *J* = 16.1, 4.3 Hz, **8**), 4.89 (2H, d, 1.8 Hz, -NC**H**$_2$Ar), 3.64 (3H, s, -NOC**H**$_3$), 2.07 (2H, t, *J* = 6.3 Hz, **4**), 1.79 (3H, d, *J* = 0.5 Hz, **18**), 1.70-1.60 (2H, m, **3**), 1.55-1.45 (2H, m, **2**), 1.09 (6H, s, **16**+**17**) ppm.

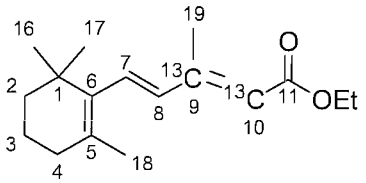
^{13}C NMR (100 MHz, $CDCl_3$) δ 167.75 (C, **9**), 143.84 (CH, **7**), 137.07 (C, **6**), 136.94 (C, -NCH$_2$**C**CHCHCH-), 134.99 (C, **5**), 128.90 (CH, -NCH$_2$CCH**C**HCH-), 128.82 (CH, -NCH$_2$**C**CHCHCH-), 127.95 (CH, -NCH$_2$CCHCH**C**H-), 119.91 (CH, **8**), 63.18 (CH_3, -NO**C**H$_3$), 49.79 (CH_2, -N**C**H$_2$Ar), 40.14

(CH_2, **2**), 34.45 (C, **1**), 33.82 (CH_2, **4**), 29.16 (CH_3, **16**+**17**), 22.07 (CH_3, **18**), 19.32 (CH_2, **3**) ppm.

LRMS (ES^+) *m/z* 315 [$M+H^+$], 337 [$M+Na^+$].

HRMS (ES^+) for $C_{19}{}^{13}CH_{28}NO_2$, calculated 315.2148, found 315.2150 Da.

[9,10-$^{13}C_2$]-(2*E*,4*E*)-Ethyl 3-methyl-5-(2,6,6-trimethylcyclohex-1-enyl)penta-2,4-dienoate (2.39)



$C_{15}{}^{13}C_2H_{26}O_2$
m.w. = 264.37 g/mol
Colourless oil

To a suspension of NaH (197 mg, 4.94 mmol) in Et_2O (4 mL) was added a solution of [2-^{13}C]-triethyl phosphonoacetate (1.000 g, 4.44 mmol) in Et_2O (3 mL) and stirred for 2 hours. To this mixture a solution of [9-^{13}C]-*β*-ionone (**2.14**) (367 mg, 1.90 mmol) in Et_2O (2 mL) was added dropwise and stirred for 60 hours. The reaction was quenched with H_2O (10 mL), this was extracted with Et_2O (3 x 40 mL). The combined organic phases were washed with brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving a yellow oil. Purification by flash column chromatography on silica gel (4.0 cm x 21 cm) eluting with hexane→1% Et_2O/hexane gave the desired product **2.39** as a colourless oil (420 mg, 1.59 mmol, 84%, 9-*E*/*Z* = 7:1).

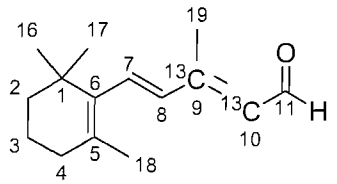
FT-IR (neat) ν_{max} 2956 (m), 2926 (m), 2863 (m), 1709 (m), 1557 (m), 1455 (m), 1223 (m), 1148 (s) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.57 (1H, dd, *J* = 16.0, 6.6 Hz, **7**), 6.15-6.05 (1H, m, **8**), 5.75 (1H, d, *J* = 159.2 Hz, **10**), 4.18 (2H, q, *J* = 7.1 Hz, -OC**H**$_2$CH$_3$), 2.34 (3H, t, *J* = 5.4 Hz, **19**), 2.04 (2H, t, *J* = 5.6 Hz, **4**), 1.70 (3H, s, **18**), 1.66-1.55 (2H, m, **3**), 1.49-1.44 (2H, m, **2**), 1.30 (3H, t, *J* = 7.1 Hz, -OCH$_2$C**H**$_3$), 1.03 (6H, s, **16**+**17**) ppm.

^{13}C NMR ($CDCl_3$, 75 MHz) Data unassignable.

LRMS (ES^+) *m/z* 265 [$M+H^+$].

[9,10-$^{13}C_2$]-(2*E*,4*E*)-3-Methyl-5-(2,6,6-trimethylcyclohex-1-enyl)penta-2,4-dienal (2.40)



$C_{13}{}^{13}C_2H_{22}O$
m.w. = 220.32 g/mol
Colourless oil

A solution of ester **2.39** (415 mg, 1.58 mmol, 9-*E*/*Z* = 7:1) in Et_2O (17 mL) was added dropwise to a slurry of $LiAlH_4$ (96 mg, 2.52 mmol) in Et_2O (4 mL) at –78 °C. This mixture was stirred for 1 hour then warmed to rt and stirred for a further 2 hours. The reaction was quenched with H_2O (0.1 mL), 15% NaOH (0.1 mL) and H_2O (0.3 mL) sequentially. The white suspension was dried ($MgSO_4$) and filtered through a pad of celite and concentrated *in vacuo*. The oil was dissolved in CH_2Cl_2 (17 mL), to the reaction was added crushed molecular sieves (94 mg), TPAP (55 mg, 0.16 mmol) and NMO (369 mg, 3.15 mmol) before the black solution was stirred for 30 minutes. The reaction was concentrated *in vacuo* to give a black oil. Purification by flash column chromatography on silica gel (3.5 cm x 15 cm) eluting with 3% EtOAc/hexane furnished the desired aldehyde **2.40** as a mixture of isomers (111 mg, 0.50 mmol, 32%, 9-*E*/*Z* = 3:1).

FT-IR (neat) ν_{max} 2928 (m), 2865 (m), 1628 (s), 1610 (s), 1561 (w), 1457 (w) cm^{-1}.

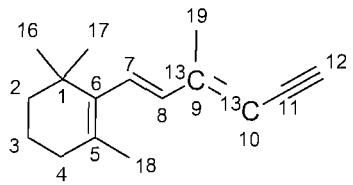
^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.14 (1H, dd, *J* = 24.5, 8.0 Hz, **11**), 6.75 (1H, br dd, *J* = 16.1, 6.5 Hz, **7**), 6.22 (1H, dt, *J* = 16.1, 4.0 Hz, **8**), 5.94 (1H, dd, *J* = 157.4, 8.6 Hz, **10**), 2.32 (3H, t, *J* = 4.6 Hz, **19**), 2.06 (2H, t, *J* = 5.5 Hz, **4**), 1.73 (3H, s,

18), 1.56-1.45 (2H, m, **3**), 1.40-1.33 (2H, m, **2**), 0.94 (6H, s, **16**+**17**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 191.68 (CH, dd, *J* = 56.9, 2.9 Hz, **11**), 155.33 (C, d, *J* = 67.1 Hz, **9**), 137.18 (C, dd, *J* = 48.6, 5.8 Hz, **6**), 136.02 (CH, dd, *J* = 52.5, 2.0 Hz, **8**), 135.91 (C, d, *J* = 7.8 Hz, **5**), 133.03 (CH, d, *J* = 9.7 Hz, **7**), 129.07 (CH, d, *J* = 67.1 Hz, **10**), 39.90 (CH_2, **2**), 34.62 (C, **1**), 33.58 (CH_2, **4**), 29.28 (CH_3, **16**+**17**), 22.08 (CH_3, **18**), 19.41 (CH_2, **3**), 13.29 (CH_3, d, *J* = 40.8 Hz, **19**) ppm.

LRMS (CI, NH_3) *m/z* 221 [$M+H^+$].

[9,10-$^{13}C_2$]-1,3,3-Trimethyl-2-((1*E*,3*E*)-3-methylhexa-1,3-dien-5-ynyl)cyclohex-1-ene (2.42)



$C_{14}{}^{13}C_2H_{22}$

m.w. = 216.33 g/mol

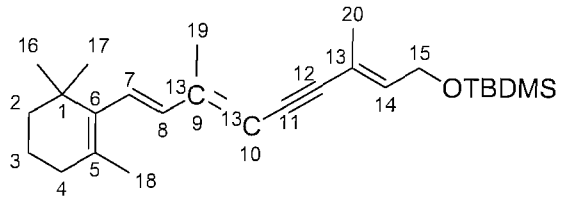
Amber oil

To a solution of LDA (1.8 M in THF/heptane/ethylbenzene, 0.31 mL, 0.60 mmol) in THF (4 mL) at –78 °C was added $TMSCHN_2$ (2.0 M in Et_2O, 0.31 mL, 0.60 mmol) dropwise and stirred for 45 minutes. To this solution was added a solution of aldehyde **2.40** (110 mg, 0.50 mmol, 9-*E*/*Z* = 3:1) in THF (1 mL), this was stirred for 30 minutes then allowed to warm to rt and stir for a further 2.5 hours. The reaction was quenched with H_2O (2 mL) and diluted with Et_2O (15 mL), then separated. The aqueous phase was extracted with Et_2O (3 x 10 mL) before the combined organic phases were washed with brine (5 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving a brown oil. Purification by flash column chromatography on silica gel (2.5 cm x 14 cm) eluting with hexane furnished the desired alkyne **2.42** as an amber oil (61 mg, 0.28 mmol, 57%, 9-*E*/*Z* = 12:1).

^{1}H NMR (300 MHz, C_6D_6) δ 6.24 (1H, br d, J = 16.1 Hz, **7**), 6.05 (1H, dd, J = 16.1, 2.9 Hz, **8**), 5.42 (1H, d, J = 163.1 Hz, **10**), 3.04 (1H, br s, **12**), 2.04 (3H, q, J = 3.2 Hz, **19**), 1.88 (2H, m, **4**), 1.62 (3H, s, **18**), 1.59-1.47 (2H, m, **3**), 1.45-1.35 (2H, m, **2**), 1.01 (6H, s, **16**+**17**) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) Data unassignable.

[9,10-$^{13}C_2$]-((2*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,6,8-trien-4-ynyloxy)(*tert*-butyl)dimethylsilane (2.43)



$C_{24}{}^{13}C_2H_{42}OSi$
m.w. = 400.68 g/mol
Amber oil

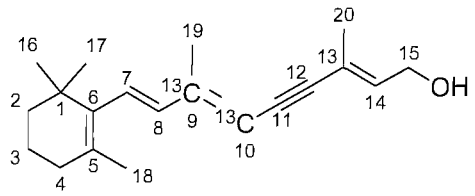
TBDMS protected dehydro retinol **2.43** was prepared by a method described by Borhan *et al.*[6] To a solution of iodide **1.32** (143 mg, 0.46 mmol) in ^{i}PrNH$_2$ (1.6 mL) was added $Pd(PPh_3)_4$ (4 mg, 4 μmol) in one portion and stirred for 5 minutes. CuI (1 mg, 4 μmol) was then added to the mixture and stirred for 5 minutes. Alkyne () (76 mg, 0.35 mmol) in ^{i}PrNH$_2$ (1.6 mL) was added dropwise before stirring for 4.5 hours. The reaction was concentrated *in vacuo* then dissolved in Et_2O (20 mL) and a saturated solution of NH_4Cl (5 mL). The phases were separated and the organic phase was washed with brine (5 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving an amber oil. Purification by flash column chromatography on silica gel (2.5 cm x 15 cm) eluting with hexane gave the desired product **2.43** as an unseparated mixture of product **2.43** (107 mg, 0.27 mmol, 76%) and iodide **1.32** (38 mg, 0.12 mmol, 26%) determined by ^{1}H NMR.

FT-IR (neat) ν_{max} 2954 (s), 2928 (m), 2856 (m), 1639 (w), 1472 (w), 1462 (w), 1255 (m), 1089 (s), 835 (s) cm^{-1}.

^{1}H NMR (300 MHz, C_6D_6) δ 6.40-6.08 (3H, m, **7**+**8**+**14**), 5.66 (1H, d, J = 162.6 Hz, **10**), 3.81 (2H, br t, J = 5.3 Hz, **15**), 2.06 (3H, dd, J = 4.8, 0.8 Hz, **19**), 1.95-1.86 (2H, m, **4**), 1.76 (3H, s, **20**), 1.66 (3H, s, **18**), 1.60-1.49 (2H, m, **3**), 1.48-1.39 (2H, m, **2**), 1.04 (6H, s, **16**+**17**), 0.90 (9H, s, -SiC(CH_3)$_3$), -0.03 (6H, s, -Si(CH_3)$_2$) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) Data unassignable.

[9,10-$^{13}C_2$]-3,7-Dimethyl-9-(2,6,6-trimethyl-cyclohex-1-enyl)-nona-2,6,8-trien-4-yn-1-ol (2.44)



$C_{18}{}^{13}C_2H_{28}O$
m.w. = 286.42 g/mol
Amber oil

Alcohol **2.44** was prepared by a method described by Borhan *et al.*[6] To a solution of alkyne **2.43** as an unseparated mixture of alkyne **2.43** (107 mg, 0.27 mmol, 76%) and iodide **1.32** (38 mg, 0.12 mmol, 26%) (determined by ^{1}H NMR) in THF (1 mL) was added TBAF (1.0 M in THF, 0.43 mL, 0.43 mmol) dropwise. The reaction was stirred for 2 hours before being quenched with a saturated solution of NH_4Cl (3 mL) and diluted with Et_2O (15 mL) and separated. The aqueous phase was extracted with Et_2O (3 x 15 mL) before the combined organic phases were washed with brine (5 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving a yellow oil. Purification by flash column chromatography on silica gel (3.5 cm x 15 cm) eluting with 15% EtOAc/hexane gave the desired product **2.44** (35 mg, 0.12 mmol, 44%).

FT-IR (neat) ν_{max} 3420 (br), 2933 (s), 2868 (m), 1728 (m), 1368 (m) cm^{-1}.

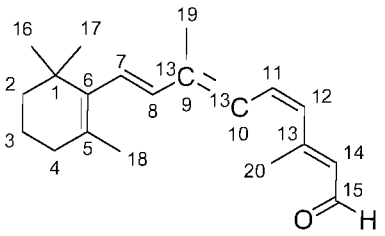
^{1}H NMR (300 MHz, C_6D_6) δ 6.36-6.22 (1H, m, **7**), 6.22-6.09 (1H, m, **8**), 6.08-5.97 (1H, m, **14**), 5.68 (1H, d, J = 162.0 Hz, **10**), 3.84 (2H, q, J = 5.8 Hz, **15**), 2.12 (3H, q, J = 5.8 Hz, **19**), 1.95-

1.86 (2H, m, **4**), 1.68 (6H, s, **18**+**20**), 1.61-1.49 (2H, **3**), 1.48-1.39 (2H, m, **2**), 1.04 (6H, s, **16**+**17**) ppm.

^{13}C NMR ($CDCl_3$, 75 MHz) Data unassignable.

LRMS (CI, NH_3) *m/z* 287 [$M+H^+$].

[9,10-$^{13}C_2$]-11*Z*-Retinal (2.45)



$C_{18}{}^{13}C_2H_{28}O$

m.w. = 286.42 g/mol

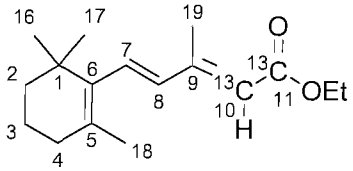
Yellow oil

Argon was bubbled through a suspension of zinc dust (1.000 g, 15.29 mmol) in H_2O (6 mL) and stirred for 15 minutes. After this time $Cu(OAc)_2$ (100 mg, 0.55 mmol) was added and the mixture was stirred for a further 15 minutes. $AgNO_3$ (100 mg, 0.59 mmol) was added and the reaction mixture was stirred for a further 30 minutes. The resulting black suspension was filtered and washed with H_2O, MeOH and Et_2O, allowing the black solid to stay moist with Et_2O. The activated zinc catalyst was immediately transferred to a mixture of H_2O (2 mL) and MeOH (2 mL), before a solution of allylic alcohol **2.44** (35 mg, 0.12 mmol) in MeOH (2 mL) was added dropwise. The reaction mixture was warmed to 40 °C and stirred for 24 hours before the mixture was filtered through a pad of celite, washing with H_2O and Et_2O. The organic phase was separated and washed with brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo* giving an orange oil. The orange oil (24 mg, 0.08 mmol) was stirred in CH_2Cl_2 (3 mL), before the addition of crushed molecular sieves (100 mg), NMO (26 mg, 0.226 mmol) and TPAP (15 mg, 44 μmol). This mixture was stirred for 30 minutes before filtration through a pad of celite and neutral alumina, flushing with Et_2O. The solution was concentrated *in vacuo* to give the title compound () as a yellow oil (15 mg). Purification by HPLC eluting with Et_2O (2.00 mL/minute) and hexane (7.99 mL/minute) gave [9,10-$^{13}C_2$]-11*Z*-retinal **(2.45)** (4 mg, 14 μmol, 17 % over 2

steps), [9,10-$^{13}C_2$]-all-*E*-retinal (**2.46**) as a yellow oil (3 mg, 11 μmol, 13 % over 2 steps).

No data available, isomers were identified by HPLC analysis.[22,172]

[10,11-$^{13}C_2$]-(2*E*,4*E*)-Ethyl 3-methyl-5-(2,6,6-trimethylcyclohex-1-enyl)penta-2,4-dienoate (2.48)



$C_{15}{}^{13}C_2H_{26}O_2$
m.w. = 264.37 g/mol
Pale yellow oil

To a slurry of NaH (207 mg, 5.19 mmol) in Et_2O (5 mL) was added a solution of [1,2-$^{13}C_2$]-triethylphosphonoacetate (**1.95**) (1.17 g, 5.19 mmol) in Et_2O (1 mL) slowly dropwise. This mixture was stirred for 2 hours giving a yellow solution. To the reaction was added a solution of *β*-ionone (**1.36**) (665 mg, 3.46 mmol) in Et_2O (2 mL) dropwise and the yellow solution was stirred for 62 hours forming an amber mixture. The reaction was quenched with H_2O (15 mL) and the reaction mixture was extracted with hexane (4 x 25 mL). The combined organic phases were washed with brine (5 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving pale yellow oil. Purification by flash column chromatography on silica gel (5.0 cm x 11 cm) eluting with 3% EtOAc/hexane afforded the desired ester **2.48** as pale yellow oil (819 mg, 3.10 mmol, 90%, *E*/*Z* = 11:1).

FT-IR (neat) ν_{max} 2929 (m), 2865 (w), 1669 (s), 1614 (w), 1581 (m), 1131 (s) cm^{-1}.

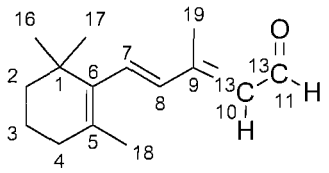
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.57 (1H, d, *J* = 16.1 Hz, **7**), 6.10 (1H, dd, *J* = 16.1, 5.3 Hz, **8**), 5.75 (1H, d, *J* = 159.4 Hz, **10**), 4.18 (2H, dq, *J* = 7.0, 3.0 Hz, -OC**H$_2$**CH$_3$), 2.34 (3H, d, *J* = 4.8 Hz, **19**), 2.03 (2H, t, *J* = 6.3 Hz, **4**), 1.70 (3H, s, **18**), 1.67-1.59 (2H, m, **3**) 1.50-1.46 (2H, m, **2**), 1.30 (3H, t, *J* = 7.2 Hz, -OCH$_2$C**H$_3$**), 1.03 (6H, s, **16**+**17**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 167.63 (C, d, J = 77.8 Hz, **11**), 153.10 (C, d, J = 69.0 Hz, **9**), 137.55 (C, **5**), 136.58 (CH, dd, J = 8.8, 2.9 Hz, **8**), 133.96 (CH, d, J = 8.8 Hz, **7**), 131.41 (C, **6**), 118.39 (CH, d, J = 77.8 Hz, **10**), 59.94 (CH_2, -O**C**H_2CH$_3$), 39.88 (CH_2, **2**), 34.57 (C, **1**), 33.41 (CH_2, **4**), 29.29 (CH_3, **16**+**17**), 21.98 (CH_3, **18**), 19.50 (CH_2, **3**), 14.72 (CH_3, d, J = 1.9 Hz, -OCH$_2$**C**H$_3$), 14.00 (CH_3, **19**) ppm.

LRMS (ES^+) *m/z* 265 [$M+H^+$].

HRMS (ES^+) for $C_{15}{}^{13}C_2H_{27}O_2$, calculated 265.2072, found 265.2073 Da.

[10,11-$^{13}C_2$]-(2*E*,4*E*)-3-Methyl-5-(2,6,6-trimethylcyclohex-1-enyl)penta-2,4-dienal (2.49)



$C_{13}{}^{13}C_2H_{22}O$
m.w. = 220.32 g/mol
Pale yellow oil

To a slurry of $LiAlH_4$ (186 mg, 4.90 mmol) in Et_2O (10 mL) at −78 °C was added a solution of [10,11-$^{13}C_2$]-ethyl-β-ionylidene acetate **(2.48)** (810 mg, 3.07 mmol, 9-*E*/*Z* = 11:1) in Et_2O (30 mL) dropwise. After complete addition the reaction was stirred for 1 hour at −78 °C before the reaction was warmed to rt and stirred for a further 2.5 hours. The reaction was quenched with H_2O (0.2 mL), 15% NaOH (0.2 mL) and H_2O (0.6 mL) sequentially. Then stirred for 20 minutes producing a white precipitate. The suspension was dried ($MgSO_4$) and the precipitate was removed by filtration and the filtrate was concentrated *in vacuo* giving a colourless oil. The oil was dissolved in CH_2Cl_2 (32 mL). To this solution was added crushed molecular sieves (4.2 g), NMO (718 mg, 6.13 mmol) and TPAP (108 mg, 0.31 mmol) producing a black suspension. After stirring at rt for 30 minutes the black suspension was filtered and concentrated *in vacuo* giving a black oil. Purification by flash column chromatography (4.0 cm x 13

cm) eluting with 4% EtOAc/hexane afforded the desired aldehyde **2.49** (514 mg, 2.33 mmol, 76%) and 9*Z*-aldehyde **2.49** (82 mg, 0.37 mmol, 12%).

FT-IR (neat) ν_{max} 2928 (m), 2865 (m), 1628 (s), 1610 (s), 1561 (w), 1457 (w) cm^{-1}.

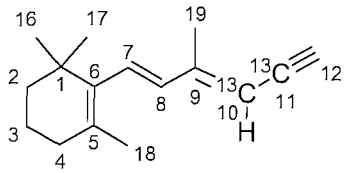
^{1}H NMR (400 MHz, $CDCl_3$) δ 10.13 (1H, ddd, J = 169.7, 24.5, 8.0 Hz, **11**), 6.74 (1H, d, J = 16.3 Hz, **7**), 6.22 (1H, dd, J = 16.1, 5.0 Hz, **8**), 5.94 (1H, dd, J = 157.4, 8.0 Hz, **10**), 2.32 (3H, d, J = 4.0 Hz, **19**), 2.05 (2H, t, J = 6.3 Hz, **4**), 1.73 (3H, s, **18**), 1.67-1.59 (2H, m, **3**), 1.51-1.46 (2H, m, **2**), 1.05 (6H, s, **16**+**17**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 191.67 (CH, d, J = 57.3 Hz, **11**), 157.95 (C, dd, J = 66.1, 2.9 Hz, **9**), 137.41 (C, **6**), 136.02 (CH, dd, J = 6.8, 1.9 Hz, **8**), 135.91 (C, d, J = 7.8 Hz, **5**), 133.38 (1H, d, J = 2.9 Hz, **7**), 129.06 (CH, d, J = 57.3 Hz, **10**), 39.90 (CH_2, **2**), 34.61 (C, **1**), 33.57 (CH_2, **4**), 29.27 (CH_3, **16**+**17**), 22.07 (CH_3, **18**), 19.40 (CH_2, **3**), 13.28 (CH_3, d, J = 4.9 Hz, **19**) ppm.

LRMS (ES^+) *m/z* 221 [$M+H^+$].

HRMS (ES^+) for $C_{13}{}^{13}C_2H_{23}O$, calculated 221.1811, found 221.1812 Da.

[10,11-$^{13}C_2$]-1,3,3-Trimethyl-2-((1*E*,3*E*)-3-methylhexa-1,3-dien-5-ynyl)cyclohex-1-ene (2.50)



$C_{14}{}^{13}C_2H_{22}$

m.w. = 216.33 g/mol

Pale yellow oil

To a solution of LDA (1.8 M in THF/heptane/ethylbenzene, 1.53 mL, 2.76 mmol) in THF (23 mL) at –78 °C was added $TMSCHN_2$ (2.0 M in Et_2O, 1.38 mL, 2.76 mmol) dropwise. This mixture was stirred at –78 °C for 2 hours before a solution of aldehyde **2.49** (507 mg, 2.31 mmol) in THF (5 mL) was added dropwise. The brown solution was stirred for 30 minutes before warming to rt and stirring for 3 hours. The reaction was quenched with H_2O (20 mL) and the phases separated. The aqueous phase was extracted with Et_2O (3 x 25 mL) and the combined organic phases were washed with brine (10 mL), dried ($MgSO_4$) then concentrated *in vacuo* giving a brown oil. Purification by flash column chromatography on silica gel (3.5 cm x 6 cm) eluting with hexane→10% Et_2O/hexane afforded the desired alkyne **2.50** as a pale yellow oil (212 mg, 0.98 mmol, 42%) and aldehyde **2.49** (36 mg, 0.16 mmol, 7%).

FT-IR (neat) ν_{max} 3310 (w), 2958 (m), 2928 (m), 2864 (m), 2826 (w), 1442 (w), 965 (m) cm^{-1}.

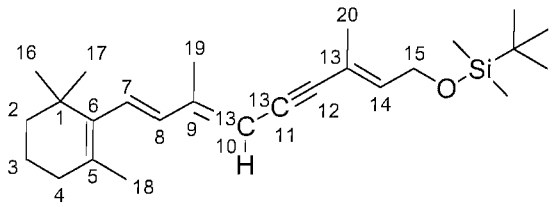
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.29 (1H, d, *J* = 16.1 Hz, **7**), 6.10 (1H, dd, *J* = 16.1, 5.5 Hz, **8**), 5.41 (1H, d, *J* = 163.4 Hz, **10**), 3.29 (1H, ddd, *J* = 48.9, 4.5, 2.4 Hz, **12**), 2.09 (3H, d, *J* = 5.8 Hz, **19**), 2.02 (2H, t, *J* = 6.3 Hz, **4**), 1.70 (3H, s, **18**), 1.66-1.58 (2H, m, **3**), 1.50-1.45 (2H, m, **2**), 1.02 (6H, s, **16**+**17**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 149.61 (C, d, *J* = 74.8 Hz, **9**), 137.69 (C, **6**), 135.43 (CH, dd, *J* = 9.2, 4.4 Hz, **8**), 130.58 (CH, d, *J* = 8.7 Hz, **7**), 130.54 (C, **5**), 107.60 (CH, *J* = 89.4 Hz, **10**), 82.78 (C, d, *J* = 89.4 Hz, **11**), 39.91 (CH_2, **2**), 34.56 (C, **1**), 33.37 (CH_2, **4**), 29.24 (CH_3, **16**+**17**), 21.98 (CH_3, **18**),

19.56 (CH_2, **3**), 15.39 (CH_3, d, J = 15.4 Hz, **19**) ppm. C12 was obscured.

LRMS (CI, NH_3) *m/z* 217 [$M+H^+$].

[10,11-$^{13}C_2$]-((2*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,6,8-trien-4-ynyloxy)(*tert*-butyl)dimethylsilane (2.51)



$C_{24}{}^{13}C_2H_{42}OSi$

m.w. = 400.68 g/mol

Yellow oil

To a solution of ((*E*)-3-iodobut-2-enyloxy)(*tert*-butyl)dimethylsilane (**1.32**) (365 mg, 1.17 mmol) in $^{i}PrNH_2$ (3 mL) was added $Pd(PPh_3)_4$ (11 mg, 0.01 mmol), this was then stirred for 5 minutes. After which CuI (2 mg, 0.01 mmol) was added in one portion and the mixture was stirred for a further 5 minutes. A solution of alkyne **2.50** (211 mg, 0.98 mmol) in $^{i}PrNH_2$ (1 mL) was added and the resultant solution was stirred 3.5 hours. The reaction was quenched by concentration *in vacuo* giving a viscous amber oil. Purification by flash column chromatography (3.5 cm x 9 cm) eluting with 0.1%→1% Et_2O/hexane affording the TBS ether **2.51** as a yellow oil (349 mg, 0.87 mmol, 89%, 13-*E*/*Z* = 1.5:2).

All-*E*-isomer

FT-IR (neat) ν_{max} 2955 (m), 2928 (m), 2857 (m), 1255 (m), 836 (s) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.26 (1H, d, J = 16.1 Hz, **7**), 6.11 (1H, d, J = 16.1, 5.2 Hz, **8**), 5.93 (1H, t, J = 6.4 Hz, **14**), 5.53 (1H, d, J = 162.6 Hz, **10**), 4.29 (2H, d, J = 6.4 Hz, **15**), 2.06 (3H, d, J = 5.7 Hz, **19**), 2.02 (2H, t, J = 6.0 Hz, **4**), 1.85 (3H, d, J = 1.3 Hz, **20**), 1.70 (3H, s, **18**), 1.02 (6H, s,

16+**17**), 0.92 (9H, s, -SiC(C**H**$_3$)$_3$), 0.09 (6H, s, -Si(C**H**$_3$)$_2$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 147.45 (C, d, *J* = 16.5 Hz, **9**), 137.85 (C, **6**), 135.92 (CH, dd, *J* = 8.8, 2.9 Hz, **14**), 130.32 (C, **5**), 129.63 (CH, d, *J* = 9.7 Hz, **7**), 119.66 (C, **13**), 108.99 (CH, d, *J* = 91.4 Hz, **10**), 86.48 (C, d, *J* = 91.4 Hz, **11**), 60.47 CH_2, **15**), 39.95 (CH_2, **2**), 34.59 (C, **1**), 33.42 (CH_2, **4**), 29.27 (CH_3, **16**+**17**), 26.29 (CH_3, -SiC(**C**H_3)$_3$), 22.02 (CH_3, **18**), 19.58 (CH_2, **3**), 18.72 (C, -Si**C**(CH$_3$)$_3$), 18.08 (CH_3, **20**), 15.40 (CH_3, **9**), –4.80 (CH_3, -Si(**C**H$_3$)$_2$) ppm. C8 and C12 were obscured.

LRMS (ES^+) *m/z* 424 [M+Na^+].

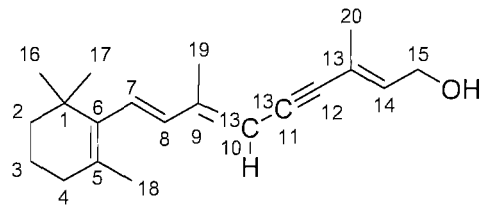
13*Z*-Isomer

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.28 (1H, d, *J* = 16.1 Hz, **7**), 6.13 (1H, dd, *J* = 16.1, 5.6 Hz, **8**), 5.77 (1H, t, *J* = 6.5 Hz, **14**), 5.58 (1H, d, *J* = 162.4 Hz, **10**), 4.42 (2H, d, *J* = 6.0 Hz, **15**), 2.08 (3H, dt, *J* = 5.5, 1.3 Hz, **19**), 2.02 (2H, t, *J* = 6.3 Hz, **4**), 1.92 (3H, d, *J* = 1.3 Hz, **20**), 1.71 (3H, d, *J* = 0.8 Hz, **18**), 1.66-1.58 (2H, m, **3**), 1.50-1.45 (2H, m, **2**), 1.03 (6H, s, **16**+**17**), 0.92 (9H, s, -SiC(C**H**$_3$)$_3$), 0.10 (6H, s, -Si(C**H**$_3$)$_2$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 147.83 (C, t, *J* = 4.9 Hz, **9**), 137.82 (C, 6), 135.79 (CH, dd, *J* = 9.7, 3.9 Hz, **14**), 130.46 (C, **5**), 129.95 (CH, d, *J* = 8.8 Hz, **7**), 119.59 (C, **13**), 108.94 (CH, d, *J* = 91.4 Hz, **10**), 93.61 (C, d, *J* = 91.4 Hz, **11**), 62.88 (CH_2, **15**), 39.95 (CH_2, **2**), 34.59 (C, **1**), 33.42 (CH_2, **4**), 29.27 (CH_3, **16**+**17**), 26.36 (CH_3, -SiC(**C**H$_3$)$_3$), 22.02 (CH_3, **18**), 19.58 (CH_2, **3**), 18.75 (C, -Si**C**(CH$_3$)$_3$), 18.08

(CH_3, **20**), 15.49 (CH_3, **19**), –4.74 (CH_3, -Si(CH_3)$_2$) ppm. C8 and C12 were obscured.

[10,11-$^{13}C_2$]-3,7-Dimethyl-9-(2,6,6-trimethyl-cyclohex-1-enyl)-nona-2,6,8-trien-4-yn-1-ol (2.52)



$C_{18}{}^{13}C_2H_{28}O$

m.w. = 286.42 g/mol

Pale yellow oil

To a solution of TBS protected dehydroretinol **2.51** (345 mg, 0.86 mmol, 13-*E*/*Z* = 1.5:2) in THF (4.5 mL) at 0 °C was added TBAF (1.0 M in THF, 0.95 mL, 0.95 mmol) slowly dropwise. The resultant mixture was warmed to rt and stirred for 1 hour. After which, the reaction was quenched with H_2O (5 mL) and the amber solution was extracted with Et_2O (4 x 20 mL). The combined organic phases were washed with brine (5 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving a yellow oil. Purification by flash column chromatography on silica gel (3.5 cm x 12 cm) eluting with 10%→20%EtOAc/hexane afforded the desired dehydroretinol **2.52** as a pale yellow oil (87 mg, 0.30 mmol, 35%) and the undesired 13*Z* dehydroretinol (151 mg, 0.53 mmol, 61 %).

All-*E*-isomer

FT-IR (neat) ν_{max} 3319 (br), 2956 (w), 2927 (m), 2864 (w), 1441 (w) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.27 (1H, d, *J* = 16.1 Hz, **7**), 6.12 (1H, dd, *J* = 16.1, 5.3 Hz, **8**), 6.01 (1H, t, *J* = 6.9 Hz, **14**), 5.53 (1H, d, *J* = 162.6 Hz, **10**), 4.27 (2H, d, *J* = 6.8 Hz, **15**), 2.07 (3H, d, *J* = 4.5 Hz, **19**), 2.02 (2H, t, *J* = 6.2 Hz, **4**), 1.90 (3H, d, *J* = 1.5 Hz, **20**), 1.70 (3H, s, **18**), 1.66-1.58 (2H, m,

3), 1.56 (1H, br s, -O**H**), 1.50-1.43 (2H, m, **2**), 1.03 (6H, s, **16**+**17**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 147.80 (C, d, *J* = 72.9 Hz, **9**), 137.81 (C, **6**) 135.81 (CH, dd, *J* = 9.2, 4.4 Hz, **8**), 134.68 (CH, dd, *J* = 4.9, 2.0 Hz, **14**), 130.44 (C, **5**), 129.91 (CH, d, *J* = 9.7 Hz, **7**), 122.01 (C, dd, *J* = 17.5, 2.9 Hz, **13**), 108.77 (CH, d, *J* = 91.4 Hz, **10**), 87.31 (C, d, *J* = 91.4 Hz, **11**), 59.63 (CH_2, **15**), 39.94 (CH_2, **2**), 34.59 (C, **1**), 33.41 (CH_2, **4**), 29.26 (CH_3, **16**+**17**), 22.01 (CH_3, **18**), 19.57 (CH_2, **3**), 18.03 (CH_3, **20**), 15.41 (CH_3, d, *J* = 2.9 Hz, **19**) ppm. C12 was obscured.

LRMS (ES^+) *m/z* 596 [$2M+Na^+$].

13*Z*-Isomer

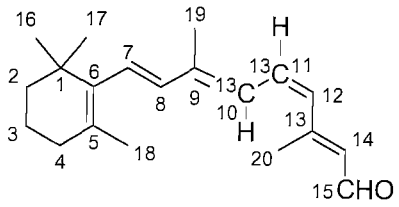
FT-IR (neat) ν_{max} 3324 (br), 2926 (m), 2864 (w), 2826 (w), 1456 (w) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.29 (1H, d, *J* = 16.1 Hz, **7**), 6.13 (1H, dd, *J* = 16.1, 5.5 Hz, **8**), 5.87 (1H, t, *J* = 6.8 Hz, **14**), 5.57 (1H, d, *J* = 162.6 Hz, **10**), 4.37 (2H, t, *J* = 6.4 Hz, **15**), 2.08 (3H, d, *J* = 5.5 Hz, **19**), 2.03 (2H, t, *J* = 6.2 Hz, **4**), 1.95 (3H, d, *J* = 1.5 Hz, **20**), 1.66-1.58 (2H, m, **3**), 1.50-1.41 (2H, m, **2**), 1.03 (6H, s, **16**+**17**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 147.98 (C, d, *J* = 74.0 Hz, **9**), 137.79 (C, **6**), 135.70 (CH, dd, *J* = 9.2, 4.4 Hz, **8**), 134.82 (C, **14**), 130.31 (C, **5**), 130.26 (CH, d, *J* = 8.8 Hz, **7**), 122.05 (C, **13**), 108.62 (CH, d, *J* = 91.4 Hz, **10**), 93.95 (C, d, *J* = 91.4 Hz, **11**), 93.74 (C, dd, *J* = 82.6, 7.3 Hz, **12**), 61.94 (CH_2, **15**), 39.93 (CH_2, **2**), 34.59 (C, **1**), 33.42 (CH_2,

4), 29.27 (CH_3, **16**+**17**), 23.64 (CH_3, **18**), 22.02 (CH_2, **3**), 19.57 (CH_3, **20**), 15.52 (CH_3, d, J = 2.9 Hz, **19**) ppm.

[10,11-$^{13}C_2$]-11*Z*-Retinal (2.53)



$C_{18}{}^{13}C_2H_{28}O$

m.w. = 286.42 g/mol

Yellow oil

[10,11-$^{13}C_2$]-retinal (**2.53**) was prepared by a method described by Borhan *et al.*[6] Argon was bubbled through a suspension of zinc dust (6.83 g, 0.104 mol) in H_2O (41 mL) for 15 minutes. To the suspension was added $Cu(OAc)_2$ (683 mg, 3.76 mmol) in one portion giving a black suspension. The mixture was stirred for 15 minutes before the addition of $AgNO_3$ (683 mg, 4.02 mmol) in one portion, leading to an exothermic reaction. After stirring for 30 minutes the activated zinc was filtered and washed with H_2O, MeOH, acetone and Et_2O sequentially. The moist zinc catalyst was suspended in H_2O (11 mL) and ^{i}PrOH (11 mL). To the suspension was added a solution of dehydro retinol **2.52** (81 mg, 0.28 mmol) in ^{i}PrOH (11 mL) and the reaction was warmed to 40 °C and stirred for 26 hours. After this time the reaction was filtered through a pad of celite flushing with H_2O and Et_2O. The phases were separated and the aqueous phase was extracted with Et_2O (4 x 20 mL) before the combined organic phases were washed with brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo* giving crude isomericially pure [10,11-$^{13}C_2$]-11*Z*-retinol as an oil (88 mg).

The crude retinol (82 mg, ~0.28 mmol) was dissolved in CH_2Cl_2 (3 mL) before the addition of crushed molecular sieves (167 mg), NMO (66 mg, 0.57 mmol) and TPAP (30 mg, 0.09 mmol) sequentially. The black solution was stirred for 30 minutes before being passed through a short column of neutral alumina topped with a pad of celite, flushing with Et_2O. The yellow solution was concentrated *in vacuo* giving isomericially pure crude [10,11-$^{13}C_2$]-11*Z*-retinal (**2.53**) (79 mg) as a yellow oil. Purification by HPLC eluting with Et_2O (2.00 mL/minute) and hexane (7.99 mL/minute) gave [10,11-$^{13}C_2$]-11*Z*-retinal (**2.53**) (40 mg, 0.14 mmol, 50% over 2 steps), [11,12-$^{13}C_2$]-all-*E*-

retinal (8 mg, 0.03 mmol, 10% over 2 steps) and [10,11-$^{13}C_2$]-retinal as a mixture of isomers (18 mg, 0.06 mmol, 22% over 2 steps).

FT-IR (neat) ν_{max} 2926 (m), 2863 (w), 1660 (s), 1552 (m) cm^{-1}.

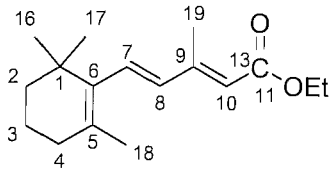
^{1}H NMR (400 MHz, C_6D_6) δ 9.91 (1H, d, *J* = 7.8 Hz, **15**), 6.57 (1H, br dd, *J* = 153.1, 12.8 Hz, **10**), 6.37 (1H, dt, *J* = 151.6, 11.9 Hz, **11**), 6.34 (1H, d, *J* = 16.1 Hz, **7**), 6.22 (1H, dd, *J* = 16.1, 5.3 Hz, **8**), 6.10 (1H, br d, *J* = 8.0 Hz, **14**), 5.58 (1H, t, *J* = 11.2 Hz, **12**), 1.91 (2H, t, *J* = 6.2 Hz, **4**), 1.76 (3H, s, **20**), 1.74 (3H, d, *J* = 5.0 Hz, **19**), 1.68 (3H, s, **18**), 1.60-1.51 (2H, m, **3**), 1.47-1.40 (2H, m, **2**), 1.07 (6H, s, **16**+**17**) ppm.

^{13}C NMR (100 MHz, C_6D_6) δ 190.51 (CH, d, *J* = 56.4 Hz, **15**), 154.64 (C, **13**), 141.40 (C, d, *J* = 70.9 Hz, **9**), 138.82 (CH, dd, *J* = 6.8, 2.9 Hz, **8**), 138.56 (C, **6**), 131.66 (CH, dd, *J* = 48.6, 9.7 Hz, **12**), 131.31 (CH, d, *J* = 57.8 Hz, **11**), 130.66 (C, **5**), 130.07 (CH, d, *J* = 8.8 Hz, **7**), 127.06 (CH, d, *J* = 57.8 Hz, **10**), 40.39 (CH_2, **2**), 35.09 (C, **1**), 33.81 (CH_2, **4**), 29.68 (CH_3, **16**+**17**), 22.43 (CH_3, **18**), 20.22 (CH_2, **3**), 17.98 (CH_3, **20**), 12.84 (CH_3, d, *J* = 4.9 Hz, **19**) ppm. C14 was obscured.

LRMS (ES^+) *m/z* 287 [$M+H^+$], 309 [$M+Na^+$].

HRMS (ES^+) for $C_{18}{}^{13}C_2H_{29}O$, calculated 287.2280, found 287.2280 Da.

[11-^{13}C]-(2*E*,4*E*)-Ethyl 3-methyl-5-(2,6,6-trimethylcyclohex-1-enyl)penta-2,4-dienoate (2.54)



$C_{16}{}^{13}CH_{26}O_2$

m.w. = 263.38 g/mol

Pale yellow oil

To a slurry of NaH (178 mg, 4.44 mmol) in Et_2O (5 mL) was added [1-^{13}C]-triethylphosphonoacetate (1.00 g, 4.44 mmol) slowly dropwise. This mixture was stirred for 2 hours giving a colourless solution. To the reaction was added a solution of *β*-ionone (**1.36**) (569 mg, 2.96 mmol) in Et_2O (2 mL) dropwise, the yellow solution was stirred for 62 hours forming a white suspension. The reaction was quenched with H_2O (10 mL) and the reaction was extracted with hexane (4 x 25 mL). The combined organic phases were washed with brine (5 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving a pale yellow oil. Purification by flash column chromatography on silica gel (5.0 cm x 11 cm) eluting with 2%→3% EtOAc/hexane afforded the desired ester **2.54** as pale yellow oil (713 mg, 2.71 mmol, 91%, *E*:*Z* = 13:1).

FT-IR (neat) ν_{max} 2929 (w), 2865 (w), 1669 (s), 1606 (m), 1445 (w), 1131 (s) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.60 (1H, d, *J* = 16.1 Hz, **7**), 6.10 (1H, d, *J* = 16.1 Hz, **8**), 5.75 (1H, s, **10**), 4.18 (2H, dqd, *J* = 7.0, 7.0, 3.0 Hz, -OC**H$_2$**CH$_3$), 2.34 (3H, s, **19**), 2.03 (2H, t, *J* = 6.0 Hz, **4**), 1.70 (3H, s, **18**), 1.67-1.59 (2H, m, **3**), 1.51-1.45 (2H, m, **2**), 1.30 (3H, t, *J* = 7.2 Hz, -OCH$_2$C**H$_3$**), 1.03 (6H, s, **16**+**17**) ppm.

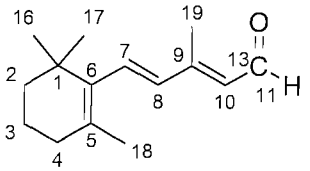
^{13}C NMR (75 MHz, $CDCl_3$) δ 167.63 (C, **11**), 153.13 (C, **9**), 137.55 (C, **5**), 136.58 (CH, d, *J* = 8.7 Hz, **8**), 133.96 (CH, **7**), 131.41 (C, **6**), 118.39 (CH, d, *J* = 77.8 Hz, **10**), 59.94 (CH$_2$, d, *J* = 1.9 Hz, -O**C**H$_2$CH$_3$), 39.88 (CH$_2$, **2**), 34.57 (C, **1**), 33.41 (CH$_2$, **4**), 29.23 (CH$_3$, **16**+**17**), 21.98 (CH$_3$, **18**), 19.50

(CH_2, **3**), 14.73 (CH_2, d, J = 1.9 Hz, -OCH_2CH_3), 13.99 (CH_3, **9**) ppm.

LRMS (ES^+) *m/z* 264 [$M+H^+$].

HRMS (ES^+) for $C_{16}{}^{13}CH_{27}O_2$, calculated 264.2040, found 264.2039 Da.

[11-^{13}C]-(2*E*,4*E*)-3-Methyl-5-(2,6,6-trimethylcyclohex-1-enyl)penta-2,4-dienal (2.55)



$C_{14}{}^{13}CH_{22}O$
m.w. = 219.33 g/mol
Pale yellow oil

To a slurry of $LiAlH_4$ (162 mg, 4.28 mmol) in Et_2O (8 mL) at –78 °C was added a solution of ester **2.54** (705 mg, 2.68 mmol, 9-*E*/*Z* = 13:1) in Et_2O (27 mL) dropwise. After complete addition the reaction was stirred for 1 hour at –78 °C. The reaction was then warmed to rt and stirred for a further 2.5 hours. The reaction was quenched with H_2O (0.2 mL), 15% NaOH (0.2 mL) and H_2O (0.6 mL) sequentially and stirred for 20 minutes producing a white precipitate. The heterogeneous mixture was dried ($MgSO_4$) and the precipitate was removed by filtration and the solution was concentrated *in vacuo* giving a colourless oil. The oil was taken up in CH_2Cl_2 (28 mL), to this solution was added crushed molecular sieves (1.6 g), NMO (628 mg, 5.36 mmol) and TPAP (94 mg, 0.27 mmol) producing a black suspension. After stirring at rt for 30 minutes the black suspension was filtered through celite and concentrated *in vacuo* giving a black oil. Purification by flash column chromatography (4.0 cm x 13 cm) eluting with 4% EtOAc/hexane afforded the desired aldehyde **2.55** as a pale yellow oil (431 mg, 1.97 mmol, 73%) and 9*Z*-aldehyde (44 mg, 0.20 mmol, 7%).

FT-IR (neat) ν_{max} 2928 (m), 2864 (w), 2825 (w), 1629 (s), 1446 (m), 1104 (m) cm^{-1}.

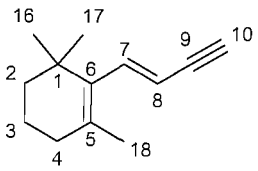
^{1}H NMR (400 MHz, $CDCl_3$) δ 10.14 (1H, dd, *J* = 169.8, 8.0 Hz, **11**), 6.75 (1H, d, *J* = 16.1 Hz, **7**), 6.22 (1H, d, *J* = 16.1 Hz, **8**), 5.94 (1H, d, *J* = 8.0 Hz, **10**), 2.32 (3H, s, **19**), 2.06 (2H, t, *J* = 6.3 Hz, **4**), 1.73 (3H, s, **18**), 1.68-1.59 (2H, m, **3**), 1.52-1.45 (2H, m, **2**), 1.05 (6H, s, **16**+**17**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 191.64 (CH, **11**), 155.29 (C, d, *J* = 2.9 Hz, **9**), 137.42 (C, **6**), 136.04 (CH, d, *J* = 6.8 Hz, **8**), 135.88 (C, **5**), 133.06 (CH, **7**), 129.09 (CH, d, *J* = 56.4 Hz, **10**), 39.90 (CH_2, **2**), 34.62 (C, **1**), 33.58 (CH_2, **4**), 29.28 (CH_3, **16**+**17**), 22.08 (CH_3, **18**), 19.41 (CH_2, **3**), 13.29 (CH_3, d, *J* = 4.9 Hz, **19**) ppm.

LRMS (ES^+) *m/z* 220 [$M+H^+$].

HRMS (ES^+) for $C_{14}{}^{13}CH_{23}O$, calculated 220.1777, found 220.1780 Da.

2-But-1-en-3-ynyl-1,3,3-trimethylcyclohexene (2.56)



$C_{13}H_{18}$
m.w. = 174.28 g/mol
Yellow oil

Terminal alkyne **2.56** was prepared using a method described by Negishi *et al.*[173] To a solution of LDA (1.8 M in THF/heptane/ethylbenzene, 3.18 mL, 5.72 mmol) in THF (10 mL) at –78 °C was added a solution of *β*-ionone (**1.36**) (1.00 g, 5.20 mmol) in THF (1 mL) dropwise and stirred for 1.5 hours at –78 °C. Chlorodiethyl phosphonate (0.83 mL, 5.72 mmol) was added dropwise and the reaction was stirred at rt for 3.5 hours. The reaction mixture was added slowly to a solution of LDA (1.8 M in THF/heptane/ethylbenzene, 6.50 mL, 11.70 mmol) in THF (10 mL) at –78 °C and stirred for 3.5 hours whilst warming to rt and stirred for a further 14.5 hours. The reaction was quenched with water (15 mL) and diluted with Et_2O (20 mL) then separated. The aqueous phase was extracted with Et_2O (3 x 20 mL) before the

combined organic phases were washed with 1 N HCl (15 mL), water (15 mL), $NaHCO_3$ (15 mL) and brine (15 mL) sequentially. The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* giving an amber oil. Purification by flash column chromatography on silica gel (3.0 cm x 7 cm) eluting with hexane afforded the title compound **2.56** as a yellow oil (588 mg, 3.37 mmol, 65%). Spectroscopic details were consistent with those observed in the literature.[173]

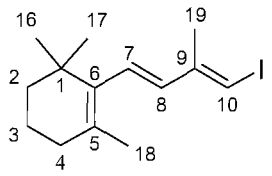
FT-IR (neat) ν_{max} 3311 (w), 2928 (m), 2865 (m), 2100 (w), 1456 (w), 1361 (w), 1204 (w), 957 (m) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.69 (1H, d, J = 16.3 Hz, **7**), 5.45 (1H, dd, J = 16.3, 2.0 Hz, **8**), 2.93 (1H, d, J = 2.0 Hz, **10**), 2.02 (2H, t, J = 6.0 Hz, **4**), 1.72 (3H, s, **18**), 1.65-1.57 (2H, m, **3**), 1.49-1.42 (2H, m, **2**), 1.03 (6H, s, **16**+**17**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 143.22 (CH, **7**), 137.15 (C, **6**), 132.20 (C, **5**), 111.18 (CH, **8**), 83.73 (C, **9**), 77.26 (CH, **10**), 39.90 (CH_2, **2**), 34.35 (C, **1**), 33.44 (CH_2, **4**), 29.08 (CH_3, **16**+**17**), 21.85 (CH_3, **18**), 19.42 (CH_2, **3**) ppm.

LRMS (CI, NH_3) *m/z* 175 [$M+H^+$].

2-(4-Iodo-3-methyl-buta-1,3-dienyl)-1,3,3-trimethyl-cyclohexene (2.57)



$C_{14}H_{21}I$

m.w. = 316.22 g/mol

Pale yellow oil

Iodide **2.57** was prepared by a method described by Vaz *et al.*[174] To a solution of Cp_2ZrCl_2 (923 mg, 3.16 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added $AlMe_3$ (2.0 M in hexane, 4.73 mL, 9.47 mmol) and a solution of terminal alkyne **2.56** in CH_2Cl_2 (10 mL) slowly. The reaction was allowed to warm to rt and stir for 17 hours before being cooled to –50 °C. To the solution was added a solution of iodine (2.40 g, 9.47 mmol) in

THF (15 mL) very slowly and stirred for 1 hour at −50 °C. The reaction was quenched with water (10 mL) and diluted with THF (10 mL), the phases were separated before the aqueous phase was extracted with EtOAc (4 x 20 mL). The combined organic phases were washed with water (15 mL) and brine (20 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving an orange oil. Purification by flash column chromatography on silica gel (3.0 cm x 10 cm) eluting with hexane afforded the title compound **2.57** as a pale yellow oil (821 mg, 2.60 mmol, 82%). Spectroscopic details were consistent with those observed in the literature.[175]

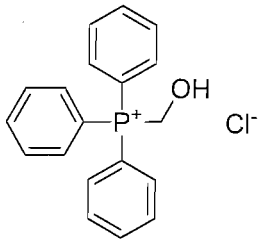
FT-IR (neat) ν_{max} 2956 (m), 2925 (m), 2863 (m), 2826 (m), 1572 (w), 1453 (m), 1377 (m), 1359 (m), 1295 (m), 1161 (m), 962 (s), 757 (m) cm^{-1}.

^{1}H NMR (400 MHz, C_6D_6) δ 6.33 (1H, d, J = 16.3 Hz, **7**), 6.25 (1H, s, **10**), 6.20 (1H, d, J = 16.3 Hz, **8**), 2.09 (2H, t, J = 6.0 Hz, **4**), 2.07 (3H, s, **19**), 1.83 (3H, s, **18**), 1.80-1.70 (2H, m, **3**), 1.66-1.59 (2H, m, **2**), 1.21 (6H, s, **16**+**17**) ppm.

^{13}C NMR (100 MHz, C_6D_6) δ 145.90 (C, **9**), 137.96 (C, **6**), 134.85 (CH, **8**), 129.80 (C, **5**), 128.48 (CH, **7**), 83.01 (CH, **10**), 40.09 (CH_2, **2**), 34.63 (C, **1**), 33.46 (CH_2, **4**), 29.33 (CH_3, **16**+**17**), 22.10 (CH_3, **18**), 20.29 (CH_3, **19**), 19.96 (CH_2, **3**) ppm.

LRMS (CI, NH_3) *m/z* 317 [$M+H^+$].

Hydroxymethyl triphenylphosphonium chloride (2.58)



$C_{19}H_{18}ClOP$
m.w. = 328.77 g/mol
White solid

Hydroxymethyl phosphonium chloride (**2.58**) was prepared using a method described by Frank *et al.*[90] Paraformaldehyde (253 mg, 8.38 mmol) was added in one portion to a solution of HCl (2 M in Et_2O, 3.81 mL, 7.62 mmol) in Et_2O (4 mL). This mixture was stirred for 10 minutes before PPh_3 (2.00 g, 7.62 mmol) in Et_2O (4 mL) was added dropwise and the resulting white suspension was stirred for 96 hours. After this time the white suspension was filtered and washed with Et_2O, dissolved in CH_2Cl_2 and concentrated *in vacuo*. This gave the title compound **2.58** as a white solid (2.01 g, 6.10 mmol, 80%). The spectroscopic data was in good accordance to the literature.[90]

Mpt 185-188 °C, (Lit: 190-192 °C)[176]

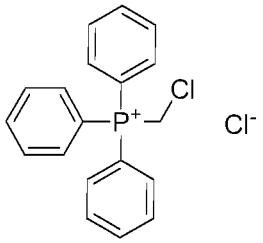
FT-IR (neat) ν_{max}/cm^{-1} 3494 (w), 3407 (w), 3047 (m), 3024 (m), 2814 (w), 1587 (m), 1484 (m), 1435 (s), 1116 (s), 1055 (s), 997 (m), 875 (m), 742 (s), 720 (s).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90-7.60 (15H, m, Ar**H**), 5.46 (2H, s, -$P^+C\mathbf{H_2}OH$), 3.50 (1H, brs, -O**H**) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 135.46 (CH, -PCCHCH**C**H-), 134.30 (CH, d, J = 26.1 Hz, -PCCH**C**HCH-), 130.65 (CH, d, J = 34.77 Hz, -PC**C**HCHCH-), 117.76 (C, d, J = 249.2 Hz, -P**C**CHCHCH-), 58.33 (CH_2, d, J = 191.3 Hz, -$P^+\mathbf{C}H_2OH$) ppm.

LRMS (ES^+) *m/z* 293 [M^+-Cl^-].

Chloromethyl triphenylphosphonium chloride (2.59)



$C_{19}H_{17}Cl_2P$

m.w. = 347.22 g/mol

White solid

Chloromethyl phosphonium chloride (**2.59**) was prepared by a method described by Lawrence *et al.*[91] To a solution of hydroxymethyl phosphonium chloride (**2.58**) (2.00 g, 6.08 mmol) in CH_2Cl_2 (9 mL) was added thionyl chloride (0.93 mL, 12.77 mmol) dropwise and heated to reflux and stirred for 2 hour. The reaction mixture was cooled then concentrated *in vacuo* and dried under high vacuum to give a cream solid. Purification by flash column chromatography on silica gel (5.0 cm x 6 cm) eluting with $CH_2Cl_2 \rightarrow 10\%$ MeOH/CH_2Cl_2 afforded the title compound **2.59** as a white solid (1.81 g, 5.21 mmol, 86%). The spectroscopic data was in good accordance to the literature.[91]

Mpt 257-260 °C, (Lit: 262-264 °C).[91]

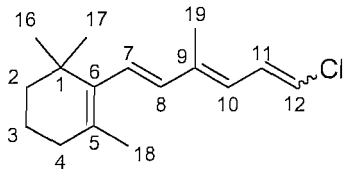
FT-IR (neat) ν_{max} 3508 (w), 3411 (w), 3023 (m), 2814 (m), 1586 (m), 1484 (m), 1434 (s), 1114 (s), 1055 (m), 997 (m), 875 (m), 840 (w), 742 (s), 720 (s) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00-7.88 (6H, m, -PCCHC**H**CH-), 7.86-7.76 (3H, m, -PCCHCHC**H**-), 7.75-7.63 (6H, m, -PCC**H**CHCH-), 6.22 (2H, d, J = 5.9 Hz, -$P^+C\mathbf{H_2}Cl$) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 135.82 (CH, d, J = 11.8 Hz, -PCCHCH**C**H-), 134.66 (CH, d, J = 41.3 Hz, -PCC**H**CHCH-), 130.76 (CH, d, J = 51.2 Hz, -PC**C**HCHCH-), 116.77 (C, d, J = 350.6 Hz, -P**C**CHCHCH-), 34.03 (CH_2, d, J = 222.5 Hz, -$P^+\mathbf{C}H_2Cl$) ppm.

LRMS (ES^+) *m/z* 311 [$M(^{35}Cl)^+$-Cl^-], 313 [$M(^{37}Cl)^+$–Cl^-].

2-((1*E*,3*E*)-6-Chloro-3-methylhexa-1,3,5-trienyl)-1,3,3-trimethylcyclohex-1-ene (2.60)



$C_{16}H_{23}Cl$

m.w. = 316.22 g/mol

Pale yellow oil

To a slurry of phosphonium chloride **2.59** (175 mg, 0.50 mmol) Et_2O (4 mL) was added piperidine (0.05 mL, 0.50 mmol) followed by *n*-BuLi (1.85 M in hexane, 0.27 mL, 0.50 mmol). The orange brown suspension was stirred at rt for 2 hours before a solution of aldehyde **1.14** (100 mg, 0.46 mmol) in Et_2O (1 mL) was added dropwise and stirred for 17 hours. The brown suspension was quenched with H_2O (5 mL) and the mixture extracted with Et_2O (4 x 15 mL). The combined organic phases were washed with brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving a brown solid and oil. Purification by flash column chromatography on neutral alumina (2.0 cm x 10 cm) eluting with 5% Et_2O/hexane gave the desired vinyl chloride **2.60** as a pale yellow oil (69 mg, 0.28 mmol, 60%, 11-*E*/*Z* = 1:1).

FT-IR (neat) ν_{max} 2957 (m), 2926 (m), 2863 (m), 2826 (w), 1604 (w), 1557 (m), 1444 (m) cm^{-1}.

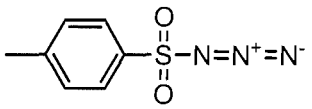
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84 (1H, dd, *J* = 13.1, 11.8 Hz, **11**(11-*E*)), 6.67 (1H, dd, *J* = 11.0, 7.3 Hz, **11**(11-*Z*)), 6.43 (1H, d, *J* = 11.0 Hz, **10**(11-*Z*)), 6.29 (1H, d, *J* = 16.1 Hz, **7**(11-*E*/*Z*)), 6.23 (1H, d, *J* = 16.1 Hz, **7**(11-*E*/*Z*)), 6.21 (1H, d, *J* = 16.1 Hz, **8**(11-*E*/*Z*)), 6.20 (1H, d, *J* = 13.1 Hz, **10**(11-*E*)), 6.07 (1H, d, *J* = 16.1 Hz, **8**(11-*E*/*Z*)), 6.05 (1H, d, *J* = 7.3 Hz, **12**(11-*Z*)), 5.97 (1H, d, *J* = 11.8 Hz, **12**(11-*E*)), 2.04 (2H, t, *J* = 6.2 Hz, **4**(11-*E*/*Z*)), 2.03 (2H, t, *J* = 6.2 Hz, **4**(11-*E*/*Z*)), 1.96 (3H, d, *J* = 1.5 Hz, **19**(11-*E*/*Z*)), 1.92 (3H, d, *J* = 1.3 Hz, **19**(11-*E*/*Z*)), 1.74 (3H, s, **18**(11-*E*/*Z*)), 1.71 (3H, s, **18**(11-*E*/*Z*)), 1.68-1.60 (4H, m, 2 x **3**(11-*E*/*Z*)), 1.52-1.46 (4H, m, 2 x **2**(11-

E/*Z*)), 1.05 (6H, s, **16**+**17**(11-*E*/*Z*)), 1.04 (6H, s, **16**+**17**(11-*E*/*Z*)) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 139.33 (C, **9**), 138.02 (C, **9**), 137.98 (C, **6**), 137.61 (CH, **8**), 137.26 (CH, **8**), 137.02 (C, **6**), 130.88 (CH, **11**), 130.07 (C, **5**), 129.85 (C, **5**), 128.99 (CH, **7**), 128.35 (CH, **7**), 126.40 (CH, **11**), 125.37 (CH, **12**), 122.93 (CH, **10**), 120.38 (CH, **10**), 118.44 (CH, **12**), 39.95 (CH_2, **2**), 34.59 (C, **1**), 34.57 (C, **1**), 33.38 (CH_2, **4**), 29.27 (CH_3, **16**+**17**), 22.05 (CH_3, **18**), 22.02 (CH_3, **18**), 19.60 (CH_2, **3**), 13.33 (CH_3, **19**), 12.96 (CH_3, **19**) ppm.

LRMS (CI, NH_3) *m/z* 251 [$M^{35}Cl+H^+$], 253 [$M^{37}Cl+H^+$].

Tosyl azide (2.62)



$C_7H_7N_3O_2S$
m.w. = 197.21 g/mol
Colourless oil

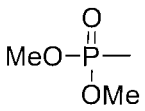
Tosyl azide was prepared by a method described by Ghosh *et al.*[92] To a solution of tosyl chloride (4.00 g, 20.98 mmol) in acetone (60 mL) and H_2O (60 mL) at 0 °C was added NaN_3 (1.36 g, 20.98 mmol) in one portion. The solution was stirred at 0 °C for 2 hours before being warmed to rt and stirred for a further 2 hours. After this time, the acetone was removed by concentration *in vacuo* and the resultant mixture was extracted with Et_2O (4 x 25 mL). The combined organic phases were washed with brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo* giving a colourless oil. Purification by distillation under reduced pressure (0.4 mbar, 60-65 °C) afforded tosyl azide (**2.62**) as a colourless oil (3.65 g, 18.50 mmol, 88%). Spectroscopic details are consistent with those reported in the literature.[92]

FT-IR (neat) ν_{max} 2121 (s), 1595 (w), 1494 (w), 1366 (m), 1160 (s) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (2H, d, J = 8.2 Hz, -SO_2CC**H**-), 7.41 (2H, d, J = 8.2 Hz, -SO_2CCHC**H**-), 2.49 (3H, s, C**H$_3$**-) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 146.53 (C, CH_3**C**-), 135.89 (C, -SO_2**C**-), 130.60 (CH, CH_3**C**CH-), 127.86 (CH, -SO_2CCH**C**H-), 22.07 (CH_3, **C**H_3-) ppm.

Dimethyl methylphosphonate (2.65)



$C_3H_9O_3P$

m.w. = 124.08 g/mol

Colourless oil

Phosphonate **2.65** was prepared by a method described by Kiddle *et al.*[93] Methyl iodide (1.00 mL, 16.06 mmol) and trimethyl phosphite (1.89 mL, 16.06 mmol) were mixed before being irradiated with microwaves (70 °C, 300 W). At ~70 °C the reaction became exothermic causing the temperature to rise to ~155 °C, the reaction was stopped using the emergency stop button at ~2 minutes. The colourless oil was purified by distillation under reduced pressure (10 mbar, 50-60 °C) affording the desired phosphonate **2.65** as a colourless oil (1.90 g, 15.34 mmol, 96%). Spectroscopic details were consistent with the literature.[93]

FT-IR (neat) ν_{max} 3486 (br), 2957 (w), 2852 (w), 1463 (w), 1238 (s), 1019 (s) cm^{-1}.

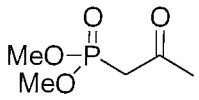
^{1}H NMR (300 MHz, $CDCl_3$) δ 3.71 (6H, d, J = 11.0 Hz, C**H$_3$**O-), 1.45 (3H, d, J = 17.4 Hz, -PC**H$_3$**) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 52.46 (CH_3, d, J = 6.1 Hz, **C**H_3O-), 10.18 (CH_3, d, J = 144.3 Hz, -P**C**H_3) ppm.

^{31}P NMR (121 MHz, $CDCl_3$) δ 33.76 (s) ppm.

LRMS (ES^+) *m/z* 125 [$M+H^+$], 188 [$M+MeCN+Na^+$].

Dimethyl 2-oxopropylphosphonate (2.66)



$C_5H_{11}O_4P$

m.w. = 166.11 g/mol

Colourless oil

Phosphonate **2.66** was prepared using a method described by Mathey *et al.*[95] To a solution of *n*-BuLi (2.06 M in hexane, 0.86 mL, 1.77 mmol) in THF (1 mL) at –60 °C was added a solution of methyl phosphonate **2.65** (200 mg, 1.61 mmol) in THF (1 mL). The mixture was stirred for 10 minutes at –60 °C before the addition of CuI (338 mg, 1.77 mmol) and the reaction was warmed slowly to –30 °C before stirring for 1 hour. This gave a brown solution which was cooled to –40 °C before a solution of acetyl chloride (0.12 mL, 1.69 mmol) in Et_2O (1 mL) was added dropwise. The resultant solution was stirred for 3 hours at –35 °C then allowed to warm slowly to rt and stirred for 15 hours. The reaction was quenched with H_2O (10 mL) then filtered through a pad of celite flushing with CH_2Cl_2. The phases were separated and the aqueous phase extracted with CH_2Cl_2 (3 x 15 mL). The combined organic phases were dried ($MgSO_4$) and concentrated *in vacuo* giving a colourless oil. Purification by flash column chromatography (2.0 cm x 8 cm) eluting with EtOAc gave the desired *β*-keto phosphonate **2.66** as a colourless oil (161 mg, 0.97 mmol, 60%).

FT-IR (neat) ν_{max} 3477 (br), 2960 (w), 2855 (w), 1712 (s), 1245 (s), 1019 (s) cm^{-1}.

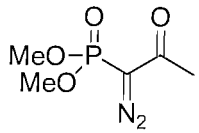
^{1}H NMR (300 MHz, $CDCl_3$) δ 3.78 (6H, d, *J* = 11.3 Hz, -P(OC**H$_3$**)$_2$), 3.09 (2H, d, *J* = 22.9 Hz, -PC**H$_2$**-), 2.31 (3H, s, -C(O)C**H$_3$**) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 199.96 (C, d, *J* = 6.6 Hz, -**C**=O), 53.35 (CH_3, d, *J* = 6.6 Hz, -P(O**C**H$_3$)$_2$), 42.58 (CH_2, d, *J* = 127.7 Hz, -P**C**H$_2$-), 31.71 (CH_3, -C(O)**C**H$_3$) ppm.

^{31}P NMR (121 MHz, $CDCl_3$) δ 23.01 (s) ppm.

LRMS (ES^+) *m/z* 167 [$M+H^+$], 189 [$M+Na^+$].

Dimethyl (1-diazo-2-oxopropyl)phosphonate (2.67)



$C_5H_9N_2O_4P$

m.w. = 192.11 g/mol

Pale yellow oil

Diazo compound **2.67** was prepared using a combination of methods described by Ghosh *et al.* and Goundry *et al.*[92,96] To a suspension of NaH (118 mg, 2.95 mmol) in THF (9 mL) at 0 °C was added a solution of *β*-keto phosphonate **2.66** (446 mg, 2.68 mmol) in THF (3 mL) dropwise. The reaction was stirred for 2 hours at 0 °C before the dropwise addition of a solution of tosyl azide (**2.62**) (582 mg, 2.95 mmol) in benzene (3 mL). After complete addition the reaction was allowed to warm to rt and stir for 20 hours giving a brick red suspension. This suspension was filtered through a pad of celite flushing with benzene and EtOAc. The red solution was concentrated *in vacuo* giving a brown red oil. Purification by flash column chromatography on silica gel (4.0 cm x 6 cm) eluting with 50%→100% EtOAc/hexane afforded the desired diazo compound **2.67** as a pale yellow oil (400 mg, 2.08 mmol, 77%). Spectroscopic details are consistent with those reported in the literature.[92]

FT-IR (neat) ν_{max} 3502 (br), 2960 (w), 2856 (w), 2123 (s), 1657 (s), 1267 (s), 1023 (s) cm^{-1}.

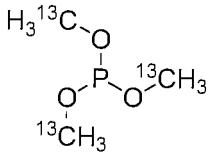
^{1}H NMR (300 MHz, $CDCl_3$) δ 3.85 (6H, d, *J* = 11.9 Hz, -P(OC**H**$_3$)$_2$), 2.28 (3H, s, -C(O)C**H**$_3$) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 190.20 (C, d, *J* = 13.6 Hz, -**C**=O), 53.91 (CH_3, d, *J* = 5.5 Hz, -P(O**C**H$_3$)$_2$), 27.49 (CH_3, -C(O)**C**H$_3$) ppm. -**C**=N_2 was not observed.

^{31}P NMR (121 MHz, $CDCl_3$) δ 14.92 (s) ppm.

LRMS (ES^+) *m/z* 165 [M-N_2+ H^+], 193 [M+H^+].

[1-$^{13}C_3$]-Trimethyl phosphite (2.71)



$^{13}C_3H_9O_3P$

m.w. = 127.05 g/mol

Colourless oil

To a solution of ^{13}MeOH (1.00 g, 30.26 mmol) and tributylamine (7.21 mL, 30.26 mmol) in tetralin (4 mL) at 0 °C was added a solution of PCl_3 (0.83 mL, 9.46 mmol) in tetralin (2 mL) slowly dropwise. The reaction was stirred at 0 °C for 5 minutes before being allowed to warm to rt and stirred for 44 hours giving a colourless solution. Purification under reduced pressure (0.4 mbar, rt) gave an inseparable mixture of [$^{13}C_3$]-trimethyl phosphite **(2.71)** (808 mg, 6.36 mmol, 67%, estimated by ^{1}H NMR) and tetralin (4.59 g).

FT-IR (neat) ν_{max} Due to stench, the FT-IR could not be obtained.

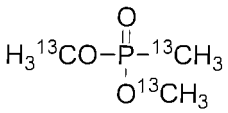
^{1}H NMR (300 MHz, $CDCl_3$) δ 3.57 (9H, dd, *J* = 145.2, 10.6 Hz, P(OC**H$_3$**)$_3$) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 49.40 (CH_3, d, *J* = 10.5 Hz, P(O**C**H$_3$)$_3$) ppm.

^{31}P NMR (121 MHz, $CDCl_3$) δ 141.99 (q, *J* = 11.1 Hz) ppm.

LRMS (ES^+) *m/z* 128 [M+H^+].

[1,2-$^{13}C_3$]-Dimethyl methylphosphonate (2.72)



$^{13}C_3H_9O_3P$

m.w. = 127.05 g/mol

Colourless oil

[$^{13}C_3$]-trimethyl phosphate (**2.71**) (as a 1:3 mixture with tetralin, 971 mg, ~1.85 mmol of phosphite) was mixed with $^{13}CH_3I$ (264 mg, 1.85 mmol). The mixture was irradiated with microwaves for 15 minutes (120 °C, 300 W). Purification by flash column chromatography on silica gel (3.0 cm x 3 cm) eluting with $Et_2O \rightarrow$EtOAc gave the desired phosphonate **2.72** as a colourless oil (165 mg, 1.30 mmol, ~70%).

FT-IR (neat) ν_{max} 3432 (br), 3016 (w), 2927 (m), 2858 (w), 2838 (w), 1494 (m) cm^{-1}.

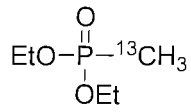
^{1}H NMR (300 MHz, $CDCl_3$) δ 3.74 (6H, dd, J = 147.5, 11.0 Hz, -P(OC**H**$_3$)$_2$), 1.48 (3H, dd, J = 128.4, 17.5 Hz, -PC**H**$_3$) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 52.53 (CH_3, d, J = 6.1 Hz, -P(O**C**H_3)$_2$), 10.26 (CH_3, d, J = 144.9 Hz, -P**C**H_3) ppm.

^{31}P NMR (121 MHz, $CDCl_3$) δ 33.79 (dt, J = 144.8, 6.1 Hz) ppm.

LRMS (ES^+) *m/z* 128 [$M+H^+$], 150 [$M+Na^+$], 191 [M+MeCN+Na^+].

[1-^{13}C]-Diethyl methylphosphonate (2.74)



$C_4{}^{13}CH_{13}O_3P$

m.w. = 153.12 g/mol

Colourless oil

To a mixture of diethyl phosphate (**2.73**) (0.70 mL, 5.38 mmol) and K_2CO_3 (1.49 g, 10.76 mmol) was added $^{13}CH_3I$ (1.00 g, 6.99 mmol) dropwise and the vessel was sealed. The reaction was stirred at 35 °C for 24 hours. After which crushed molecular

sieves and K_2CO_3 (740 mg, 5.38 mmol) were added. The reaction was stirred for a further 24 hours at 35 °C. After this time the mixture was transferred to a microwave tube washing with $CHCl_3$ (~2 mL), before the mixture was irradiated with microwaves (110 °C, 300 W), the reaction was stopped at 9 minutes as the reaction had became unstable. The suspension was filtered washing with $CHCl_3$ and CH_2Cl_2 and the solution was concentrated *in vacuo* giving a yellow oil. Purification by distillation under reduced pressure (0.4 mbar, rt) yielded the desired phosphonate **2.74** as a colourless oil (663 mg, 4.33 mmol, 80%).

FT-IR (neat) ν_{max} 3433 (br), 2986 (m), 1305 (m), 1227(m), 1026 (s) cm^{-1}.

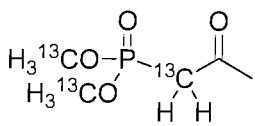
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.10 (4H, dqd, J = 8.2, 7.0, 4.6 Hz, -P(OC**H**$_2$CH$_3$)$_2$), 1.47 (3H, dd, J = 128.2, 17.5 Hz, -PC**H**$_3$), 1.33 (6H, t, J = 7.1 Hz, -P(OCH$_2$C**H**$_3$)$_2$) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 61.79 (CH_2, d, J = 6.1 Hz, -P(O**C**H$_2$CH$_3$)$_2$), 16.74 (CH_3, d, J = 6.1 Hz, -P(OCH$_2$**C**H$_3$)$_2$), 11.58 (CH_3, d, J = 144.9 Hz, -P**C**H$_3$) ppm.

^{31}P NMR (121 MHz, $CDCl_3$) δ 31.07 (d, J = 144.8 Hz) ppm.

LRMS (ES^+) *m/z* 154 [M+H$^+$].

[1,2-^{13}C$_3$]-Dimethyl (2-oxopropyl)phosphonate (2.75)



$C_2{}^{13}C_3H_{11}O_4P$

m.w. = 169.09 g/mol

Pale yellow oil

[$^{13}C_2$]-Phosphonate **2.75** was prepared using a method described by Mathey *et al.*[177] To a solution of *n*-BuLi (1.39 mL, 3.26 mmol) in THF (2.5 mL) at –60 °C was slowly added a solution of phosphonate **2.72** (377 mg, 2.97 mmol) in THF (1.2 mL), forming a brown solution. The brown solution was stirred for 10 minutes at –60 °C before the

addition of CuI (622 mg, 3.26 mmol). The reaction was slowly warmed to –30 °C and stirred for 1 hour giving a dark brown solution. After cooling to –40 °C a solution of acetyl chloride (0.22 mL, 3.12 mmol) in Et_2O (2 mL) was slowly added to the mixture and the reaction warmed to –35 °C then stirred for 3 hours. After this period the reaction mixture was allowed to warm slowly to rt and stir for 15 hours giving a brown biphasic solution. The reaction was quenched with H_2O (5 mL), producing a cream suspension, which was filtered through a pad of celite flushing with H_2O and CH_2Cl_2. The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (2 x 15 mL). The combined organic phases were washed with brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving an amber oil. Purification by distillation under reduced pressure (0.4 mbar, rt) gave the product as an amber oil. This oil was further purified by flash column chromatography (3.0 cm x 4 cm) eluting with EtOAc afforded the desired phosphonate **2.75** as a pale yellow oil (180 mg, 1.06 mmol, 36%).

FT-IR (neat) ν_{max} 3432 (br), 2956 (w), 2853 (w), 1713 (s), 1238 (s), 1015 (s) cm^{-1}.

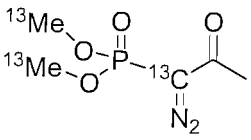
^{1}H NMR (300 MHz, $CDCl_3$) δ 3.79 (6H, dd, J = 148.3, 11.3 Hz, -P(OC**H**$_3$)$_2$), 3.10 (2H, dd, J = 128.6, 22.7 Hz, -PC**H**$_2$-), 2.32 (3H, s, -C(O)C**H**$_3$) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 199.95 (C, dd, J = 37.9, 6.8 Hz, -**C**=O), 53.38 (CH_3, d, J = 6.6 Hz, -P(O**C**H_3)$_2$), 42.62 (CH_2, d, J = 128.3 Hz, -P**C**H_2-), 31.72 (CH_3, d, J = 14.9 Hz, -C(O)**C**H_3) ppm.

^{31}P NMR (121 MHz, $CDCl_3$) δ 23.01 (dt, J = 128.1, 6.3 Hz) ppm.

LRMS (ES^+) *m/z* 170 [$M+H^+$].

[1,2-$^{13}C_3$]-Dimethyl (1-diazo-2-oxopropyl)phosphonate (2.76)



$C_2{}^{13}C_3H_9N_2O_4P$

m.w. = 195.09 g/mol

Colourless oil

Phosphonate **2.76** was prepared by a combination of methods described by Ghosh and Goundry *et al.*[178,179] To a slurry of NaH (22 mg, 0.54 mmol) in benzene (0.8 mL) and THF (1.1 mL) at 0 °C was added a solution of phosphonate **2.75** (83 mg, 0.49 mmol) in THF (1.1 mL) dropwise. The mixture was stirred for 1 hour at 0 °C before the addition of TsN_3 (0.08 mL, 0.54 mmol) slowly dropwise. The resultant mixture was warmed to rt and stirred for 14 hours giving a brick red suspension. The suspension was filtered through a pad of celite flushing with benzene (3 x 10 mL) then EtOAc (5 x 10 mL), the filtrate was concentrated *in vacuo* giving a dark amber oil. Purification by flash column chromatography on silica gel (2.5 cm x 7 cm) eluting with EtOAc gave the desired labelled Bestman reagent **2.76** as a colourless oil (70 mg, 0.36 mmol, 73%).

FT-IR (neat) ν_{max} 3496 (br), 2955 (w), 2852 (w), 2175 (m), 2116 (s), 1655 (s) cm^{-1}.

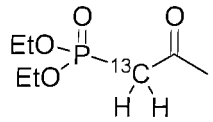
^{1}H NMR (300 MHz, $CDCl_3$) δ 3.85 (6H, dd, J = 148.8, 11.8 Hz, -P(OC**H$_3$**)$_2$), 2.28 (3H, d, J = 2.2 Hz, -C(O)C**H$_3$**) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 53.91 (CH_3, d, J = 5.5 Hz, -P(O**C**H$_3$)$_2$), 27.48 (CH_3, d, J = 21.0 Hz, -C(O)**C**H$_3$) ppm. **C**=O and **C**=N_2 not observed. C=O and C=N_2 not observed.

^{31}P NMR (121 MHz, $CDCl_3$) δ 14.92 (d, J = 220.5 Hz) ppm.

LRMS (ES^+) *m/z* 196 [M+H^+], 218 [M+Na^+].

[1-$^{13}C_3$]-Diethyl (2-oxopropyl)phosphonate (2.77)



$C_6{}^{13}CH_{15}O_4P$

m.w. = 195.16 g/mol

Colourless oil

Phosphonate **2.77** was prepared by a method described by Mathey *et al.*[95] To a solution of phosphonate **2.74** (303 mg, 1.98 mmol) in THF (2.5 mL) at –60 °C was added *n*-BuLi (2.34 M in hexane, 0.93 mL, 2.18 mmol) dropwise. The mixture was stirred at –60 °C for 5 minutes before the addition of CuI (415 mg, 2.18 mmol) in one portion. The brown solution was slowly warmed to –30 °C and stirred at this temperature for 1.5 hours, then cooled to –40 °C. A solution of acetyl chloride (0.15 mL, 2.08 mmol) in Et_2O (1.5 mL) was added to the brown mixture slowly before the reaction was stirred at –35 °C for 2.5 hours. The brown solution was slowly warmed to rt and stirred for 17 hours giving an amber biphasic solution. The reaction was quenched with H_2O (2 mL) giving an off white suspension and colourless solution. This mixture was filtered through a pad of celite, flushing with THF followed by CH_2Cl_2. The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 25 mL), before the combined organic phases were dried ($MgSO_4$) and concentrated *in vacuo* giving a yellow green oil. Purification by distillation under reduced pressure (0.4 mbar, 60 °C) gave the desired phosphonate **2.77** as a colourless oil (323 mg, 1.66 mmol, 84%).

FT-IR (neat) ν_{max} 3475 (br), 2985 (m), 2912 (w), 1714 (s), 1246 (s), 1022 (s) cm^{-1}.

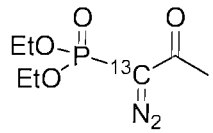
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.16 (4H, dqd, J = 8.2, 7.1, 1.3 Hz, -P(OC**H$_2$**CH$_3$)$_2$), 3.09 (2H, dd, J = 128.8, 22.9 Hz, -PC**H$_2$**-), 2.33 (3H, d, J = 1.5 Hz, -C(O)C**H$_3$**), 1.35 (6H, td, J = 7.0, 0.6 Hz, -P(OCH$_2$C**H$_3$**)$_2$) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 62.91 (CH_2, d, J = 6.6 Hz, -P(O**C**H$_2$CH$_3$)$_2$), 43.79 (CH_2, d, J = 127.2 Hz, -P**C**H$_2$-, 31.68 (CH_3, d, J = 14.9 Hz, -C(O)**C**H$_3$), 16.65 (CH_3, d, J = 6.1 Hz, -P(OCH$_2$**C**H$_3$)$_2$) ppm. **C**=O not observed.

^{31}P NMR (121 MHz, $CDCl_3$) δ 20.29 (d, J = 127.0 Hz) ppm.

LRMS (ES^+) *m/z* 196 [$M+H^+$], 218 [$M+Na^+$], 413 [$2M+Na^+$].

[1-^{13}C]-Diethyl (1-diazo-2-oxopropyl)phosphonate (2.78)



$C_6{}^{13}CH_{13}N_2O_4P$

m.w. = 221.16 g/mol

Colourless oil

To a slurry of NaH (42 mg, 1.06 mmol) in benzene (1.5 mL) and THF (2 mL) at 0 °C was added a solution of phosphonate **2.77** (188 mg, 0.96 mmol) in THF (2 mL). After stirring for 1 hour at 0 °C, TsN_3 (209 mg, 1.06 mmol) in THF (1 mL) was added slowly dropwise. The reaction was warmed to rt and stirred for 16 hours giving a brick red suspension. This suspension was filtered through a pad of celite flushing with benzene (3 x 10 mL) followed by EtOAc (6 x 10 mL), the filtrate was concentrated *in vacuo* giving a dark amber oil. Purification by flash column chromatography on silica gel (4.0 cm x 7 cm) eluting with EtOAc afforded the desired diazo compound **2.78** as a colourless oil (156 mg, 0.71 mmol, 73 %).

FT-IR (neat) ν_{max} 3502 (br, 2986 (w), 2934 (w), 2910 (w), 2175 (w), 2113 (s), 1655 (s), 1260 (s), 1014 (s) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.22 (4H, m, -$P(OCH_2CH_3)_2$), 2.29 (3H, d, J = 2.2 Hz, -$C(O)CH_3$), 1.40 (6H, td, J = 7.0, 0.7 Hz, -$P(OCH_2CH_3)_2$) ppm.

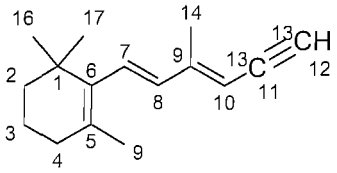
^{13}C NMR (75 MHz, $CDCl_3$) δ 64.88 (C, d, J = 218.4 Hz, -**C**=N_2), 53.74 (CH_2, d, J = 5.5 Hz, -$P(OCH_2CH_3)_2$), 27.59 (CH_3, d, J = 21.6 Hz, -$C(O)CH_3$), 16.50 (CH_3, d, J = 6.6 Hz, -$P(OCH_2CH_3)_2$) ppm. **C**=O not observed.

^{31}P NMR (121 MHz, $CDCl_3$) δ 11.66 (d, J = 218.3 Hz) ppm.

LRMS (ES^+) *m/z* 222 [$M+H^+$], 244 [$M+Na^+$].

HRMS (ES^+) for $C_6{}^{13}CH_{14}N_2O_4P$, calculated 222.0719, found 222.0718 Da.

[11,12-$^{13}C_2$]-1,3,3-Trimethyl-2-((1*E*,3*E*)-3-methylhexa-1,3-dien-5-ynyl)cyclohex-1-ene (2.79)



$C_{14}{}^{13}C_2H_{22}$
m.w. = 216.33 g/mol
Pale yellow oil

To a solution of ^{13}C labelled diethoxy Bestman reagent **2.78** (391 mg, 1.77 mmol) in THF (20 mL) at −78 °C was added NaOMe (0.5 M in methanol, 3.54 mL, 1.77 mmol) dropwise over 15 minutes. Stirring for 1 hour at −78 °C gave a slightly cloudy mixture, to it was added a solution of aldehyde **2.54** (259 mg, 1.18 mmol) in THF (8 mL) dropwise over 10 minutes. The yellow solution was stirred at −78 °C for 30 minutes before being warmed to rt over 30 minutes and stirred for 7.5 hours. The reaction was quenched with H_2O (5 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving an amber oil. Purification by flash column chromatography on silica gel (3.0 cm x 7 cm) eluting with 5% EtOAc/hexane gave the desired alkyne **2.79** as a pale yellow oil (162 mg, 0.75 mmol, 63%) and starting aldehyde **2.54** (39 mg, 0.18 mmol, 15%).

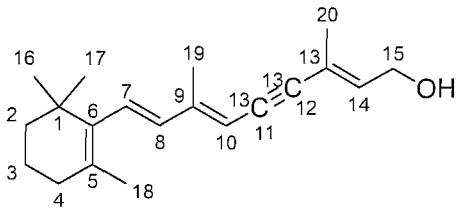
FT-IR (neat) ν_{max} 3293 (m), 2958 (m), 2927 (s), 2864 (m), 2826 (w), 2010 (w), 1443 (m) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.29 (1H, d, J = 16.1 Hz, **7**), 6.10 (1H, d, J = 16.1 Hz, **8**), 5.41 (1H, br s, **10**), 3.29 (1H, dd, J = 299.2, 2.3 Hz, **12**), 2.09 (3H, s, **19**), 2.02 (2H, t, J = 6.0 Hz, **4**), 1.70 (3H, br d, J = 1.0 Hz, **18**), 1.66-1.58 (2H, m, **3**), 1.50-1.45 (2H, m, **2**), 1.02 (6H, s, **16**+**17**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 149.62 (C, **9**), 137.69 (C, **6**), 135.44 (CH, d, J = 8.8 Hz, **8**), 130.58 (CH, **7**), 130.54 (C, **5**), 107.60 (CH, dd, J = 70.0, 30.1 Hz, **10**), 84.13 (CH, d, J = 200.2 Hz, **12**), 82.40 (C, d, J = 200.2 Hz, **11**), 39.91 (CH_2, **2**), 34.57 (C, **1**), 33.38 (CH_2, **4**), 29.24 (CH_3, **16**+**17**), 21.99 (CH_3, **18**), 19.56 (CH_2, **3**), 15.42 (CH_3, **19**) ppm.

LRMS (CI, NH_3) *m/z* 217 [$M+H^+$].

[11,12-$^{13}C_2$]-3,7-Dimethyl-9-(2,6,6-trimethyl-cyclohex-1-enyl)-nona-2,6,8-trien-4-yn-1-ol (2.80)



$C_{18}{}^{13}C_2H_{28}O$

m.w. = 286.42 g/mol

Pale yellow oil

To a solution of iodide **1.32** (405 mg, 1.30 mmol) in $^{i}PrNH_2$ (3 mL) was added $Pd(PPh_3)_4$ (13 mg, 10.82 μmol) and the reaction was stirred for 5 minutes. After which CuI (2 mg, 10.82 μmol) was added and the reaction was stirred for a further 5 minutes. Then a solution of alkyne **2.79** (234 mg, 1.08 mmol) in $^{i}PrNH_2$ (1.6 mL) was added dropwise producing a deep amber solution. The solution was stirred for 3.5 hours before being concentrated *in vacuo*, then dissolved in Et_2O (30 mL) and washed with H_2O (3 x 5 mL) and brine (5 mL). The organic phase was concentrated *in vacuo* giving a dark amber oil (569 mg). The residue as a mixture of alkyne **2.79** and iodide **1.32** (4:1, 465 mg, ~1.21 mmol) was dissolved in THF (6 mL) and cooled to 0 °C. To the solution was added TBAF (1.0 M in THF, 1.34 mL, 1.34 mmol) dropwise. After complete addition the reaction was warmed to r.t and stirred for 1 hour. The reaction was quenched with H_2O (5 mL) and the dark brown solution was extracted with Et_2O (3 x 15 mL). The combined organic phases were washed with brine (5 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving a dark amber oil. The crude material was filtered through a plug of neutral alumina eluting with 20% EtOAc/hexane and concentrated *in vacuo* giving an amber oil. Purification by flash column

chromatography on silica gel (3.0 cm x 15 cm) eluting with 15% EtOAc/hexane gave the desired dehydro retinol **2.80** (191 mg, 0.67 mmol, 69% over 2 steps) and the 13*Z*-isomer (23 mg, 0.08 mmol, 8%).

All-*E*-isomer

FT-IR (neat) ν_{max} 3313 (br), 2926 (m), 2864 (m), 1441 (m) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.27 (1H, d, *J* = 16.1 Hz, **7**), 6.12 (1H, d, *J* = 16.1 Hz, **8**), 6.01 (1H, br q, *J* = 7.5 Hz, **14**), 5.53 (1H, d, *J* = 4.8 Hz, **10**), 4.27 (2H, br t, *J* = 5.4 Hz, **15**), 2.07 (3H, s, **19**), 2.02 (2H, t, *J* = 6.3 Hz, **4**), 1.90 (3H, ddt, *J* = 5.3, 1.5, 0.8 Hz, **20**), 1.70 (3H, s, **18**), 1.66-1.58 (2H, m, **3**), 1.51-1.44 (2H, m, **2**), 1.03 (6H, s, **16**+**17**) ppm. -OH was not observed.

^{13}C NMR (100 MHz, $CDCl_3$) δ 147.80 (C, **9**), 137.81 (C, **6**), 135.81 (CH, d, *J* = 9.3 Hz, **8**), 134.68 (CH, d, *J* = 3.9 Hz, **14**), 130.44 (C, **5**), 129.91 (CH, **7**), 121.93 (C, dd, *J* = 105.9, 38.9 Hz, **13**), 108.76 (CH, dd, *J* = 91.4, 11.7 Hz, **10**), 98.79 (C, d, *J* = 177.9 Hz, **12**), 87.23 (C, d, *J* = 177.9 Hz, **11**), 59.64 (CH_2, d, *J* = 6.8 Hz, **15**), 39.94 (CH_2, **2**), 34.59 (C, **1**), 33.41 (CH_2, **4**), 29.37 (CH_3, **16**+**17**), 22.02 (CH_3, **18**), 19.57 (CH_3, **20**), 18.03 (CH_2, **3**), 15.41 (CH_3, d, *J* = 3.9 Hz, **19**) ppm.

LRMS (ES^+) *m/z* 269 [$M-H_2O+H^+$].

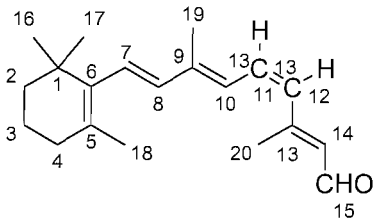
13*Z*-Isomer

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.29 (1H, d, *J* = 16.1 Hz, **7**), 6.12 (1H, d, *J* = 16.1 Hz, **8**), 5.86 (1H, dtq, *J* = 13.8, 6.8, 1.5 Hz, **14**), 5.57 (1H, br s, **10**), 4.36 (2H, d, *J* = 6.8 Hz, **15**), 2.08 (3H, t, *J* =

0.8 Hz, **19**), 2.02 (2H, t, J = 5.9 Hz, **4**), 1.95 (3H, tt, J = 3.1, 1.5 Hz, **20**), 1.70 (3H, q, J = 0.9 Hz, **18**), 1.67-1.55 (2H, m, **3**), 1.52-1.43 (2H, m, **2**), 1.03 (6H, s, **16**+**17**) ppm. -OH was not observed.

^{13}C NMR (75 MHz, $CDCl_3$) δ 147.94 (C, **9**), 137.77 (C, **6**), 135.68 (CH, dd, J = 5.5, 3.3 Hz, **8**), 134.84 (CH, **14**), 130.54 (C, **5**), 130.23 (CH, **7**), 121.87 (C, dd, J = 58.1, 38.7 Hz, **13**), 108.62 (CH, dd, J = 61.1, 41.2 Hz, **10**), 95.44 (C, d, J = 176.9 Hz, **12**), 93.02 (C, d, J = 176.9 Hz, **11**), 61.90 (CH_2, **15**), 39.92 (CH_2, **2**), 34.56 (C, **1**), 33.39 (CH_2, **4**), 29.24 (CH_3, **16**+**17**), 23.62 (CH_3, **18**), 21.99 (CH_3, **20**), 19.55 (CH_2, **3**), 15.51 (CH_3, **19**) ppm.

[11,12-$^{13}C_2$]-11*Z*-Retinal (2.81)



$C_{18}{}^{13}C_2H_{28}O$

m.w. = 286.42 g/mol

Yellow oil

[11,12-$^{13}C_2$]-11*Z*-retinal (**2.81**) was prepared by a method described by Borhan *et al.*[6] Argon was bubble through a suspension of zinc dust (11.42 g, 0.175 mol) in H_2O (68 mL) for 15 minutes. To the suspension was added $Cu(OAc)_2$ (1.141 g, 6.28 mmol) in one portion producing a black suspension. The mixture was stirred for 15 minutes before the addition of $AgNO_3$ (1.141 g, 6.72 mmol) in one portion leading to an exothermic reaction. After stirring for 30 minutes the activated zinc was filtered and washed with H_2O, MeOH, acetone and Et_2O sequentially. The moist zinc catalyst was suspended in H_2O (19 mL) and ^{i}PrOH (19 mL). To the suspension was added a solution of dehydro retinol **2.80** (134 mg, 0.47 mmol) in ^{i}PrOH (19 mL) and the reaction was warmed to 40 °C and stirred for 26 hours. After this time the reaction was filtered through a pad of celite flushing with H_2O and Et_2O and the phases were separated. The aqueous was extracted with Et_2O (4 x 20 mL) and the combined organic phases were

washed with brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo* giving crude isomericially pure 11,12-$^{13}C_2$-11*Z*-retinol as an oil (139 mg).

The crude retinol (136 mg, ~0.47 mmol) was dissolved in CH_2Cl_2 (5 mL). To the solution was added crushed molecular sieves (280 mg), NMO (110 mg, 0.94 mmol) and TPAP (49 mg, 0.14 mmol) sequentially. The black solution was stirred for 30 minutes before being passed through a short column of neutral alumina topped with a pad of celite, flushing with Et_2O. The yellow solution was concentrated *in vacuo* giving isomericially pure crude [11,12-$^{13}C_2$]-retinal (**2.81**) (127 mg) as a yellow oil. Purification by HPLC eluting with Et_2O (2.00 mL/minute) and hexane (7.99 mL/minute) gave [11,12-$^{13}C_2$]-11*Z*-retinal (**2.81**) as a yellow oil (69 mg, 0.24 mmol, 51% over 2 steps), [11,12-$^{13}C_2$]-all-*E*-retinal as a yellow oil (5 mg, 0.02 mmol, 4%) and [11,12-$^{13}C_2$]-retinal as a mixture of isomers (22 mg, 0.08 mmol, 16%).

FT-IR (neat) ν_{max} 2956 (w), 2926 (m), 2862 (m), 2826 (w), 1657 (s), 1566 (m), 1444 (m) cm^{-1}.

^{1}H NMR (400 MHz, C_6D_6) δ 9.91 (1H, d, *J* = 7.8 Hz, **15**), 6.58 (1H, br d, *J* 11.1 Hz, **10**), 6.38 (1H, dq, *J* = 148.3, 12.1 Hz, **11**), 6.34 (1H, d, *J* = 16.1 Hz, **7**), 6.22 (1H, d, *J* = 16.1 Hz, **8**), 6.10 (1H, br t, *J* = 6.5 Hz, **14**), 5.58 (1H, dd, *J* = 154.7, 11.8 Hz, **12**), 1.91 (2H, t, *J* = 6.4 Hz, **4**), 1.77 (3H, dd, *J* = 4.1, 1.4 Hz, **20**), 1.74 (3H, s, **19**), 1.68 (3H, s, **18**), 1.60-1.52 (2H, m, **3**), 1.46-1.42 (2H, m, **2**), 1.07 (6H, s, **16**+**17**) ppm.

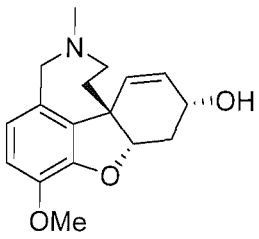
^{13}C NMR (100 MHz, C_6D_6) δ 190.50 (CH, **15**), 154.60 (C, dd, *J* = 39.4, 13.1 Hz, **13**), 141.42 (C, d, *J* = 5.8 Hz, **9**), 138.82 (CH, d, *J* = 4.9 Hz, **8**), 138.58 (C, **6**), 131.87 (1H, d, *J* = 70.0 Hz, **12**), 131.06 (1H, d, *J* = 70.0 Hz, **11**), 131.10 (CH, dd, *J* = 3.9, 2.0 Hz, **14**), 130.66 (C, **5**), 130.08 (CH, **7**), 127.05 (CH, dd, *J* = 41.8, 13.6 Hz, **10**), 40.41 (CH_2, **2**), 35.10 (C, **1**), 33.82 (CH_2, **4**), 29.69 (CH_3, **16**+**17**), 22.43

(CH_3, **18**), 20.23 (CH_2, **3**), 18.00 (CH_3, **20**), 12.85 (CH_3, d, J = 3.4 Hz, **19**) ppm.

LRMS (ES^+) *m/z* 287 [$M+H^+$], 309 [$M+Na^+$].

HRMS (ES^+) for $C_{18}{}^{13}C_2H_{29}O$, calculated 287.2280, found 287.2279 Da.

(–)-Galanthamine (3.1)



$C_{17}H_{21}NO_3$
m.w. = 287.35 g/mol
White solid

To a solution of mesylates **4.9** (10 mg, 20.68 μmol, dr = 4.8:1) in CH_2Cl_2 (0.2 mL) at 0 °C was added TFA (31 μL, 414μmol) dropwise. The mixture was warmed to rt and stirred for 1.5 hours. The reaction was cooled to 0 °C before the addition of $CHCl_3$ (0.3 mL) and $NaHCO_3$ (0.3 mL), then allowed to warm to rt and stirred for 3 hours. The reaction was diluted with H_2O (3 mL) and $CHCl_3$ (5 mL) and the organic phase was separated, re-extracting the aqueous with $CHCl_3$ (3 x 5 mL). The combined organic phase was dried ($MgSO_4$) and concentrated *in vacuo* giving a waxy solid. Purification by flash column chromatography on silica gel (1.2 cm x 8 cm) eluting with 5%→10% MeOH/CH_2Cl_2 afforded (–)-galanthamine (**3.1**) as a white solid (4 mg, 13.92 μmol, 67%, 92% *e.e.*) and *epi*-galanthamine (**3.7**) (1 mg, 3.48 μmol, 17%). Spectroscopic and analytical data were consistent with those reported previously (NMR[138]).

Mpt 125-127 °C, (Lit: 125-127 °C, 128-129 °C).[111,180]

$[\alpha]_D$ = –81.3 (*c* 1.5, $CHCl_3$, 24 °C), (Lit: –93.4 (*c* 1.0, $CHCl_3$, 25 °C).[111]

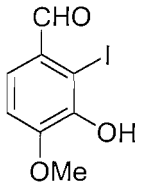
FT-IR (neat) ν_{max} 3384 (br), 3025 (w), 2916 (m), 1623 (m), 1591 (w), 1507 (s), 1438 (s), 1282 (s) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.67 (1H, d, *J* = 8.0 Hz, -NCH_2CCHCH-), 6.63 (1H, d, *J* = 8.0 Hz, -NCH_2CCHCH-), 6.08 (1H, dd, *J* = 10.3, 1.3 Hz, -CCH=CH-), 6.01 (1H, ddd, *J* = 10.3, 5.0, 1.3 Hz, -CCH=$CHCH_2$-), 4.62 (1H, s, $ArOCHCH_2$-), 4.15 (1H, br t, *J* = 5.0 Hz, -$CHCH$(OH)-), 4.10 (1H, d, *J* = 15.1 Hz, -NCH_2Ar), 3.84 (3H, s, $ArOCH_3$), 3.69 (1H, d, *J* = 15.1 Hz, -NCH_2Ar), 3.28 (1H, ddd, *J* = 14.3, 12.8, 1.8 Hz, -CH_2CH_2N-), 3.06 (1H, dt, *J* = 14.3, 3.3 Hz, -CH_2CH_2N-), 2.70 (1H, ddd, *J* = 15.6, 3.5, 1.5 Hz, $ArCHCH_2CH(OH)$-), 2.41 (3H, s, -NCH_3), 2.10 (1H, td, *J* = 13.6, 3.3 Hz, -CH_2CH_2N-), 2.02 (1H, ddd, *J* = 15.6 Hz, 5.0, 2.5 Hz, -$CH_2CH(OH)$-), 1.65 (1H, br s, -OH), 1.59 (1H, ddd, *J* = 13.6, 4.0, 1.8 Hz, -CH_2CH_2N-) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 146.23 (C, -$COCH_3$), 144.51 (C, -$COCHCH_2CH(OH)$-), 133.44 (C, -NCH_2CCCCH=CH-), 129.69 (C, -NCH_2C-), 128.00 (CH, -CH=$CHCCH_2CH_2N$-), 127.24 (CH, -CH=$CHCCH_2CH_2N$-), 122.42 (CH, -NCH_2CCHCH-), 111.64 (CH, -NCH_2CCHCH-), 89.11 (CH, $ArOCHCH_2$-), 62.46 (CH, $ArOCHCH_2CH(OH)$-), 61.01 (CH_2, -NCH_2Ar), 56.30 (CH_3, $ArOCH_3$), 54.22 (CH_2, -NCH_2CH_2-), 48.60 (C, -CCH=CH-), 42.50 (CH_3, -NCH_3), 34.22 (CH_2, $ArOCHCH_2CH(OH)$-), 30.33 (CH_2, -NCH_2CH_2-) ppm.

LRMS (ES^+) *m/z* 288 [$M+Na^+$], 310 [$M+H^+$].

3-Hydroxy-iodo-4-methoxybenzaldehyde (3.70)



$C_8H_7IO_3$

m.w. = 278.04 g/mol

Peach Solid

Iodide **3.70** was prepared following a procedure described by Markovich *et al.*[146] To a solution of *iso*vanillin (4.000 g, 26.29 mmol) in pyridine (16 mL) at 0 °C was added a solution of ICl (4.268 g, 26.29 mmol) in dioxane (26 mL) dropwise. The reaction was allowed to warm to rt and stir for 7 days. The reaction mixture was concentrated *in vacuo* giving a brown oil, which was treated with H_2O (50 mL) and 6 N HCl (20 mL). The mixture was extracted with EtOAc (4 x 50 mL) before the combined organic phases were washed with a saturated solution of $Na_2S_2O_3$ (2 x 20 mL), H_2O (2 x 25 mL) and brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving a peach solid. Purification by recrystallisation from EtOAc gave the desired iodide **3.70** as a peach solid (3.978 g, 14.31 mmol, 54%). Spectroscopic details are consistent with those reported in the literature.[146]

Mpt 168-174 °C, (lit: 169-172 °C).[146]

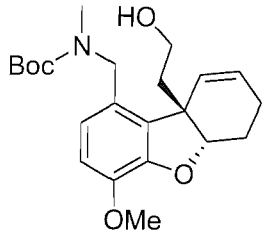
FT-IR (neat) ν_{max} 3213 (br), 3015 (w), 2942 (w), 2873 (w), 2841 (w), 1666 (s), 1582 (s), 1556 (s) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.04 (1H, s, -C**H**O), 7.56 (1H, d, J = 8.4 Hz, CHOCC**H**-), 6.93 (1H, d, J = 8.4 Hz, CHOCCHC**H**-), 6.30 (1H, s, -O**H**), 4.01 (3H, s, -OC**H$_3$**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 195.07 (CH, -**C**HO), 151.02 (C, MeO**C**-), 146.09 (C, -**C**OH), 129.14 (C, -**C**CHO), 124.18 (CH, -**C**HCCHO), 110.32 (CH, -**C**HCHCCHO), 88.38 (C, -**C**I), 56.92 (CH_3, -O**C**H$_3$) ppm.

LRMS (ES^+) *m/z* 279 [$M+H^+$].

***Tert*-butyl-*N*-[((5a*S*,9a*S*)-9a-(hydroxyethyl)-4-(methyloxy)-5a,6,7,9a-tetrahydrodibenzo[*b*,*d*]furan-1yl)methyl]-*N*-methylcarbamate (3.81)**



$C_{22}H_{31}NO_5$

m.w. = 389.49 g/mol

Pale yellow oil

NMR spectra exhibited broadening / doubling of peaks due to restricted rotation

To a solution of iodide **4.8** (388 mg, 0.75 mmol) in toluene (24 mL) was added Ag_2CO_3 (620 mg, 2.25 mmol), dppp (46 mg, 0.11 mmol) and $Pd(OAc)_2$ (25 mg, 0.11 mmol). The green heterogeneous mixture was heated to reflux and stirred for 16 hours giving a black solution. The solution was concentrated *in vacuo* giving a black oil. Purification by flash column chromatography on silica gel (3.5 cm x 12 cm) eluting with 60% EtOAc/hexane afforded the desired tricyclic product **3.81** as a pale yellow oil (169 mg, 0.43 mmol, 58%).

$[\alpha]_D = -26.5$ (*c* 1.2, $CHCl_3$, 26 °C).

FT-IR (neat) ν_{max} 3442 (br), 2972 (w), 2931 (m), 2839 (w), 1690 (s), 1622 (m), 1581 (w), 1505 (s) cm^{-1}.

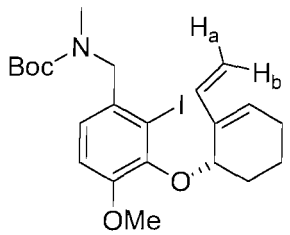
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.72 (1H, d, *J* = 8.5 Hz, -NCH_2CCHCH-), 6.58 (1H, d, *J* = 8.5 Hz, -NCH_2CCHCH-), 5.87 (1H, ddd, *J* = 11.0, 4.3, 2.8 Hz, -CCH=C**H**-), 5.81 (1H, d, *J* = 11.0 Hz, -C**H**=CH-), 4.85 (1H, dd, *J* = 5.5, 3.6 Hz, Ar$OCHCH_2CH_2$-), 4.58 (1H, br d, *J* = 15.7 Hz, -NCH_2Ar), 4.43 (1H, d, *J* = 15.7 Hz, -NCH_2Ar), 3.86 (3H, s, ArOCH_3), 3.69-3.57 (2H, m, -CH_2OH), 2.82 (3H, s, -NCH_3), 2.27-1.92 (5H, m, Ar$OCHCH_2CH_2CH$=CHCCH_2CH_2OH), 1.85 (1H, m, -CH_2-), 1.48 (9H, s, -$OC(CH_3)_3$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 156.40 (C, -**C**=O), 147.86 (C, -**C**OCHCH$_2$CH$_2$-), 144.51 (C, -**C**OMe), 131.58 (C, -NCH$_2$C**C**-), 129.39 (CH, -C**C**H=CH-), 128.65 (CH, -CCH=**C**H-), 126.54 (C, -NCH$_2$**C**CHCH-), 120.64 (CH, br, -NCH$_2$C**C**HCH-), 111.46 (CH, -NCH$_2$CCH**C**H-), 85.76 (CH, ArO**C**HCH$_2$CH$_2$-), 80.23 (C, -O**C**(CH$_3$)$_3$), 59.96 (CH$_2$, -**C**H$_2$OH), 56.18 (CH$_3$, ArO**C**H$_3$), 49.53 (C, -**C**CH=CH-), 48.90 (CH$_2$, -N**C**H$_2$Ar), 40.84 (CH$_2$, -**C**H$_2$CH$_2$OH), 34.32 (CH$_3$, -N**C**H$_3$), 28.78 (CH$_3$, -OC(**C**H$_3$)$_3$), 24.87 (CH$_2$, ArOCH**C**H$_2$CH$_2$-), 20.11 (CH$_2$, ArOCHCH$_2$**C**H$_2$-) ppm.

LRMS (ES$^+$) *m/z* 390 [M+H$^+$], 412 [M+Na$^+$], 801 [2M+Na$^+$].

HRMS (ES$^+$) for $C_{22}H_{32}NO_5$, calculated 390.2275, found 390.2264 Da.

***Tert*-butyl-3-((*S*)-2-vinylcyclohex-2-enyloxy)-2-iodo-4-methoxybenzylmethylcarbamate (3.85)**



$C_{22}H_{30}INO_4$
m.w. = 499.38 g/mol
Colourless oil
NMR spectra exhibited broadening / doubling of peaks due to restricted rotation

To a solution of enyne **3.87** (532 mg, 1.07 mmol) in CH_2Cl_2 (32 mL) was added Grubb's 1st generation catalyst (26 mg, 0.03 mmol) in one portion and the resultant purple solution was stirred for 3 hours at rt. The reaction was concentrated *in vacuo* giving a brown oil. Purification by flash column chromatography on silica gel (4.0 cm x 10 cm) eluting with 10% EtOAc/hexane gave the desired diene **3.85** as a colourless oil (453 mg, 0.91 mmol, 85%).

$[\alpha]_D = -140.2$ (*c* 0.6, $CHCl_3$, 26 °C).

FT-IR (neat) ν_{max} 3003 (w), 2973 (w), 2935 (w), 2866 (w), 2835 (w), 1694 (s), 1644 (w), 1587 (w), 1475 (m) cm^{-1}.

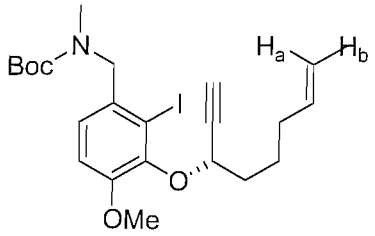
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.87 (1H, d, *J* = 8.3 Hz, -NCH_2CCHCH-), 6.78 (1H, m, -NCH_2CCHCH-), 6.33 (1H, dd, *J* = 17.7, 11.2 Hz, H_2C=CH-), 6.03 (1H, dd, *J* = 4.9, 2.9 Hz, -CH_2CH=CCH=CH_2), 5.45 (1H, t, *J* = 3.4 Hz, Ar$OCHCH_2$-), 5.24 (1H, br d, *J* = 17.7 Hz, $\mathbf{H_b}$), 4.84 (1H, d, *J* = 11.2 Hz, $\mathbf{H_a}$), 4.39 (2H, br, -NCH_2Ar), 3.87 (3H, s, ArOCH_3), 2.85 & 2.80 (3H, br s, -NCH_3), 2.34 (1H, m, Ar$OCHCH_2CH_2CH_2$-), 2.26-2.00 (5H, m, Ar$OCHCH_2CH_2CH_2$-), 1.51 & 1.43 (9H, br s, -$OC(CH_3)_3$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 156.32 & 155.88 (C, -C=O), 151.17 (C, -CHCOMe), 147.16 (-$CHCCOCHCH_2CH_2$-), 138.57 (CH, H_2C=CHC-), 136.42 (C, H_2C=CHC-), 133.21 (C, -NCH_2CCHCH-), 132.86 (CH, -CH_2CH=CCH=CH_2), 122.17 & 121.61 (CH, -NCH_2CCHCH-), 112.53 (CH, -NCH_2CCHCH-), 111.96 (CH_2, H_2C=$CHCH$-), 80.00 (C, -$OC(CH_3)_3$), 73.04 (CH, Ar$OCHCH_2$-), 58.10 & 57.18 (CH_2, -NCH_2Ar), 56.21 (CH_3, ArOCH_3), 34.48 (CH_3, -NCH_3), 28.78 (CH_3, -$OC(CH_3)_3$), 28.56 (CH_2, Ar$OCHCH_2$-), 26.22 (CH_2, Ar$OCHCH_2CH_2CH_2CH$-), 18.11 (CH_2, Ar$OCHCH_2CH_2CH_2CH$-) ppm. -CI was not observed.

LRMS (ES^+) *m/z* 500 [$M+H^+$], 522 [$M+Na^+$].

HRMS (ES^+) for $C_{22}H_{30}INNaO_4$, calculated 522.1112, found 522.1111 Da.

***Tert*-butyl-3-((*S*)-oct-7-en-1-yn-3-yloxy)-2-iodo-4-methoxybenzylmethylcarbamate (3.87)**



$C_{22}H_{30}INO_4$
m.w. = 499.38 g/mol
Pale yellow oil
NMR spectra exhibited broadening / doubling of peaks due to restricted rotation

To a solution of phenolic ether **4.7** (800 mg, 1.40 mmol) in MeOH (8 mL) was added K_2CO_3 (213 mg, 1.54 mmol) in one portion. The heterogeneous mixture was stirred for 4 hours at rt giving a white opaque mixture. The reaction was quenched with a saturated solution of NH_4Cl (10 mL) then concentrated *in vacuo* before dissolving in EtOAc (30 mL) and H_2O (10 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3 x 40 mL). The combined organic phases were washed with brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving the title compound **3.87** as a pale yellow oil which required no further purification (675 mg, 1.35 mmol, 97%).

$[\alpha]_D = -1.1$ (*c* 0.5, $CHCl_3$, 26 °C).

FT-IR (neat) ν_{max} 3290 (w), 3236 (w), 3076 (w), 2974 (w), 2932 (w), 2866 (w), 2838 (w), 2111 (w), 1690 (s), 1640 (w), 1589 (m), 1476 (m) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92-6.80 (2H, m, -C**H**C**H**COMe), 5.87 (1H, ddt, *J* = 17.1, 10.3, 6.7 Hz, H_2C=C**H**-), 5.23 (1H, br t, *J* = 5.5 Hz, ArOC**H**CH_2-), 5.07 (1H, dq, *J* = 17.1, 1.8 Hz, **H_a**), 5.00 (1H, ddt, *J* = 10.3, 2.0, 1.3 Hz, **H_b**), 4.60-4.30 (2H, br, -NC**H_2**-), 3.85 (3H, s, ArOC**H_3**), 2.86 (3H, br s, -NC**H_3**), 2.35 (1H, d, *J* = 2.0 Hz, -C≡C**H**), 2.18 (2H, q, *J* = 7.0 Hz, H_2C=CHC**H_2**-), 2.07-2.00 (2H, m,

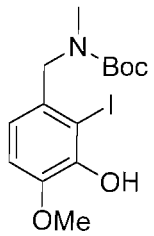
ArOCHC**H**$_2$-), 1.88-1.65 (2H, m, ArOCHCH$_2$C**H**$_2$-), 1.50 & 1.43 (9H, br s, -OC(C**H**$_3$)$_3$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 156.41 (C, -**C**=O), 151.66 (C, -**C**OMe), 146.34 (C, -**C**OCHCH$_2$), 138.84 (CH, H$_2$C=**C**H-), 133.15 (C, -NCH$_2$**C**CH-), 122.95 (CH, -NCH$_2$C**C**H-), 115.12 (CH$_2$, H$_2$**C**=CH-), 112.69 (CH, -NCH$_2$C**C**H-), 82.25 (C, -**C**≡CH), 80.06 (C, -**C**(CH$_3$)$_3$), 75.54 (CH, -C≡**C**H), 71.55 (CH, ArO**C**HCH$_2$-), 57.91 & 57.03 (CH$_2$, -N**C**H$_2$-), 56.39 (CH$_3$, ArO**C**H$_3$), 35.68 (CH$_2$, ArOCH**C**H$_2$-), 34.56 (CH$_3$, -N**C**H$_3$), 33.74 (H$_2$C=CH**C**H$_2$-), 28.78 (CH$_3$, -OC(**C**H$_3$)$_3$), 24.80 (CH$_2$, H$_2$C=CHCH$_2$**C**H$_2$-) ppm. -**C**I was not observed.

LRMS (ES$^+$) *m/z* 500 [M+H$^+$], 522 [M+Na$^+$].

HRMS (ES$^+$) for $C_{22}H_{30}INNaO_4$, calculated 522.1112, found 522.1119 Da.

***Tert*-butyl-3-hydroxy-2-iodo-4-methoxybenzylmethylcarbamate (3.89)**



$C_{14}H_{20}INO_4$

m.w. = 393.22 g/mol

White Solid

NMR spectra exhibited broadening due to restricted rotation

To a suspension of aldehyde **3.70** (2.00 g, 7.19 mmol) in MeOH (10 mL) was added methylamine (2.0 M in methanol, 7.19 mL, 14.39 mmol) dropwise producing a red solution which was stirred for 20 hours leading to an orange suspension. The reaction was concentrated *in vacuo* then redissolved in MeOH (100 mL). To the solution were added 4 Å molecular sieves and the solution was stirred 15 minutes before the addition of $NaBH_4$ (598 mg, 15.81 mmol). The resultant amber solution was stirred for 22 hours before concentration *in vacuo* giving a beige solid. The solid was dissolved in dioxane

(35 mL) and 1 N NaOH (18 mL) before the addition of $(Boc)_2O$ (1.82 mL, 7.91 mmol) and the reaction was stirred for 16 hours. The crude mixture was passed through a pad of celite flushing with EtOAc and the phases were separated. The aqueous was extracted with EtOAc (4 x 30 mL) then CH_2Cl_2 (3 x 30 mL). The combined organic phases were dried ($MgSO_4$) and concentrated *in vacuo* giving a brown solid. Purification by flash column chromatography on silica gel (4.0 cm x 6 cm) eluting with 30% EtOAc/hexane afforded the desired Boc protected benzylic amine **3.89** as a white solid (2.47 g, 6.28 mmol, 87%).

Mpt 108-111 °C.

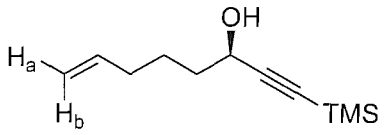
FT-IR (neat) ν_{max} 3337 (br), 3003 (w), 2974 (w), 2935 (w), 2839 (w), 1678 (s), 1597 (w), 1481 (s) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.83 (1H, d, J = 6.8 Hz, -C**H**$COCH_3$), 6.71 (1H, m, -C**H**$CHCOCH_3$), 6.24 (1H, br s, -O**H**), 4.42 (2H, br s, -NCH_2**$_2$**Ar), 3.90 (3H, s, ArOCH_3), 2.85 (3H, br s, -NCH_3), 1.45 (9H, br s, -$OC(CH_3)_3$) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 156.32 (C, -**C**=O), 145.94 (C, -**C**OCH_3), 145.29 (C, -**C**OH), 132.76 (C, -NCH_2**C**-), 119.17 & 118.57 (CH, -NCH_2C**C**HCH-), 110.73 (C, -NCH_2CCH**C**H-), 80.07 (C, -O**C**$(CH_3)_3$), 57.42 (CH_2, -N**C**H_2C-), 56.69 (CH_3, -CO**C**H_3), 34.53 (CH_3, -N**C**H_3), 28.76 (CH_3, -OC(**C**$H_3)_3$) ppm. -**C**I was not observed.

LRMS (ES^+) *m/z* 416 [$M+Na^+$].

(*R*)-1-(Trimethylsilyl)oct-7-en-1-yn-3-ol (3.90)



$C_{11}H_{20}OSi$

m.w. = 196.36 g/mol

Colourless oil

Ketone **3.92** (1.00 g, 5.15 mmol) was cooled to 0 °C before treatment with *R*-Alpine borane (0.5 M in THF, 20.6 mL, 10.3 mmol) followed by immediate concentrated *in vacuo* giving a yellow oil. The oil was stirred for 18 hours at rt before quenching at 0 °C with acetaldehyde (1.5 mL). The mixture was stirred for 15 minutes at 0 °C before Et_2O (50 mL) and ethanol amine (0.68 mL, 11.32 mmol) were added. The reaction mixture was warmed to rt and stirred for 1.5 hours producing a white precipitate and yellow solution. The heterogeneous mixture was filtered through a pad of celite, flushing with Et_2O. The organic phase was washed with H_2O (10 mL) and brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving a yellow oil. Purification by flash column chromatography on silica gel (5.0 cm x 8 cm) eluting with 5% EtOAc/hexane afforded the desired alcohol **3.90** as a colourless oil (901 mg, 4.59 mmol, 89%). The enantiomeric excess was estimated to be 92% *ee* from chiral HPLC analysis (OD-H chiral column) of phenyl ether derivative prepared by Mitsunobu coupling with benzaldehyde **3.70**. NMR data for alcohol **3.90** are consistent with those reported in the literature for the racemic material.[181]

$[\alpha]_D$ +1.5 (*c* 1.0, $CHCl_3$, 22 °C, 92% *e.e.*).

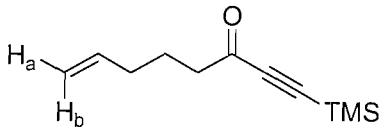
FT-IR (neat) ν_{max} 3331 (br), 2957 (w), 2172 (w), 1641 (w), 1250 (m) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82 (1H, ddt, *J* = 17.1, 10.3, 6.7 Hz, H_2C=C**H**-), 5.04 (1H, dq, *J* = 17.1, 1.8 Hz, $\mathbf{H_b}$), 4.97 (1H, ddt, *J* = 10.3, 2.0, 1.1 Hz, $\mathbf{H_a}$), 4.37 (1H, q, *J* = 7.0 Hz, -C**H**OH), 2.11 (2H, qt, *J* = 6.8, 1.5 Hz, H_2C=CHC$\mathbf{H_2}$-), 1.77-1.67 (2H, m, -C$\mathbf{H_2}$C(OH)-), 1.64-1.50 (2H, m, H_2C=CHCH_2C$\mathbf{H_2}$-), 0.18 (9H, s, -Si(C$\mathbf{H_3}$)$_3$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 138.76 (CH, H_2C=**C**H-), 115.13 (CH_2, H_2**C**=CH-), 107.10 (C, -**C**≡CTMS), 89.83 (C, -C≡**C**TMS), 63.12 (CH, -**C**OH), 37.45 (CH_2, -**C**H_2C(OH)-), 24.71 (CH_2, H_2C=CHCH_2**C**H_2-), 0.23 (CH_3, -Si(**C**H_3)$_3$) ppm.

LRMS (ES^+) *m/z* 219 [$M+Na^+$], 431 [$2M+K^+$].

1-(Trimethylsilyl)oct-7-en-1-yn-3-one (3.92)



$C_{11}H_{18}OSi$
m.w. = 194.35 g/mol
Colourless oil

A solution of TMS acetylene (3.456 g, 35.19 mmol) in THF (110 mL) at –40 °C was treated with *n*-BuLi (2.28 M in hexane, 15.43 mL, 35.19 mmol) dropwise. This solution was stirred for 2 hours allowing the mixture to warm to 0 °C. The mixture was cooled to –10 °C before the dropwise addition of a solution of Weinreb amide **4.5** (3.688 g, 23.46 mmol) in THF (28 mL) giving a colourless solution. After stirring at –10 °C for 3 hours the reaction was quenched with a saturated solution of NH_4Cl (30 mL). The phases were separated before the aqueous phase was extracted with Et_2O (3 x 25 mL). The combined organic phases were washed with brine (15 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving a yellow oil. Purification by flash column chromatography on silica gel (5.0 cm x 9 cm) eluting with CH_2Cl_2 gave the desired ketone **3.92** as a colourless oil (3.879 g, 19.93 mmol, 85%).

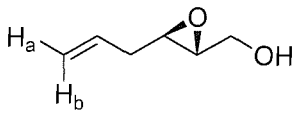
FT-IR (neat) ν_{max} 3078 (w), 2961 (w), 1676 (s), 1642 (m) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.78 (1H, ddt, *J* = 17.1, 10.3, 6.7 Hz, H_2C=C**H**-), 5.08-4.97 (2H, m, **H_2**C=CH-), 2.57 (2H, t, *J* = 7.3 Hz, TMSC≡CC(O)C**H_2**-), 2.10 (2H, br q, *J* = 7.0 Hz, H_2C=CHC**H_2**-), 1.78 (2H, qn, *J* = 7.3 Hz, H_2C=CHCH_2C**H_2**-), 0.25 (9H, s, -Si(C**H_3**)$_3$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 187.97 (C, -**C**=O), 137.95 (CH, H_2C=**C**H-), 115.83 (CH_2, H_2**C**=CH-), 102.40 (C, -**C**≡CTMS), 98.05 (C, -C≡**C**TMS), 44.83 (CH_2, TMSC≡CC(O)**C**H_2-), 33.15 (CH_2, H_2C=CH**C**H_2-), 23.37 (CH_2, H_2C=CHCH_2**C**H_2-), -0.41 (CH_3, -Si(**C**H_3)$_3$) ppm.

LRMS (ES^+) *m/z* 195 [$M+H^+$].

((2*R*,3*R*)-3-Allyloxiran-2-yl)methanol (3.97)



$C_6H_{10}IO_2$

m.w. = 114.14 g/mol

Colourless oil

Epoxide **3.97** was prepared by a method described by Ma *et al.*[159] To a suspension of 4 Å molecular sieves (100 mg) in CH_2Cl_2 (18 mL) at –20 °C was added *D*-DET (0.11 mL, 0.61 mmol), Ti(^{i}OPr)$_4$ (0.14 mL, 0.51 mmol) and ^{t}BuOOH (2.04 mL, 10.19 mmol). The mixture was stirred for 1 hour at –20 °C before the addition of a solution of alcohol **3.98** (500 mg, 5.09 mmol) in CH_2Cl_2 (1 mL). The reaction was stirred for 16 hours at –20 °C. Ti(^{i}OPr)$_4$ (0.14 mL, 0.51 mmol) was added to the reaction and stirring was continued for a further 3 hours at –20 °C before being quenched with 15% NaOH (0.4 mL), $MgSO_4$ and celite. The quenched reaction was filtered through a pad of celite then concentrated *in vacuo* giving a pale yellow oil. Purification by flash column chromatography on silica gel (4.0 cm x 7 cm) eluting with 30% EtOAc/hexane afforded the desired epoxide **3.97** as a colourless oil (247 mg, 2.16 mmol, 43%). Spectroscopic details were consistent with the literature.[159]

$[\alpha]_D$ = +25.0 (*c* 0.5, $CHCl_3$, 24 °C), (Lit: + 23.2, *c* 10.0, MeOH, 20 °C).[182]

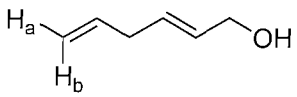
FT-IR (neat) ν_{max} 3416 (br), 3082 (w), 2982 (m), 2916 (w), 1642 (m), 1417 (m) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82 (1H, ddt, *J* = 17.1, 10.3, 6.8 Hz, H_2C=C**H**-), 5.16 (1H, dq, *J* = 17.1, 1.5 Hz, $\mathbf{H_b}$), 5.12 (1H, dq, *J* = 10.3, 1.5 Hz, $\mathbf{H_a}$), 3.92 (1H, br d, *J* = 12.8 Hz, -C**H**$_2$OH), 3.65 (1H, dt, *J* = 12.8, 4.5 Hz, -C**H**$_2$OH), 3.06 (1H, td, *J* = 5.4, 2.3 Hz, -C**H**(O)CHCH$_2$OH), 2.97 (1H, dt, *J* = 4.5, 2.3 Hz, -CH(O)C**H**CH$_2$OH), 2.37 (2H, ddt, *J* = 6.8, 5.4, 1.5 Hz, H_2C=CHC$\mathbf{H_2}$-), 1.86 (1H, br s, -O**H**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 133.11 (CH, H_2C=**C**H-), 118.04 (CH_2, H_2**C**=CH-), 61.89 (CH_2, -**C**H$_2$OH), 58.20 (CH, -CH(O)**C**HCH$_2$OH), 55.06 (CH, -**C**H(O)CHCH$_2$OH), 35.93 (CH_2, H_2C=CH**C**H$_2$-) ppm.

LRMS (ES^+) *m/z* 480 [$4M+Na^+$].

(*E*)-Hexa-2,5-dien-1-ol (3.98)



$C_6H_{10}O$

m.w. = 98.14 g/mol

Colourless oil

Allylic alcohol **3.98** was prepared by a method described by Rao *et al.*[158] To a grey suspension of $LiAlH_4$ (2.16 g, 56.91 mmol) in THF (150 mL) was added a solution of alcohol **4.12** (4.21 g, 56.91 mmol) in THF (50 mL) dropwise. The mixture was heated to reflux and stirred for 4 hours before the reaction was cooled to rt then quenched with H_2O (2.2 mL), 15% NaOH (2.2 mL) and H_2O (6.6 mL) sequentially. Stirring for 20 minutes produced a white precipitate. The heterogeneous mixture was dried ($MgSO_4$) and the precipitate was removed by filtration through a pad of celite. The filtrate was concentrated *in vacuo* giving a yellow oil. Purification by distillation under reduced pressure (10 mbar, 50-60 °C) gave the desired product **3.98** as a colourless oil (3.65 g, 37.15 mmol, 85%). Spectroscopic details were consistent with the literature.[158]

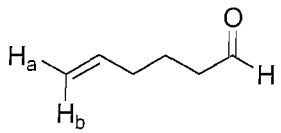
FT-IR (neat) ν_{max} 3314 (br), 2871 (m), 1638 (m), 1429 (m), 1087 (m) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.83 (1H, ddt, J = 17.1, 10.3, 6.5 Hz, H_2C=C**H**-), 5.77-5.63 (2H, m, -CH_2C**H**=C**H**CH_2-), 5.06 (1H, dq, J = 17.1, 1.8 Hz, $\mathbf{H_b}$), 5.02 (1H, ddt, J = 10.3, 1.8, 1.5 Hz, $\mathbf{H_b}$), 4.11 (2H, d, J = 4.5 Hz, -C**H$_2$**OH), 2.84-2.77 (2H, m, H_2C=CHC**H$_2$**CH=CH-) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 136.64 (CH, H_2C=**C**H-), 130.84 (CH, -CH_2**C**H=**C**HCH_2-), 130.44 (CH, -CH_2**C**H=**C**HCH_2-), 115.88 (CH_2, H_2**C**=CH-), 63.92 (CH_2, -**C**H_2OH), 36.62 (CH_2, H_2C=CH**C**H_2CH=CH-) ppm.

LRMS (CI, NH_3) *m/z* 81 [M-H_2O+H^+], 98 [M-H_2O+NH_4^+], 116 [M+NH_4^+].

Hex-5-enal (4.2)



$C_6H_{10}O$
m.w. = 98.14 g/mol
Colourless oil

Aldehyde was prepared by a method described by Meyer *et al.*[151] To a suspension of PCC (16.14 g, 74.88 mmol) in CH_2Cl_2 (125 mL) was added hex-5-en-1-ol (6.00 mL, 49.92 mmol) dropwise. The resultant black solution was stirred for 4 hours before dilution with Et_2O (200 mL) and trituration. The solution was decanted and the remaining black gum was triturated with Et_2O (3 x 50 mL). The combined solutions were filtered through a plug of silica before the solvents were removed by distillation at atmospheric pressure. Purification by distillation under reduced pressure (10 mbar, 40-45 °C) provided the desired aldehyde **4.2** as a colourless oil (3.44 g, 35.03 mmol, 70%). Spectroscopic details are consistent with those reported in the literature.[183]

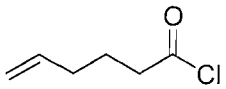
FT-IR (neat) ν_{max} 3078 (w), 2936 (w), 2824 (w), 2721 (w), 1722 (s), 1641 (m), 912 (s) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.78 (1H, t, J = 1.6 Hz, -C**H**O), 5.78 (1H, ddt, J = 17.0, 10.2, 6.7 Hz, -C**H**=CH_2), 5.03 (1H, dq, J = 17.0, 2.0 Hz, $\mathbf{H_b}$), 5.01 (1H, ddt, J = 10.2, 2.0, 1.2 Hz, $\mathbf{H_a}$), 2.45 (2H, dt, J = 7.3, 1.6 Hz, -C$\mathbf{H_2}$CHO), 2.11 (2H, br q, J = 6.8 Hz, H_2C=CHC$\mathbf{H_2}$-), 1.74 (2H, qn, J = 7.1 Hz, -C$\mathbf{H_2}CH_2CHO$) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 202.70 (CH, -**C**HO). 137.89 (CH, H_2C=**C**H-), 115.89 (CH_2, H_2**C**=CH-), 43.45 (CH_2, -**C**H_2CHO), 33.30 (CH_2, H_2C=CH**C**H_2-), 21.55 (CH_2, H_2C=$CHCH_2$**C**H_2-) ppm.

LRMS (ES^+) *m/z* 140 [$M+H^+$+MeCN].

Hex-5-enoyl chloride (4.4)



C_6H_9ClO
m.w. = 132.59 g/mol
Pale yellow oil

Acid chloride **4.4** was prepared by a method reported by Ahrendt *et al.*[154] To hexenoic acid **4.3** (400 mg, 3.50 mmol) was added one drop of DMF, followed by the slow dropwise addition of oxalyl chloride (0.37 mL, 4.20 mmol). After the cessation of gas evolution, the reaction was stirred for 20 minutes before it was diluted with Et_2O (10 mL) and filtered. The filtrate was concentrated *in vacuo* giving the desired acid chloride as a pale yellow oil **4.4** (349 mg, 2.63 mmol, 75%). Spectroscopic details are consistent with those reported in the literature.[154]

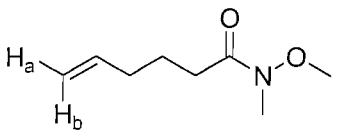
FT-IR (neat) ν_{max} 3079 (w), 2937 (w), 2865 (w), 1798 (s), 1747 (m), 1642 (w) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.76 (1H, ddt, J = 17.0, 10.3, 6.8 Hz, H_2C=C**H**CH_2-), 5.11-5.02 (2H, m, **H_2**C=CHCH_2-), 2.91 (2H, t, J = 7.2 Hz, -C**H_2**C(O)Cl), 2.14 (2H, q, J = 7.1 Hz, H_2C=CHC**H_2**-), 1.83 (2H, qn, J = 7.3 Hz, -**CH_2**CH_2C(O)Cl) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 174.04 (C, -CH_2**C**(O)Cl), 136.99 (CH, H_2C=**C**HCH_2-), 116.60 (CH_2, H_2**C**=CH-), 46.58 (CH_2, -**C**H_2C(O)Cl), 32.59 (CH_2, H_2C=CH**C**H_2-), 24.45 (CH_2, -**C**H_2CH_2C(O)Cl) ppm.

LRMS (EI) *m/z* (relative intensity) 97 (69%) [M·-^{35}Cl].

***N*-Methoxy-*N*-methylhex-5-enamide (4.5)**



$C_8H_{15}NO_2$
m.w. = 157.21 g/mol
Colourless oil

To a solution of hexenoic acid (200 mg, 1.75 mmol) in CH_2Cl_2 (10 mL) was added EDCI (503 mg, 2.63 mmol), DMAP, (321 mg, 2.63 mmol) and *N,O*-dimethylhydroxyamine hydrochloride (256 mg, 2.63 mmol). The resultant mixture was stirred for 2.5 hours before being quenched with brine (10 mL). The phases were separated and the aqueous phase extracted with EtOAc (3 x 20 mL). The combined organic phases were washed with 2 M HCl (10 mL) and brine (10 mL) before being dried ($MgSO_4$) and concentrated *in vacuo* giving a pale yellow oil. Purification by flash column chromatography on silica gel (3.0 cm x 6 cm) eluting with 40% EtOAc/hexane yielded the desired Weinreb amide **4.5** as a colourless oil (257 mg, 1.63 mmol, 93%).

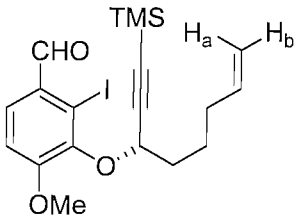
FT-IR (neat) ν_{max} 3077 (w), 2938 (w), 1661 (s) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81 (1H, ddt, *J* = 17.1, 10.3, 6.8 Hz, H_2C=C**H**-), 5.04 (1H, dq, *J* = 17.1 Hz, 2.0 Hz, $\mathbf{H_a}$), 4.98 (1H, ddt, *J* = 10.3, 2.0, 1.3 Hz, $\mathbf{H_b}$), 3.68 (3H, s, -NOC**H**$_3$), 3.18 (3H, s, -NC**H**$_3$), 2.43 (2H, t, *J* = 7.5 Hz, -C**H**$_2$C(O)N-), 2.12 (2H, q, *J* = 7.1 Hz, H_2C=CHC**H**$_2$-), 1.75 (2H, qn, *J* = 7.5 Hz, H_2C=CHCH$_2$C**H**$_2$-) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 181.92 (C, -**C**=O), 138.49 (CH, H_2C=**C**H-), 115.38 (CH_2, H_2**C**=CH-), 61.54 (CH_3, -NO**C**H$_3$), 33.64 (CH_2, H_2C=CH**C**H$_2$-), 32.60 (CH_3, -N**C**H$_3$), 31.49 (CH_2, -**C**H$_2$C(O)N-), 24.05 (CH_2, H_2C=CHCH$_2$**C**H$_2$-) ppm.

LRMS (ES^+) *m/z* 180 [$M+Na^+$], 221 [$M+MeCN+Na^+$].

3-((*S*)-1-trimethylsilyl)oct-7-en-1-yn-3-yloxy)-2-iodo-4-methoxybenzaldehyde (4.6)



$C_{19}H_{25}IO_3Si$

m.w. = 456.39 g/mol

Colourless oil

To a solution of aldehyde **3.89** (200 mg, 0.72 mmol) in THF (6 mL) was added PPh_3 (315 mg, 1.20 mmol) and a solution of alcohol **3.90** (118 mg, 0.60 mmol) in THF (1.5 mL) dropwise. The resultant mixture was stirred for 5 minutes before the addition of DIAD (0.24 mL, 1.20 mmol) dropwise. The mixture was heated to reflux and stirred for 14 hours before the reaction was concentrated *in vacuo* giving a yellow oil. Purification by flash column chromatography on silica gel (2.0 cm x 9 cm) eluting with 3% EtOAc/hexane gave the desired phenolic ether as a colourless oil **4.6** (91 mg, 0.20 mmol, 33%, 92% *ee*).

$[\alpha]_D$ +30.0 (*c* 0.6, $CHCl_3$, 21 °C).

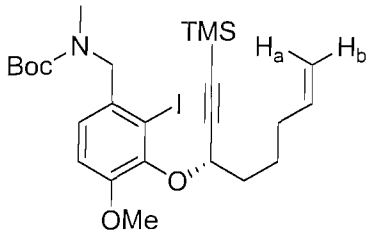
FT-IR (neat) ν_{max} 2953 (w), 2843 (w), 1683 (s), 1640 (w), 1573 (s), 1475 (m) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.07 (1H, s, -C**H**O), 7.72 (1H, d, *J* = 8.6 Hz, CHOCC**H**CH-), 6.96 (1H, d, *J* = 8.6 Hz, CHOCCHC**H**-), 5.88 (1H, ddt, *J* = 17.0, 10.3, 6.6 Hz, H_2C=C**H**-), 5.22 (1H, t, *J* = 6.4 Hz, ArOC**H**CH_2-), 5.08 (1H, dq, *J* = 17.0, 1.7 Hz, $\mathbf{H_a}$), 5.01 (1H, ddt, *J* = 10.3, 2.0, 1.5 Hz, $\mathbf{H_b}$), 3.94 (3H, s, ArOC$\mathbf{H_3}$), 2.20 (2H, q, *J* = 7.0 Hz, -C$\mathbf{H_2}$CH=CH_2), 2.15-1.95 (2H, m, ArOCHC$\mathbf{H_2}$-), 1.85-1.65 (2H, m, ArOCHCH_2C$\mathbf{H_2}$-), 0.02 (9H, s, -Si(C$\mathbf{H_3}$)$_3$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 195.93 (CH, -**C**HO), 158.04 (C, -**C**OMe + CHOC**C**O-), 138.85 (CH, -**C**H=CH_2), 129.47 (C, CHO**C**-), 127.40 (CH, CHOC**C**HCH-), 115.15 (CH_2, -CH=**C**H_2), 111.85 (CH, CHOCCH**C**H-), 103.26 (C, -**C**I), 103.02 (C, -**C**≡CTMS), 93.61 (C, -C≡**C**TMS), 72.53 (CH, ArO**C**HCH_2), 56.50 (CH_3, ArO**C**H_3), 35.63 (CH_2, ArOCH**C**H_2-), 33.71 (CH_2, -**C**H_2CH=CH_2), 24.90 (CH_2, ArOCHCH_2**C**H_2-), -0.03 (CH_3, -Si(**C**H_3)$_3$) ppm.

LRMS (ES^+) *m/z* 479 [M+Na^+], 495 [M+K^+], 936 [2M+Na^+].

***Tert*-butyl-3-((*S*)-1-(trimethylsilyl)oct-7-en-1-yn-3-yloxy)-2-iodo-4-methoxybenzylmethylcarbamate (4.7)**



$C_{25}H_{38}INO_4Si$

m.w. = 571.56 g/mol

Colourless oil

NMR spectra exhibited broadening / doubling of peaks due to restricted rotation

To a solution of phenol **3.89** (774 mg, 1.97 mmol) in THF (18 mL) was added PPh_3 (938 mg, 3.58 mmol) and a solution of propargylic alcohol **3.90** (351 mg, 1.79 mmol) in THF (4 mL). This solution was stirred for 5 minutes before the dropwise addition of DIAD (0.70 mL, 3.58 mmol). The reaction was then heated to reflux and stirred for 3.5 hours. The reaction mixture was cooled and concentrated *in vacuo* giving a brown orange oil. Purification by flash column chromatography on silica gel (5.0 cm x 8 cm) eluting with 5%→10% EtOAc/hexane afforded the desired ether **4.7** as a colourless oil (835 mg, 1.46 mmol, 74%).

$[\alpha]_D$ = +11.5 (*c* 0.5, $CHCl_3$, 27 °C).

FT-IR (neat) ν_{max} 2957 (w), 2934 (w), 2865 (w), 2838 (w), 2175 (w), 1696 (s), 1640 (w), 1589 (w), 1476 (m), 1389 (m) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.87 (1H, br d, *J* = 6.7 Hz, -C**H**COMe), 6.81 (1H, m, -C**H**CHCOMe), 5.87 (1H, ddt, *J* = 17.1, 10.3, 6.7 Hz, H_2C=C**H**-), 5.20 (1H, m, ArOC**H**CH_2-), 5.07 (1H, dq, *J* = 17.1, 1.8 Hz, $\mathbf{H_a}$), 4.99 (1H, ddt, *J* = 10.3, 2.0, 1.3 Hz, $\mathbf{H_b}$), 4.46 (2H, br, -NC$\mathbf{H_2}$Ar-), 3.84 (3H, s, ArOC$\mathbf{H_3}$), 2.83 (3H, br, -NC$\mathbf{H_3}$), 2.18 (2H, br q, *J* = 6.7 Hz, H_2C=CHC$\mathbf{H_2}$-), 2.10-1.93 (2H, m, ArOCHC$\mathbf{H_2}$-), 1.86-1.65 (2H, m, ArOCHCH_2C$\mathbf{H_2}$-),

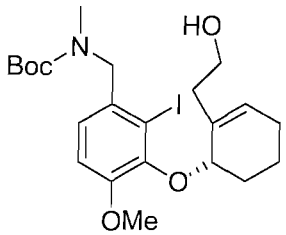
1.51 & 1.44 (9H, br s, -OC(CH_3)$_3$), 0.04 (9H, s, -Si(CH_3)$_3$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 156.34 (C, -**C**=O), 151.83 (C, -**C**OMe), 146.63 (C, -**C**OCHCH$_2$), 138.98 (CH, H_2C=**C**H-), 132.71 (C, -NCH$_2$**C**CH-), 123.52 & 122.49 (CH, -NCH$_2$C**C**H-), 115.00 (CH_2, H_2**C**=CH-), 112.46 (CH, -NCH$_2$CCH**C**H-), 104.07 (C, -**C**≡CTMS), 92.60 (C, -C≡**C**TMS), 80.06 (C, -**C**(CH_3)$_3$), 72.21 (CH, ArO**C**H-), 57.99 & 56.92 (CH_2, -N**C**H$_2$-), 56.36 (CH_3, ArO**C**H$_3$), 35.67 (CH_2, ArOCH**C**H$_2$), 34.44 (CH_3, -N**C**H$_3$), 28.79 (CH_3, -C(**C**H$_3$)$_3$), 24.90 (CH_2, H_2C=CHCH$_2$**C**H$_2$-), 0.06 (CH_3, -Si(**C**H$_3$)$_3$) ppm. -**C**I was not observed.

LRMS (ES$^+$) *m/z* 572 [M+H$^+$], 594 [M+Na$^+$].

HRMS (ES$^+$) for $C_{25}H_{38}INNaO_4Si$, calculated 594.1507, found 594.1504 Da.

***Tert*-butyl-3-((*S*)-2-(2-hydroxyethyl)cyclohex-2-enyloxy)-2-iodo-4-methoxybenzylmethylcarbamate (4.8)**



$C_{22}H_{32}INO_5$
m.w. = 517.40 g/mol
Colourless oil
NMR spectra exhibited broadening / doubling of peaks due to restricted rotation

To a solution of diene **3.85** (436 mg, 0.87 mmol) in THF (6 mL) was added 9-BBN (0.5 M in THF, 2.62 mL, 1.31 mmol) dropwise, the resultant pale yellow solution was stirred for 17 hours. To the mixture was added 3 M NaOH (5 mL) followed by H_2O_2 (5 mL) leading to an exothermic reaction. The reaction was stirred for 3 hours before the

addition of H_2O (15 mL). The mixture was extracted with Et_2O (4 x 25 mL) and the combined organic phases were washed with brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving a colourless oil. Purification by flash column chromatography on silica gel (3.5 cm x 9 cm) eluting with 50% EtOAc/hexane gave the desired alcohol **4.8** as a colourless oil (410 mg, 0.79 mmol, 91%).

$[\alpha]_D = -50.4$ (*c* 1.2, $CHCl_3$, 26 °C).

FT-IR (neat) ν_{max} 3436 (br), 2973 (w), 2933 (m), 2867 (w), 2835 (w), 1678 (s), 1586 (m), 1474 (m) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.89 (1H, d, *J* = 8.5 Hz, -$NCH_2CC\mathbf{H}CH$-), 6.81 (1H, br d, *J* = 8.5 Hz, -$NCH_2CCHC\mathbf{H}$-), 5.84 (1H, dd, *J* = 3.8, 3.5 Hz, -$ArOCHCH_2CH_2CH_2C\mathbf{H}$-), 5.07 (1H, br t, *J* = 4.1 Hz, $ArOC\mathbf{H}CH_2$-), 4.41 (2H, br, -$NC\mathbf{H_2}Ar$), 3.85 (3H, s, $ArOC\mathbf{H_3}$), 3.84-2.70 (2H, m, -$C\mathbf{H_2}OH$), 2.86 (3H, br, -$NC\mathbf{H_3}$), 2.66 (1H, dt, *J* = 13.9, 5.3 Hz, -$C\mathbf{H_2}CH_2OH$), 2.46 (1H, dt, *J* = 13.9, 6.5 Hz, -$C\mathbf{H_2}CH_2OH$), 2.20 (1H, m, -$C\mathbf{H_2}$-), 2.12-1.97 (2H, m, -$C\mathbf{H_2}$-), 1.96-1.84 (2H, m, -$C\mathbf{H_2}$-), 1.60-1.34 (10H, m, -$C\mathbf{H_2}$- and -$OC(C\mathbf{H_3})_3$) ppm.

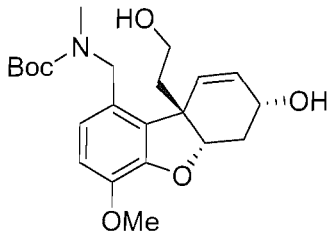
^{13}C NMR (100 MHz, $CDCl_3$) δ 156.29 (C, -**C**=O), 151.23 (C, -**C**OCH_3), 146.61 (C, -**C**$OCHCH_2CH_2CH_2$-), 134.52 (C, -**C**CH_2=CH_2OH), 133.23 (C, -NCH_2**C**-), 130.53 (CH, -**C**$HCCH_2CH_2OH$), 122.43 & 122.05 (CH, -NCH_2C**C**HCH-), 112.66 (CH, -NCH_2CCH**C**H-), 80.08 (C, -**O**$C(CH_3)_3$), 77.01 (CH, ArO**C**HCH_2CH_2-), 62.15 (CH_2, -**C**H_2OH), 58.20 & 57.36 (CH_2, -N**C**H_2Ar), 56.16 (CH_3, $ArOCH_3$), 38.40 (CH_2, -**C**H_2CH_2OH), 34.64 (CH_3, -N**C**H_3), 28.77 (CH_3, -$OC(\mathbf{C}H_3)_3$), 28.51 (CH_2, $ArOCH\mathbf{C}H_2CH_2CH_2$-), 25.89 ($CH_2$,

ArOCHCH$_2$CH$_2$**C**H$_2$-), 19.06 (CH$_2$, ArOCHCH$_2$**C**H$_2$CH$_2$-) ppm. -**C**I was not observed.

LRMS (ES$^+$) *m/z* 540 [M+Na$^+$].

HRMS (ES$^+$) for $C_{22}H_{32}INNaO_5$, calculated 540.1217, found 540.1230 Da.

***Tert*-butyl-*N*-[((5a*S*,7*R*,9a*S*)-7-hydroxy-9a-(2-hydroxyethyl)-4-(methyloxy)-5a,6,7,9a-tetrahydrodibenzo[*b,d*]furan-1-yl)methyl]-*N*-methylcarbamate (4.9)**



$C_{22}H_{31}NO_6$

m.w. = 405.48 g/mol

Peach oil

NMR spectra exhibited broadening / doubling of peaks due to restricted rotation

To a solution of cyclohexene **3.81** (25 mg, 64.19 μmol) in dioxane (0.6 mL) was added NaH_2PO_4 (8 mg, 64.19 μmol), SeO_2 (7 mg, 64.19 μmol) and quartz sand (29 mg). The yellow heterogeneous mixture was flushed with argon and sealed before heating to 150 °C and stirring for 1 hour. A further addition of SeO_2 (7 mg, 64.19 μmol) and quartz sand (29 mg) was made and the tube was flushed with argon, resealed then heated to 150 °C for an additional 1 hour. A final addition of SeO_2 (7 mg, 64.19 μmol) and quartz sand (29 mg) was made before the tube was flushed with argon, resealed then heated to 150 °C for a further 1 hour. After cooling the reaction mixture was passed through a plug of celite eluting with EtOAc and the resulting solution was concentrated *in vacuo* giving an orange oil. The oil was dissolved in CH_2Cl_2 (30 mL) and washed with 1 N NaOH (10 mL). The aqueous phase was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic was washed with a mixture of brine (5 mL) and H_2O (5 mL), dried (Na_2SO_4) and concentrated *in vacuo* giving a brown oil. Purification by flash column chromatography on silica gel (1.5 cm x 6 cm) eluting with 50% EtOAc/hexane gave the desired diol **4.9** (16 mg, 39.46 μmol, 61%, dr = 4.8:1) as an unseparated

mixture of diastereoisomers. Data were obtained from the mixture of diastereoisomers (4.8:1).

$[\alpha]_D = -0.1$ (*c* 0.35, $CHCl_3$, 26 °C).

FT-IR (neat) ν_{max} 3399 (br), 2931 (m), 1670 (s), 1623 (m), 1581 (w), 1505 (m) cm^{-1}.

For major diastereoisomer:

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.75 (1H, d, *J* = 8.5 Hz, -NCH_2CCHCH-), 6.64 (1H, d, *J* = 8.3 Hz, -NCH_2CCHCH-), 5.96 (1H, dd, *J* = 10.0, 4.0 Hz, -CCH=C**H**-), 5.85 (1H, d, *J* = 10.0 Hz, -CC**H**=CH-), 4.90 (1H, dd, *J* = 6.3, 3.4 Hz, Ar$OCHCH_2$-), 4.55 (1H, d, *J* = 15.8 Hz, -NCH_2Ar), 4.46 (1H, d, *J* = 15.8 Hz, -NCH_2Ar), 4.20 (1H, dt, *J* = 4.6, 4.0 Hz, Ar$OCHCH_2CH$(OH)-), 3.85 (3H, s, ArOCH_3), 3.72-3.58 (2H, m, -CH_2CH_2OH for both diastereoisomers), 2.81 (3H, s, -NCH_3), 2.31 (1H, ddd, *J* = 14.3, 6.3, 4.6 Hz, Ar$OCHCH_2CH$(OH)-), 2.22-2.12 (2H, m, Ar$OCHCH_2CH$(OH)- and -CH_2CH_2OH), 1.96 (1H, dt, *J* = 14.3, 7.0 Hz, -CH_2CH_2OH), 1.47 (9H, s, -$OC(CH_3)_3$) ppm.

Selected data for minor diastereoisomer:

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.60 (1H, d, *J* = 8.3 Hz, -NCH_2CCHCH-), 5.04 (1H, dd, *J* = 5.4, 4.0 Hz, Ar$OCHCH_2$-), 2.44 (1H, dt, *J* = 5.4 Hz, -CH_2CH(OH)-), 1.89 (1H, ddd, *J* = 13.5, 8.0, 3.5 Hz, -CH_2CH_2OH) ppm.

For major diastereoisomer:

^{13}C NMR (100 MHz, $CDCl_3$) δ 156.34 (C, -C=O), 147.15 (C, -$COCHCH_2$-), 144.91 (C, -COMe), 130.02 (CH, -CCH=CH-), 129.80 (CH, -CCH=CH-), 126.34 (CH, -NCH_2CCHCH-), 121.14

(CH, br, -NCH_2**C**$CHCH$-), 111.82 (CH, -NCH_2CCH**C**H-), 85.15 (CH, Ar**O**$CHCH_2$-), 80.42 (C, -**O**$C(CH_3)_3$), 63.16 (CH, $ArOCHCH_2$**C**H(OH)-), 59.59 (CH_2, -CH_2**C**H_2OH), 56.23 (CH_3, -CO**C**H_3), 49.88 (C, -**C**CH_2CH_2OH), 49.11 (CH_2, -N**C**H_2Ar), 39.25 (CH_2, -**C**H_2CH_2OH), 34.27 (CH_3, -N**C**H_3), 32.67 (CH_2, $ArOCH$**C**H_2-), 28.77 (CH_3, -OC(**C**$H_3)_3$) ppm. -NCH_2**C**C- was not observed.

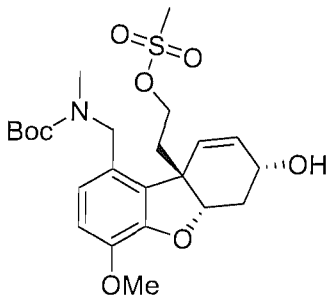
Selected data for minor diastereoisomer:

^{13}C NMR (100 MHz, $CDCl_3$) δ 111.60 (CH, -NCH_2CCH**C**H-), 84.24 (CH, $ArOCH$**C**H_2-), 62.92 (CH, -**C**H(OH)-), 59.90 (CH_2, -CH_2**C**H_2OH), 49.68 (C, -**C**CH=CH-) ppm.

LRMS (ES^+) *m/z* 428 [$M+Na^+$].

HRMS (ES^+) for $C_{22}H_{31}NNaO_6$, calculated 428.2044, found 428.2041 Da.

2-[(3*R*4a*S*,9b*S*)-9-([[(1,1-Dimethylethyl)oxy]carbonyl)(methyl)amino]methyl)-3-hydroxy-6-(methyloxy)-3,4,4a,9b-tetrahydrodibenzo[*b*,*d*]furan-9-yl]ethyl methanesulfonate (4.10)



$C_{23}H_{33}NO_8S$

m.w. = 483.58 g/mol

Colourless oil

NMR spectra exhibited broadening / doubling of peaks due to restricted rotation

To a solution of diols **4.9** (30 mg, 73.90 μmol, *dr* = 4.8:1) in THF (1.6 mL) at 0 °C was added Et_3N (11 μL, 78.30 μmol) and MsCl (6 μL, 78.30 μmol) dropwise. The beige cloudy mixture was stirred for 1 hour at 0 °C before the addition of Et_3N (21 μL, 147.8 μmol) and the reaction was stirred for a further 4.5 hours at 0 °C. After this time a

further addition of MsCl (2 μL, 26.10 μmol) was made and the reaction was warmed to rt then stirred for 2 hours. After which two further additions of MsCl (2 μL, 26.10 μmol) at 2 hours and 48 hours were made respectively. H_2O (5 mL) was added and the mixture was extracted with EtOAc (4 x 10 mL), the combined organic was dried ($MgSO_4$) and concentrated *in vacuo*. It was found that the crude was a 3:1 mixture of starting material and product. The crude material was dissolved in CH_2Cl_2 (1.6 mL) and treated with Et_3N (31 μL, 222 μmol) and MsCl (4 μL, 51.73 μmol) and stirred for 16 hours at rt before a further addition of MsCl (2 μL, 25.87 μmol) was made and the reaction was stirred for 16 hours. H_2O (5 mL) was added and the mixture was extracted with EtOAc (4 x 10 mL), the combined organic was dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography on silica gel (2.0 cm x 10 cm) eluting with 70% EtOAc/hexane afforded the desired mesylate **4.10** (12 mg, 24.81 μmol, 34%), starting material **4.9** (3 mg, 7.39 μmol, 10%) and dimesylate (7 mg, 12.46 μmol, 17%). Data was obtained from the mixture of diastereoisomers (4.8:1).

$[\alpha]_D = -2.3$ (*c* 0.5, $CHCl_3$, 26 °C).

FT-IR (neat) ν_{max} 3421 (br), 2973 (w), 2935 (w), 2838 (w), 1682 (s), 1623 (w), 1581 (w), 1506 (m), 1355 (s), 1173 (s) cm^{-1}.

For major diastereoisomer:

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.77 (1H, d, *J* = 8.4 Hz, -NCH_2CCH**C**H-), 6.67 (1H, d, *J* = 8.4 Hz, -NCH_2**C**CHCH-), 6.01 (1H, dd, *J* = 10.3, 3.8 Hz, -CCH=C**H**-), 5.90 (1H, br d, *J* = 10.3 Hz, -CC**H**=CH-), 4.84 (1H, dd, *J* = 6.5, 4.0 Hz, ArOC**H**CH_2-), 4.55 (1H, br d, *J* = 15.3 Hz, -NC**H**$_2$Ar), 4.44 (1H, d, *J* = 15.3 Hz, -NC**H**$_2$Ar), 4.27-4.14 (3H, m, -C**H**(OH)- and -CH_2C**H**$_2$OMs), 3.86 (3H, s, ArOC**H**$_3$), 2.94 (3H, s, -CH_2OSO_2C**H**$_3$), 2.81 (3H, s, -NC**H**$_3$), 2.40 (1H, m, -C**H**$_2CH_2$OMs), 2.31-2.18 (2H, m, ArOCHC**H**$_2$CH(OH)-), 2.13 (1H, dt, *J* = 14.8, 7.3 Hz, -C**H**$_2CH_2$OMs), 1.48 (9H, s, -OC(C**H**$_3$)$_3$) ppm.

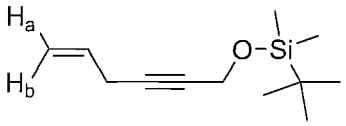
For major diastereoisomer:

^{13}C NMR (100 MHz, $CDCl_3$) δ 156.19 (C, -**C**=O), 147.19 (C, -**C**OCHCH$_2$-), 145.09 (C, -**C**OMe), 141.09 (C, -NCH$_2$**C**CCO-), 131.30 (CH, -CCH=**C**H-), 128.70 (CH, -**C**CH=CH-), 126.31 (C, -NCH$_2$**C**CHCH-), 121.68 (CH, br, -NCH$_2$**C**CHCH-), 112.68 (CH, -NCH$_2$CCH**C**H-), 84.68 (CH, ArO**C**HCH$_2$-), 80.45 (C, -**C**(CH$_3$)$_3$), 66.68 (CH$_2$, -**C**H$_2$OMs), 63.21 (CH, -**C**H(OH)-), 56.30 (CH$_3$, ArO**C**H$_3$), 49.54 (C, -**C**CH=CH-), 49.29 (CH$_2$, -N**C**H$_2$Ar), 37.75 (CH$_3$, -CH$_2$OSO$_2$**C**H$_3$), 36.03 (CH$_2$, -C**C**H$_2$CH$_2$OMs), 34.17 (CH$_3$, -N**C**H$_3$), 33.13 (CH$_2$, ArOCH**C**H$_2$-), 28.78 (CH$_3$, -OC(**C**H$_3$)$_3$) ppm.

LRMS (ES$^+$) *m/z* 506 [M+Na$^+$], 990 [2M+Na$^+$].

HRMS (ES$^+$) for $C_{23}H_{33}NNaO_8S$, calculated 506.1819, found 506.1825 Da.

***Tert*-butyl(hex-5-en-2-ynyloxy)dimethylsilane (4.11)**



$C_{12}H_{22}OSi$

m.w. = 210.39 g/mol

Colourless oil

A solution of TBDMS protected propargyl alcohol **2.5** (2.50 g, 14.68 mmol) in THF (50 mL) at −78 °C was treated with *n*-BuLi (7.72 mL, 14.68 mmol) dropwise. After stirring at −78 °C for 2 hours, allyl bromide (6.35 mL, 73.39 mmol) was added dropwise and stirred at −78 °C for 1 hour before warming to rt and stirring for 1 hour. To the reaction was added HMPA (5.11 mL, 29.35 mmol) dropwise and the reaction was stirred 15 hours. The reaction was quenched with H_2O (25 mL) and extracted with Et_2O (4 x 25 mL). The combined organic phases were washed with H_2O (2 x 10 mL) and brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* giving a pale yellow oil. Purification by flash column chromatography on silica gel (4.0 cm x 6 cm) eluting with hexane gave the desired enyne **4.11** as a colourless oil (1.88 g, 8.92 mmol, 61%).

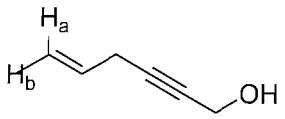
FT-IR (neat) ν_{max} 2955 (w), 2929 (w), 2857 (w), 1472 (w), 1463 (w) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82 (1H, ddt, J = 17.0, 10.1, 5.4 Hz, H_2C=C**H**-), 5.32 (1H, dq, J = 17.0, 1.8 Hz, $\mathbf{H_b}$), 5.11 (1H, dq, J = 10.0, 1.7 Hz, $\mathbf{H_a}$), 4.35 (2H, t, J = 2.2 Hz, -C**H$_2$**OTBDMS), 3.00 (2H, dtt, J = 5.4, 2.1, 1.8 Hz, H_2C=CHC**H$_2$**-), 0.92 (9H, s, -OSiC(C**H$_3$**)$_3$), 0.13 (6H, s, -OSi(C**H$_3$**)$_2$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 132.82 (CH, H_2C=**C**H-), 116.46 (CH_2, H_2**C**=CH-), 82.16 (C, H_2C=CHCH_2**C**≡CCH_2-), 81.38 (C, H_2C=CHCH_2C≡**C**CH_2-), 52.31 (CH_2, -**C**H_2OTBDMS), 26.23 (CH_3, -SiC(**C**H_3)$_3$), 23.49 (CH_2, H_2C=CH**C**H_2C≡C-), 18.70 (C, -Si**C**$(CH_3)_3$), –4.76 (CH_3, -Si(**C**H_3)$_2$) ppm.

LRMS (CI, NH_4) *m/z* 211 [M+H$^+$].

Hex-5-en-2-yn-1-ol (4.12)



C_6H_8O
m.w. = 96.13 g/mol
Colourless oil

Alcohol **4.12** was prepared by a method described by Taber *et al.*[157] To a solution of propargyl alcohol (**2.4**) (3.14 mL, 53.51 mmol) in THF (55 mL) at 0 °C was added ethylmagnesium bromide (3.00 M in Et_2O, 39.24 mL, 117.73 mmol) dropwise producing a white suspension. The reaction was diluted with THF (55 mL) and warmed to 50 °C then stirred for 2 hours. The reaction was cooled to 0 °C before the addition of CuBr.Me_2S (880 mg, 4.28 mmol) and allyl bromide (5.56 mL, 64.22 mmol). The reaction was warmed to rt and stirred for 15 hours before being quenched with a saturated solution of NH_4Cl (50 mL) and H_2O (10 mL). The phases were separated and

the organic phase was washed with 2 M HCl (10 mL), H_2O (15 mL) and brine (10 mL) sequentially. The organic phase was dried (Na_2SO_4) and concentrated *in vacuo* giving a brown oil. Purification by distillation under reduced pressure (10 mbar, 60-70 °C) afforded the desired alcohol **4.12** as a colourless oil (4.22 g, 43.90 mmol, 82%). Spectroscopic details were consistent with the literature.[157]

FT-IR (neat) ν_{max} 3332 (br), 2872 (w), 1642 (m), 1419 (m) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.82 (1H, ddt, J = 17.0, 10.1, 5.4 Hz, H_2C=C**H**-), 5.31 (1H, dq, J = 17.0, 1.8 Hz, $\mathbf{H_a}$), 5.12 (1H, dq, J = 10.1, 1.6 Hz, $\mathbf{H_b}$), 4.29 (2H, t, J = 2.2 Hz, -C$\mathbf{H_2}$OH), 3.01 (2H, dtt, J = 5.3, 2.2, 1.8 Hz, H_2C=CHC$\mathbf{H_2}$-) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 132.58 (CH, H_2C=**C**H-), 116.61 (CH_2, H_2**C**=CH-), 83.30 (C, -**C**≡CCH_2OH), 80.99 (C, -C≡**C**CH_2OH), 51.65 (CH_2, -C≡C**C**H_2OH), 23.38 (CH_2, H_2C=CH**C**H_2-) ppm.

LRMS (CI, NH_3) *m/z* 97 [$M+H^+$].

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