



Streamlined synthesis of taxol analogues

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ASLIB ABSTRACT

STREAMLINED SYNTHESIS OF TAXOL ANALOGUES

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This thesis centres on the synthesis of taxol analogues *via* late-stage hydroxylation with P450 enzymes. To accomplish this, the taxane core, specifically taxa-4(5),11(12)-dien-2-one, was synthesised by classical synthetic methods, and subsequently oxidised using P450_{BM3} mutants.

Chapter 1 introduces enzymatic catalysis, and the advantages and disadvantages of its application to organic synthesis. Additionally, an overview of taxol, including its discovery, mode of action, biosynthesis and large-scale production, and a summary of the previously reported approaches to the taxane core are described.

Chapter 2 details the problems encountered and solutions implemented when reproducing Baran's route to taxa-4(5),11(12)-dien-2-one. Furthermore, approaches to some of its intermediates and an alternative route to taxa-4(5),11(12)-dien-2-one, which is based on Baran's, are discussed.

Chapter 3 describes the development of a new, practical and short synthetic route to taxa-4(5),11(12)-dien-2-one which, ultimately, led to 1,3-di-*epi*-taxa-4(5),11(12)-dien-2-one. Additionally, the application of this route to the synthesis of a model compound and attempts to convert this racemic synthesis into an enantioselective route are reported.

Finally, the enzymatic oxidation of taxa-4(5),11(12)-dien-2-one and related molecules using P450_{BM3} mutants is explored in Chapter 4. A preliminary study to determine the substrate enantioselectivity of the mutants is also described, along with the biological assays of the oxidised compounds produced during the study.

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ABBREVIATIONS AND ACRONYMS

°C	degrees Celcius
Ac	acetyl
ACN	acetonitrile
AIBN	azobisisobutyronitrile
Ala	alanine
app.	apparent
aq.	aqueous
Arg	arginine
Bn	benzyl
Boc	<i>tert</i> -butoxycarbonyl
br	broad
Bu	butyl
Bz	benzoyl
δ	chemical shift
calcd.	calculated
CI	chemical ionisation
cm	centimetre
COSY	correlation spectroscopy
Cys	cysteine
d	doublet
DA	Diels–Alder
DABCO	1,4-diazabicyclo[2.2.2]octane
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DCE	1,2-dichloroethane
DDQ	2,3-dichloro-5,6-dicyano- <i>p</i> -benzoquinone
DMAP	4-(dimethylamino)pyridine
DME	dimethoxyethane
DMF	dimethylformamide
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1 <i>H</i>)-pyrimidinone
DNA	deoxyribonucleic acid
dr	diastereomeric ratio
EA	ethyl acetate
ee	enantiomeric excess
eq.	equivalent(s)
ESI	electrospray ionisation

Abbreviations and Acronyms

Et	ethyl
ether	diethyl ether
EQ	external quench
FAD	flavin adenine dinucleotide
FDA	food and drug administration
FI	field ionisation
FMN	flavin mononucleotide
g	gram
GC	gas chromatography
GDH	glucose dehydrogenase
GDP	guanosine-5'-diphosphate
GTP	guanosine-5'-triphosphate
Glu	glutamine
Gly	glycine
GPP	geranylgeranyl pyrophosphate
h	hours
His	histidine
HMBC	heteronuclear multiple-bond correlation spectroscopy
HMPA	hexamethylphosphoramide
HRMS	high-resolution mass spectrometry
HSQC	heteronuclear single-quantum correlation spectroscopy
Hz	Hertz
h ν	irradiation
Ile	isoleucine
IMDA	Intramolecular Diels–Alder
IPP	isopentenyl pyrophosphate
IQ	internal quench
IR	infrared spectroscopy
<i>J</i>	coupling constant
L	litre
LDA	lithium diisopropylamide
Leu	leucine
Lys	lysine
m	medium
mg	milligram
mL	millilitre
mmol	millimole
m	multiplet

M	mega
M	molar
<i>m</i> -CPBA	3-chloroperbenzoic acid
Me	methyl
min	minute(s)
mol	mole
MOM	methoxymethyl
m.p.	melting point
MS	mass spectrometry
Ms	methanesulfonyl
m/ z	mass-to-charge ratio
MTOC	microtubule organising centre
NADH	nicotinamide adenine dinucleotide
NADP ⁺	nicotinamide adenine dinucleotide phosphate
NADPH	nicotinamide adenine dinucleotide phosphate
NBS	<i>N</i> -bromosuccinimide
NCI	national cancer institute
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser effect spectroscopy
PCC	pyridinium chlorochromate
PCR	polymerase chain reaction
Ph	phenyl
Phe	phenylalanine
ppm	parts-per-million
Pro	proline
Pyr	pyridine
q	quartet
quin	quintet
R _f	retention factor
R _t	retention time
RT	room temperature
S	spin quantum number
s	second
s	singlet
satd.	saturated
t	triplet
TBAF	tetrabutylammonium fluoride
TBS	<i>tert</i> -butyldimethylsilyl

Abbreviations and Acronyms

TEA	triethylamine
TES	triethylsilyl
TEMPO	(2,2,6,6-tetramethylpiperidin-1-yl)oxyl
Tf	trifluoromethanesulfonyl
THF	tetrahydrofuran
Thr	threonine
TIPS	triisopropylsilyl
TLC	thin-layer chromatography
TMS	trimethylsilyl
Ts	4-toluenesulfonyl
Tyr	tyrosine
UV	ultraviolet
WT	wild type
μ	micro

1 INTRODUCTION

1.1 Contextualisation of the project

This project is part of the 'Oxford Innovative Organic Synthesis for Cancer Research (OxIOSCR)' programme, an EU-funded Marie Curie Innovative Doctoral Programme (IDP). The programme was established to develop synthetic routes to natural products and their analogues with potent anti-cancer activity and to apply underdeveloped synthetic methods and techniques, such as biocatalysis, electrosynthesis and flow chemistry. Despite evidence through the literature of the manifold advantages of these techniques, there is still a reticence to implement them in organic synthesis.

1.2 Enzymatic catalysis in organic synthesis

One of the main research topics in organic synthesis is the preparation of natural products and drugs. If chiral, these molecules should ideally be enantiopure compounds. Although enantiomers have the same chemical structure, their biological activity may vary hugely, owing to differences in binding affinity with the target receptor. Pertinent properties of these compounds, such as pharmacology, toxicology, pharmacokinetics and metabolism can therefore be quite disparate, which is a serious consideration in the commercialisation of drugs.^[1,2] The thalidomide scandal is an infamous example of the importance of these differences.^[3] Thalidomide (**1**) is a sedative drug widely used in the 1950's to combat morning sickness in pregnant women. It was commercialised as a racemic drug, and subsequent studies identified that only the (*R*)-enantiomer is desirably bioactive, the (*S*)-enantiomer being toxic and producing teratogenic deformities in foetuses. This case is even more problematic because the two enantiomers racemise *in vivo*, due to the acidic hydrogen at the stereogenic centre (**Figure 1.1**). Approximately 2,000 children died and more than 10,000 had serious birth defects as a result of its administration.

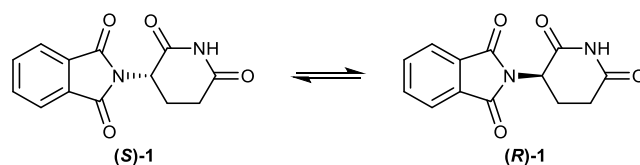
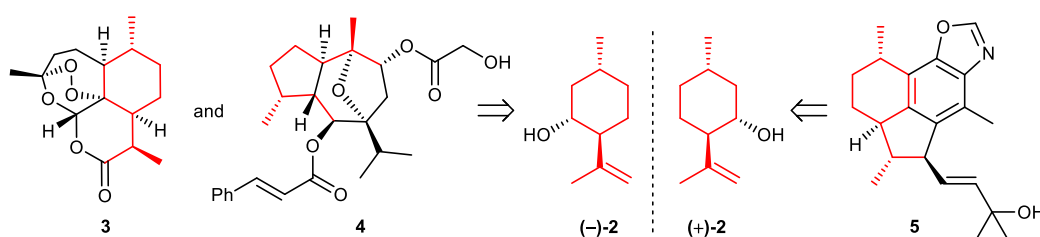


Figure 1.1. (*S*)- and (*R*)-enantiomers of thalidomide (**1**).

One of the main aims of organic chemistry is focussed on the synthesis of enantiomerically pure compounds. To accomplish this, organic chemists make use of different synthetic methods, such as chiral pool synthesis, asymmetric synthesis and chiral resolution.

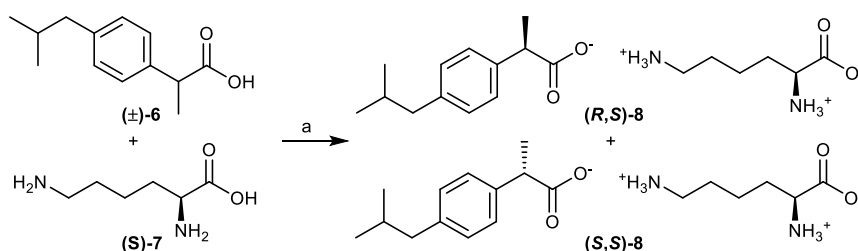
In chiral pool synthesis, a naturally occurring enantiopure compound is used as a starting material in the synthesis of a complex molecule, and its stereogenic centres, which often remain unchanged during the synthesis, influence the formation of new ones. For example, (–)-isopulegol [(–)-**2**], a monoterpene alcohol present in the essential oils of various plants, has been used as a starting point in the synthesis of several natural products, such as artemisinin (**3**)^[4] and englerin A (**4**)^[5] both relevant due to their anticancer properties. Its unnatural enantiomer [(+)-**2**], which is commercially available, has likewise been used in natural product synthesis, the antitubercular agent ileabethoxazole (**5**)^[6] being an example (**Scheme 1.1**).



Scheme 1.1. Chiral pool syntheses of artemisinin (**3**), englerin A (**4**) and ileabethoxazole (**5**) from (–)- and (+)-isopulegol (**2**).

In contrast, in asymmetric synthesis, an enantiopure reagent which will not be included in the final product guides the selective formation of the new stereogenic centres. Chiral auxiliaries, ligands and catalysts, and the desymmetrisation of *meso*-compounds are both included under this category.

On the other hand, chiral resolution involves the separation of racemic compounds into their enantiomers through reaction with an enantiopure compound, using either chemical derivatisation or kinetic resolution strategies. In chemical derivatisation, both enantiomers react with the chiral compound, and thus an inseparable mixture of enantiomers is converted into a separable mixture of diastereomers. In kinetic resolution, the chiral compound reacts preferentially with one of the enantiomers, leaving the other enantiomer unchanged. Chiral resolution is usually a last resort to achieve enantiopurity as only a maximum 50% yield of the desired enantiomer can be obtained. However, this method is used a lot commercially, especially if both enantiomers can be sold. For example, ibuprofen (**6**) is an anti-inflammatory drug which is sold as a racemic mixture, even though studies demonstrate that the (*S*)-enantiomer is 160 times more potent than the (*R*)-enantiomer *in vitro*,^[7] and also more effective than the racemate.^[8] Consequently, pharmaceutical companies are interested in producing enantiomerically pure (*S*)-**6**. Practical and economical industrial syntheses of racemic ibuprofen **6** have been developed previously, hence resolution is the most used technique for producing (*S*)-**6**. Merck described the industrial resolution of racemic ibuprofen **6** using (*S*)-lysine **7** as a resolving agent; generating the diastereomeric salts (*R,S*)-**8** and (*S,S*)-**8**, which are separable due to different solubility (**Scheme 1.2**).^[9]



Scheme 1.2. Resolution of racemic ibuprofen (**6**). *Reagents and conditions:* a) water/ethanol, RT, (*S,S*)-**8** (50%).

Biocatalysis is another method used to synthesise enantiomerically pure compounds, counted among asymmetric synthesis. That is the use of enzymes to catalyse chemical reactions; they increase the reaction rate by providing an alternative reaction

pathway of lower activation energy. Enzymes are well-known for their high stereoselectivity and stereospecificity when introducing chiral centres into a molecule, and this is the main reason for their application in organic synthesis. Their complex three dimensional structure allows them to differentiate between structural and stereochemical features of the substrate and product.

The first enzyme to be isolated and crystallised was *urease* extracted from the jack bean by Sumner in 1926.^[10,11] This was later followed by the isolation of *pepsin*, *trypsin* and *chymotrypsin* by Northrop and Stanley in the period 1930 to 1936.^[12-14] These scientists were awarded the 1946 Nobel Prize in Chemistry for their work isolating these enzymes and proving that they are proteins.

Enzymes catalyse a broad range of reactions and for almost every described chemical reaction, there is an enzymatic analogue. Their highly selective nature means that each enzyme only catalyses a specific reaction. They are classified into six main categories, according to the type of reaction they catalyse (**Table 1.1**).

Table 1.1. Enzyme classification according to the type of reaction catalysed.

Enzyme class	Catalysed reaction
Oxido-reductases	Oxidation-reduction reactions by oxygenation or by removal/addition of H-atoms
Transferases	Transfer of functional groups (one carbon, aldehydic or ketonic, acyl, phosphoryl, glycosyl, amino, nitro and sulfur-containing groups)
Hydrolases	Hydrolysis reactions (esters, amides, anhydrides, epoxides, nitriles, etc.)
Lyases	Addition of small molecules to double bonds, such as C=C, C=O and C=N
Isomerases	Isomerisation reactions (racemisations, epimerisations, <i>cis-trans</i> isomerisation and C=C bond migration)
Ligases	Formation of bonds (C-O, C-S, C-N and C-C) with ATP cleavage

The application of enzymatic catalysis in organic synthesis has other advantages aside from high stereoselectivity and stereospecificity.^[15-17] Most enzymatic reactions occur under gentle reaction conditions, mild temperatures (20–50 °C) and near neutral pH (6–10), to ensure the stability and activity of the enzyme. In turn, these mild conditions minimise

problems with isomerisation, racemisation, epimerisation and rearrangements. The multiple functional group protection and deprotection strategies usually required in chemical synthesis are not necessary in enzymatic catalysis, since their reactions are highly specific and chemo- and regio-selective. Consequently, enzymes operate independently on their own substrate, even in the presence of other enzymes and their respective substrates, allowing multiple and sequential enzymatic reactions to be performed in one-pot or flow systems.

Although there are many advantages to the application of enzymatic catalysis in organic synthesis, there are also some limitations, including extreme specificity, solubility, cost, activity, and stability; many of which have been overcome.^[15] For example, organic compounds are usually hydrophobic and difficult to solubilise in aqueous media, which is the natural environment for enzymes. These solubility issues were predominantly solved when it was discovered that many enzymes are tolerant towards the presence of organic solvents. Since then, water-miscible and water-immiscible organic solvent mixtures, ensuring solvation of the substrate, have been used for enzymatic catalysis.

One of the main costs associated with enzymatic catalysis is the price of the cofactor, a compound often required by many enzymes for their catalytic activity (**Figure 1.2**). Cofactors can be either organic molecules or inorganic ions and they can bond to the enzyme by a covalent bond (prosthetic groups), or by a non-covalent bond (coenzymes). The coenzyme is modified during the reaction by transferring electrons or chemical groups to the substrate and is therefore used in stoichiometric amounts. However, coenzymes are usually too expensive and the use of stoichiometric amounts would not be cost-efficient. In order to make the system economically attractive, cofactor regeneration systems have been developed.^[15,18–20] This enables the use of substoichiometric quantities of cofactor, and only the co-substrate, the substrate required for cofactor regeneration, is used in stoichiometric amounts; thus, it must be cheap and readily available.

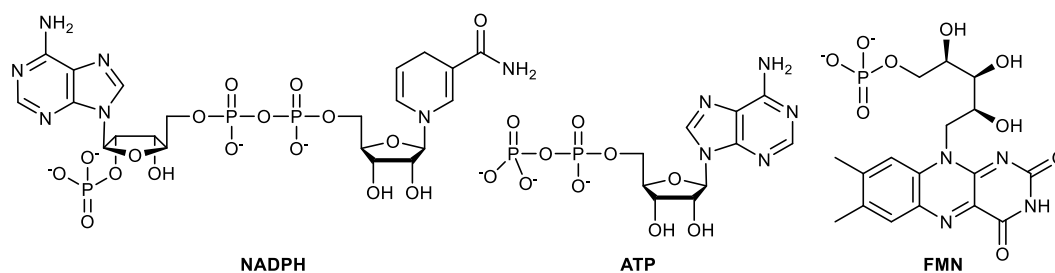


Figure 1.2. Cofactors often used in enzymatic reactions.

The enzyme's production cost also has to be taken into consideration. Enzymes can be used as whole-cell systems or as free enzymes to carry out a biotransformation. When whole-cells are used, the enzyme is over-expressed in a host organism inside which the biotransformation will take place. This method has the disadvantage of low efficacy, and the outcome is often difficult to predict, control and manipulate due to the presence of other enzymes in the cell. In contrast, when a free enzyme is used, it is also over-expressed in a host organism but the cell is subsequently disrupted, and the enzyme isolated and purified. The production of enzymes by fermentation is a cheap process, although their purification makes them expensive. To make free enzymes economically attractive, techniques have been developed to reduce the cost, such as: i) immobilisation of enzymes onto solid-supports, which allows recovery and re-use of the enzyme; ii) biphasic reaction media (water and a water-immiscible organic solvent), where the enzymatic reaction takes place in the aqueous phase, while the product(s) accumulates in the organic phase; and iii) enzyme-membrane reactors, where the enzyme reacts as a free enzyme and is prevented from leaving the reactor by a membrane with a specific molecular-weight cut-off.^[15]

However, the main limitations that have prevented organic chemists from implementing enzymatic transformations in organic synthesis are low activity, low efficiency when using wild-type strains and limited stability under organic reaction conditions. But, even these limitations have been overcome due to the tremendous progress in protein engineering.

Protein engineering is the process of developing useful and valuable proteins by modification of their primary structure. First, specific amino acid residues are substituted by changing the codon of the corresponding DNA; then the “mutated” gene sequence is expressed in a host organism, which will synthesise the mutated enzyme (**Figure 1.3**). Protein engineering uses three different approaches to design “new” enzymes: rational design, directed evolution, and *in-silico* design.^[21]

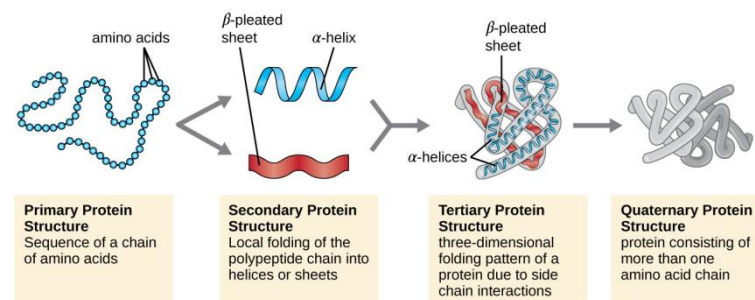


Figure 1.3. Protein structure has four levels of organisation. Mutations in the primary protein structure produce modifications in the subsequent structures. Retrieved from <https://courses.lumenlearning.com/microbiology/chapter/proteins/>.

Rational design is usually used when the enzyme under study has been purified, crystallised and co-crystallised with the substrate and product. Thus, a lot of information about its three dimensional structure is available. In this case, the key amino acid residues in the active site, which interact with the substrate by hydrogen bonding, hydrophobic or electrostatic effects, are identified and exchanged with other residues (**Figure 1.4**). These mutations produce structural alterations in the active site, ideally improving the enzyme functionality by giving access to hindered substrates or by aiding the exit of product(s). This approach is often unsuccessful because of the difficulty in predicting the exact relationship between enzyme function and crystal structure.

On the other hand, directed evolution is used when there is limited information available about the enzyme structure. The enzyme is randomly mutated in an amino acid residue using the error-prone polymerase chain reaction (PCR), in which the polymerase makes mistakes in the base pairing during DNA synthesis (**Figure 1.4**). A pool of mutated enzymes is generated (the mutant library) and their enzymatic activity is tested. The

mutations which produce the most active enzymes are selected to make up the first-generation mutants, which can yield an improved active enzyme or a starting point for further mutagenesis. However, random enzymatic mutation has the risk of affording enzymes that are non-functional or unable to fold competently.

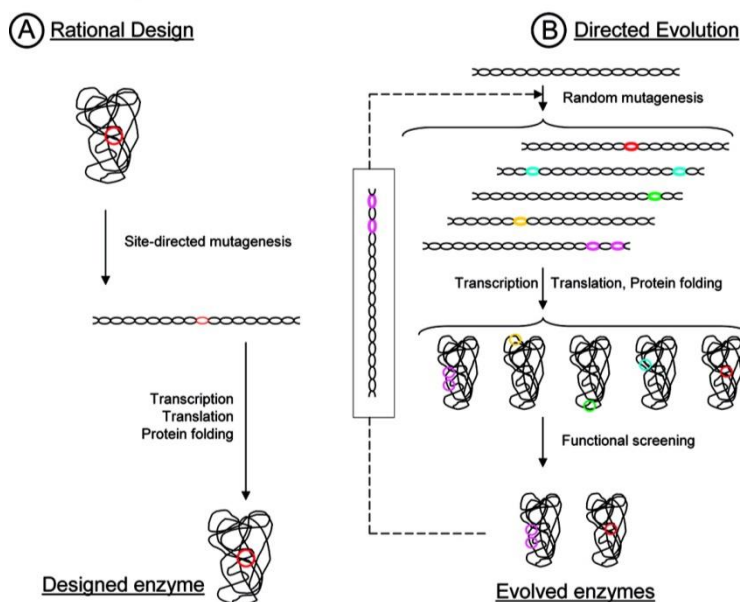


Figure 1.4. Enzyme preparation by: A) rational design; B) directed evolution.^[21]

In-silico design consists of applying computational methods to design new enzymes for which no natural equivalent is believed to exist. Since the enzyme is created from scratch, an existing protein is usually used as starting point; its sequence is then redesigned to introduce functionality whilst maintaining a rigid backbone. Computational methods allow the design of an extensive library of virtual enzymes and evaluation of their activity, without the requirement to synthesise all of them.

1.3 Enzymatic catalysis in the Robertson group

The application of enzymatic catalysis in organic synthesis is known and its advantages have been demonstrated previously. Although its use has increased as a result of advances in enzyme discovery, enzyme engineering and process development, it is still not considered a “standard synthetic tool” and organic chemists are often reluctant to

introduce enzymatic transformations into their syntheses. This is mainly because organic chemistry and biochemistry are perceived as two different and immiscible worlds. More effort should be made to convince organic chemists about the advantages of biocatalysis and its applicability to organic synthesis. The study of natural product biosynthesis could allow the development of chemical syntheses which take inspiration from Nature. As a result, these approaches would contain strategically similar steps to those present in the biosynthesis, and similar, or even the same enzymes could be employed in these syntheses.

One of the main research lines of the Robertson group is focussed on the biomimetic synthesis of cytotoxic natural products and their analogues. In Nature, terpenoids are produced *via* a three-step process in which a hydrocarbon skeleton is synthesised first by linking isopentenyl pyrophosphate units (IPP) together, followed by a cyclisation step which furnishes a large amount of diverse cyclic hydrocarbon products; these are subsequently subjected to enzymatic modifications to introduce the required functional groups, often oxidations with P450 enzymes. Taking the biosynthesis of terpenoids as an example, a similar two-step approach is followed by the Robertson group to synthesise natural products. This consists of: (1) synthesis of a hydrocarbon (or barely oxidised) skeleton using classical chemical methods and (2) enzymatic oxidation using P450_{BM3} mutants. This late-stage hydroxylation step gives easy access to a wide range of natural product analogues with different oxidative patterns.

The aforementioned cytochrome P450 enzymes are a family of mono-oxygenases. They catalyse the oxidation of organic molecules by insertion of a single oxygen atom from molecular dioxygen into a C–H bond in a highly regio- and stereo-specific manner. They are environmentally friendly, particularly when compared with classical oxidative methods which typically require highly toxic reagents and harsh conditions, and they also avoid the difficulties of undesirable side-products and formation of racemic mixtures. The Robertson

group works in collaboration with the Wong group, who have developed a panel of >200 P450_{BM3} mutants, to perform the late-stage hydroxylations.

Since P450 enzymes catalyse oxidative transformations, the Robertson group works on highly oxidised natural products, such as alvocidib (**9**), trigoxyphin K (**10**) and L (**11**), viridiol (**12**), eleutherobin (**13**) and taxol (**14**) (**Figure 1.5**). Given that these natural products have very different structures, the results of the enzymatic oxidation of the non- or minimally oxidised precursors will provide more information about the selectivity of the P450_{BM3} mutants, and thus enable the identification of oxidative patterns (mutated enzymes which preferentially oxidise at specific positions). Therefore, the primary focus of the Robertson group is to develop a toolbox of P450_{BM3} mutants, each of which would predictably oxidise a specific site in a complex molecule. This late-stage hydroxylation methodology will tremendously simplify and shorten the synthesis of complex molecules, and will give straightforward access to a large range of analogues with different oxidation patterns.

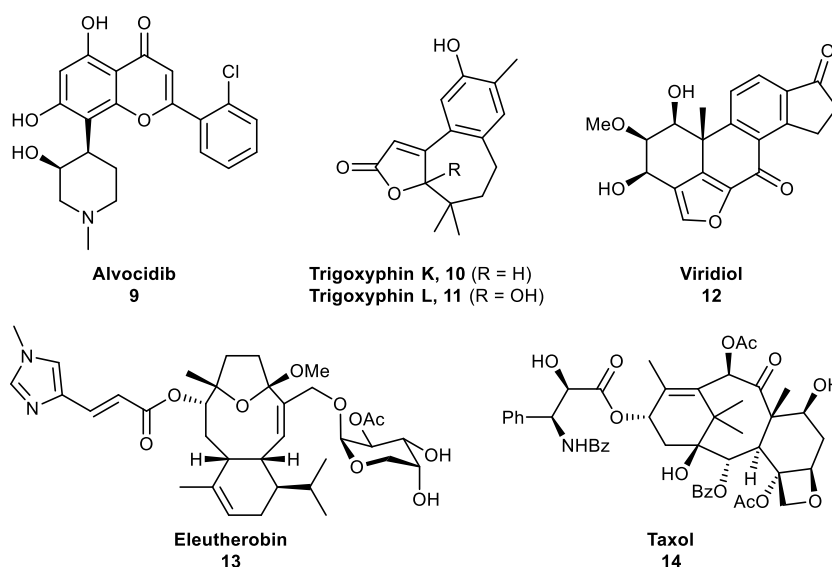
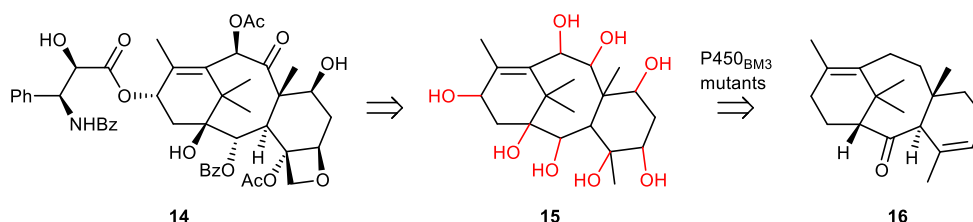


Figure 1.5. Natural product targets in the Robertson group.

1.4 Aims of the project

Taxol (**14**), also known by the generic name of paclitaxel, is a chemotherapy drug widely used to treat several types of cancer. Due to its potent anticancer activity, taxol was selected as a target molecule in the OxIOSCR project. Additionally, its highly oxidised state made it ideal to apply the late-stage enzymatic oxidation methodology.

The aim of the project centres on the synthesis of taxol analogues *via* late-stage hydroxylation with P450 enzymes. Therefore, two well differentiated aims were identified at the outset: (1) the synthesis of the hydrocarbon taxane skeleton, specifically taxadienone **16**, by classical synthetic methods and (2) the enzymatic oxidation of taxadienone **16** and other synthetic intermediates using P450_{BM3} mutants (**Scheme 1.3**). The use of P450 enzymes will allow chemo-, regio- and stereo-selective oxidations, without the need for protecting groups in the synthesis. In addition, it is an economic and sustainable method in which oxygen is used as oxidising agent and water is the only by-product.



Scheme 1.3. Retrosynthetic analysis of taxol (**14**) and analogues incorporating late-stage hydroxylation.

This approach was expected to give access to a broad range of taxol analogues with different oxidation patterns, which will then be submitted to biological assays in an attempt to identify which oxidised positions are responsible and indispensable for cytotoxic activity. Thus, the complex structure of taxol would be reduced to less functionalised but still active taxane structures, with much simpler and shorter syntheses. Precedents of bioactive taxol derivatives with reduced functionalities have been reported in studies aiming to determine structure-activity relationships in taxol.^[22–24]

1.5 Discovery and classification of taxol and taxane-type compounds

Yew trees of the genus *Taxus* (Taxaceae) are slow-growing shrubs distributed across the northern hemisphere and well-known since ancient times for their poisonous properties, evidenced by the use of the bark and yew extracts as a poison, disinfectant, abortifacient and treatment of headaches and snakebites.

Lucas discovered “taxine” in 1856, a white alkaloidal non-crystalline powder, isolated in 1% yield from the needles of the English yew *Taxus baccata* L.^[25] Although it was responsible for the toxicity of the plant, further purification or structure elucidation was not possible at the time, due to the lack of analytical instruments. Several degradative and constructive studies were performed to determine its composition, but its structure was not elucidated until 1956, when Graf^[26,27] claimed that taxine was a mixture of alkaloids, the main components being taxine A (17) and taxine B (18) (Figure 1.6).

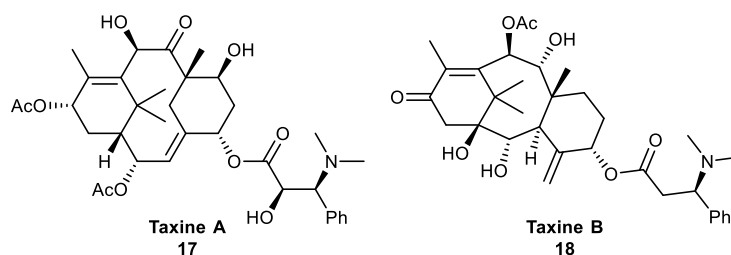


Figure 1.6. Main components of taxine: taxine A (17) and taxine B (18).

The chemical study of yew trees did not progress further until the 1950's, when the National Cancer Institute (NCI) in the United States started a screening programme of plant extracts using model tumour systems *in vivo* and tumour cells *in vitro*. In 1964, an extract from the bark of the pacific yew *Taxus brevifolia* Nutt. showed cytotoxicity to KB cells, and taxol was isolated as the bioactive component by Wall and Wani in 1966, in a yield of 0.014%.^[28] The structure was finally elucidated in 1971 by a combination of NMR spectroscopic analysis, X-ray crystallography and degradative studies. Later taxol was also isolated from other *Taxus* species, including *Taxus baccata* L. and *Taxus cuspidata*.

At the time, the scientific community was not interested in further pursuing the study of taxol as an anticancer agent, mainly because its cytotoxic activity was not remarkable in comparison to other natural products, such as vinblastine (**19**), vincristine (**20**) and colchicine (**21**) (**Figure 1.7**), and because of its poor solubility, modest isolation yield and purification difficulty.

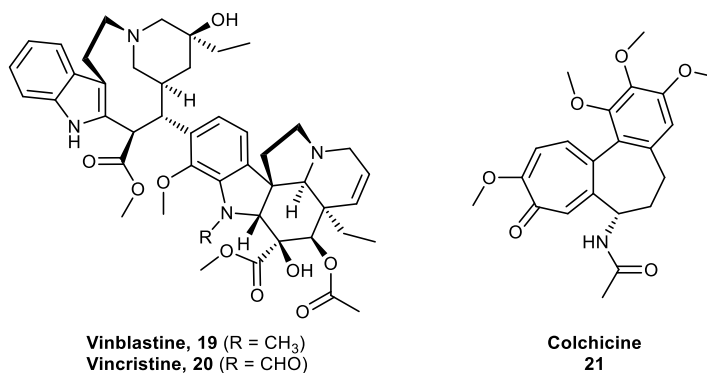


Figure 1.7. Natural products with cytotoxic activity.

A major turning point in the development of taxol was the discovery of its unique mode of action. All previously identified natural products whose cytotoxic activity involved an interaction with tubulin, prevented the assembly of tubulin into microtubules, thus inhibiting mitosis and cell proliferation. Taxol was the first compound shown to promote microtubule assembly. In 1978, Fuchs and Johnson^[29] reported taxol to be antimetabolic and in 1979, Horwitz and Schiff^[30] discovered that it disturbs cell division through enhancement of the polymerization of tubulin and consequent microtubule stabilisation.

The pharmacological study of taxol was then progressed to drug development, culminating in its approval for ovarian cancer by the Food and Drug Administration (FDA) in 1992 and for breast cancer in 1994. Its clinical use has increased since then, and today it is also routinely used to treat lung, head and neck, prostate, and cervical cancers.

Since the discovery of its unique mode of action, significant effort and resources have been invested in the study of all *Taxus* species, in order to isolate potentially more effective taxol derivatives or an abundant starting material for the semi-synthesis of taxol

and related compounds. For example, 10-deacetylbaccatin III (**22**) was isolated by the Potier group in France, as the main taxane compound from the European *Taxus baccata* in 0.1% yield.^[31] Currently it is used in the semi-synthesis of taxol (**14**) and taxotere (**23**) (also known by the generic name of docetaxel), a taxol analogue approved to treat breast cancer by the FDA in 1996 (**Figure 1.8**).

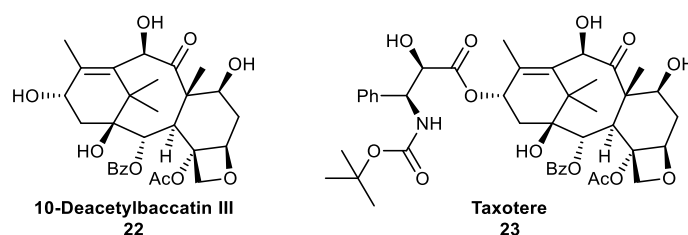


Figure 1.8. Taxol-like compounds.

So far, more than 550 taxanes have been isolated from various *Taxus* plants with some of them possessing interesting anticancer activities, and thus it was necessary to find a system to classify them. Nowadays they are classified into ten groups based on the carbon ring system and the substitution pattern (**Figure 1.9**).^[32]

Taxane compounds with the 6/8/6-ring system, known as the taxane skeleton, such as taxol (**14**), 10-deacetylbaccatin III (**22**) and taxotere (**23**), are the most abundant.

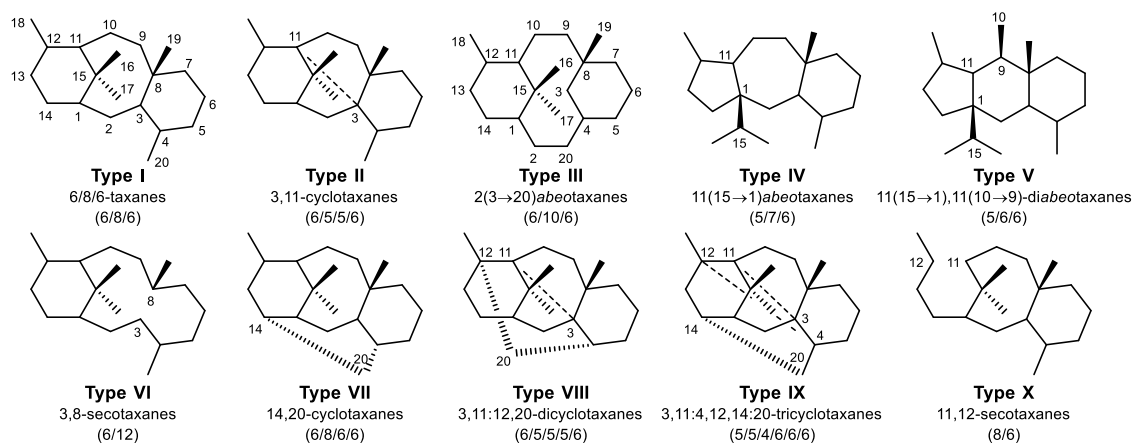


Figure 1.9. Structural classification of taxanes based on the carbon ring system.

1.6 Taxol mode of action^[33,34]

Tubulin is a small globular protein synthesised by eukaryotic cells. There are six different types of tubulin proteins (α -, β -, γ -, δ -, ϵ - and ζ -tubulin), with each one having a

different cellular location and function. α - and β -tubulin monomers bind to one another to form a heterodimer, which, in turn, assembles into linear protofilaments when intracellular conditions favour their assembly. In addition, the protofilaments associate laterally into cylindrical structures, known as microtubules (**Figure 1.10**).

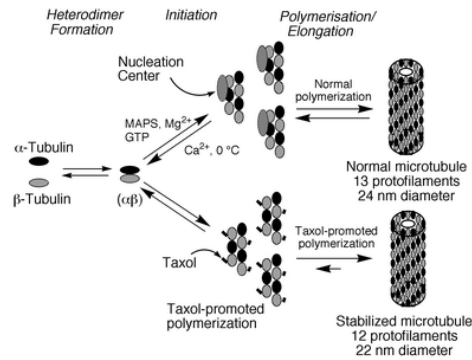


Figure 1.10. Schematic representation of normal microtubule assembly (upper) and taxol-promoted microtubule assembly (lower).^[34]

The protofilaments are composed of head-to-tail arrays of tubulin heterodimers and, consequently, microtubules are polar structures with two distinct ends; one has α -tubulin subunits exposed (slow-growing minus end) while the other has β -tubulin subunits exposed (fast-growing plus end).

Microtubules are dynamic structures; they are always in continuous assembly and disassembly. Their formation consists of three fundamental steps: nucleation, polymerisation and steady state. The nucleation step requires a microtubule organising centre (MTOC) which contains γ -tubulin and, when combined with other proteins, forms a complex that acts as a template for tubulin heterodimers to initiate the polymerisation.

The polymerisation step consists of the addition of tubulin dimers into the plus end of the microtubule. At this point, tubulin heterodimers are in a GTP-bound state, meaning that both α - and β -tubulin monomers are bound to a molecule of guanosine-5'-triphosphate (GTP). While the GTP bound to α -tubulin is stable and plays a structural function, the GTP bound to β -tubulin is susceptible to hydrolysis to guanosine-5'-diphosphate (GDP) and plays an important role in microtubule assembly. GTP hydrolysis weakens the binding affinity of tubulin for adjacent molecules, thereby favouring

depolymerisation. However, if the GDP-bound tubulin is in the middle of the microtubule, the heterodimer cannot spontaneously eject from the polymer but, if it is at the tip of a microtubule, it tends to fall off. Therefore, if the GTP bound to the β -tubulin subunit at the plus end of the microtubule hydrolyses before another heterodimer is added, the GDP-bound tubulin will dissociate, resulting in rapid depolymerisation and shrinkage of the microtubule.

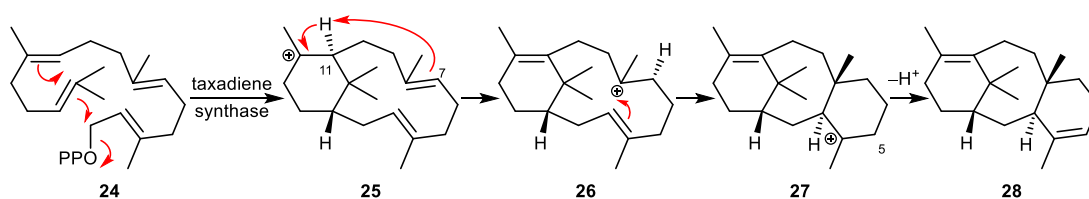
Microtubules are basic components of the cell's cytoskeleton, and are involved in cell shape and movement, including cell locomotion and intracellular transport. Microtubules also play a key role in cell division; composing the mitotic spindle, which provides the structural framework for the physical segregation of sister chromatids during cell division. A wide variety of cytotoxic drugs interfere with microtubule dynamics by binding to the β -tubulin monomer, and thus modifying its assembling properties and interfering with cell division. These drugs can either inhibit the assembly of tubulin heterodimers into microtubules, known as microtubule depolymerising agents, or stabilise microtubules under normally destabilising conditions, known as microtubule polymerising agents.

Taxol and other taxane-type compounds are microtubule polymerising agents. They stabilise the GDP-bound tubulin in the microtubule preventing their depolymerisation, even when the hydrolysed GDP-bound tubulin has reached the tip of the microtubule.

1.7 Taxol biosynthesis^[35]

In 1995, Croteau^[36] elucidated the first step in the biosynthesis of taxol: the assembly of the taxane skeleton. Overall, geranylgeranyl pyrophosphate (GPP, **24**) cyclises into taxadiene **28** promoted by the enzyme taxadiene synthase. The cyclisation starts with GPP ionisation and taxane A-ring closure, leading to carbocation **26**. Subsequently, the intramolecular $11\alpha,7\alpha$ -proton transfer initiates the transannular B/C-ring closure and

generates taxenyl cation **27** which, upon deprotonation at C5, yields the endocyclic C4–C5 double bond and thus, taxadiene **28** (Scheme 1.4).



Scheme 1.4. Cyclisation of geranylgeranyl pyrophosphate (**24**) to taxadiene **28**.

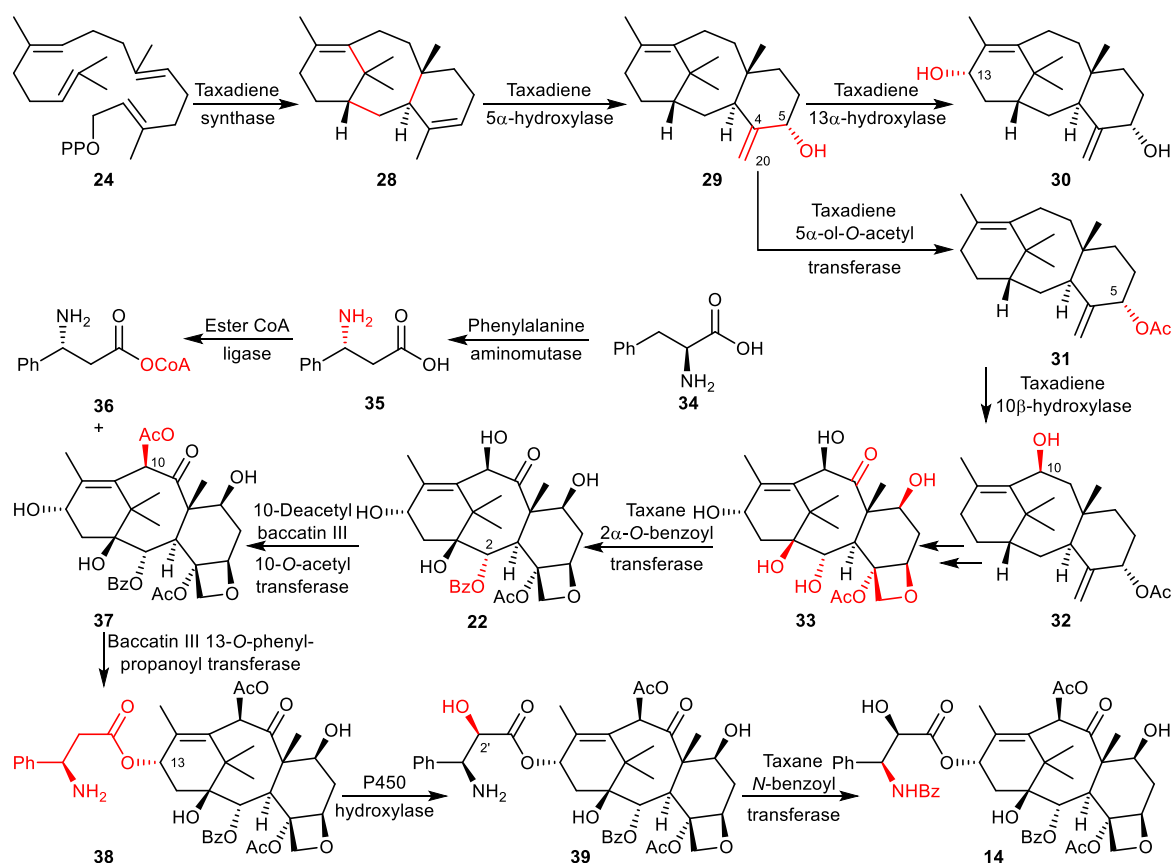
The second step in the taxol biosynthesis consists of the hydroxylation at the C5 position with concomitant migration of the endocyclic C4–C5 double bond into the exocyclic C4–C20 position by taxadiene-5 α -hydroxylase. Taxadienol **29** can either be hydroxylated at the C13 position by the taxadiene-13 α -hydroxylase and afford taxadienediol **30**, or be acetylated by the action of taxane-5 α -ol-*O*-acetyl transferase to give **31** and, subsequently, hydroxylated at the C10 position by taxadiene-10 β -hydroxylase, affording taxane **32** (Scheme 1.5). The opposite substrate selectivities of the 10 β -hydroxylase and the 13 α -hydroxylase suggest that taxol biosynthesis is not a linear pathway and there are branch points that can lead to other related taxoids.

At this point, the biosynthesis continues with several hydroxylations at the C1, C2, C7 and C13 positions, oxidation at C9, and epoxidation at the C4–C20 double bond, followed by migration of the α -acetoxy group from C5 to the C4 position together with the expansion of the oxirane to an oxetane group. Neither the exact order nor the enzymes involved are yet known but, based on the relative frequency of oxygenation at various positions in the natural taxanes, it appears likely that oxidation occurs first at C9, followed by C2 and C7, and later at C1, C4 and C20.

From the polyhydroxylated taxane **33**, the biosynthesis proceeds with benzoylation at the C2 position by taxane-2 α -*O*-benzoyl transferase, affording 10-deacetylbaaccatin III (**22**). This is subsequently acetylated at the C10 position by the enzyme 10-deacetylbaaccatin III-*O*-acetyl transferase to afford baaccatin III (**37**).

Next, coupling of the amide side-chain and the taxane skeleton is achieved by the esterification of baccatin III (**37**) at the C13 position with β -phenylalanyl-CoA (**36**), catalysed by baccatin III 13-*O*-phenylpropanoyl transferase. The required β -phenylalanyl-CoA (**36**) is obtained from *L*-phenylalanine (**34**), by rearrangement to β -phenylalanine (**35**) with phenylalanine aminomutase and activation with a so far undiscovered ester CoA ligase.

The final steps of taxol biosynthesis comprise hydroxylation at the C2' position to give taxane **39**, by an unknown cytochrome P450 dependent hydroxylase, then *N*-benzylation, catalysed by taxane *N*-benzoyl transferase, to finally afford taxol (**14**).



Scheme 1.5. Taxol biosynthetic pathway.

1.8 Large-scale taxol production^[35]

Taxol is one of the most effective and successful drugs discovered in the last 50 years. For this reason, since its approval as a drug there has been a high demand and an urgent need to find an efficient production method.

Extraction from *Taxus* yew trees was the first production method to be considered. The concentration of taxol in yew trees is very low (0.001–0.05%); only 1 kg of taxol could be isolated from 3,000 yew trees or from 10,000 kg of *Taxus* bark. Given that each patient needs approximately 2.5–3.0 g of taxol for a full course of treatment, it is estimated that eight sixty-year-old yew trees would be necessary for the treatment of only a single patient.^[35] Therefore, taxol production by plant extraction is not an efficient process, neither environmentally sustainable and, in addition, it requires complex systems and specific purification techniques which use advanced and expensive technology.

Chemical synthesis was then contemplated as a production method. Taxol is a complex and highly functionalised molecule bearing a hindering *gem*-dimethyl group, a bridgehead olefin, an oxetane ring, two hydroxyl groups, one benzoyl group, two acetyl groups and a side chain with hydroxyl and benzoylamino functional groups. As a result of its structural complexity, the seven total and three formal reported syntheses of taxol are complex, long and low yielding, thus making them impractical for large-scale manufacture.^[37–49]

Hence, semi-synthetic methods arose as an alternative for taxol production. For these, simpler and readily available taxanes intermediates are required. Bristol-Myers Squibb, a leading global supplier of taxol, used to produce taxol by semi-synthesis from 10-deacetylbaccatin III (**22**),^[50] which is extracted from the renewable needles of *Taxus baccata* in a yield six to tenfold greater than the yield of taxol. Despite this method proved to be viable, it still relied on plant extraction.

Plant cell-culture processes appeared as the ideal method for large-scale production of taxol.^[35] These are environmentally sustainable, not depended on plant extraction neither on weather, season or contamination, and the material can be grown independently of its original location. Additionally, the productivity of plant cell cultures can be enhanced by cell engineering. All these advantages resulted in Bristol-Myers Squibb changing taxol production, which is currently based on a plant cell culture method developed by Phyton Inc.^[51]

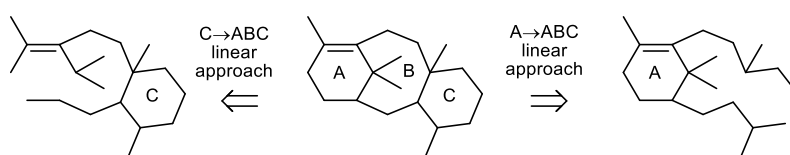
1.9 Precedents for the synthesis of the taxane skeleton

The first aim of the project centres on the synthesis of taxadienone **16**, which contains the taxane skeleton and almost no functionality except for a ketone group at the C2 position. For this reason, an overview of the reported approaches to the taxane skeleton is more relevant than a detailed description of all the long and low yielding total and formal taxol syntheses.

In the following discussion, the synthetic approaches towards the taxane skeleton have been classified into two main groups: linear and convergent syntheses. In turn, these have been further divided into subgroups, based on the key reaction step(s) used to establish the taxane core.

1.9.1 Linear approaches towards the taxane skeleton

A linear synthesis consists of a sequence of transformations, in which the product of one reaction is the reactant in the following step. In particular, a linear synthesis of the taxane skeleton involves sequential building of the three rings (A, B and C). Either an A-ring precursor (A→ABC linear approach) or a C-ring precursor (C→ABC linear approach) can be used as the starting point for the taxane skeleton synthesis (**Scheme 1.6**).



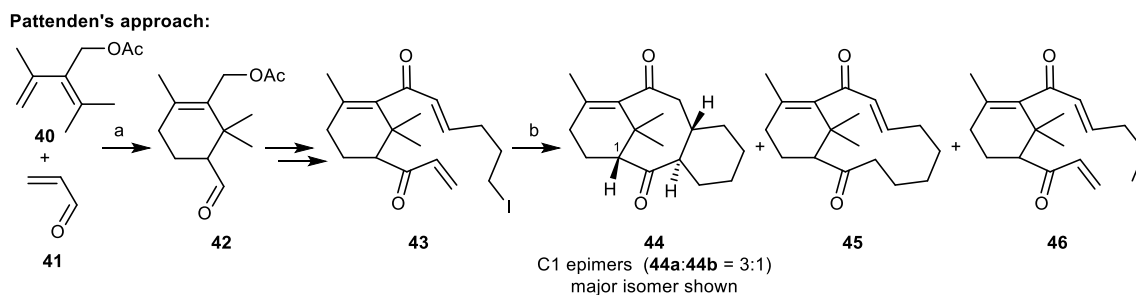
Scheme 1.6. Linear approaches towards the ABC tricyclic taxane skeleton.

1.9.1.1 Bioinspired strategy

Pattenden,^[52] who was inspired by the biosynthesis in which the tricyclic taxane skeleton is afforded by cyclisation of geranylgeranyl pyrophosphate, envisaged the synthesis of the taxane BC-ring by a tandem radical macrocyclisation-transannulation (**Scheme 1.7**).

In Pattenden's approach, the functionalised A-ring **42**, obtained by the intermolecular Diels–Alder (DA) reaction between diene **40** and acrolein (**41**), was

subsequently modified to afford the radical precursor **43** which, upon treatment with tributyltin hydride and AIBN, gave the cyclised product **44** as a 3:1 mixture of C1 epimers (25%), and the reduced products **45** and **46**.



Scheme 1.7. Pattenden's approach towards the taxane skeleton. **Reagents and conditions:** a) $\text{BF}_3 \cdot \text{OEt}_2$, $-78\text{ }^\circ\text{C}$, 79%; b) Bu_3SnH , AIBN, benzene, reflux, **44** (25%), **45** (20%) and **46** (30%).

1.9.1.2 Cycloaddition strategies

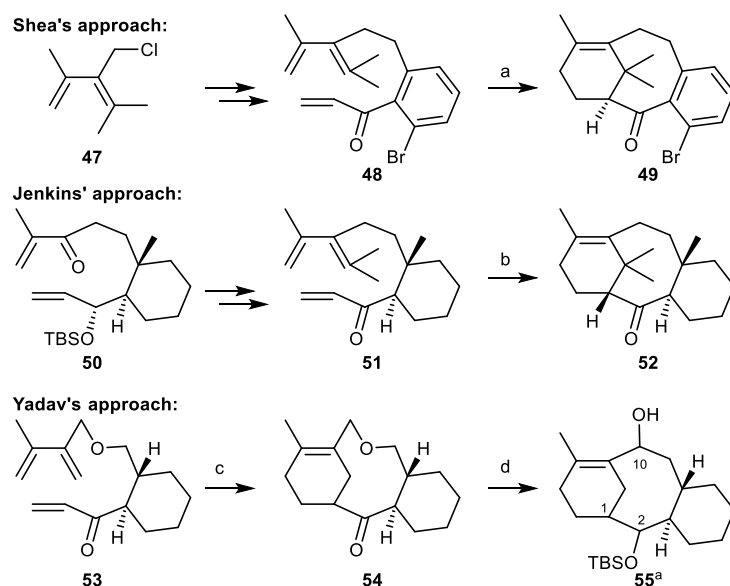
Cycloaddition reactions constitute an attractive approach toward taxanes because they allow the direct construction of the tricyclic taxane skeleton. As a result, a significant proportion of taxane syntheses incorporate cycloaddition reactions as the key step of the synthesis. For example, due to the occurrence of the 6-membered A- and C-rings, intramolecular Diels–Alder (IMDA) reactions are the cycloadditions most widely used in taxane synthesis and they can be applied to generate the taxane AB- or BC-ring systems. Cycloaddition reactions have also been applied in the construction of the 8-membered taxane B-ring. These include [2+2] and [4+2] cycloadditions with a subsequent fragmentation step, and also direct [4+4] cycloadditions.

Shea, Jenkins, and Yadav applied IMDA reactions to the direct construction of the taxane AB-ring system, providing access to the A-ring with the bridgehead double bond (**Scheme 1.8**).

The approaches of Shea^[53–56] and Jenkins^[57,58] are quite similar; however, there are three main differences: (1) The strategy used to introduce the diene structure differs; Shea used the prefabricated diene structure **47**, whereas Jenkins assembled it in a few steps. (2) Different taxane C-ring precursors were used; Shea used an aromatic C-ring precursor

(48), which favoured the cyclisation but needed to be reduced after cyclisation, whereas Jenkins used an alicyclic C-ring precursor (51). (3) The IMDA reactions were performed under different conditions; Shea applied thermal conditions and Jenkins used Lewis acidic conditions instead.

In turn, Yadav^[59,60] employed an interesting variation of the DA approach towards the taxane skeleton. As with Shea's and Jenkins' strategies, an IMDA reaction was used to produce a tricyclic structure (54) containing the taxane A- and C-rings and a 9-membered oxygen heterocycle; this subsequently underwent a Wittig rearrangement to afford the taxane B-ring (55).

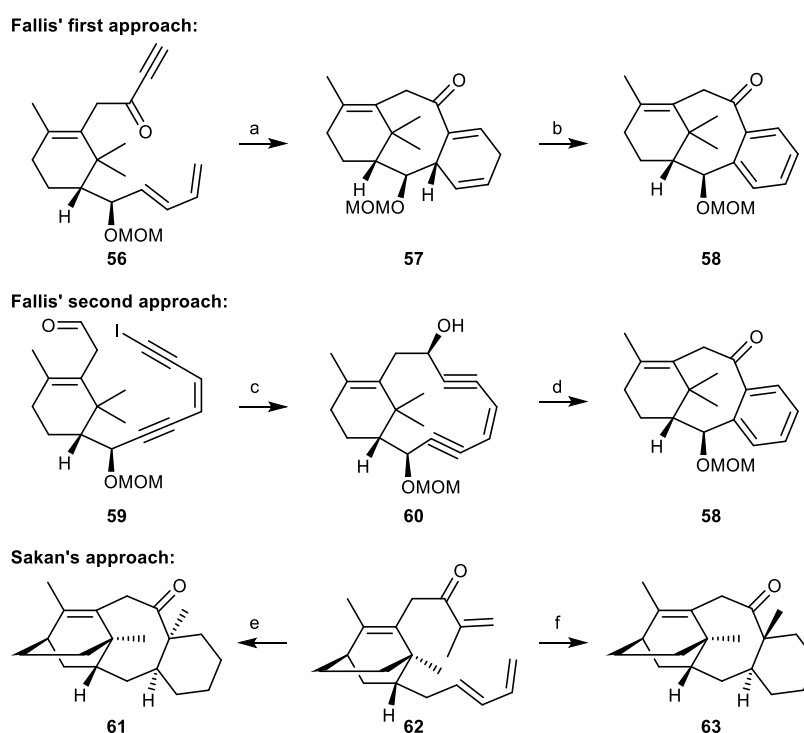


Scheme 1.8. IMDA reactions to construct the taxane AB-rings. **Reagents and conditions:** a) xylene, 150 °C, 76%; b) BF₃·OEt₂, toluene, -40 °C, 58%; c) Et₂AlCl, DCM, 0 °C, 45%; d) i. NaBH₄, EtOH; ii. TBSCl, imidazole, DMF; iii. *n*-BuLi, THF, -78 °C to RT; 40%. ^a Stereochemistry for C1, C2 and C10 was not reported.

IMDA reactions have also been applied to generate the taxane BC-ring (**Scheme 1.9**). In Fallis' first approach,^[61] a microwave assisted IMDA reaction afforded taxane 57, which was converted into the aromatic C-ring 58 after treatment with DDQ. Fallis reported that this cyclisation could only be performed under thermal conditions because, under acidic conditions, the double bond of the cyclohexene migrated into conjugation with the acetylenic ketone, preventing access to the conformation required for the IMDA reaction.

In his second approach,^[62] Fallis followed a similar strategy. The 12-membered ring **60** was afforded by a CrCl₂-NiCl₂-induced intramolecular coupling between the iodoacetylene and the aldehyde groups in **59**. Subsequently, **60** was cycloaromatised to afford the taxane **58** with an aromatic C-ring.

Although both of Fallis' approaches accomplished the synthesis of the taxane skeleton, neither of them was promising due to the low yielding cyclisation step.

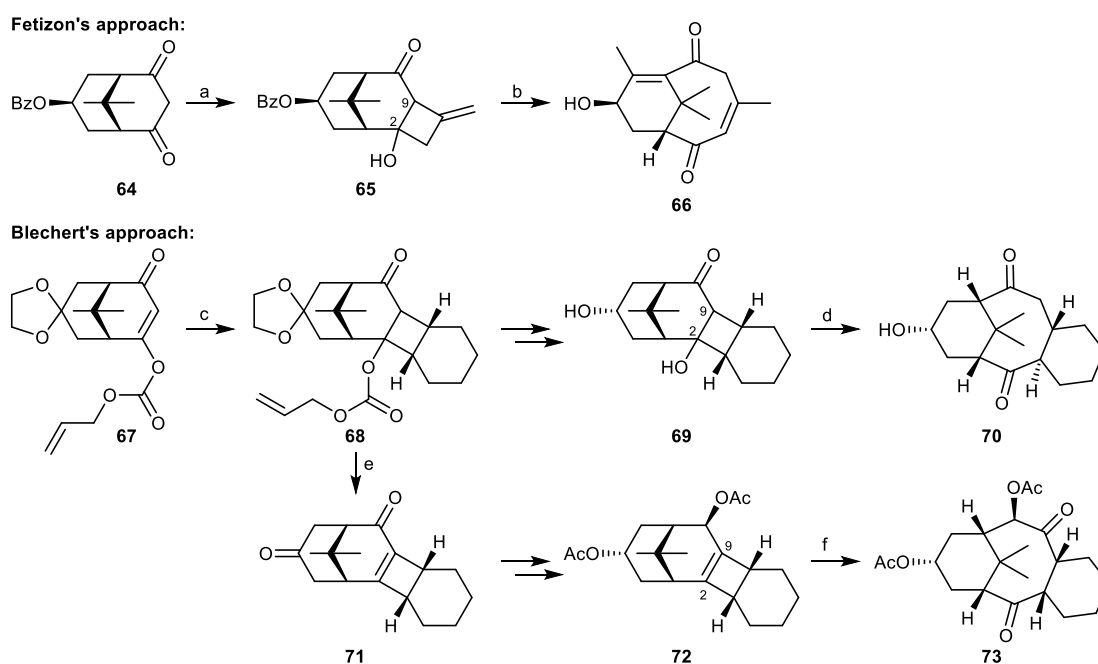


Scheme 1.9. IMDA reactions to construct the taxane BC-rings. **Reagents and conditions:** a) hv, toluene, reflux, 35–40%; b) DDQ, benzene, reflux, low yield (not specified); c) CrCl₂, NiCl₂, benzene, RT, 60%; d) i. hv, toluene, reflux; ii. PDC, DCM, RT, 3–10% (over 2 steps); e) Me₂AlCl, benzene, RT, 85%; f) toluene, 160 °C, 70%.

In a similar fashion, Sakan^[63,64] reported an IMDA reaction but, in this case, the A-ring formed part of a bicyclo[2.2.2]octane system **62**, hence it was conformationally restricted to an orientation that favoured the cycloaddition. This IMDA reaction could be performed under Lewis acidic and thermal conditions, but different isomers were afforded in each case. The *cis*-fused product **61** was obtained under Lewis acidic conditions; whereas, under thermal conditions, the *trans*-fused product **63** was generated instead. Considering that Lewis acid catalysis of DA reactions improved *endo* selectivity, a possible explanation

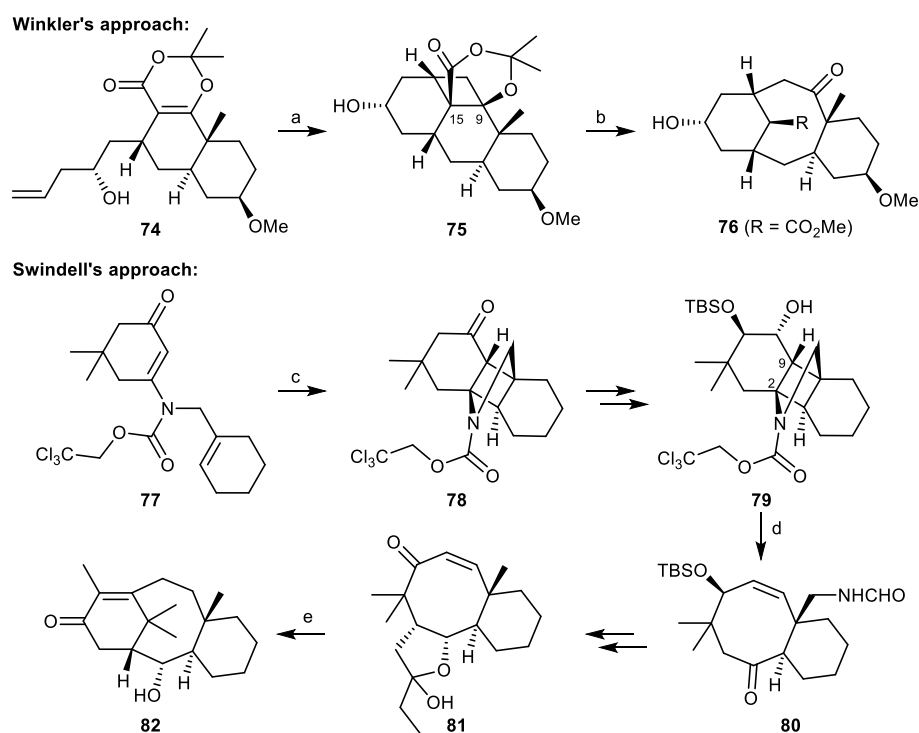
for the opposite stereochemical outcome was that the *cis*-fused **61** arises from an *endo* transition state and the *trans*-fused **63** from an *exo* one.

Fetizon^[65–67] and Blechert^[68,69] both applied a photochemical [2+2] cycloaddition-retroaldol strategy with C2–C9 bond cleavage to form the 8-membered taxane B-ring (Scheme 1.10). Fetizon generated the taxane AB-ring system **66** by the [2+2] cyclisation of β -diketone **64** and allene. However, **66** was afforded in a low yield and would require a subsequent C-ring annulation. In contrast, Blechert used cyclohexene in the [2+2] cycloaddition instead and afforded the taxane ABC-ring system **70** in a more efficient and straightforward manner. In addition, in order to introduce further oxygen functionality on the B-ring, Blechert^[70] exchanged the retroaldol fragmentation step for dehydration and C=C bond oxidative cleavage. This sequence afforded taxane **73**, which contained three oxidised positions on the taxane B-ring.



Scheme 1.10. [2+2] photocycloaddition-fragmentation strategies towards the taxane B-ring. **Reagents and conditions:** a) allene, hv, MeOH, $-78\text{ }^{\circ}\text{C}$; b) KOH, EtOH, $0\text{ }^{\circ}\text{C}$, 35% (over 2 steps); c) cyclohexene, hv, DCM, $-60\text{ }^{\circ}\text{C}$, 80%; KO t -Bu, t -BuOH, $70\text{ }^{\circ}\text{C}$, 86%; e) i. K₂CO₃, MeOH, RT; ii. H₃O⁺, 75% (over 2 steps); f) O₃, DCM, MeOH, $-78\text{ }^{\circ}\text{C}$; then (CH₃)₂S, $-78\text{ }^{\circ}\text{C}$ to RT, 40%.

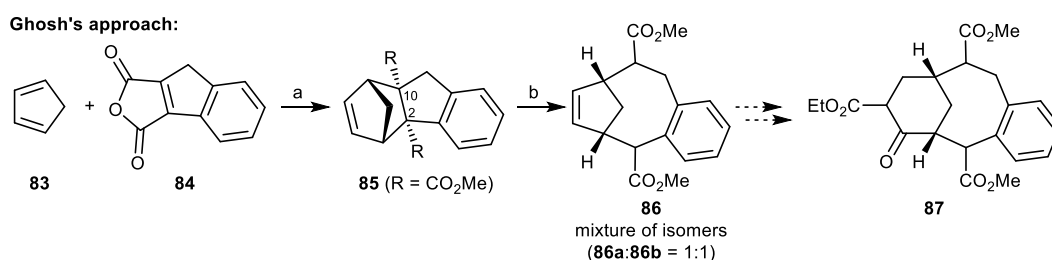
A [2+2] photocycloaddition-fragmentation sequence towards the synthesis of the 8-membered taxane B-ring has also been reported by both Winkler and Swindell (**Scheme 1.11**). Winkler^[71] furnished the taxane ABC-ring system **76** by an intramolecular dioxenone [2+2] photocycloaddition/base-fragmentation sequence, resulting in C9–C15 bond cleavage. In contrast, Swindell^[72,73] assembled first the taxane BC-ring system **80** *via* a [2+2] photocycloaddition-fragmentation strategy, with C2–C9 bond cleavage, and subsequently, introduced the taxane A-ring to finally achieve taxane **82**.



Scheme 1.11. [2+2] photocycloaddition-fragmentation strategies towards the taxane B-ring. **Reagents and conditions:** hv, ACN, acetone, RT, 75%; b) i. KOH, MeOH, RT; ii. CH₂N₂, ether, 82% (over 2 steps); c) hv, benzene, RT, 71%; d) i. MsCl, TEA, 0 °C; ii. Zn, THF, RT; iii. AcOH, THF, H₂O, RT; iv. AcOCHO, pyr, 0 °C to RT, 61% (over 4 steps); e) i. H₂, Pd-C; ii. KO^tBu, 74% (over 2 steps).

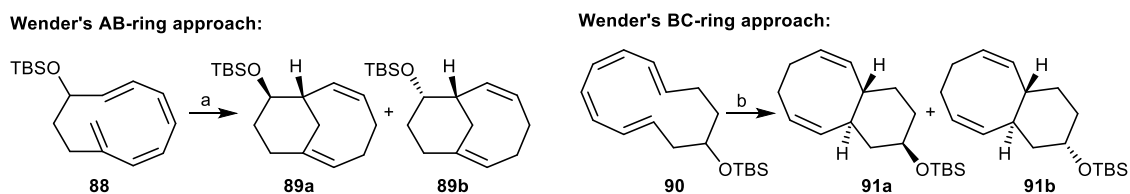
Ghosh^[74] also generated the 8-membered taxane B-ring *via* a cycloaddition-fragmentation sequence but, in this case, an intermolecular DA was employed instead of a [2+2] photocycloaddition (**Scheme 1.12**). The tricyclic taxane system **86**, containing a contracted 5-membered A-ring, was afforded by the DA reaction between cyclopentadiene **83** and maleic anhydride **84**, followed by reductive C2–C10 bond cleavage. Taxane **87**

would finally be achieved after ring expansion with ethyl diazoacetate, with the procedure reported by Ghosh in similar substrates.



Scheme 1.12. DA-fragmentation strategy towards the taxane B-ring. **Reagents and conditions:** a) i. AlCl₃, THF, 0 °C; ii. NaHCO₃, EtOH, H₂O, reflux; iii. CH₂N₂, ether, RT, 81% (over 3 steps); b) Na, NH₃(l), -55 °C, 33%.

Alternatively, Wender constructed the taxane B-ring by expanding his methodology developed for the synthesis of polycycles containing 8-membered rings. As a result, the taxane AB-ring^[75] **89** and BC-ring^[75,76] **91** systems were afforded by the nickel-catalysed intramolecular [4+4] cycloaddition of the tethered 1,3-diene units **88** and **90**, respectively (**Scheme 1.13**). In principle, this approach could also be used for the one-step formation of the taxane ABC-tricycle core from a suitable macrocyclic tetraene. However, this approach has not yet been explored.

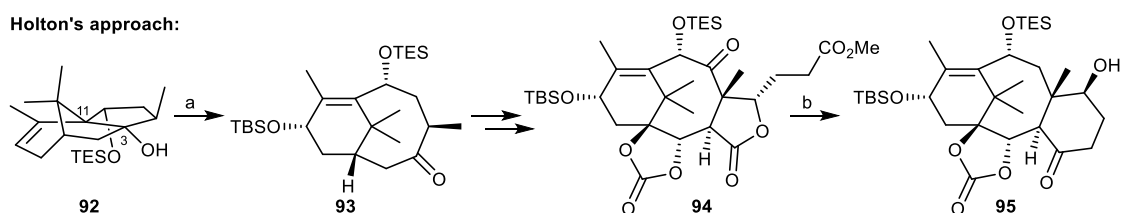


Scheme 1.13. Wender's approach towards the taxane AB- and BC-ring systems *via* an intramolecular [4+4] cycloaddition. **Reagents and conditions:** a) PPh₃, Ni(COD)₂, toluene, 110 °C, 52%, (89a:89b = 1.3:1); b) PPh₃, Ni(COD)₂, toluene, 110 °C, 92%, (91a:91b = 1.7:1).

1.9.1.3 Epoxy-alcohol fragmentation strategies

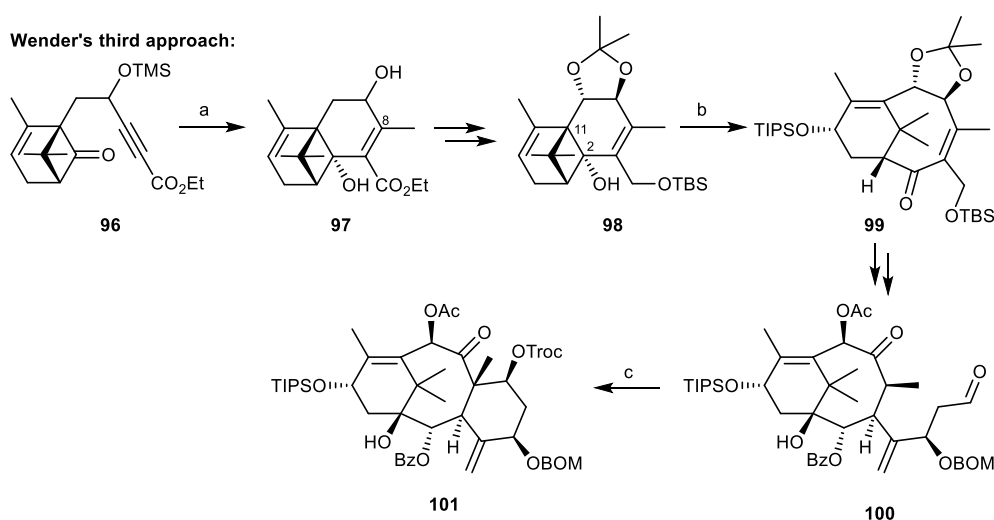
Holton^[38,39] applied his study of the fragmentation of bicyclic epoxy-alcohols to the construction of the taxane skeleton, which led to the first total synthesis of taxol (**Scheme 1.14**). The olefinic alcohol **92**, obtained from (1*S*,4*S*)-(-)-camphor, was subjected to epoxy-alcohol fragmentation with C3–C11 bond cleavage to furnish the taxane AB-ring system **93** which, after functionalisation into the cyclisation substrate **94**, underwent a Dieckman cyclisation to afford the C-ring, and thus establish the tricyclic taxane core. Holton's

synthesis finished with a series of steps to introduce the required functionalities into **95** to finally achieve taxol.



Scheme 1.14. Key steps in Holton's total synthesis of taxol. **Reagents and conditions:** a) i. *t*-BuOOH, $\text{Ti}(\text{O}i\text{-Pr})_4$, DCM, 0 °C; then $(\text{CH}_3)_2\text{S}$, reflux; ii. TBSOTf, pyr, 0 °C, 94% (over 2 steps); b) i. LDA, THF, -78 °C; then AcOH; ii. 2-methoxypropene, *p*-TsOH, THF, 0 °C; iii. KSPh, DMF, 86 °C; iv. H_3O^+ , 77% (over 4 steps).

Apart from the mentioned approaches towards the AB- and BC-ring systems, Wender^[41,42] also reported a total synthesis of taxol, in which an epoxy-alcohol fragmentation sequence was applied to the construction of the 8-membered taxane B-ring (**Scheme 1.15**). In this case, the alkynyl ester **96**, obtained from pinene, underwent methyl conjugate addition to the C8 position, followed by cyclisation *in situ* to afford the tricyclic alcohol **97**. The taxane AB-ring system **99** was obtained after chemoselective epoxidation of the trisubstituted alkene **98** with *m*-CPBA treatment, and epoxy-alcohol fragmentation with C2–C11 bond cleavage. Wender's synthesis followed with taxane C-ring assembly *via* an intramolecular aldol cyclisation to afford **101**, and finished with introduction of the required functionalities to finally achieve taxol.



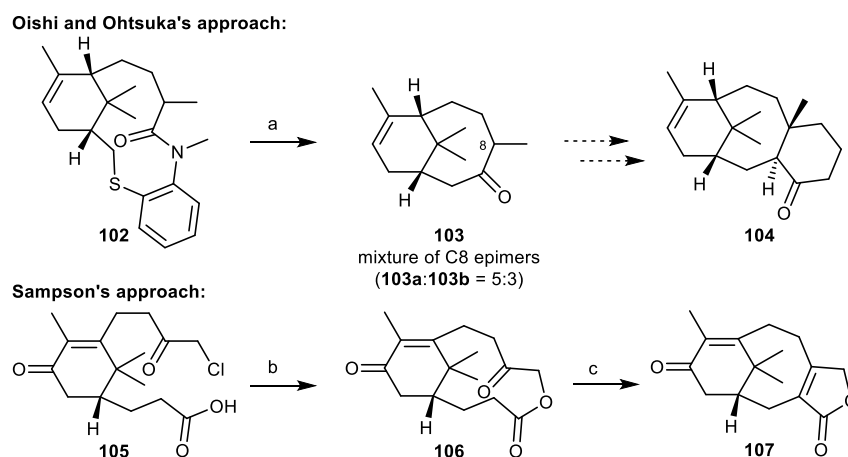
Scheme 1.15. Key steps in Wender's total synthesis of taxol. **Reagents and conditions:** a) Me_2CuLi , ether, -78 °C to RT; then AcOH, H_2O , 97%; b) i. *m*-CPBA, Na_2CO_3 , DCM, -78 °C to -15 °C; ii. DABCO, DCM, reflux; then TIPSOTf, 2,6-lutidine, -78 °C, 85%; c) DMAP, DCM, RT; then TrocCl, 62%.

1.9.1.4 Other strategies

Some attractive linear approaches towards the construction of the taxane skeleton, which do not fit in any of the previous categories, are summarised below.

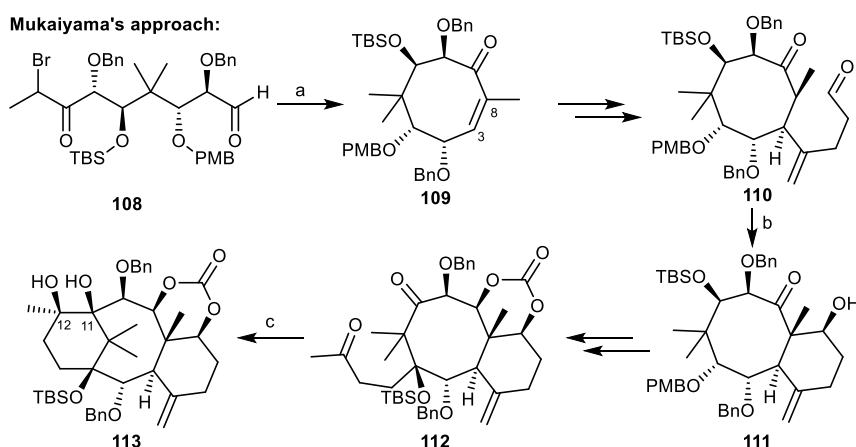
Oishi and Ohtsuka^[77–79] implemented their studies on the assembly of 8- to 12-membered rings, by contraction of lactam sulfoxides and sulfones,^[80–82] in the construction of the taxane B-ring (**Scheme 1.16**). The contraction of lactam sulfide **102** into ketone **103** started with oxidation of the sulfide to sulfone which, upon deprotonation at the sulfone α -carbon, underwent transannular acylation and gave ketone **103** after reductive cleavage of the sulfone group. Oishi and Ohtsuka reported the conversion of ketone **103**, already containing the taxane AB-ring system, to the tricyclic taxane system **104** without specifying the steps involved. Even though this is an attractive approach, its application is limited since the synthesis of the lactam sulfide is lengthy and low yielding.

Sampson^[83] reported the construction of the taxane AB-ring system **107** via macrolactonisation of the chloro keto acid **105** and subsequent intramolecular transannular aldol condensation (**Scheme 1.16**). Lactone **107** has not been further utilised in the synthesis of the taxane skeleton, but a DA reaction could be envisaged for taxane C-ring construction.



Scheme 1.16. Approaches involving intramolecular transannulation for taxane B-ring construction. **Reagents and conditions:** a) i. NaIO₄, MeOH, RT; b) ii. LDA, THF, –65 °C to 0 °C; iii. Na-Hg, Na₂HPO₄, MeOH, ether, RT, 49% (over 3 steps); b) NaOH (aq.), DMSO, 100 °C, 59%; c) i. NaH, DMSO; ii. NH₄Cl (aq.), 51% (over 2 steps).

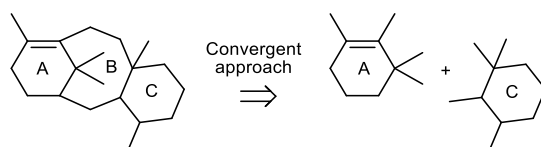
Mukaiyama^[43,84,85] reported a linear total synthesis of taxol, where the B-ring was furnished first and the A and C-ring were subsequently assembled into the framework (**Scheme 1.17**). The 8-membered cyclic enone **109** was afforded by the SmI₂-mediated intramolecular aldol cyclisation of ketoaldehyde **108**, followed by acetylation and treatment with DBU to introduce the C3–C8 double bond. Mukaiyama's synthesis continued with C-ring construction *via* the intramolecular aldol reaction of ketoaldehyde **110**, prepared by organocuprate Michael addition, affording aldol **111**. The taxane **113** was finally achieved after assembling the taxane A-ring using an intramolecular pinacol coupling with C11–C12 bond formation. Mukaiyama's synthesis finished with the introduction of the required functionalities to convert taxane **113** into taxol.



Scheme 1.17. Key steps in Mukaiyama's total synthesis of taxol. **Reagents and conditions:** a) i. SmI₂, THF, –78 °C; ii. Ac₂O, DMAP, pyr, RT; iii. DBU, benzene, 60 °C, 54% (3 steps); b) NaOMe, MeOH, THF, 0 °C, 98%; c) TiCl₂, LiAlH₄, THF, 35 °C, 52%.

1.9.2 Convergent approaches towards the taxane skeleton

A convergent synthesis is a strategic approach in which a complex molecule is assembled from several fragments with smaller size and reduced complexity. These fragments are called pre-assembled compounds because they are synthesised first and then combined to furnish the final product. In a convergent approach towards the taxane skeleton, the taxane B-ring is furnished by first coupling together A- and C-ring precursors and closing the taxane B-ring subsequently (**Scheme 1.18**).

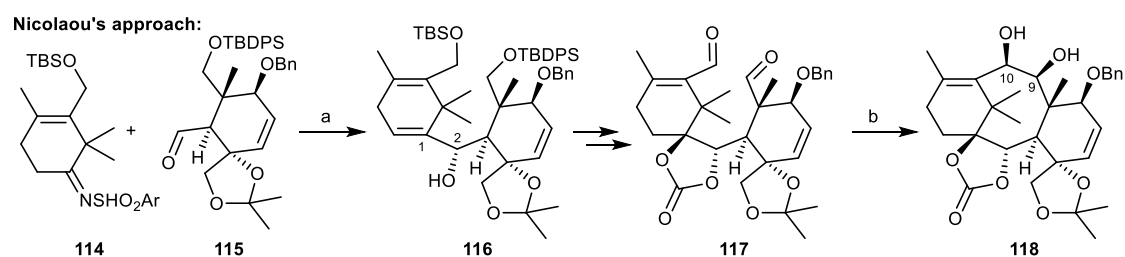


Scheme 1.18. Convergent approach towards the taxane skeleton.

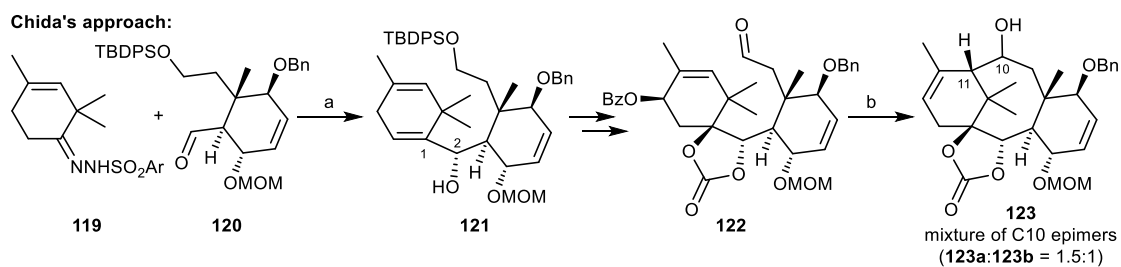
The convergent approaches towards the taxane core have been classified based on the reaction used as key step in achieving the taxane B-ring. The most widely used strategy to join suitably functionalised A- and C-ring precursors is based on the vinyl lithium-aldehyde coupling, applied in six of the ten reported total and formal syntheses of taxol. These approaches have been classified, in turn, depending on the method used to generate the vinyl lithium precursor: treatment of aryl hydrazone with *n*-BuLi (Shapiro coupling), which has been used in two total syntheses of taxol, or lithium-halogen exchange, used in four taxol approaches. Attractive strategies involving anionic oxy-Cope rearrangements have also been applied to convergent taxane skeleton syntheses.

1.9.2.1 Shapiro coupling tactics

Nicolaou^[37,86–88] and Chida^[48] have both reported total syntheses of taxol in which the A- and C-ring precursors were joined by a Shapiro coupling reaction with C1–C2 bond formation (**116** and **121**). However, different strategies were used to close the 8-membered taxane B-ring. Nicolaou reported a pinacol coupling between C9–C10 to afford diol **118** (**Scheme 1.19**), whereas Chida generated tricyclic alcohol **123** using an SmI₂-mediated reductive cyclisation with C10–C11 bond formation (**Scheme 1.20**).

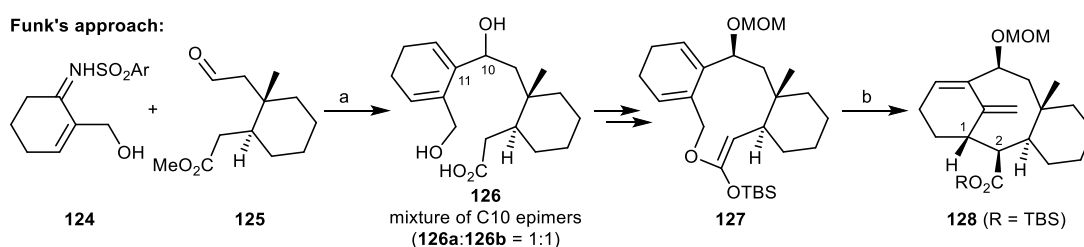


Scheme 1.19. C1–C2 Shapiro coupling reactions applied to the total synthesis of taxol. **Reagents and conditions:** a) **114**, *n*-BuLi, THF, –78 °C to RT; then **115**, 0 °C, 82%; b) (TiCl₃)₂–(DME)₃, DME, 70 °C, 82%;



Scheme 1.20. C1–C2 Shapiro coupling reactions applied to the total synthesis of taxol. **Reagents and conditions:** a) **119**, *n*-BuLi, THF, $-78\text{ }^{\circ}\text{C}$ to $0\text{ }^{\circ}\text{C}$; then **120**, $-78\text{ }^{\circ}\text{C}$, 92%; b) SmI₂, HMPA, THF, $40\text{ }^{\circ}\text{C}$, 66% (**123a**:**123b** = 1.5:1).

Funk's approach^[89,90] towards the taxane skeleton also made use of a Shapiro coupling reaction, to join the taxane A- and C-ring precursors (**126**) with C10–C11 bond formation, as opposed to C1–C2 as shown previously. Subsequently, Funk applied his previously developed methodology for ring contractions, using an Ireland–Claisen rearrangement in TBS-enol ether **127** to close the taxane B-ring *via* C1–C2 bond formation and afford taxane **128** (Scheme 1.21).



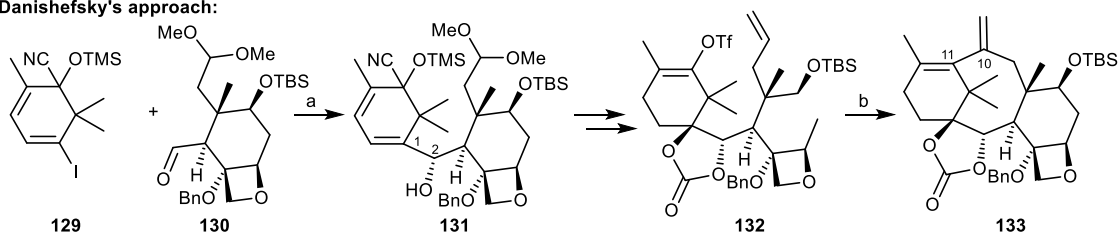
Scheme 1.21. C10–C11 Shapiro coupling reaction applied to the synthesis of the taxane skeleton. **Reagents and conditions:** A) **124**, *t*-BuLi, THF, $-78\text{ }^{\circ}\text{C}$ to $-10\text{ }^{\circ}\text{C}$; then **125**, $-10\text{ }^{\circ}\text{C}$, 56% (**126a**:**126b** = 1:1); b) toluene, reflux, 82%.

1.9.2.2 Lithium-halogen exchange tactics

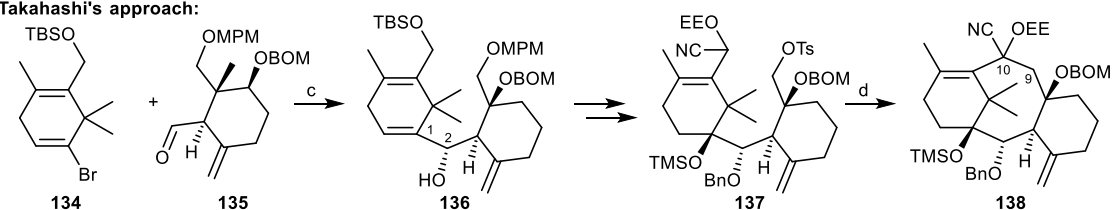
Total and formal syntheses of taxol reported by Danishefsky,^[40,91] Takahashi,^[46] Kuwajima,^[44,92,93] and Nakada^[47,94–96] all involve a lithium-halogen exchange to generate the lithium species required for the vinyllithium-aldehyde coupling of the taxane A- and C-ring precursors (Scheme 1.22). While Danishefsky and Takahashi implemented this methodology in C1–C2 coupling, affording allylic alcohols **131** and **136**, Kuwajima and Nakada applied it to C2–C3 coupling, generating allylic alcohols **141** and **146**, respectively. Subsequently, different strategies were used to close the 8-membered taxane B-ring: Danishefsky applied a Heck reaction with C10–C11 bond formation (**133**); Nakada also closed the B-ring *via* C10–C11 coupling but, in this case, using a Pd-catalysed

intramolecular alkenylation of a methyl ketone (**148**). Both Kuwajima and Takahashi described a C9–C10 B-ring closure, *via* an intramolecular Mukaiyama aldol reaction (**143**) and an intramolecular alkylation (**138**), respectively.

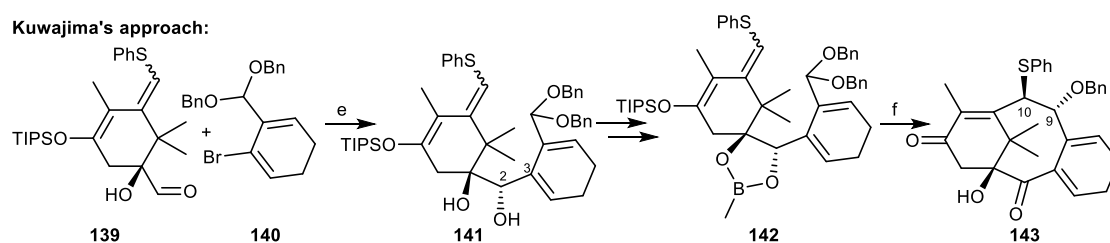
Danishefsky's approach:



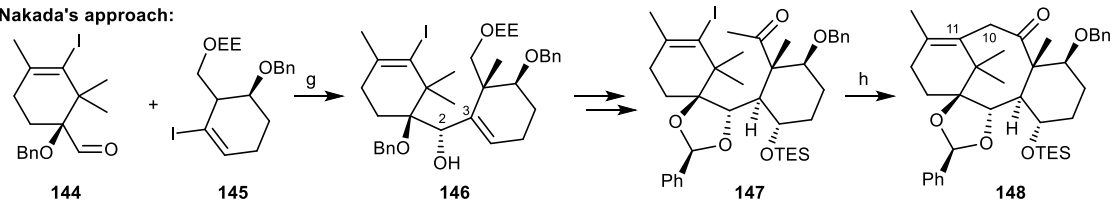
Takahashi's approach:



Kuwajima's approach:



Nakada's approach:

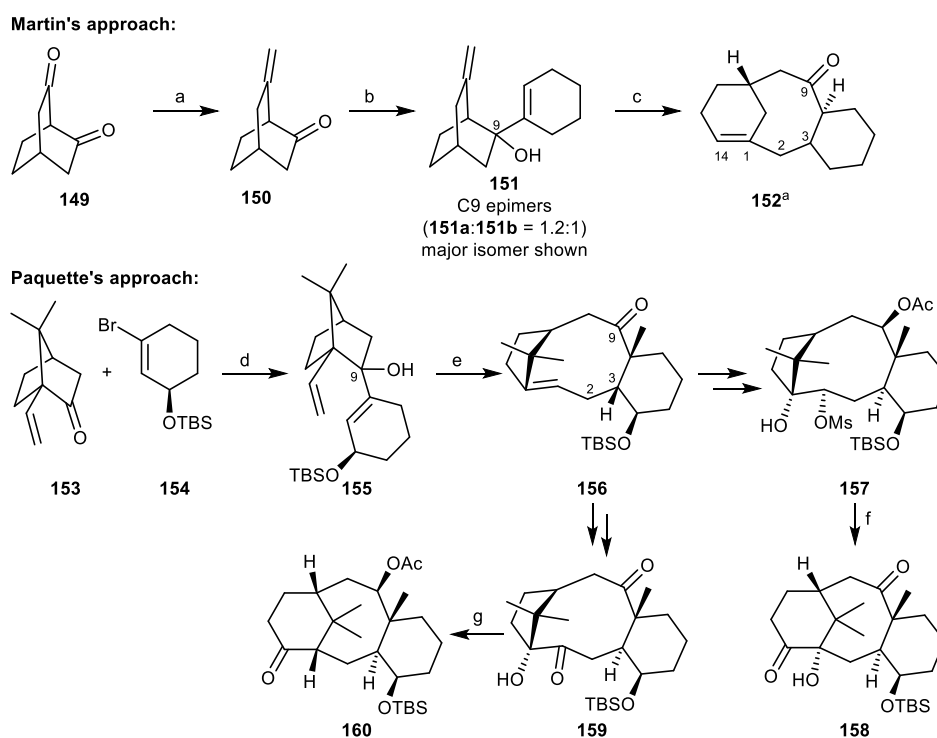


Scheme 1.22. Lithium-halogen exchange applied to total and formal syntheses of taxol. **Reagents and conditions:** a) **129**, *t*-BuLi, THF, $-78\text{ }^{\circ}\text{C}$; then **130**, $-78\text{ }^{\circ}\text{C}$, 93%; b) Pd(PPh₃)₄, K₂CO₃, ACN, $90\text{ }^{\circ}\text{C}$, 49%; c) **134**, *t*-BuLi, CeCl₃, THF, $-78\text{ }^{\circ}\text{C}$; then **135**, $-78\text{ }^{\circ}\text{C}$, 78%; d) LiN(TMS)₂, dioxane, hv, $145\text{ }^{\circ}\text{C}$, 49%; e) **140**, *t*-BuLi, *t*-BuMgCl, THF, $-94\text{ }^{\circ}\text{C}$; then **139**, $-78\text{ }^{\circ}\text{C}$, 68%; f) i. TiCl₂(*O*-i-Pr)₂, DCM, $-78\text{ }^{\circ}\text{C}$ to $0\text{ }^{\circ}\text{C}$; ii. Pinacol, DMAP, benzene, RT, 59% (over 2 steps); g) **145**, *n*-BuLi, THF, $-78\text{ }^{\circ}\text{C}$; then **144**, $-78\text{ }^{\circ}\text{C}$, 95%; h) Pd(PPh₃)₄, KOPh, toluene, $100\text{ }^{\circ}\text{C}$, 97%.

1.9.2.3 Oxy-Cope rearrangement strategies

Martin's approach^[97] towards the taxane skeleton relied on an anionic oxy-Cope rearrangement (**Scheme 1.23**), which consisted of a three-step process starting from diketone **149**. A Wittig olefination was used to introduce the terminal double bond (**150**), followed by cyclohexenyllithium addition to afford allylic alcohol **151**, which underwent an anionic oxy-Cope rearrangement to finally furnish the taxane system **152**. This

methodology led to the carbon-carbon connectivity of the taxane core with the bridgehead double bond incorrectly located at the C1–C14 position instead of the C11–C12.



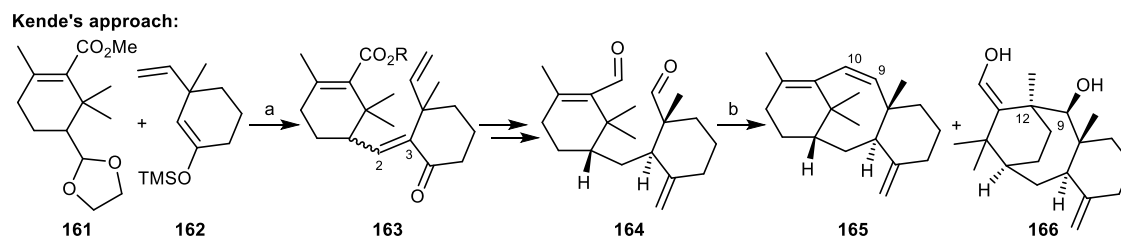
Scheme 1.23. Anionic oxy-Cope rearrangements applied to the synthesis of the taxane skeleton. **Reagents and conditions:** a) $\text{CH}_2=\text{PPh}_3$, THF, $-78\text{ }^\circ\text{C}$ to RT, 80%; b) cyclohexenyllithium, THF, $0\text{ }^\circ\text{C}$, 72% (**151a:151b** = 1.2:1); c) KH, THF, RT; d) **154**, $t\text{-BuLi}$, CeCl_3 , THF, $-78\text{ }^\circ\text{C}$; then **153**, $-78\text{ }^\circ\text{C}$, 59%; e) KHMDS, THF, $-60\text{ }^\circ\text{C}$; then CH_3I , $-60\text{ }^\circ\text{C}$, 74%; f) Et_2AlCl , DCM, $-78\text{ }^\circ\text{C}$ to $-15\text{ }^\circ\text{C}$, 90%; g) $\text{Al}(\text{O}t\text{-Bu})_3$, benzene, RT, 54%. ^a Taxane **152** was obtained as a single product but its stereochemistry was not determined.

Paquette^[98] also employed an anionic oxy-Cope rearrangement to furnish the taxane B-ring (**Scheme 1.23**). Addition of vinyl bromide **154** to ketone **153**, by generating the corresponding organocerium species, generated allylic alcohol **155** which, upon base treatment, underwent anionic oxy-Cope rearrangement to afford ketone **156** after methylation *in situ*. Tricyclic ketone **156** contains a contracted 5-membered A-ring and an expanded 9-membered B-ring and hence Paquette developed several strategies for conversion to the taxane skeleton, including: pinacol-like^[99] (**157**→**158**) and α -hydroxy ketone^[100,101] (**159**→**160**) rearrangements.

1.9.2.4 Other strategies

Kende^[102] reported a convergent approach towards the tricyclic taxane structure that does not fit into any of the strategies already mentioned (**Scheme 1.24**). In this

method, the taxane A- and C-ring precursors were coupled *via* a Mukaiyama aldol reaction, with C2–C3 bond formation (**163**), followed by B-ring closure through McMurry coupling between C9 and C10 to afford the taxane skeleton. However, taxane **165** was obtained in low yield and diol **166** was the major product, arising from 1,4-coupling.



Scheme 1.24. Key steps in Kende's approach towards the taxane skeleton synthesis. **Reagents and conditions:** a) i. TiCl_4 , DCM, $-50\text{ }^\circ\text{C}$; ii. *p*-TsOH, benzene, $80\text{ }^\circ\text{C}$, 90% (over 2 steps); b) TiCl_3 , Zn–Cu, DME, reflux, **165** (20%) and **166** (40%).

Having now gained a comprehensive knowledge of the relevant existing approaches towards the taxane hydrocarbon skeleton, different methods of synthesising the taxane core could be assessed to find the most suitable route, reaching the desired product in a short, practical and efficient manner.

2 APPROACHES TO TAXADIENONE 16

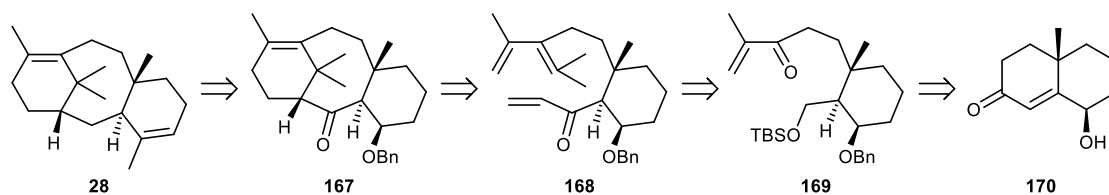
2.1 Taxadienone: target molecule for oxidation with P450_{BM3} mutants

As stated in the Introduction, the primary aim of this project centred on the application of enzymatic catalysis to the synthesis of taxol analogues. To this end, the taxane hydrocarbon skeleton would be assembled first using classical synthetic methods, and subsequently oxidised with P450_{BM3} mutants. This approach would provide a quick access to taxol-type compounds with different hydroxylation profiles.

The selection of the most suitable compound for the enzymatic oxidation was the first decision to make. The target molecule must obviously contain the taxane skeleton and none or minimal oxidative adornments. Additionally, other factors such as the ease of the synthesis and the oxidation potential must be taken into account during target selection.

Taxadiene **28** was the first molecule to be considered during target selection because it is the least oxidised taxane and also the substrate for oxidation in the biosynthesis of taxol-type compounds. However, its synthesis and oxidation feasibility were deemed inconvenient and thus, it was rejected, for the reasons described below.

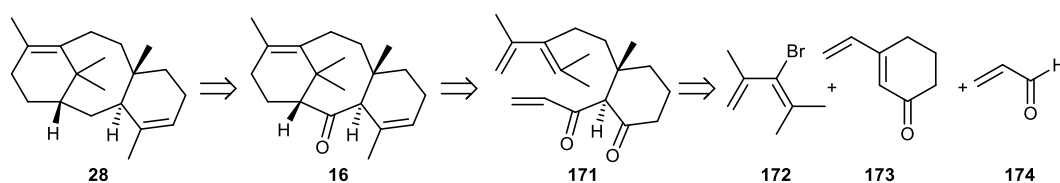
The synthesis of taxadiene **28** has only been reported twice, by Williams^[103] (26 steps) and Baran^[104] (10 steps). In both approaches, the AB-rings were forged by an IMDA reaction and the bare taxane carbon skeleton was achieved by deoxygenation of oxidised taxane intermediates (**Schemes 2.1 and 2.2**). Considering that the aim of the project was to introduce oxidation to the taxane skeleton by late-stage hydroxylation using P450_{BM3} mutants, it did not make sense to choose a molecule for which the synthesis would involve deoxygenation steps. Moreover, taxadiene **28** was not a particularly suitable substrate for enzymatic oxidation either because it was expected to be insufficiently soluble in the aqueous media required by the enzymes.



Scheme 2.1. Williams' retrosynthetic analysis of taxadiene 28.

As a result, other taxane compounds with only one oxidised position were considered and, taxadienone **16**, which is oxidised at the C2 position, was selected as the most suitable target. On the one hand, its oxidation seemed more feasible due to the presence of a functional group in each of the taxane A-, B- and C-rings (one heteroatom and two olefins). On the other, shorter synthetic approaches could be developed owing to the oxidised C2 position, whilst also giving access to the natural (C2 α -hydroxy) and unnatural (C2 β -hydroxy) taxane compounds.

The synthesis of taxadienone **16** has only been described by Baran,^[105,106] in an 8 step sequence, with the longest linear sequence of 7 steps and 18–20% yield (Scheme 2.2). This synthesis was later optimised and scaled-up by Krasutsky^[107] for application in large scale production. This route appears short, convergent and scalable; the tricyclic taxane skeleton is forged by means of an IMDA reaction and all the stereochemical information derives from just one enantioselective reaction, with others set diastereoselectively.



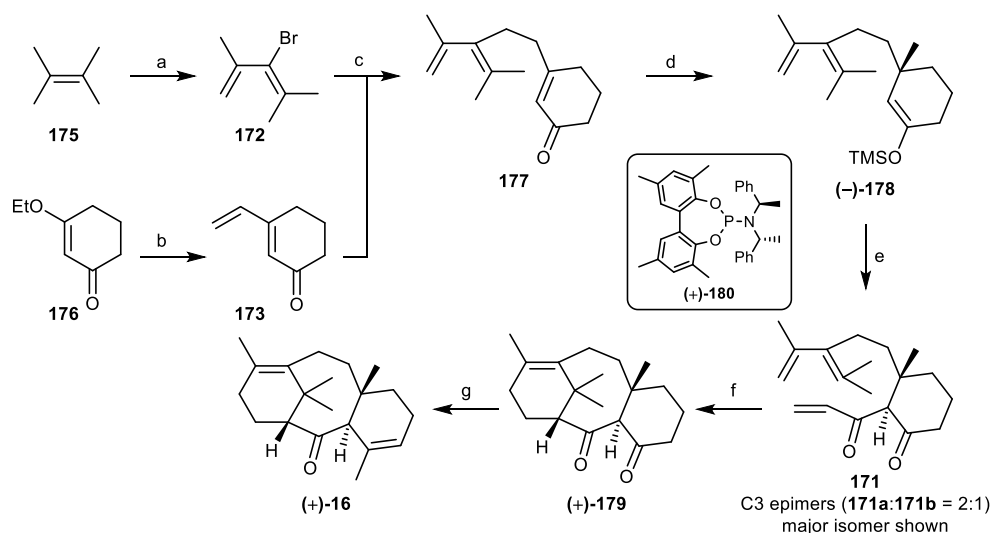
Scheme 2.2. Baran's retrosynthetic analysis of taxadiene 28 and taxadienone 16.

As the primary interest of the project was the application of P450_{BM3} mutants into the synthesis of taxol-type compounds and not the development of synthetic approaches to taxanes, it was decided to follow Baran's synthesis to obtain taxadienone **16** quickly.

Even though Baran emphasised the scalability and robustness of his synthesis to taxadienone **16**, he also remarked that most of the steps had initially been difficult to scale-

up and suffered from low and inconsistent yields prior to optimisation.^[106] Indeed, reproducing this synthesis proved troublesome. Every single step required optimisation; making the synthesis of taxadienone **16** more challenging and time-consuming than expected at the outset of the project.

In this chapter, Baran's synthesis of taxadienone **16** (**Scheme 2.3**) and the problems faced in reproducing it, along with their respective solutions, are introduced. In addition, further approaches towards taxadienone **16** and intermediates which have their basis in Baran's route are presented.



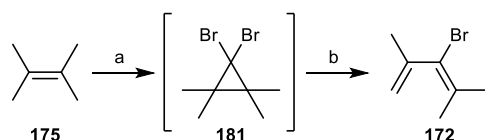
Scheme 2.3. Baran's route to taxadienone **16**. **Reagents and conditions:** a) CHBr_3 , *t*-BuOK, pentane, 0 °C to RT; then PhNMe_2 , 150 °C, 67%; b) vinylmagnesium bromide, ether, 0 °C to RT, 75%; c) *s*-BuLi, ether, -78 °C; then $\text{CuBr}\cdot\text{SMe}_2$, TMSCl, -78 °C; then **173**, -78 °C to RT, 86%; d) Me_3Al , CuTC, ligand **(+)-180**, ether, -78 °C to -30 °C; then THF, TMSCl, -30 °C to RT, 89%, 93% e.e.; e) acrolein, $\text{Gd}(\text{OTf})_3$, $\text{H}_2\text{O}/\text{EtOH}/\text{toluene}$ (1:10:4), 4 °C; then CrO_3 , H_2SO_4 , acetone, 0 °C, 85%; f) $\text{BF}_3\cdot\text{OEt}_2$, DCM, 0 °C, **171a** (47%) and **171b** (29%); g) i. PhNTf_2 , KHMDS, THF, 0 °C; ii. Me_2Zn , $\text{Pd}(\text{PPh}_3)_4$, THF, 0 °C to RT, 84% (over 2 steps).

2.2 Reproduction of Baran's route to taxadienone 16

Baran's synthesis started with the preparation of bromodiene **172** and dienone **173**. Cyclopropanation of 2,3-dimethyl-2-butene (**175**) with dibromocarbene and subsequent dehydrohalogenative ring-opening induced by *N,N*-dimethylaniline afforded bromodiene **172**. Baran modified this known two-step transformation^[108,109] into a one-pot procedure, emphasising that 2.0 eq. of butene **175** were required to avoid the presence of bromoform

in the thermal step because, being rather unvolatile, it must be consumed entirely in the cyclopropanation step.

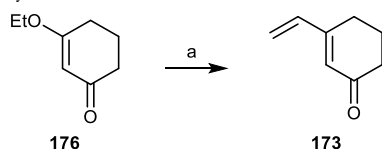
However, when this reaction was performed as reported, bromoform was always present in the crude product mixture, and was difficult to remove due to its low volatility (b.p. 151 °C) and similar R_f to bromodiene **172**. When the two steps were separated, and cyclopropane **181** was isolated, bromoform was also present; thus, the crude product was placed under high vacuum until the bromoform was completely removed. *N,N*-dimethylaniline was then added to the reaction mixture and, in this case, pure bromodiene **172** was obtained.



Scheme 2.4. Synthesis of bromodiene **172**. **Reagents and conditions:** a) CHBr_3 , *t*-BuOK, pentane, 0 °C to RT; b) PhNMe_2 , 150 °C, 61% (over 2 steps).

Baran used a previously described method^[110] to prepare dienone **173**. The reported 1,2-addition of vinylmagnesium bromide to ethoxy-enone **176** followed by enol ether hydrolysis with dilute acid afforded dienone **173** in 75% yield. However, lower yields were achieved following this methodology (maximum 69%) and therefore, other reaction conditions used on similar substrates^[111] were tested (**Table 2.1**), affording dienone **173** in a 97% yield.

Table 2.1. Optimisation of dienone **173** synthesis.

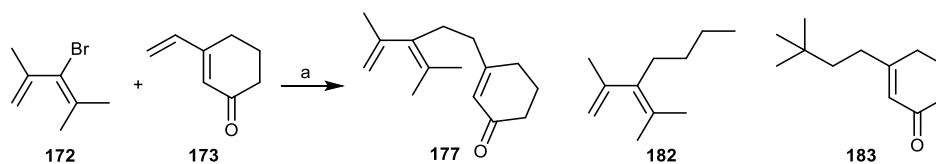


Reagents and conditions: a) vinylmagnesium bromide, solvent, T (°C) to RT.

Entry	Vinylmagnesium bromide (eq.)	Solvent	T (°C)	Yield (%)
1 ^{a,b}	1.1	ether	0	–
2	1.1	THF	0	69
3	1.4	THF	–78	97

^a Conditions reported by Baran; ^b Ethoxy-enone **176** was recovered unchanged.

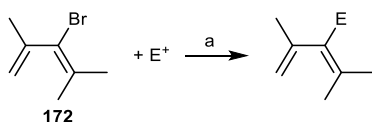
With bromodiene **172** and dienone **173** in hand, the preparation of enone **177** *via* a Cu-mediated 1,6-addition was studied. As reported by Baran, the lithiated species of bromodiene **172** was generated *in situ* upon treatment with *s*-BuLi by lithium-bromine exchange and subsequently coupled to dienone **173** by means of a Lewis acid-modified organocopper 1,6-addition, using CuBr·SMe₂ and TMSCl. Baran also noted that this reaction was initially plagued with inconsistent yields due to side-product formation in which the lithiating agent played an important role (**Scheme 2.5**): when *n*-BuLi was used, diene **182** was afforded by alkylation with *n*-BuBr; and, when *t*-BuLi was used instead, the 1,6-addition of *t*-BuLi to dienone **173** occurred in preference to the lithium-bromine exchange, affording enone **183**. As a result, Baran reported that *s*-BuLi was the most convenient lithiating agent due to its less nucleophilic character, allowing enone **177** to be prepared in decagram quantities per reaction batch.



Scheme 2.5. Results reported by Baran on the preparation of enone **177**. **Reagents and conditions:** a) **172**, BuLi, ether, -78°C ; then CuBr₂·SMe₂, TMSCl, -78°C ; then **173**, -78°C to RT; **177** was afforded using *s*-BuLi; **182**, with *n*-BuLi; and **183**, with *t*-BuLi.

However, in our hands it was difficult to make the 1,6-addition reaction work reliably. Following Baran's conditions, bromodiene **172** was recovered unchanged which denoted a problem in the lithiation step. For this reason, different BuLi sources were explored (**Table 2.2**); in these cases, commercially available electrophiles were used because, reportedly, dienone **173** decomposes quickly even at -20°C .^[104]

After testing *n*-BuLi, *s*-BuLi and *t*-BuLi, lithiation of bromodiene **172** only proceeded with *t*-BuLi and even though enone **177** was afforded, the yield was low and inconsistent (**entry 6**). Baran's conditions were then re-tested using new *s*-BuLi (**entry 7**) and despite enone **177** was now always obtained, the yield remained variable and significantly different from the reported one (86%).

Table 2.2. Tested conditions to metalate bromodiene **172**.

Reagents and conditions: a) **172**, BuLi, THF, $-78\text{ }^{\circ}\text{C}$; then $\text{CuBr}_2\cdot\text{SMe}_2$, TMSCl^a , $-78\text{ }^{\circ}\text{C}$; then E^+ , $-78\text{ }^{\circ}\text{C}$ to RT.

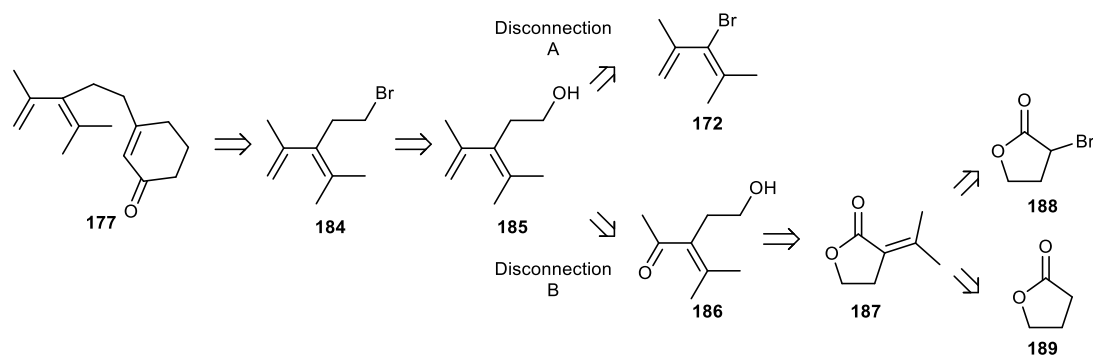
Entry	E^+	Lithiating agent	Bromodiene 172	Product	Yield (%)
1 ^b	173	<i>s</i> -BuLi	Recovered	–	–
2	Benzyl bromide	<i>n</i> -BuLi	Recovered	1,2-diphenylethane	91
3	Benzyl bromide	<i>s</i> -BuLi	Recovered	–	–
4	1-Chloro-3-iodopropane	<i>t</i> -BuLi	Reacted	–	–
5	Cyclohex-2-en-1-one	<i>t</i> -BuLi	Reacted	–	–
6 ^c	173	<i>t</i> -BuLi	Reacted	177	8, –, 20, –
7 ^{b,c}	173	<i>s</i> -BuLi	Reacted	177	31, 6, 36, 3

^a TMSCl was used only in entries 1, 5, 6 and 7; ^b Conditions reported by Baran; ^c Yields obtained in different reactions.

The organocopper 1,6-addition of bromodiene **172** to dienone **173** was unreliable, and hence a practical approach to enone **177** was required.

2.3 Alternative syntheses of enone **177**

At this point, it was envisaged that the methodology used by Baran to prepare dienone **173** could be applied to enone **177**; that is, the preparation of **177** *via* addition of the Grignard reagent derived from **184** to ethoxy-enone **176**. The required bromodiene **184** would be obtained from alcohol **185** which, in turn, would be prepared following two different approaches. Firstly, with many grams of bromodiene **172** available, alcohol **185** would be afforded by a two-carbon extension sequence involving the addition of the lithiated species of **172** to ethylene oxide^[112] (**Scheme 2.6**, disconnection A). In the second approach, alcohol **185** would be obtained by means of an olefination reaction of enone **186**, which would arise from the ring-opening of lactone **187**. Finally, the synthesis of lactone **187** was envisaged from a Horner–Wadsworth–Emmons reaction^[113] or an aldol condensation^[114] (**Scheme 2.6**, disconnection B).



Scheme 2.6. Retrosynthetic analysis of enone 177.

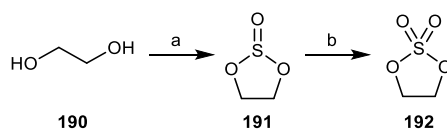
2.3.1 Disconnection A

Despite the low yields on the metalation of bromodiene **172** when reproducing Baran's route (**Table 2.2**), the lithiation and subsequent addition to ethylene oxide to generate alcohol **185** were studied (**Table 2.3**). In these reactions, no bromodiene **172** was recovered, so low yields were attributed to adventitious water present in the reaction media, which would quench the lithiated species of **172** and generate volatile diene **194** (**entry 1**). In case the ethylene oxide was wet, drying agents (CaCl_2 and NaOH) were tested but alcohol **185** was not afforded in any case and reaction of the drying agent with ethylene oxide was evident (**entries 2 and 3**). Slightly improved results were obtained using *s*-BuLi rather than *t*-BuLi (**entries 1 and 4**).

In order to make the reaction more practical, safer, and easier to reproduce, a commercial solution of ethylene oxide in THF was used. Similar results were achieved when using this solution as had been obtained with ethylene oxide gas (**entry 5**). Then, based on the known activation of epoxides with Lewis acids,^[115,116] $\text{BF}_3 \cdot \text{OEt}_2$ was included, giving a slightly improved yield (**entry 6**). However, when the amounts of epoxide and Lewis acid were increased, alcohol **185** was afforded in lower yield (**entries 7 and 8**). The reaction with the Grignard reagent derived from **172** afforded alcohol **185** in a very low yield (**entry 9**).

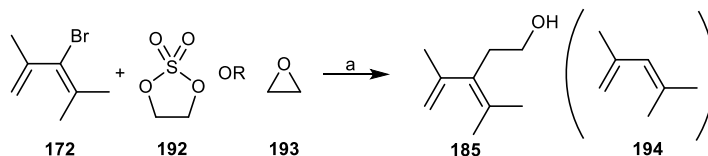
1,2-Cyclic sulfates are synthetic equivalents of epoxides,^[117] hence the two-carbon extension of metalated bromodiene **172** using sulfate **192** was also attempted. The required

sulfate **192** was prepared from ethylene glycol (**190**) (Scheme 2.7).^[118,119] Alcohol **185** was generated from sulfate **192**, but again the yield was poor (entry 10).



Scheme 2.7. Synthesis of sulfate **192**. **Reagents and conditions:** a) SOCl_2 , DCM, RT, 100%; b) NaIO_4 , $\text{RuCl}_3 \cdot x\text{H}_2\text{O}$, $\text{H}_2\text{O}/\text{CHCl}_3$ (3:1), RT, 37%.

Table 2.3. Tested conditions in the two-carbon extension of bromodiene **172**.



Reagents and conditions: a) **172**, BuLi, THF, -78°C ; then $\text{BF}_3 \cdot \text{OEt}_2$; then **192** or **193**, -78°C to RT.

Entry	Reagent (eq.)	Metalating agent	$\text{BF}_3 \cdot \text{OEt}$ (eq.)	Drying agent	Yield (%)
1	193 gas (excess)	<i>t</i> -BuLi	–	–	28
2	193 gas (excess)	<i>t</i> -BuLi	–	CaCl_2 (s)	–
3	193 gas (excess)	<i>t</i> -BuLi	–	NaOH (s)	–
4	193 gas (excess)	<i>s</i> -BuLi	–	–	40
5	193 sol. (1.0)	<i>s</i> -BuLi	–	–	40
6	193 sol. (1.0)	<i>s</i> -BuLi	1.0	–	55
7	193 sol. (2.0)	<i>s</i> -BuLi	1.0	–	33
8	193 sol. (3.5)	<i>s</i> -BuLi	1.4	–	50
9	193 sol. (1.0)	Mg	–	–	7
10	192 (1.4)	<i>s</i> -BuLi	–	–	29

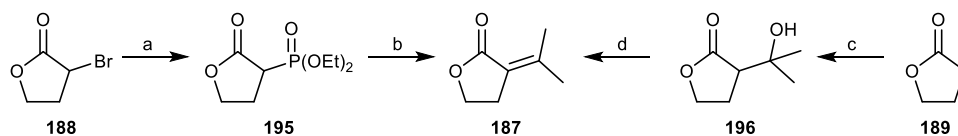
The chain extension of bromodiene **172** using ethylene oxide **193** or its equivalent sulfate **193** successfully afforded alcohol **185**, but yields were modest at best and this was deemed to be an unsuitable approach to synthesise enone **177**.

2.3.2 Disconnection B

In this approach, the ring opening of lactone **187** was intended to afford alcohol **185**; initial studies therefore focussed on identifying an efficient synthesis of **187** (Scheme 2.8).

Lactone **187** was first prepared *via* the Horner–Wadsworth–Emmons reaction of acetone and phosphonate **195** which, in turn, was obtained *via* an Arbusov reaction of α -bromo- γ -butyrolactone (**188**).^[113] This sequence was low yielding (6%) because an impurity

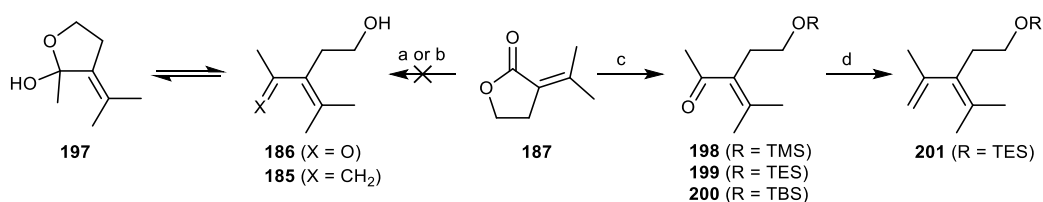
generated in the first step proved impossible to remove, and consequently this interfered with the second step. Alternatively, aldol condensation of γ -butyrolactone (**189**) and acetone afforded lactone **187** in 90% yield.^[114]



Scheme 2.8. Syntheses of lactone **187**. **Reagents and conditions:** a) $\text{P}(\text{OEt})_3$, reflux, 39%; b) NaH , THF, 0 °C; then acetone, RT, 6%; c) LDA , THF, -78 °C; then acetone, -78 °C to RT; d) H_2SO_4 , toluene, reflux, 90% (over 2 steps).

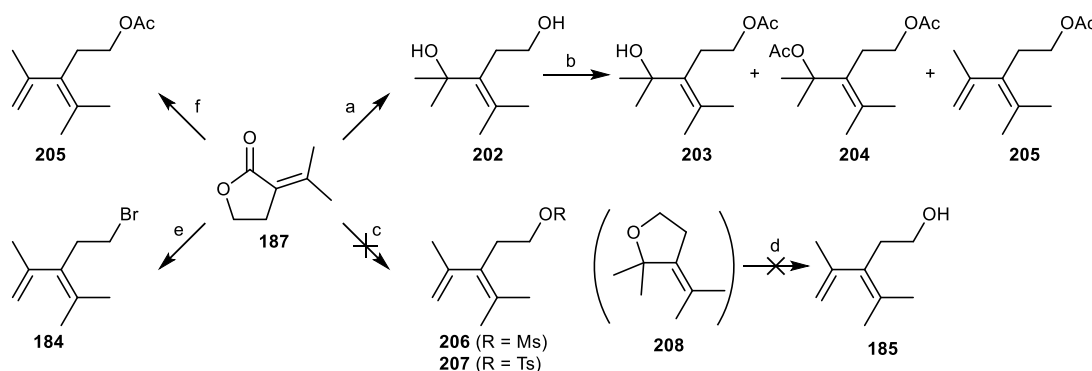
With lactone **187** in hand, its ring-opening with both methyllithium and methylmagnesium bromide was studied.

When ring-opening of lactone **187** with methyllithium was attempted (**Scheme 2.9**), the obtained product could not be identified but, as no starting material was recovered and only 1.0 eq. of methyllithium was used, the addition of only one methyl group must have occurred. Therefore, enone **186** may have been generated but impossible to isolate and characterise, probably due to dynamic equilibrium with hemiketal **197**. For this reason, the ring-opening of lactone **187** and subsequent Wittig reaction *in situ* were attempted. Under these conditions, the same unidentified product was isolated, suggesting that the Wittig reaction had failed. Convinced that enone **186** was being generated, an *in situ* quench using a chlorotrialkylsilane was envisaged and, as predicted, silyl ethers **198**, **199** and **200** were afforded. The Wittig reaction was tested with silyl ether **199**, affording diene **201** in an unoptimised 20% yield (63% recovery of **199**), confirming that dienes could be afforded by the ring-opening approach with methyllithium.



Scheme 2.9. Ring-opening of lactone **187** with methyllithium. **Reagents and conditions:** a) MeLi , THF, -78 °C to RT; b) MeLi , THF, -78 °C to RT; then CH_2PPh_3 , 0 °C to RT; c) MeLi , THF, -78 °C; then chlorotrialkylsilane, pyr or imidazole, -78 °C to RT, **198** (33%), **199** (90%), **200** (77%); d) CH_2PPh_3 , THF, 0 °C to RT, 20% (+ 62% of **199**).

When ring-opening of lactone **187** with methylmagnesium bromide was attempted (Scheme 2.10), double methyl addition occurred and diol **202** was isolated. With these results in mind, it was envisaged that lactone **187** could be transformed into a diene-type structure by double addition to the carbonyl group, protection of the obtained diol and subsequent elimination of the tertiary alcohol. Hence, the conversion of diol **202** into diene **205** upon treatment with acetyl chloride and pyridine was attempted but, in this case, acetate **205** was afforded in very low yield (1%), with acetate **203** (56%) and diacetate **204** (12%) being the major products. Unexpectedly, and pleasingly, when acetyl chloride was used as the quenching agent following the methylation step, acetate **205** was obtained as the only product in a 90% yield.

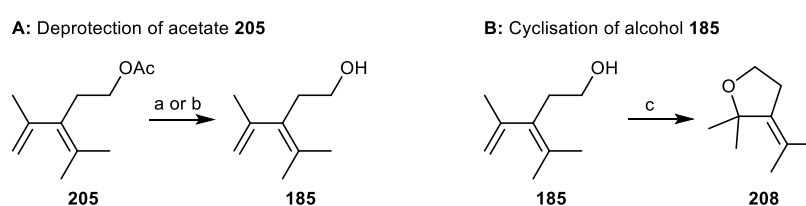


Scheme 2.10. Ring-opening of lactone **187** with methylmagnesium bromide. **Reagents and conditions:** a) MeMgBr, THF, $-78\text{ }^{\circ}\text{C}$ to RT, 66%; b) pyr, DCM, $0\text{ }^{\circ}\text{C}$; then AcCl, RT, **203** (56%), **204** (12%) and **205** (1%); c) MeMgBr, ether, $-78\text{ }^{\circ}\text{C}$ to RT; then MsCl or Ts₂O, $0\text{ }^{\circ}\text{C}$ to RT, **208** (100%, using MsCl) and (91%, using Ts₂O); d) BBr₃, DCM, RT; then pyr, RT; e) MeMgBr, ether, $-78\text{ }^{\circ}\text{C}$ to RT; then PBr₃, $0\text{ }^{\circ}\text{C}$, 1%; f) MeMgBr, ether, $-78\text{ }^{\circ}\text{C}$ to RT; then AcCl, RT, 90%;

These results suggested that alcohol **185** or bromodiene **184** might be obtained directly by using other quenching agents: phosphorus tribromide, methanesulfonyl chloride and *p*-toluenesulfonyl anhydride. Under these conditions, either the product was obtained in a very low yield (bromodiene **184**) or not obtained at all (sulfonates **206** and **207**). However, tetrahydrofuran derivative **208** was generated with both methanesulfonyl chloride and *p*-toluenesulfonyl anhydride. This suggested that sulfonylation of the primary alcohol and subsequent cyclisation occurred faster than sulfonylation of the tertiary alcohol. The ring-opening of tetrahydrofuran **208** was then attempted following some

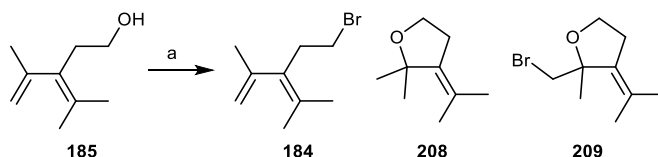
precedents in BBr_3 -assisted cleavage of ethers,^[120–123] but alcohol **185** was not afforded, probably due to decomposition of **208** under the reaction conditions.

At this stage, only two diene compounds had been afforded from lactone **187**: silyl ether **201** in 2 steps in an unoptimised yield of 20% using methyllithium, and acetate **205** in one step and a 90% yield using methylmagnesium bromide. As the synthesis of acetate **205** was already optimised, one step shorter and more efficient, it was decided to concentrate efforts on the synthetic route using methylmagnesium bromide.



Scheme 2.11. Deprotection of acetate **205** (A) and cyclisation of alcohol **185** under acidic conditions (B). *Reagents and conditions:* a) K_2CO_3 , MeOH, RT, 98%; b) NaOH (aq), THF, RT, 24% (+ 61% of **205**); c) CDCl_3 , RT, 100%.

From acetate **205**, the synthesis of enone **177** continued with the conversion of **205** into bromodiene **184**. Firstly, the deprotection of acetate **205** was studied by transesterification (99% yield) and basic hydrolysis (20% yield, with 61% recovery of **205**) (**Scheme 2.11**), followed by the direct transformation of the obtained alcohol **185** into bromodiene **184** (**Table 2.4**). Initially, the Appel reaction using tetrabromomethane as bromide donor was performed and, even though **184** was afforded, the yield was low (25%) and the bromoform by-product was impossible to remove due to its similar b.p. and R_f (**entry 1**). Consequently, other bromide donors were considered. When NBS was used, bromide **184** was generated but the major product was tetrahydrofuran **208** (**entry 2**). As the cyclisation of alcohol **185** into **208** had been observed in deuterated chloroform (**Scheme 2.11**), it was inferred that when using NBS, the reaction mixture became sufficiently acidic to protonate the diene and promote the cyclisation.

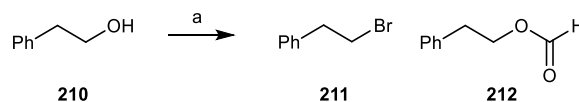
Table 2.4. Appel reaction to convert alcohol **185** into bromodiene **184**.

Reagents and conditions: a) “Br⁻”, PPh₃, solvent, T (°C).

Entry	Bromide donor	Solvent	T (°C)	Product (yield)
1	CBr ₄	DMF	0	184 (21%)
2	NBS	ACN	-78 to RT	184 (17%), 208 (57%) ^a
3	1,3-dibromo-5,5-dimethylhydantoin	DCM	0 to RT	209 (10%)

^a Yields calculated from the ¹H-NMR spectrum of the crude product; **X** could not be purified because decomposes on SiO₂.

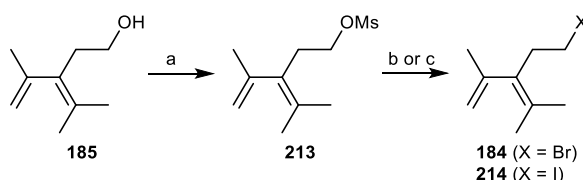
Next, 1,3-dibromo-5,5-dimethylhydantoin was considered as a replacement for tetrabromomethane; its use in the Appel reaction was unprecedented, therefore the reaction was tested with the model compound **210** (Table 2.5). When the “optimised” conditions were applied to alcohol **185**, bromide **209** was afforded instead of **184** (entry 3); arising from electrophilic bromination of the diene to initiate the cyclisation.

Table 2.5. Appel reaction using 1,3-dibromo-5,5-dimethylhydantoin.

Reagents and conditions: a) 1,3-dibromo-5,5-dimethylhydantoin, PPh₃, solvent, T (°C).

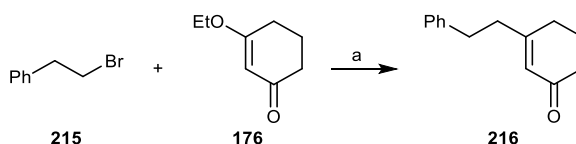
Entry	Solvent	T (°C)	Reaction time (h)	Product (Yield)
1	DMF	0	1	210 (8%), 212 (15%)
2	CH ₃ CN	0	1	–
3	CH ₃ CN	0 to RT	12	211 (33%)

Due to the multiple failures in the direct transformation of alcohol **185** into bromodiene **184**, a two-step mesylation and substitution was used.^[124,125] Following this sequence, bromodiene **184** was finally afforded efficiently and iododiene **214** was prepared analogously (Scheme 2.12).



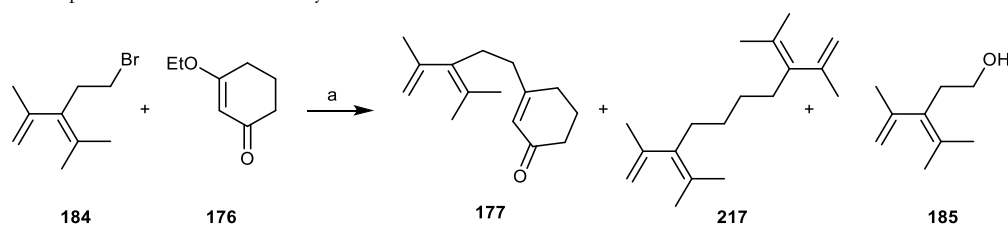
Scheme 2.12. Conversion of alcohol **185** into bromodiene **184** and iododiene **214**. *Reagents and conditions:* a) Et_3N , MsCl , DCM , $0\text{ }^\circ\text{C}$, 94%; b) LiBr , THF , reflux, **184** (70%); c) NaI , acetone, reflux, **214** (84%).

From bromodiene **184**, the synthesis of enone **177** was continued by the generation of the Grignard reagent from **184** and its addition to ethoxy-enone **176**. This sequence was first tested and optimised using the model compound **215** (Scheme 2.13) and then, attempted with bromodiene **184**, which required further optimisation (Table 2.6).



Scheme 2.13. Synthesis of enone **216** *via* Grignard reagent addition. *Reagent and conditions:* a) Mg , THF , RT ; then **176**, $-78\text{ }^\circ\text{C}$ to RT , 76%.

When the optimised conditions for bromide **215** were applied to bromodiene **184**, enone **177**, dimer **217** and alcohol **185** were obtained. The initial low yields (entry 1) and the isolation of **185** suggested the presence of O_2 and water in the reaction media. After screening several reaction conditions, it was found that activation of the magnesium metal (stirring for 2 h under an inert atmosphere), the addition of a small iodine crystal, and slightly heating improved the preparation of the Grignard reagent and thus, the synthesis of enone **177** (entry 5). In an attempt to minimise the formation of dimer **217**, the number of eq. of bromodiene **184** was reduced and, although the amount of **217** did not decrease, the desired enone **177** was afforded in higher yield (entries 2 and 4). Finally, it was noted that the use of an activating agent was critical to the preparation of the Grignard reagent (entry 7) and, although the reaction worked better with iodine, this could be exchanged for others such as 1,2-dibromoethane (entry 3).

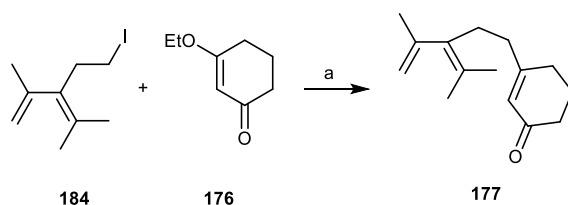
Table 2.6. Optimisation of enone **177** synthesis.

Reagents and conditions: a) Mg, THF, RT; then **176**, 0 °C to RT.

Entry	Mg (eq.)	184 (eq.)	Activating agent ^a	Mg activation ^b	Heat activation ^c	177 (%)	217 (%)	185 (%)
1	1.7	1.8	I ₂	–	–	6	10	5
2	1.7	1.8	I ₂	–	Yes	36	16	1
3	1.7	1.8	1,2-dibromo-ethane	–	Yes	20	12	–
4	1.4	1.4	I ₂	–	Yes	46	22	–
5	1.4	1.4	I ₂	2 h	Yes	65	20	–
6	1.4	1.4	I ₂	12 h	Yes	30	26	–
7	1.4	1.4	–	2 h	Yes	–	–	–

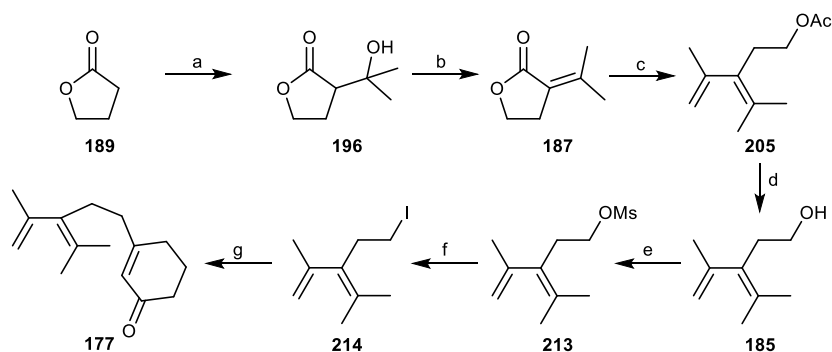
^a A drop or a small crystal was added to the reaction mixture; ^b Stirring under an inert atmosphere of N₂ for the specified time; ^c Heat for 5 min to initiate the formation of the Grignard reagent;

Despite enone **177** being obtained in up to 65% yield, the preparation of the Grignard reagent was complicated and somewhat unreliable. This, and the straightforward access to iododiene **214** (**Scheme 2.12**), led us to instead examine the reactivity of lithium species derived from **214**. Treatment of iododiene **214** with *t*-BuLi to generate the lithiated species^[124,125] which was added directly to ethoxy-enone **176** afforded enone **177**, after acidic hydrolysis, in a high and reproducible yield (79%) (**Scheme 2.14**).



Scheme 2.14. Synthesis of enone **177** via lithium species derived from iodide **184**. **Reagents and conditions:** a) *t*-BuLi, THF, –78 °C to RT; then **176**, 0 °C to RT, 79%

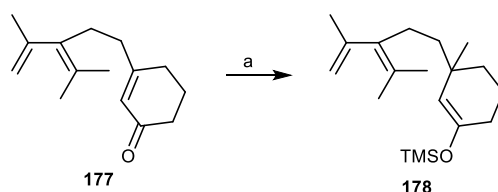
The results presented in this section allowed the development of an efficient synthesis of enone **177** (**Scheme 2.15**) which was aimed for the continuation of Baran's route to taxadienone **16**.



Scheme 2.15. Developed synthesis of enone **177** from butyrolactone **189**. *Reagents and conditions:* a) LDA, THF, -78 °C; then acetone, -78 °C to RT; b) H_2SO_4 , toluene, reflux, 90% (over 2 steps); c) MeMgBr, ether, -78 °C to RT; then AcCl, RT, 90%; d) K_2CO_3 , MeOH, RT, 98%; e) Et_3N , MsCl, DCM, 0 °C, 94%; f) NaI, acetone, reflux, 84%; g) *t*-BuLi, THF, -78 °C to RT; then 3-ethoxycyclohex-2-en-1-one (**176**), 0 °C to RT, 79%.

2.4 Continuation of the reproduction of Baran's route to taxadienone 16

With a practical and reproducible synthesis of enone **177**, Baran's route to taxadienone **16** was continued. The next step consisted of the enantioselective 1,4-addition of a methyl group to enone **177** under Alexakis conditions,^[126] to establish the C8 quaternary centre, and subsequent enolate trapping as a TMS-enol ether. At this stage, the reproducibility of the asymmetric reaction was a subject of concern; this reaction would likely require optimisation and, given the delays encountered in the synthesis so far, the racemic alternative was pursued. Therefore, a modified literature procedure^[127] was used and a racemic mixture of TMS-enol ether **178** was afforded (**Scheme 2.16**). At the time, the intention was to return to the asymmetric reaction once the entire route had been optimised. The synthesis of racemic TMS-enol ether **178** was ultimately advantageous because it enabled us to study the selective oxidation by the P450_{BM3} mutants of the two enantiomers of taxadienone **16**.



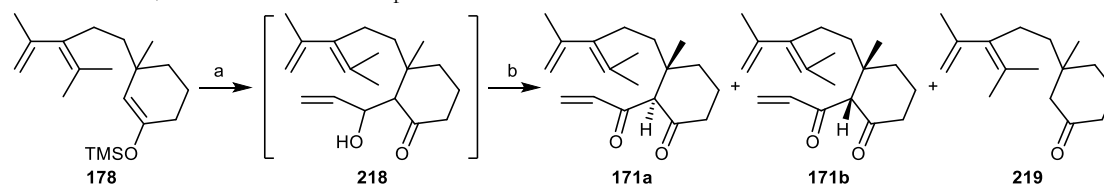
Scheme 2.16. Synthesis of racemic TMS-enol ether **178**. *Reagents and conditions:* a) LiCl (30 mol%), CuI (15 mol%), TMSCl, THF, -78 °C; then MeMgBr, -78 °C, 99%;

In continuation of the route, Baran had attempted the Mukaiyama aldol reaction of TMS-enol ether **178** with acrolein using commonly employed Lewis acids (TiCl_4 , SnCl_4 , $\text{Sn}(\text{OTf})_2$, $\text{Sc}(\text{OTf})_3$, $\text{BF}_3 \cdot \text{OEt}_2$, TMSOTf , ZnCl_2 , $\text{MgBr}_2 \cdot \text{OEt}_2$) and other catalysts (LiCl_4 , $\text{Zr}(\text{O}i\text{-Bu})_4$, $\text{Bi}(\text{OTf})_3$, AgOTf , SiCl_4) but desilylated ketone **219** was the only product isolated under these conditions. Regeneration of TMS-enol ether **178** or C3 functionalisation from ketone **219** was not feasible because of the difficulty of selecting for the correct regioisomer.

Baran discovered that water facilitates the aldol reaction and aldol **218** was generated under Kobayashi conditions, using $\text{Gd}(\text{OTf})_3$ and very specific solvent systems (water/ethanol/toluene, 1:10:4). Aldol **218** was then oxidised in the same reaction flask with Jones' reagent to afford keto-enone **171**, as a diastereomeric mixture (**171a**:**171b** = 2:1).

By this stage it was unsurprising that several problems were encountered in our attempts to reproduce the synthesis of keto-enone **171**, revealing that the crucial aldol/oxidation sequence was complicated. First, aldol **218** could not be purified by flash column chromatography because retro-aldol reaction took place on silica, returning ketone **219**. Then, when the aldol reaction and subsequent oxidation were attempted, an inseparable mixture of keto-enones **171a**, **171b** (always in a 2:1 ratio) and ketone **219** was obtained, with ketone **219** being the major product in some cases (**Table 2.7**).

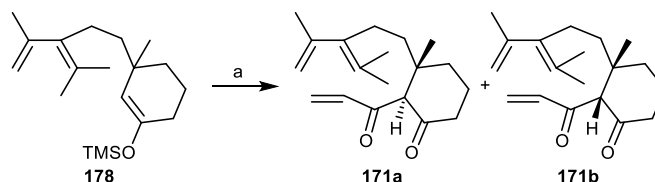
At this stage, the origin of ketone **219** was studied and two possibilities were contemplated: hydrolysis of TMS-enol ether **178** in the reaction medium, due to the presence of water, or retro-aldol reaction of the aldol adduct **218**. TMS-enol ether **178** was recovered unchanged after re-submitting it to the aldol reaction conditions (without acrolein) and this suggested that competing fast retro-aldol reaction during the oxidation step was the most likely origin of ketone **219**.

Table 2.7. Aldol/oxidation reaction attempts to afford keto-enone **171**.

Reagents and conditions: a) acrolein, Gd(OTf)₃, H₂O/EtOH/toluene (1:10:4), 4 °C; b) CrO₃, H₂SO₄, acetone, 0 °C.

Entry	Keto-enone 171a (%)	Keto-enone 171b (%)	Ketone 219 (%)
1	38	17	11
2	10	4	50
3	40	15	21
4	18	9	33
5	31	15	16

Given literature precedent for the aldol reaction of lithium cyclohexenolates with acrolein,^[128–132] and that lithium enolates are more reactive than their TMS-enol ether counterparts, it was deemed that the regeneration of the lithium enolate from TMS-enol ether **178** might facilitate the aldol reaction and enable formation of aldol adduct **218**. In addition, as the oxidation could then be carried out under anhydrous conditions, Jones' reagent was exchanged for the less toxic Dess–Martin periodinane. Under these conditions, keto-enone **171** was afforded in a reproducible yield of 43% over the two steps as a diastereomeric mixture (**171a**:**171b** = 2:1) (**Scheme 2.17**). Although ketone **219** was not generated under these conditions, other side-products were obtained, probably arising from 1,4-addition to acrolein, inferred from the presence of aldehyde proton signals in ¹H-NMR spectra.

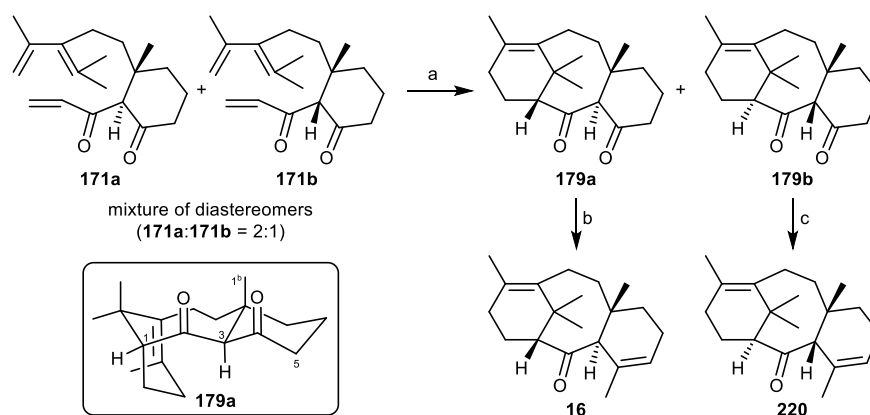


Scheme 2.17. Synthesis of keto-enone **171** *via* regeneration of the lithium enolate from TMS-enol ether **178**. **Reagents and conditions:** a) i. MeLi, THF, –20 °C; then acrolein, –78 °C; ii. NaHCO₃, DMP, DCM, RT, 43% (**171a**:**171b** = 2:1).

From keto-enone **171**, Baran's synthesis continued with an IMDA reaction under Lewis acidic conditions to forge the tricyclic taxane skeleton. This IMDA reaction was

successfully reproduced with no further optimisation, and a separable mixture of diketones **179a** and **179b** was afforded in a 2:1 ratio (**Scheme 2.18**).

Baran's synthesis of taxadienone **16** finished with installation of the C4 methyl group by enol triflate formation and Negishi coupling. Even though diketone **179** is a β -diketone and C3 would be expected to be the most acidic position, deprotonation at this carbon does not occur, possibly owing to steric hindrance, against the approach of the bulky base (see conformation of **179a** in **Scheme 2.18**), or a predominance of conformations in which there is little overlap of $(C-H)\sigma$ and $(C=O)\pi^*$. Consequently, diketone **179** may be deprotonated selectively at the C5 position, the enolate trapped as a triflate and subsequently subjected to standard Negishi conditions using Me_2Zn to introduce the C4 methyl. This sequence was reproduced efficiently and taxadienone **16** was achieved as a racemic mixture from diketone **179a**. Additionally, di-*epi*-taxadienone **220** was prepared analogously from diketone **179b** (**Scheme 2.18**).



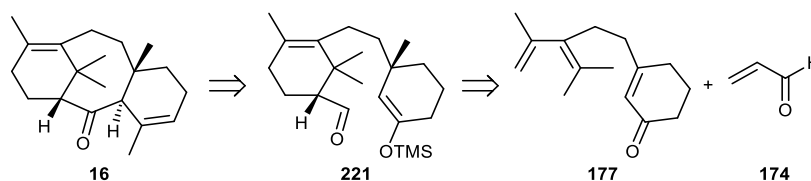
Scheme 2.18. Synthesis of taxadienone **16** and di-*epi*-taxadienone **220**. **Reagents and conditions:** a) $BF_3 \cdot OEt_2$, DCM, 0 °C, **179a** (37%) and **179b** (27%); b) i. $PhNTf_2$, KHMDS, THF, 0 °C; ii. Me_2Zn , $Pd(PPh_3)_4$, THF, 0 °C to RT, 67% (over 2 steps); c) i. $PhNTf_2$, KHMDS, THF, 0 °C; ii. Me_2Zn , $Pd(PPh_3)_4$, THF, 0 °C to RT, 31% (over 2 steps) (+27% recovered **179b**).

In summary, many problems were encountered and overcome in the reproduction of Baran's route but, after optimising almost every step, the synthesis of taxadienone **16**, and di-*epi*-taxadienone **220**, was finally achieved; by developing an alternative synthesis to enone **177** and modifying the aldol/oxidation sequence to afford keto-enone **171**.

2.5 New approach to taxadienone 16 inspired by Baran's route

As reproducing Baran's synthesis to taxadienone **16** was proving to be troublesome and time consuming, other approaches were studied simultaneously. In this section, a synthesis based on a re-examination of Baran's route is presented.

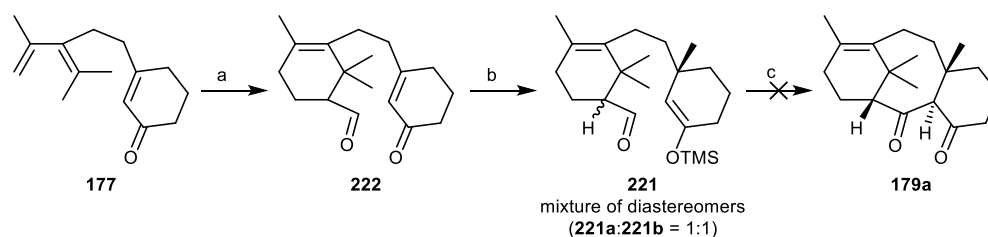
The most problematic step in Baran's route was the Mukaiyama aldol reaction of TMS-enol ether **178** and acrolein, then oxidation to afford keto-enone **171**. An intramolecular version of this reaction was considered to be potentially more efficient, with the retro-aldol being less of an issue. The synthesis of the taxane AB-rings was envisaged by furnishing first the A-ring *via* a DA reaction,^[133,134] and then closing the B-ring *via* an intramolecular aldol reaction (**Scheme 2.19**). However, the disadvantage of this approach was that the desired diastereomer, taxadienone **16**, could only be achieved in a maximum 50% yield if no asymmetric reaction was used.



Scheme 2.19. Retrosynthetic analysis of taxadienone **16** based on Baran's route.

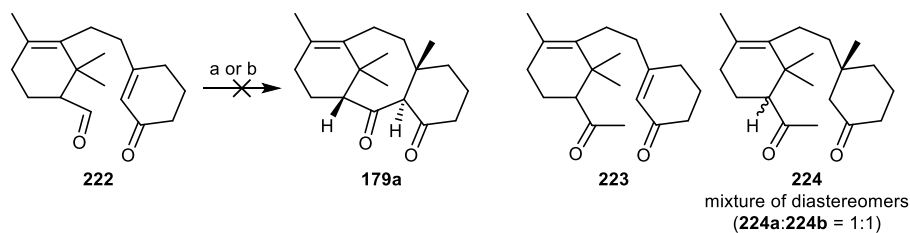
The new route started with the preparation of enone-carbaldehyde **222**, achieved in a 46% yield, by means of the DA reaction of enone **177** and acrolein under Lewis acidic conditions. Problems arose early in the synthesis, whilst attempting to close the taxane B-ring in **222**. Firstly, the Cu-catalysed 1,4-methyl addition to enone-carbaldehyde **222** was studied with enolate trapping as the TMS-enol ether, followed by intramolecular Mukaiyama aldol reaction, and then oxidation of the so-formed aldol. In practise, a 5:2 mixture of TMS-enol ether **221** (as a diastereomeric mixture, **221a:221b** = 1:1) and unreacted enone-carbaldehyde **222** was obtained after the methyl conjugate addition to **222** and TMS-enolate trapping step. This crude product was directly subjected to the

cyclisation/oxidation conditions, but only a low recovery of unchanged enone-carbaldehyde **222** was afforded (**Scheme 2.20**).



Scheme 2.20. First attempt to cyclise enone-carbaldehyde **222**. **Reagents and conditions:** a) acrolein, $\text{BF}_3 \cdot \text{OEt}_2$, DCM, -78°C , 46%; b) LiCl (30 mol%), CuI (15 mol%), TMSCl, THF, -78°C ; then MeMgBr, -78°C , 67% (**221a:221b** = 1:1) (+33% of **222**); c) i. TiCl_4 , DCM, -78°C ; ii. NaHCO_3 , DMP, DCM, RT.

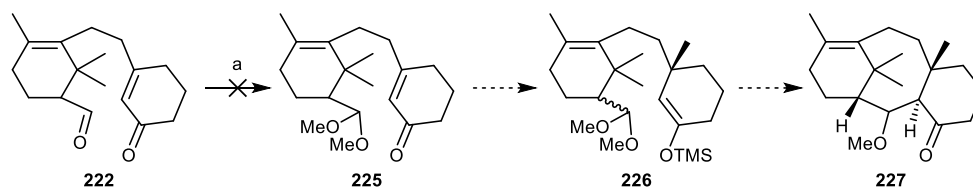
Due to the proximity of reacting groups in intramolecular reactions, the enolate resulting from the methyl conjugate addition to **222** could react directly with the aldehyde group to close the B-ring. However, when this sequence was performed under Cu-catalysed conditions, diketone **179** was not obtained, instead affording keto-enone **223** and diketone **224** (**Scheme 2.21**).



Scheme 2.21. Second attempt to cyclise enone-carbaldehyde **222**. **Reagents and conditions:** a) i. CuI (22 mol%), Me_2S , THF, -78°C ; then MeMgBr, -78°C to RT; ii. NaHCO_3 , DMP, DCM, RT, **223** (28%) and **224** (36%, **224a:224b** = 1:1); b) i. CuI, Me_2S , MeMgBr, THF, -78°C to RT; ii. NaHCO_3 , DMP, DCM, RT, **223** (13%), **224** (12%, **224a:224b** = 1:1).

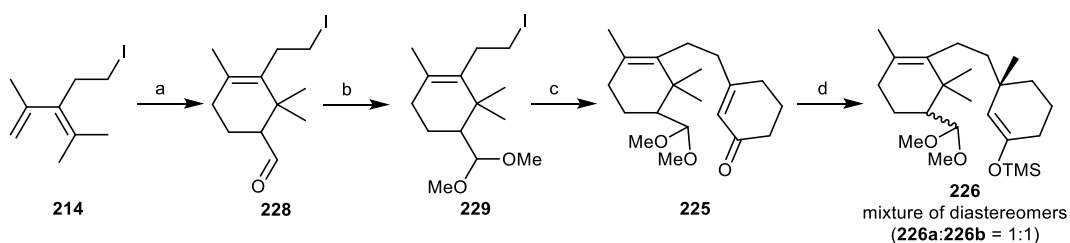
At this stage, the origin of keto-enone **223** and diketone **224** was studied and two theories emerged. Firstly, the organocuprate was not being formed *in situ* and consequently, MeMgBr was the actual reactive species; or alternatively, the conjugate addition was less favourable than the addition to the aldehyde, probably due to steric hindrance or conformational issues. However, when Cu-stoichiometric conditions were used, keto-enone **223** and diketone **224** were once again isolated (**Scheme 2.21**). Therefore, from these results it was inferred that the methyl addition to the aldehyde proceeded preferentially to the conjugate addition in enone-carbadehyde **222**. Consequently, it was necessary to protect the aldehyde group before introducing the C8 methyl group.

Based on literature precedents for the condensation of acetals with TMS-enol ethers under the influence of SnCl_4 ,^[135,136] the B-ring closure was envisaged *via* a related intramolecular condensation. Since aldehydes are typically more reactive than ketones, the regioselective protection of the aldehyde as a dimethyl acetal under Noyori conditions^[137] was attempted but **222** was recovered unchanged (**Scheme 2.22**).



Scheme 2.22. Third attempt to cyclise enone-carbaldehyde **222**. **Reagents and conditions:** a) TMSOTf (10 mol%), TMSOMe, DCM, $-78\text{ }^\circ\text{C}$.

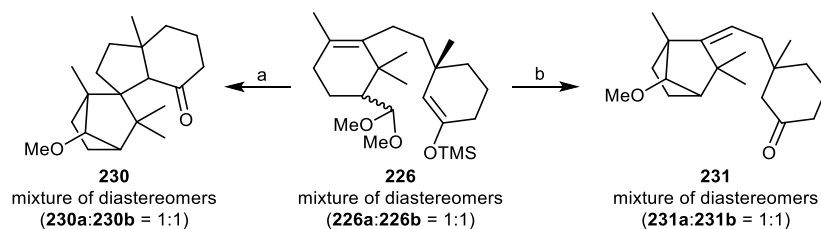
Consequently, a longer route towards enone-acetal **225** was studied, in which the protection of the aldehyde group would take place prior to the taxane A- and C-ring coupling. DA reaction of iododiene **214** and acrolein under Lewis acidic conditions furnished the taxane A-ring. The obtained aldehyde **228** was protected as its dimethyl acetal (**229**) and subjected to lithium-iodine exchange upon treatment with *t*-BuLi. This organolithium was then added to 3-ethoxycyclohex-2-en-1-one (**176**), affording enone-acetal **225** after hydrolysis. Next, the Cu-catalysed methyl conjugate addition and TMS-enolate trapping sequence was used again in the synthesis of TMS-enol ether **226** (**Scheme 2.23**).



Scheme 2.23. Synthesis of TMS-enol ether **226**. **Reagents and conditions:** a) acrolein, $\text{BF}_3 \cdot \text{OEt}_2$, DCM, $-78\text{ }^\circ\text{C}$, 92%; b) TMSOTf (10 mol%), TMSOMe, DCM, $-78\text{ }^\circ\text{C}$, 81%; c) *t*-BuLi, THF, $-78\text{ }^\circ\text{C}$ to RT; then 3-ethoxycyclohex-2-en-1-one, $0\text{ }^\circ\text{C}$ to RT, 60%; d) LiCl (30 mol%), CuI (15 mol%), TMSCl, THF, $-78\text{ }^\circ\text{C}$; then MeMgBr, $-78\text{ }^\circ\text{C}$, 94% (**226a:226b** = 1:1).

With TMS-enol ether **226** in hand, the modified intramolecular Mukaiyama acetal aldol reaction was attempted (**Scheme 2.24**). Under Lewis acidic conditions (TiCl_4 and

TMSOTf), only the unexpected, and tentatively assigned, cyclised products **230** and **231** were obtained; most likely arising by a Prins-type reaction followed by silyl enol ether alkylation (\rightarrow **230**) or elimination (\rightarrow **231**).



Scheme 2.24. Cyclisation of TMS-enol ether **226** under Lewis acidic conditions. **Reagents and conditions:** a) TiCl_4 , THF, -78°C to -23°C , 59% (**230a:230b** = 1:1); b) TMSOTf cat., CH_2Cl_2 , -78°C , 30% (**231a:231b** = 1:1).

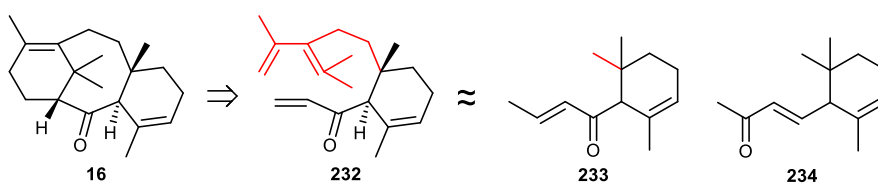
Although enone-aldehyde **222** and enone-acetal **225** were structurally close to the taxane skeleton, and further strategies could have been attempted to close the B-ring, this synthesis was abandoned due to its length and production of complicated diastereomeric mixtures. Accordingly, efforts were redirected towards more productive routes.

3. NEW APPROACH TO DI-*EPI*-TAXADIENONE 220

3.1 Designing a new route to taxadienone 16

As stated in the Introduction, taxadienone **16** was selected as the starting point for late-stage hydroxylation with P450_{BM3} mutants to generate taxol analogues. In Chapter 2, the problems encountered and solutions found in reproducing Baran's route to taxadienone **16** were outlined. However, despite the tedious optimisation performed, some reactions were still insufficiently reliable or efficient to be used repeatedly and other approaches to **16** were simultaneously studied. In this chapter, a new and reproducible approach to taxadienone **16** is presented, which ultimately, produced di-*epi*-taxadienone **220**.

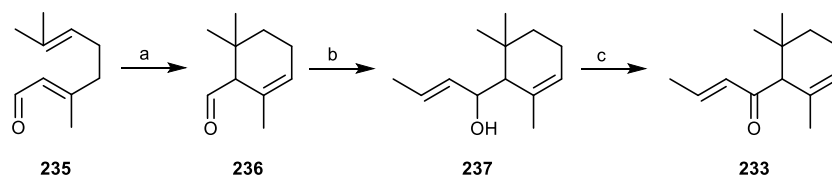
IMDA reactions have been reported extensively in the synthesis of the taxane AB-ring system^[56,59,61,64,138] because of the ease in which the bridged double bond is introduced and the high stereoselectivity and atom economy of the process. Consequently, when devising a new approach to taxadienone **16**, an IMDA reaction was the first retrosynthetic disconnection considered, leading to enone **232** (Scheme 3.1). The structural similarities of **232** and α -damascone (**233**), in which the diene side chain has been replaced by a methyl group, shifted our attention to the reported syntheses of α -damascone (**233**).



Scheme 3.1. IMDA disconnection of taxadienone **16** and the structural similarities of enone **232** to α -damascone (**233**) and α -ionone (**234**).

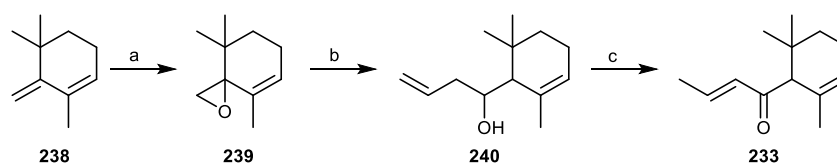
α -Damascone (**233**) and α -ionone (**234**) belong to the family of rose ketones that are a group of structurally very closely related compounds, which have been used extensively in the perfume industry due to their pleasant flower fragrance. Several approaches to α -damascone involve the interconversion of α -ionone derivatives,^[139–144] and acid-catalysed cyclisation of polyenic precursors^[145,146] previously used in the synthesis of α -

ionone (**234**). However, approaches which involved the assembly of a C₁₀ unit and the subsequent addition of a C₃ chain^[147–151] were more interesting due to their feasibility to be applied to the synthesis of enone **232**.



Scheme 3.2. Demole's synthesis of α -damascone (**233**). **Reagents and conditions:** a) i. aniline, ether, RT; ii. H₂SO₄, ether, –20 °C, 70% (over 2 steps); b) CH₃CH=CH-MgBr, THF, –5 °C to RT, 69%; c) CrO₃, pyr, RT, 50%.

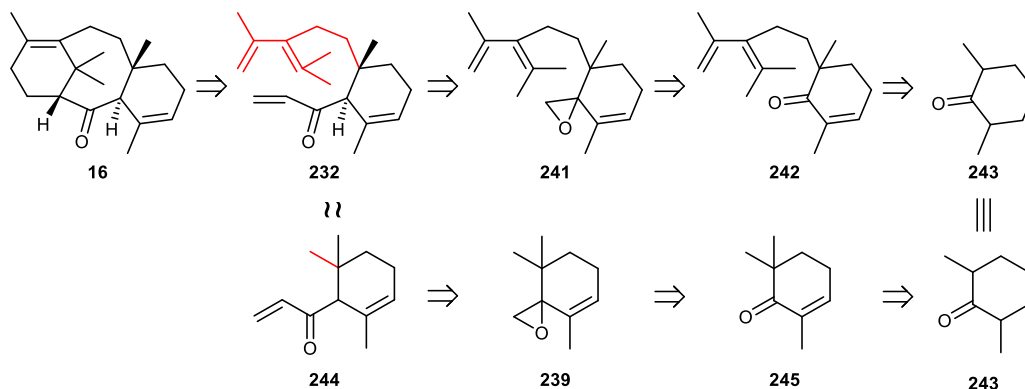
Demole^[152] was the first to apply the three-carbon homologation strategy to the synthesis of α -damascone (**233**). Citral (**235**) was converted into α -cyclocitral (**236**)^[153] *via* Schiff base formation and subsequent cyclisation under acidic conditions. Addition of 1-propenylmagnesium bromide to **236** and oxidation of the allylic alcohol **237** afforded α -damascone (**233**) (**Scheme 3.2**). Boulin^[154] reported a similar homologation strategy, in which **233** was obtained *via* allylmagnesium bromide addition to α -cyclocitral (**236**), which was generated *in situ* by epoxide-aldehyde isomerisation of **239**, and followed by alcohol oxidation and double bond isomerisation. In this case, epoxide **239** was prepared by regioselective alkene epoxidation of γ -pyronene (**238**) (**Scheme 3.3**).



Scheme 3.3. Boulin's synthesis of α -damascone (**233**). **Reagents and conditions:** a) *m*-CPBA, ether, RT, 30%; b) CH₂=CHCH₂-MgBr, ether, RT 91%; c) i. PCC, DCM, RT; ii. Al₂O₃, ether, RT, 90% (over 2 steps).

A similar strategy to Boulin's was then envisaged for the synthesis of enone **232** from epoxide **241**. In this case, **241** would be afforded *via* the Corey–Chaykovsky epoxidation of enone **242**, thus establishing a ketone group in a strategic position which would facilitate the introduction of the diene side chain by alkylation of ketone **243** with iododiene **214**. A bromination-dehydrobromination strategy would be used to introduce the C4–C5 double bond in the early stages of the synthesis (**Scheme 3.4**).

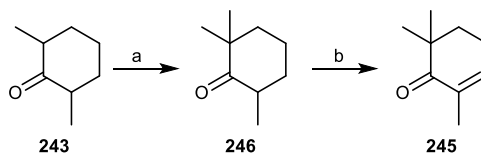
Before beginning this new synthesis to taxadienone **16**, the feasibility of the route was examined. Since enones **232** and **244** differ by only the diene side chain, the proposed new route was firstly applied to the synthesis of enone **244** as a model compound for **232** (**Scheme 3.4**).



Scheme 3.4. Retrosynthetic analysis of the new route to taxadienone **16**, and enone **244** as a model compound.

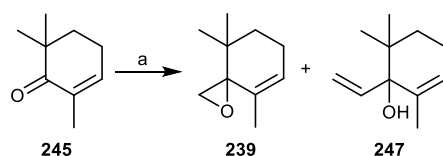
3.2 Application of the new proposed route to the synthesis of enone **244**

The synthesis of enone **245** was achieved by methylation of 2,6-dimethylcyclohexanone (**243**) and introduction of the double bond *via* bromination with NBS and subsequent thermal-induced dehydrobromination^[155] with Li_2CO_3 and LiBr (**Scheme 3.5**).



Scheme 3.5. Synthesis of enone **245**. **Reagents and conditions:** a) LDA, THF, $-78\text{ }^\circ\text{C}$; then MeI, $-78\text{ }^\circ\text{C}$ to RT, 92%; b) i. NBS, CCl_4 , reflux; ii. Li_2CO_3 , LiBr, DMF, reflux, 97% (over 2 steps).

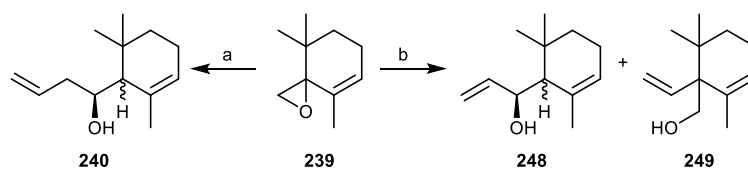
When the reaction conditions reported for the Corey–Chaykovsky epoxidation of a similar substrate^[156] were applied to enone **245**, epoxide **239** was obtained, along with unwanted allylic alcohol **247**. Assuming that **247** was generated by the second addition of a methylene group, the number of eq. of trimethylsulfonium iodide was reduced in an attempt to minimise its formation (**Table 3.1**). It was found that when 2.5 eq. of trimethylsulfonium iodide were used, the formation of allylic alcohol **247** was completely suppressed and epoxide **239** was the only product isolated, in a relatively high yield (**entry 2**).

Table 3.1. Screened conditions to epoxidise enone **239**.

Reagents and conditions: a) *n*-BuLi, (CH₃)₃Si, THF, 0°C.

Entry	(CH ₃) ₃ Si (eq.)	Epoxide 239 (%)	Allylic alcohol 247 (%)	Recovered enone 245 (%)
1	2.0	8	–	41
2	2.5	79	–	–
3	3.0	26	6	–

The epoxide-aldehyde isomerisation of epoxide **239** and the subsequent Grignard addition were then studied. Boulin^[154] reported the conversion of epoxide **239** to alcohol **240** as a one-pot procedure upon treatment with allylmagnesium bromide. When these conditions were attempted, alcohol **240** was obtained as a separable mixture of two diastereomers (**240a** and **240b**) (Scheme 3.6). In contrast, when vinylmagnesium bromide was used, only a small amount of allylic alcohol **248** was afforded, as a separable diastereomeric mixture (**248a** and **248b**), along with alcohol **249** (Scheme 3.6). Given that **249** arose from the direct addition of vinylmagnesium bromide to epoxide **239**, the epoxide-aldehyde isomerisation did not occur efficiently under the reaction conditions, suggesting that the nature of the Grignard reagent may have some influence in the isomerisation.

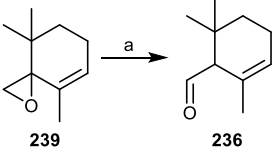


Scheme 3.6. Syntheses of alcohol **240** and **248** via epoxide-aldehyde isomerisation of **239** and Grignard addition *in situ*. *Reagents and conditions:* a) CH₂=CHCH₂-MgBr, ether, 0 °C to RT, **240a** (11%) and **240b** (19%); b) CH₂=CH-MgBr, ether, 0 °C to RT; **248a** (8%), **248b** (1%) and **249** (12%).

Consequently, the epoxide isomerisation and the Grignard addition were performed as separate steps. Firstly, different Lewis acids were tested to isomerise epoxide **239** to aldehyde **236** (Table 3.2). Despite **239** partly isomerising during purification by flash

column chromatography on silica, when treatment with SiO₂ was attempted, epoxide **239** was recovered unchanged (**entry 1**). Treatment with MgBr₂·OEt₂ led to epoxide decomposition (**entry 2**); finally, aldehyde **236** was obtained upon treatment with BF₃·OEt₂ (**entry 3**).

Table 3.2. Screened conditions to isomerise epoxide **239** to aldehyde **236**.

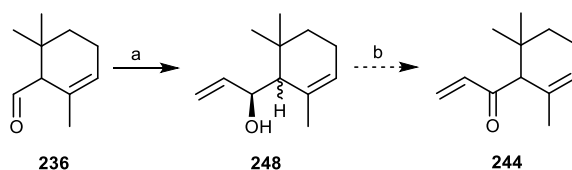


239 $\xrightarrow{\text{a}}$ **236**

Reagents and conditions: a) Lewis acid, solvent, RT.

Entry	Lewis acid	Solvent	Yield (%)
1	SiO ₂	ether	–
2	MgBr ₂ ·OEt ₂	ether	–
3	BF ₃ ·OEt ₂	benzene	90

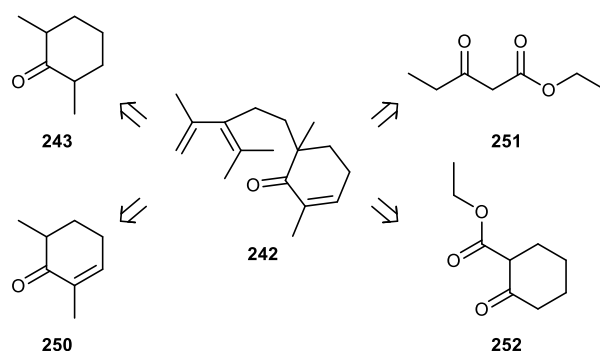
The addition of vinylmagnesium bromide to aldehyde **236** afforded allylic alcohol **248** as a single diastereomer (**248a**) in an unoptimised yield of 39% (**Scheme 3.7**). Although the synthesis of enone **244** would still require the oxidation of allylic alcohol **248** and further optimisation of some steps, the preparation of **248** had already fulfilled the main objective of the synthesis of enone **244**, establishing the feasibility of the new route.



Scheme 3.7. Synthesis of enone **244**. *Reagents and conditions:* a) CH₂=CH-MgBr, THF, RT, **248a** (39%); b) DMP, NaHCO₃, DCM, RT.

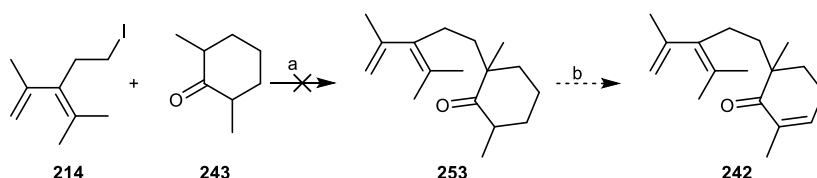
3.3 Application of the new proposed route to the synthesis of taxadienone 16

The synthesis of allylic alcohol **248** proved that all the key steps of the new route were viable, and thus its application to the synthesis of taxadienone **16** was studied. However, problems were encountered early on when attempting to prepare enone **242**. Several approaches toward **242** were examined, including the alkylation of ketone **243**, enone **250**, and acyclic and cyclic β-keto esters **251** and **252** respectively (**Scheme 3.8**).



Scheme 3.8. Retrosynthetic analysis of the approaches studied to synthesise enone **242**.

The alkylation of 2,6-dimethylcyclohexanone (**243**) with iododiene **214**, and subsequent introduction of the double bond was the first approach attempted to prepare enone **242** (Scheme 3.9). Despite the use of different bases (KHMDS, LDA and KH), ketone **243** was always recovered unchanged, along with traces of **253** and decomposition products of iododiene **214**. Given that ketone **246** had been prepared successfully by alkylation of ketone **243** with LDA and iodomethane (Scheme 3.5), the unsuccessful results for the synthesis of ketone **253** suggested that the alkylation with iododiene **214** was the problematic step and not the enolate generation from **243**.



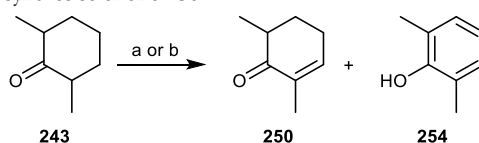
Scheme 3.9. Synthesis of enone **242** via alkylation of ketone **243** and subsequent introduction of the double bond. **Reagents and conditions:** a) Base (LDA, KHMDS or KH), THF, $-78\text{ }^{\circ}\text{C}$; then iododiene **214**, $-78\text{ }^{\circ}\text{C}$ to RT; b) i. NBS, CCl_4 , reflux; ii. Li_2CO_3 , LiBr, DMF, reflux.

The alkylation of enone **250** with iododiene **214** was deemed more likely than the alkylation of ketone **243** due to the presence of the double bond, which could give access to a conformation which facilitates the alkylation step. Therefore, the synthesis of enone **242** from ketone **243** via the introduction of the double bond and subsequent alkylation with iododiene **214** was studied next.

The preparation of enone **250** via bromination-dehydrobromination^[155] of 2,6-dimethylcyclohexanone (**243**) with NBS and subsequent Li_2CO_3 and LiBr treatment was

optimised to minimise the formation of unwanted phenol **254** (Table 3.3). Given that **254** was formed by further oxidation of **250**, enone **250** may be generated efficiently after the NBS treatment, and thus the subsequent Li₂CO₃ and LiBr treatment, which should help in the dehydrobromination step, may not be required, and may actually be promoting the overoxidation of **250** to **254**. As predicted, enone **250** was the only product isolated after just NBS treatment^[157] (entry 3). Additionally, in an attempt to avoid the reported use of tetrachloromethane, several chlorinated solvents were screened (entry 3, 4 and 5). It was thus established that chloroform could be used without affecting the yield.

Table 3.3. Screened conditions to synthesise enone **250**.

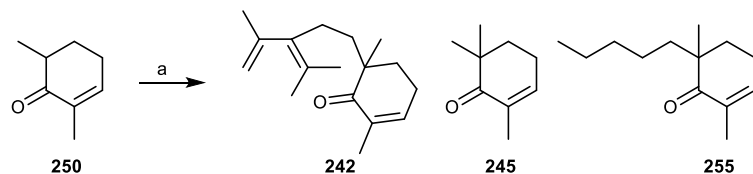


Reagents and conditions: a) i. NBS, solvent, reflux; ii. Li₂CO₃, LiBr, DMF, reflux; b) NBS, solvent, reflux.

Entry	Procedure	NBS (eq.)	Solvent	Enone 250 (%)	Phenol 254 (%)
1	a	2.0	CCl ₄	18	56
2	a	1.0	CCl ₄	52	3
3	b	1.0	CCl ₄	80	–
4	b	1.0	CHCl ₃	77	–
6	b	1.0	DCE	58	9

Alkylation of enone **250** was first attempted with simple iodoalkanes, affording enones **245** and **255** successfully *via* enolate generation with KHMDS and subsequent alkylation with iodomethane and 1-iodohexane respectively (Table 3.4). However, when these reaction conditions were applied using iododiene **214**, enone **242** was obtained only in low yield, unreacted enone **250** was recovered and decomposition products of iododiene **214** were observed (Table 3.4). These results reaffirmed the hypothesis that the alkylation with iododiene **214** was the problematic step.

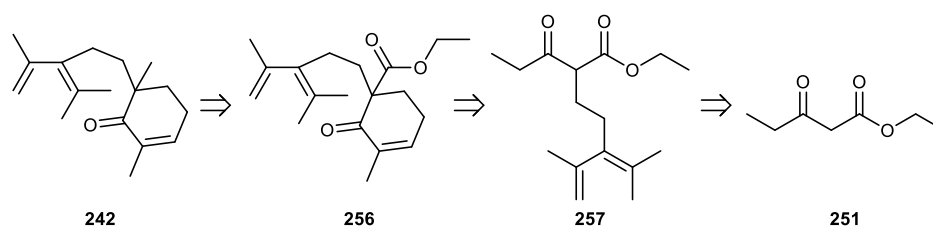
Table 3.4. Alkylation of enone 250.



Reagents and conditions: a) KHMDS, THF, $-78\text{ }^{\circ}\text{C}$; then iodoalkane, $-78\text{ }^{\circ}\text{C}$ to RT.

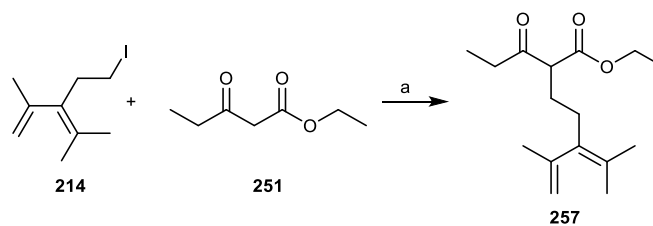
Entry	Iodoalkane	Iodoalkane (eq.)	Product	Yield (%)
1	Iodomethane	1.0	245	11
2	Iodomethane	2.0	245	33
3	1-Iodohexane	2.0	255	59
4	Iododiene 214	2.0	242	12

With many difficulties in synthesising enone 242 *via* alkylation of either ketone 243 or enone 250 with iododiene 214, a completely different synthetic plan was attempted. Given β -keto esters can be alkylated with relatively unreactive electrophiles, it was hypothesised that alkylation of the acyclic β -keto ester 251, with iododiene 214, followed by a Robinson annulation with acrolein would forge β -keto ester 256. Subsequent decarboxylation and methylation would afford enone 242 (Scheme 3.10).



Scheme 3.10. Retrosynthetic analysis of enone 242 *via* alkylation of β -keto ester 251.

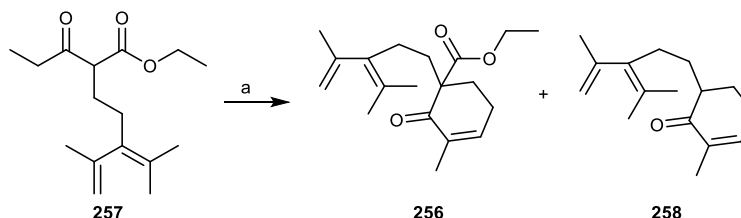
In this case, the alkylation of 251 with iododiene 214 was deemed more feasible than the previous alkylations, due to the use of the more reactive and less hindered enolate of 251. This, in turn, could be prepared with a weaker base, which would also minimise base-induced decomposition of iododiene 214. Initially, β -keto ester 257 was afforded as predicted using a weak base, K_2CO_3 , and the more reactive enolate of 251; after further optimisation, the yield of 257 was improved (Table 3.5).

Table 3.5. Alkylation of β -keto ester 251.

Reagents and conditions: a) K_2CO_3 , acetone, RT; then iododiene 214, reflux.

Entry	β -Keto ester 251 (eq.)	K_2CO_3 (eq.)	Yield (%)	Recovered iododiene 214 (%)
1	1.0	0.5	16	75
2	1.0	1.0	32	47
3	3.0	3.0	75	15

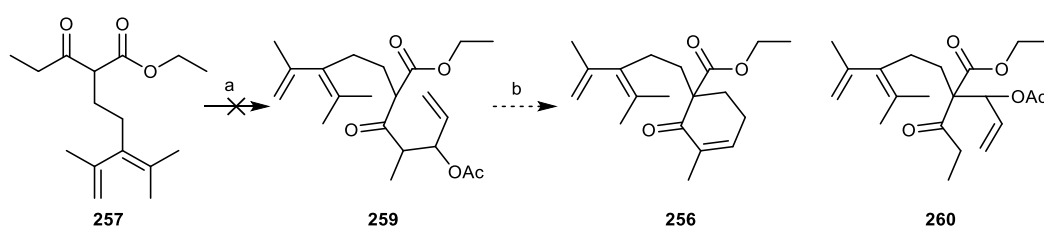
Applying the reported procedure for the synthesis of cyclohexenones *via* the tandem Michael addition/aldol condensation between β -keto esters and enones (or enals) using potassium *tert*-butoxide, followed by decarboxylation *in situ*,^[158] the direct cyclisation of β -keto ester 257 and acrolein was attempted. β -Keto ester 256 and enone 258 were afforded but, unfortunately, the yields were very low (Scheme 3.11).



Scheme 3.11. Synthesis of β -keto ester 256 *via* Robinson annulation of β -keto ester 257 and acrolein. **Reagents and conditions:** a) acrolein, *t*-BuOK, *t*-BuOH, reflux, 256 (6%) and 258 (7%).

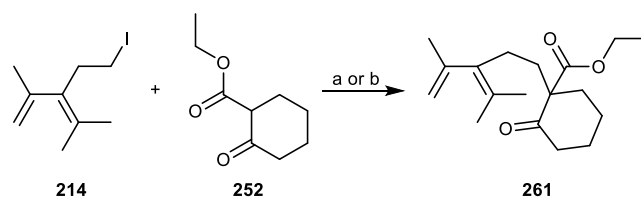
The low yields of 256 and 258 were assumed to arise from the steric hindrance around the methylene carbon of β -keto ester 257. Consequently, a strategy based on literature precedents for the preparation of cyclohexenones using the dienolate of β -keto esters^[159,160] was envisaged as an alternative. Acetate 259 would be generated by addition of the dienolate of β -keto ester 257 to acrolein and the intermediate aldol adduct trapped *in situ* with acetic anhydride. The Mn(III)-mediated cyclisation of 259 and subsequent acetate elimination would then generate β -keto ester 256 (Scheme 3.12). However, when this

strategy was attempted, acetate **259** was not afforded and the tentatively assigned acetate **260** was isolated instead, suggesting that the dienolate of **257** was not formed.



Scheme 3.12. Synthesis of β -keto ester **256** via the dienolate of β -keto ester **257**. **Reagents and conditions:** a) NaH, THF, RT; then *n*-BuLi, -78 °C; then acrolein, -78 °C, then Ac₂O, -78 °C, **260** (13%); b) i. Mn(OAc)₃·2H₂O, AcOH, 80 °C; ii. DBU, THF, RT.

The lack of success with the cyclisation of β -keto ester **257** and acrolein led us to attempt the direct alkylation of cyclic β -keto ester **252** with iododiene **214**. When K₂CO₃ was used,^[161] β -keto ester **261** was afforded in very low yield (4%), along with mainly unreacted **252** (**Scheme 3.13**). The yield of **261** was slightly improved using NaH (16%) but still not high enough to continue using this strategy.

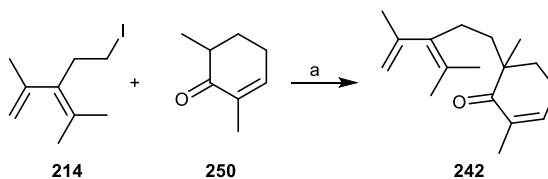


Scheme 3.13. Alkylation of β -keto ester **252**. **Reagents and conditions:** a) K₂CO₃, acetone, RT; then iododiene **214**, reflux, 4%; b) NaH, THF, RT; then iododiene **214**, reflux, 16%.

Since the alkylation of acyclic and cyclic β -keto esters strategy had reached a dead-end, the studied approaches to **242** were re-examined, focussing on the reactions that seemingly should have worked. Since enone **250** was successfully alkylated with simple iodoalkanes (**Table 3.4**), and enone **242** was also afforded using iododiene **214**, albeit in low yield (**Table 3.4**), it was decided to attempt again the synthesis of **242** via alkylation of enone **250**. There are literature precedents for the use of HMPA and DMPU as a co-solvent to improve alkylation reactions,^[162–165] and hence the alkylation of enone **250** and iododiene **214** was attempted with these additives. Pleasingly, enone **242** was finally achieved in a modest yield (69%) using DMPU and in a lower yield (23%) using HMPA

(**Table 3.6**). These results represented a turning point in the project and allowed the new route to taxadienone **16** to be continued.

Table 3.6. Alkylation of enone **250**.

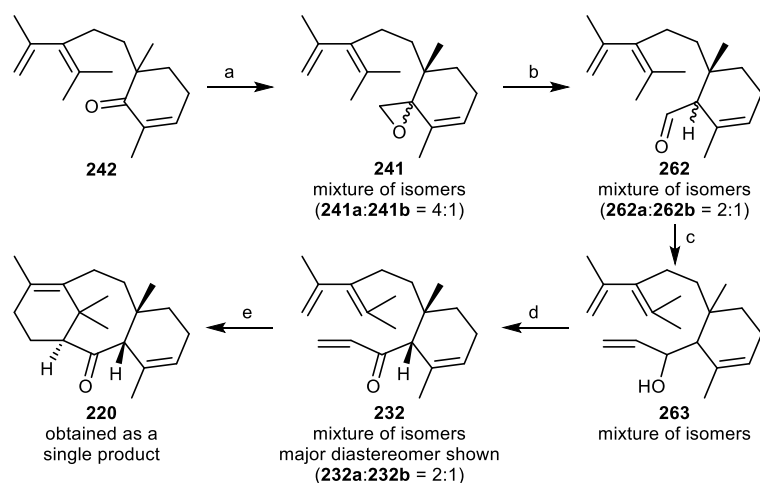


Reagents and conditions: a) LDA, co-solvent, THF, $-78\text{ }^{\circ}\text{C}$; then iododiene **214**, $-78\text{ }^{\circ}\text{C}$ to RT.

Entry	Co-solvent	Yield (%)
1	DMPU	69
2	HMPA	23

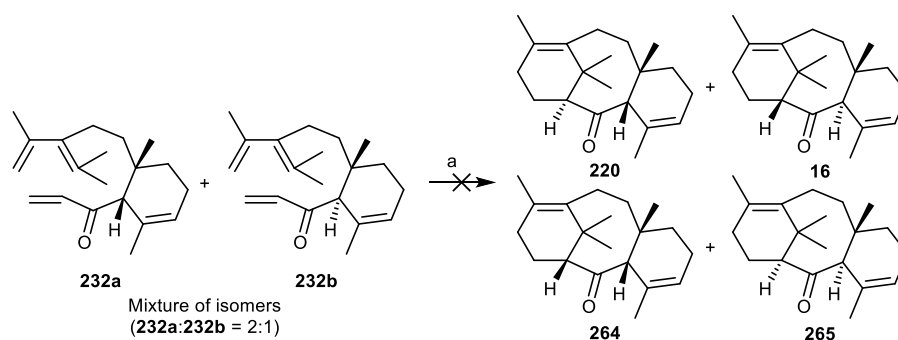
Epoxide **241** was prepared by means of Corey–Chaykovsky epoxidation of enone **242**, producing **241** as an inseparable mixture of two diastereomers (**241a**:**241b** = 4:1). Isomerisation of epoxide **241** to aldehyde **262** upon treatment with $\text{BF}_3 \cdot \text{OEt}_2$, then addition of vinylmagnesium bromide generated allylic alcohol **263**; this was then directly oxidised with Dess–Martin periodinane to obtain enone **232** as an inseparable mixture of two diastereomers (**232a**:**232b** = 2:1). When the diastereomeric mixture of **232** was subjected to the IMDA reaction upon treatment with $\text{BF}_3 \cdot \text{OEt}_2$, di-*epi*-taxadienone **220** was the only product isolated (**Scheme 3.14**), identical with that previously synthesised following Baran’s procedure (**Scheme 2.18**).

Our interest was then focussed on understanding the stereochemical outcome of the IMDA reaction, particularly in identifying the reaction behaviour of the minor diastereomer **232b**, which had the stereochemistry required to give access to taxadienone **16** itself. Several strategies were then put in action to shed some light on this issue.



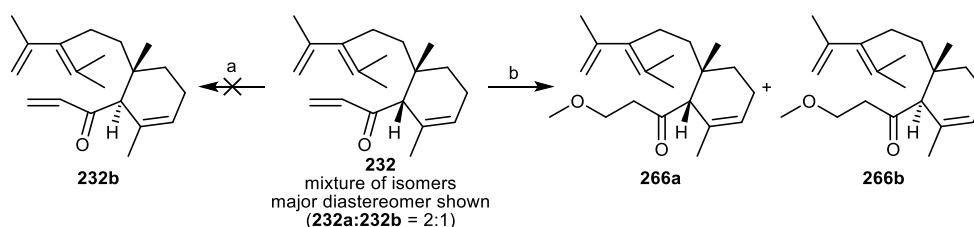
Scheme 3.14. Synthesis of di-*epi*-taxadienone **220**. **Reagents and conditions:** a) *n*-BuLi, (CH₃)₃SiI, THF, 0 °C, 100% (241a:241b = 4:1); b) BF₃·OEt₂, THF, RT; c) CH₂=CH-MgBr, THF, RT; d) DMP, NaHCO₃, DCM, RT, 59% (over 3 steps) (232a:232b = 2:1); e) BF₃·OEt₂, DCM, 0 °C, 42%.

Preliminary computational studies of the IMDA reaction of **232** under thermal conditions were performed (**Scheme 3.15**, see **Appendix A** for details). The free energy barriers for the formation of the four possible diastereomers (**16**, **220**, **264** and **265**) were estimated and, considering the similarity between these values, it was concluded that all four diastereomers should be accessible under thermal conditions. However, taxadienone **16** and di-*epi*-taxadienone **220** showed a slightly lower free energy barrier than their respective C1 epimers, **265** and **264**, and thus their formation should be preferential. Additionally, taxadienone **16** displayed the lowest free energy barrier, slightly lower than that of di-*epi*-taxadienone **220**, and consequently its formation should be the most favourable. In contrast, when the IMDA reaction of the diastereomeric mixture of enone **232** (232a:232b = 2:1) was performed under thermal conditions, no cyclised product was isolated and only decomposition of **232** was observed at temperatures higher than 150 °C (**Scheme 3.15**).



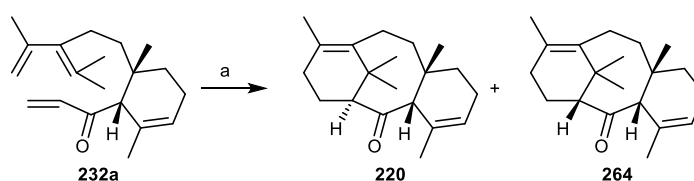
Scheme 3.15. IMDA reaction of enone **232** under thermal conditions. **Reagents and conditions:** a) C₆D₆, 25–200 °C.

Considering that the IMDA reaction under thermal conditions was not achievable, further studies on the reaction under Lewis acidic conditions were performed. To determine the behaviour of the minor diastereomer **232b**, it was crucial to perform the IMDA reaction with only this diastereomer. Given that the *trans*-diastereomer was expected to be more stable than the *cis*-diastereomer on steric grounds, the epimerisation of enone **232a** to **232b** was attempted (Scheme 3.16). When sodium methoxide was used, 1,4-addition of methoxide to the enone took place instead, affording ketone **266** as a mixture of separable diastereomers **266a** and **266b**. When the bulkier and less nucleophilic base DBU was used, enone **232** was recovered unchanged.

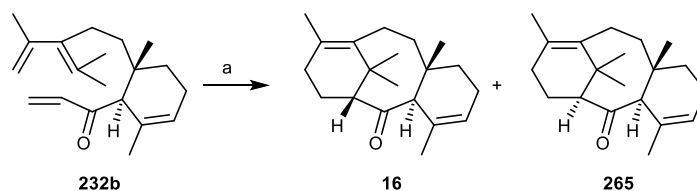


Scheme 3.16. Tested conditions to epimerise enone **232a** to **232b**. **Reagents and conditions:** a) DBU, THF, RT; b) NaOMe, MeOH, 30 °C, **266a** (46%) and **266b** (22%).

Since the epimerisation of enone **232a** to **232b** proved unsuccessful, preparative TLC was used to separate both diastereomers. Subsequently, the IMDA reaction with $\text{BF}_3 \cdot \text{OEt}_2$ was attempted with each diastereomer separately. Reaction with enone **232a** was consistent and led to an inseparable mixture of di-*epi*-taxadienone **220** and the tentatively assigned *epi*-taxadienone **264** (64%, **220:264** = 7:5) (Scheme 3.17). In contrast, the results of the reaction with enone **232b** were ambiguous. The first time the reaction was performed, taxadienone **16** and the tentatively assigned *epi*-taxadienone **265** were obtained as an inseparable mixture (11%, **16:265** = 17:10) but, on a second attempt, only *epi*-taxadienone **265** (50%) was afforded (Scheme 3.18).



Scheme 3.17. IMDA reaction of enone **232a** with $\text{BF}_3 \cdot \text{OEt}_2$. **Reagents and conditions:** a) $\text{BF}_3 \cdot \text{OEt}_2$, DCM, 0 °C, 64% (**220:264** = 7:1).



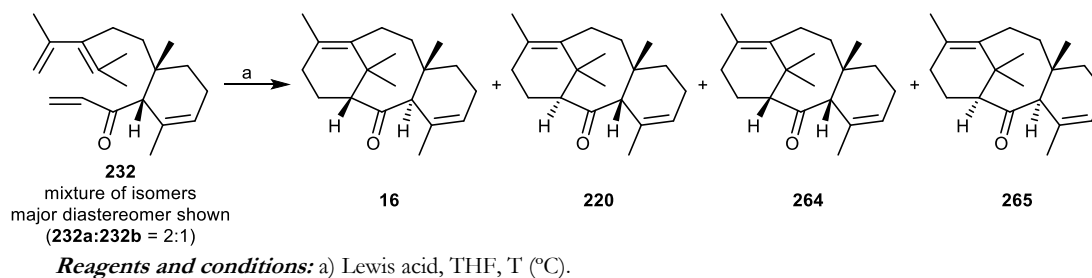
Scheme 3.18. IMDA reaction of enone **232b** with $\text{BF}_3 \cdot \text{OEt}_2$. **Reagents and conditions:** a) $\text{BF}_3 \cdot \text{OEt}_2$, DCM, 0 °C, 1st attempt: 11% (**16**:**265** = 17:10) and 2nd attempt 50% (**265**).

Despite these inconclusive results, several ideas arose from them. Firstly, they were in accordance with the preliminary computational studies, which stated that all four diastereomers (**16**, **220**, **264**, and **265**) should be accessible. Whilst these results suggested that enone **232a** cyclised preferentially into di-*epi*-taxadienone **220**, enone **232b** could cyclise into taxadienone **16** and *epi*-taxadienone **265**, if any preference still remained unclear. However, since neither **220** nor **264** were afforded in the IMDA reaction of **232b**, it was confirmed that epimerisation of **232b** to **232a** did not occur under the reaction conditions.

In addition, from these results, it was inferred that the IMDA reaction of enone **232** displayed a maximum yield of ~50%. Considering that this reaction was performed with a diastereomeric mixture of enone **232** (**232a**:**232b** = 2:1) and, assuming that **232a** cyclised completely into **220** and **232b** into **16**, it was estimated that di-*epi*-taxadienone **220** could be obtained with only a maximum yield of 33%, and taxadienone **16** with only a maximum yield of 16%. Given that this reaction was performed on a very small scale (0.21 mmol), a maximum amount of 20 mg of **220**, and 9 mg of **16** could be obtained. The tiny amount of **16** generated could have been lost during the crude product purification; hence, this would explain why di-*epi*-taxadienone **220** was the only product isolated. To clarify this, the IMDA reaction of enone **232** (**232a**:**232b** = 2:1) was performed on a bigger scale (1.90 mmol) and, in this case, di-*epi*-taxadienone **220** and *epi*-taxadienone **264** were afforded as an inseparable mixture (54%, **220**:**264** = 5:1). Even though taxadienone **16** and *epi*-taxadienone **265** were not isolated, a closer examination of the ¹H-NMR spectrum of the crude reaction mixture showed traces of **16** and **265**, supporting the hypothesis of their loss during the purification process.

Since the IMDA reaction of enone **232** was only achieved upon treatment with $\text{BF}_3 \cdot \text{OEt}_2$, other Lewis acid which have been previously used in IMDA reactions of similar substrates^[57,166,167] were screened (**Table 3.7**). These results were in accordance with the previous findings that enone **232a** cyclised preferentially into di-*epi*-taxadienone **220**. In addition, these revealed that **232a** cyclises faster than **232b**, since enone **232b** was recovered unchanged in almost all cases.

Table 3.7. IMDA reaction of enone **232** under Lewis acidic conditions.

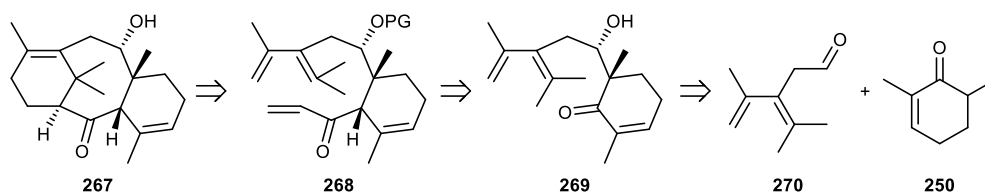


Entry	Lewis acid	T (°C)	Product	Yield (%)	Recovered enone 232	
					(%)	(232a : 232b)
1	$\text{BF}_3 \cdot \text{OEt}_2$	0	220	42	–	–
2	$\text{BF}_3 \cdot \text{OEt}_2$	–78	220	10	42	2:1
3	Et_2AlCl	–78	220	54	18	5:13
4	Et_2AlCl	–78 to 0	220	42	7	0:1
5	EtAlCl_2	–78	220	7	10	1:0
6	MeAlCl_2	–78	220	32	10	0:1

Even though this new route was designed to synthesise taxadienone **16**, which was only afforded in a very low yield, a practical and short synthetic route to the taxane framework was developed that produced di-*epi*-taxadienone **220** in six steps and 13% yield from iododiene **214**. Our work then focussed on modifying this route to develop an enantioselective synthesis of **220**.

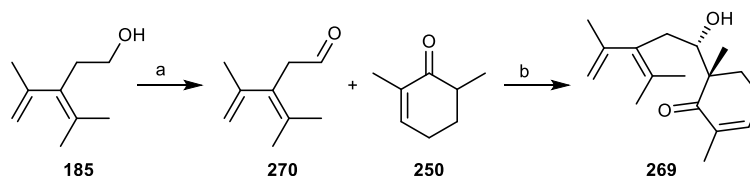
3.4 Approaches studied to convert the new route to di-*epi*-taxadienone 220 into an enantioselective synthesis

In attempting to convert the developed racemic synthesis of di-*epi*-taxadienone 220 into an enantioselective route, the installation of the diene side chain seemed an obvious point for establishing the absolute stereochemistry. Firstly, our studies focussed on the enantioselective synthesis of aldol 269, an analogue of enone 242, *via* an asymmetric aldol reaction of enone 250 and aldehyde 270 (Scheme 3.19). In this case, a hydroxyl group would be introduced at C9 but, considering that the main aim of the project was the late stage hydroxylation of the taxane framework with P450_{BM3} mutants, the prior C9 oxidation was not a problem. However, due to expected reactivity interferences, this hydroxyl group would need to be protected for the synthesis to continue, and thus two more steps (alcohol protection and deprotection) would be added to the sequence.



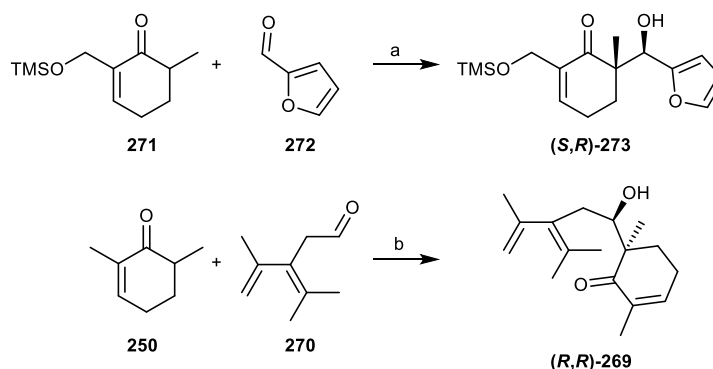
Scheme 3.19. First approach studied on the development of an asymmetric synthesis of di-*epi*-taxadienone 220.

Firstly, the racemic aldol reaction of enone 250 and aldehyde 270 was studied. For this, alcohol 185, used in the synthesis of iododiene 214 (Scheme 2.12), was oxidised to aldehyde 270 using Dess–Martin periodinane and subsequently, the aldol reaction between enone 250 and aldehyde 270 using LDA was performed, affording aldol 269 diastereoselectively (Scheme 3.20). Whilst the use of DMPU or HMPA as a co-solvent was crucial in the alkylation of enone 250 with iododiene 214, these were found not to be required for the aldol reaction of 250 with aldehyde 270.



Scheme 3.20. Racemic synthesis of aldol **269**. **Reagents and conditions:** a) DMP, NaHCO₃, DCM, RT, 91%; b) LDA, THF, -78 °C; then aldehyde **270**, -78 °C, 77%.

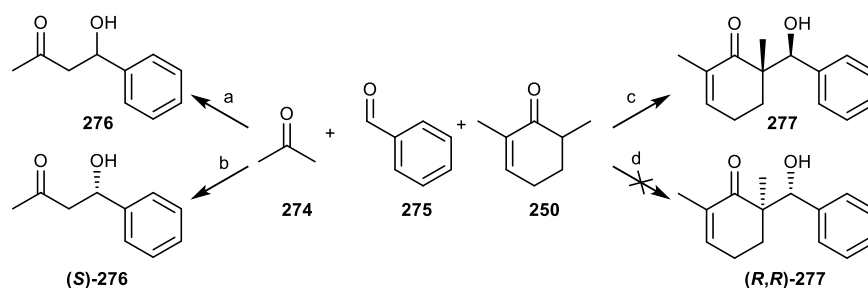
Next, asymmetric versions of the aldol reaction between enone **250** and aldehyde **270** were studied. The SAMP-hydrazone methodology,^[168–170] in which the asymmetric C–C bond formation is facilitated by the pyrrolidine chiral auxiliary of (*S*)-1-amino-2-methoxymethylpyrrolidine, was the first to be considered. However, the failure of the formation of the SAMP-hydrazone of the similar enone **271** reported by Williams^[171,172] prevented us from attempting this strategy and led us to instead study the aldol reaction of chiral enol borinates with diisopinocampheyl chloroborane (DIP-Cl), which reportedly permitted the preparation of aldol **273**, giving an 80–90% ee (**Scheme 3.21**).^[172]



Scheme 3.21. Reported asymmetric synthesis of aldol **273**,^[172] and its application to the asymmetric synthesis of aldol **269**. **Reagents and conditions:** a) KHMDS, (–)-DIP-Cl, THF -78 °C; then aldehyde **272**, -78 °C, 33–40%, 80–90% ee; b) KHMDS, (+)-DIP-Cl, THF -78 °C; then aldehyde **270**, -78 °C to RT, 9%. The configuration of aldol **269** was assigned by correlation with the reported configuration of aldol **273** when using (–)-DIP-Cl.^[172]

When the conditions reported for the use of KHMDS and (+)-DIP-Cl^[172,173] were applied to the aldol reaction between enone **250** and aldehyde **270**, aldol **269** was afforded in low yield, with **250** remaining largely unreacted (**Scheme 3.21**). Although other reported conditions to perform asymmetric aldol reactions with DIP-Cl and TEA^[174] or DIPEA^[175] were tested, aldol **269** was not obtained in any case and enone **250** was recovered.

With the unsuccessful attempts to achieve enantiomerically enriched aldol **269** using (+)-DIP-Cl, it seemed necessary to confirm the feasibility of the asymmetric aldol reaction with chiral enol borinates of (+)-DIP-Cl, and thus the reported asymmetric aldol reaction between acetone **274** and benzaldehyde **275** was reproduced.^[175] Aldol **276** was afforded in a 79% yield, with a 67% ee (determined by the Mosher ester method), comparable to the described 78% yield and 57% ee (**Scheme 3.22**). These reaction conditions were then applied to the aldol reaction between enone **250** and benzaldehyde **275** but, once again, **250** was recovered unreacted (**Scheme 3.22**).

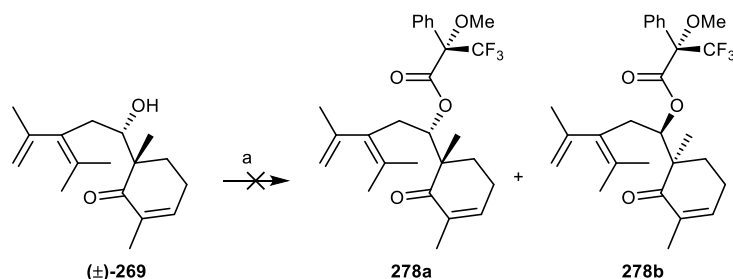


Scheme 3.22. Aldol reaction between ketone **274** and aldehyde **275**, and between enone **250** and aldehyde **275** under racemic and asymmetric conditions. **Reagents and conditions:** a) LDA, THF, $-78\text{ }^{\circ}\text{C}$; then aldehyde **275**, $-78\text{ }^{\circ}\text{C}$, 42%; b) DIPEA, (+)-DIP-Cl, DCM $-78\text{ }^{\circ}\text{C}$; then aldehyde **275**, $-78\text{ }^{\circ}\text{C}$ to $-15\text{ }^{\circ}\text{C}$, 78%, 67% ee; c) LDA, THF, $-78\text{ }^{\circ}\text{C}$; then aldehyde **275**, $-78\text{ }^{\circ}\text{C}$, 90%; d) DIPEA, (+)-DIP-Cl, DCM $-78\text{ }^{\circ}\text{C}$; then aldehyde **275**, $-78\text{ }^{\circ}\text{C}$ to $-15\text{ }^{\circ}\text{C}$. The configuration of aldol **276** was assigned by correlation with the reported specific rotation of **276**,^[175] and the ee of aldol **276** was determined by the Mosher ester method.

In view of the failure of the asymmetric aldol reaction between enone **250** and benzaldehyde **275** using (+)-DIP-Cl, and that the synthesis of enantioenriched aldol **269** was not as efficient as reported for aldol **273**,^[172] it appeared that the oxygen atom of the trimethylsilyloxy group in enone **271** may play an important role, interacting with the chiral borane enolate complex and giving access to a conformation which favours the aldol reaction.

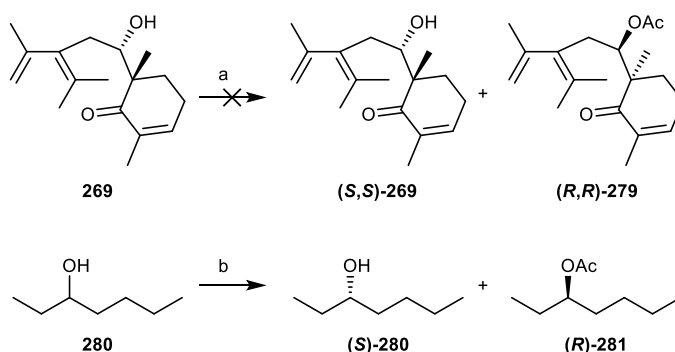
Despite enantiomerically enriched aldol **269** only having been achieved in low yield, this was prepared efficiently racemically, thus chiral derivatisation and enzymatic resolution methods were considered for the development of an enantioselective synthesis of di-*epi*-taxadienone **220**.

When the chiral derivatisation of aldol **269** using (*R*)-Mosher's acid chloride was performed, only traces of Mosher ester derivatives **278a** and **278b** were observed by analysis of the $^1\text{H-NMR}$ spectrum of the crude reaction mixture (**Scheme 3.23**). Therefore, the enantiomers of racemic aldol **269** could not be separated by chemical derivatisation.



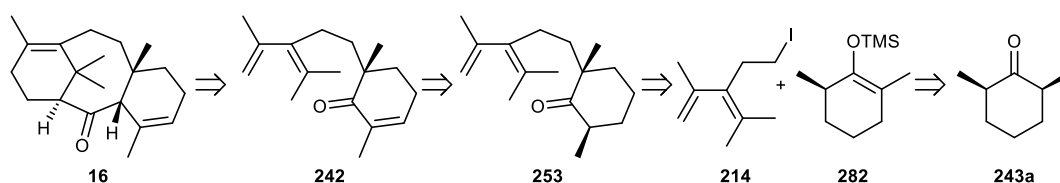
Scheme 3.23. Chiral derivatisation of racemic aldol **269** with (*R*)-Mosher's acid chloride. **Reagents and conditions:** a) (*R*)-MTPA-Cl, pyr, C_6D_6 , RT.

Additionally, when the widely reported enzymatic resolution of alcohols with lipases^[176] was applied to aldol **269**, this was recovered unchanged (**Scheme 3.24**). Once again, to confirm the viability of the procedure used, the reported enzymatic resolution of racemic 3-heptanol **280** was performed,^[177] recovering (*S*)-**280** in a 20% yield, with an ee > 99% (determined by the Mosher ester method), comparable to the 35% and ee > 99% reported (**Scheme 3.24**). From the unsuccessful results on the chiral derivatisation and enzymatic resolution of aldol **269**, it was inferred that the hydroxyl group in **269** may be too hindered and inaccessible for derivatisation, or have reduced nucleophilicity.



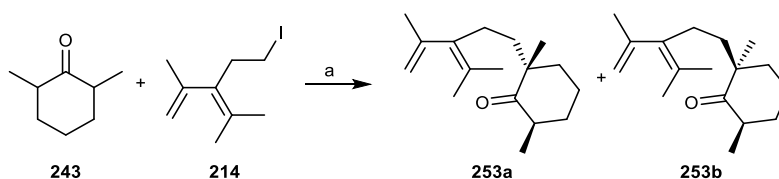
Scheme 3.24. Enzymatic resolution of racemic aldol **269** and racemic alcohol **280**. **Reagents and conditions:** a) *C. Antarctica* lipase B, vinyl acetate, hexane, RT; b) *C. Antarctica* lipase B, vinyl acetate, hexane, RT, (*S*)-**280** (20%, 99% ee) and (*R*)-**281** (42%). The configuration of acetate **281** was assigned by correlation with the reported specific rotation of **281**.^[177] The configuration of alcohol **280** was assigned by correlation with the configuration of acetate **281**^[177] and the ee of alcohol **280** was determined by the Mosher ester method.

Considering the unsuccessful attempts developing an enantioselective synthesis of di-*epi*-taxadienone **220** *via* aldol **269**, a different strategy was put in action and the desymmetrisation of *cis*-2,6-dimethylcyclohexanone (**243a**) was investigated instead (**Scheme 3.25**). The two α -protons of ketone **243a** are enantiotopic, and thus a chiral base should be able to distinguish between them and preferentially generate one enantiomer of silyl enol ether **282**. Subsequent alkylation of enantiomerically pure **282** with iododiene **214** would give access to enantiopure ketone **253**, assuming alkylation *anti* to the methyl group, which would be taken forward in the synthesis to afford enantiopure di-*epi*-taxadienone **220**.



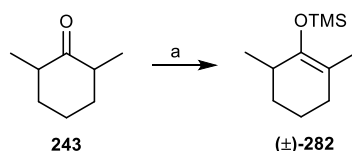
Scheme 3.25. Second approach studied on the development of an asymmetric synthesis to di-*epi*-taxadienone **220**.

Alkylation of ketone **243** with iododiene **214** had been explored when attempting to synthesise racemic enone **253** (**Scheme 3.9**); although the reaction failed at the time, DMPU or HMPA had not been tested as a co-solvent (see **section 3.3**). Hence, the alkylation of ketone **243** and iododiene **214** was attempted again, with DMPU added, and ketone **253** was obtained as an inseparable mixture of two diastereomers (**253a**:**253b** = 5:2) (**Scheme 3.26**). These results reaffirmed the crucial use of DMPU in the reaction.



Scheme 3.26. Racemic synthesis of ketone **253**. **Reagents and conditions:** a) LDA, DMPU, THF, -78 °C; then iododiene **214**, -78 °C to RT, 29% (**253a**:**253b** = 5:2).

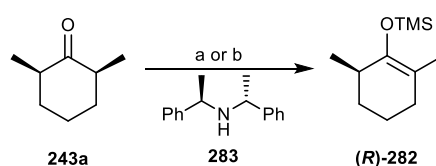
Once the feasibility of the alkylation of ketone **243** with iododiene **214** was established, the asymmetric synthesis of ketone **253** *via* desymmetrisation of ketone **243a** was studied. Firstly, TMS-enol ether **282** was prepared under racemic conditions (**Scheme 3.27**), and subsequently its asymmetric synthesis was investigated.



Scheme 3.27. Racemic synthesis of TMS-enol ether **282**. **Reagents and conditions:** a) LDA, TMSCl, THF, $-78\text{ }^{\circ}\text{C}$; then ketone **240**, $-78\text{ }^{\circ}\text{C}$, 83%.

After an examination of the literature on the desymmetrisation of prochiral ketones, it was established that three main research groups had worked on this area: Simpkins,^[178–180] Koga^[180–183] and Henderson.^[184,185] After examining their methodologies, Simpkins' conditions were deemed more practical: the chiral amines used are easier to synthesise than the ones used by Koga and Henderson, and some are commercially available; furthermore, the reaction conditions are less toxic, since HMPA, crucial in Koga's and Henderson's procedures, is not required.

Table 3.8. Asymmetric synthesis of TMS-enol ether **282**.



Reagents and conditions: a) **IQ:** **283**, *n*-BuLi, TMSCl, THF, $-78\text{ }^{\circ}\text{C}$; then ketone **243a**, T ($^{\circ}\text{C}$); b) **EQ:** **283**, *n*-BuLi, THF, $-78\text{ }^{\circ}\text{C}$; then ketone **243a**, $-78\text{ }^{\circ}\text{C}$; then TMSCl, T ($^{\circ}\text{C}$).

Entry	Quench	T ($^{\circ}\text{C}$)	Reaction time	Additive	Yield (%)	ee (%) ^a
1	IQ	-78	45 min	–	78	15
2	IQ	-78	45 min	DMPU	30	18
3	IQ	-20	14 h	–	76	37
4	EQ	-78	15 min	–	71	50
5	EQ	-78	15 min	LiCl	46	40
6	EQ	-20	14 h	–	57	50

^a ee's determined by chiral GC analysis.

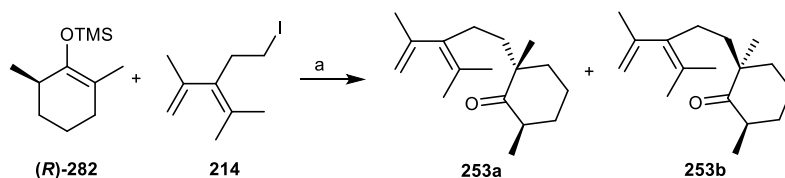
The preparation of enantioenriched TMS-enol ether **282** using the enantiomerically pure chiral base **283** and Simpkins' conditions was then examined^[179] (**Table 3.8**). Two different methodologies were studied: an internal quench (IQ), in which the base and trimethylchlorosilane are mixed prior to addition of the ketone; and an external quench (EQ), in which the base reacts with the ketone before the trimethylchlorosilane is added.

Additionally, the reported improvement of the enantioselectivity when using different additives, such as LiCl^[186] or DMPU,^[184] was also explored (**entries 2 and 5**).

The internal quench method is reported to give higher enantioselectivity than the external quench method,^[186] but, in my hands, higher enantioselectivities (50% ee) were obtained using an external quench and no additive. Even though the enantioselectivity of TMS-enol ether **282** was still moderate, and the use of alternative chiral bases would need to be tested, it was deemed sensible to investigate first the alkylation of **282** with iododiene **214** before optimising the enantioselective preparation of **282**.

From TMS-enol ether **282**, the lithium enolate was regenerated upon treatment with methyllithium, and iododiene **214** added, using DMPU as a co-solvent; ketone **253** was obtained as an inseparable mixture of two diastereomers (**253a** and **253b**). When this reaction was performed using enantioenriched TMS-enol ether **282** (ee 50%), racemic ketones **253a** and **253b** were isolated, and hence, the enantioselectivity was lost (**Table 3.9**).

Table 3.9. Synthesis of ketone **253**.



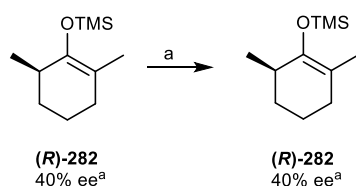
Reagents and conditions: a) MeLi, THF, RT; then DMPU, -78 °C; then iododiene **214**, -78 °C to RT.

Entry	TMS-enol ether 282 (ee %)	Yield (%)	Ketone 253	
			(253a : 253b)	ee (%) ^a
1	0	30	5:2	0
2	50	22	17:10	0

^a ee's determined by chiral GC analysis.

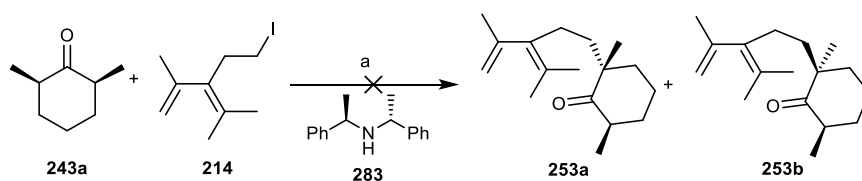
This reaction can be separated into two steps: regeneration of the enolate of **282**, and alkylation with **214**. In an attempt to determine if chirality was lost during the first step, the lithium enolate of enantioenriched TMS-enol ether **282** was regenerated and quenched again with chlorotrimethylsilane. In this case, the resulting TMS-enol ether **282** had the same ee as the starting material and thus, chirality remained unchanged (**Scheme 3.28**). These results suggested that in the preparation of ketone **253** from enantioenriched TMS-

enol ether **282**, chirality was lost during the alkylation step, which might be slow enough to permit racemisation of the enantioenriched lithium enolate of **282** *in situ*.



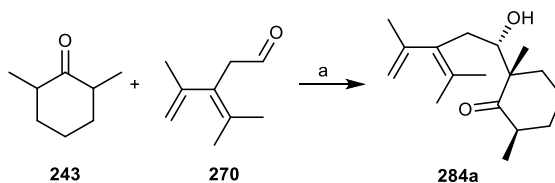
Scheme 3.28. Regeneration of TMS-enol ether **282**. **Reagents and conditions:** a) MeLi, THF, RT; then TMSCl, $-78\text{ }^{\circ}\text{C}$, 26%. ^a ee's determined by chiral GC analysis.

Given the unsuccessful synthesis of enantioenriched ketone **253** *via* enantioenriched TMS-enol ether **282**, and that previously racemic ketone **253** had been prepared *via* direct alkylation of ketone **243** and iododiene **214** using LDA (**Scheme 3.25**), the synthesis of enantioenriched **253** using the enantiomerically pure chiral base **283** was attempted but, in this case, **243** was recovered unreacted (**Scheme 3.29**).



Scheme 3.29. Asymmetric synthesis of ketone **253**. **Reagents and conditions:** a) **283**, *n*-BuLi, THF, $-78\text{ }^{\circ}\text{C}$; then DMPU, $-78\text{ }^{\circ}\text{C}$; then iododiene **214**, $-78\text{ }^{\circ}\text{C}$.

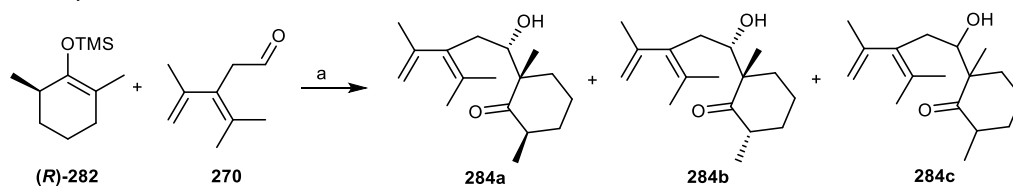
Considering that the loss of chirality during the preparation of ketone **253** from enantioenriched **282** might be due to the slow alkylation with iododiene **214**, it seemed sensible to attempt the aldol reaction of TMS-enol ether **282** and aldehyde **270**, since aldehyde **270** was expected to be more reactive than iodide **214**.



Scheme 3.30. Racemic synthesis of aldol **284**. **Reagents and conditions:** a) LDA, THF, $-78\text{ }^{\circ}\text{C}$; then aldehyde **270**, $-78\text{ }^{\circ}\text{C}$, 17%.

Firstly, the direct aldol reaction of ketone **243** with aldehyde **270** was attempted and aldol **284** was afforded as a single product (**284a**) (**Scheme 3.30**). In contrast, the regeneration of the lithium enolate of TMS-enol ether **282** upon methylolithium treatment

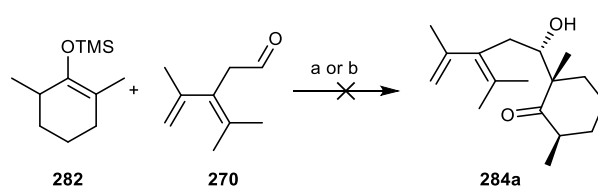
and subsequent addition of aldehyde **270** afforded aldol **284** as a separable mixture of three diastereomers (**284a**, **284b** and **284c**), **284a** being the major product (Table 3.10). When this reaction was performed using enantiomerically enriched TMS-enol ether **282** (37% ee) (entry 2), aldols **284a**, **284b** and **284c** were also obtained, **284a** being afforded in a 40% yield, with a 30% ee, without losing chirality.

Table 3.10. Synthesis of aldol **284**.

Reagents and conditions: a) MeLi, THF, RT; then aldehyde **270**, $-78\text{ }^{\circ}\text{C}$.

Entry	TMS-enol ether 282 (ee %)	Aldol 284		
		284a (%), ee %)	284b (%)	284c (%)
1	0	39, 0	2	4
2	37	40, 34	2	4

Additionally, the synthesis of aldol **284** *via* the Mukaiyama aldol reaction of TMS-enol ether **282** and aldehyde **270** was examined but no successful results were obtained. When TiCl_4 was used, only decomposition was observed, and with $\text{BF}_3 \cdot \text{OEt}_2$, only traces of aldol **284a** were observed (Scheme 3.31).

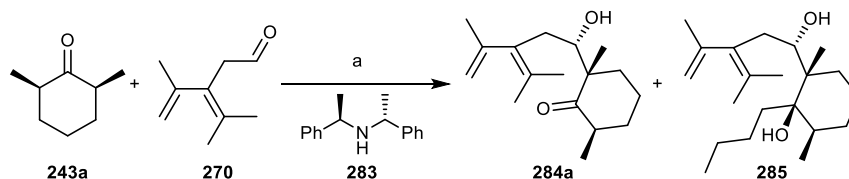


Scheme 3.31. Racemic synthesis of aldol **284** *via* Mukaiyama aldol reaction. **Reagents and conditions:** a) TiCl_4 , DCM, $-78\text{ }^{\circ}\text{C}$; b) $\text{BF}_3 \cdot \text{OEt}_2$, DCM, $-78\text{ }^{\circ}\text{C}$.

Following from the preparation of racemic aldol **284a** *via* the aldol reaction between ketone **243** and iododiene **214** using LDA, the synthesis of enantioenriched **284a** was attempted using ketone **243a** and the enantiomerically pure chiral base **283**, but ketone **243** was recovered unreacted (Table 3.11). It was speculated that the lithium enolate of **243a** was generated and formed a complex with the chiral base which, in turn, hindered the approach of the aldehyde **270**, and thus prevented the aldol reaction from occurring.

Consequently, in an attempt to disassemble the hypothesised enolate-chiral base complex, *n*-BuLi was added prior to the addition of aldehyde **270**, but diol **285** was isolated, the product of addition of *n*-BuLi to aldol **284a** (**entry 3**). In an attempt to minimise the formation of **285**, the number of eq. of *n*-BuLi used were reduced, but diol **285** was still the only product isolated (**entry 2**).

Table 3.11. Asymmetric synthesis of aldol **284**.



Reagents and conditions: **283**, *n*-BuLi, THF, $-78\text{ }^{\circ}\text{C}$; then *n*-BuLi (eq.), $-78\text{ }^{\circ}\text{C}$; then aldehyde **270**, $-78\text{ }^{\circ}\text{C}$.

Entry	<i>n</i> -BuLi (eq.)	Product	Yield (%)
1	0.0	–	–
2	0.6	285	39
3	1.4	285	65

At this point, several approaches to convert the racemic new route to di-*epi*-taxadienone **220** into enantioselective had been studied, including asymmetric aldol reaction of chiral enol borinates, chiral derivatisation, enzymatic resolution and desymmetrisation of prochiral compounds. From all of them, the desymmetrisation of *cis*-2,6-dimethyl-cyclohexanone (**243a**) emerged as the most effective, affording enantioenriched aldol **284a** in a 30% ee; which could be further optimised testing alternative chiral bases in the enantioselective preparation of TMS-enol ether **282**.

Even though the enantioselective synthesis of taxane **267**, analogue of di-*epi*-taxadienone **220**, from enantioenriched **284a** was not taken further, the application of the route to aldol **284a** was deemed feasible, after alcohol protection.

4 LATE-STAGE HYDROXYLATION WITH P450 MUTANTS

4.1 Synthesis of taxol analogues *via* late-stage hydroxylation of taxanes

In the previous chapter, the approaches studied towards taxadienone **16** were outlined. These resulted in the synthesis of four compounds containing the taxane core: diketones **179a** and **179b**, taxadienone **16**, and di-*epi*-taxadienone **220** (Figure 4.1). The following step in the preparation of taxol analogues consisted of transforming these compounds into more complex hydroxylated taxanes *via* enzymatic oxidation using P450_{BM3} mutants. Additionally, this study would provide more information about the capabilities of P450 mutants in organic synthesis.

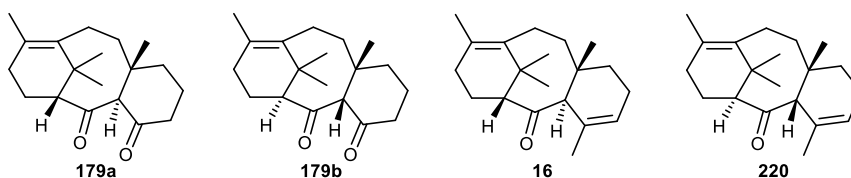
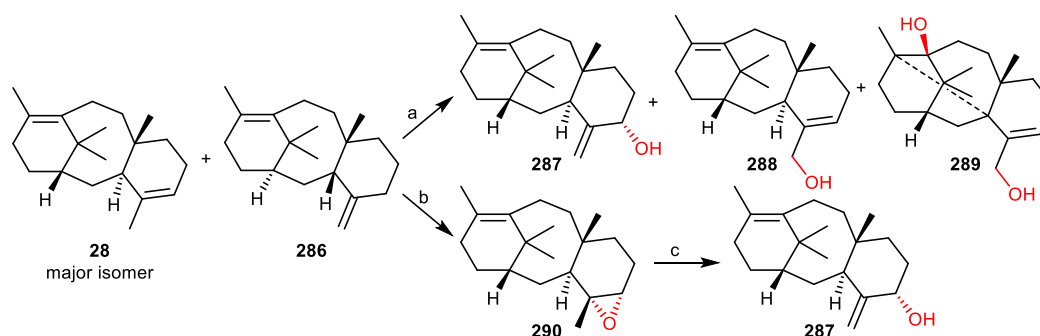


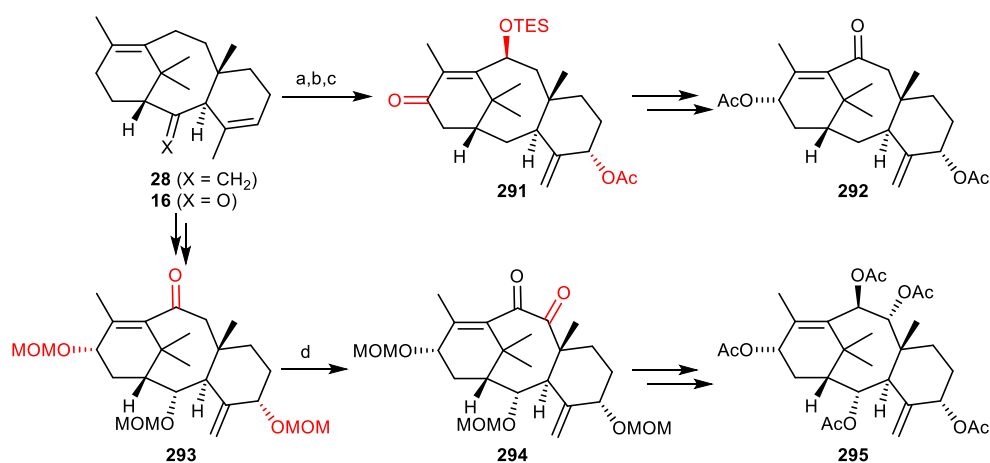
Figure 4.1. Taxane compounds synthesised *via* conventional synthetic methods.

Oxidation of C–H bonds situated on the taxane core has previously been reported using chemical methods. For example, both Williams and Hayes reported the oxidation of taxadiene **28** at the C5 position; Williams^[187] utilised an allylic oxidation method, using SeO₂, whilst Hayes^[188] employed olefin epoxidation, with subsequent epoxide–allylic alcohol rearrangement (Scheme 4.1). Both studies were performed to garner information on the biomimetic interconversion of **28** to taxadienol **287**.



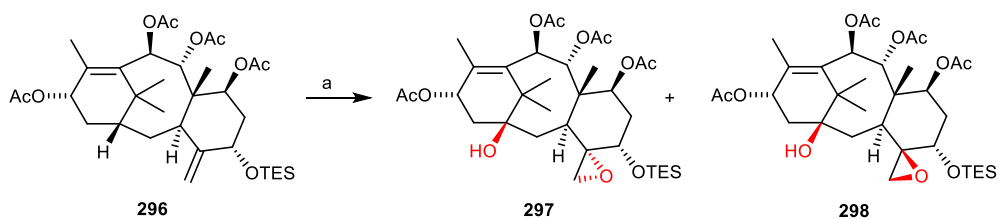
Scheme 4.1. C–H bond oxidation of taxadiene **28**. **Reagents and conditions** a) i. SeO₂, *t*-BuOOH, DCM, RT; ii. NaBH₄, EtOH, RT; **287** (11%), **288** (17%) and **289** (30%); b) DMDO, acetone/DCM, 0 °C to RT, 63%; c) *n*-BuLi, TMP, Et₂AlCl, 0 °C, toluene, 60%.

Similarly, in an attempt to mimic the biosynthesis of taxol in the laboratory, Baran^[189] reported the synthesis of (–)-taxuyunnanine D (**292**) from taxadiene **28** *via* a series of allylic oxidations at the C5, C10 and C13 positions, using palladium-catalysed and chromium(V)-based oxidations, and radical halogenation (**Scheme 4.2**). Later, Baran^[190] applied a similar strategy to the synthesis of taxabaccatin III (**295**) from taxadienone **16**, and in this case the additional oxidation at the C1 position was performed *via* enolate oxygenation (**Scheme 4.2**).



Scheme 4.2. C–H bond oxidation of taxadiene **28** and taxadienone **16**. **Reagents and conditions:** a) Pd(OAc)₂, *p*-benzoquinone, anisole, acetic acid, 50 °C, 49%; b) bis[2-ethyl-2-hydroxy-butanoato(2-)]oxochromate(V), MnO₂, 15-crown-5, trifluorotoluene, 80 °C, 53%; c) NBS, benzoyl peroxide, CCl₄, reflux; then AgOTf, Et₃SiOH, toluene, 0 °C, 80%; d) Et₂NLi, MoOPh, THF, RT to –20 °C to RT; then Cu(OAc)₂, MeOH, RT, 77%.

C–H bond oxidation using chemical methods has also been used in more advanced taxanes, to oxidise positions which are neither activated nor proximal to a directing group. For example, Oritani^[191] reported the oxidation of taxane **296** at the C1 position, along with epoxidation at the C4–C20 double bond, by treatment with a large excess of dimethyldioxirane (**Scheme 4.3**).



Scheme 4.3. C–H bond oxidation of taxane **296**. **Reagents and conditions:** a) DMDO (30 eq), DCM, RT, 90%.

However, the oxidation of C–H bonds *via* chemical methods usually requires strong oxidants, such as Cr(VI), Mn(VII), or Se(IV), and harsh reaction conditions. Despite these being widely applied in synthesis, they often have poor tolerance with other functional groups, reduced regioselectivity of the oxidation, and over-oxidation of the product. For these reasons, enzymatic oxidation has emerged as an ideal alternative, owing to the high efficacy, and chemo-, regio- and stereo-selectivity demonstrated by enzymes. In particular, the use of P450 enzymes was envisaged to oxidise the taxane hydrocarbon skeleton, as this occurs in the biosynthesis of taxol. Oxidation at the C6, C10 and C13 positions was deemed more likely to occur, due to the relative ease by which allylic C–H bonds can be broken. However, particular interest was focussed on enzymes that achieve oxidation at positions that are inaccessible to traditional chemical reagents (**Figure 4.2**).

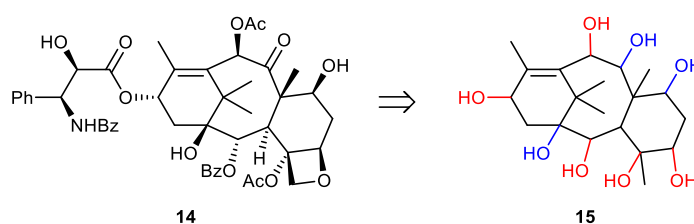


Figure 4.2. Oxidised positions of the taxane skeleton in taxol (**14**); positions in red are accessible by chemical oxidation methods and positions in blue are not accessible by chemical oxidation methods.

The enzymatic oxidation of taxanes **16**, **179a**, **179b** and **220** was conducted in collaboration with Prof. Luet Wong's group, who have developed a panel of >200 P450_{BM3} mutants, spanning a range of oxidation profiles and a broad substrate spectrum.

4.2 Cytochrome P450s

Cytochrome P450s are a family of heme b-dependent monooxygenases with a characteristic absorption band at 450 nm, corresponding to the CO-bound ferrous form, from which the family takes the name.

In 1958, P450s were discovered by Klingenberg^[192] and Garfinkel^[193] independently, in microsomes isolated from rat and pig livers, respectively. Initially, they were identified as CO-binding pigments, and later Omura and Sato^[194,195] discovered that

they are heme proteins, and named them P450 (Pigment 450, in reference to their distinctive absorption band). Since this discovery, P450s have been found in virtually every organism, including animals, plants, bacteria, fungi, and even archaea and viruses. However, they are not omnipresent and indeed *Escherichia coli*, the most common organism in which P450s are expressed, has no native P450.

Although the P450 family has >4000 members and their sequence identity is often $\leq 20\%$, all P450s have a highly conserved tertiary structure and share a characteristic protein fold with 12 α -helices and 5 β -sheets. Their prosthetic group consists of an iron porphyrin(IX) complex (heme b), which is axially ligated to the protein *via* the thiolate side chain of a proximal cysteine residue, the only residue which is invariant (**Figure 4.3**).

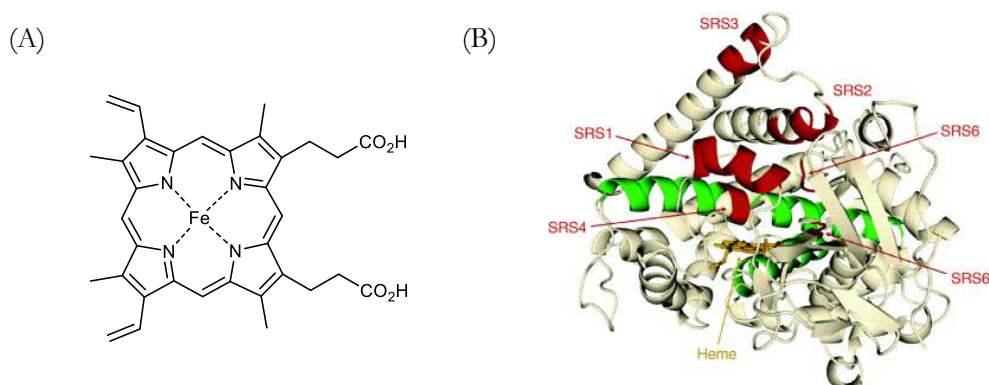


Figure 4.3. (A) Structure of heme b; (b) Model of the conserved tertiary structure of P450 monooxygenases. The protoporphyrin IX (heme) is coloured orange, the substrate recognition site (SRS1–SRS6) is coloured red, and the heme coordinating I and L helices are shown in green.^[196]

P450s are widely known for their extraordinary ability to introduce oxygen into non-activated C–H bonds, which is typically known as the signature reaction of P450s. Molecular dioxygen is activated using two electrons, typically from NADPH, and one oxygen atom is then inserted into a C–H bond of the substrate, whilst the other is reduced to water (**Figure 4.4**).

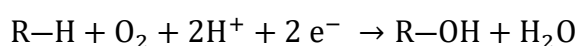


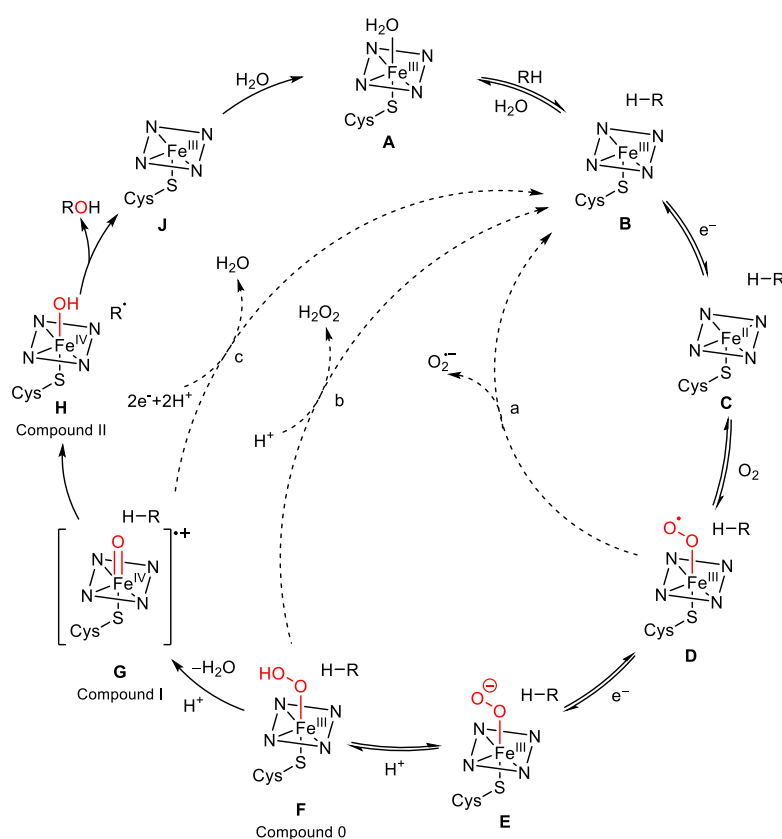
Figure 4.4. Equation representing the signature reaction of P450 enzymes.

However, P450s also exhibit other modes of action; including: C=C epoxidation, *N*- and *O*-dealkylation, C–C bond formation and cleavage, ring expansion and heteroatom oxidation.

P450s work in the presence of oxygen, the cofactor NADPH and a corresponding electron transfer system. The P450 catalytic cycle^[197] was initially widely studied and its intricacies were extensively debated due to the vast variety of reactions catalysed by P450s. However, the general features and steps are now broadly agreed upon (**Scheme 4.4**).

In the resting state, the heme iron is in a low spin state ($S = 1/2$) and arranged in a hexa-coordinate iron(III) complex (**A**), bearing the four nitrogen atoms of the porphyrin system in the equatorial plane, and a water and the proximal thiolate ligands in axial positions. The arrival of the substrate (R–H) in the active site results in the loss of the axial water ligand and leads to the formation of the penta-coordinate iron(III) complex **B**. This process is accompanied by a change in the spin state of the iron from low-spin to high-spin ($S = 1/2 \rightarrow S = 5/2$), and also by the heme reduction potential becoming more oxidising. This facilitates a single electron transfer (SET) from a reduced pyridine nucleotide (NADPH), to the heme iron *via* electron transfer partners, reducing the metal to the iron(II) state **C**. Molecular dioxygen then binds to the heme group, producing the iron(III) oxy-complex **D**. A second single electron transfer generates the iron(III) peroxy-complex **E**, which is rapidly protonated to give the iron(III) hydroperoxy-complex **F** (Compound 0). A second protonation at the terminal oxygen and subsequent heterolytic O–O bond cleavage generates the active iron(IV) oxo-species **G** (Compound I), accompanied with loss of water. This radical cation complex is highly electrophilic, and thus, in hydroxylation reactions, **G** abstracts a hydrogen atom from the substrate (R–H) to generate an alkyl radical (R[•]) and the iron(IV) hydroxide-complex **H**. Recombination of the alkyl radical with the iron(IV)-bound OH group produces the alcohol product, which is then released from the active site, returning the heme group to the penta-coordinate iron(III) complex **J**. At

this stage, the enzyme can bind a water ligand and return to the resting state **A**, or bind another substrate molecule and restart the cycle.



Scheme 4.4. The P450 catalytic cycle and its uncoupling mechanism: a) Superoxide uncoupling; b) Peroxide uncoupling; c) Oxidase uncoupling.

The P450 catalytic cycle can be disrupted by three different mechanisms, which hinder the oxidation of the substrate (shown by dashed arrows in **Scheme 4.4**). When dioxygen binding is hindered, the second electron transfer could be too slow and lead to the loss of superoxide from complex **D** (superoxide uncoupling). If the substrate is loosely bound in the active site, then water may approach too closely to the iron atom, and protonate the iron-oxygen bond of complex **F**, leading to the loss of hydrogen peroxide (peroxide uncoupling). In contrast, if the substrate is tightly bound but no hydrogen atom is conveniently positioned for abstraction, the iron(IV) oxo-species **G** is reduced to iron (III) and water is lost.

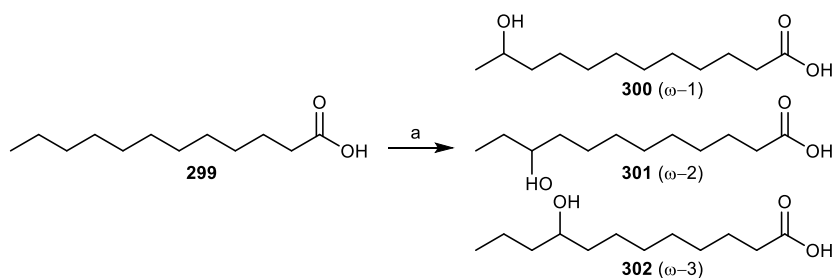
P450s catalyse the oxidation of a broad array of substrates and are vital in a large number of biological processes. Whilst some have anabolic functions and are substrate-

specific, such as the synthesis of steroids in mammals, and natural products in plants; others have catabolic functions, involved in the detoxification and metabolism of xenobiotics, and are more substrate promiscuous, with versatility being an asset in such roles, for instance, microsomal-derived P450.

Although the use of microsomal-derived P450 may seem a more sensible choice due to their broad range of substrates tolerated, eukaryotic P450s are slow-acting and more difficult to handle in a laboratory. These enzymes are membrane bound, and thus the use of whole-cells is necessary; this requires the consideration of additional parameters, such as membrane permeability to enable hydrophobic substrates to enter the cytoplasm, life-time of cells, and the isolation of the organic compounds from the cells. Consequently, bacterial P450s, which are typically soluble, easier to isolate from cells and show higher stability, activity and better expression rates in recombinant hosts, are preferred by researchers. For instance, the Wong group works with bacterial P450_{BM3}.

4.3 Cytochrome P450_{BM3}

P450_{BM3} was the third P450 enzyme isolated from *Bacillus megaterium*, from which it takes the name. It was discovered in the early 1970s by Fulco,^[198–200] who identified it as a medium- to long-chain fatty acid hydroxylase (**Scheme 4.5**), acting selectively at subterminal positions ($\omega-1$, $\omega-2$, $\omega-3$); however, its precise physiological role is still unclear.



Scheme 4.5. Product distribution in the oxidation of lauric acid by P450_{BM3}. **Reagents and conditions:** a) P450_{BM3}, O₂, NADPH, RT, **300** (36%), **301** (30%), and **302** (34%).

Whilst the majority of P450s require an external reductase cofactor to supply electrons to the active site, P450_{BM3} is self-sufficient, only requiring NADPH and molecular dioxygen to function. This is because it is composed of two domains:^[201,202] a heme domain (BMP), which contains the active site and is of similar size to other non-self-sufficient P450; and a reductase domain (BMR), which is fused to the C-terminus of the heme domain and consists of two prosthetic flavin groups (FMN and FAD) in an equimolar ratio. The reductase domain mediates the sequential delivery of the two electrons from NADPH, which are passed first to FAD, then FMN and finally to the heme iron in the heme domain (**Figure 4.5**). Despite P450_{BM3} being the first self-sufficient P450 to be discovered, it is not the only one, for example, the P450_{foxy}^[203,204] isolated from *Fusarium oxysporum* (a fungal pathogen), has a similar fusion-redox protein.

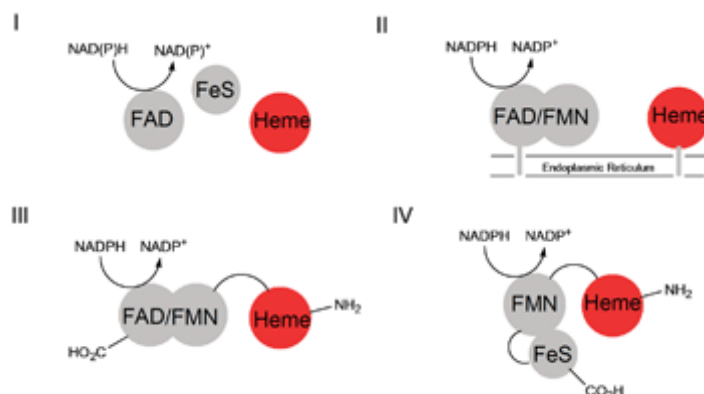


Figure 4.5. Typical topologies of P450s, all containing the common heme domain but different electron transfer components. Representatives of I are P450_{cam}; of II human P450 involved in drug metabolism; of III P450_{BM3} and of IV P450_{RhF}. III and IV are of particular interest because they are self-sufficient.^[205]

Additionally, P450_{BM3}s show a higher level of catalytic activity compared to other fatty acid hydroxylases. This is partly because the heme and reductase domains are connected, hence the activity of the enzyme is not dependent on encountering a redox partner, and the electron transfer becomes a fast and efficient process.

Apart from its self-sufficiency and high level of activity, P450_{BM3} is among the most well studied bacterial P450 due to the ease of handling in the laboratory, its high level of expression in a host organism, but more remarkably, due to its substrate promiscuity and

activity variability. However, whilst the natural substrate range of P450_{BM3} is useful for industrial purposes, such as fragrances and pharmaceuticals, a much wider variety of substrates is desirable for oxidation. In this context, the reported crystal structures of the enzyme in its substrate-free (SF) and substrate-bound (SB) forms (**Figure 4.6.**), along with site-specific mutagenesis experiments, helped with identifying key amino acid residues that play important roles in determining enzyme activity and selectivity.

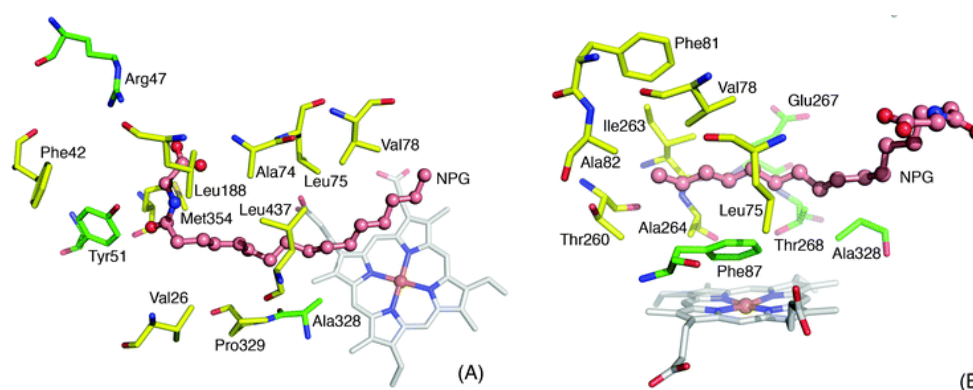


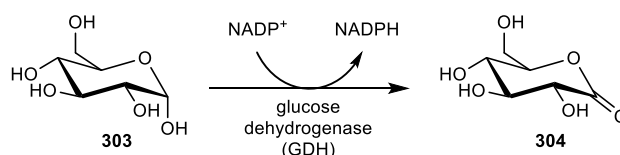
Figure 4.6. Crystal structure of *N*-palmitoylglycine (NPG)-bound P450_{BM3} centred in: (A) the substrate access channel, and (B) the active site.^[197]

As discussed earlier, the axial thiolate is the most important residue for retention of the monooxygenase activity. Additionally, the role of other key amino acid residues situated in the active site and in the hydrophobic channel, through which the substrate must pass to access the active site, has also been identified. For example, arginine-47 (Arg47) and tyrosine-51 (Tyr51) are two polar amino acid residues situated close to the entrance to the substrate channel and they regulate the admission of potential substrates and water.^[206–208] Leucine-437 (Leu437) is also located at the access channel and it has been suggested that it acts as a “safety catch”, keeping the enzyme conformation away from the SB form when no substrate is bound.^[209] Phenylalanine-87 (Phe87) is responsible for the displacement of the axial water ligand upon binding the substrate in the active site, and thus plays a pivotal role in substrate specificity.^[206,210] Alanine-264 (Ala264) also assists in drawing the water ligand away from the axial binding site.^[206] Glutamic acid-267 (Glu267) and threonine-268 (Thr268) are the highly conserved acid-alcohol pair in the P450 family for P450_{BM3}. Whilst

it has also been suggested that Glu267 plays an important role in activation of dioxygen, potentially taking part in the proton transfer,^[207] Thr268 plays a more complex role, being involved in proton delivery, electron transfer and dioxygen activation.^[211]

These findings assisted the development of new P450_{BM3}s *via* protein engineering,^[212] in which rational design, directed evolution, or a combination of both processes, known as rational evolution, have been used to introduce mutations in the DNA sequence of the protein to, ultimately, generate a variety of mutant P450_{BM3}s. These studies allowed the development of new P450_{BM3}s with a broad substrate spectrum, as well as enhanced enzyme activity, selectivity (chemo-, regio-, enantio-), robustness, longevity and stability, and thus improving and broadening the applicability of P450_{BM3}s in C–H functionalisation in organic synthesis.

The only conceivable disadvantage of P450_{BM3} is that it is pyridine nucleotide dependent, and thus requires the expensive cofactor NADPH to operate. However, two solutions have already been reported to reduce the cofactor cost. One option consists of using whole-cells, thus the cofactor production is undertaken by the host organism; however, this has some disadvantages (previously mentioned). The second option, which is more convenient, consists of using enzymatic cofactor regeneration,^[18–20] in this case, the cofactor is recycled *in situ via* a dehydrogenase and at the expense of a co-substrate, which is usually cheaper than NADPH (**Scheme 4.6**).



Scheme 4.6. NADPH regeneration *via* glucose dehydrogenase (GDH), which oxidises D-glucose (303) to D-gluconolactone (304), thus recycling NADPH.

4.4 Research in the Wong group and the library of P450_{BM3} mutants

The Wong group have developed a library of P450_{BM3} mutants, with the aim of broadening the application of P450s in performing oxidations in organic synthesis. The

library contains >200 members spanning a range of oxidation profiles and a broad substrate spectrum, including alkanes, drugs, terpenes and polycyclic aromatic hydrocarbons. Additionally, this library has been used as a resource for identifying key amino acid residues and collecting information about mutation-selectivity trends which, in turn, were later used to guide further protein engineering.

To introduce a mutation in the amino acid sequence of a protein, a mutation in the DNA sequence which encodes this protein (gene) has to be introduced first. The Wong group used site-directed mutagenesis using the polymerase chain reaction (PCR)-method to introduce mutations in the gene of the wild-type P450_{BM3}. Additionally, some mutations were added by error-prone PCR. The mutated P450_{BM3} gene was then inserted into a plasmid (a small piece of DNA) from *Escherichia coli*, the recipient host organism. This plasmid was then inoculated with *E. coli* cells, which then synthesised the P450_{BM3} mutant after inducing its expression with IPTG (Figure 4.7).

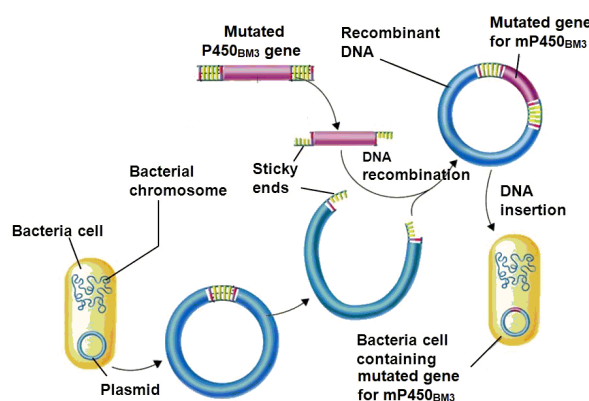
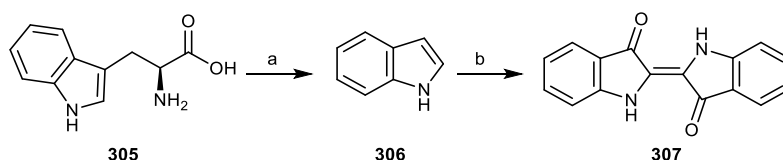


Figure 4.7. General approach to generate a P450_{BM3} mutant. Retrieved from https://moodle.clsd.k12.pa.us/district_videos/Biology/iText/products/0-13-115540-7/ch13/ch13_s3_1.html. Copyright 2004 by Pearson Education, Inc.

The indigo method^[213] was used to identify P450_{BM3} mutants with promising activity during colony growth, without isolation and purification being necessary. With this method, any active P450_{BM3} mutant would turn the complex media blue, due to the formation of the blue-dye indigo (307), by oxidation and dimerisation of indole (306), which, is produced in the growth media by degradation of L-tryptophan (307) (Scheme 4.7). This is an easy, visual method of identifying P450_{BM3} mutants capable of oxidising

substrates which have a large degree of structural difference to the native substrates. Active P450_{BM3} mutants were then isolated, purified using ammonium sulfate precipitation and then subjected to further mutations. The activity and selectivity of the P450_{BM3} mutants were then evaluated using propylbenzene and naphthalene.^[214,215]



Scheme 4.7. Formation of indigo (307) from L-tryptophan (305) metabolism. *Reagents and conditions:* a) Tryptophanase; b) P450_{BM3}, glucose, GDH, NADP⁺, phosphate buffer, RT.

This methodology was used to develop the actual library of P450_{BM3} mutants in which each member typically contains at least one point of mutation, including amino acid insertions and/or deletions. These mutations were introduced based on directed-evolution, thus performing substitutions at specific amino acid residues which are known to be in contact or in close proximity to a bound substrate. Initially, four variants of mutations, simply derived from wild-type P450_{BM3}, were identified to possess increased oxidation activity for a wide range of organic compounds, including A330P (single mutation variant), KT2 (5 mutations, A191T/N239H/I259V/A267T/L353I), KSK19 (4 mutations, F87A/H171L/Q307H/N319Y), and KT5 (4 mutations, F87A/A330P/E377A/D425N). Next, some of these mutants were also combined with variants already established to increase activity (RLYF, R47L/Y51F or F87A) to finally afford the actual library of P450_{BM3} mutants. A list with all the members of the library, along with the mutations, can be found in the **Experimental section**.

4.5 Enzymatic oxidation of the synthesised taxanes with P450_{BM3} mutants

4.5.1 General procedure for the enzymatic oxidations

The study of the enzymatic oxidation of the synthesised taxanes **16**, **179a**, **179b** and **220** was performed in collaboration with Prof. Luet Wong's group, which gave access to

the library of P450_{BM3} mutants. Firstly, a general procedure for the enzymatic oxidations was designed, which consisted of:

1. *Screening*: the substrate was screened against 24 or 48 P450_{BM3} mutants on small scale (0.5 μmol).
2. *Analysis of the results*: each enzymatic reaction was analysed by GC/FID to identify the active enzymes and give a rough idea of the number of oxidised products generated.
3. *Enzyme selection*: promising enzymatic reactions were selected for scale-up. For this, different criteria were taken into consideration, such as the conversion of the reaction, the number of products generated and their selectivities.
4. *Reproducibility of the results*: before scaling up, the selected enzymatic reactions were repeated three times to confirm the reproducibility of the results obtained during the screening.
5. *Enzyme preparation*: the required enzymes were prepared by growing an *E. coli* cell culture, already containing a mutated P450_{BM3} gene. Its expression was induced by addition of IPTG; then the enzyme was isolated by cell lysis, extracted and purified, using ammonium sulfate precipitation.
6. *Scale-up*: the selected enzymatic reactions were scaled up from 0.5 μmol to 50 μmol . The reaction was followed by GC/FID and worked up when appropriate.
7. *Isolation and structure elucidation of the products*: The crude reaction mixture was purified by flash column chromatography and the molecular structure of the isolated products was elucidated using NMR and MS experiments.
8. *Enzyme optimisation*: in principle, the active enzymes would be subjected to further mutations to improve their enzymatic activity and selectivity with the studied substrate.

4.5.2 Enzymatic oxidation of diketone **179a**

This protocol was first applied for the oxidation of diketone **179a**. The Wong group have studied the oxidation of poorly functionalised steroids using P450_{BM3} mutants and found that the inclusion of methyl- β -cyclodextrin improved the conversion in the enzymatic oxidations, suggesting that this additive may help to solubilise non-polar steroids in the aqueous medium required by the enzymes. Taking into consideration the structural similarities between the steroids and diketone **179a**, both polycyclic hydrocarbons being fairly functionalised, it was deemed that methyl- β -cyclodextrin may also be necessary for the enzymatic oxidation of **179a** and, thus, it was used in the initial screening.

Table 4.1. Active enzymes in the screening of diketone **179a** with 24 P450_{BM3} mutants using methyl- β -cyclodextrin ([c] = 10 mM).

Entry	Enzyme	Retention time (min) ^a		
		4.45	5.02	5.06
1	KSK19/AM	67%	7%	26%
2	KSK19/AI/AI	92%	2%	6%
3	KSK19/I263A	93%	–	7%
4	KSK19/AI/V78I	95%	3%	2%

^a GC R_t: diketone **179a** (4.45 min), oxidised products of **179a** (5.02 and 5.06 min).

Diketone **179a** was first screened against 24 P450_{BM3} mutants using methyl- β -cyclodextrin as an additive (10 mM). Four P450_{BM3} mutants were found to be active, with only one having a conversion of >30% and GC analysis of these reactions indicated the generation of two oxidised products, along with unreacted **179a** (Table 4.1).

Given these unsatisfactory results, it seemed logical to question the necessity of methyl- β -cyclodextrin in these enzymatic reactions. The KSK19/AI/AI mutant had proven to be active in the initial screening so a repeat oxidation of diketone **179a** was performed at different concentrations of methyl- β -cyclodextrin (Table 4.2). The highest conversion (87%) was achieved when no methyl- β -cyclodextrin was used, falling to 23% when it was added at the lowest concentration (5.0 mM). Additionally, in the absence of methyl- β -cyclodextrin, a new oxidised product was observed by GC. From these results it

was clear that the presence of methyl- β -cyclodextrin was detrimental to the enzymatic oxidation of diketone **179a**, suggesting that it should not be used for the oxidation of taxanes.

Table 4.2. Oxidation of diketone **179a** with the P450_{BM3} mutant KSK19/AI/AI at different concentration of methyl- β -cyclodextrin.

Entry	[Cyclodextrin] (mM)	Retention time (min) ^a			
		4.45	5.02	5.06	5.18
1	0	13%	19%	63%	5%
2	5.0	78%	4%	17%	1%
3	10.0	79%	5%	15%	1%
4	20.0	91%	2%	7%	–
5	35.0	94%	2%	4%	–
6	50.0	97%	1%	2%	–

^a GC R_t: diketone **179a** (4.45 min), oxidised products of **179a** (5.02, 5.06 and 5.18 min).

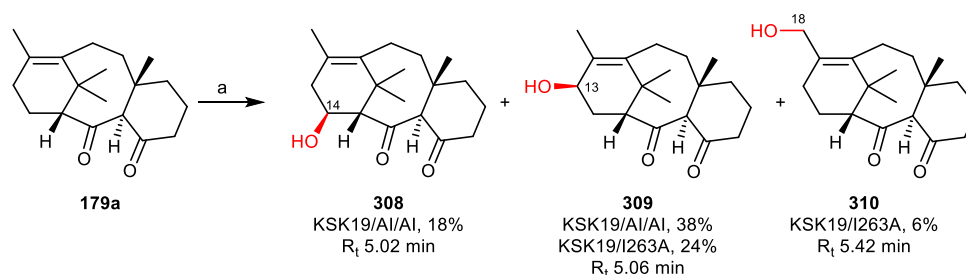
Diketone **179a** was then screened against 48 P450_{BM3} mutants without methyl- β -cyclodextrin and, despite 36 enzymes being active, only nine achieved a conversion of >50%. GC analysis indicated the generation of five oxidised products (**Table 4.3**, see **Appendix C** for full details of the screening). From these results, three enzymes were selected for scale-up: KSK19/AI/AI because it generated four of the five oxidised products, and KSK19/I263A and RP/HL because the 5.42 min and the 4.88 min products were afforded with the highest yields respectively. Before scale-up, the reproducibility of these reactions was verified.

Table 4.3. Active enzymes in the screening of diketone **179a** with 48 P450_{BM3} mutants without methyl- β -cyclodextrin.

Entry	Enzyme	Retention time (min) ^a					
		4.45	4.88	5.02	5.06	5.18	5.42
1	KSK19/AM	48%	–	6%	46%	–	–
2	KSK19/AI/AI	20%	–	22%	51%	5%	2%
3	KSK19/I263A	33%	–	4%	50%	–	13%
4	KSK19/AI/V78I	17%	–	44%	37%	–	2%
5	RLYF/KSK19	15%	–	44%	39%	–	2%
6	RP/AM/IA	18%	–	38%	43%	–	1%
7	RLYF	45%	–	–	52%	–	3%
8	RP/HL	45%	16%	6%	32%	–	1%
9	RT2/SW/AW	44%	–	2%	53%	–	1%

^a GC R_t: diketone **179a** (4.45 min), and oxidised products of **179a** (4.88, 5.02, 5.06, 5.18 and 5.42 min).

The required enzymes were prepared following a general procedure, using the Wong group's cell bank which contains *E. coli* cell cultures with the P450_{BM3} gene mutated. The three chosen reactions were scaled up, the oxidised products isolated, and their molecular structures elucidated by NMR and MS studies (**Scheme 4.8**). KSK19/AI/AI yielded mono-oxidised products at the C14 and C13 positions (**308** and **309**, respectively), along with unreacted diketone **179a** (29%). When KSK19/I263A was used, taxanes **309** and **310** were obtained, **310** being mono-oxidised at the C18 position. In contrast, the scale-up of RP/HL only returned unreacted diketone **179** and hence, the 4.882 min product was not identified.



Scheme 4.8. Identified products from the oxidation of diketone **179a** with P450_{BM3} mutants KSK19/AI/AI and KSK19/I263A. **Reagents and conditions:** a) P450_{BM3} mutant, glucose, GDH, NADP⁺, phosphate buffer, RT. See **Appendix D** for details of elucidation of the oxidised product structures and relative stereochemistry.

The generation of taxanes **308**, **309** and **310** *via* the enzymatic oxidation of diketone **179a** demonstrated the value of the procedure, and thus the study continued with the oxidation of taxanes **19**, **179b** and **220**.

4.5.2 Enzymatic oxidation of diketone **179b**

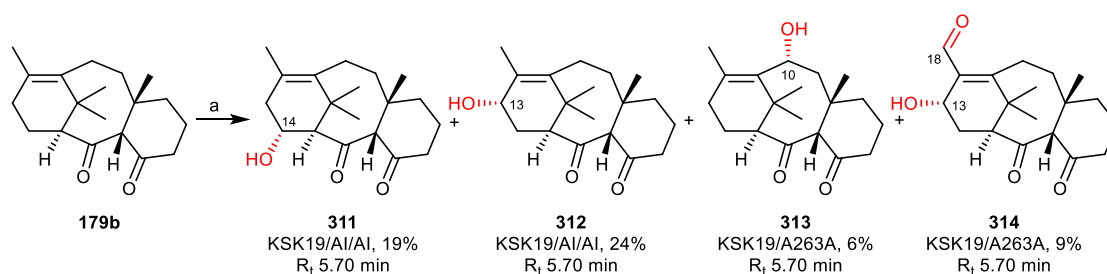
Diketone **179b** was screened against 24 P450_{BM3} mutants; eleven displayed activity but only five achieved a conversion of >50% (**Table 4.4**, see **Appendix C** for full details of the screening). GC analysis showed only one new peak at 5.70 min, indicating that only one oxidised product had been generated. However, this peak was broad and had different shapes between individual enzymatic reactions, suggesting the formation of different oxidised products with similar retention times and, consequently, overlapping peaks.

Table 4.4. Active enzymes in the screening of diketone **179b** with 24 P450_{BM3} mutants without methyl- β -cyclodextrin.

Entry	Enzyme	Retention time (min) ^a		
		4.81	4.92	5.70
1	RLYF/KSK19/AI	3%	13%	84%
2	KSK19/AM	–	–	100%
3	KSK19/AI/Y51L	4%	40%	56%
4	KSK19/AI/AI	–	–	100%
5	KSK19/I263A	–	–	100%

^a GC R_t: impurity present in the starting material (4.81 min), diketone **179b** (4.92 min), oxidised products of **179b** (5.702 min).

Given their high conversions during screening, the KSK19/AI/AI and KSK19/I263A mutants were selected for scale-up. After checking their reproducibility, these enzymatic reactions were effected on larger scale. Contrary to the GC results, which showed only one new peak, four different oxidised products were isolated (**311**, **312**, **313** and **314**), confirming the hypothesis of peak overlap of the products. Whilst only mono-oxidised products at the C14 and C13 positions were obtained with the KSK19/AI/AI mutant (taxanes **311** and **312** respectively), with KSK19/I263A oxidation occurred at the C10, C13 and C18 positions, affording mono- and di-oxidised products **313** and **314** (Scheme 4.9).



Scheme 4.9. Identified products from the oxidation of diketone **179b** with P450_{BM3} mutants KSK19/AI/AI and KSK19/I263A. **Reagents and conditions:** a) P450_{BM3} mutant, glucose, GDH, NADP⁺, phosphate buffer, RT. See Appendix D for details of elucidation of the oxidised product structures and relative stereochemistry.

4.5.3 Enzymatic oxidation of taxadienone **16**

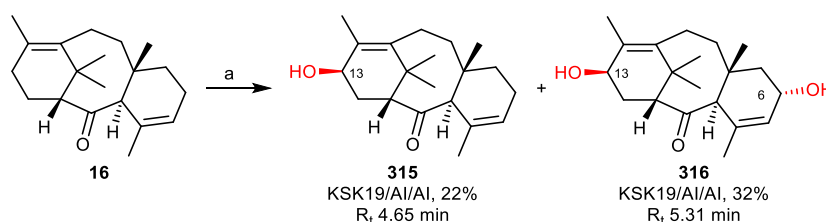
Taxadienone **16** was screened against 24 P450_{BM3} mutants, with sixteen enzymes active and only four with a conversion of >50%. Five oxidised products were observed by GC analysis (Table 4.5, see Appendix C for full details of the screening).

Table 4.5. Active enzymes in the screening of taxadienone **16** with 24 P450_{BM3} mutants without methyl- β -cyclodextrin.

Entry	Enzyme	Retention time (min) ^a						
		3.86	4.07	4.45	4.65	4.70	5.31	5.37
1	RLYF/KSK19/AI	4%	8%	10%	21%	2%	37%	19%
2	KSK19/AI/Y51L	7%	41%	9%	22%	1%	18%	2%
3	KSK19/AI/AI	5%	17%	4%	15%	–	44%	15%
4	KSK19/I263A	6%	49%	–	22%	–	17%	6%

^a GC R_i: impurity present in the starting material (3.86 min), taxadienone **16** (4.07 min), oxidised products of **16** (4.45, 4.65, 4.70, 5.31 and 5.37 min).

The reaction with KSK19/AI/AI mutant was again selected for scale-up because it showed high conversion and reproducibility. For this, oxidation occurred at the C6 and C13 positions, generating mono- and di-oxidised products **315** and **316**, whilst also returning unreacted taxadienone **16** (30%) (**Scheme 4.10**). Despite the observation of five oxidised products by GC during screening, only two were identified (**315** and **316**).



Scheme 4.10. Identified products from the oxidation of taxadienone **16** with P450_{BM3} mutant KSK19/AI/AI. **Reagents and conditions:** a) P450_{BM3} mutant, glucose, GDH, NADP⁺, phosphate buffer, RT. **Appendix D** for details of the oxidised products.

4.5.4 Enzymatic oxidation of di-*epi*-taxadienone **220**

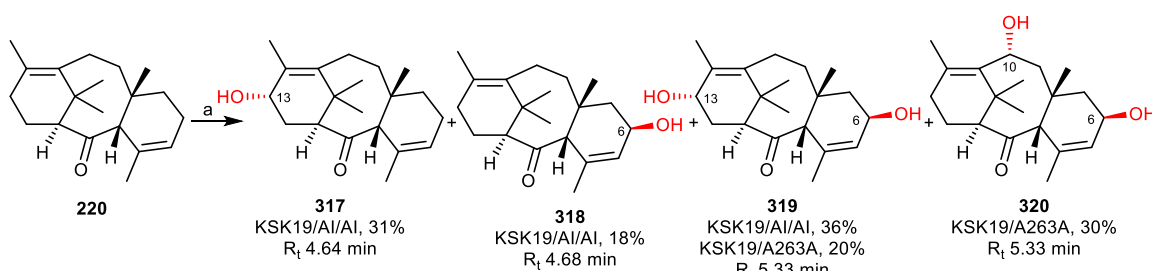
When di-*epi*-taxadienone **220** was screened against 24 P450_{BM3} mutants, seventeen enzymes were active but only four achieved a conversion of >50%. GC analysis of the enzymatic reactions indicated the generation of three oxidised products (**Table 4.6**, see **Appendix C** for full details of the screening).

Table 4.6. Active enzymes in the screening of di-*epi*-taxadienone **220** with 24 P450_{BM3} mutants without methyl- β -cyclodextrin.

Entry	Enzyme	Retention time (min) ^a				
		3.90	4.06	4.64	4.68	5.33
1	RLYF/KSK19/AI	3%	11%	19%	44%	23%
2	KSK19/AI/AI	8%	40%	13%	33%	6%
3	KSK19/I263A	3%	27%	13%	25%	32%

^a GC R_i: impurity present in the starting material (3.90 min), di-*epi*-taxadienone **220** (4.06 min), oxidised products of **220** (4.64, 4.68 and 5.33 min).

After checking their reproducibility, the reactions with KSK19/AI/AI and KSK19/I263A mutants were scaled up. Oxidation at the C6 and C13 positions was observed with KSK19/AI/AI, affording mono-oxidised products **317** and **318**, and di-oxidised product **319**. With KSK19/I263A, oxidation occurred at the C6, C10 and C13 positions and only the di-oxidised products **319** and **320** were isolated. These have very similar retention times and appeared as one peak in GC analysis (Scheme 4.11).



Scheme 4.11. Identified products from the oxidation of di-*epi*-taxadienone **220** using P450_{BM3} mutant KSK19/AI/AI and KSK19/I263A. **Reagents and conditions:** a) P450_{BM3} mutant, glucose, GDH, NADP⁺, phosphate buffer, RT. See Appendix D for details of elucidation of the oxidised product structures and relative stereochemistry.

4.5.5 Lessons learned from the enzymatic oxidation of taxanes **16**, **179a**, **179b** and **220**

The study of the enzymatic oxidation of taxanes **16**, **179a**, **179b** and **220** using P450_{BM3} mutants afforded thirteen oxidised products with different oxidation patterns. From these results, a few general trends were identified.

Firstly, allylic positions were more prone to oxidation, as expected at the outset of the project, with oxidation observed at the C10, C13 and C18 positions, and additionally at C6 for taxadienone **16** and di-*epi*-taxadienone **220**. However, oxidation of unactivated positions was also detected, for instance, oxidation at the C14 position of diketones **179a** and **179b**.

The presence of an additional unsaturation led to an increased propensity towards oxidation. For this reason, di-oxidised products were afforded with taxadienone **16** and di-*epi*-taxadienone **220**, whilst only mono-oxidised products resulted from reaction with diketones **179a** and **179b**.

For all four substrates, the KSK19/AI/AI and KSK19/I263A mutants were among the most active enzymes, always having a conversion of >50%. Additionally, RLYF/KSK19/AI, KSK19/AM, KSK19/AI/Y51L and KSK19/AI/V78I also showed high activity for at least one of the substrates. As indicated by the similarity in their names, all these mutants have the KSK19 modification, which consists of four mutations. A deeper study may enable a correlation to be established between mutation and oxidised position, potentially giving great insight into the conformation of these taxanes in the active site, thus guiding the development of new P450_{BM3} mutants with higher activity *via* rational design.

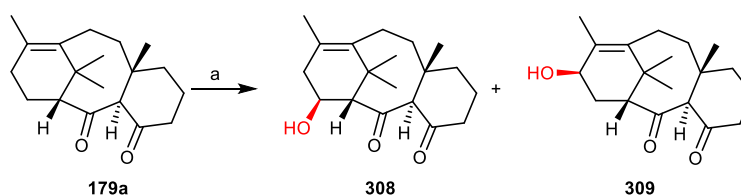
4.5.6 One step further in the enzymatic oxidation of taxanes

One of the aims of the project was to synthesise highly oxidised taxanes *via* a series of sequential enzymatic oxidations. Therefore, after studying the oxidation of taxanes **16**, **179a**, **179b** and **220**, the project was taken forward and the oxidation of one of the products obtained was attempted. Taxane **319** was selected as the most suitable substrate for this study because it is already oxidised at the most reactive positions (C6 and C13), and thus any further oxidation would be unexpected.

However, when taxane **319** was screened against 24 P450_{BM3} mutants, none were active. It should not be inferred from this that the oxidation of taxane **319** using P450_{BM3} mutants is not viable. These unsuccessful results only mean that the screened enzymes are not active in taxane **319**, and other P450_{BM3} mutants with completely different mutations should be screened. Compared to the taxanes initially studied (**16**, **179a**, **179b** and **220**), taxane **319** has two additional hydroxyl groups, and thus is expected to interact differently with the active site of the enzyme. For this reason, it is not surprising that the enzymes which were active in the first screening were not in the second. Consequently, these results encourage the development of new P450_{BM3} mutants.

4.5.7 Study to determine the substrate enantioselectivity of P450_{BM3} mutants

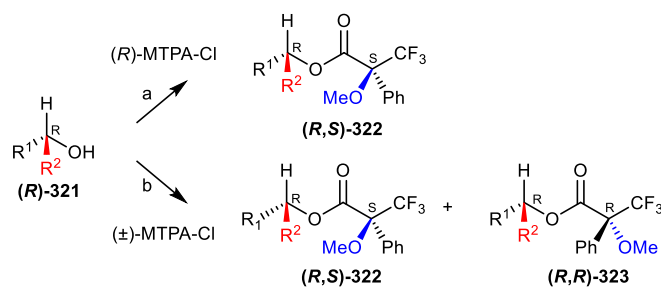
Due to the high enantioselectivity demonstrated by enzymes and the use of racemic mixtures of taxanes **16**, **179a**, **179b** and **220** for the enzymatic oxidations studied, it was of interest to probe the substrate enantioselectivity of the P450_{BM3} mutants, and determine if the enzymes reacted preferentially with a single enantiomer. To accomplish this, the oxidation of diketone **179a** using the KSK19/AI/AI mutant, which generated taxanes **308** and **309**, was examined (Scheme 4.12). Two methods were employed for analysis: a study of the recovered starting material, and a study of the generated products.



Scheme 4.12. Oxidation of diketone **179a** with P450_{BM3} mutant KSK19/AI/AI. **Reagents and conditions:** a) P450_{BM3} mutant, glucose, GDH, NADP⁺, phosphate buffer, RT, **308** (18%), **309** (38%) and **179a** (29%).

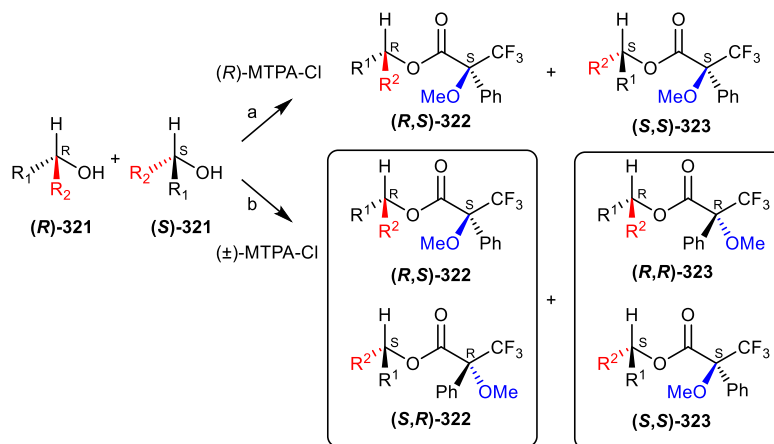
Firstly, analysis to determine the ee of recovered diketone **179a** was attempted. Unfortunately, separation of the enantiomers of a racemic mixture of **179a** *via* chiral GC was not achieved, despite a thorough screening of different chiral columns and conditions. The enantiopurity of taxanes **308** and **309** was then examined using the Mosher ester method. For this, each product was reacted separately with (*R*)-MTPA-Cl and (±)-MTPA-Cl, and ¹H and ¹⁹F NMR spectra were recorded for each reaction.

Prior to analysing the results obtained in the derivatisation of **308** and **309**, it was necessary to first determine the expected results in the two situations: when the product of the enzymatic oxidation is enantiopure or is racemic. If the product is enantiopure (alcohol (*R*)-**321**), the ¹H and ¹⁹F NMR spectra of the two reaction mixtures will differ. When using (±)-MTPA-Cl, two products will be observed in the NMR spectra, owing to the two diastereomers generated [(*R,S*)-**322** and (*R,R*)-**323**], whilst in the NMR spectra when using (*R*)-MTPA-Cl, only one product will be observed, since only one diastereomer [(*R,S*)-**322**] is generated (Scheme 4.13).



Scheme 4.13. Results expected in the chiral derivatisation of enantiopure alcohol **(R)-321**. *Reagents and conditions:* a) (R) -MTPA-Cl, pyr, C_6D_6 , RT; b) (\pm) -MTPA-Cl, pyr, C_6D_6 , RT.

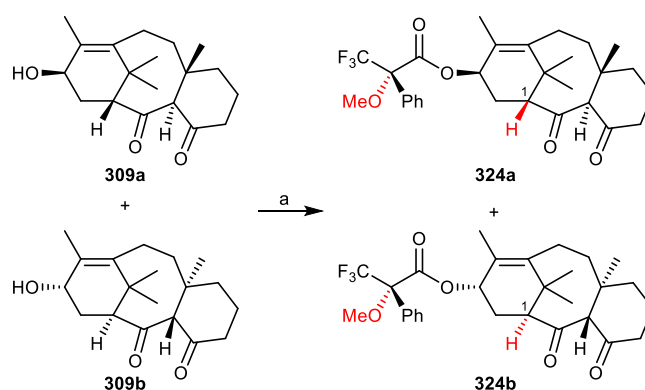
In contrast, if the initial product is racemic (alcohol **(±)-321**), the NMR spectra of the two reaction mixtures will, in principle, be identical, considering that enantiomers have identical spectroscopic properties. When (R) -MTPA-Cl is used, two diastereomers are generated [**(R,S)-322** and **(S,S)-323**], and thus two products will be observed by NMR. When using (\pm) -MTPA-Cl, four diastereomers will be generated [**(R,S)-322**, **(S,R)-322**, **(R,R)-323** and **(S,S)-323**]; however, they are two pairs of enantiomers [**(R,S)-322**–**(S,R)-322**, and **(R,R)-323**–**(S,S)-323**], and hence only two products will be observed in the NMR spectra (**Scheme 4.14**).



Scheme 4.14. Results expected in the chiral derivatisation of racemic alcohol **321**. *Reagents and conditions:* a) (R) -MTPA-Cl, pyr, C_6D_6 , RT; b) (\pm) -MTPA-Cl, pyr, C_6D_6 , RT.

When taxane **309** was analysed using the Mosher ester method (oxidation reaction with a conversion of 87%), it was found that **309** was enantioenriched, with an ee of 57%, suggesting that the enzyme has a slight preference for a single enantiomer when oxidising the C13 position (**Scheme 4.15** and **Figure 4.8**). In contrast, when taxane **308** was analysed (oxidation reaction with a conversion of 100%), it was found to be enantiopure

(Scheme 4.16 and Figure 4.9), indicating that the enzyme is substrate enantioselective when oxidising the C14 position, reacting exclusively with one enantiomer.



Scheme 4.15. Chiral derivatisation of taxane **309**. *Reagents and conditions:* a) (R)-MTPA-Cl, pyr, C₆D₆, RT.

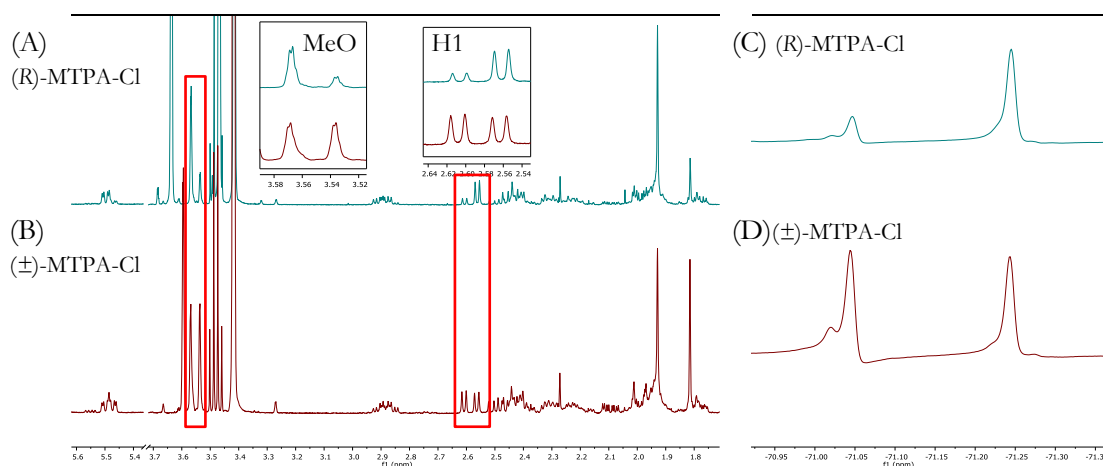
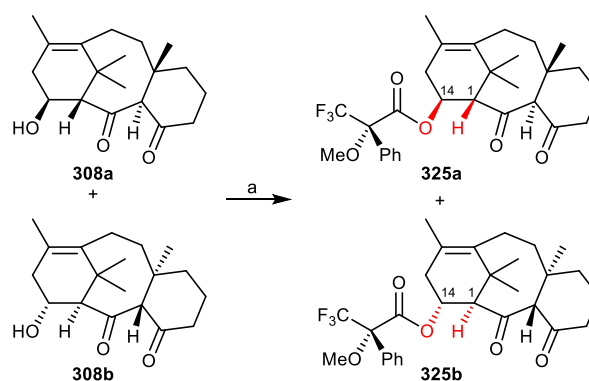


Figure 4.8. NMR spectra of the chiral derivatisation of taxane **309**: A) ¹H NMR when using (R)-MTPA-Cl; B) ¹H NMR when using (±)-MTPA-Cl; C) ¹⁹F NMR when using (R)-MTPA-Cl; D) ¹⁹F NMR when using (±)-MTPA-Cl.



Scheme 4.16. Chiral derivatisation of taxane **308**. *Reagents and conditions:* a) (R)-MTPA-Cl, pyr, C₆D₆, RT.

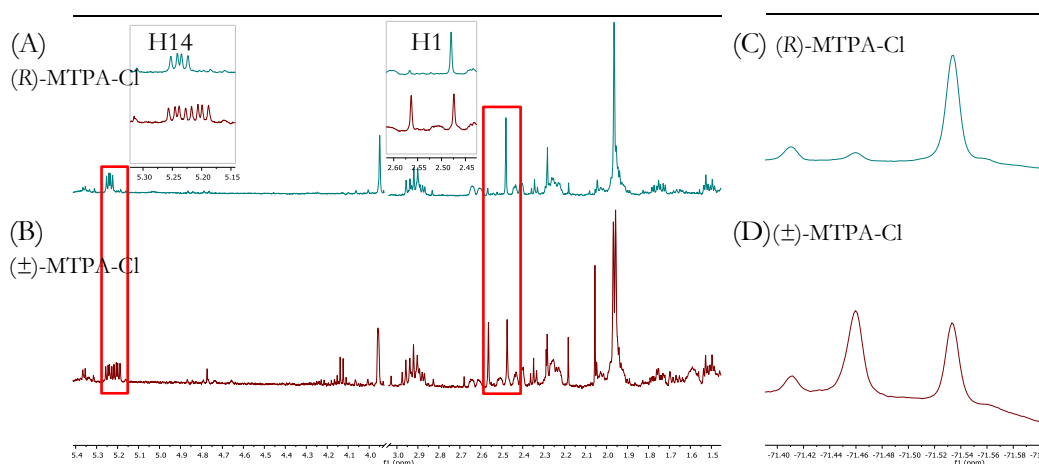


Figure 4.9. NMR spectra of the chiral derivatisation of taxane **308**: A) ^1H NMR when using (R)-MTPA-Cl; B) ^1H NMR when using (\pm)-MTPA-Cl; C) ^{19}F NMR when using (R)-MTPA-Cl; D) ^{19}F NMR when using (\pm)-MTPA-Cl.

Although the results for taxane **308** and **309** are not directly comparable because the samples used were from different reactions, it seemed reasonable to believe that the enzyme shows different substrate enantioselectivity for each oxidation position. Whilst it is highly substrate enantioselective for oxidation at the C14 position, since **308** is generated as a single enantiomer, it shows lower enantioselectivity for oxidation at the C13 position, generating **309** in an ee of 57%. However, further experiments, with different enzymes and different substrates, should be carried out to garner more information about the substrate enantioselectivity of the enzymes before generalising.

Additionally, a preliminary study to determine the absolute configuration of the only enantiomer obtained for **308** and the major enantiomer obtained for **309** was performed. With the Mosher ester data previously acquired, the method for deducing the configuration of stereogenic, secondary alcohol centers reported by Hoye^[216] was applied to determine the configuration of the alcohol center at C14 and C13, respectively. Subsequently, the absolute configuration of the molecule was established using the already determined relative stereochemistry *via* correlation between ^1H signals in the NOESY spectrum. In both case, the only and the major enantiomer of the obtained oxidised products, **308b** and **309b** respectively, had the opposite configuration to the natural taxanes (see **Appendix E** for full details of the study).

4.5.8 Biological assays of the oxidised taxanes obtained

A key aim of the project was to determine whether any of the produced taxol analogues retained any cytotoxic activity. Therefore, six of the thirteen oxidised products were subjected to biological assays (**Figure 4.10**). Their viability was tested against two cell lines: MCF7 (breast cancer) and H460 (lung cancer); however, all compounds showed IC_{50} values greater than 50 μ M, and thus were not active enough to take forward for further biological assays.

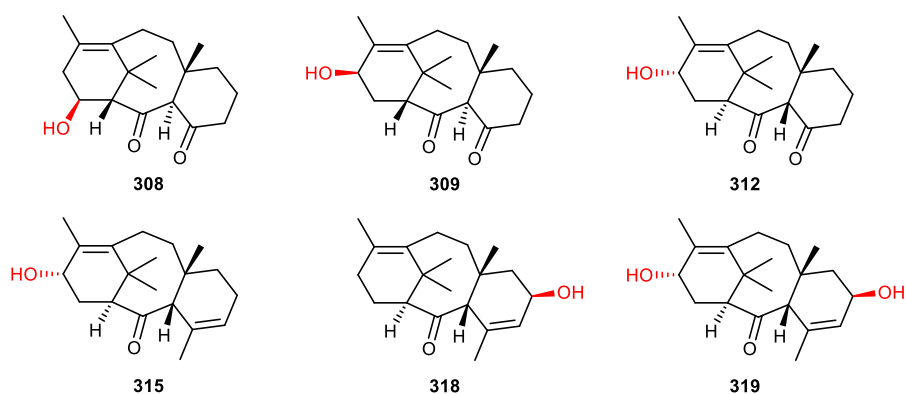


Figure 4.10. Oxidised taxanes tested against MCF7 and H460 cell lines $IC_{50} > 50 \mu$ M in all cases.

5 CONCLUSIONS AND FUTURE WORK

This project targeted the synthesis of taxol analogues. To accomplish this, enzymatic oxidation was applied *via* late-stage hydroxylation of low-oxidation state taxanes with P450 enzymes. Two well differentiated aims were identified at the outset of the project: (1) the synthesis of taxa-4(5),11(12)-dien-2-one using classical synthetic methods, and (2) the enzymatic oxidation of taxa-4(5),11(12)-dien-1-one and related molecules with P450_{BM3} mutants.

As the primary interest of the project was the application of P450_{BM3} mutants to the synthesis of taxol-type compounds, Baran's route was initially followed to generate taxa-4(5),11(12)-dien-2-one quickly. Despite ultimately being prepared by following this route, extensive modification and optimisation was required in almost every step of the synthesis. Consequently, other approaches to some of the synthetic intermediates, and also to taxa-4(5),11(12)-dien-2-one itself, were examined.

The first alternative route to taxa-4(5),11(12)-dien-2-one that was examined was based on Baran's, and even though advanced intermediates were successfully synthesised, when the last step was performed (an intramolecular Mukaiyama aldol reaction to close the taxane B ring) unexpected cyclised products were obtained, none of them being taxa-4(5),11(12)-dien-2-one.

A new, short and practical route to the taxane core was then developed, which provided 1,3-di-*epi*-taxa-4(5),11(12)-dien-2-one. The IMDA reaction used to generate the taxane AB ring was studied in order to understand the stereochemical outcome, and why taxa-4(5),11(12)-dien-2-one was not afforded. Additionally, several approaches were examined to convert this racemic synthesis into an enantioselective route, with the desymmetrisation of *cis*-2,6-dimethylcyclohexanone and subsequent aldol reaction proving

to be the most promising strategy. Future work should focus on improving the ee of this reaction and on carrying forward the aldol product into the synthesis of the taxane core.

Regarding the enzymatic oxidation using P450_{BM3} mutants, four taxanes were studied, and thirteen oxidised products were identified, with the allylic positions being, as expected, the most prone to oxidation, but oxidation at non-activated positions also being observed. The most active enzymes were the same for all four compounds and these share some common mutations. Therefore, a future study to determine any correlation between oxidised position and enzyme mutation would be useful for the development of new P450_{BM3} mutants with improved activity and selectivity. Moreover, the unsuccessful enzymatic oxidation of one of the “first generation” oxidised taxanes suggests that other P450_{BM3} mutants bearing completely different mutations should be used in future oxidations.

Additionally, a study to identify the substrate enantioselectivity of the P450_{BM3} mutants was performed. So far, the results suggest that the enzymes have different substrate enantioselectivity for each oxidation position. However, before general conclusions can be reached, experiments with different enzymes and substrates should be performed in order to garner more information.

Finally, six of the thirteen oxidised taxanes were evaluated in biological assays. All of them proved to be insufficiently active to be taken forward for mechanism-specific biological assays, and it is too early to draw conclusions about correlations between oxidised position and bioactivity.

EXPERIMENTAL

6.1 General experimental

The compounds are presented in the order in which they appear in the main text and described with a diagram of the compound and an atom numbering. These atom numbers are used to illustrate the NMR peak assignment and do not necessarily correspond to the IUPAC nomenclature.

Solvents and reagents: All reactions were conducted in oven-dried reaction vessels under an inert atmosphere of argon with anhydrous solvents. “Petrol” refers to the fraction of petroleum ether boiling in the range 30–40 °C. Dry diethyl ether (ether), dichloromethane (DCM), tetrahydrofuran (THF), acetonitrile (ACN), ethyl acetate (EA) and benzene were obtained from solvent dispenser units having been passed through an activated alumina column under argon. THF was additionally distilled over sodium and benzophenone before use. Pyridine, triethylamine, benzaldehyde and dimethyl sulfide were distilled prior to use and stored over 4Å MS, KOH, or powdered CaH₂ as appropriate.^[217] Carbon tetrabromide and *N*-bromosuccinimide were purified using standard procedures.^[217]

Chromatography: Thin layer chromatography (TLC) was carried out using Merck aluminium-backed DC60 F₂₅₄ plates (particle size 0.2 mm). Spots were visualised by ultraviolet light ($\lambda_{\text{max}} = 254 \text{ nm}$) and then stained and heated with anisaldehyde, potassium permanganate, or ammonium molybdate solutions as appropriate. Retention factors (R_f) are reported along with the solvent system used in parenthesis. Flash column chromatography was performed using Merck 60 silica gel (particle size 40–63 μm) and the solvent system used is reported in parentheses.

Melting points: Melting points were determined using a Griffin MFB-700-010U melting point apparatus and are uncorrected.

NMR spectroscopy: Proton (^1H) and carbon (^{13}C) NMR spectra were recorded on a Bruker AVX500 (500/126 MHz), Bruker AVX400 (400/101 MHz), or Bruker DPX200 (200 MHz) spectrometers in deuterated solvents. Proton and carbon chemical shifts (δ_{H} , δ_{C}) are quoted in ppm and referenced to the residual protonated solvent peak as the internal reference (CDCl_3 , δ 7.26 and 77.16 ppm respectively; C_6D_6 , δ 7.15 and 128.06 ppm respectively). Peak assignments were made on the basis of chemical shifts, integrations and coupling constants, using COSY, HSQC, HMBC, NOE and NOESY data, and by comparison with spectra of related compounds. Multiplicities are described as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quin), sextet (sxt), septet (sept), multiplet (m) and broad (br). Coupling constants (J) were rounded to the nearest 0.5 Hz. When the relative stereochemistry of the molecule is specified, selected NOESY data is shown: correlation between ^1H signals is indicated by a black arrow and the lack of correlation between ^1H signals is shown by a dashed arrow.

Infrared spectroscopy: Infrared spectra were recorded using a Bruker Tensor 27 FT-IR spectrometer as a thin film on a diamond ATR module. All assignable absorption maxima are reported in wavenumbers (cm^{-1}) and are described as strong (s), medium (m), weak (w), and broad (br) relative to the most intense peak.

Mass spectrometry: Low resolution mass spectra were recorded on a Micromass LCT Premier spectrometer (ESI). High resolution mass spectra (HRMS) were recorded by the Chemistry Research Laboratory staff on a Bruker Daltonics MicroTOF spectrometer (ESI/FI). Mass to charge ratios (m/z) are reported in Daltons.

6.2 General procedures

6.2.1 General procedures for enzymatic oxidations

- **Growth media and buffer:**

Deionised water ($\rho \sim 15 \text{ M}\Omega \cdot \text{cm}^{-1}$) from a Millipore ELIX 5-UV system was used to prepare all the growth media and buffers. Media were autoclaved at 121 °C for 30 min. All components added for bacterial culture growths were first sterilised via a syringe filter (0.22 μm).

Table 6.1. Media components.

Media	Components (per litre of water)
Trace elements	CaCl ₂ ·H ₂ O (740 mg), CoCl ₂ ·6H ₂ O (250 mg), CuSO ₄ ·5H ₂ O (100 mg), FeCl ₃ ·6H ₂ O (16.7 g), MnSO ₄ ·4H ₂ O (132 mg), Na ₂ EDTA (20.1 g), ZnSO ₄ ·7H ₂ O (180 mg)
FB _{glycerol}	K ₂ HPO ₄ (13.8 g), NaH ₂ PO ₄ (3.5 g), (NH ₄) ₂ SO ₄ (2.5 g), Na ₂ SO ₄ (500 mg), Na ₃ citrate·2H ₂ O (2.0 g), NH ₄ Cl (1.2 g) and glycerol (6.0 g); autoclave and then add an aq. solution of MgSO ₄ (1.0 M, 2.0 mL), trace elements solution (3.0 mL), thiamine (10% w/v in aq. HCl, 1.0 mL) and kanamycin (30 mg/mL, 1.0 mL)
Phosphate buffer	KH ₂ PO ₄ (5.38 g), K ₂ HPO ₄ (27.8 g)

- **Enzyme production and purification:**

A 2 L conical flask containing FB_{glycerol} (600 mL, components shown in **Table 6.1**) was autoclaved for 2 h. After cooling to RT, an aq. solution of MgSO₄ (1.0 M, 1.2 mL), a trace elements solution (1.8 mL, components shown in **Table 6.1**), thiamine (10% w/v in aq. HCl, 0.6 mL) and kanamycin (30 mg/mL, 0.6 mL) were added sequentially to the flask. The solution was then inoculated with a starter culture containing *E. coli* Bl21.DE3 (500 μL) that had been transformed with the vector. The flask was shaken at 180 rpm and 37 °C for 24 h. The growth media was then cooled to RT for 1 h. Protein expression was induced by the sequential addition of tryptone powder (1.0 g), aq. solution of IPTG (0.4 M, 60 μL), aq. solution of FeCl₂ (10 mM, 600 μL) and aq. solution δ -ALA (0.1 M, 600 μL). The culture was then shaken at 120 rpm and 20 °C for 48 h.

The cells were harvested by centrifugation of the growth media at 4,250 rpm for 10 min at 4 °C. The supernatant was discarded and the pellet was re-suspended in phosphate buffer

Experimental

(20 mL per 600 mL culture). The suspension was transferred to a beaker and the cells were lysed by sonication on ice. The sonication procedure consisted of 10 cycles of 15 sec pulses followed by a 30 sec cooling period. The lysed suspension was centrifuged for 15 min at 9,500 rpm and 4 °C to remove the cell debris. The supernatant containing the enzyme was purified using $(\text{NH}_4)_2\text{SO}_4$ fractionation. Sufficient $(\text{NH}_4)_2\text{SO}_4$ to achieve 40% saturation was added (2.33 g per 10 mL solution), the mixture was stirred at 4 °C and once the salt was fully dissolved, the mixture was centrifuged at 9,500 rpm and 4 °C for 30 min. More $(\text{NH}_4)_2\text{SO}_4$ was then added to achieve 60% saturation (1.24 g per 10 mL solution) and when all the salt had dissolved, the mixture was centrifuged at 9,500 rpm and 4 °C for 20 min. The supernatant was discarded and the pellet was re-suspended in phosphate buffer (5 mL).

- **Enzyme quantification:**

A CO-difference assay was used to determine the concentration of mP450 enzyme harvested. The mP450 enzyme stock was diluted by adding 100 μL to 900 μL of phosphate buffer. A few mg of $\text{Na}_2\text{S}_2\text{O}_4$ was added to reduce the heme iron and the UV-Vis spectrum (200–700 nm) was then recorded and used as the baseline. CO was slowly bubbled into the solution and the spectrum of the solution was recorded indicating the protein peak intensity at 450 nm.

The concentration of the protein was determined using the following equation:

$$[P450]\mu\text{M} = \frac{10 \times (A_{450} - A_{490})}{\epsilon} \times 10^6$$

where ϵ is the extinction coefficient ($91,000 \text{ M}^{-1} \cdot \text{cm}^{-1}$).

- **General procedure A: screening of compounds using P450_{BM3} mutants**

Small scale screens of the substrates were conducted on a 0.5 mL scale in a 24 well plate containing 24 P450_{BM3} mutants (1 per well). These reactions were performed using

phosphate buffer (200 mM, pH 7.5) containing glucose (100 mM), NADP⁺ (40 μM), GDH (20 U/mL), substrate (1 mM) and enzyme (2 μM).

The screening plates were prepared by adding the mixture detailed in **Table 6.2** (400 μL) to each well containing a mP450 enzyme (100 μL).

Table 6.2. Components for screening reaction.

Component	Stock concentration	Volume (μL)	Final concentration
Glucose	1 M	50	100 mM
GDH	2 U/μL	5	20 U/mL
NADP ⁺	4 mM	5	40 μM
Substrate	100 mM	5	1 mM
Enzyme	10 μM	100	2 μM
Phosphate buffer	–	335	200 mM
Reaction volume		500	

The plates were shaken on a platform shaker for 24 h at 120 rpm and RT. Each reaction mixture was then transferred into a 1.5 mL microcentrifuge tube, and EA (300 μL) was added. The biphasic solution was shaken for 15 min at RT and then centrifuged for 2 min at 13,000 rpm. The organic layer (100 μL) was separated and transferred into a 200 μL glass GC insert. This was analysed by a Thermo Finnigan Trace1300 gas chromatograph equipped with a flame ionisation detector, an AS3000 autosampler and a DB-1 fused silica column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). Helium was used as the carrier gas (1.5 mL/min). The initial oven temperature was 200 °C which was held for 1 min, and then raised at 30 °C/min to 300 °C and kept at this final temperature for 2 min.

- **General procedure B: preparative scale reactions using P450_{BM3} mutants**

All preparative scale reactions were performed in a beaker at RT using the same ratios of reagents as in *General Procedure A* (**Table 6.2**). The mixture was stirred at RT and monitored via gas chromatography at regular intervals until deemed appropriate to work up, typically

Experimental

2–3 days. Isopropanol was added and the mixture was stirred for 5 min at RT; K_2CO_3 was added and then stirring continued for an additional 5 min at RT. The organic layer was separated and concentrated under reduced pressure. The crude residue was re-dissolved in EA and water, the aqueous layer was extracted with EA ($\times 3$) and the organic extracts were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography to separate and purify the products.

- **P450_{BM3} mutants and the mutations in each:**

Table 6.3. P450_{BM3} mutants in plate A and the mutations in each.

NA	Enzyme	Mutation(s)
1	RLYF/KSK19/AI	R47L/Y51F/F87A/H171L/Q307H/N319Y/A328I
2	KSK19/AM	F87A/A82M/H171L/Q307H/N319Y
3	KSK19/AI/Y51L	F87A/ H171L/I263A/Q307H/N319Y/A328I
4	KSK19/AI/AI	F87A/H171L/A184I/Q307H/N319Y/A328I
5	RP/FV/EV/L437VL	R47L/Y51F/F87V/E267V/I401P/L437VL
6	KSK19/I263A	F87A/H171L/I263A/Q307H/N319Y
7	RP	R47L/Y51F/I401P
8	RP/EV	R47L/Y51F/E267V/I401P
9	RT2/F81W	R47L/Y51F/F81W/A191T/N239H/I259V/A276T/L353I
10	RP/FV/EV	R47L/Y51F/F87V/E267V/I401P
11	KSK19/AI/F87V	F87V/H171L/Q307H/N319Y/A328I
12	RP/FV/EV/L437LV	R47L/Y51F/F87V/E267V/I401P/L437LV
13	KSK19/AI/V78I	V78I/F87A/H171L/Q307H/N319Y/A328I
14	RT2/AP/I401M	R47L/Y51F/A191T/N239H/I259V/A276T/ A330P/L353I/I401M
15	RT2/AP/AM	R47L/Y51F/A82M/A191T/N239H/I259V/ A276T/A330P/L353I
16	RT2/IP	R47L/Y51F/A191T/N239H/I259V/A276T/L353I/I401P
17	RT2/AP	R47L/Y51F/A191T/N239H/I259V/A276T/ A330P/L353I
18	RT2/AP/A184I	R47L/Y51F/A184I/A191T/N239H/I259V/ A276T/A330P/L353I
19	RT2/AP/V78I	R47L/Y51F/V78I/A191T/N239H/I259V/ A276T/A330P/L353I
20	RT2	R47L/Y51F/A191T/N239H/I259V/A276T/L353I

NA	Enzyme	Mutation(s)
21	RP/IA/EV	R47L/Y51F/I263A/E267V/I401P
22	WT	–
23	RP/AP	R47L/Y51F/A330P/I401P
24	RP/FV	R47L/Y51F/F87V/I401P

Table 6.4. P450_{BM3} mutants in plate B and the mutations in each.

NB	Enzyme	Mutation(s)
1 (25)	A330P	A330P
2 (26)	RP/FW	R47L/Y51F/I401P/ F81W
3 (27)	RT2/AP/AI/SA	R47L/Y51F/S72A/A184I/A191T/N239H/ I259V/A276T/A330P/L353I
4 (28)	RP/HL/IG	R47L/Y51F/H171L/I263G/I401P
5 (29)	RT2/AP/PV	R47L/Y51F/A191T/N239H/I259V/A276T/ P329V/A330P/L353I
6 (30)	GVQ	A74G/F87V/L188Q
7 (31)	RT2/AW	R47L/Y51F/A191T/N239H/I259V/A276T/ A330W/L353I
8 (32)	F87A	F87A
9 (33)	RLYF/KSK19	R47L/Y51F/F87A/H171L/Q307H/N319Y
10 (34)	RP/AM/IA	R47L/Y51F/A82M/I263A/I401P
11 (35)	RT2/VF	R47L/Y51F/V78F/A191T/N239H/I259V/ A276T/L353I
12 (36)	RLYF	R47L/Y51F
13 (37)	KSK19/AM/EF	A82M/F87A/H171L/E267F/Q307H/N319Y
14 (38)	RP/HL	R47L/Y51F/H171L/I401P
15 (39)	RP/EG	R47L/Y51F/E267G/I401P
16 (40)	KSK48	R179H/L233V/S270I/Q307H/N319Y
17 (41)	KSK19/QP/FV	F87V/H171L/Q307H/N319Y/Q403P
18 (42)	RT2/AP/VI/AI	R47L/Y51F/V78I/A184I/A191T/N239H/ I259V/A276T/A330P/L353I
19 (43)	RP/FV/EV/LQ	R47L/Y51F/F87V/L188Q/E267V/I401P
20 (44)	RP/FV/EV/VF	R47L/Y51F/V78F/F87V/E267V/I401P
21 (45)	RT2/FW/AI	R47L/Y51F/F81W/A191T/N239H/I259V/ A276T/A328I/L353I
22 (46)	RP/EM	R47L/Y51F/E267M/I401P
23 (47)	KSK19/AI/IA	F87A/ H171L/I263A/Q307H/N319Y/A328I
24 (48)	RT2/SW/AW	R47L/Y51F/S72W/A191T/N239H/I259V/ A276T/A330W/L353I

6.2.2 General procedure for Mosher ester method

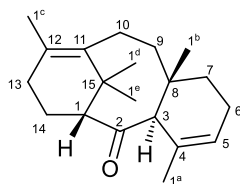
- **General procedure C:**

In a 2 mL glass vial, the alcohol (racemic or enantioenriched, 1.0 eq.) and dry pyridine (3.1 eq.) were dissolved in CDCl_3 ($[\text{alcohol}] = 3.2 \text{ mM}$) at RT. MTPA-Cl (racemic or enantiomerically pure, 1.9 eq.) was added, the vial was closed with the cap and the mixture was stirred at RT. The reaction was monitored by TLC and when it was completed, the mixture was diluted with CDCl_3 (0.6 mL). The entire CDCl_3 solution was transferred to an NMR tube and the required NMR experiments were recorded, usually proton (^1H) and fluorine (^{19}F) NMR spectra.

6.3 Experimental procedures and characterisation

(1*R**,3*S**,8*S**)-4,8,12,15,15-Pentamethyltricyclo[9.3.1.0^{3,8}]pentadec-4,11-diene-2-one

(**16**)^[105]

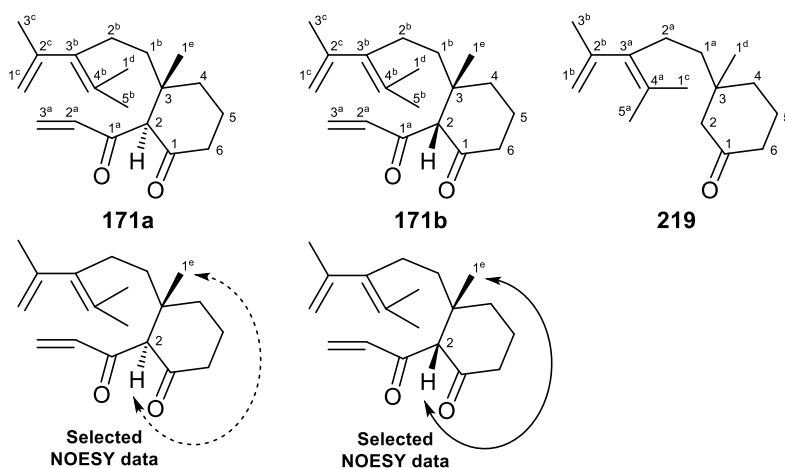


A solution of diketone **179a** (48 mg, 0.17 mmol) in THF (2.3 mL) was added dropwise to a solution of *N*-phenyl-bis(trifluoromethanesulfonimide) (118 mg, 0.33 mmol) in THF (1.5 mL) at 0 °C. Subsequently, potassium bis(trimethylsilyl)amide (0.5 M in toluene, 0.50 mL, 0.25 mmol) was added dropwise at 0 °C. The mixture was stirred for 1 h at 0 °C and then, poured into a cold satd. aq. solution of NaHCO₃ (4 mL) and diluted with hexane (4 mL). The two layers were separated and the organic layer was washed sequentially with aq. KOH (3.0 M, 4 × 3 mL), water (2 × 3 mL), and brine (3 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The triflate (103 mg) was unstable and therefore was used immediately and without further purification. The triflate (103 mg) was redissolved in THF (1.7 mL), cooled to 0 °C and then, a solution of tetrakis(triphenylphosphine) palladium(0) (10 mg, 9 μmol) in THF (0.4 mL) was added. After stirring for 5 min at 0 °C, dimethylzinc (2.0 M in toluene, 0.41 mL, 0.82 mmol) was added dropwise and stirring continued for an additional 5 min at 0 °C. The cooling bath was removed and the mixture was warmed to RT and then stirred for 14 h. The reaction was quenched with aq. HCl (1.0 M, 2 mL). The two layers were separated, the aqueous layer was extracted with hexane (2 × 2 mL) and the organic extracts were combined, washed with brine (2 × 2 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (hexane/EA, 9:1), affording taxadienone **16** (32 mg, 66%) as a white solid. *R_f* = 0.52 (hexane/EA, 9:1); *m.p.* 72–92 °C; *IR* (ν_{max}/cm⁻¹): 1677m

Experimental

(C=O), 1388s (CH₃), 1190s (CH₃); ¹H NMR (500 MHz, CDCl₃) δ 5.56 (d, *J* = 6.0, 1H, *H*₅), 3.31 (s, 1H, *H*₃), 2.59 (t, *J* = 4.5, 1H, *H*₁), 2.56–2.44 (m, 2H, *H*₁₀ and *H*₁₃), 2.26 (ddd, *J* = 15.5, 12.5, 4.0, 1H, *H*₁₀), 2.19–2.04 (m, 2H, *H*₆ and *H*₁₃), 2.03–1.98 (m, 2H, *H*₁₄), 1.81 (s, 3H, *H*₁^α), 1.86–1.76 (m, 2H, *H*₆ and *H*₉), 1.49 (dq, *J* = 2.5, 1.0, 3H, *H*₁^α), 1.35 (dt, *J* = 14.0, 4.0, 1H, *H*₉), 1.35–1.29 (m, 1H, *H*₇), 1.21 (s, 3H, *H*₁^β), 1.20–1.17 (m, 1H, *H*₇), 1.09 (s, 3H, *H*₁^α), 0.91 (s, 3H, *H*₁^β); ¹³C NMR (126 MHz, CDCl₃) δ 218.2 (C₂), 136.9 (C₄), 133.5 (C₁₁), 130.8 (C₁₂), 124.9 (C₅), 65.0 (C₁), 56.1 (C₃), 43.7 (C₉), 40.9 (C₇), 38.7 (C₈), 37.5 (C₁₅), 29.1 (C₁^ε), 28.4 (C₁₃), 27.7 (C₁^δ), 23.9 (C₁^α), 23.7 (C₁₀), 22.3 (C₁^ε), 21.9 (C₆), 20.7 (C₁^β), 18.8 (C₁₄); MS (ESI⁺): *m/z* 287.2 ([M+H]⁺, 88%), 309.2 ([M+Na]⁺, 56%); HRMS (ESI⁺): *m/z* found 287.2369, calcd. for C₂₀H₃₁O [M+H]⁺ 287.2369. Data consistent with the literature.^[105]

(2*S,3*S**)-** and **(2*R**,3*S**)-**(2-Acryloyl-3-methyl-3-[4-methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl]cyclohexan-1-one (171a and 171b)^[105]



- **Method A:**

MeLi (1.6 M in ether, 1.04 mL, 1.67 mmol) was added all at once to a solution of TMS-enol ether **178** (467 mg, 1.52 mmol) in THF (10.9 mL) at –20 °C and the mixture was stirred for 15 min at –20 °C. After cooling to –78 °C, freshly distilled acrolein (140 μL, 2.10 mmol)

was added dropwise and the mixture was stirred for 30 min at $-78\text{ }^{\circ}\text{C}$. The reaction was quenched at $-78\text{ }^{\circ}\text{C}$ by the sequential addition of NH_4Cl (s) and a satd. aq. solution of NH_4Cl (10 mL) added dropwise. After warming to RT, the two layers were separated, the aqueous layer was extracted with ether ($3 \times 6\text{ mL}$) and the organic extracts were combined, washed with brine (10 mL), dried over MgSO_4 , filtered and concentrated under reduced pressure. Aldol **218** was unstable on silica, the retro-aldol reaction takes place, and therefore was used without further purification. The aldol **218** was redissolved in DCM (7.6 mL) and then NaHCO_3 (1.28 g, 15.2 mmol) and Dess-Martin periodinane (772 mg, 1.82 mol) were added sequentially at RT. The mixture was stirred for 20 min at RT and then, diluted with ether (7 mL). The reaction was quenched by the sequential addition of a satd. aq. solution of $\text{Na}_2\text{S}_2\text{O}_3$ (7 mL) and a satd. aq. solution of NaHCO_3 (7 mL), and the mixture was stirred for 20 min at RT. The two layers were separated, the aqueous layer was extracted with ether ($4 \times 5\text{ mL}$) and the organic extracts were combined, dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (hexane/EA, 10:1), affording keto-enone **171** (190 mg, 43%) as an inseparable mixture of two diastereomers (**171a**:**171b** = 2:1). **Data for both diastereomers 171a and 171b:** $R_f = 0.41$ (hexane/EA, 10:1); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 1710s (C=O), 1684s (C=O), 1668m (C=O), 1609w (C=C), 892m (C=C); **MS** (ESI⁺): m/z 311.2 ([M+Na]⁺, 100%), 289.2 ([M+H]⁺, 56%); **HRMS** (ESI⁺): m/z found 289.2162, calcd. for $\text{C}_{19}\text{H}_{29}\text{O}_2$ [M+H]⁺ 289.2162; **NMR data for diastereomer 171a:** **¹H NMR** (500 MHz, CDCl_3) δ 6.35 (dd, $J = 17.5, 10.5$, 1H, $H2^a$), 6.24 (dd, $J = 17.5, 1.0$, 1H, $H3^a$), 5.81 (dd, $J = 10.5, 1.0$, 1H, $H3^a$), 4.90 (dq, $J = 3.0, 1.5$, 1H, $H1^c$), 4.52 (dq, $J = 3.0, 1.0$, 1H, $H1^c$), 3.77 (t, $J = 1.5$, 1H, $H2$), 2.68 (ddd, $J = 14.0, 10.5, 7.0$, 1H, $H6$), 2.32 (dt, $J = 14.0, 5.0$, 1H, $H6$), 2.10–2.06 (m, 1H, $H4$), 2.05–2.00 (m, 2H, $H2^b$), 1.99–1.90 (m, 2H, $H5$), 1.73 (dd, $J = 1.5, 1.0$, 3H, $H3^c$), 1.64 (s, 3H, $H5^b$), 1.63 (s, 3H, $H1^d$), 1.55–1.51 (m, 1H, $H4$), 1.33–1.23 (m, 2H, $H1^b$), 0.98 (s, 3H, $H1^e$); **¹³C NMR** (126 MHz, CDCl_3) δ 208.1 (C1), 196.6 (C1^a), 146.3

Experimental

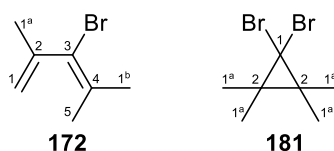
(C2^c), 137.9 (C2^a), 135.8 (C3^b), 129.3 (C3^a), 125.6 (C4^b), 113.6 (C1^c), 70.4 (C2), 42.1 (C3), 39.4 (C6), 37.9 (C1^b), 31.6 (C4), 24.7 (C2^b), 23.5 (C1^c), 22.8 (C3^c), 21.9 (C5^b), 21.6 (C5), 19.6 (C1^d). Data consistent with the literature.^[105] **NMR data for diastereomer 171b:** **¹H NMR** (500 MHz, CDCl₃) δ 6.37 (dd, *J* = 17.5, 10.5, 1H, H2^a), 6.28 (dd, *J* = 17.5, 1.0, 1H, H3^a), 5.82 (dd, *J* = 10.5, 1.0, 1H, H3^a), 4.87 (dq, *J* = 3.0, 1.5, 1H, H1^c), 4.48 (dq, *J* = 3.0, 1.0, 1H, H1^c), 3.78 (t, *J* = 1.5, 1H, H2), 2.64 (ddd, *J* = 14.5, 11.0, 7.0, 1H, H6), 2.39–2.32 (m, 1H, H4), 2.33–2.29 (m, 1H, H6), 2.05–1.98 (m, 2H, H2^b), 1.98–1.94 (m, 2H, H5), 1.69 (dd, *J* = 1.5, 1.0, 3H, H3^c), 1.65 (s, 3H, H5^b), 1.58 (s, 3H, H1^d), 1.50–1.46 (m, 1H, H1^b), 1.45–1.39 (m, 1H, H4), 1.25–1.20 (m, 1H, H1^b), 0.95 (s, 3H, H1^c); **¹³C NMR** (126 MHz, CDCl₃) δ 207.8 (C1), 196.1 (C1^a), 146.5 (C2^c), 137.6 (C2^a), 136.0 (C3^b), 129.7 (C3^a), 125.4 (C4^b), 113.5 (C1^c), 71.4 (C2), 42.6 (C3), 39.0 (C6), 38.2 (C1^b), 32.3 (C4), 25.1 (C2^b), 24.0 (C1^c), 22.9 (C3^c), 22.1 (C5), 21.9 (C5^b), 19.6 (C1^d). Data consistent with the literature.^[105]

- **Method B:**

Acrolein (0.88 mL, 13.2 mmol) and a solution of Gd(OTf)₃ (40 mg, 66.2 μmol) in water/ethanol (1:10, 3.1 mL) were added sequentially dropwise to a solution of TMS-enol ether **178** (203 mg, 0.66 mmol) in toluene (1.1 mL) at 0 °C. The mixture was stirred for 24 h at 4 °C and then concentrated under reduced pressure over a short period of less than 10 min. Aldol **218** was unstable on silica, the retro-aldol reaction takes place, and therefore was used without further purification. Aldol **218** was redissolved in non-dry acetone (7.7 mL), the mixture was cooled to 0 °C and a solution of CrO₃ (323 mg, 3.23 mmol) in aq. H₂SO₄ (97%, 0.3 mL) and water (1.3 mL) was added dropwise at 0 °C. After stirring for 10 min at 0 °C, the reaction was quenched with isopropanol (1 mL) and stirring continued for an additional 10 min at 0 °C. The solution was diluted with water (23 mL) and EA (23 mL). The two layers were separated, the aqueous layer was extracted with EA (3 × 14 mL) and the organic extracts were combined, washed sequentially with water (20 mL) and brine (20

mL), dried over anhydrous Na_2SO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (hexane/EA, 10:1), affording an inseparable mixture (155 mg) of keto-enone **171** (55%), as two diastereomers **171a** and **171b** (**171a**:**171b** = 2:1) (data as above), and 3-methyl-3-[4-methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl]-cyclohexan-1-one **219** (21%) as a yellow oil. Yields were calculated from the ^1H -NMR spectrum of the mixture. **Data for 219:** $R_f = 0.41$ (hexane/EA, 10:1); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 1711s (C=O), 1683w (C=C), 1631w (C=C), 891m (C=C); **^1H NMR** (400 MHz, CDCl_3) δ 4.90 (dq, $J = 3.0, 1.5$, 1H, $H1^b$), 4.53 (dq, $J = 3.0, 1.0$, 1H, $H1^b$), 2.27 (t, $J = 6.5$, 2H, $H6$), 2.20 (d, $J = 13.5$, 1H, $H2$), 2.12 (d, $J = 13.5$, 1H, $H2$), 2.10–1.92 (m, 2H, $H2^a$), 1.92–1.82 (m, 2H, $H5$), 1.74 (dd, $J = 1.5, 1.0$, 3H, $H3^b$), 1.65 (s, 3H, $H5^a$), 1.64 (s, 3H, $H1^c$), 1.64–1.61 (m, 1H, $H4$), 1.59–1.55 (m, 1H, $H4$), 1.28 (app. ddd, $J = 9.5, 8.0, 3.0$, 2H, $H1^a$), 0.93 (s, 3H, $H1^d$); **^{13}C NMR** (101 MHz, CDCl_3) δ 212.4 (C1), 146.5 (C2^b), 136.4 (C3^a), 125.2 (C4^a), 113.3 (C1^b), 53.9 (C2), 41.2 (C6), 40.2 (C1^a), 38.7 (C3), 35.9 (C4), 25.1 (C2^a), 24.9 (C1^d), 22.9 (C3^b), 22.3 (C5), 21.9 (C5^a), 19.6 (C1^c); **MS** (ESI⁺): m/z 257.2 ($[\text{M}+\text{Na}]^+$, 36%); **HRMS** (CI⁺): m/z found 135.2061, calcd. for $\text{C}_{16}\text{H}_{27}\text{O}$ $[\text{M}+\text{H}]^+$ 235.2056. Data consistent with the literature.^[105]

3-Bromo-2,4-dimethylpenta-1,3-diene (**172**)^[108]

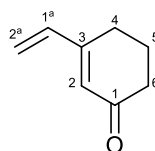


Freshly distilled bromoform (0.52 mL, 5.95 mmol) was added in three equal portions over the course of 1 h to a solution of potassium *tert*-butoxide (0.74 g, 6.59 mmol) and 2,3-dimethyl-2-butene (1.40 mL, 11.8 mmol) in pentane (8.7 mL) at 0 °C. The reaction mixture was warmed to RT and stirred for 12 h. The volatile components were evaporated under

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reduced pressure and the product was dried under high vacuum for 6 h, affording crude 1,1-dibromo-2,2,3,3-tetramethylcyclopropane **181** (1.22 g) as a yellow oil. $R_f = 0.76$ (petrol); $^1\text{H NMR}$ (200 MHz, CDCl_3) δ 1.25 (s, 12H, $H1^a$). A mixture of the crude cyclopropane **181** (1.22 g) and *N,N*-dimethylaniline (3.30 mL, 26.0 mmol) was heated to reflux for 20 min. After cooling to RT, the reaction was quenched by the addition of ether (20 mL) and aq. HCl (3.0 M, 10 mL). The layers were separated and the organic layer was washed sequentially with aq. HCl (3.0 M, 2×10 mL), water (10 mL) and brine (10 mL), dried over anhydrous MgSO_4 , filtered, and concentrated under reduced pressure. Bromodiene **172** was obtained as a colourless oil (0.633 g, 61% from 2,3-dimethyl-2-butene) and was used without further purification. A small sample of **172** was purified by filtration through a small pad of silica for characterisation purposes. $R_f = 0.82$ (petrol); IR ($\nu_{\text{max}}/\text{cm}^{-1}$): 3089w ($=\text{CH}_2$), 1626w (C=C), 1092m (C-Br), 852s (C= CH_2); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.04 (dq, $J = 2.0, 1.5$, 1H, $H1$), 4.91 (dq, $J = 2.0, 1.0$, 1H, $H1$), 1.90 (s, 3H, $H5$), 1.89 (dd, $J = 1.5, 1.0$, 3H, $H1^a$), 1.81 (s, 3H, $H1^b$); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 144.2 (C2), 131.2 (C4), 120.3 (C3), 116.6 (C1), 24.6 (C5), 22.0 (C1^a), 21.8 (C1^b); HRMS (TOF FI^+): m/z found 174.0049, calcd. for $\text{C}_7\text{H}_{11}^{79}\text{Br}$ [M^+] 174.0044. Data consistent with the literature.^[108]

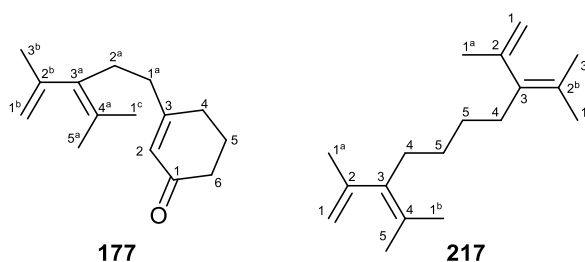
3-Vinylcyclohex-2-en-1-one (**173**)^[110]



Vinylmagnesium bromide (1.0 M in THF, 0.96 mL, 0.96 mmol) was added dropwise to a solution of 3-ethoxycyclohex-2-en-1-one (0.10 mL, 0.69 mmol) in THF (0.7 mL) at -78 °C. After stirring for 30 min at -78 °C, the acetone/dry ice bath was removed and the mixture was warmed to RT and then stirred for 3 h. The reaction was quenched with aq. HCl (1.0

M, 0.6 mL) and the mixture was stirred for 30 min at RT. The layers were separated, the aqueous layer was extracted with ether (2×2 mL) and the organic extracts were combined, dried over anhydrous MgSO_4 , filtered, and concentrated under reduced pressure. After purification by flash column chromatography (DCM), dienone **173** (82 mg, 97%) was obtained as a yellow oil. $R_f = 0.21$ (DCM); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 6.49 (dd, $J = 17.5, 10.5$, 1H, $H1^a$), 5.95 (s, 1H, $H2$), 5.69 (dd, $J = 17.5, 0.5$, 1H, $H2^a$), 5.47 (dd, $J = 10.5, 0.5$, 1H, $H2^b$), 2.47 (t, $J = 6.0$, 2H, $H4$), 2.42 (t, $J = 6.5$, 2H, $H6$), 2.05 (app. quin., $J = 6.5$, 2H, $H5$); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 200.5 (C1), 157.0 (C3), 138.1 (C1^a), 128.4 (C2), 120.8 (C2^a), 37.9 (C6), 24.4 (C4), 22.4 (C5); **MS** (ESI⁺): m/z 145.1 ($[\text{M}+\text{Na}]^+$, 100%), 123.2 ($[\text{M}+\text{H}]^+$, 42%), 267.1 ($[\text{2M}+\text{Na}]^+$, 25%). Data consistent with the literature.^[110]

3-[4-Methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl]cyclohex-2-en-1-one (**177**)^[105]



- **Method A:**

t-BuLi (1.7 M in pentane, 9.90 mL, 16.8 mmol) was added dropwise to a solution of iododiene **214** (2.00 g, 8.00 mmol) in freshly distilled ether (30.8 mL) at -78 °C. The obtained mixture was stirred for 15 min at -78 °C, the cooling bath was removed and the reaction was warmed to RT then stirred for 1 h. After cooling to 0 °C, 3-ethoxycyclohex-2-en-1-one (2.30 mL, 15.8 mmol) was added and the mixture was stirred for 30 min at 0 °C, warmed to RT and then stirred for 3 h. The reaction was quenched with aq. HCl (1.0 M, 15 mL). The two layers were separated, the aqueous layer was extracted with ether (4×8 mL) and the organic extracts were combined, dried over anhydrous MgSO_4 , filtered and

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concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 2:1), affording enone **177** (1.38 g, 79 %) as a pale yellow oil. $R_f = 0.42$ (petrol/ether, 2:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 3074w (=CH₂), 1668s (C=O), 1625w (C=C); **¹H NMR** (400 MHz, CDCl₃) δ 5.88 (t, $J = 1.5$, 1H, *H2*), 4.96 (dq, $J = 3.0, 1.5$, 1H, *H1^b*), 4.57 (dq, $J = 3.0, 1.0$, 1H, *H1^b*), 2.35 (t, $J = 6.5$, 2H, *H6*), 2.30 (td, $J = 6.5, 1.5$, 2H, *H4*), 2.27–2.18 (m, 4H, *H1^a* and *H2^a*), 1.98 (quin, $J = 6.5$, 2H, *H5*), 1.76 (dd, $J = 1.5, 1.0$, 3H, *H3^b*), 1.67 (s, 6H, *H5^a* and *H1^c*); **¹³C NMR** (101 MHz, CDCl₃) δ 200.1 (C1), 166.7 (C3), 146.0 (C2^b), 135.3 (C3^a), 126.4 (C4^a), 125.7 (C2), 114.0 (C1^b), 37.5 (C6), 36.9 (C1^a), 30.0 (C4), 28.6 (C2^a), 22.9 (C5), 22.8 (C3^b), 21.9 (C5^a), 19.8 (C1^c); **MS** (ESI⁺): m/z 241.1 ([M+Na]⁺, 100%), 459.3 ([2M+Na]⁺, 80%), 219.2 ([M+H]⁺, 60%); **HRMS** (ESI⁺): m/z found 241.1557, calcd. for C₁₅H₂₂O²³Na [M+Na]⁺ 241.1563. Data consistent with the literature.^[105]

- **Method B:**

Dry magnesium turnings (39 mg, 1.60 mmol) were stirred vigorously under an inert atmosphere of Ar for 2 h then suspended in THF (1.2 mL). A small crystal of iodine and a solution of bromodiene **184** (328 mg, 1.61 mmol) in THF (0.3 mL) were added sequentially to the suspension at RT. The reaction mixture was heated slightly until it became colourless and then stirred for 30 min at RT. After cooling to 0 °C, a solution of 3-ethoxycyclohex-2-en-1-one (161 mg, 1.15 mmol) in THF (0.4 mL) was added and the mixture was stirred for 30 min at 0 °C. The acetone/dry ice bath was removed and the reaction was warmed to RT and then stirred for 3 h. The reaction was quenched with aq. HCl (1.0 M, 0.5 mL). The two layers were separated, the aqueous layer was extracted with ether (5 × 2 mL) and the organic extracts were combined, dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 5:1 to 2:1), affording enone **177** (164 mg, 65%) (data as above) and

2,9-dimethyl-8-(prop-1-en-2-yl)-3-(propan-2-ylidene)deca-1,8-diene **217** (40 mg, 20%) as a colourless oil. $R_f = 0.86$ (petrol); **IR** ($\nu_{\max}/\text{cm}^{-1}$) 3074w (=CH₂), 1631m (C=C), 892s (C=CH₂); **¹H NMR** (400 MHz, CDCl₃) δ 4.90 (dq, $J = 3.0, 1.5, 2\text{H}, H1$), 4.53 (dq, $J = 3.0, 1.0, 2\text{H}, H1$), 2.05 (t, $J = 7.0, 4\text{H}, H4$), 1.75 (dd, $J = 1.5, 1.0, 6\text{H}, H1^a$), 1.65 (s, 12H, $H1^b$ and $H3^b$), 1.34–1.25 (m, 4H, $H5$); **¹³C NMR** (101 MHz, CDCl₃) δ 146.9 (C2), 137.0 (C3), 124.7 (C2^b), 112.9 (C1), 31.2 (C4), 28.6 (C5), 22.9 (C1^a), 21.9 (C1^b), 19.8 (C3^b); **MS** (ESI⁺): m/z 264.0 ([M+NH₄]⁺, 50%), 247.2 ([M+H]⁺, 33%).

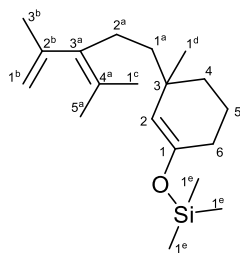
- **Method C:**

t-BuLi (1.7 M in pentane, 0.83 mL, 1.41 mmol) was added dropwise to a solution of bromodiene **172** (132 mg, 0.75 mmol) in THF (2.9 mL) at $-78\text{ }^\circ\text{C}$. After stirring for 30 min at $-78\text{ }^\circ\text{C}$, CuBr·SMe₂ (145 mg, 0.71 mmol) was added. The resulting mixture was stirred for 30 min at $-78\text{ }^\circ\text{C}$, chlorotrimethylsilane (0.15 mL, 1.18 mmol) was added dropwise, and stirring continued for an additional 5 min at $-78\text{ }^\circ\text{C}$. A solution of dienone **173** (73.1 mg, 0.60 mmol) in THF (0.8 mL) was added dropwise and the obtained mixture was stirred at $-78\text{ }^\circ\text{C}$ for 2 h, warmed slowly to RT and then stirred for 12 h. The reaction mixture was poured into a solution of glacial acetic acid (1.6 mL) and ether (6.7 mL) and the mixture was stirred for 30 min at RT. Aq. HCl (3.0 M, 4.0 mL) was added and stirring continued for an additional 30 min at RT. The layers were separated and the aqueous layer was extracted with ether (2 × 4 mL). The organic extracts were combined in an Erlenmeyer flask and a satd. aq. solution of NaHCO₃ (4 mL) was added in portions. The layers were separated and the organic layer was washed sequentially with water (6 mL) and brine (6 mL), dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/EA, 10:1), affording enone **177** (25.6 mg, 20%). Data as above.

- **Method D:**

n-BuLi (1.4 M in cyclohexane, 0.58 mL, 0.81 mmol) was added dropwise to a solution of bromodiene **172** (150 mg, 0.86 mmol) in THF (3.4 mL) at $-78\text{ }^{\circ}\text{C}$. After stirring for 30 min at $-78\text{ }^{\circ}\text{C}$, CuBr \cdot SMe₂ (167 mg, 0.81 mmol) was added. The resulting mixture was stirred for 30 min at $-78\text{ }^{\circ}\text{C}$, chlorotrimethylsilane (0.17 mL, 1.34 mmol) was added dropwise, and stirring continued for an additional 5 min at $-78\text{ }^{\circ}\text{C}$. A solution of dienone **173** (84 mg, 0.69 mmol) in THF (0.8 mL) was added dropwise and the obtained mixture was stirred at $-78\text{ }^{\circ}\text{C}$ for 2 h, warmed slowly to RT and then stirred for 14 h. The reaction mixture was poured into a solution of glacial acetic acid (1.9 mL) and ether (7.7 mL) and the mixture was stirred for 30 min at RT. Aq. HCl (3.0 M, 4.8 mL) was added and stirring was continued for an additional 30 min at RT. The layers were separated and the aqueous layer was extracted with ether (3 \times 4 mL). The organic extracts were combined in an Erlenmeyer flask and a satd. aq. solution of NaHCO₃ (6 mL) was added in portions. The layers were separated and the organic layer was washed sequentially with water (5 mL) and brine (5 mL), dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 3:1), affording enone **177** (53.7 mg, 36%). Data as above.

({3-Methyl-3-[4-methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl]cyclohex-1-en-1-yl}oxy) trimethylsilane (178)^[105]

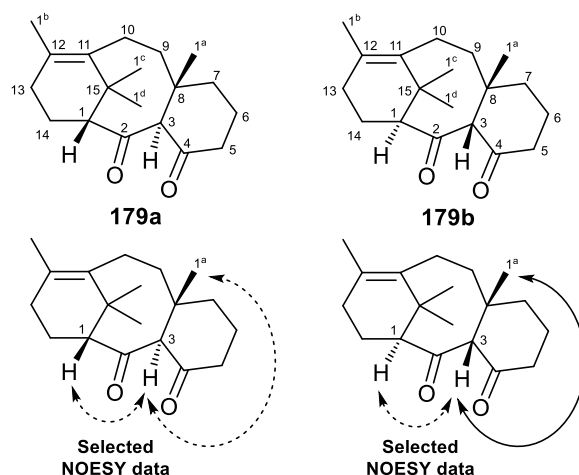


A suspension of pre-dried LiCl (under high vacuum at 100 °C for 14 h) (25 mg, 0.59 mmol) and CuI (55 mg, 0.29 mmol) in THF (11.8 mL) was stirred for 10 min at RT. After cooling to –78 °C, chlorotrimethylsilane (1.31 mL, 10.3 mmol) and a solution of enone **177** (426 mg, 1.95 mmol) in THF (2.0 mL) were sequentially added dropwise. The mixture was stirred for 10 min at –78 °C, MeMgBr (3.0 M in ether, 0.78 mL, 2.34 mmol) was added dropwise and stirring continued for an additional 20 min at –78 °C. The reaction was quenched at this temperature with anhydrous trimethylamine (3.34 mL, 24.0 mmol) injected in all at once. The mixture was warmed to RT and then diluted with pentane, filtered through cotton wool and the residues were washed with pentane (7 mL). The filtrate was washed with a satd. aq. solution of NaHCO₃ (2 × 7 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. TMS-enol ether **178** (586 mg, 99%) was afforded as a pale brown oil and was used without further purification. $R_f = 0.97$ (petrol/ether, 2:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 1661w (C=C), 1251m (Si-CH₃), 839s (Si-(CH₃)₃); **¹H NMR** (500 MHz, CDCl₃) δ 4.89 (dq, $J = 3.0, 1.5$, 1H, $H1^b$), 4.66 (s, 1H, $H2$), 4.53 (dq, 1H, $J = 3.0, 1.0$, 1H, $H1^b$), 2.09–1.94 (m, 2H, $H2^a$), 1.96–1.91 (m, 2H, $H6$), 1.75 (dd, $J = 1.5, 1.0$, 3H, $H3^b$), 1.69–1.66 (m, 2H, $H5$), 1.65 (s, 6H, $H5^a$ and $H1^c$), 1.45–1.38 (m, 1H, $H4$), 1.34–1.21 (m, 3H, $H4$ and $H1^d$), 0.95 (s, 3H, $H1^d$), 0.18 (s, 9H, $H1^e$); **¹³C NMR** (126 MHz, CDCl₃) δ 149.5 (C1), 146.9 (C2^b), 137.2 (C3^a), 124.6 (C4^a), 114.5 (C2), 112.9 (C1^b), 41.9 (C1^a), 34.7 (C3), 34.6 (C4), 30.1 (C6), 28.1 (C1^d), 26.2 (C2^a), 23.0 (C3^b), 21.9 (C5^a), 19.8

Experimental

(C5), 19.6 (C1^o), 0.5 (C1^o); **HRMS** (TOF FI⁺): m/z found 306.2378, calcd. for C₁₉H₃₄OSi [M]⁺ 306.2379. Data consistent with the literature.^[105]

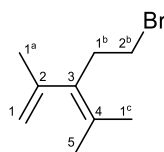
(1*R,3*S**,8*S**)-** and **(1*S**,3*R**,8*S**)-** 8,12,15,15-Tetramethyltricyclo[9.3.1.0^{3,8}]pentadec-11-ene-2,4-dione (**179a** and **179b**)^[105]



A solution of keto-enone **171** as a diastereomeric mixture (123 mg, 0.43 mmol, **171a:171b** = 2:1) in DCM (2.7 mL) was added dropwise over 1 h to a solution of BF₃·OEt₂ (160 μL, 1.30 mmol) in DCM (6.7 mL) at 0 °C. The mixture was stirred for 1 h at 0 °C and then, poured into a cold satd. aq. solution of NaHCO₃ (10 mL). The two layers were separated, the aqueous layer was extracted with DCM (3 × 7 mL) and the organic extracts were combined, washed sequentially with water (15 mL) and brine (15 mL), dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (hexane/EA, 10:1), affording diketone **179** as a separable mixture of two diastereomers: **179a** (46 mg, 37%) as a beige solid and **179b** (33 mg, 27%) as a pale yellow oil. **Data for diastereomer 179a:** R_f = 0.46 (hexane/EA, 3:1); **m.p.** 113–118 °C; **IR** (ν_{\max} /cm⁻¹): 1724s (C=O), 1689w (C=O); **¹H NMR** (500 MHz, CDCl₃) δ 4.22 (s, 1H, *H*3), 2.90 (ddd, J = 14.5, 12.0, 5.5, 1H, *H*10), 2.58–2.47 (m, 1H, *H*13), 2.45 (d, J = 8.5, 1H, *H*1), 2.40–2.33 (m, 1H, *H*5), 2.25–2.14 (m, 2H, *H*5 and *H*10),

2.08–1.97 (m, 3H, *H7*, *H13* and *H14*), 1.98–1.91 (m, 2H, *H6*), 1.91 (t, $J = 1.0$, 3H, *H1^b*), 1.76 (ddd, $J = 15.5, 12.0, 5.0$, 1H, *H9*), 1.67 (ddd, $J = 15.5, 10.0, 5.5$, 1H, *H14*), 1.44 (ddd, $J = 15.5, 5.5, 4.0$, 1H, *H9*), 1.36 (dt, $J = 13.0, 4.0$, 1H, *H7*), 1.26 (s, 3H, *H1^c*), 1.11 (s, 3H, *H1^d*), 1.02 (s, 3H, *H1^e*); **¹³C NMR** (126 MHz, CDCl₃) δ 210.0 (C2), 207.0 (C4), 137.2 (C11), 131.6 (C12), 64.2 (C3), 62.1 (C1), 44.2 (C8), 39.6 (C5), 39.5 (C9), 38.5 (C15), 37.6 (C7), 29.6 (C1^d), 29.0 (C13), 25.5 (C1^e), 25.1 (C10), 24.2 (C1^a), 22.4 (C1^b), 21.2 (C6), 18.0 (C14); **MS** (ESI⁺): m/z 311.2 ([M+Na]⁺, 100%), 289.2 ([M+H]⁺, 73%); **HRMS** (ESI⁺): m/z found 289.2162, calcd. for C₁₉H₂₉O₂ [M+H]⁺ 289.2162. Data consistent with the literature.^[105]

Data for diastereomer 179b: $R_f = 0.68$ (hexane/EA, 3:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 1704m (C=O), 1678s (C=O); **¹H NMR** (400 MHz, CDCl₃) δ 3.66 (s, 1H, *H3*), 2.85 (td, $J = 14.0, 7.0$, 1H, *H5*), 2.81 (td, $J = 13.5, 5.5$, 1H, *H10*), 2.55–2.43 (m, 1H, *H13*), 2.43 (d, $J = 7.5$, 1H, *H1*), 2.30–2.16 (m, 2H, *H5* and *H13*), 2.18–2.05 (m, 2H, *H7* and *H10*), 2.03–1.87 (m, 4H, *H6*, *H9* and *H14*), 1.85 (t, $J = 1.0$, 3H, *H1^b*), 1.79–1.69 (m, 1H, *H6*), 1.33–1.29 (m, 1H, *H9*), 1.28 (s, 3H, *H1^c*), 1.19–1.11 (m, 1H, *H7*), 1.11 (s, 3H, *H1^d*), 0.96 (s, 3H, *H1^e*); **¹³C NMR** (101 MHz, CDCl₃) δ 211.0 (C2), 209.5 (C4), 136.2 (C11), 131.7 (C12), 69.1 (C3), 62.6 (C1), 43.7 (C8), 40.1 (C9), 39.4 (C5), 38.4 (C15), 36.0 (C7), 29.6 (C1^d), 28.8 (C13), 25.0 (C1^e), 24.8 (C10), 24.3 (C1^a), 22.1 (C1^b), 21.2 (C6), 17.9 (C14); **MS** (ESI⁺): m/z 311.2 ([M+Na]⁺, 63%), 289.2 ([M+H]⁺, 33%); **HRMS** (ESI⁺): m/z found 289.2161, calcd. for C₁₉H₂₉O₂ [M+H]⁺ 289.2162. Data consistent with the literature.^[105]

3-(2-Bromoethyl)-2,4-dimethylpenta-1,3-diene (184)^[166]**• Method A:**

A mixture of mesylate **213** (1.52 g, 7.0 mmol) and LiBr (1.21 g, 13.9 mmol) in THF (21 mL) was heated to reflux for 5 h. After cooling to RT, the reaction was quenched with water (10 mL). The two layers were separated, the aqueous layer was extracted with ether (5 × 15 mL) and the organic extracts were combined, dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 5:1), affording bromodiene **184** (1.03 g, 70%) as a colourless oil. $R_f = 0.70$ (petrol); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 1632w (C=C); **¹H NMR** (400 MHz, CDCl₃) δ 4.97 (dq, $J = 3.0, 1.5$, 1H, $H1$), 4.60 (dq, $J = 3.0, 1.0$, 1H, $H1$), 3.34 (t, $J = 8.0$, 2H, $H2^b$), 2.65 (t, $J = 8.0$, 2H, $H1^b$), 1.76 (dd, $J = 1.5, 1.0$, 3H, $H1^a$), 1.70 (s, 3H, $H5$), 1.68 (s, 3H, $H1^c$); **¹³C NMR** (101 MHz, CDCl₃) δ 145.3 (C2), 133.7 (C3), 128.9 (C4), 114.4 (C1), 34.7 (C1^b), 31.5 (C2^b), 22.6 (C1^a), 21.9 (C1^c), 20.0 (C5); **HRMS** (TOF FI⁺): m/z found 202.0363, calcd. for C₉H₁₅⁷⁹Br [M]⁺ 202.0357.

• Method B:

MeMgBr (3.0 M in ether, 0.58 mL, 1.74 mmol) was added dropwise to a solution of lactone **187** (100 mg, 0.79 mmol) in ether (1.1 mL) at -78 °C. The obtained mixture was stirred for 30 min at -78 °C, warmed to RT and then stirred for 2 h. After cooling to 0 °C, PBr₃ (0.22 mL, 2.34 mmol) was added dropwise. The reaction mixture was stirred for 1 h at 0 °C and subsequently poured into cold water (1 mL). The two layers were separated, the aqueous layer was extracted with ether (5 × 1 mL) and the organic extracts were combined, washed

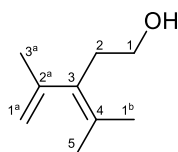
sequentially with water (2 mL) and brine (2 mL), dried over anhydrous MgSO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 10:1), affording bromodiene **184** (6 mg, 1%). Data as above.

- **Method C:**

Tetrabromomethane (331 mg, 1.00 mmol) and triphenylphosphine (262 mg, 1.00 mmol) were added sequentially to a solution of alcohol **185** (70 mg, 0.50 mmol) in DMF (0.8 mL) at 0 °C. After stirring for 1 h at 0 °C, the reaction was quenched with a satd. aq. solution of NH_4Cl (1 mL). The two layers were separated, the aqueous layer was extracted with petrol (3×1 mL) and the organic extracts were combined, dried over anhydrous MgSO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol), affording bromodiene **184** (21 mg, 21%). Data as above.

- **Method D:**

N-Bromosuccinimide (55 mg, 0.31 mmol) was added in portions to a solution of alcohol **185** (37 mg, 0.26 mmol) and triphenylphosphine (81 mg, 0.31 mmol) in DCM (0.3 mL) at -78 °C. The acetone/dry ice bath was removed and the reaction mixture was warmed to RT, stirred for 1 h at RT and subsequently quenched by the addition of a satd. aq. solution of NH_4Cl (1 mL). The two layers were separated, the aqueous layer was extracted with petrol (3×1 mL) and the organic extracts were combined, dried over anhydrous MgSO_4 , filtered and concentrated under reduced pressure. The crude product (26.3 mg) was a mixture of bromodiene **184** (17%) (data as above) and tetrahydrofuran **208** (57%) (data as below). Tetrahydrofuran **208** was unstable on silica and therefore the crude product was not purified. Yields were calculated from the $^1\text{H-NMR}$ spectrum of the crude product.

4-Methyl-3-(prop-1-en-2-yl)pent-3-en-1-ol (**185**)^[109]

- **Method A:**

A suspension of acetate **205** (11.85 g, 65.0 mmol) and K_2CO_3 (10.79 g, 78.1 mmol) in MeOH (89.1 mL) was stirred for 1 h at RT, and then filtered and concentrated under reduced pressure. The residue was redissolved in a satd. aq. solution of NH_4Cl (50 mL) and ether (50 mL). Then, the two layers were separated, the aqueous layer was extracted with ether (5×25 mL) and the organic extracts were combined, washed with brine (50 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 2:1) affording alcohol **185** (8.90 g, 98 %) as a light yellow oil. $R_f = 0.39$ (petrol/ether, 2:1); **IR** (ν_{max}/cm^{-1}): 3319br (O–H), 1632w (C=C), 1041s (C–O); **1H NMR** (400 MHz, $CDCl_3$) δ 4.96 (dq, $J = 3.0, 1.5$, 1H, $H1^a$), 4.58 (dq, $J = 3.0, 1.0$, 1H, $H1^b$), 3.62 (q, $J = 6.0$, 2H, $H2$), 2.40 (t, $J = 6.0$, 2H, $H2$), 1.78 (dd, $J = 1.5, 1.0$, 3H, $H3^a$), 1.72 (s, 3H, $H5$), 1.70 (s, 3H, $H1^b$), 1.41 (t, $J = 6.0$, 1H, OH); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 146.5 ($C2^b$), 132.7 (C3), 128.7 (C4), 113.7 ($C1^a$), 61.4 (C1), 34.0 (C2), 22.7 ($C3^a$), 22.0 ($C1^b$), 20.0 (C5); **MS** (ESI⁺): m/z 163.1 ($[M+Na]^+$, 100%); **HRMS** (CI⁺): m/z found 141.1274, calcd. for $C_9H_{17}O$ $[M+H]^+$ 141.1273. Data consistent with the literature.^[109]

- **Method B:**

An aq. solution of sodium hydroxide (1.0 M, 0.66 mL, 0.66 mmol) was added to a solution of acetate **205** (100 mg, 0.55 mmol) in THF (1.3 mL) at RT. The mixture was stirred for 1 h at RT and then the reaction was quenched with a satd. aq. solution of $NaHCO_3$ (1.4 mL).

The two layers were separated, the aqueous layer was extracted with ether (5×2 mL) and the organic extracts were combined, dried over anhydrous MgSO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 2:1), affording alcohol **185** (19 mg, 20%) (data as above) and acetate 205 (61 mg, 61% recovery).

- **Method C:**

n-BuLi (1.4 M in cyclohexane, 1.47 mL, 2.06 mmol) was added dropwise to a stirred solution of bromodiene **172** (300 mg, 1.71 mmol) in THF (1.0 mL) at -78 °C. After stirring for 30 min at -78 °C, ethylene oxide (2.5–3.3 M in THF, 0.69 mL, 1.73 mmol) and $\text{BF}_3 \cdot \text{OEt}_2$ (0.21 mL, 1.70 mmol) were added sequentially. The reaction was stirred for 1 h at -78 °C, warmed to 0 °C and then quenched with MeOH (1 mL). The two layers were separated, the aqueous layer was extracted with ether (3×1 mL) and the organic extracts were combined, washed with a satd. aq. solution of NH_4Cl , dried over anhydrous MgSO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 2:1), affording alcohol **185** (133 mg, 55%). Data as above.

- **Method D:**

Dry magnesium turnings (42 mg, 1.73 mmol) were stirred vigorously under an inert atmosphere of Ar for 2 h then suspended in THF (1.3 mL). A small crystal of iodine and a solution of bromodiene **172** (300 mg, 1.71 mmol) in THF (0.4 mL) were added sequentially to the suspension at RT. The reaction mixture was heated slightly until it became colourless and then stirred for 30 min at RT. After cooling to 0 °C, ethylene oxide (2.5–3.3 M in THF, 0.5 mL, 1.25 mmol) was added dropwise. The ice bath was removed and the mixture was warmed to RT and then stirred for 1 h. The reaction was quenched with a satd. aq.

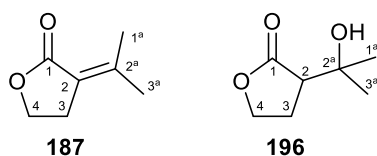
Experimental

solution of NH_4Cl (2 mL). The two layers were separated, the aqueous layer was extracted with ether (5×2 mL) and the organic extracts were combined, dried over anhydrous MgSO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol to petrol/ether, 5:1), affording alcohol **185** (13 mg, 7%). Data as above.

- **Method E:**

n-BuLi (1.4 M in cyclohexane, 1.72 mL, 2.41 mmol) was added dropwise to a solution of bromodiene **172** (300 mg, 1.71 mmol) in THF (25 mL) at -78 °C. After stirring for 30 min at -78 °C, a solution of sulfate **192** (256 mg, 2.06 mmol) in THF (3.6 mL) was added dropwise. The obtained mixture was slowly warmed to RT and then stirred for 12 h. The reaction was quenched by the addition of THF with 1% of H_2SO_4 and 0.5% of water (50 mL) and the mixture was stirred at RT until the hydrolysis was completed. The two layers were separated, the aqueous layer was extracted with DCM (2×10 mL) and the organic extracts were combined, dried over anhydrous MgSO_4 , filtered and concentrated under reduced pressure. After purification by flash column chromatography (petrol/ether, 2:1), alcohol **185** (69 mg, 29%) was obtained. Data as above.

2-(Propan-2-ylidene)- γ -butyrolactone (**187**)^[113,114]



- **Method A:**

n-BuLi (1.6 M in hexane, 95.6 mL, 153 mmol) was added dropwise to a solution of diisopropylamine (22.4 mL, 160 mmol) in THF (76.2 mL) at -78 °C. The obtained mixture

was warmed to 0 °C then stirred for 30 min. After cooling again to -78 °C, a solution of γ -butyrolactone (12.0 g, 139 mmol) in THF (54.9 mL) was added dropwise. The mixture was stirred for 1 h at -78 °C, acetone (20.4 mL, 278 mmol) was added dropwise, and stirring continued for 4 h at -78 °C. The mixture was warmed to RT and then stirred for 14 h. The reaction was quenched by the addition of water (60 mL) and then diluted with THF (30 mL). The two layers were separated and the organic layer was extracted with aq. HCl (1 M, 6 \times 25 mL). The aqueous extracts were combined and extracted with chloroform (9 \times 25 mL). The organic extracts were combined, dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. 2-(2-Hydroxypropan-2-yl)- γ -butyrolactone **196** (18.4 g) was obtained as a yellow oil and was used without further purification. R_f = 0.09 (petrol/ether, 2:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 3465br (O-H), 1754s (C=O), 1163m (C-O), 1017m (C-O); **¹H NMR** (400 MHz, CDCl₃) δ 4.37 (td, J = 10.0, 2.0, 1H, H_4), 4.19 (dddd, J = 10.0, 9.0, 7.0, 0.5, 1H, H_4), 2.72 (dd, J = 11.5, 9.0, 1H, H_2), 2.35 (dddd, J = 13.0, 9.0, 7.0, 2.0, 1H, H_3), 2.12 (dddd, J = 13.0, 11.5, 10.0, 9.0, 1H, H_3), 1.31 (s, 3H, H_1^a), 1.27 (s, 3H, H_3^a); **¹³C NMR** (101 MHz, CDCl₃) δ 178.9 (C1), 70.9 (C2^a), 66.5 (C4), 49.7 (C2), 28.1 (C1^a), 25.7 (C3), 25.5 (C3^a); **MS** (ESI⁺): m/z 167.1 ([M+Na]⁺, 100%), 311.1 ([2M+Na]⁺, 38%); **HRMS** (ESI⁺): m/z found 167.0678, calcd. for C₇H₁₂O₃²³Na [M+Na]⁺ 167.0678. Aq. H₂SO₄ (97%, 0.99 mL, 18 mmol) was added to a solution of aldol **196** (18.4 g from previous step) in toluene (273 mL) and the mixture was heated to reflux for 14 h. After cooling to RT, the reaction mixture was diluted with THF (150 mL) and washed sequentially with aq. HCl (1.0 M, 100 mL), a satd. aq. solution of NaHCO₃ (100 mL) and brine (100 mL). The obtained aqueous layers were combined, adapted to pH 7 and extracted with DCM (3 \times 100 mL). All the organic extracts were combined, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 2:1 to 1:1), affording lactone **187** (15.7 g, 90% from γ -butyrolactone) as a yellow oil. R_f = 0.32 (petrol/ether, 2:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 1738s (C=O),

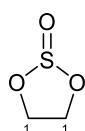
Experimental

1666m (C=C), 1184s (C–O), 1029s (C–O); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 4.28 (t, $J = 7.5$, 2H, $H4$), 2.92–2.82 (m, 2H, $H3$), 2.26 (s, 3H, $H1^a$), 1.88 (s, 3H, $H3^a$); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 170.7 (C1), 150.4 (C2^a), 118.5 (C2), 64.3 (C4), 27.8 (C3), 24.7 (C3^a), 19.8 (C1^a); **MS** (ESI⁺): m/z 149.1 ([M+Na]⁺, 100%), 275.1 ([2M+Na]⁺, 76%), 127.1 ([M+H]⁺, 43%); **HRMS** (ESI⁺): m/z found 127.0753, calcd. for $\text{C}_7\text{H}_{11}\text{O}_2$ [M+H]⁺ 127.0753. Data consistent with the literature.^[113]

- **Method B:**

A solution of phosphonate **195** (100 mg, 0.45 mmol) in THF (6.6 mL) was added dropwise to a slurry of sodium hydride (60 % dispersion in mineral oil, 20 mg, 0.50 mmol) in THF (1.0 mL) at 0 °C. After stirring for 30 min at 0 °C, a solution of acetone (33 μL , 0.45 mmol) in THF (3.2 mL) was added and the obtained mixture was warmed to RT then stirred for 36 h. The reaction was quenched by the addition of ether (0.2 mL) and water (0.5 mL) at 0 °C. THF was removed under reduced pressure, the aqueous layer was extracted with chloroform (3×5 mL) and the organic extracts were combined, washed with water (2×5 mL), dried over anhydrous MgSO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 1:1), affording lactone **187** (3 mg, 6%). Data as above.

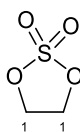
Ethylene sulfite (191)^[118]



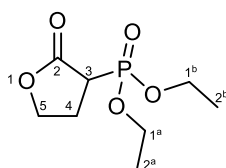
Thionyl chloride (3.50 mL, 48.0 mmol) was added slowly via syringe to a stirred solution of ethylene glycol (2.20 mL, 39.4 mmol) in DCM (10 mL) at RT. The evolved HCl gases were led into a trap with a satd. aq. solution of NaOH and the reaction mixture was stirred until

the gas evolution was ceased. The solvent was then evaporated under reduced pressure and sulfite **191** (4.26 g, 100%) was afforded as colourless oil. $R_f = 0.36$ (petrol/ether, 1:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 2980w (C-H), 1193s (S=O); **$^1\text{H NMR}$** (400 MHz, CDCl_3) δ 4.77–4.63 (m, 2H, *Ht*), 4.40–4.29 (m, 2H, *Hl*); **$^{13}\text{C NMR}$** (101 MHz, CDCl_3) δ 67.4 (C1). Data consistent with the literature.^[118]

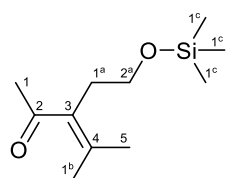
Ethylene sulfate (**192**)^[118,119]



$\text{RuCl}_3 \cdot x\text{H}_2\text{O}$ (0.1 M in water, 0.92 mL, 92 μmol) was added to a solution of NaIO_3 (1.28 g, 5.98 mmol) in water (2.9 mL). After cooling to 0 $^\circ\text{C}$, a solution of sulfite **191** (500 mg, 4.62 mmol) in chloroform (1 mL) was added and the obtained mixture was stirred for 3 h at 0 $^\circ\text{C}$. The two layers were separated, the aqueous layer was extracted with chloroform (3×3 mL) and the organic extracts were combined, washed sequentially with a satd. aq. solution of $\text{Na}_2\text{S}_2\text{O}_3$ (6 mL) and brine (6 mL), dried over anhydrous MgSO_4 , filtered and concentrated under reduced pressure. After filtering through a small pad of Florisil, sulfate **192** (211 mg, 37%) was obtained as a white solid. $R_f = 0.15$ (petrol/ether, 1:1); **$^1\text{H NMR}$** (400 MHz, CDCl_3) δ 4.75 (s, 4H, *Ht*); **$^{13}\text{C NMR}$** (101 MHz, CDCl_3) δ 68.5 (C1). Data consistent with the literature.^[118,119]

Diethyl (2-oxotetrahydrofuran-3-yl)phosphonate (**195**)^[113]

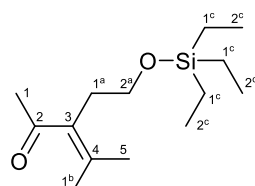
A mixture of α -bromo- γ -butyrolactone (1.00 mL, 10.8 mmol) and triethylphosphite (2.22 mL, 12.9 mmol) was heated to reflux for 4 h. After cooling to RT, ethyl bromide was evaporated under reduced pressure. The crude product was purified by flash column chromatography (EA), affording phosphonate **195** (937 mg, 39%) as a colourless oil. R_f = 0.23 (EA); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 1767m (C=O), 1250m (P=O), 1016s (C-O), 960m (C-O); **¹H NMR** (400 MHz, CDCl_3) δ 4.50–4.39 (m, 1H, H_5), 4.38–4.30 (m, 1H, H_5), 4.29–4.15 (m, 4H, $H1^a$ and $H1^b$), 3.05 (dddd, $J = 23.5, 10.0, 6.0, 1.0$, 1H, H_3), 2.68–2.50 (m, 2H, H_4), 1.39–1.32 (m, 6H, $H2^a$ and $H2^b$); **¹³C NMR** (101 MHz, CDCl_3) δ 172.2 (d, $J_{C-P} = 3.6$, C2), 67.6 (d, $J_{C-P} = 5.5$, C5), 63.7 (d, $J_{C-P} = 7.0$, C1^a), 63.1 (d, $J_{C-P} = 7.0$, C1^b), 38.8 (d, $J_{C-P} = 142.5$, C3), 24.9 (d, $J_{C-P} = 4.0$, C4), 16.5 (d, $J_{C-P} = 3.0$, C2^a), 16.4 (d, $J_{C-P} = 2.5$, C2^b); **MS** (ESI⁺): m/z 467.0 ($[2M+Na]^+$, 100%), 245.0 ($[M+Na]^+$, 63%), 223.0 ($[M+H]^+$, 20%). Data consistent with the literature.^[113]

4-Methyl-3-{2-[(trimethylsilyl)oxy]ethyl}pent-3-en-2-one (**198**)

MeLi (1.6 M in ether, 0.49 mL, 0.78 mmol) was added dropwise to a solution of lactone **187** (100 mg, 0.79 mmol) in THF (1.6 mL) at -78 °C. The mixture was stirred for 15 min at -78 °C, chlorotrimethylsilane (0.30 mL, 2.36 mmol) was added dropwise and stirring continued for an additional 30 min at -78 °C. Then, pyridine (70 μL , 87 mmol) was added

dropwise and the reaction mixture was stirred for 30 min at $-78\text{ }^{\circ}\text{C}$, warmed to RT and then stirred for 14 h. After concentrating under reduced pressure, the residue was redissolved in water (2 mL) and petrol (2 mL). The two layers were separated, the aqueous layer was extracted with petrol ($3 \times 2\text{ mL}$) and the organic extracts were combined, dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol to petrol/ether, 10:1), affording silyl ether **198** (57 mg, 33%) as a pale yellow oil. $R_f = 0.24$ (petrol/ether, 10:1); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 1685m (C=O), 1622m (C=C), 1250m (Si-CH₃), 1080m (Si-O); **¹H NMR** (400 MHz, CDCl₃) δ 3.56 (t, $J = 7.5$, 2H, $H2^a$), 2.53 (t, $J = 7.5$, 2H, $H1^a$), 2.27 (s, 3H, $H1$), 1.83 (s, 3H, $H5$), 1.78 (s, 3H, $H1^b$), 0.09 (s, 9H, $H1^c$); **¹³C NMR** (101 MHz, CDCl₃) δ 205.9 (C2), 138.6 (C4), 134.1 (C3), 61.6 (C2^a), 33.5 (C1^a), 30.5 (C1), 22.8 (C5), 21.5 (C1^b), -0.5 (C1^c); **MS** (ESI⁺): m/z 237.1 ([M+Na]⁺, 100%), 215.2 ([M+H]⁺, 38%); **HRMS** (ESI⁺): m/z found 237.1276, calcd. for C₁₁H₂₂O₂²³Na [M+Na]⁺ 237.1281.

4-Methyl-3-{2-[(triethylsilyl)oxy]ethyl}pent-3-en-2-one (**199**)

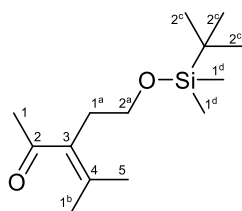


MeLi (1.6 M in ether, , 0.49 mL, 0.78 mmol) was added dropwise to a solution of lactone **187** (100 mg, 0.79 mmol) in THF (1.6 mL) at $-78\text{ }^{\circ}\text{C}$. The mixture was stirred for 15 min at $-78\text{ }^{\circ}\text{C}$, chlorotriethylsilane (0.40 mL, 2.38 mmol) was added dropwise and stirring continued for an additional 30 min at $-78\text{ }^{\circ}\text{C}$. Then, pyridine (70 μL , 87 mmol) was added dropwise and the reaction mixture was stirred for 30 min at $-78\text{ }^{\circ}\text{C}$, warmed to RT and then stirred for 14 h. After concentrating under reduced pressure, the residue was redissolved in water (2 mL) and petrol (2 mL). The two layers were separated, the aqueous

Experimental

layer was extracted with petrol (3×2 mL) and the organic extracts were combined, dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 10:1), affording silyl ether **199** (182 mg, 90%) as a pale yellow oil. $R_f = 0.35$ (petrol/ether, 10:1); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 1686m (C=O), 1621w (C=C), 1095s (Si–O); **$^1\text{H NMR}$** (400 MHz, CDCl_3) δ 3.59 (t, $J = 7.0$, 2H, $H2^a$), 2.54 (t, $J = 7.0$, 2H, $H1^a$), 2.27 (s, 3H, $H1$), 1.83 (s, 3H, $H5$), 1.78 (s, 3H, $H1^b$), 0.94 (t, $J = 8.0$, 9H, $H2^c$), 0.58 (q, $J = 8.0$, 6H, $H1^c$); **$^{13}\text{C NMR}$** (101 MHz, CDCl_3) δ 205.9 (C2), 138.6 (C4), 134.2 (C3), 61.9 (C2^a), 33.6 (C1^a), 30.5 (C1), 22.8 (C5), 21.5 (C1^b), 6.9 (C2^c), 4.5 (C1^c); **MS** (ESI⁺): m/z 279.2 ($[\text{M}+\text{Na}]^+$, 100%), 257.2 ($[\text{M}+\text{H}]^+$, 38%); **HRMS** (ESI⁺): m/z found 279.1742, calcd. for $\text{C}_{14}\text{H}_{28}\text{O}_2^{23}\text{NaSi}$ $[\text{M}+\text{Na}]^+$ 279.1751.

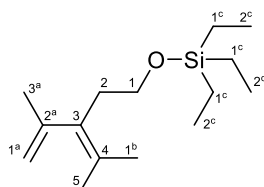
4-Methyl-3-{2-[(*tert*-butyldimethylsilyl)oxy]ethyl}pent-3-en-2-one (200)



MeLi (1.6 M in ether, 0.49 mL, 0.78 mmol) was added dropwise to a solution of lactone **187** (100 mg, 0.79 mmol) in THF (1.6 mL) at -78 °C. After stirring for 15 min at -78 °C, *tert*-butyl(chloro)dimethylsilane (143 mg, 0.95 mmol) and imidazole (108 mg, 1.59 mmol) were added sequentially. The obtained mixture was stirred for 30 min, warmed to RT and then stirred for 14 h. After concentrating under reduced pressure, the residue was redissolved in petrol (2 mL) and water (2 mL). The two layers were separated, the aqueous layer was extracted with petrol (3×2 mL) and the organic extracts were combined, dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 20:1), affording silyl ether **200** (156 mg, 77%) as a pale yellow oil. $R_f = 0.54$ (petrol/ether, 10:1); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 1686m (C=O),

1254m (Si-CH₃), 1094m (Si-O); **¹H NMR** (400 MHz, CDCl₃) δ 3.60 (t, *J* = 7.0, 2H, *H*^{2*}), 2.53 (t, *J* = 7.0, 2H, *H*^{1*}), 2.27 (s, 3H, *H*¹), 1.84 (s, 3H, *H*⁵), 1.78 (s, 3H, *H*^{1*}), 0.88 (s, 9H, *H*^{2*}), 0.03 (s, 6H, *H*^{1*}); **¹³C NMR** (101 MHz, CDCl₃) δ 205.9 (C2), 138.6 (C4), 134.3 (C3), 62.3 (C2^a), 33.6 (C1^a), 30.6 (C1), 26.1 (C2^c), 22.8 (C5), 21.6 (C1^b), 18.5 (C1^c), -5.3 (C1^d); **MS** (ESI⁺): *m/z* 279.2 ([M+Na]⁺, 100%), 257.2 ([M+H]⁺, 40%); **HRMS** (ESI⁺): *m/z* found 279.1743, calcd. for C₁₄H₂₈O₂²³NaSi [M+Na]⁺ 279.1751.

{[4-Methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl]oxy}triethylsilane (201)

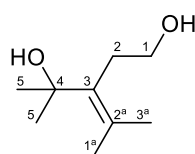


n-BuLi (2.5 M in hexane, 0.47 mL, 1.18 mmol) was added dropwise to a suspension of methyltriphenylphosphonium bromide (418 mg, 1.17 mmol) in THF (1.9 mL) at -78 °C. The mixture was warmed to RT and then stirred for 20 min. After cooling to 0 °C, a solution of silyl ether **199** (100 mg, 0.39 mmol) in THF (0.4 mL) was added and the mixture was stirred for 10 min at 0 °C, warmed to RT, stirred for 1 h at RT and then heated to reflux for 14 h. After cooling to RT, the reaction was quenched by the addition of a satd. aq. solution of NH₄Cl (2 mL). The two layers were separated, the aqueous layer was extracted with ether (3 × 2 mL) and the organic extracts were combined, washed with brine (2 mL), dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. Purification by flash column chromatography (petrol/ether, 100:1) afforded silyl ether **199** (62 mg, 63% recovery) (data as above) and silyl ether **201** (20 mg, 20%) as a pale yellow oil. **R_f** = 0.64 (petrol/ether, 100:1); **IR** (ν_{max}/cm⁻¹): 3075m (=CH₂), 1632w (C=C), 1084s (Si-O), 740s (Si-C); **¹H NMR** (400 MHz, CDCl₃) δ 4.90 (dq, *J* = 3.0, 1.5, 1H, *H*^{1*}), 4.53 (dq, *J* = 3.0, 1.0, 1H, *H*^{1*}), 3.55 (t, *J* = 8.0, 2H, *H*¹), 2.36 (t, *J* = 8.0, 2H, *H*²), 1.76 (dd,

Experimental

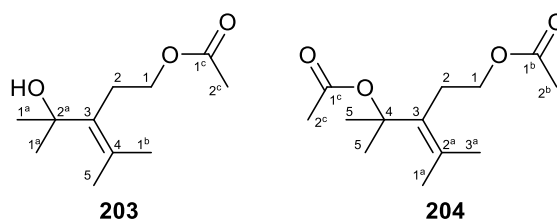
$J = 1.5, 1.0, 3\text{H}, H3^a$), 1.69 (s, 3H, $H5$), 1.66 (s, 3H, $H1^b$), 0.96 (t, $J = 8.0, 9\text{H}, H2^c$), 0.59 (q, $J = 8.0, 6\text{H}, H1^c$); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 146.8 ($\text{C}2^a$), 132.9 ($\text{C}3$), 127.4 ($\text{C}4$), 113.2 ($\text{C}1^a$), 61.7 ($\text{C}1$), 35.0 ($\text{C}2$), 22.7 ($\text{C}3^a$), 21.8 ($\text{C}1^b$), 19.9 ($\text{C}5$), 6.9 ($\text{C}2^c$), 4.6 ($\text{C}1^c$); **MS** (ESI⁺): m/z 293.0 ($[\text{M}+\text{K}]^+$, 100%); **HRMS** (TOF FI⁺): m/z found 254.2061, calcd. for $\text{C}_{15}\text{H}_{30}\text{OSi}$ $[\text{M}]^+$ 254.2066.

4-Methyl-3-(propan-2-ylidene)pentane-1,4-diol (**202**)



MeMgBr (3.0 M in ether, 0.53 mL, 1.59 mmol) was added dropwise to a solution of lactone **187** (100 mg, 0.79 mmol) in THF (1.1 mL) at $-78\text{ }^\circ\text{C}$. After stirring for 30 min at $-78\text{ }^\circ\text{C}$, the acetone/dry ice bath was removed and the mixture was warmed to RT and then stirred for 2 h. The reaction was quenched by the addition of a satd. aq. solution of NH_4Cl (1 mL). The two layers were separated, the aqueous layer was extracted with ether ($7 \times 1\text{ mL}$) and the combined organic extracts were dried over anhydrous MgSO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 1:1), affording diol **202** (83 mg, 66%) as a pale yellow oil. $R_f = 0.08$ (petrol/ether, 2:1); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 3312br (O–H), 1638w (C=C), 1113m (C–O); $^1\text{H NMR}$ (400 MHz, C_6D_6) δ 3.52 (t, $J = 6.0, 2\text{H}, H1$), 2.51 (t, $J = 6.0, 2\text{H}, H2$), 1.68 (s, 3H, $H1^a$), 1.51 (s, 3H, $H3^a$), 1.48 (s, 6H, $H5$); $^{13}\text{C NMR}$ (101 MHz, C_6D_6) δ 139.5 ($\text{C}3$), 126.3 ($\text{C}2^a$), 72.8 ($\text{C}4$), 62.8 ($\text{C}1$), 32.2 ($\text{C}2$), 30.6 ($\text{C}5$), 23.1 ($\text{C}1^a$), 22.8 ($\text{C}3^a$); **MS** (ESI⁺): m/z 181.1 ($[\text{M}+\text{Na}]^+$, 100%); **HRMS** (ESI⁺): m/z found 181.1196, calcd. for $\text{C}_9\text{H}_{18}\text{O}_2^{23}\text{Na}$ $[\text{M}+\text{Na}]^+$ 181.1199.

3-(2-Hydroxypropan-2-yl)-4-methylpent-3-en-1-yl acetate (203) and 4-methyl-3-(propan-2-ylidene)pentane-1,4-diyl diacetate (204)

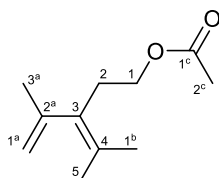


Pyridine (0.21 mL, 2.60 mmol) was added to a solution of diol **202** (83 mg, 0.52 mmol) in DCM (0.7 mL) at RT. After cooling to 0 °C, acetyl chloride (0.11 mL, 1.55 mmol) was added dropwise and the mixture was warmed to RT and then stirred for 1 h. The reaction was quenched by the addition of a satd. aq. solution of NH₄Cl (1 mL). The two layers were separated, the aqueous layer was extracted with ether (5 × 1 mL) and the organic extracts were combined, dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol to petrol/ether, 1:1), affording acetate **203** (59 mg, 56 %) as a pale yellow oil, diacetate **204** (16 mg, 12%) as a pale yellow oil and acetate **205** (1 mg, 1%) (data as below). **Data for 203:** R_f = 0.36 (petrol/ether, 2:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 3468br (O–H), 1738m (C=O), 1238s (C–O); **¹H NMR** (400 MHz, CDCl₃) δ 4.06 (t, J = 8.0, 2H, $H1$), 2.52 (t, J = 8.0, 2H, $H2$), 2.05 (s, 3H, $H2'$), 1.88 (s, 3H, $H5$), 1.73 (s, 3H, $H1^b$), 1.43 (s, 6H, $H1^a$); **¹³C NMR** (101 MHz, CDCl₃) δ 171.2 (C1^c), 134.7 (C3), 130.5 (C4), 73.8 (C2^a), 64.4 (C1), 30.9 (C1^a), 29.6 (C2), 23.2 (C1^b), 23.1 (C5), 21.2 (C2^c); **MS** (ESI⁺): m/z 223.1 ([M+Na]⁺, 100%); **HRMS** (ESI⁺): m/z found 223.1299, calcd. for C₁₁H₂₀O₃²³Na [M+Na]⁺ 223.1305. **Data for 204:** R_f = 0.71 (petrol/ether, 2:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 1732s (C=O), 1236m (C–O), 1138m (C–O); **¹H NMR** (400 MHz, CDCl₃) δ 4.09 (t, J = 8.0, 2H, $H1$), 2.50 (t, J = 8.0, 2H, $H2$), 2.04 (s, 3H, $H2^b$), 1.97 (s, 3H, $H2'$), 1.80 (s, 3H, $H1^a$), 1.72 (s, 3H, $H3^a$), 1.55 (s, 6H, $H5$); **¹³C NMR** (101 MHz, CDCl₃) δ 171.2 (C1^b), 169.4 (C1^c), 131.2 (C3), 130.6 (C2^a), 82.5 (C4), 63.5 (C1), 30.3 (C2), 28.3 (C5), 22.8 (C3^a), 22.3 (C1^a), 21.7 (C2^c), 21.2 (C2^b); **MS** (ESI⁺): m/z 265.1

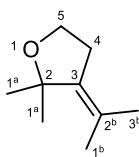
Experimental

([M+Na]⁺, 100%), 507.3 ([2M+Na]⁺, 50%); **HRMS** (ESI⁺): m/z found 265.1403, calcd. for C₁₃H₂₂O₄²³Na [M+Na]⁺ 265.1410.

4-Methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl acetate (**205**)^[112]



MeMgBr (3.0 M in ether, 47.7 mL, 143.0 mmol) was added dropwise to a solution of lactone **187** (8.20 g, 65.0 mmol) in ether (86.7 mL) at -78 °C. After stirring for 30 min at -78 °C, the acetone/dry ice bath was removed and the reaction mixture was warmed to RT and then stirred for 2 h. The mixture was cooled with a water bath and acetyl chloride (13.9 mL, 195.4 mmol) was added dropwise. The water bath was removed and the mixture was stirred for 1 h at RT. The reaction was quenched with a satd. aq. solution of NH₄Cl (60 mL). The two layers were separated, the aqueous layer was extracted with ether (5 × 40 mL) and the organic extracts were combined, dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. Flash column chromatography (petrol/ether, 20:1) afforded acetate **205** (10.70 g, 90%) as a pale yellow oil. R_f = 0.36 (petrol/ether, 20:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 3076w (=CH₂), 1740s (C=O), 1632w (C=C), 1236s (C–O), 1031m (C–O); **¹H NMR** (400 MHz, CDCl₃) δ 4.94 (dq, J = 3.0, 1.5, 1H, $H1^a$), 4.57 (dq, J = 3.0, 1.0, 1H, $H1^b$), 4.03 (t, J = 7.5, 2H, $H1$), 2.42 (t, J = 7.5, 2H, $H2$), 2.03 (s, 3H, $H2^c$), 1.77 (dd, J = 1.5, 1.0, 3H, $H3^a$), 1.70 (s, 3H, $H5$), 1.67 (s, 3H, $H1^b$); **¹³C NMR** (101 MHz, CDCl₃) δ 171.3 (C1^c), 146.0 (C2^a), 131.9 (C3), 128.4 (C4), 113.9 (C1^a), 63.2 (C1), 30.3 (C2), 22.6 (C3^a), 21.9 (C1^b), 21.2 (C2^c), 19.9 (C5); **MS** (ESI⁺): m/z 205.1 ([M+Na]⁺, 100%); **HRMS** (CI⁺): m/z found 183.1377, calcd. for C₁₁H₁₉O₂ [M+H]⁺ 183.1385. Data consistent with the literature.^[112]

2,2-Dimethyl-3-(propan-2-ylidene)tetrahydrofuran (**208**)

- **Method A:**

MeMgBr (3.0 M in ether, 0.58 mL, 1.74 mmol) was added dropwise to a solution of lactone **187** (100 mg, 0.79 mmol) in ether (1 mL) at $-78\text{ }^{\circ}\text{C}$. After stirring for 30 min at $-78\text{ }^{\circ}\text{C}$, the acetone/dry ice bath was removed and the mixture was warmed to RT, stirred for 2 h at RT and cooled to $0\text{ }^{\circ}\text{C}$. Methanesulfonyl chloride (0.18 mL, 2.33 mmol) was added dropwise and the mixture was stirred for 2 h at $0\text{ }^{\circ}\text{C}$, slowly warmed to RT and then stirred for 14 h. The reaction was quenched with a satd. aq. solution of NaHCO_3 (2 mL). The two layers were separated, the aqueous layer was extracted with ether ($5 \times 2\text{ mL}$) and the organic extracts were combined, dried over anhydrous MgSO_4 , filtered and concentrated under reduced pressure, affording tetrahydrofuran **208** (110 mg, 100%) as a pale yellow oil. Tetrahydrofuran **208** was unstable on silica and therefore was not purified. $R_f = 0.89$ (petrol/ether, 2:1); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 1169s (C-O-C), 966m (C-O-C); **$^1\text{H NMR}$** (400 MHz, C_6D_6) δ 3.71 (t, $J = 7.0$, 2H, $H5$), 2.33–2.25 (m, 2H, $H4$), 1.53 (tq, $J = 2.0, 0.5$, 3H, $H1^b$), 1.49 (tq, $J = 1.5, 0.5$, 3H, $H3^b$), 1.43 (s, 6H, $H1^a$); **$^{13}\text{C NMR}$** (101 MHz, C_6D_6) δ 140.2 (C3), 120.4 (C2^b), 80.8 (C2), 64.0 (C5), 33.5 (C4), 26.7 (C1^a), 23.3 (C3^b), 19.9 (C1^b); **HRMS** (TOF FI^+): m/z found 140.1199, calcd. for $\text{C}_9\text{H}_{16}\text{O}$ $[\text{M}]^+$ 140.1201.

- **Method B:**

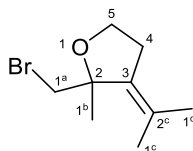
MeMgBr (3.0 M in ether, 0.58 mL, 1.74 mmol) was added dropwise to a solution of lactone **187** (100 mg, 0.79 mmol) in ether (1 mL) at $-78\text{ }^{\circ}\text{C}$. After stirring for 30 min at $-78\text{ }^{\circ}\text{C}$, the acetone/dry ice bath was removed and the reaction mixture was warmed to RT and then

Experimental

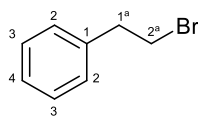
stirred for 2 h. *p*-Toluenesulfonic anhydride (774 mg, 2.37 mmol) was added and the mixture was stirred for 1 h at RT. The reaction was quenched with a satd. aq. solution of NH₄Cl (2 mL). The two layers were separated, the aqueous layer was extracted with ether (5 × 2 mL) and the organic extracts were combined, dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure, affording tetrahydrofuran **208** (100 mg, 91%).

Data as above.

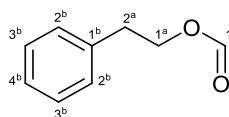
2-(Bromomethyl)-2-methyl-3-(propan-2-ylidene)tetrahydrofuran (**209**)



1,3-Dibromo-5,5-dimethylhydantoin (389 mg, 1.36 mmol) and triphenylphosphine (357 mg, 1.36 mmol) were added sequentially to a solution of alcohol **185** (96 mg, 0.68 mmol) in ACN (1.2 mL) at 0 °C. The mixture was stirred for 1 h at 0 °C, warmed to RT and then stirred for 14 h. The reaction was quenched with a satd. aq. solution of NH₄Cl (1 mL). The two layers were separated, the aqueous layer was extracted with petrol (3 × 1 mL) and the combined organic extracts were dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 10:1), affording bromide **209** (15 mg, 10%) as a pale yellow oil. Bromide **209** was tentatively assigned. R_f = 0.84 (petrol/ether, 10:1); ¹H NMR (200 MHz, CDCl₃) δ 3.88 (app. td, J = 7.0, 2.0, 2H, $H5$), 3.60 (s, 2H, $H1^a$), 2.67–2.48 (m, 2H, $H4$), 1.70 (m, 6H, $H1^c$), 1.48 (s, 3H, $H1^b$).

(2-Bromoethyl)benzene (211)^[218]

1,3-Dibromo-5,5-dimethylhydantoin (480 mg, 1.68 mmol) and triphenylphosphine (440 mg, 1.68 mmol) were added sequentially to a solution of 2-phenylethanol (100 mg, 0.82 mmol) in ACN (1.4 mL) at 0 °C. The mixture was stirred for 1 h at 0 °C, warmed to RT and then stirred for 14 h. The reaction was quenched with a satd. aq. solution of NH₄Cl (1 mL), the aqueous layer was extracted with ether (2 × 1 mL) and the organic extracts were combined, dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol), affording bromide **211** (51 mg, 33%) as a colourless oil. *R_f* = 0.47 (petrol); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 1496m (arC-C), 1453m (arC-C), 1031w (C-Br); **¹H NMR** (400 MHz, CDCl₃) δ 7.41–7.31 (m, 2H, *H*3), 7.33–7.27 (m, 1H, *H*4), 7.29–7.21 (m, 2H, *H*2), 3.61 (t, *J* = 7.5, 2H, *H*2^a), 3.20 (t, *J* = 7.5, 2H, *H*1^a); **¹³C NMR** (101 MHz, CDCl₃) δ 139.0 (C1), 128.8 (C2), 128.7 (C3), 127.1 (C4), 39.6 (C1^a), 33.1 (C2^a); **MS** (ESI⁺): *m/z* 318.2 ([M+NH₄]⁺, 30%).

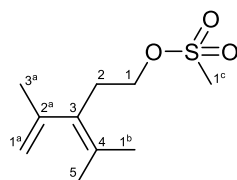
2-Phenylethyl formate (212)^[219]

1,3-Dibromo-5,5-dimethylhydantoin (469 mg, 1.64 mmol) and triphenylphosphine (430 mg, 1.64 mmol) were added sequentially to a solution of 2-phenylethanol (100 mg, 0.82 mmol) in DMF (1.4 mL) at 0 °C and the mixture was stirred for 1 h at 0 °C. The reaction was quenched with a satd. aq. solution of NH₄Cl (1 mL). The two layers were separated, the aqueous layer was extracted with ether (2 × 1 mL) and the organic layers extracts were

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combined, dried over anhydrous MgSO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 2:1 to 1:1), affording 2-phenylethanol (8 mg, 8% recovery) and ester **212** (19 mg, 15%) as a colourless oil. $R_f = 0.94$ (petrol/ether, 2:1); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 1719s (C=O), 1160s (C-O); **$^1\text{H NMR}$** (400 MHz, CDCl_3) δ 8.97 (s, 1H, $H1$), 7.29–7.22 (m, 2H, $H3^b$), 7.18–7.15 (m, 3H, $H2^b$ and $H4^b$), 4.32 (td, $J = 7.0, 1.0, 2\text{H}$, $H1^a$), 2.91 (t, $J = 7.0, 2\text{H}$, $H2^a$); **$^{13}\text{C NMR}$** (101 MHz, CDCl_3) δ 161.1 (C1), 137.5 (C1^b), 129.0 (C3^b), 128.7 (C2^b), 126.9 (C4^b), 64.5 (C1^a), 35.1 (C2^a); **HRMS** (TOF FI⁺): m/z found 150.0680, calcd. for $\text{C}_9\text{H}_{10}\text{O}_2$ $[\text{M}]^+$ 150.0681.

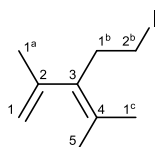
4-Methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl methanesulfonate (**213**)^[124]



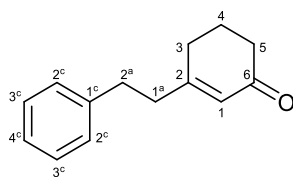
Methanesulfonyl chloride (4.70 mL, 60.7 mmol) was added dropwise to a solution of alcohol **185** (6.10 g, 43.5 mmol) and triethylamine (18.2 mL, 130.6 mmol) in DCM (145 mL mL) at 0 °C. The mixture was stirred for 2 h at 0 °C and then, the reaction was quenched with a satd. aq. solution of NaHCO_3 (80 mL). The two layers were separated, the aqueous layer was extracted with ether (5×50 mL) and the organic extracts were combined, dried over anhydrous MgSO_4 , filtered and concentrated under reduced pressure. Mesylate **213** (8.97 g, 94%) was obtained as a dark orange oil and was used without further purification. $R_f = 0.38$ (petrol/ether, 2:1); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 1352s (SO_2), 1171s (SO_2); **$^1\text{H NMR}$** (400 MHz, CDCl_3) δ 4.99 (dq, $J = 3.0, 1.5, 1\text{H}$, $H1^a$), 4.59 (dq, $J = 3.0, 1.0, 1\text{H}$, $H1^a$), 4.17 (t, $J = 7.5, 2\text{H}$, $H1$), 2.98 (s, 3H, $H1^c$), 2.56 (t, $J = 7.5, 2\text{H}$, $H2$), 1.77 (dd, $J = 1.5, 1.0, 3\text{H}$, $H3^a$), 1.71 (s, 3H, $H5$), 1.69 (s, 3H, $H1^b$); **$^{13}\text{C NMR}$** (101 MHz, CDCl_3) δ 145.4 (C2^a), 130.5 (C3), 129.9 (C4), 114.6 (C1^a), 68.4 (C1), 37.6 (C1^c), 30.8 (C2), 22.5 (C3^a), 21.9 (C1^b), 20.0 (C5);

MS (ESI⁺): m/z 241.1 ([M+Na]⁺, 100%), 219.1 ([M+H]⁺, 68%); **HRMS** (ESI⁺): m/z found 219.1051, calcd. for C₁₀H₁₉O₃³²S [M+H]⁺ 219.1049. Data consistent with the literature.^[124]

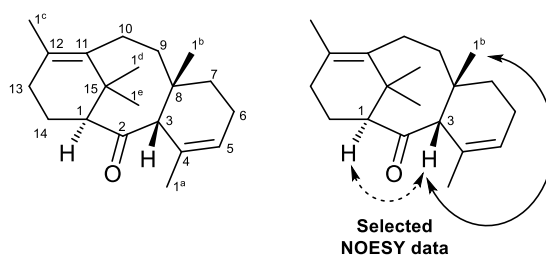
3-(2-Iodoethyl)-2,4-dimethylpenta-1,3-diene (214)^[124]



A mixture of mesylate **213** (8.38 g, 38.4 mmol) and NaI (17.27 g, 115.2 mmol) in acetone (80 mL) was heated to reflux for 4 h. The mixture was cooled to RT and then, the solvent was removed by reduced pressure. The residue was redissolved in ether (80 mL) and washed with brine (3 × 50 mL), dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol), affording iododiene **214** (8.03 g, 84%) as a yellow oil. R_f = 0.86 (petrol); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 1632w (C=C); **¹H NMR** (400 MHz, CDCl₃) δ 4.97 (dq, J = 3.0, 1.5, 1H, $H1$), 4.62 (dq, J = 3.0, 1.0, 1H, $H1$), 3.12 (t, J = 8.0, 2H, $H2^b$), 2.67 (t, J = 8.0, 2H, $H1^b$), 1.76 (dd, J = 1.5, 1.0, 3H, $H1^a$), 1.68 (s, 3H, $H5$), 1.66 (s, 3H, $H1^c$); **¹³C NMR** (101 MHz, CDCl₃) δ 145.1 (C2), 135.6 (C3), 128.5 (C4), 114.4 (C1), 35.6 (C1^b), 22.7 (C1^a), 22.0 (C1^c), 20.0 (C5), 4.8 (C2^b); **HRMS** (TOF FI⁺): m/z found 250.0218, calcd. for C₉H₁₅I [M]⁺ 250.0219. Data consistent with the literature.^[124]

3-(2-Phenylethyl)cyclohex-2-en-1-one (216)^[220]

A solution of 1-bromo-2-phenylethane (180 μ L, 1.30 mmol) in THF (0.3 mL) was added dropwise to a suspension of magnesium turnings (41 mg, 1.69 mmol) and a small crystal of iodine in THF (0.9 mL) at RT. After stirring for 30 min at RT, the mixture was cooled to -78 $^{\circ}$ C and a solution of 3-ethoxycyclohex-2-en-1-one (100 mg, 0.71 mmol) in THF (0.3 mL) was added. The mixture was stirred for 30 min at -78 $^{\circ}$ C, the acetone/dry ice bath was removed and the mixture was warmed to RT and then stirred for 3 h. The reaction was quenched with aq. HCl (1.0 M, 2 mL). The two layers were separated, the aqueous layer was extracted with ether (5×1 mL) and the organic extracts were combined, dried over anhydrous MgSO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 1:1), affording enone **216** (109 mg, 76%) as a yellow oil. $R_f = 0.51$ (petrol/ether, 1:1); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 3061w (arC–H), 3027w (=CH), 1664s (C=O), 1625w (C=C), 1603w (arC–C); **$^1\text{H NMR}$** (400 MHz, CDCl_3) δ 7.34–7.27 (m, 2H, $H3^b$), 7.25–7.16 (m, 3H, $H2^b$ and $H4^b$), 5.90 (quin, $J = 1.5$, 1H, $H2$), 2.82 (t, $J = 8.0$, 2H, $H2^a$), 2.53 (t, $J = 8.0$, 2H, $H1^a$), 2.36 (t, $J = 6.5$, 2H, $H6$), 2.30 (t, $J = 6.5$, 2H, $H4$), 1.99 (quin, $J = 6.5$, 2H, $H5$); **$^{13}\text{C NMR}$** (101 MHz, CDCl_3) δ 200.0 (C1), 165.5 (C3), 140.8 (C1^b), 128.7 (C3^b), 128.4 (C2^b), 126.4 (C4^b), 126.1 (C2), 39.8 (C1^a), 37.5 (C6), 33.5 (C2^a), 30.0 (C4), 22.8 (C5); **MS** (ESI⁺): m/z 223.1 ($[\text{M}+\text{Na}]^+$, 100%), 201.1 ($[\text{M}+\text{H}]^+$, 90%), 423.2 ($[\text{2M}+\text{Na}]^+$, 60%); **HRMS** (CI⁺): m/z found 201.1274, calcd. for $\text{C}_{14}\text{H}_{17}\text{O}$ $[\text{M}+\text{H}]^+$ 201.1273. Data consistent with the literature.^[220]

(1*S,3*R**,8*S**)-4,8,12,15,15-Pentamethyltricyclo[9.3.1.0^{3,8}]pentadec-4,11-diene-2-one (220)**

- **Method A:**

A solution of diketone **179b** (30 mg, 0.10 mmol) in THF (1.5 mL) was added dropwise to a solution of *N*-phenyl-bis(trifluoromethanesulfonylimide) (77 mg, 0.22 mmol) in THF (1.0 mL) at 0 °C. Subsequently, potassium bis(trimethylsilyl)amide (0.5 M in toluene, 0.34 mL, 0.17 mmol) was added dropwise at 0 °C. The mixture was stirred for 1 h at 0 °C and then, poured into a cold satd. aq. solution of NaHCO₃ (4 mL) and diluted with hexane (4 mL). The two layers were separated and the organic layer was washed sequentially with aq. KOH (3.0 M, 4 × 3 mL), water (2 × 3 mL), and brine (3 mL), dried over MgSO₄, filtered and concentrated under reduced pressure, affording a mixture of triflate and diketone **179b** (173 mg). The triflate was unstable and therefore the crude product was used without further purification. The crude product (173 mg) was redissolved in THF (1.1 mL), cooled to 0 °C and then a solution of tetrakis(triphenyl-phosphine)palladium(0) (6 mg, 5 μmol) in THF (0.3 mL) was added. After stirring for 5 min at 0 °C, dimethylzinc (2.0 M in toluene, 0.27 mL, 0.54 mmol) was added dropwise and stirring continued for an additional 5 min at 0 °C. The cooling bath was removed and the mixture was warmed to RT and then stirred for 5 h. The reaction was quenched with aq. HCl (1.0 M, 2 mL). The two layers were separated, the aqueous layer was extracted with hexane (2 × 2 mL) and the organic extracts were combined, washed with brine (2 × 2 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (hexane/EA, 15:1), affording diketone **179b** (8 mg, 27% recovery) (data as

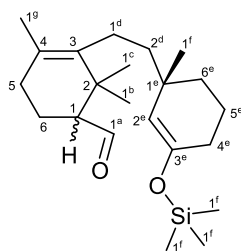
Experimental

above) and di-*epi*-taxadienone **220** (10 mg, 31%) as a white solid. $R_f = 0.33$ (hexane/EA, 40:1); **m.p.** 73–84 °C; **IR** ($\nu_{\max}/\text{cm}^{-1}$): 1683s (C=O), 1658w (C=C), 1441m (CH₃), 1440m (CH₃); **¹H NMR** (500 MHz, CDCl₃) δ 5.54(s, 1H, H5), 2.95 (s, 1H, H3), 2.83 (td, $J = 13.5$, 5.5, 1H, H10), 2.53–2.48 (m, 1H, H13), 2.46 (dd, $J = 5.5$, 2.5, 1H, H1), 2.16–2.08 (m, 2H, H10 and H13), 2.09–1.95 (m, 4H, H6 and H14), 1.85 (s, 3H, H1'), 1.85–1.75 (m, 1H, H7), 1.78–1.71 (m, 1H, H9), 1.55 (app. q, $J = 2.0$, 3H, H1^a), 1.28 (s, 3H, H1^d), 1.17 (ddd, $J = 15.0$, 5.5, 2.5, 1H, H9), 1.11 (s, 3H, H1^c), 0.92 (s, 3H, H1^b), 0.89–0.86 (m, 1H, H7); **¹³C NMR** (126 MHz, CDCl₃) δ 218.0 (C2), 136.8 (C11), 131.2 (C4), 129.9 (C12), 123.9 (C5), 62.8 (C1), 57.1 (C3), 40.5 (C9), 37.9 (C8), 37.8 (C15), 33.0 (C7), 30.0 (C1^c), 28.7 (C13), 25.1 (C1^d), 25.0 (C10), 24.8 (C1^a), 23.6 (C1^b), 22.2 (C6), 22.1 (C1^e), 18.4 (C14); **MS** (ESI⁺): m/z 309.1 ([M+Na]⁺, 100%), 287.2 ([M+H]⁺, 21%), 295.5 ([2M+Na]⁺, 13%); **HRMS** (ESI⁺): m/z found 309.2190, calcd. for C₂₀H₃₀O²³Na [M+Na]⁺ 309.2188.

- **Method B:**

A solution of enone **232** as an inseparable mixture of two diastereomers **232a** and **232b** (73 mg, 0.25 mmol, **232a:232b** = 2:1) in DCM (4.0 mL) was added to a solution of BF₃·OEt₂ (120 μ L, 0.97 mmol) in DCM (16.4 mL) at 0 °C. The mixture was stirred for 1 h at 0 °C and then poured into a satd. aq. solution of NaHCO₃ (20 mL). The two layers were separated, the aqueous layer was extracted with DCM (3 \times 10 mL) and the organic extracts were combined, washed sequentially with water (10 mL) and brine (10 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/EA, 36:1), affording di-*epi*-taxadienone **220** (31 mg, 42%) as a white solid. Data as above.

**2,2,4-Trimethyl-3-(2-{1-methyl-3-[(trimethylsilyl)oxy]cyclohex-2-en-1-yl}ethyl)
cyclohex-3-ene-1-carbaldehyde (**221**)**

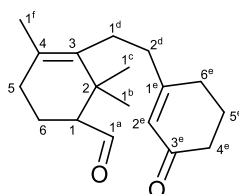


A suspension of pre-dried LiCl (under high vacuum at 100 °C for 14h) (4 mg, 94 μ mol) and CuI (9 mg, 47 μ mol) in THF (1.8 mL) was stirred for 10 min at RT. After cooling to -78 °C, chlorotrimethylsilane (200 μ L, 1.58 mmol) and a solution of enone-carbaldehyde **222** (83 mg, 0.30 mmol) in THF (0.3 mL) were added sequentially and dropwise. The mixture was stirred for 10 min at -78 °C, MeMgBr (3.0 M in ether, 110 μ L, 0.33 mmol) was added dropwise and stirring continued for an additional 20 min at -78 °C. The reaction was quenched at this temperature with anhydrous trimethylamine (0.57 mL, 4.09 mmol) injected in all at once. The mixture was warmed to RT and then diluted with pentane (2 mL), filtered through cotton wool and the residues were washed with pentane (2 mL). The filtrate was washed with a satd. aq. solution of NaHCO₃ (2 \times 2 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product (105 mg) was a pale yellow oil consisting in a mixture of enone-carbaldehyde **222** (33% recovery) (data as below) and TMS-enol ether **221** (67%) as a mixture of two diastereomers: **221a** and **221b** (**221a**:**221b** = 1:1). The crude product was used without further purification and yields were calculated from the ¹H-NMR spectrum of the crude product. **Data for both diastereomers 221a and 221b:** R_f = 0.79 (petrol/ether, 2:1); **IR** (ν_{\max} /cm⁻¹): 1717w (C=O), 1660w (C=C), 1249m (Si-CH₃); **¹H NMR** (500 MHz, CDCl₃) δ 9.83 (d, J = 3.0, 2H, $H1^a$) 4.69 (q, J = 1.5, 2H, $H2^a$), 2.15 (app. dtd, J = 10.0, 3.0, 1.5, 2H, $H1$), 2.03–1.97 (m, 8H, $H5$ and $H1^b$), 1.97–1.92 (m, 4H, $H4^b$), 1.88–1.71 (m, 4H, $H6$), 1.71–1.65 (m, 4H,

Experimental

H5^e), 1.61 (s, 6H, H1^f), 1.45 (app. q, $J = 6.5$, 2H, H6^e), 1.37–1.28 (m, 4H, H2^a), 1.29–1.24 (m, 2H, H6^e), 1.19 (s, 3H, H1^b), 1.18 (s, 3H, H1^b), 1.05 (s, 3H, H1^c), 1.04 (s, 3H, H1^c), 0.99 (s, 6H, H1^f), 0.18 (s, 18H, H1^h); **¹³C NMR** (126 MHz, CDCl₃) δ 207.03 (C1^a), 207.01 (C1^a), 149.68 (C3^e), 136.73 (C3), 127.14 (C4), 114.05 (C2^e), 57.86 (C1), 57.85 (C1), 43.30 (C2^d), 43.29 (C2^d), 37.38 (C1^c), 37.34 (C1^c), 35.14 (C2), 34.56 (C6^e), 30.63 (C5), 30.59 (C5), 30.09 (C4^e), 30.08 (C4^e), 28.10 (C1^f), 28.09 (C1^b), 28.01 (C1^b), 23.92 (C1^e), 23.88 (C1^e), 22.91 (C1^d), 22.89 (C1^d), 20.01 (C1^e), 19.98 (C6), 19.82 (C5^e), 0.52 (C1^h); **MS** (ESI⁺): m/z 385.3 ([M+Na]⁺, 100%); **HRMS** (CI⁺): m/z found 363.2715, calcd. for C₂₂H₃₉O₂²⁸Si [M+H]⁺ 363.2713.

2,2,4-Trimethyl-3-[2-(3-oxocyclohex-1-en-1-yl)ethyl]cyclohex-3-ene-1-carbaldehyde (**222**)

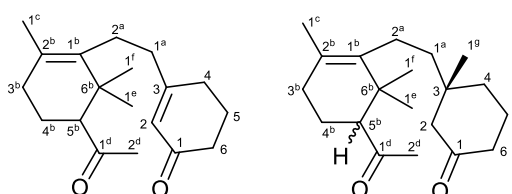


Acrolein (192 μ L, 2.87 mmol) was added to a solution of enone **177** (180 mg, 0.82 mol) in DCM (3.3 mL) at RT. After cooling to -78 °C, BF₃·OEt₂ (0.30 mL, 2.42 mmol) was added dropwise and the mixture was stirred for 2 h at -78 °C. The reaction was quenched at this temperature with a satd. aq. solution of NaHCO₃ (3 mL) and then warmed to RT. The two layers were separated, the aqueous layer was extracted with ether (2 \times 2 mL) and the organic layers were combined, washed with a satd. aq. solution of NaHCO₃ (2 \times 2 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 2:1), affording enone-carbaldehyde **222** (103 mg, 46%) as a colourless oil. $R_f = 0.35$ (petrol/ether, 1:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 1716m (C=O), 1666s (C=O), 1623w (C=C); **¹H NMR** (500 MHz, CDCl₃) δ 9.84 (d, $J = 3.0$, 1H, H1^a), 5.92 (quin, $J = 1.5$, 1H, H2^a), 2.38 (t, $J = 6.5$, 2H, H4^a), 2.33 (td, J

= 6.0, 1.5, 2H, $H6^c$), 2.30–2.23 (m, 2H, $H2^d$), 2.23–2.16 (m, 3H, $H1$ and $H1^d$), 2.06–1.98 (m, 4H, $H5$ and $H5^c$), 1.85 (dddd, $J = 14.0, 6.0, 5.0, 3.0$, 1H, $H6$), 1.78–1.72 (m, 1H, $H6$), 1.64 (s, 3H, $H1^f$), 1.22 (s, 3H, $H1^b$), 1.06 (s, 3H, $H1^c$); $^{13}\text{C NMR}$ (126 MHz, CDCl_3) δ 206.3 (C1^a), 200.1 (C3^c), 166.3 (C1^c), 135.4 (C3), 128.8 (C4), 125.4 (C2^c), 57.6 (C1), 38.6 (C2^d), 37.5 (C4^c), 37.3 (C2), 30.7 (C5), 29.9 (C6^c), 27.8 (C1^b), 26.0 (C1^d), 23.7 (C1^c), 22.9 (C5^c), 20.0 (C1^f), 19.8 (C6); **MS** (ESI^+): m/z 297.1 ($[\text{M}+\text{Na}]^+$, 100%), 275.2 ($[\text{M}+\text{H}]^+$, 31%); **HRMS** (ESI^+): m/z found 297.1824, calcd. for $\text{C}_{18}\text{H}_{26}\text{O}_2^{23}\text{Na}$ $[\text{M}+\text{Na}]^+$ 297.1825.

3-[2-(5-Acetyl-2,6,6-trimethylcyclohex-1-en-1-yl)ethyl]cyclohex-2-en-1-one (223) and

3-{2-[5-acetyl-2,6,6-trimethylcyclohex-1-en-1-yl]ethyl}-3-methylcyclohexan-1-one (224)



223

224

- **Method A:**

CuI (12 mg, 63 μmol) and dimethyl sulfide (200 μL , 2.72 mmol) were added sequentially to a solution of enone-carbaldehyde **222** (76 mg, 0.28 mmol) in THF (2.0 mL) at RT. After cooling to $-78\text{ }^\circ\text{C}$, MeMgBr (3.0 M in ether, 100 μL , 0.30 mmol) was added dropwise. The mixture was slowly warmed to RT and then stirred for 14 h. The reaction was quenched with a satd. aq. solution of NH_4Cl (2 mL). The two layers were separated, the aqueous layer was extracted with ether ($3 \times 2\text{ mL}$) and the organic extracts were combined, washed sequentially with a satd. aq. solution of NaHCO_3 (2 mL) and brine (2 mL), dried over MgSO_4 , filtered and concentrated under reduced pressure. The crude product was used without further purification. The crude product was redissolved in DCM (1.4 mL) and NaHCO_3 (235 mg, 2.80 mmol) and Dess-Martin periodinane (144 mg, 0.34 mmol) were

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added sequentially at RT. The mixture was stirred for 20 min at RT and then diluted with ether (2 mL). The reaction was quenched by the sequential addition of a satd. aq. solution of $\text{Na}_2\text{S}_2\text{O}_3$ (2 mL) and a satd. aq. solution of NaHCO_3 (2 mL) and the obtained mixture was stirred for 20 min at RT. The two layers were separated, the aqueous layer was extracted with ether (4×5 mL) and the organic extracts were combined, dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (hexane/EA, 3:1), affording keto-enone **223** (23 mg, 28% from enone-carbaldehyde **222**) as a colourless oil and diketone **224** (31 mg, 36% from enone-carbaldehyde **222**) as an inseparable mixture of two diastereomers **224a** and **224b** (**224a:224b** = 1:1) as a colourless oil. **Data for 223:** R_f = 0.22 (hexane/EA, 3:1); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 1707m (C=O), 1667s (C=O), 1624w (C=O); **$^1\text{H NMR}$** (400 MHz, CDCl_3) δ 5.91 (t, J = 1.5, 1H, H_2), 2.54 (dd, J = 10.5, 3.5, 1H, H_5^b), 2.37 (t, J = 6.0, 2H, H_6), 2.33 (td, J = 6.0, 1.5, 2H, H_4), 2.29–2.23 (m, 2H, H_2^a), 2.18 (d, J = 0.5, 3H, H_2^d), 2.17–2.14 (m, 2H, H_1^a), 2.02–1.95 (m, 4H, H_5 and H_3^b), 1.84–1.76 (m, 1H, H_4^b), 1.76–1.70 (m, 1H, H_4^b), 1.61 (s, 3H, H_1^c), 1.12 (s, 3H, H_1^c), 1.01 (s, 3H, H_1^f); **$^{13}\text{C NMR}$** (101 MHz, CDCl_3) δ 212.6 (C_1^d), 200.0 (C1), 166.6 (C3), 135.6 (C_1^b), 127.8 (C_2^b), 125.4 (C2), 58.6 (C_5^b), 38.6 (C_2^a), 37.9 (C_6^b), 37.5 (C6), 31.8 (C_2^d), 31.3 (C_3^b), 29.9 (C4), 27.8 (C_1^e), 26.1 (C_1^a), 23.3 (C_1^f), 22.9 (C5), 22.1 (C_4^b), 20.0 (1^g); **MS** (ESI⁺): m/z 311.2 ($[\text{M}+\text{Na}]^+$, 100%), 289.2 ($[\text{M}+\text{H}]^+$, 58%), 599.4 ($[\text{2M}+\text{Na}]^+$, 50%); **HRMS** (ESI⁺): m/z found 289.2162, calcd. for $\text{C}_{19}\text{H}_{29}\text{O}_2$ $[\text{M}+\text{H}]^+$ 289.2162. **Data for both diastereomers 224a and 224b:** R_f = 0.28 (hexane/EA, 3:1); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 1709s (C=O); **$^1\text{H NMR}$** (400 MHz, CDCl_3) δ 2.52 (dd, J = 11.5, 2.5, 2H, H_5^b), 2.32–2.26 (m, 4H, H_6), 2.25–2.20 (m, 2H, H_2), 2.17 (s, 6H, H_2^d), 2.15–2.10 (m, 2H, H_2), 2.03–1.92 (m, 4H, H_3^b), 1.92–1.85 (m, 8H, H_5 and H_2^b), 1.84–1.73 (m, 2H, H_4^b), 1.73–1.62 (m, 6H, H_4 and H_4^b), 1.51 (s, 6H, H_1^c), 1.38–1.30 (m, 4H, H_1^a), 1.07 (s, 6H, H_1^c), 0.98 (s, 6H, H_1^f), 0.97 (s, 6H, H_1^f); **$^{13}\text{C NMR}$** (101 MHz, CDCl_3) δ 213.0 (C_1^d), 212.3 (C1), 136.29 (C_1^b), 136.28 (C_1^b), 126.8 (C_2^b), 126.7 (C_2^b), 58.8 (C_5^b), 58.7(C_5^b), 53.7 (C2), 53.6 (C2),

41.58 (C1^a), 41.55 (C1^a), 41.2 (C6), 39.3 (C3), 38.02 (C6^b), 38.00 (C6^b), 35.9 (C4), 31.91 (C2^d), 31.89 (C2^d), 31.5 (C3^b), 27.81 (C1^e), 27.79 (C1^e), 24.81 (C1^f), 24.78 (C1^f), 23.38 (C1^f), 23.36 (C1^f), 22.4 (C2^b), 22.1 (C4^b), 22.0 (C5), 20.0 (C1^g), 19.9 (C1^g); **MS** (ESI⁺): m/z 327.2 ([M+Na]⁺, 100%), 305.3 ([M+H]⁺, 21%); **HRMS** (ESI⁺): m/z found 327.2294, calcd. for C₂₀H₃₂O₂²³Na [M+Na]⁺ 327.2294.

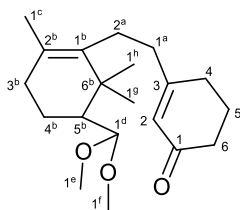
- **Method B:**

MeMgBr (3.0 M in ether, 190 μ L, 0.57 mol) was added dropwise to a suspension of CuI (145 mg, 0.76 mmol) and dimethyl sulfide (160 μ L, 2.18 mmol) in THF (0.8 mL) at -78 °C. After stirring for 30 min at -78 °C, a solution of enone-carbaldehyde **222** (103 mg, 0.38 mmol) in THF (0.4 mL) was added dropwise. The mixture was slowly warmed to RT and then stirred for 14 h. The reaction was quenched by the addition of a satd. aq. solution of NH₄Cl (1 mL). The two layers were separated, the aqueous was extracted with ether (3 \times 2 mL) and the organic extracts were combined, washed sequentially with a satd. aq. solution of NaHCO₃ (2 mL) and brine (2 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product (255 mg) was used without further purification. The crude product was redissolved in DCM (1.9 mL) and NaHCO₃ (319 mg, 3.80 mmol) and Dess-Martin periodinane (195 mg, 0.46 mmol) were added sequentially at RT. the mixture was stirred for 20 min at RT and then diluted with ether (2 mL). The reaction was quenched by the addition of a satd. aq. solution of Na₂S₂O₃ (2 mL) and a satd. aq. solution of NaHCO₃ (2 mL). The obtained mixture was stirred for 20 min at RT. The two layers were separated, the aqueous layer was extracted with ether (4 \times 5 mL) and the organic extracts were combined, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (hexane/EA, 3:1), affording keto-enone **223** (15 mg, 13% from enone-carbaldehyde **222**), and diketone

Experimental

224 (14 mg, 12% from enone-carbaldehyde **222**) as an inseparable mixture of two diastereomers **224a** and **224b** (**224a:224b** = 1:1). Data as above.

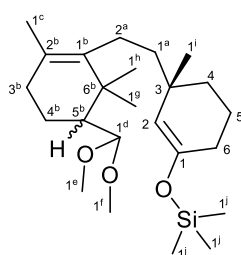
3-{2-[5-(Dimethoxymethyl)-2,6,6-trimethylcyclohex-1-en-1-yl]ethyl}cyclohex-2-en-1-one (**225**)



t-BuLi (1.7 M in pentane, 2.04 mL, 3.47 mmol) was added dropwise to a solution of iodide **229** (583 mg, 1.66 mmol) in freshly distilled ether (6.3 mL) at -78 °C and the mixture was stirred for 15 min at -78 °C. The cooling bath was removed, the mixture was warmed to RT, stirred for 1 h at this temperature and then cooled to 0 °C. 3-Ethoxycyclohex-2-en-1-one (480 μ L, 3.30 mmol) was added dropwise at 0 °C and the mixture was stirred for 30 min at 0 °C, warmed to RT and then stirred for 3 h. The reaction was quenched with a satd. aq. solution of NH_4Cl (5 mL). The two layers were separated, the aqueous layer was extracted with ether (4×5 mL) and the organic extracts were combined, dried over anhydrous MgSO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 4:1 to 2:1), affording enone-acetal **225** (318 mg, 60 %) as a colourless oil. R_f = 0.25 (petrol/ether, 2:1); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 1668s (C=O), 1624w (C=C), 1071m (C-O-C), 1050m (C-O-C); **$^1\text{H NMR}$** (500 MHz, C_6D_6) δ 6.08 (s, 1H, H_2), 4.26 (d, J = 4.5, 1H, H_{1^d}), 3.20 (s, 3H, H_{1^e}), 3.16 (s, 3H, H_{1^f}), 2.16 (t, J = 6.5, 2H, H_6), 2.09–1.98 (m, 5H, H_{1^a} , H_{2^a} and H_{4^b}), 1.94 (dt, J = 10.0, 6.0, 1H, H_{3^b}), 1.89–1.82 (m, 1H, H_{3^b}), 1.72 (td, J = 6.5, 1.5, 2H, H_4), 1.69 (dd, J = 4.5, 2.0, 1H, H_{5^b}), 1.67–1.60 (m, 1H, H_{4^b}), 1.47 (quin, J = 6.5, 2H, H_5), 1.47 (s, 3H, H_{1^g}), 1.16 (s, 3H, H_{1^h}), 1.01 (s, 3H, H_{1^i}); **$^{13}\text{C NMR}$** (126 MHz, C_6D_6) δ 197.6 (C1), 164.2 (C3), 136.8 (C1^b), 128.4

(C2^b), 125.8 (C2), 106.5 (C1^d), 53.9 (C1^f), 53.5 (C1^e), 47.9 (C5^b), 38.9 (C1^a), 38.0 (C6^b), 37.7 (C6), 32.6 (C3^b), 29.5 (C4), 27.8 (C1^g), 26.7 (C2^a), 23.0 (C5), 22.5 (C1^h), 20.3 (C4^b), 20.2 (C1^c); **MS** (ESI⁺): m/z 343.3 ([M+Na]⁺, 100%); **HRMS** (ESI⁺): m/z found 343.2243, calcd. for C₂₀H₃₂O₃²³Na [M+Na]⁺ 343.2243.

3-Methyl-3-{2-[5-dimethoxymethyl-2,6,6-trimethylcyclohex-1-en-1-yl]ethyl}-1-[(trimethylsilyl)oxy]cyclohex-1-ene (226)

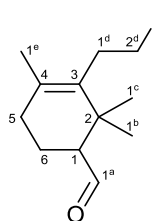


A suspension of pre-dried LiCl (under high vacuum at 100 °C for 14h) (13 mg, 0.31 mmol) and CuI (29 mg, 0.15 mmol) in THF (3.6 mL) was stirred for 10 min at RT. After cooling to -78 °C, chlorotrimethylsilane (0.67 mL, 5.28 mmol) and a solution of enone-acetal **225** (318 mg, 0.99 mmol) in THF (3.5 mL) were added sequentially and dropwise. The mixture was stirred for 10 min at -78 °C, MeMgBr (3.0 M in ether, 0.36 mL, 1.08 mmol) was added dropwise and stirring continued for an additional 20 min at -78 °C. The reaction was quenched at this temperature with anhydrous trimethylamine (1.70 mL, 12.2 mmol) injected in all at once. The mixture was warmed to RT and then diluted with pentane (5 mL), filtered through cotton wool and the residues were washed with pentane (5 mL). The filtrate was washed with a satd. aq. solution of NaHCO₃ (2 × 5 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. TMS-enol ether **226** (380 mg, 94%) was afforded as an inseparable mixture of two diastereomers: **226a** and **226b** (**226a**:**226b** = 1:1) as a yellow oil. The crude mixture was used without further purification. **Data for both diastereomers 226a and 226b**: R_f = 0.86 (petrol/ether, 2:1); **IR** (ν_{\max} /cm⁻¹): 2830w (C-H),

Experimental

1660w (C=C), 1262w (Si-CH₃), 839s (C=C); **¹H NMR** (500 MHz, C₆D₆) δ 4.92 (q, *J* = 1.5, 2H, H₂), 4.30 (d, *J* = 4.5, 2H, H_{1^d}), 3.20 (s, 3H, H_{1^e}), 3.19 (s, 3H, H_{1^f}), 3.16 (s, 3H H_{1^f}), 3.15 (s, 3H H_{1^f}), 2.28–2.10 (m, 4H, H_{2^a}), 2.05 (app. dt, *J* = 6.5, 1.5, 8H, H₆ and H_{3^b}), 2.03–1.97 (m, 2H, H_{4^b}), 1.97–1.88 (m, 2H, H_{4^b}), 1.77 (app. ddd, *J* = 11.5, 4.5, 2.0, 2H, H_{5^b}), 1.75–1.64 (m, 2H, H₅), 1.65 (s, 6H, H_{1^e}), 1.65–1.59 (m, 2H, H₅), 1.60–1.47 (m, 4H, H_{1^e}), 1.30 (s, 6H, H_{1ⁱ}), 1.30–1.25 (m, 2H, H₄), 1.14 (s, 6H, H_{1^g}), 1.10 (s, 6H, H_{1^h}), 0.24 (s, 9H, H_{1ⁱ}), 0.23 (s, 9H, H_{1ⁱ}); **¹³C NMR** (126 MHz, C₆D₆) δ 150.3 (C₁), 150.2 (C₁), 138.0 (C_{1^b}), 137.9 (C_{1^b}), 127.4 (C_{2^b}), 113.5 (C₂), 113.4 (C₂), 106.7 (C_{1^d}), 106.6 (C_{1^d}), 53.9 (C_{1^f}), 53.5 (C_{1^e}), 53.4 (C_{1^e}), 48.1 (C_{5^b}), 48.0 (C_{5^b}), 44.1 (C_{1^a}), 44.0 (C_{1^a}), 38.2 (C_{6^b}), 35.5 (C₃), 35.4 (C₃), 35.0 (C₄), 34.9 (C₄), 32.8 (C_{4^b}), 30.6 (C_{3^b}), 30.5 (C_{3^b}), 28.4 (C_{1^h}), 28.2 (C_{1ⁱ}), 28.1 (C_{1ⁱ}), 23.8 (C_{2^a}), 22.9 (C_{1^g}), 20.5 (C₆), 20.4 (C_{1^e}), 20.3 (C_{1^e}), 20.1 (C₅), 0.6 (C_{1ⁱ}); **MS** (ESI⁺): *m/z* 431.3 ([M+Na]⁺, 72%); **HRMS** (ESI⁺): *m/z* found 431.2952, calcd. for C₂₄H₄₄O₃²³NaSi [M+Na]⁺ 431.2951.

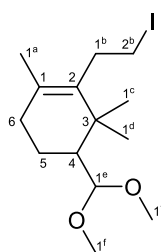
3-(2-Iodoethyl)-2,2,4-trimethylcyclohex-3-ene-1-carbaldehyde (228)



Acrolein (0.56 mL, 8.38 mmol) was added to a solution of iododiene **214** (600 mg, 2.40 mmol) in DCM (9.6 mL) at RT. After cooling to –78 °C, BF₃·OEt₂ (0.89 mL, 7.21 mmol) was added dropwise and the reaction mixture was stirred for 2 h at –78 °C. The reaction was quenched at –78 °C with a satd. aq. solution of NaHCO₃ (6 mL) and the mixture was warmed to RT. The two layers were separated, the aqueous layer was extracted with ether (3 × 3 mL) and the organic extracts were combined, washed with a satd. aq. solution of

NaHCO₃ (2 × 4 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 9:1) affording aldehyde **228** (677 mg, 92%) as a pale yellow oil. $R_f = 0.48$ (petrol/ether, 9:1); **IR** ($\nu_{\max} / \text{cm}^{-1}$): 1717s (C=O), 1671m (C=C); **¹H NMR** (400 MHz, CDCl₃) δ 9.76 (d, $J = 3.0$, 1H, $H1^a$), 3.12–2.90 (m, 2H, $H2^b$), 2.68–2.49 (m, 2H, $H1^d$), 2.11 (dt, $J = 10.5, 3.0$, 1H, $H1$), 1.92 (t, $J = 6.5$, 2H, $H5$), 1.83–1.71 (m, 1H, $H6$), 1.71–1.60 (m, 1H, $H6$), 1.57 (s, 3H, $H1^c$), 1.15 (s, 3H, $H1^b$), 0.98 (s, 3H, $H1^e$); **¹³C NMR** (101 MHz, CDCl₃) δ 205.9 (C1^a), 137.2 (C3), 130.8 (C4), 57.4 (C1), 36.9 (C2), 34.4 (C1^d), 30.7 (C5), 27.8 (C1^b), 23.5 (C1^e), 20.3 (C1^c), 19.6 (C6), 4.1 (C2^d); **HRMS** (EI⁺): m/z found 306.0489, calcd. for C₁₂H₁₉IO [M]⁺ 306.0481.

4-(Dimethoxymethyl)-2-(2-iodoethyl)-1,3,3-trimethylcyclohex-1-ene (**229**)

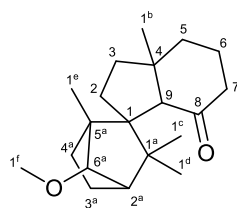


Trimethylsilyl trifluoromethanesulfonate (15 μL , 83 μmol) and methoxytrimethylsilane (230 μL , 1.67 mmol) were added sequentially to a solution of aldehyde **228** (250 mg, 0.82 mmol) in DCM (4.6 mL) at -78 °C. After stirring for 1.5 h at -78 °C, the reaction was quenched at this temperature by the addition of pyridine (1 mL). The mixture was poured into a satd. aq. solution of NaHCO₃ (3 mL) and the two layers were separated. The aqueous layer was extracted with ether (5 × 3 mL) and the organic extracts were combined, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 90:7), affording acetal **229** (235 mg, 81%) as a colourless oil. $R_f = 0.48$ (petrol/ether, 9:1); **IR** ($\nu_{\max} / \text{cm}^{-1}$): 2828w (C-H), 1375w (O-C-O), 1361w (O-C-O), 1072s (O-C-O), 1050s (O-C-O); **¹H NMR** (500 MHz, C₆D₆) δ 4.13

Experimental

(d, $J = 4.0$, 1H, $H1^f$), 3.13 (s, 3H, $H1^f$), 3.08 (s, 3H, $H1^g$), 2.99–2.87 (m, 2H, $H2^b$), 2.62 (app. quint, $J = 14.0$, 5.5, 2H, $H1^b$), 1.92–1.85 (m, 1H, $H5$), 1.79–1.64 (m, 2H, $H6$), 1.54 (ddd, $J = 11.5$, 4.0, 2.0, 1H, $H4$), 1.52–1.43 (m, 1H, $H5$), 1.30 (s, 3H, $H1^a$), 1.00 (s, 3H, $H1^c$), 0.85 (s, 3H, $H1^d$); $^{13}\text{C NMR}$ (126 MHz, C_6D_6) δ 138.5 (C2), 130.7 (C1), 106.4 (C1 e), 53.9 (C1 g), 53.4 (C1 f), 47.6 (C4), 37.4 (C3), 35.3 (C1 b), 32.5 (C6), 27.6 (C1 c), 22.2 (C1 d), 20.2 (C1 a), 20.0 (C5), 4.7 (C2 b). HRMS could not be obtained for this compound.

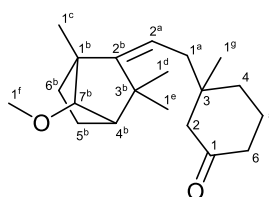
7-Methoxy-1,3,3,3a'-tetramethylhexahydrospiro[bicyclo[2.2.1]heptane-2,1'-inden]-7'(4'H)-one (**230**)



A solution of TiCl_4 (1.0 M in DCM, 0.24 mL, 0.24 mmol) was added dropwise to a solution of TMS-enol ether **226** (**226a:226b** = 1:1) (89 mg, 0.22 mmol) in DCM (2.2 mL) at -78 °C. The mixture was stirred for 15 min at -78 °C, warmed to -23 °C and stirring continued for 2 h at -23 °C. The reaction was quenched at this temperature with a satd. aq. solution of NaHCO_3 (2 mL). After warming to RT, the two layers were separated, the aqueous layer was extracted with ether (3×2 mL) and the organic extracts were combined, dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 4:1 to 2:1), affording ketone **230** (39 mg, 59%) as an inseparable mixture of two diastereomers (**230a:230b** = 1:1) as a white solid. Ketone **230** was tentatively assigned and only key data is assigned. **Data for both diastereomers 230a and 230b** : $R_f = 0.51$ (petrol/ether, 4:1); **m.p.** 63–74 °C; **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 1678m (C=O), 1104 (O-C-O); $^1\text{H NMR}$ (500 MHz, C_6D_6) δ 4.54 (s, 1H, $H6^a$), 4.30 (s, 1H, $H6^a$), 3.34 (s, 3H, $H1^f$), 3.30 (s, 3H, $H1^f$), 2.68 (s, 1H, $H9$), 2.40–2.16 (m, 4H), 2.29 (s, 1H,

$H1^f$), 1.83–1.69 (m, 4H, $H2^a$), 1.62–1.43 (m, 15H, $H2^a$), 1.42 (s, 3H, $H1^f$), 1.33 (s, 3H, $H1^f$), 1.01 (s, 3H, $H1^f$), 0.94 (s, 3H, $H1^d$), 0.89 (s, 6H, $H1^c$ and $H1^d$), 0.80 (s, 3H, $H1^b$), 0.75 (s, 3H, $H1^b$); $^{13}\text{C NMR}$ (126 MHz, C_6D_6) δ 215.0 (C8), 214.5 (C8), 86.6 (C6), 86.3 (C6), 66.3 (C1), 65.1 (C1), 63.0 (C9), 62.5 (C9), 56.9 (C1^f), 56.8 (C1^f), 54.6 (C5^a), 54.2 (C5^a), 52.2 (C2^a), 51.8 (C2^a), 42.4 (C1^a), 42.2 (C1^a), 33.2 (C1^c), 33.0 (C1^c), 31.0 (C1^b), 28.4 (C1^b), 24.0 (C1^d), 23.1 (C1^d), 18.7 (C1^c), 17.0 (C1^c); **MS** (ESI⁺): m/z 327.2 ($[\text{M}+\text{Na}]^+$, 100%), 305.3 ($[\text{M}+\text{H}]^+$, 63%); **HRMS** (ESI⁺): m/z found 305.2475, calcd. for $\text{C}_{20}\text{H}_{33}\text{O}_2$ $[\text{M}+\text{H}]^+$ 305.2475.

3-[2-(7-Methoxy-1,3,3-trimethylbicyclo[2.2.1]heptan-2-ylidene)ethyl]-3-methylcyclohexan-1-one (231)

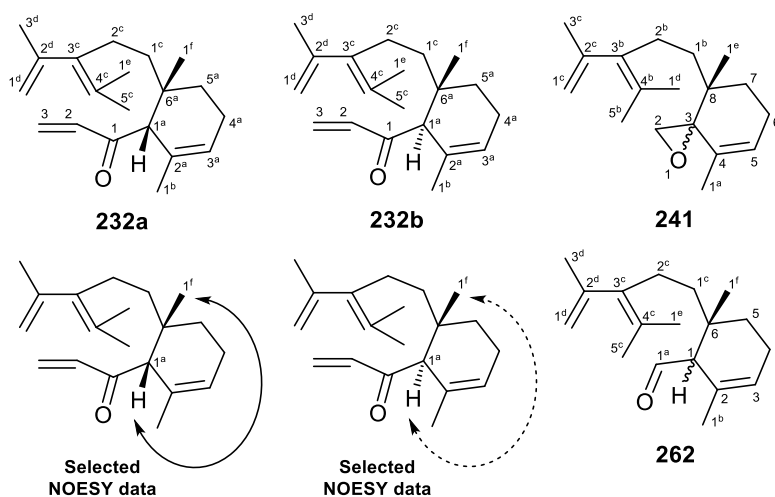


Trimethylsilyl trifluoromethanesulfonate (4 μL , 22 μmol) was added dropwise to a solution of TMS-enol ether **226** (**226a:226b** = 1:1) (100 mg, 0.24 mmol) in DCM (0.7 mL) at -78 °C and the mixture was stirred for 1 h at -78 °C. The reaction was quenched at this temperature with water (1 mL). After warming to RT, the two layers were separated, the aqueous layer was extracted with DCM (3×1 mL) and the organic extracts were combined, dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 2:1), affording ketone **231** (22 mg, 30%) as a mixture of two diastereomers (**231a:231b** = 1:1) as a pale yellow oil. Ketone **231** was tentatively assigned. **Data for both diastereomers 231a:231b**: R_f = 0.78 (petrol/ether, 2:1); $^1\text{H NMR}$ (500 MHz, C_6D_6) δ 5.02 (app. t, J = 7.0, 2H, $H2^a$), 3.29 (s, 2H, $H7^b$), 3.15 (s, 6H, $H1^f$), 2.11–2.04 (m, 2H, $H2$), 1.98 (d, J = 7.5, 4H, $H1^a$), 1.93–1.86 (m, 2H, $H2$), 1.72 (d, J = 4.0, 2H, $H4^b$), 1.51–1.44 (m, 4H, $H6$), 1.21 (s, 6H, $H1^c$), 1.13 (s,

Experimental

6H, $H1^d$), 1.07 (d, $J = 2.0$, 6H, $H1^e$), 0.77 (s, 6H, $H1^f$); ^{13}C NMR (126 MHz, C_6D_6) δ 209.0 (C1), 156.5 (C2^b), 112.3 (C2^a), 89.0 (C7^b), 56.4 (C1^f), 53.2 (C1^b), 51.0 (C4^b), 41.1 (C2), 41.0 (C2), 40.6 (C1^a), 40.5 (C1^a), 38.8 (C3^b), 27.8 (C1^c), 26.1 (C1^d), 26.0 (C1^d), 24.8 (C1^e), 24.7 (C1^e), 22.2 (C6), 16.2 (C1^g); **MS** (ESI+): m/z 327.2 ($[\text{M}+\text{Na}]^+$, 100%), 305.3 ($[\text{M}+\text{H}]^+$, 64%).

1-**{(1*R**,6*S**)-2,6-Dimethyl-6-[4-methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl]cyclohex-2-en-1-yl}prop-2-en-1-one (232a)** and 1-**{(1*S**,6*S**)-2,6-dimethyl-6-[4-methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl]cyclohex-2-en-1-yl}prop-2-en-1-one (232b)**



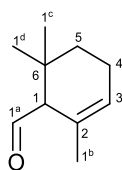
n-BuLi (2.5 M in hexane, 1.57 mL, 3.93 mmol) was added dropwise to a suspension of trimethylsulfonium iodide (802 mg, 3.93 mmol) in THF (4.4 mL) at -5 °C controlling that the temperature did not become higher than 0 °C. After stirring for 10 min at 0 °C, a solution of enone **242** (646 mg, 2.62 mmol) in THF (2.9 mL) was added dropwise and stirring continued for an additional 30 min at 0 °C. The solution was poured into a brine solution (5 mL) with ice and warmed to RT. The two layers were separated, the aqueous layer was extracted with ether (3×5 mL) and the organic extracts were combined, washed with brine (2×10 mL), dried over MgSO_4 , filtered and concentrated under reduced pressure. 4,8-Dimethyl-8-[4-methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl]-1-oxaspiro[2.5]oct-4-ene

241 was obtained as a mixture of two diastereomers **241a** and **241b** (688 mg, **241a:241b** = 4:1) as a pale yellow oil and was used without further purification because it isomerises on silica. Therefore, only the major diastereomer **241a** could be fully characterised and only key data could be assigned for the minor diastereomer **241b**. $R_f = 0.80$ (petrol/ether, 20:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 1446 (CH_2); **MS** (ESI^+): m/z 261.3 ($[\text{M}+\text{H}]^+$, 47%). **NMR data for diastereomer 241a:** $^1\text{H NMR}$ (500 MHz, CDCl_3) δ 5.75–5.68 (m, 1H, $H5$), 4.88 (dq, $J = 3.0, 1.5, 1\text{H}, H1'$), 4.53 (dq, $J = 3.0, 1.0, 1\text{H}, H1'$), 2.86 (d, $J = 5.0, 1\text{H}, H2$), 2.70 (d, $J = 5.0, 1\text{H}, H2$), 2.18–2.05 (m, 2H, $H6$ and $H2^b$), 2.05–1.95 (m, 2H, $H6$ and $H2^b$), 1.75 (dd, $J = 1.5, 1.0, 3\text{H}, H3^c$), 1.65 (s, 3H, $H5^b$), 1.64 (s, 3H, $H1^d$), 1.67–1.62 (m, 1H, $H7$), 1.63–1.55 (m, 1H, $H7$), 1.50 (td, $J = 2.0, 1.0, 3\text{H}, H1^e$), 1.42 (app. td, $J = 12.5, 5.0, 2\text{H}, H1^b$), 0.81 (s, 3H, $H1^e$); $^{13}\text{C NMR}$ (126 MHz, CDCl_3) δ 146.8 ($\text{C}2^e$), 137.1 ($\text{C}3^b$), 132.5 ($\text{C}4$), 128.4 ($\text{C}5$), 124.8 ($\text{C}4^b$), 113.0 ($\text{C}1^e$), 63.5 ($\text{C}3$), 47.6 ($\text{C}2$), 34.7 ($\text{C}8$), 33.3 ($\text{C}7$), 33.0 ($\text{C}1^b$), 25.9 ($\text{C}2^b$), 22.9 ($\text{C}3^e$), 22.7 ($\text{C}6$), 21.9 ($\text{C}1^d$), 21.1 ($\text{C}1^e$), 19.6 ($\text{C}5^b$), 16.9 ($\text{C}1^a$). **NMR data for diastereomer 241b:** $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.83–5.79 (m, 1H, $H5$), 4.92 (dq, $J = 3.0, 1.5, 1\text{H}, H1'$), 4.55 (qd, $J = 3.0, 1.0, 1\text{H}, H1'$), 2.93 (d, $J = 4.5, 1\text{H}, H2$), 2.87 (d, $J = 4.5, 1\text{H}, H2$), 1.72 (dd, 1.5, 1.0, 3H, $H3^c$), 1.66–1.63 (m, 6H, $H5^b$ and $H1^d$), 1.53 (td, $J = 2.0, 1.5, 3\text{H}, H1^e$), 0.86 (s, 3H, $H1^e$). $\text{BF}_3 \cdot \text{OEt}_2$ (50 μL , 0.41 mmol) was added dropwise to a solution of epoxide **241** (688 mg from the previous step) in THF (2.6 mL) at RT and the obtained mixture was stirred for 5 min at RT. If the reaction was quenched at this point by the addition of water, 2,6-dimethyl-6-[4-methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl]cyclohex-2-ene-1-carbaldehyde **262** as a pale yellow oil as an inseparable mixture of two diastereomers **262a** and **262b** (**262a:262b** = 2:1). **Data for both diastereomers 262a and 262b:** $R_f = 0.74$ (petrol/ether, 20:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 1715 ($\text{C}=\text{O}$); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 9.39 (d, $J = 5.0, 1\text{H}, H1^e$), 9.34 (d, $J = 5.5, 2\text{H}, H1^e$), 5.65–5.60 (m, 3H, $H5$), 4.82–4.77 (m, 3H, $H1^d$), 4.42–4.39 (m, 3H, $H1^d$), 2.37 (d, $J = 5.0, 1\text{H}, H1$), 2.32 (d, $J = 5.5, 2\text{H}, H1$), 2.08–1.85 (m, 15H, $H4, H1^e$ and $H2$), 1.92–1.85 (m, 8H, $H8$ and $H1^e$), 1.64–1.62

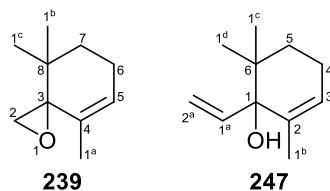
Experimental

(m, 9H, $H3^d$), 1.54 (s, 9H, $H5^e$), 1.53 (s, 9H, $H1^f$), 1.48 (app. q, 9H, $H1^b$), 1.37–1.29 (m, 8H, $H3$ and $H1^f$), 0.87 (s, 3H, $H1^f$), 0.79 (s, 6H, $H1^f$); **MS** (ESI⁺): m/z 283.1 ([M+Na]⁺, 100%), 261.1 ([M+H]⁺, 50%). However, it was more convenient to run the addition of the Grignard reagent without the isolation of aldehyde **262**; thus, it was only done once to confirm the formation of **262**. Without working up the reaction, vinylmagnesium bromide (1.0 M in THF, 3.93 mL, 3.93 mmol) was added dropwise at RT and the mixture was stirred for an additional 30 min at RT. The reaction was quenched with a satd. aq. solution of NH₄Cl (5 mL). The two layers were separated, the aqueous layer was extracted with ether (5 × 5 mL), the organic extracts were combined, washed sequentially with a satd. aq. solution of NaHCO₃ (15 mL) and brine (15 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. Allylic alcohol **263** (815 mg) was used without further purification. Allylic alcohol (825 mg) was redissolved in DCM (13.1 mL) and NaHCO₃ (2.20 g, 226.2 mmol) and Dess-Martin periodinane (1.33 g, 3.14 mmol) were added sequentially at RT. After stirring for 20 min at RT, the reaction was diluted with ether (7 mL) and quenched by the addition of a satd. aq. solution of Na₂S₂O₃ (10 mL) and subsequently a satd. aq. solution of NaHCO₃ (10 mL). The mixture was stirred for 20 min at RT and then the two layers were separated, the aqueous layer was extracted with DCM (2 × 10 mL) and the organic extracts were combined, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 40:1), affording enone **232** (462.4 mg, 59%) as an inseparable mixture of two diastereomers **232a** and **232b** (**232a**:**232b** = 2:1) as a pale yellow oil. The two diastereomers were separated by preparative TLC (petrol/ether, 40:3) for characterisation purposes. **Data for diastereomer 232a**: pale yellow oil. R_f = 0.45 (petrol/ether, 20:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 1688m (C=O), 1673m (C=C), 893m (C=C); **¹H NMR** (500 MHz, CDCl₃) δ 6.56 (dd, J = 17.0, 10.5, 1H, $H2$), 6.22 (dd, J = 17.0, 1.5, 1H, $H3$), 5.66 (dd, J = 10.5, 1.5, 1H, $H3$), 5.63–5.59 (m, 1H, $H3^a$), 4.84 (dq, J = 3.0, 1.5, 1H, $H1^d$),

4.45 (dq, $J = 3.0, 1.0, 1\text{H}, H1^d$), 2.96 (s, 1H, $H1^e$), 2.20–2.11 (m, 1H, $H4^e$), 2.10–1.99 (m, 3H, $H4^e$ and $H2^e$), 1.78–1.70 (m, 1H, $H5^e$), 1.69 (dd, $J = 1.5, 1.0, 3\text{H}, H3^d$), 1.60 (s, 3H, $H5^e$), 1.59 (s, 3H, $H1^e$), 1.58–1.56 (m, 3H, $H1^b$), 1.32 (ddt, $J = 13.0, 7.0, 2.0, 1\text{H}, H5^e$), 1.26 (ddd, $J = 13.5, 11.5, 7.0, 1\text{H}, H1^e$), 1.07 (ddd, $J = 13.5, 11.0, 6.0, 1\text{H}, H1^e$), 0.93 (s, 3H, $H1^f$); **^{13}C NMR** (126 MHz, CDCl_3) δ 202.8 (C1), 146.5 (C2^d), 136.8 (C2), 136.7 (C3^e), 130.2 (C2^a), 127.6 (C3), 124.8 (C4^e), 124.1 (C3^a), 113.1 (C1^d), 61.3 (C1^a), 39.2 (C1^e), 35.7 (C6^a), 28.6 (C5^a), 25.1 (C2^e), 23.7 (C1^f), 23.6 (C1^b), 22.9 (C3^d), 22.6 (C4^a), 21.8 (C5^e), 19.5 (C1^e); **MS** (ESI⁺): m/z 309.2 ([M+Na]⁺, 100%), 287.2 ([M+H]⁺, 35%); **HRMS** (ESI⁺): m/z found 287.23710, calcd. for $\text{C}_{20}\text{H}_{31}\text{O}$ [M+H]⁺ 287.23694. **Data for diastereomer 232b:** pale yellow oil. $R_f = 0.34$ (petrol/ether, 20:1); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 1689m (C=O), 1673m (C=C), 1632w (C=C), 1609m (C=C), 893m (C=C); **^1H NMR** (500 MHz, CDCl_3) δ 6.56 (dd, $J = 17.5, 10.5, 1\text{H}, H2$), 6.24 (dd, $J = 17.5, 1.5, 1\text{H}, H3$), 5.70 (dd, $J = 10.5, 1.5, 1\text{H}, H3$), 5.65–5.62 (m, 1H, $H3^e$), 4.89 (dq, $J = 3.0, 1.5, 1\text{H}, H1^d$), 4.52 (dq, $J = 3.0, 1.0, 1\text{H}, H1^d$), 3.06 (s, 1H, $H1^e$), 2.15–2.05 (m, 2H, $H4^e$), 2.05–2.00 (m, 2H, $H2^e$), 1.74 (dd, $J = 1.5, 1.0, 3\text{H}, H3^d$), 1.71–1.65 (m, 1H, $H5^e$), 1.64 (s, 6H, $H5^e$ and $H1^e$), 1.57–1.54 (m, 3H, $H1^b$), 1.37 (ddd, $J = 14.0, 11.0, 7.5, 1\text{H}, H1^e$), 1.35–1.29 (m, 1H, $H5^e$), 1.28–1.20 (m, 1H, $H1^e$), 0.84 (s, 3H, $H1^f$); **^{13}C NMR** (126 MHz, CDCl_3) δ 203.0 (C1), 146.7 (C2^d), 137.1 (C2), 136.6 (C3^e), 130.2 (C2^a), 127.8 (C3), 125.0 (C4^e), 124.2 (C3^a), 113.2 (C1^d), 59.8 (C1^a), 37.6 (C1^e), 35.2 (C6^a), 29.0 (C5^a), 25.3 (C2^e), 24.4 (C1^f), 23.5 (C1^b), 22.9 (C3^d), 22.6 (C4^a), 21.9 (C5^e), 19.6 (C1^e); **MS** (ESI⁺): m/z 309.2 ([M+Na]⁺, 67%), 287.1 ([M+H]⁺, 43%); **HRMS** (ESI⁺): m/z found 287.23711, calcd. for $\text{C}_{20}\text{H}_{31}\text{O}$ [M+H]⁺ 287.23694.

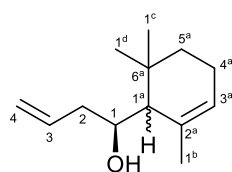
2,6,6-Trimethylcyclohex-2-ene-1-carbaldehyde (**236**)^[221]

$\text{BF}_3 \cdot \text{OEt}_2$ (50 μL , 0.41 mmol) was added dropwise to a solution of epoxide **239** (160 mg, 1.05 mmol) in benzene (1 mL) at RT. After stirring for 5 min at RT, the reaction was quenched with water (1 mL). The two layers were separated, the aqueous layer was extracted with ether (5×1 mL) and the organic extracts were combined, washed with water (3 mL), dried over anhydrous MgSO_4 , filtered and concentrated under reduced pressure. Aldehyde **236** (144 mg, 90 %) was obtained as a yellow oil and was used without further purification. $R_f = 0.57$ (petrol/ether, 20:1); IR ($\nu_{\text{max}}/\text{cm}^{-1}$): 1716s (C=O); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 9.47 (d, $J = 5.0$, 1H, $H1^a$), 5.70–5.74 (m, 1H, $H\beta$), 2.36 (d, $J = 5.0$, 1H, $H1$), 2.19–2.08 (m, 2H, $H4$), 1.65 (ddd, $J = 13.5, 10.0, 7.0$, 1H, $H5$), 1.59 (tdd, $J = 2.5, 1.5, 0.5$, 3H, $H1^b$), 1.35 (dddd, $J = 13.5, 6.0, 3.0, 1.5$, 1H, $H5$), 0.99 (s, 3H, $H1^c$), 0.90 (s, 3H, $H1^d$); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 202.6 ($\text{C}1^a$), 127.2 (C2), 125.6 (C3), 63.9 (C1), 32.1 (C5), 31.7 (C6), 27.6 ($\text{C}1^c$), 27.1 ($\text{C}1^d$), 23.2 (C4), 22.7 ($\text{C}1^b$); HRMS (CI^+): m/z found 153.1279, calcd. for $\text{C}_{10}\text{H}_{17}\text{O}$ $[\text{M}+\text{H}]^+$ 153.1274. Data consistent with the literature.^[221]

4,8,8-Trimethyl-1-oxaspiro[2.5]oct-4-ene (**239**)^[222]

n-BuLi (2.5 M in hexane, 1.25 mL, 3.13 mmol) was added dropwise to a suspension of trimethylsulfonium iodide (639 mg, 3.13 mmol) in THF (2.8 mL) at -5 °C controlling that

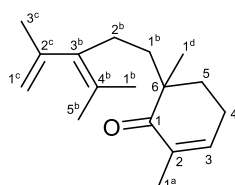
the temperature did not become higher than 0 °C. After stirring for 10 min at 0 °C, a solution of enone **245** (172 mg, 1.24 mmol) in THF (0.9 mL) was added dropwise. The mixture was stirred for 30 min at 0 °C and then poured into a brine solution (3 mL) with ice. After warming to RT, the two layers were separated, the aqueous layer was extracted with ether (3 × 3 mL) and the organic extracts were combined, dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 20:1), affording epoxide **239** (150 mg, 79%) as a colourless oil. **R_f** = 0.62 (petrol/ether, 20:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 1719s (C=C); **¹H NMR** (400 MHz, CDCl₃) δ 5.78–5.74 (m, 1H, *H*5), 2.88 (d, *J* = 4.5, 1H, *H*2), 2.84 (d, *J* = 4.5, 1H, *H*2), 2.20–2.05 (m, 2H, *H*6), 1.61–1.56 (m, 2H, *H*7), 1.52 (tdd, *J* = 2.0, 1.5, 0.5, 3H, *H*1^a), 0.92 (s, 3H, *H*1^b), 0.87 (s, 3H, *H*1^c); **¹³C NMR** (101 MHz, CDCl₃) δ 131.8 (C4), 129.3 (C5), 62.6 (C3), 48.3 (C2), 35.6 (C7), 32.5 (C8), 24.4 (C1^c), 22.9 (C6), 22.8 (C1^b), 17.3 (C1^a); **MS** (ESI⁺): *m/z* 153.1 ([M+H]⁺, 20%); **HRMS** (CI⁺): *m/z* found 153.1278, calcd. for C₁₀H₁₇O [M+H]⁺ 153.1274. Data consistent with the literature.^[222] During the optimisation of the reaction conditions, the side-product 2,6,6-trimethyl-1-vinylcyclohex-2-en-1-ol **247**^[223] was afforded as a pale yellow oil. **R_f** = 0.25 (petrol/ether, 20:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 3507br (O-H), 1703w (C=C), 1659w (C=C), 1636w (C=C), 1128m (C-O), 963s (C=C), 920s (C=C); **¹H NMR** (400 MHz, CDCl₃) δ 5.87 (dd, *J* = 17.0, 10.5, 1H, *H*1^a), 5.53–5.48 (m, 1H, *H*3), 5.23 (dd, *J* = 17.0, 1.5, 1H, *H*2^a), 5.17 (dd, *J* = 10.5, 1.5, 1H, *H*2^b), 2.12–1.93 (m, 2H, *H*4), 1.61 (ddd, *J* = 2.5, 2.0, 1.5, 3H, *H*1^b), 1.62–1.55 (m, 1H, *H*5), 1.41 (ddd, *J* = 13.5, 6.5, 4.5, 1H, *H*5), 0.97 (s, 3H, *H*1^c), 0.89 (s, 3H, *H*1^d); **¹³C NMR** (101 MHz, CDCl₃) δ 140.8 (C1^a), 135.5 (C2), 124.4 (C3), 113.8 (C2^a), 78.9 (C1), 36.7 (C6), 33.5 (C5), 24.5 (C1^d), 22.8 (C4), 22.4 (C1^c), 18.4 (C1^b); **HRMS** (CI⁺): *m/z* found 167.1434, calcd. for C₁₁H₁₉O [M+H]⁺ 167.1436. Data consistent with the literature.^[223]

1-(2,6,6-Trimethylcyclohex-2-en-1-yl)but-3-en-1-ol (**240**)^[148,154]

Allylmagnesium bromide (1.0 M in ether, 2.70 mL, 2.70 mmol) was added dropwise to a solution of epoxide **239** (205 mg, 1.35 mmol) in ether (0.6 mL) at 0 °C. The mixture was warmed to RT and then stirred for 4 h. The reaction was quenched with aq. HCl (1.0 M, 1.5 mL). The two layers were separated, the aqueous layer was extracted with EA (5 × 2 mL) and the organic extracts were combined, washed sequentially with a satd. aq. solution of NaHCO₃ (5 mL) and brine (5 mL), dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 20:1), affording alcohol **240** as a separable mixture of two diastereomers: **240a** (30 mg, 11%) as a colourless oil and **240b** (49 mg, 19%) as a colourless oil. **Data for diastereomer 240a:** $R_f = 0.44$ (petrol/ether, 10:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 3454br (OH), 1640w (C=C), 996s (C-O), 911s (C-O); **¹H NMR** (400 MHz, CDCl₃) δ 5.93–5.78 (m, 1H, *H*3), 5.48–5.44 (m, 1H, *H*3^a), 5.21–5.10 (m, 2H, *H*4), 3.88 (app. dq, $J = 10.5, 2.5$, 1H, *H*1), 2.29 (dddt, $J = 14.0, 6.0, 3.0, 1.5$, 1H, *H*2), 2.11 (dddt, $J = 14.0, 10.5, 9.0, 1.0$, 1H, *H*2), 2.04–1.96 (m, 2H, *H*4^a), 1.82–1.80 (m, 1H, *H*1^a), 1.78 (tdd, $J = 2.0, 1.5, 0.5$, 3H, *H*1^b), 1.52–1.44 (m, 1H, *H*5^a), 1.21–1.13 (m, 1H, *H*5^a), 1.04 (s, 3H, *H*1^c), 0.91 (s, 3H, *H*1^d); **¹³C NMR** (126 MHz, CDCl₃) δ 136.6 (C3), 133.3 (C2^a), 122.8 (C3^a), 118.1 (C4), 71.6 (C1), 54.9 (C1^a), 40.4 (C2), 32.4 (C6^a), 31.6 (31.6), 28.9 (C1^c), 28.6 (C1^d), 25.9 (C1^b), 23.1 (C4^a). **HRMS** (CI): m/z found 231.1387, calcd. for C₁₅H₁₉O₂ [M–H][−] 231.1390. Data consistent with the literature.^[154] **Data for diastereomer 240b:** $R_f = 0.53$ (petrol/ether, 10:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 3487br (O–H), 1640w (C=C), 995m (C=C), 911s (C=C); **¹H NMR** (400 MHz, CDCl₃) δ 5.86 (ddt, $J = 17.0, 10.0, 7.0$, 1H, *H*3), 5.70–5.66 (m, 1H, *H*3^a), 5.14–5.07 (m, 2H, *H*4),

3.90–3.80 (m, 1H, *Hf*), 2.39 (dddt, $J = 14.0, 8.0, 7.0, 1.5$, 1H, *H2*), 2.27 (dddt, $J = 14.0, 7.0, 6.0, 1.5$, 1H, *H2*), 2.07–2.00 (m, 2H, *H4^a*), 1.80 (tdd, $J = 2.0, 1.5, 0.5$, 3H, *H1^b*), 1.73 (br. s, 1H, *H1^a*), 1.71–1.66 (m, 1H, *H5^a*), 1.63 (d, $J = 6.0$, 1H, *OH*), 1.18–1.11 (m, 1H, *H5^a*), 0.97 (s, 3H, *H1^c*), 0.86 (s, 3H, *H1^d*); ¹³C NMR (101 MHz, CDCl₃) δ 136.3 (C3), 131.9 (C2^a), 126.1 (C3^a), 117.1 (C4), 69.6 (C1), 54.3 (C1^a), 44.3 (C2), 32.8 (C6^a), 30.8 (C5^a), 28.4 (C1^c), 27.7 (C1^d), 26.2 (C1^b), 23.5 (C4^a); MS (ESI⁺): m/z 217.2 ([M+Na]⁺, 100%); HRMS (CI⁺): m/z found 195.1742, calcd. for C₁₃H₂₃O [M+H]⁺ 195.1743. Data consistent with the literature.^[154]

2,6-Dimethyl-6-[4-methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl]cyclohex-2-en-1-one (242)



- **Method A:**

n-BuLi (1.6 M in hexane, 1.76 mL, 2.82 mmol) was added dropwise to a solution of diisopropylamine (0.40 mL, 2.85 mmol) in THF (4.2 mL) at -78 °C. After stirring for 15 min at -78 °C, a solution of enone **250** (350 mg, 2.82 mmol) in THF (3.2 mL) was added dropwise and stirring continued for an additional 15 min at -78 °C. DMPU (1.55 mL, 12.82 mmol) was added dropwise at -78 °C, the mixture was stirred for 15 min at -78 °C and then iododiene **214** (1.30 g/mL, 0.25 mL, 1.30 mmol) was added dropwise. The mixture was slowly warmed to RT and then stirred for 14 h. The reaction was quenched with a satd. aq. solution of NH₄Cl (5 mL). The two layers were separated, the aqueous layer was extracted with ether (5 × 4 mL) and the organic extracts were combined, washed with brine (15 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 4:1), affording

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enone **242** (219 mg, 69%) as a colourless oil. $R_f = 0.32$ (petrol/ether, 40:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 1713m (C=O), 1667s (C=C), 892m (C=C-H); **$^1\text{H NMR}$** (500 MHz, CDCl_3) δ 6.63–6.56 (m, 1H, $H3$), 4.89 (dq, $J = 3.0, 1.5$, 1H, $H1^a$), 4.52 (dq, $J = 3.0, 1.0$, 1H, $H1^b$), 2.42–2.31 (m, 1H, $H4$), 2.31–2.23 (m, 1H, $H4$), 2.05–1.89 (m, 3H, $H5$ and $H2^b$), 1.77 (ddd, $J = 15.5, 8.0, 5.5$, $H5$, 1H), 1.75 (td, $J = 2.0, 1.5$, 3H, $H1^c$), 1.74 (dd, $J = 1.5, 1.0$, 3H, $H3^c$), 1.65 (s, 3H, $H5^b$), 1.64 (s, 3H, $H1^d$), 1.54 (ddd, $J = 13.5, 12.5, 5.0$, 1H, $H1^b$), 1.44 (ddd, $J = 13.5, 12.5, 5.0$, 1H, $H1^b$), 1.08 (s, 3H, $H1^c$); **$^{13}\text{C NMR}$** (126 MHz, CDCl_3) δ 204.6 (C1), 146.6 (C2^c), 143.3 (C3), 136.4 (C3^b), 134.1 (C2), 125.3 (C4^b), 113.2 (C1^c), 44.4 (C6), 35.0 (C1^b), 34.0 (C5), 25.8 (C2^b), 23.0 (C4), 22.8 (C1^c), 22.0 (C3^c), 21.9 (C5^b), 19.6 (C1^d), 16.7 (C1^a); **MS** (ESI⁺): m/z 269.2 ($[\text{M}+\text{Na}]^+$, 100%), 247.2 ($[\text{M}+\text{H}]^+$, 41%), 515.4 ($[\text{2M}+\text{Na}]^+$, 14%); **HRMS** (CI⁺): m/z found 247.2058, calcd. for $\text{C}_{17}\text{H}_{27}\text{O}$ $[\text{M}+\text{H}]^+$ 247.2056.

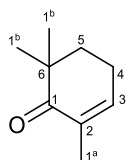
- **Method B:**

n-BuLi (1.6 M in hexane, 0.83 mL, 1.33 mmol) was added dropwise to a solution of diisopropylamine (189 μL , 1.35 mmol) in THF (2.0 mL) at -78 °C. After stirring for 15 min at -78 °C, a solution of enone **250** (164 mg, 1.32 mmol) in THF (1.5 mL) was added dropwise and stirring continued for an additional 15 min at -78 °C. HMPA (1.00 mL, 5.75 mmol) was added dropwise at -78 °C, the mixture was stirred for 15 min at -78 °C and then a solution of iododiene **214** (150 mg, 0.60 mmol) in THF (1.1 mL) was added dropwise. The mixture was slowly warmed to RT and then stirred for 14 h. The reaction was quenched with a satd. aq. solution of NH_4Cl (4 mL). The two layers were separated, the aqueous layer was extracted with ether (5×3 mL) and the organic extracts were combined, washed with brine (10 mL), dried over MgSO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 4:1), affording enone **242** (35 mg, 23%). Data as above.

- **Method C:**

A solution of enone **250** (75 mg, 0.60 mmol) in THF (1.7 mL) was added dropwise to a solution of potassium bis(trimethylsilyl)amide (0.5 M in toluene, 1.20 mL, 0.60 mmol) in THF (0.3 mL) at -78 °C. After stirring for 30 min at -78 °C, a solution of iododiene **214** (300 mg, 1.20 mmol) in THF (1.2 mL) was added dropwise at -78 °C and the mixture was slowly warmed to RT and then stirred for 14 h. The reaction was quenched with a satd. aq. solution of NH_4Cl (3 mL). The two layers were separated, the aqueous layer was extracted with ether (5×3 mL) and the organic extracts were combined, washed with brine (5 mL), dried over MgSO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 25:1), affording enone **242** (18 mg, 12%). Data as above.

2,6,6-Trimethylcyclohex-2-en-1-one (**245**)^[224]



- **Method A:**

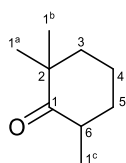
A mixture of ketone **246** (1.00 g, 7.13 mmol) and *N*-bromosuccinimide (2.54 g, 14.3 mmol) in CCl_4 (7.0 mL) was heated to reflux for 14 h. After cooling to RT, the orange suspension was filtered, the white solid was washed with cold CCl_4 (3 mL) and the filtrate was concentrated under reduced pressure. The residue was redissolved in DMF (3.6 mL) and Li_2CO_3 (527 mg, 7.13 mmol) and LiBr (310 mg, 3.57 mmol) were added sequentially at RT. The mixture was heated to reflux for 1h and after cooling to RT, diluted with water (4 mL). The two layers were separated, the aqueous layer was extracted with petrol (2×4 mL) and the organic extracts were combined, dried over anhydrous MgSO_4 and concentrated under

Experimental

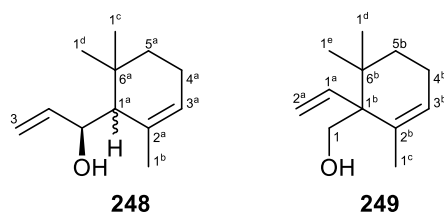
reduced pressure. The crude product was purified by distillation under reduced pressure (100 °C, 10 mmHg), affording enone **245** (958 mg, 97%) as a colourless oil. $R_f = 0.49$ (petrol/ether, 20:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 1667s (C=O); **$^1\text{H NMR}$** (400 MHz, CDCl_3) δ 6.65–6.59 (m, 1H, $H\beta$), 2.32 (tdq, $J = 6.0, 4.0, 2.0$, 2H, $H\alpha$), 1.80 (t, $J = 6.0$, 2H, $H\gamma$), 1.76–1.73 (m, 3H, $H\delta$), 1.09 (s, 6H, $H\epsilon$); **$^{13}\text{C NMR}$** (101 MHz, CDCl_3) δ 204.9 (C1), 143.6 (C3), 133.9 (C2), 41.4 (C6), 36.8 (C5), 24.5 (C1^b), 23.2 (C4), 16.6 (C1^a); **HRMS** (EI⁺): m/z found 138.1039, calcd. for $\text{C}_9\text{H}_{14}\text{O}$ [M]⁺ 138.1045. Data consistent with the literature.^[224]

- **Method B:**

A solution of enone **250** (100 mg, 0.81 mmol) in THF (2.3 mL) was added dropwise to a solution of potassium bis(trimethylsilyl)amide (0.5 M in toluene, 1.62 mL, 0.81 mmol) in THF (0.4 mL) at -78 °C. After stirring for 30 min at -78 °C, iodomethane (100 μL , 1.61 mmol) was added dropwise at -78 °C and the mixture was slowly warmed to RT and then stirred for 14 h. The reaction was quenched with a satd. aq. solution of NH_4Cl (2 mL). The two layers were separated, the aqueous layer was extracted with ether (5×2 mL) and the organic extracts were combined, washed with brine (7 mL), dried over anhydrous MgSO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 20:1) affording enone **245** (37 mg, 33%). Data as above.

2,2,6-Trimethylcyclohexan-1-one (246)^[225]

n-BuLi (2.5 M in hexane, 50.4 mL, 126.0 mmol) was added dropwise to a solution of diisopropylamine (20.7 mL, 147.7 mmol) in THF (155 mL) at -78 °C. The mixture was warmed to 0 °C and then stirred for 30 min. After cooling to -78 °C, a solution of 2,6-dimethylcyclohexan-1-one (as a mixture of isomers) (15.0 g, 118.9 mmol) in THF (30 mL) was added dropwise. The obtained mixture was stirred for 1.5 h at -78 °C, iodomethane (11.1 mL, 178.3 mmol) was added dropwise and stirring continued for an additional 1 h at -78 °C. The acetone/dry ice bath was removed and the mixture was warmed to RT, stirred for 14 h and then poured into a mixture of a satd. aq. solution of NH_4Cl (90 mL), water (9 mL) and ether (45 mL) with vigorously stirring. The two layers were separated, the aqueous layer was extracted with ether (5×75 mL) and the organic extracts were combined, washed with brine (200 mL), dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The crude product was purified by distillation under reduced pressure (140 °C, 34 mmHg), affording ketone **246** (15.4 g, 92%) as a colourless oil. $R_f = 0.59$ (petrol/ether, 20:1); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 2930m (C-H), 1705s (C=O); **$^1\text{H NMR}$** (400 MHz, CDCl_3) δ 2.66 (app. sept, $J = 6.5$, 1H, $H6$), 2.05 (dsxt, $J = 13.5$, 3.5, 1H, $H5$), 1.88 (qt, $J = 13.5$, 3.5, 1H, $H4$), 1.77 (dq, $J = 13.5$, 3.5, 1H, $H3$), 1.64 (dq, $J = 13.5$, 3.5, 1H, $H4$), 1.55 (td, $J = 13.5$, 3.5, 1H, $H3$), 1.32 (qd, $J = 13.5$, 3.5, 1H, $H5$), 1.18 (s, 3H, $H1^a$), 1.04 (s, 3H, $H1^b$), 0.99 (d, $J = 6.5$, 3H, $H1^c$); **$^{13}\text{C NMR}$** (101 MHz, CDCl_3) δ 217.5 (C1), 45.4 (C2), 42.0 (C3), 40.9 (C6), 36.9 (C5), 25.8 ($C1^b$), 25.5 ($C1^a$), 21.7 (C4), 15.2 ($C1^c$); **HRMS** (EI^+): m/z found 140.1199 calcd. for $\text{C}_9\text{H}_{16}\text{O}$ $[\text{M}]^+$ 140.1201. Data consistent with the literature.^[225]

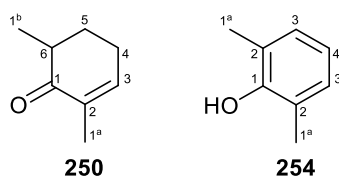
1-(2,6,6-Trimethylcyclohex-2-en-1-yl)prop-2-en-1-ol (**247**)^[143]

- **Method A:**

Vinylmagnesium bromide (1.0 M in THF, 6.76 mL, 6.76 mmol) was added dropwise to a solution of aldehyde **236** (209 mg, 1.37 mmol) in THF (2.3 mL) at RT. After stirring for 1 h at RT, the mixture was poured into a satd. aq. solution of NH₄Cl (5 mL). The two layers were separated, the aqueous layer was extracted with ether (5 × 5 mL) and the organic extracts were combined, washed sequentially with a satd. aq. solution of NaHCO₃ (15 mL) and brine (15 mL), dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 20:1) affording alcohol **248** as the single diastereomer **248a** (98 mg, 39%) as a pale yellow oil. **Data for diastereomer 248a:** $R_f = 0.46$ (petrol/ether, 20:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 3524br (O-H), 1673s (C=C), 1611w (C=C), 988s (C=C), 916s (C=C); **¹H NMR** (400 MHz, CDCl₃) δ 5.98 (ddd, $J = 17.0, 10.5, 4.0, 1\text{H}, H_2$), 5.70–5.65 (m, 1H, H_{3^a}), 5.26 (ddd, $J = 17.0, 2.5, 1.5, 1\text{H}, H_3$), 5.05 (ddd, $J = 10.5, 2.5, 1.5, 1\text{H}, H_3$), 4.43 (app. ddq, $J = 8.0, 4.0, 1.5, 1\text{H}, H_1$), 2.08–1.99 (m, 2H, H_{4^a}), 1.87–1.82 (m, 1H, H_{5^a}), 1.80 (br. s, 1H, H_{1^a}), 1.70 (tdd, $J = 2.0, 1.5, 0.5, 3\text{H}, H_{1^b}$), 1.56 (d, $J = 8.0, 1\text{H}, OH$), 1.20–1.12 (m, 1H, H_{5^a}), 1.04 (s, 3H, H_{1^c}), 0.88 (s, 3H, H_{1^d}); **¹³C NMR** (101 MHz, CDCl₃) δ 144.1 (C2), 131.2 (C2^a), 126.5 (C3^a), 112.2 (C3), 70.0 (C1), 56.0 (C1^a), 32.7 (C6^a), 31.1 (C5^a), 28.7 (C1^c), 27.9 (C1^d), 25.8 (C1^b), 23.4 (C4^a); **MS** (ESI⁺): m/z 203.1 ([M+Na]⁺, 78%); **HRMS** (CI⁺): m/z found 181.1587, calcd. for C₁₂H₂₁O [M+H]⁺ 181.1587. Data consistent with the literature.^[143]

- **Method B:**

Vinylmagnesium bromide (1.0 M in THF, 2.66 mL, 2.66 mmol) was added dropwise to a solution of epoxide **239** (203 mg, 1.33 mmol) in ether (0.6 mL) at 0 °C. The mixture was warmed to RT and then stirred for 4 h. The reaction was quenched with aq. HCl (3.0 M, 0.5 mL) and diluted with ether (0.5 mL). The two layers were separated, the aqueous layer was extracted with ether (5 × 1 mL) and the organic extracts were combined, washed sequentially with a satd. aq. solution of NaHCO₃ (3 mL) and brine (3 mL), dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 20:1), affording allylic alcohol **248** as a separable mixture of two diastereomers: **248a** (18 mg, 8%) and **248b** (1 mg, 1%) as a pale yellow oil, and (2,6,6-trimethyl-1-vinylcyclohex-2-en-1-yl)methanol **249** (28 mg, 12%) a pale yellow oil. **Data for diastereomer 248b:** $R_f = 0.29$ (petrol/ether, 20:1); $^1\text{H NMR}$ (400 MHz, CDCl₃) δ 5.96 (ddd, $J = 17.0, 10.5, 6.5$ Hz, 1H, H_2), 5.51–5.46 (m, 1H, H_3^a), 5.23 (dt, $J = 17.0, 1.5$, 1H, H_3), 5.11 (dt, $J = 10.5, 1.5$, 1H, H_3), 4.46–4.39 (m, 1H, H_1), 2.02–1.93 (m, 2H, H_4^a), 1.84 (br. s, 1H, H_1^a), 1.78 (tdd, $J = 2.0, 1.5, 0.5$, 3H, H_1^b), 1.57–1.51 (m, 1H, H_5^a), 1.12–1.05 (m, 1H, H_5^a), 1.02 (s, 3H, H_1^c), 0.89 (s, 3H, H_1^d). Data consistent with the literature.^[143] **Data for 249:** $R_f = 0.21$ (petrol/ether, 20:1); $^1\text{H NMR}$ (400 MHz, CDCl₃) δ 5.78–5.66 (m, 1H, H_3^a), 5.72 (dd, $J = 18.0, 11.0$, 1H, H_1^b), 5.20 (dd, $J = 11.0, 1.5$, 1H, H_2^b), 4.93 (dd, $J = 18.0, 1.5$, 1H, H_2^b), 3.86 (dd, $J = 11.5, 8.0$, 1H, H_1), 3.75 (dd, $J = 11.5, 4.0$, 1H, H_1), 2.05–1.96 (m, 2H, H_4^a), 1.66 (td, $J = 2.0, 1.5$, 3H, H_1^c), 1.56–1.36 (m, 2H, H_5^a), 1.23 (dd, $J = 8.0, 4.0$, 1H, OH), 0.96 (s, 3H, H_1^d), 0.89 (s, 3H, H_1^e).

2,6-Dimethylcyclohex-2-en-1-one (**250**)^[226]

- **Method A:**

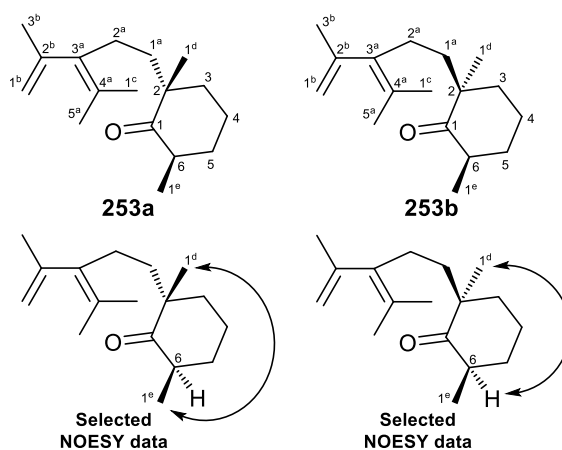
N-bromosuccinimide (705 mg, 3.96 mmol) was added to a solution of 2,6-dimethylcyclohexan-1-one (as a mixture of isomers) (500 mg, 3.96 mmol) in CCl₄ (4.0 mL) at RT and the suspension was heated to reflux for 14 h. After cooling to RT, it was filtered and the white precipitate was washed with cold CCl₄. The filtrate was concentrated under reduced pressure and then redissolved in DMF (2.0 mL). Li₂CO₃ (293 mg, 3.96 mmol) and LiBr (172 mg, 1.98 mmol) were added sequentially at RT and then the mixture was heated to reflux for 1 h. After cooling to RT, the reaction was quenched with water (2 mL). The two layers were separated, the aqueous layer was extracted with petrol (2 × 2 mL) and the organic extracts were combined, dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 20:1), affording enone **250** (258 mg, 52%) as a colourless oil and 2,6-dimethylphenol **254**^[227] (25 mg, 3%) as a yellow solid. **Data for 250:** *R_f* = 0.50 (petrol/ether, 10:1); **IR** (ν_{max}/cm⁻¹): 1710w (C=C), 1669s (C=O); **¹H NMR** (400 MHz, CDCl₃) δ 6.68 (ddq, *J* = 6.0, 3.5, 1.5, 1H, *H*₃), 2.43–2.31 (m, 3H, *H*₄ and *H*₆), 2.03 (dtdd, *J* = 13.0, 4.5, 1.5, 1H, *H*₅), 1.76 (dt, *J* = 2.0, 1.5, 3H, *H*_{1^a}), 1.75–1.66 (m, 1H, *H*₅), 1.13 (d, *J* = 7.0, 3H, *H*_{1^b}); **¹³C NMR** (101 MHz, CDCl₃) δ 202.7 (C1), 144.7 (C3), 135.2 (C2), 41.8 (C6), 31.4 (C5), 25.5 (C4), 16.3 (C1^a), 15.4 (C1^b); **MS** (ESI⁺): *m/z* 147.1 ([M+Na]⁺, 33%); **HRMS** (CI⁺): *m/z* found 125.0966, calcd. for C₈H₁₃O [M+H]⁺ 125.0961. Data consistent with the literature.^[226] **Data for 254:** *R_f* = 0.30 (petrol/ether, 10:1); m.p. 45–50 °C; **IR** (ν_{max}/cm⁻¹): 3407br (O-H), 1657w (arC-C), 1594w (arC-C), 1190s (C-O), 756s (C=C); **¹H**

NMR (400 MHz, CDCl₃) δ 6.98 (d, $J = 7.5$, 2H, H_3), 6.76 (t, $J = 7.5$, 1H, H_4), 4.59 (s, 1H, OH), 2.25 (s, 6H, $C1^a$); **¹³C NMR** (101 MHz, CDCl₃) δ 152.3 (C1), 128.7 (C3), 123.1 (C2), 120.3 (C4), 16.0 (C1^a), **HRMS** (ESI): m/z found 121.0658, calcd. for C₈H₉O [M-H]⁻ 121.0658. Data consistent with the literature.^[227]

- **Method B:**

N-bromosuccinimide (2.81 g, 15.8 mmol) was added to a solution of 2,6-dimethylcyclohexan-1-one (2.00 g, 15.8 mmol) in chloroform (15.8 mL) at RT and the suspension was heated to reflux for 14 h. After cooling to RT, and it was filtered and the white precipitate was washed with cold CHCl₃. The filtrate was concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether 4:3), affording enone **250** (1.50 g, 77%). Data as above.

2,6-Dimethyl-2-[4-methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl]cyclohexan-1-one (**253**)



- **Method A:**

n-BuLi (2.5 M in hexane, 160 μ L, 0.40 mmol) was added dropwise to a solution of diisopropylamine (57 μ L, 0.41 mmol) in THF (0.6 mL) at -78 °C. After stirring for 15 min at -78 °C, a solution of 2,6-dimethylcyclohexan-1-one (as a mixture of isomers) (50 mg,

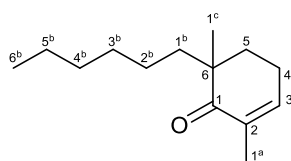
Experimental

0.40 mmol) in THF (0.6 mL) was added dropwise, and stirring continued for an additional 15 min. DMPU (0.22 mL, 1.82 mmol) was added at $-78\text{ }^{\circ}\text{C}$, the obtained mixture was stirred for 15 min at $-78\text{ }^{\circ}\text{C}$, and then iododiene **214** (30 μL , 0.18 mmol) was added dropwise at $-78\text{ }^{\circ}\text{C}$. The acetone/dry ice bath was removed and the mixture was warmed to RT, and then stirred for 14 h. The reaction was quenched with a satd. aq. solution of NH_4Cl (2 mL). The two layers were separated, the aqueous layer was extracted with ether (5 \times 1 mL) and the organic extracts were combined, washed with brine (3 mL), dried over MgSO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 40:1), affording ketone **253** (13 mg, 29%) as an inseparable mixture of two diastereomers **253a** and **253b** (**253a**:**253b** = 5:2) as a pale yellow oil. **Data for both diastereomers 253a and 253b:** R_f = 0.68 (petrol/ether, 40:1); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 1704s (C=O); 892m (C=CH₂); **MS** (ESI⁺): m/z 271.2 ([M+Na]⁺, 100%), 249.2 ([M+H]⁺, 44%); **HRMS** (ESI⁺): m/z found 249.2213, calcd. for C₁₇H₂₉O [M+H]⁺ 249.2212. **NMR data for diastereomer 253a:** **¹H NMR** (500 MHz, C₆D₆) δ 4.98 (dq, J = 3.0, 1.5, 1H, $H1^b$), 4.68 (dq, J = 3.0, 1.0, 1H, $H1^b$), 2.43 (dq, J = 13.0, 6.5, 5.0, 1H, $H6$), 2.16 (td, J = 12.5, 4.5, 1H, $H2^a$), 1.88 (td, J = 12.5, 5.0, 1H, $H1^a$), 1.77–1.74 (m, 1H, $H2^a$), 1.70 (dd, J = 1.5, 1.0, 3H, $H3^b$), 1.69 (s, 3H, $H5^a$), 1.65–1.58 (m, 3H, $H3$, $H4$ and $H5$), 1.56 (s, 3H, $H1^c$), 1.35 (td, J = 12.5, 4.5, 2H, $H1^a$), 1.29–1.22 (m, 2H, $H4$ and $H5$), 1.16 (s, 3H, $H1^d$), 1.07 (d, J = 6.5, 3H, $H1^c$), 1.06–0.99 (m, 1H, $H3$); **¹³C NMR** (126 MHz, C₆D₆) δ 214.5 (C1), 146.7 (C2^b), 136.6 (C3^a), 125.3 (C4^a), 113.6 (C1^b), 48.8 (C2), 41.4 (C6), 40.8 (C5), 36.8 (C3), 36.4 (C1^a), 25.8 (C2^a), 22.8 (C1^d), 22.7 (C3^b), 21.9 (C5^a), 21.5 (C4), 19.5 (C1^c), 15.4 (C1^c). **NMR data for diastereomer 253b:** **¹H NMR** (500 MHz, C₆D₆) δ 5.04 (dq, J = 3.0, 1.5, 2H, $H1^b$), 4.79 (dq, J = 3.0, 1.0, 1H, $H1^b$), 2.28–2.19 (m, 2H, $H6$ and $H2^a$), 1.88 (dd, J = 1.5, 1.0, 3H, $H3^b$), 1.87–1.83 (m, 2H, $H1^a$), 1.81 (s, 3H, $H5^a$), 1.78 (s, 3H, $H1^c$), 1.69–1.66 (m, 2H, $H3$), 1.49–1.44 (m, 2H, $H4$), 1.43–1.37 (m, 2H, $H5$), 1.25–1.22 (m, 1H, $H2^a$), 1.02 (d, J = 6.5, 3H, $H1^c$), 0.91 (s, 3H, $H1^d$); **¹³C NMR** (126 MHz, C₆D₆) δ 214.6 (C1), 147.1

(C2^b), 137.5 (C3^a), 125.3 (C4^a), 113.5 (C1^b), 47.7 (C2), 41.1 (C6), 38.9 (C5), 37.9 (C3), 36.5 (C1^a), 26.1 (C2^a), 23.3 (C1^d), 23.0 (C3^b), 22.1 (C1^c), 21.5 (C4), 19.8 (C5^a), 15.5 (C1^e).

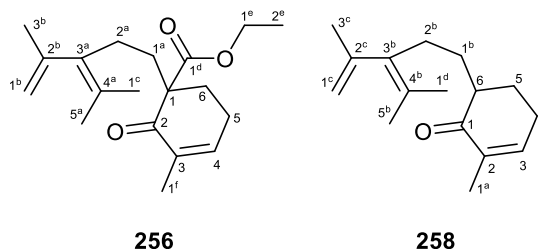
- **Method B:**

MeLi (1.6M in hexane, 0.30 mL, 0.48 mmol) was added dropwise to a solution of TMS-enol ether **282** (87 mg, 0.44 mmol) in THF (2.2 mL) at 0 °C. The mixture was warmed up to RT and then stirred for 2 h. After cooling to –78 °C, DMPU (0.48 mL, 4.00 mmol) was added, the mixture was stirred for 15 min at –78 °C and then iododiene **214** (76 µL, 0.40 mmol) was added all at once at –78 °C. The obtained mixture was stirred for 30 min at –78 °C, warmed up slowly to RT and then stirred for 14 h. The reaction was quenched with a satd. aq. solution of NH₄Cl (2 mL). The two layers were separated, the aqueous layer was extracted with ether (3 × 2 mL) and the organic extracts were combined, washed with brine (3 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 20:1), affording ketone **253** (30 mg, 30%) as an inseparable mixture of two diastereomers **253a** and **253b** (**253a**:**253b** = 5:2) as a pale yellow oil. When the reaction was performed with enantioenriched TMS-enol ether **282** (89 mg, 0.45 mmol, ee 50%), ketone **253** (22 mg, 22%) was afforded as an inseparable mixture of racemic diastereomers **253a** and **253b** (**253a**:**253b** = 17:10) [ee 0% (Chiral GC analysis)]. Enantiomeric ratios were determined by GC analysis: β-DEX 325 column; carrier gas, H₂ (80 kPa); injector/detector temperature, 200 °C; 70–120 °C, temperature gradient: 1.5 °C/min; detection method, FID; t_r = 9.998 min [(**2R***,**6S***)-**253a** and (**2S***,**6R***)-**253a**], 10.825 [(**2R***,**6R***)-**253b**], 11.114 [(**2S***,**6S***)-**253b**].

6-Hexyl-2,6-dimethylcyclohex-2-en-1-one (**255**)

A solution of enone **250** (100 mg, 0.81 mmol) in THF (2.3 mL) was added dropwise to a solution of potassium bis(trimethylsilyl)amide (0.5 M in toluene, 1.62 mL, 0.81 mmol) in THF (0.4 mL) at -78 °C. After stirring for 30 min at -78 °C, 1-iodohexane (240 μ L, 1.63 mmol) was added dropwise at -78 °C and the mixture was slowly warmed to RT and then stirred for 14 h. The reaction was quenched with a satd. aq. solution of NH_4Cl (2 mL). The two layers were separated, the aqueous layer was extracted with ether (5×2 mL) and the organic extracts were combined, washed with brine (7 mL), dried over anhydrous MgSO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 20:1), affording enone **255** (100 mg, 59%) as a colourless oil. $R_f = 0.52$ (petrol/ether, 2:1); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 1703w (C=C), 1668s (C=O); **$^1\text{H NMR}$** (400 MHz, CDCl_3) δ 6.63–6.57 (m, 1H, $H3$), 2.35–2.26 (m, 2H, $H4$), 1.90 (dt, $J = 13.0, 5.5$, 1H, $H5$), 1.80–1.69 (m, 1H, $H5$), 1.75 (td, $J = 2.0, 1.5$, 3H, $H1^a$), 1.57–1.46 (m, 1H, $H1^b$), 1.46–1.36 (m, 1H, $H1^b$), 1.32–1.15 (m, 8H, $H2^b, H3^b, H4^b$ and $H5^b$), 1.05 (s, 3H, $H1^c$), 0.86 (t, $J = 7.0$, 3H, $H6^b$); **$^{13}\text{C NMR}$** (101 MHz, CDCl_3) δ 204.8 (C1), 143.4 (C3), 134.2 (C2), 44.5 (C6), 36.6 (C1^b), 34.0 (C5), 31.9 (C4^b), 30.1 (C3^b), 24.2 (C2^b), 23.0 (C4), 22.8 (C5^b), 22.3 (C1^c), 16.7 (C1^a), 14.2 (C6^b); **MS** (ESI⁺): m/z 231.2 ($[\text{M}+\text{Na}]^+$, 100%), 209.2 ($[\text{M}+\text{H}]^+$, 38%), 439.4 ($[\text{2M}+\text{Na}]^+$, 33%); **HRMS** (ESI⁺): m/z found 209.1900, calcd. for $\text{C}_{14}\text{H}_{25}\text{O}$ $[\text{M}+\text{H}]^+$ 209.1899.

Ethyl 3-methyl-1-[4-methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl]-2-oxocyclohex-3-ene-1-carboxylate (**256**) and 2-methyl-6-[4-methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl]cyclohex-2-en-1-one (**258**)

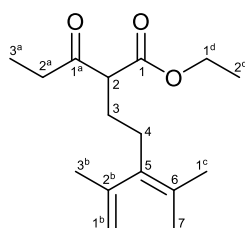


Freshly distilled acrolein (30 μ L, 0.45 mmol) was added dropwise to a solution of β -keto ester **257** (120 mg, 0.45 mol) in *t*-BuOH (0.5 mL) at RT. After cooling to 0 $^{\circ}$ C, potassium *tert*-butoxide (1.0 M in THF, 113 μ L, 0.113 mmol) was added dropwise at 0 $^{\circ}$ C. The mixture was stirred for 30 min at 0 $^{\circ}$ C and then, heated to reflux for 20 h. The reaction mixture was cooled to RT, quenched with aq. HCl (1.0 M, 1 mL) and diluted with ether (1 mL) and benzene (1 mL). The two layers were separated and the organic layer was washed sequentially with aq. NaOH (1.0 M, 3 x 2 mL) and brine (2 \times 2 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/EA, 9:1) affording β -keto ester **256** (8 mg, 6%) as a pale yellow oil and enone **258** (7 mg, 7%) as a pale yellow oil. **Data for 256:** R_f = 0.34 (petrol/ether, 9:1); **¹H NMR** (400 MHz, CDCl₃) δ 6.63–6.58 (m, 1H, *H*4), 4.93–4.89 (m, 1H, *H*1^b), 4.57–4.54 (m, 1H, *H*1^b), 4.16 (app. qd, *J* = 7.0, 2.5, 2H, *H*1^c), 2.52–2.37 (m, 2H, *H*5 and *H*6), 2.34–2.21 (m, 1H, *H*5), 2.14 (td, *J* = 13.0, 4.5, 1H, *H*2^a), 2.03 (td, *J* = 13.0, 4.5, 1H, *H*2^a), 1.99–1.94 (m, 1H, *H*6), 1.89 (td, *J* = 13.0, 4.5, 1H, *H*1^a), 1.79 (s, 3H, *H*1^f), 1.76 (s, 3H, *H*3^b), 1.76–1.68 (m, 1H, *H*1^a), 1.69 (s, 3H, *H*5^a), 1.65 (s, 3H, *H*1^f), 1.22 (m, 3H, *H*2^a); **¹³C NMR** (101 MHz, CDCl₃) δ 197.3 (C2), 172.1 (C1^d), 146.3 (C2^b), 143.7 (C4), 135.9 (C3^a), 135.2 (C3), 126.1 (C4^a), 113.5 (C1^b), 61.2 (C1^e), 56.8 (C1), 32.4 (C1^a), 30.5 (C6), 26.4 (C2^a), 23.5 (C5), 22.8 (C3^b), 21.9 (C1^c), 19.7 (C5^a), 16.7 (C1^f), 14.3 (C2^e); **MS** (ESI⁺): *m/z*

Experimental

305.2 ($[M+H]^+$, 100%), 327.2 ($[M+Na]^+$, 88%), 631.4 ($[2M+Na]^+$, 75%). **Data for 258:** R_f = 0.57 (petrol/ether, 9:1); 1H NMR (400 MHz, $CDCl_3$) δ 6.68–6.63 (m, 1H, $H3$), 4.91 (dq, J = 2.5, 1.5, 1H, $H1'$), 4.57–4.50 (m, 1H, $H1'$), 2.38–2.28 (m, 2H, $H4$), 2.28–2.19 (m, 1H, $H6$), 2.13 (t, J = 8.5, 2H, $H2^b$), 2.10–2.02 (m, 1H, $H5$), 1.89–1.77 (m, 2H, $H5$ and $H1^b$), 1.77–1.75 (m, 6H, $H1^a$ and $H3^c$), 1.68 (s, 3H, $H5^b$), 1.66 (s, 3H, $H1^d$), 1.42–1.32 (m, 1H, $H1^b$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 202.5 (C1), 146.6 (C2^c), 144.3 (C3), 136.4 (C3^b), 135.3 (C2), 125.6 (C4^b), 113.3 (C1^c), 46.5 (C6), 28.7 (C2^b), 28.5 (C5), 28.4 (C1^b), 25.1 (C4), 22.9 (C3^c), 21.9 (C1^d), 19.8 (C5^b), 16.4 (C1^a); **MS** (ESI⁺): m/z 255.2 ($[M+Na]^+$, 100%), 233.2 ($[M+H]^+$, 42%).

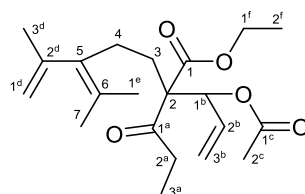
Ethyl 6-methyl-5-(prop-1-en-2-yl)-2-propionylhept-5-enoate (257)



K_2CO_3 (287 mg, 2.08 mmol) was added to a solution of ethyl 3-oxopentanoate (300 mg, 2.08 mmol) in acetone (2.1 mL) at RT. After stirring for 10 min at RT, iododiene **214** (1.30 g/mL, 130 μ L, 0.68 mmol) was added dropwise and then the reaction mixture was heated to reflux for 14 h. After cooling to RT, the mixture was diluted with ether (5 mL) and filtered. The precipitate was washed with ether (5 mL) and the filtrate was concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol to petrol/ether, 19:1), affording β -keto ester **257** (135 mg, 75%) as a colourless oil. R_f = 0.40 (petrol/ether, 9:1); **IR** (ν_{max}/cm^{-1}): 1741m (C=O), 1714s (C=O), 1631w (C=C), 1159m (C-O), 980m (C=C); 1H NMR (400 MHz, $CDCl_3$) δ 4.88 (dq, J = 2.5, 1.5, 1H, $H1^b$), 4.50 (dq, J = 2.5, 1.0, 1H, $H1^b$), 4.11 (q, J = 7.0, 2H, $H1^d$), 3.34 (dd, J = 8.0, 6.5, 1H,

H_2), 2.58–2.35 (m, 2H, H_2^a), 2.01 (t, $J = 7.5$, 2H, H_4), 1.89–1.71 (m, 2H, H_3), 1.68 (dd, $J = 1.5$, 1.0, 3H, H_3^b), 1.60 (s, 3H, H_7), 1.57 (s, 3H, H_1^c), 1.20 (t, $J = 7.0$, 3H, H_2^d), 0.99 (t, $J = 7.0$, 3H, H_3^a); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 206.2 (C_1^a), 170.1 (C1), 146.0 (C_2^b), 135.2 (C5), 126.8 (C6), 113.8 (C_1^b), 61.4 (C_1^d), 58.5 (C2), 35.1 (C_2^a), 28.5 (C4), 27.0 (C3), 22.7 (C_3^b), 21.9 (C7), 19.8 (C_1^c), 14.3 (C_2^d), 7.8 (C_3^a); **MS** (ESI $^+$): m/z 556.4 ($[\text{2M}+\text{Na}]^+$, 100%), 289.1 ($[\text{M}+\text{Na}]^+$, 43%), 267.2 ($[\text{M}+\text{H}]^+$, 28%); **HRMS** (ESI $^+$): m/z found 289.1773, calcd. for $\text{C}_{16}\text{H}_{26}\text{O}^{23}\text{Na}$ $[\text{M}+\text{Na}]^+$ 289.1774.

Ethyl 2-(1-acetoxyallyl)-6-methyl-5-(prop-1-en-2-yl)-2-propionylhept-5-enoate (**260**)

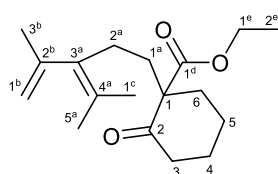


Sodium hydride (60% dispersion in mineral oil, 41 mg, 1.03 mmol) was added under an inert atmosphere of Argon to a solution of β -keto ester **257** (135 mg, 0.51 mmol) in THF (1.0 mL) at RT and the mixture was stirred for 20 min at RT. After cooling to -78 °C, *n*-BuLi (2.5 M in hexane, 240 μL , 0.60 mmol) was added dropwise. The mixture was stirred for 20 min at -78 °C, freshly distilled acrolein (34 μL , 0.51 mmol) was added dropwise and stirring continued for an additional 1 h at -78 °C. Acetic anhydride (58 μL , 0.61 mmol) was added dropwise at -78 °C, the reaction mixture was stirred for 3 h at -78 °C and then quenched at this temperature with aq. HCl (1.0 M, 0.5 mL). After warming to RT, the two layers were separated, the aqueous layer was extracted with ether (3×1 mL) and the organic extracts were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol to petro/ether, 7:3), affording β -keto ester **260** (25 mg, 13%) as a pale yellow oil. β -keto ester **260** was tentatively assigned. $R_f = 0.56$ (petrol/ether, 4:1); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ

Experimental

7.04 (dt, $J = 12.5, 1.5$, 1H, $H2^b$), 5.16 (app. dt, $J = 12.5, 8.0$, 2H, $H3^b$), 4.85 (dq, $J = 3.0, 1.5$, 1H, $H1^d$), 4.50 (dq, $J = 3.0, 1.0$, 1H, $H1^d$), 4.12 (app. q, $J = 7.0$, 3H, $H1^b$ and $H1^c$), 2.49 (dt, $J = 8.0, 1.5$, 2H, $H4$), 2.35 (q, $J = 7.5$, 1H, $H2^a$), 2.03 (s, 3H, $H2^c$), 1.83–1.78 (m, 2H, $H3$), 1.65 (dd, $J = 1.5, 1.0$, 3H, $H3^d$), 1.57 (s, 3H, $H7$), 1.55 (s, 3H, $H1^e$), 1.19 (t, $J = 7.0$, 3H, $H2^f$), 0.98 (t, $J = 7.0$, 3H, $H3^e$).

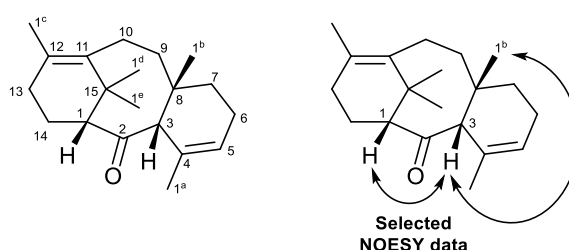
Ethyl 1-[4-methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl]-2-oxocyclohexane-1-carboxylate (**261**)



A solution of ethyl 2-oxocyclohexane-1-carboxylate (100 mg, 0.59 mmol) in DMF (0.6 mL) was added dropwise to a suspension of NaH (28 mg, 0.70 mmol) in DMF (0.6 mL) at RT. After stirring for 1 h at RT, a solution of iododiene **214** (160 mg, 0.64 mmol) in DMF (0.6 mL) was added dropwise and the obtained mixture was stirred for 6 h at RT then heated to 50 °C for 14 h. The reaction mixture was cooled to 0 °C and quenched with aq. HCl (3.0 M, 2 mL). The two layers were separated, the aqueous layer was extracted with ether (5 × 2 mL) and the organic extracts were combined, washed with brine (3 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether 19:1), affording β-keto ester **261** (28 mg, 16%) as a pale yellow oil. $R_f = 0.29$ (petrol/ether, 19:1); IR ($\nu_{\text{max}}/\text{cm}^{-1}$): 1714s (C=O), 1186m (C-O); $^1\text{H NMR}$ (400 MHz, CDCl₃) δ 4.90 (dq, $J = 3.0, 1.5$, 1H, $H1^b$), 4.55 (dq, $J = 3.0, 1.0$, 1H, $H1^c$), 4.21 (q, $J = 7.0$, 2H, $H1^d$), 2.55–2.48 (m, 1H, $H1^e$), 2.47–2.41 (m, 2H, $H3$), 2.08–1.94 (m, 2H, $H5$ and $H2^a$), 1.94–1.83 (m, 2H, $H5$ and $H6$), 1.74 (dd, $J = 1.5, 1.0$, 3H, $H3^b$), 1.73–1.67 (m, 3H, $H4$ and $H2^c$), 1.66 (s, 3H, $H5^a$), 1.65 (s, 3H, $H1^e$), 1.55–1.48 (m, 1H, $H6$), 1.47–1.42 (m, 1H, $H1^f$), 1.27 (t, $J = 7.0$, 3H, $H2^f$); $^{13}\text{C NMR}$ (101 MHz,

CDCl₃) δ 208.3 (C2), 172.2 (C1^d), 146.3 (C2^b), 135.9 (C3^a), 125.9 (C4^a), 113.5 (C1^b), 61.3 (C1^e), 60.9 (C1), 41.3 (C3), 36.1 (C1^a), 33.4 (C6), 27.8 (C2^a), 25.9 (C5), 22.7 (C3^b), 22.6 (C4), 21.9 (C1^c), 19.6 (C5^a), 14.3 (C2^c); **MS** (ESI⁺): m/z 315.2 ([M+Na]⁺, 100%), 607.4 ([2M+Na]⁺, 42%), 293.2 ([M+H]⁺, 14%); **HRMS** (CI⁺): m/z found 293.2107, calcd. for C₁₉H₂₉O₃ [M+H]⁺ 293.2111.

(1*R,3*R**,8*S**)-4,8,12,15,15-Pentamethyltricyclo[9.3.1.0^{3,8}]pentadec-4,11-diene-2-one (264)**

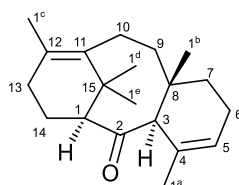


A solution of enone **232a** (56 mg, 0.20 mmol) in DCM (3.1 mL) was added to a solution of BF₃·OEt₂ (90 μL, 0.73 mmol) in DCM (12.6 mL) at 0 °C. The mixture was stirred for 1 h at 0 °C and then poured into a satd. aq. solution of NaHCO₃ (15 mL). The two layers were separated, the aqueous layer was extracted with DCM (3 × 8 mL) and the organic extracts were combined, washed sequentially with water (8 mL) and brine (8 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 20:1), affording an inseparable mixture of di-*epi*-taxadienone **220** and *epi*-taxadienone **264** (36 mg, 64%, **220:264** = 7:1) as white solid. *Epi*-taxadienone **264** was tentatively assigned. **Data for 220 and 264:** R_f = 0.47 (petrol/ether, 10:1); **m.p.** 52–64 °C; **IR** (ν_{\max} /cm⁻¹): 1684s (C=O); **MS** (ESI⁺): m/z 287.3 ([M+H]⁺, 100%), 595.4 ([2M+Na]⁺, 67%), 309.2 ([M+Na]⁺, 22%); **HRMS** (ESI⁺): m/z found 309.2190, calcd. for C₂₀H₃₀O²³Na [M+Na]⁺ 309.2188. **NMR data for 220:** data as above. **NMR data for 264** (tentatively assigned): ¹H NMR (400 MHz, CDCl₃) δ 5.61–5.56 (m, 1H, *H*₅), 3.53 (s, 1H, *H*₃), 1.86–1.82 (m, 3H, *H*_{1^a}), 1.60–1.57 (m, 3H, *H*_{1^c}), 1.19 (s, 3H,

Experimental

$H1^d$), 1.01 (s, 3H, $H1^e$), 0.76 (s, 3H, $H1^b$); $^{13}\text{C NMR}$ (126 MHz, CDCl_3) δ 218.2 (C2), 139.1 (C11), 124.9 (C4), 124.0 (C12), 120.3 (C5), 56.1 (C1), 55.0 (C3), 33.1 (C1^c), 25.04 (C1^d), 25.00 (C1^a), 23.6 (C1^b), 22.5 (C1^e).

(1*S**,3*S**,8*S**)-4,8,12,15,15-Pentamethyltricyclo[9.3.1.0^{3,8}]pentadec-4,11-diene-2-one (265)

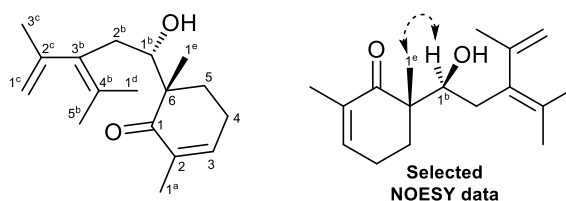


A solution of enone **332b** (28 mg, 0.10 mmol) in DCM (2.3 mL) was added to a solution of $\text{BF}_3 \cdot \text{OEt}_2$ (50 μL , 0.51 mmol) in DCM (6.0 mL) at 0 °C. The mixture was stirred for 1 h at 0 °C and then poured into a satd. aq. solution of NaHCO_3 (8 mL). The two layers were separated, the aqueous layer was extracted with DCM (3 \times 4 mL) and the organic extracts were combined, washed sequentially with water (4 mL) and brine (4 mL), dried over MgSO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 20:1), affording *epi*-taxadienone **265** (13 mg, 50%) as a pale yellow oil. *Epi*-taxadienone **265** was tentatively assigned. R_f = 0.31 (petrol/ether, 20:1); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.55–5.48 (m, 1H, $H5$), 3.51 (s, 1H, $H3$), 2.14 (t, J = 3.5, 1H, $H1$), 1.73–1.71 (m, 3H, $H1^c$), 1.47 (app. dq, J = 2.5, 1.5, 3H, $H1^a$), 1.07 (s, 3H, $H1^d$), 1.01 (s, 3H, $H1^e$), 0.78 (s, 3H, $H1^b$).

Experimental

$H1^c$), 1.09 (ddd, $J = 13.5, 10.0, 7.5$, 1H, $H1^c$), 0.91 (s, 3H, $H1^f$); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 211.9 (C1), 146.6 (C2^d), 136.7 (C3^c), 130.2 (C2^a), 125.0 (C4^c), 123.9 (C3^a), 113.2 (C1^d), 67.7 (C3), 64.7 (C1^a), 58.9 (C1^b), 44.7 (C2), 39.4 (C1^c), 35.5 (C6^a), 27.9 (C5^a), 25.0 (C2^c), 23.6 (C1^f), 23.5 (C1^b), 22.9 (C3^d), 22.6 (C4^a), 21.9 (C5^c), 19.6 (C1^e); **MS** (ESI $^+$): m/z 341.2 ($[\text{M}+\text{Na}]^+$, 100%); **HRMS** (ESI $^+$): m/z found 319.2632, calcd. for $\text{C}_{21}\text{H}_{35}\text{O}_2$ $[\text{M}+\text{H}]^+$ 319.2631. **Data for diastereomer 266b:** $R_f = 0.24$ (petrol/ether, 10:1); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 1707m (C=O), 1119s (C-O), 893m (C=C); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.61–5.56 (m, 1H, $H3^a$), 4.89 (dq, $J = 3.0, 1.5$, 1H, $H1^d$), 4.51 (dq, $J = 3.0, 1.0$, 1H, $H1^d$), 3.63 (app. td, $J = 6.5, 1.0$, 2H, $H3$), 3.32 (s, 3H, $H1^e$), 2.83 (s, 1H, $H1^a$), 2.80 (dt, $J = 17.5, 6.5$, 1H, $H2$), 2.69 (dt, $J = 17.5, 6.5$, 1H, $H2$), 2.10–1.96 (m, 4H, $H4^a$ and $H2^c$), 1.74 (dd, $J = 1.5, 1.0$, 3H, $H3^d$), 1.72–1.67 (m, 1H, $H5^a$), 1.64 (s, 3H, $H5^c$), 1.63 (s, 3H, $H1^c$), 1.58–1.55 (m, 3H, $H1^b$), 1.38–1.27 (m, 2H, $H5^a$ and $H1^c$), 1.25–1.15 (m, 1H, $H1^c$), 0.88 (s, 3H, $H1^f$); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 212.0 (C1), 146.7 (C2^d), 136.6 (C3^c), 130.0 (C2^a), 125.0 (C4^c), 124.0 (C3^a), 113.2 (C1^d), 67.7 (C3), 63.0 (C1^a), 59.0 (C1^b), 45.3 (C2), 37.4 (C1^c), 35.0 (C6^a), 28.6 (C5^a), 25.3 (C2^c), 24.4 (C1^f), 23.6 (C1^b), 22.9 (C3^d), 22.5 (C4^a), 21.9 (C5^c), 19.6 (C1^e); **MS** (ESI $^+$): m/z 341.2 ($[\text{M}+\text{Na}]^+$, 100%), 319.2 ($[\text{M}+\text{H}]^+$, 38%); **HRMS** (ESI $^+$): m/z found 319.2632, calcd. for $\text{C}_{21}\text{H}_{35}\text{O}_2$ $[\text{M}+\text{H}]^+$ 319.2631.

(*S*^{*})-6-[(*S*^{*})-1-Hydroxy-4-methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl]-2,6-dimethylcyclohex-2-en-1-one (269)

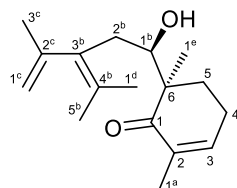


n-BuLi (1.6 M in hexane, 0.66 mL, 1.06 mmol) was added dropwise to a solution of diisopropylamine (150 μ L, 1.07 mmol) in THF (2.0 mL) at -78 $^{\circ}$ C. The mixture was warmed to 0 $^{\circ}$ C, stirred for 30 min at 0 $^{\circ}$ C and cooled again to -78 $^{\circ}$ C. A solution of enone **250** (122 mg, 0.98 mmol) in THF (2.0 mL) was added dropwise at -78 $^{\circ}$ C. The mixture was stirred for 25 min at -78 $^{\circ}$ C, a solution of aldehyde **270** (149 mg, 1.08 mmol) in THF (1.0 mL) was added dropwise and stirring continued for an additional 20 min at -78 $^{\circ}$ C. The reaction was quenched at -78 $^{\circ}$ C with a satd. aq. solution of NH_4Cl (5 mL) and then warmed to RT. The two layers were separated, the aqueous layer was extracted with ether (3×3 mL) and the organic extracts were combined, washed sequentially with water (5 mL) and brine (5 mL), dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 5:1), affording aldol **269** (199 mg, 77%) as a pale yellow solid. $R_f = 0.31$ (petrol/ether, 5:1); **m.p.** $40\text{--}47$ $^{\circ}$ C; **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 3495br (O-H), 1660s (C=O), 1194m (C-O), 894 (C=C); **^1H NMR** (400 MHz, CDCl_3) δ 6.71–6.62 (m, 1H, $H3$), 5.00 (dq, $J = 3.0, 1.5$, 1H, $H1^c$), 4.67 (dq, $J = 3.0, 1.0$, 1H, $H1^d$), 3.92 (dt, $J = 10.5, 2.5$, 1H, $H1^b$), 2.90 (d, $J = 2.5$, 1H, OH), 2.42 (dd, $J = 14.0, 10.5$, 1H, $H2^b$), 2.38–2.31 (m, 2H, $H4$), 2.08 (d, $J = 14.0$, 1H, $H2^a$), 1.92 (ddd, $J = 13.0, 9.0, 5.5$, 1H, $H5$), 1.85 (app. t, $J = 4.5$, 1H, $H5$), 1.80 (dd, $J = 1.5, 1.0$, 3H, $H3^a$), 1.76 (dt, $J = 2.0, 1.5$, 3H, $H1^a$), 1.73 (d, $J = 1.0$, 3H, $H5^b$), 1.72 (d, $J = 1.0$, 3H, $H1^d$), 1.17 (s, 3H, $H1^c$); **^{13}C NMR** (101 MHz, CDCl_3) δ 205.6 (C1), 146.7 (C2^c), 144.3 (C3), 134.4 (C3^b), 133.6 (C2), 128.6 (C4^b), 114.2 (C1^c), 73.7 (C1^b), 47.9 (C6), 32.5 (C2^b), 30.6 (C5), 22.8 (C4

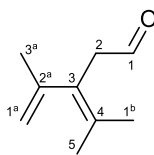
Experimental

and C3^b), 22.2 (C1^d), 20.3 (C5^b), 17.1 (C1^c), 16.6 (C1^a); **MS** (ESI⁺): m/z 285.2 ([M+Na]⁺, 100%); **HRMS** (ESI⁺): m/z found 263.2008, calcd. for C₁₇H₂₇O₂ [M+H]⁺ 263.2005.

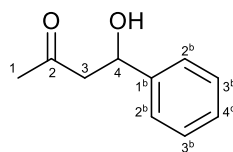
(*R*)-6-[(*R*)-1-Hydroxy-4-methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl]-2,6-dimethylcyclohex-2-en-1-one (**269**)



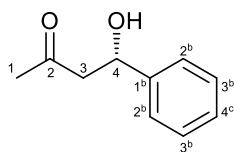
Potassium bis(trimethylsilyl)amide (0.5 M in toluene, 0.80 mL, 0.40 mmol) was added dropwise to a solution of enone **250** (41 mg, 0.33 mmol) in THF (0.7 mL) at -78 °C. The mixture was stirred for 20 min at -78 °C, a solution of (+)-B-chlorodiisopinocampheylborane (221 mg, 0.69 mmol) in THF (0.4 mL) was added and stirring continued for an additional 1 h at -78 °C. A solution of aldehyde **270** (50 mg, 0.36 mmol) in THF (0.8 mL) was added dropwise at -78 °C, the mixture was slowly warmed to RT and then stirred for 14 h. The reaction was quenched with a satd. aq. solution of NaHCO₃ (1.5 mL). The two layers were separated, the aqueous layer was extracted with DCM (3 × 1 mL) and the organic extracts were combined, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 10:1), affording aldol (***R,R***-**269**) (8 mg, 9%) as a pale yellow solid (data as above). The enantiopurity of **269** could not be determined neither by the Mosher ester method nor chiral GC. The configuration of aldol **269** was assigned by correlation with the reported configuration of aldol **273** when using (–)-DIP-Cl.^[172]

4-Methyl-3-(prop-1-en-2-yl)pent-3-enal (270)^[109]

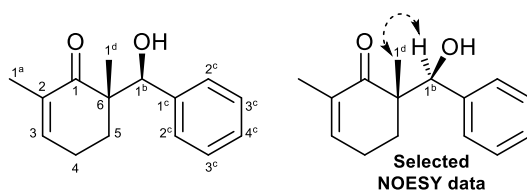
NaHCO₃ (2.39 g, 28.4 mmol) and Dess-Martin periodinane (1.45 g, 3.42 mmol) were added sequentially to a solution of the alcohol **185** (400 mg, 2.85 mmol) in DCM (14.3 mL) at RT. After stirring for 20 min at RT, the reaction was diluted with ether (10 mL), quenched by addition of a satd. aq. solution of Na₂S₂O₃ (6 mL) and a satd. aq. solution of NaHCO₃ (6 mL) and stirring continued for an additional 20 min at RT. The two layers were separated, the aqueous layer was extracted with ether (4 × 5 mL) and the organic extracts were combined, dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 5:1), affording aldehyde **270** (357 mg, 91%) as a pale yellow oil. *R_f* = 0.77 (petrol/ether, 5:1); **IR** (ν_{max}/cm⁻¹): 1722s (C=O), 1632w (C=C), 897m (C=C); **¹H NMR** (400 MHz, C₆D₆) δ 9.31 (t, *J* = 2.5, 1H, *H1*), 4.87 (dq, *J* = 2.5, 1.5, 1H, *H1^a*), 4.62 (dq, *J* = 2.5, 1.0, 1H, *H1^b*), 2.82–2.76 (m, 2H, *H2*), 1.60 (s, 3H, *H5*), 1.58 (dd, *J* = 1.5, 1.0, 3H, *H3^a*), 1.39 (s, 3H, *H1^b*); **¹³C NMR** (101 MHz, C₆D₆) δ 197.7 (C1), 146.4 (C2^a), 131.1 (C3), 128.7 (C4), 114.2 (C1^a), 46.9 (C2), 22.2 (C3^a), 21.8 (C5), 20.0 (C1^b); **HRMS** (CI⁺): *m/z* found 139.1116, calcd. for C₉H₁₅O [M+H]⁺ 139.1117. Data consistent with the literature.^[109]

4-Hydroxy-4-phenylbutan-2-one (**276**)^[175]

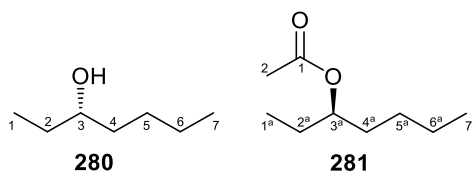
n-BuLi (2.5 M in hexane, 0.60 mL, 1.50 mmol) was added dropwise to a solution of diisopropylamine (210 μ L, 1.50 mmol) in THF (3.7 mL) at -78 $^{\circ}$ C. The mixture was warmed to 0 $^{\circ}$ C, stirred for 15 min at 0 $^{\circ}$ C and cooled again to -78 $^{\circ}$ C. Acetone (100 μ L, 1.36 mmol) was added dropwise at -78 $^{\circ}$ C. The mixture was stirred for 20 min at -78 $^{\circ}$ C, benzaldehyde (150 μ L, 1.48 mmol) was added dropwise and stirring continued for an additional 20 min at -78 $^{\circ}$ C. The reaction was quenched with a satd. aq. solution of NH_4Cl (4 mL) at -78 $^{\circ}$ C and then, warmed to RT. The two layers were separated, the aqueous layer was extracted with ether (3×4 mL) and the organic extracts were combined, washed sequentially with water (6 mL) and brine (6 mL), dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 2:1 to 1:1) affording aldol **276** (93 mg, 42%) as a white solid. $R_f = 0.30$ (petrol/ether, 1:1); **m.p.** 39–43 $^{\circ}$ C; **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 3442br (OH), 1702s (C=O), 1357m (O-H), 1089m (C-O), 1083m (C-O); **$^1\text{H NMR}$** (400 MHz, CDCl_3) δ 7.29 (d, $J = 0.5$, 2H, $H2^a$), 7.28–7.27 (m, 2H, $H3^a$), 7.23–7.19 (m, 1H, $H4^a$), 5.08 (dd, $J = 9.0$, 3.5, 1H, $H4$), 2.82 (ddq, $J = 17.5$, 9.0, 0.5, 1H, $H3$), 2.74 (ddq, $J = 17.5$, 3.5, 0.5, 1H, $H3$), 2.12 (t, $J = 0.5$, 3H, $H1$); **$^{13}\text{C NMR}$** (101 MHz, CDCl_3) δ 209.3 (C2), 142.8 (C1a), 128.7 (H3a), 127.8 (C4a), 125.7 (C2a), 70.0 (C4), 52.1 (C3), 30.9 (C1); **MS** (ESI $^+$): m/z 145.1 ($[\text{M}-\text{H}_2\text{O}-\text{H}]^-$, 84%); **HRMS** (ESI $^+$): m/z found 187.0731, calcd. for $\text{C}_{10}\text{H}_{12}\text{O}_2^{23}\text{Na}$ $[\text{M}+\text{Na}]^+$ 187.07295. Data consistent with the literature.^[175]

(4S)-4-Hydroxy-4-phenylbutan-2-one (276)^[175]

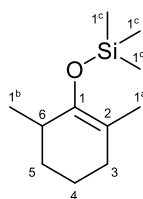
Diisopropylethylamine (0.47 mL, 2.70 mmol) and acetone (100 μ L, 1.36 mmol) were added sequentially to a solution of (+)-B-chlorodiisopinocampheylborane (568 mg, 1.77 mmol) in DCM (8.0 mL) at -78 $^{\circ}$ C. After stirring for 2 h at -78 $^{\circ}$ C, benzaldehyde (0.28 mL, 2.75 mmol) was added dropwise at -78 $^{\circ}$ C. The obtained mixture was stirred for 2 h at -78 $^{\circ}$ C, warmed to -15 $^{\circ}$ C and then stirred for 14 h at -15 $^{\circ}$ C. The reaction was quenched with aqueous buffer pH7 (8 mL). The two layers were separated, the aqueous layer was extracted with ether (3×8 mL) and the organic extracts were combined and concentrated under reduced pressure. The residue was redissolved in MeOH (6 mL) and aqueous buffer pH7 (1 mL). After cooling to 0 $^{\circ}$ C, hydrogen peroxide (30% in water, 1.6 mL) was added dropwise at 0 $^{\circ}$ C. The resulting mixture was warmed to RT, stirred for 2 h and then poured into water (8 mL). The two layers were separated, the aqueous layer was extracted with DCM (3×8 mL) and the organic extracts were combined, washed sequentially with a satd. aq. solution of NaHCO_3 (8 mL) and brine (8 mL), dried over MgSO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 2:1 to 1:1), affording aldol **(S)-276** (175 mg, 78 %) [ee 67% (Mosher ester Method), see **Appendix B**] as a white solid (data as above). $[\alpha]_{\text{D}}^{24} = -36.6^{\circ}$ (c 1.07, CHCl_3). The configuration of aldol **276** was assigned by correlation with the reported specific rotation of **279**.^[175]

(*S*^{*})-6-[(*S*^{*})-Hydroxyphenylmethyl]-2,6-dimethylcyclohex-2-en-1-one (277)

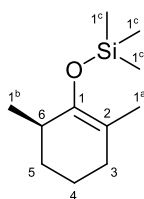
n-BuLi (2.5 M in hexane, 0.36 mL, 0.90 mmol) was added to a solution of diisopropylamine (123 μ L, 0.88 mmol) in THF (2.2 mL) at -78 $^{\circ}$ C. The mixture was warmed to 0 $^{\circ}$ C, stirred for 30 min at 0 $^{\circ}$ C and cooled again to -78 $^{\circ}$ C. A solution of enone **250** (100 mg, 0.81 mmol) in THF (2.0 mL) was added dropwise at -78 $^{\circ}$ C. The reaction was stirred for 25 min at -78 $^{\circ}$ C, benzaldehyde (90 μ L, 0.88 mmol) was added dropwise and stirring continued for an additional 20 min at -78 $^{\circ}$ C. The reaction was quenched with a satd. aq. solution of NH_4Cl (4 mL) at -78 $^{\circ}$ C and then warmed to RT. The two layers were separated, the aqueous layer was extracted with ether (3×2 mL) and the organic extracts were combined, washed sequentially with water (3 mL) and brine (3 mL), dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 5:1), affording aldol **277** (168 mg, 90%) as a white solid. $R_f = 0.29$ (petrol/ether, 5:1); **m.p.** $57\text{--}65$ $^{\circ}$ C; **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 3489br (O–H), 1649s (C=O); **^1H NMR** (400 MHz, CDCl_3) δ 7.30–7.18 (m, 5H, $H2^c$, $H3^c$ and $H4^c$), 6.69–6.64 (m, 1H, $H3$), 4.83 (s, 1H, $H1^b$), 4.80 (s, 1H, OH), 2.25–2.18 (m, 2H, $H4$), 1.75–1.74 (m, 3H, $H1^a$), 1.80–1.67 (m, 1H, $H5$), 1.25–1.15 (m, 1H, $H5$), 1.09 (s, 3H, $H1^d$); **^{13}C NMR** (101 MHz, CDCl_3) δ 207.8 (C1), 145.8 (C3), 139.2 (C1^c), 134.0 (C2), 128.3 (C3^c), 127.8 (C2^c and C4^c), 78.0 (C1^b), 47.6 (C6), 32.2 (C5), 22.8 (C4), 16.4 (C1^a), 14.1 (C1^d); **MS** (ESI⁺): m/z 253.2 ($[\text{M}+\text{Na}]^+$, 100%); **HRMS** (CI): m/z found 229.1231, calcd. for $\text{C}_{15}\text{H}_{17}\text{O}_2$ $[\text{M}-\text{H}]^-$ 229.1234.

(3S)-Heptan-3-ol (280)^[177] and **(3R)-heptan-3-yl acetate (281)**^[177]

3-Heptanol (50 mg, 0.43 mmol) was added to a suspension of vinyl acetate (0.43 mL, 4.67 mmol) and lipase B from *C. Antarctica* (68.8 mg) in hexane (8.6 mL) at RT and the mixture was stirred for 14 h at RT. The suspension was filtered and the filtrate concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 4:1 to 2:1), affording acetate **(R)-281** (29 mg, 42%) as a colourless oil and recovered alcohol **(S)-280** (10 mg, 20%) [ee > 99% (Mosher ester method), see **Appendix B**] as a colourless oil. **Data for 280:** $R_f = 0.63$ (petrol/ether, 1:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 3339br (OH), 1461m (O-H), 966s (C-O); **¹H NMR** (400 MHz, CDCl₃) δ 3.57–3.48 (m, 1H, *H*3), 1.60–1.23 (m, 8H, *H*2, *H*4, *H*5 and *H*6), 0.98–0.85 (m, 6H, *H*1 and *H*7); **¹³C NMR** (101 MHz, CDCl₃) δ 73.5 (C3), 36.8 (C4), 30.3 (C2), 28.0 (C5), 22.9 (C6), 14.2 (C7), 10.0 (C1); **MS** (ESI⁺): m/z 271.2 ([2M+K]⁺, 35%). Data consistent with the literature.^[177] The configuration of **280** was assigned by correlation with the configuration of **281**. **Data for 281:** $R_f = 0.95$ (petrol/ether, 1:1); $[\alpha]_D^{24} = +6.8^\circ$ (c 1.06, CHCl₃); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 1735s (C=O), 1236s (C-O); **¹H NMR** (400 MHz, CDCl₃) δ 4.81 (p, $J = 6.0$, 1H, *H*3^a), 2.05 (s, 3H, *H*2), 1.63–1.48 (m, 4H, *H*2^a and *H*4^a), 1.36–1.22 (m, 4H, *H*5^a and *H*6^a), 0.89 (t, $J = 7.0$, 3H, *H*1^a), 0.88 (t, $J = 7.5$, 3H, *H*7^a); **¹³C NMR** (101 MHz, CDCl₃) δ 75.7 (C3^a), 33.4 (C4^a), 27.6 (C2^a), 27.1 (C5^a), 22.8 (C6), 21.4 (C2), 14.2 (C7^a), 9.7(C1^a). Data consistent with the literature.^[177] The configuration of **281** was assigned by correlation of the reported specific rotation of **281**.^[177]

[(2,6-Dimethylcyclohex-1-en-1-yl)oxy]trimethylsilane (**282**)^[179]

n-BuLi (2.5 M in hexane, 1.24 mL, 3.10 mmol) was added dropwise to a solution of diisopropylamine (430 μ L, 310.5 mg, 3.07 mmol) in THF (4.5 mL) at -78 $^{\circ}$ C. After stirring for 15 min at -78 $^{\circ}$ C, chlorotrimethylsilane (1.54 mL, 12.13 mmol) and subsequently a solution of *cis*-2,6-dimethylcyclohexanone (300 mg, 2.38 mmol) in THF (4.7 mL) were added dropwise and stirring continued for an additional 30 min at -78 $^{\circ}$ C. The reaction was quenched by all at once addition of triethylamine (950 μ L, 6.84 mmol) and subsequently a satd. aq. solution of NaHCO₃ (9 mL) at -78 $^{\circ}$ C. After warming to RT, the two layers were separated, the aqueous layer was extracted with ether (3×5 mL) and the organic extracts were combined, dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 40:1), affording TMS-enol ether **282** (400 mg, 83%) as a colourless oil. R_f = 0.94 (petrol/ether, 20:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 1678w (C=C), 1250m (Si-CH₃), 1168s (Si-O), 837s (Si-CH₃); **¹H NMR** (500 MHz, C₆D₆) δ 2.18–2.08 (m, 1H, *H*6), 1.96–1.88 (m, 2H, *H*3), 1.72 (dddd, J = 12.5, 8.5, 6.0, 3.0, 1H, *H*5), 1.66 (dt, J = 2.0, 1.0, 3H, *H*1^a), 1.57 (ddtd, J = 17.5, 8.5, 6.0, 3.0, 1H, *H*4), 1.44–1.35 (m, 1H, *H*4), 1.31 (dddd, J = 12.5, 9.0, 6.0, 3.0, 1H, *H*5), 1.14 (d, J = 7.0, 3H, *H*1^b), 0.17 (s, 9H, *H*1^c); **¹³C NMR** (126 MHz, C₆D₆) δ 147.6 (C1), 111.5 (C2), 34.5 (C6), 32.6 (C5), 31.1 (C3), 21.0 (C4), 19.3 (C1^b), 17.2 (C1^a), 0.8 (C1^c); **HRMS** (CI⁺): m/z found 199.1512, calcd. for C₁₁H₂₂OSi [M+H]⁺ 199.1513. Data consistent with the literature.^[179]

(6*R*)-[(2,6-Dimethylcyclohex-1-en-1-yl)oxy]trimethylsilane (282)^[179]

- **Method A:**

n-BuLi (2.5 M in hexane, 0.50 mL, 1.25 mmol) was added dropwise to a solution of (+)-bis[(*R*)-1-phenylethyl]amine (268 mg, 1.19 mmol) in THF (8.0 mL) at -78 °C. The mixture was stirred for 5 min at -78 °C, warmed to RT, then stirred for 5 min at RT and cooled again to -78 °C. A solution of *cis*-2,6-dimethylcyclohexanone (100 mg, 0.79 mmol) in THF (2.0 mL) was added dropwise at -78 °C. The mixture was stirred for 15 min at -78 °C, chlorotrimethylsilane (0.50 mL, 3.94 mmol) was added dropwise -78 °C and stirring continued for an additional 30 min at -78 °C. Triethylamine (0.32 mL, 2.30 mmol) was added all at once and then the mixture was poured into a satd. aq. solution of NH_4Cl (10 mL). The two layers were separated, the aqueous layer was extracted with ether (3×8 mL) and the organic extracts were combined, dried over MgSO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 40:1), affording TMS-enol ether (**(*R*)-282**) (112 mg, 71%) [ee 50% (chiral GC analysis)] as a colourless oil (data as above, and $[\alpha]_{\text{D}}^{24} = +11.4^\circ$ (c 1.01, CHCl_3)). Enantiomeric ratios were determined by GC analysis: CP Chirasil-DEX CB column; carrier gas, H_2 (80 kPa); injector/detector temperature, 200 °C; 70–120 °C, temperature gradient: 1.5 °C/min; detection method, FID; $t_{\text{R}} = 13.824$ min [(*R*)-**282**], 14.196 min [(*S*)-**282**]. The configuration of **282** was assigned by correlation with the reported specific rotation of **282**.^[179]

- **Method B:**

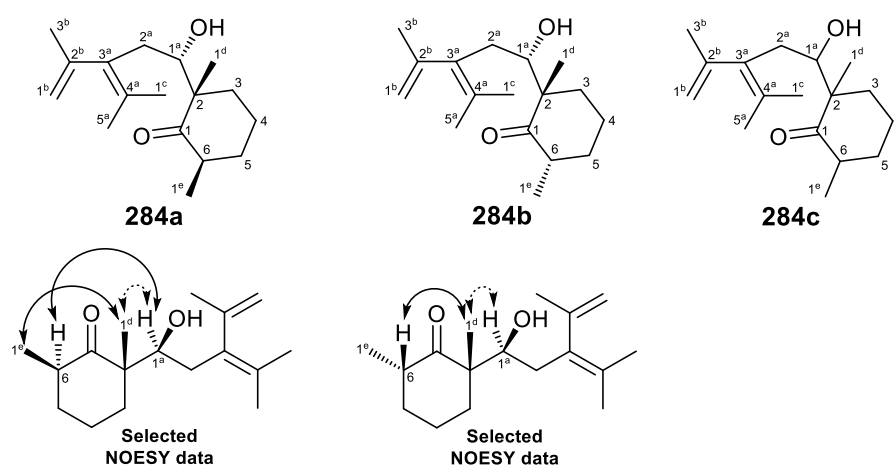
n-BuLi (2.5 M in hexane, 0.38 mL, 0.95 mmol) was added dropwise to a solution of (+)-bis[(*R*)-1-phenylethyl]amine (232 mg, 1.03 mmol) in THF (16 mL) at -78 °C. The obtained mixture was stirred for 5 min at -78 °C, warmed to RT for 5 min and cooled again to -78 °C. Chlorotrimethylsilane (0.51 mL, 4.02 mmol) and subsequently a solution of *cis*-2,6-dimethylcyclohexanone (100 mg, 0.79 mmol) in THF (4 mL) were added dropwise at -78 °C. The mixture was warmed to -40 °C and then stirred for 14 h. The reaction mixture was cooled to -78 °C and quenched by all at once addition of triethylamine (0.32 mL, 2.30 mmol) and a satd. aq. solution of NaHCO₃ (10 mL) at -78 °C. After warming to RT, the two layers were separated, the aqueous layer was extracted with ether (3 × 10 mL) and the organic extracts were combined, dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 40:1), affording TMS-enol ether (***R***)-282 (120 mg, 76%) [ee 37% (chiral GC analysis)] as a colourless oil (data as above).

- **Method C:**

MeLi (1.6 M in ether, 250 μL, 0.40 mmol) was added all at once to a solution of TMS-enol ether (***R***)-282 (72 mg, 0.36 mmol, ee 40%) in THF (1.9 mL) at 0 °C. The mixture was warmed to RT, stirred for 2 h at RT and cooled to -78 °C. Chlorotrimethylsilane (230 μL, 1.81 mmol) was added all at once at -78 °C and the mixture was stirred for 30 min at -78 °C. The reaction was quenched by the all at once addition of triethylamine (140 μL, 1.00 mmol) and subsequently a satd. aq. solution of NaHCO₃ (2 mL) at -78 °C. After warming to RT, the two layers were separated, the aqueous layer was extracted with ether (3 × 2 mL) and the organic extracts were combined, dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography

(petrol/ether, 40:1), affording TMS-enol ether **282** (19 mg, 26%) [ee 40% (chiral GC analysis)] as a colourless oil (data as above).

(2*S**,6*R**)-2-[(*S**)-1-hydroxy-4-methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl]-2,6-dimethylcyclohexan-1-one (**284a**), (2*S**,6*S**)-2-[(*S**)-1-hydroxy-4-methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl]-2,6-dimethylcyclohexan-1-one (**284b**) and 2-[1-hydroxy-4-methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl]-2,6-dimethylcyclohexan-1-one (**284c**)



- **Method A:**

n-BuLi (2.5 M in hexane, 0.29 mL, 0.73 mmol) was added to a solution of diisopropylamine (100 μ L, 0.70 mmol) in THF (1.5 mL) at -78 $^{\circ}$ C. The mixture was warmed to 0 $^{\circ}$ C, stirred for 20 min at 0 $^{\circ}$ C and cooled again to -78 $^{\circ}$ C. A solution of 2,6-dimethylcyclohexanone (82 mg, 0.65 mmol) in THF (1.5 mL) was added dropwise at -78 $^{\circ}$ C. The reaction was stirred for 25 min at -78 $^{\circ}$ C, a solution of aldehyde **270** (100 mg, 0.72 mmol) was added dropwise and stirring continued for an additional 20 min at -78 $^{\circ}$ C. The reaction was quenched with a satd. aq. solution of NH_4Cl (4 mL) at -78 $^{\circ}$ C and then warmed to RT. The two layers were separated, the aqueous layer was extracted with ether (3×2 mL) and the organic extracts were combined, washed sequentially with water (3 mL) and brine (3 mL), dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The crude

Experimental

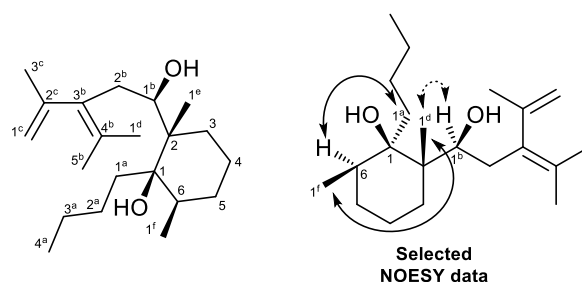
product was purified by flash column chromatography (petrol/ether, 5:1), affording aldol **284a** (29 mg, 17%) as a pale yellow solid. $R_f = 0.19$ (petrol/ether, 5:1); **m.p.** 64–72; **IR** ($\nu_{\max}/\text{cm}^{-1}$): 3485br (O–H), 1697s (C=O); **$^1\text{H NMR}$** (500 MHz, C_6D_6) δ 4.96 (dq, $J = 3.0$, 1.5, 1H, $H1^b$), 4.63 (dq, $J = 3.0$, 1.0, 1H, $H1^b$), 4.18 (ddd, $J = 10.5$, 3.0, 2.0, 1H, $H1^a$), 2.76–2.67 (m, 1H, $H6$), 2.28 (dd, $J = 14.0$, 10.5, 1H, $H2^a$), 2.08 (d, $J = 14.0$, 1H, $H2^a$), 1.78–1.70 (m, 3H, $H3$, $H4$ and $H5$), 1.69 (dd, $J = 1.5$, 1.0, 3H, $H3^b$), 1.64 (d, $J = 1.0$, 3H, $H5^a$), 1.56 (s, 3H, $H1^c$), 1.29–1.22 (m, 2H, $H3$ and $H4$), 1.19 (s, 3H, $H1^d$), 1.14 (d, $J = 6.5$, 3H, $H1^e$), 1.12–1.05 (m, 1H, $H5$); **$^{13}\text{C NMR}$** (126 MHz, C_6D_6) δ 213.1 (C1), 147.0 (C2^b), 133.5 (C3^a), 129.6 (C4^a), 114.3 (C1^b), 73.0 (C1^a), 52.9 (C2), 41.7 (C6), 38.1 (C3), 36.6 (C5), 32.9 (C2^a), 22.6 (C3^b), 22.2 (C5^a), 21.5 (C4), 20.0 (C1^c), 17.9 (C1^d), 15.6 (C1^e); **MS** (ESI⁺): m/z 287.2 ($[\text{M}+\text{Na}]^+$, 100%); **HRMS** (ESI⁺): m/z found 265.2163, calcd. for $\text{C}_{17}\text{H}_{29}\text{O}_2$ $[\text{M}+\text{H}]^+$ 265.2162.

- **Method B:**

MeLi (1.6M in hexane, 0.26 mL, 0.42 mmol) was added all at once to a solution of TMS-enol ether **282** (75 mg, 0.38 mmol) in THF (1.9 mL) at 0 °C. The mixture was warmed up to RT and then stirred for 2 h. After cooling to –78 °C, a solution of aldehyde **270** (58 mg, 0.42 mmol) in THF (0.5 mL) was added all at once. The obtained mixture was stirred for 30 min at –78 °C, and then quenched with a satd. aq. solution of NH_4Cl (2 mL). The two layers were separated, the aqueous layer was extracted with ether (3 × 2 mL) and the organic extracts were combined, washed with brine (3 mL), dried over MgSO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 10:1 to 13:1), affording aldol **284** as a separable mixture of three diastereomers: **284a** (39 mg, 39%) (data as above), **284b** (2 mg, 2%) as a pale yellow oil and **284c** (4 mg, 4%) as a colourless oil. **Data for 284b:** $R_f = 0.33$ (petrol/ether, 5:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 3523br (O–H), 1700s (C=O); **$^1\text{H NMR}$** (500 MHz,

C_6D_6) δ 4.92 (dq, $J = 3.0, 1.5, 1H, H1^b$), 4.61 (dq, $J = 3.0, 1.0, 1H, H1^b$), 4.21 (dd, $J = 10.5, 2.0, 1H, H1^a$), 2.48–2.36 (m, 3H, $H3, H6$, and $H2^a$), 1.79 (d, $J = 12.0, 1H, H2^a$), 1.79–1.74 (m, 1H, $H4$), 1.73–1.66 (m, 1H, $H5$), 1.62 (dd, $J = 1.5, 1.0, 3H, H3^b$), 1.60 (d, $J = 1.0, 3H, H5^a$), 1.52 (s, 3H, $H1^c$), 1.34–1.30 (m, 1H, $H4$), 1.28 (s, 3H, $H1^d$), 1.19 (ddd, $J = 14.0, 12.5, 5.0, 1H, H3$), 1.12 (dd, $J = 12.5, 4.5, 1H, H5$), 1.09 (d, $J = 6.5, 3H, H1^e$); ^{13}C NMR (126 MHz, C_6D_6) δ 214.2 (C1), 147.1 (C2^b), 133.7 (C3^a), 129.2 (C4^a), 114.2 (C1^b), 70.6 (C1^a), 54.2 (C2), 43.1 (C6), 37.0 (C3), 36.5 (C5), 33.5 (C2^a), 22.4 (C5^a), 22.0 (C3^b), 20.9 (C4), 19.9 (C1^c), 17.2 (C1^d), 15.5 (C1^e); HRMS (ESI⁺): m/z found 287.1982, calcd. for $C_{17}H_{28}O_2^{23}Na$ $[M+Na]^+$ 287.1981. **Data for 284c:** $R_f = 0.47$ (petrol/ether, 5:1); 1H NMR (400 MHz, C_6D_6) δ 5.08 (dq, $J = 3.0, 1.5, 1H, H1^b$), 4.89 (dq, $J = 3.0, 1.0, 1H, H1^b$), 4.03 (ddd, $J = 10.5, 3.5, 2.5, 1H, H1^a$), 3.32 (dd, $J = 3.5, 1.5, 1H, OH$), 2.54 (dd, $J = 14.0, 10.5, 1H, H2^a$), 2.22–2.10 (m, 1H, $H6$), 2.06 (ddp, $J = 14.0, 2.5, 1.5, 1H, H2^a$), 1.90 (dd, $J = 1.5, 1.0, 3H, H3^b$), 1.86 (d, $J = 1.0, 3H, H5^a$), 1.80 (d, $J = 1.5, 3H, H1^c$), 1.56–1.44 (m, 2H, $H3$ and $H5$), 1.41–1.35 (m, 2H, $H4$ and $H5$), 1.28–1.20 (m, 1H, $H4$), 1.06 (s, 3H, $H1^d$), 0.91 (d, $J = 6.5, 3H, H1^e$), 0.90–0.87 (m, 1H, $H3$); ^{13}C NMR (101 MHz, C_6D_6) δ 218.6 (C1), 147.2 (C2^b), 134.6 (C3^a), 127.6 (C4^a), 114.1 (C1^b), 74.9 (C1^a), 52.4 (C2), 41.3 (C6), 36.5 (C3), 36.3 (C5), 32.4 (C2^a), 23.1 (C3^b), 22.4 (C1^c), 21.3 (C4), 20.4 (C5^a), 18.0 (C1^d), 15.1 (C1^e). When the reaction was performed with enantioenriched TMS-enol ether (**R**)-**282** (75 mg, 0.38 mmol, ee 37%), aldol **284** as a separable mixture of three diastereomers: **284a** (40 mg, 40%) [ee 34% (chiral GC analysis)] (data as above, and $[\alpha]_D^{24} = -19.0^\circ$ (c 1.06, $CHCl_3$)), **284b** (2 mg, 2%) (data as above) and **284c** (4 mg, 4%) (data as above). Enantiomeric ratios were determined by GC analysis: Chiraldex BDA column; carrier gas, H_2 (80 kPa); injector/detector temperature, 200 °C; 150–200 °C, temperature gradient: 1.0 °C/min; detection method, FID; $t_R = 26.228$ min [(**2R***,**6S***,**1'R***)-**284a**], 26.556 min [(**2S***,**6R***,**1'S***)-**284a**].

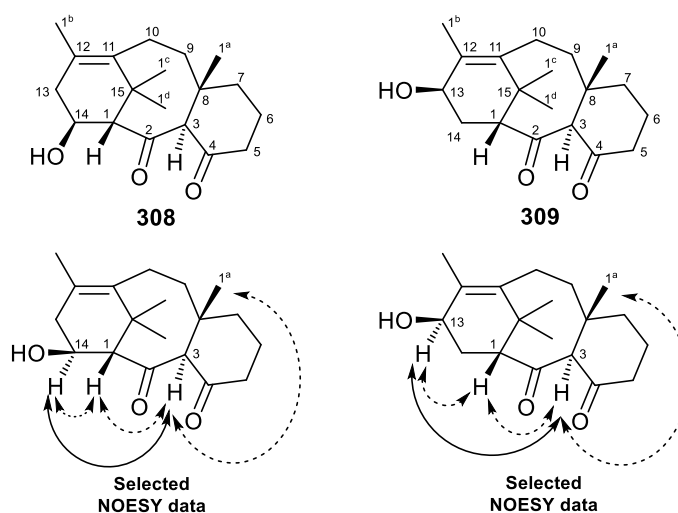
(1*R,2*S**,6*R**)-1-Butyl-2-[(*S*)-1-hydroxy-4-methyl-3-(prop-1-en-2-yl)pent-3-en-1-yl]-2,6-dimethylcyclohexan-1-ol (285)**



n-BuLi (2.5 M in hexane, 0.18 mL, 0.45 mmol) was added dropwise to a solution of (+)-bis[(*R*)-1-phenylethyl]amine (95 mg, 0.42 mmol) in THF (2.5 mL) at -78 °C. The obtained mixture was stirred for 5 min at -78 °C, warmed to RT for 5 min and cooled again to -78 °C. A solution of *cis*-2,6-dimethylcyclohexanone (38 mg, 0.30 mmol) in THF (0.8 mL) was added dropwise at -78 °C. The mixture was stirred for 1 h at -78 °C, then *n*-BuLi (2.5 M in hexane, 0.17 mL, 0.42 mmol) was added dropwise, and stirring continued for an additional 5 min at -78 °C. A solution of aldehyde 270 (46 mg, 0.33 mmol) in THF (0.8 mL) was added dropwise at -78 °C, the reaction mixture was stirred for 20 min at -78 °C and then quenched with a satd. aq. solution of NH_4Cl (3 mL) at -78 °C. After warming to RT, the two layers were separated, the aqueous layer was extracted with ether (3×2 mL) and the organic extracts were combined, washed sequentially with water (3 mL) and brine (3 mL), dried over MgSO_4 , filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petrol/ether, 10:1 to 5:1), affording diol **285** (62 mg, 65%) as a yellow oil. R_f = 0.44 (petrol/ether, 5:1); IR ($\nu_{\text{max}}/\text{cm}^{-1}$): 3462br (O–H), 1446m (C=C–H); $^1\text{H NMR}$ (500 MHz, C_6D_6) δ 4.96 (dq, J = 3.0, 1.5, 1H, $H1^f$), 4.65 (dq, J = 3.0, 1.0, 1H, $H1^e$), 3.72 (ddt, J = 9.0, 7.5, 4.0, 1H, $H1^b$), 2.36 (dd, J = 14.0, 9.0, 1H, $H2^b$), 2.15 (dd, J = 14.0, 4.0, 2H, $H2^a$), 1.72 (dd, J = 1.5, 1.0, 3H, $H3^a$), 1.67 (s, 3H, $H5^b$), 1.63 (s, 3H, $H1^d$), 1.62–1.59 (m, 1H, $H4$), 1.56–1.43 (m, 2H, $H3$ and $H5$), 1.43–1.37 (m, 2H, $H1^c$), 1.37–1.28 (m, 3H, $H3$, $H5$ and $H6$), 1.28–1.15 (m, 3H, $H4$ and $H3^b$), 1.06–0.97 (m, 2H,

$H2^a$), 0.91 (app. t, $J = 7.5$, 3H, $H1^e$), 0.89 (t, $J = 7.5$, 3H, $H4^e$), 0.84 (d, $J = 6.5$, 3H, $H1^f$); ^{13}C NMR (126 MHz, C_6D_6) δ 147.0 ($\text{C}2^e$), 134.2 ($\text{C}3^b$), 128.6 ($\text{C}4^b$), 114.0 ($\text{C}1^e$), 74.4 ($\text{C}1$), 70.3 ($\text{C}1^b$), 39.5 ($\text{C}2^b$), 37.3 ($\text{C}2$), 37.2 ($\text{C}6$), 36.3 ($\text{C}1^a$), 30.8 ($\text{C}5$), 28.5 ($\text{C}3$), 26.6 ($\text{C}4$), 26.2 ($\text{C}2^a$), 23.7 ($\text{C}3^a$), 22.7 ($\text{C}3^e$), 22.1 ($\text{C}5^b$), 20.2 ($\text{C}1^d$), 15.4 ($\text{C}1^f$), 14.4 ($\text{C}1^e$), 14.3. ($\text{C}4^a$). HRMS could not be obtained for this compound.

(1*S, 3*S**, 8*S**, 14*S**)-14-hydroxy-8,12,15,15-tetramethyltricycl[9.3.1.0^{3,8}]pentadec-11-ene-2,4-dione (308) and (1*R**, 3*S**, 8*S**, 13*R**)-13-Hydroxy-8,12,15,15-tetramethyltricyclo[9.3.1.0^{3,8}]pentadec-11-ene-2,4-dione (309)**

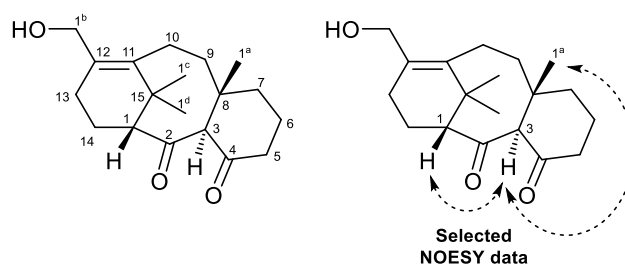


Taxanes **308** and **309** were synthesised following the *General Procedure B*, using diketone **179a** (20.0 mg, 69.4 μmol) and the P450_{BM3} mutant KSK19/AI/AI. The crude product was purified by flash column chromatography (petrol/ether, 2:1 to 1:4), affording taxane **309** (8.1 mg, 38%) as a white solid, taxane **308** (3.8 mg, 18%) as a pale yellow oil and recovered unchanged diketone **179a** (5.7 mg, 29%) as a white solid (data as above). **Data for 308:** $R_f = 0.24$ (petrol/ether, 1:4); IR ($\nu_{\text{max}}/\text{cm}^{-1}$): 3482br (OH), 1721s (C=O), 1685w (C=O); ^1H NMR (500 MHz, CDCl_3) δ 4.10 (dd, $J = 8.5, 6.0$, 1H, $H14$), 3.94 (s, 1H, $H3$), 2.89 (ddd, $J = 14.5, 11.5, 5.5$, 1H, $H10$), 2.65 (dd, $J = 18.5, 8.5$, 1H, $H13$), 2.58 (dd, $J =$

Experimental

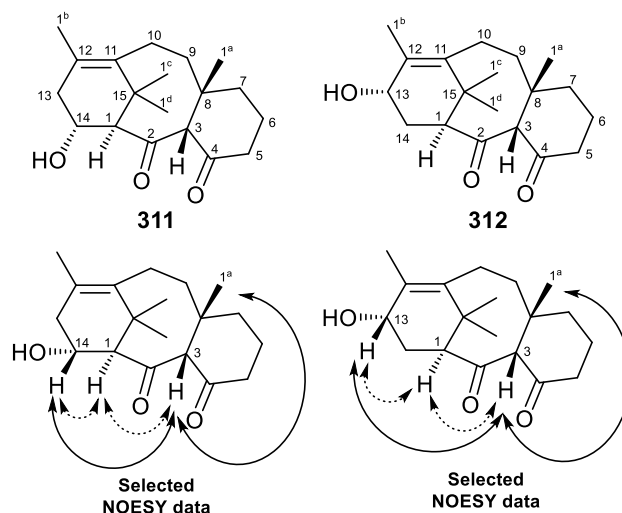
18.5, 6.0, 1H, *H13*), 2.46 (s, 1H, *H1*), 2.41–2.35 (m, 1H, *H5*), 2.27–2.20 (m, 1H, *H10*), 2.20–2.10 (m, 1H, *H5*), 1.97–1.95 (m, 1H, *H7*), 1.94 (s, 3H, *H1^b*), 1.94–1.86 (m, 2H, *H6*), 1.75 (ddd, $J = 15.5, 11.5, 5.0$, 1H, *H9*), 1.47 (dt, $J = 15.5, 5.5$, 1H, *H9*), 1.36 (dt, $J = 9.0, 1.5$, 1H, *H7*), 1.28 (s, 3H, *H1^c*), 1.23 (s, 3H, *H1^d*), 1.02 (s, 3H, *H1^e*); **¹³C NMR** (126 MHz, CDCl₃) δ 207.8 (C1), 206.8 (C4), 138.8 (C11), 130.3 (C12), 73.6 (C1), 65.9 (C3), 65.2 (C14), 44.1 (C8), 40.6 (C13), 39.8 (C9), 39.4 (C5), 39.1 (C15), 37.9 (C7), 29.6 (C1^d), 26.1 (C1^e), 24.9 (C10), 23.9 (C1^a), 22.3 (C1^b), 20.8 (C6); **MS** (ESI⁺): m/z 327.2 ([M+Na]⁺, 100%); **HRMS** (ESI⁺): m/z found 327.1941, calcd. for C₁₉H₂₈O₃²³Na [M+Na]⁺ 327.1936. **Data for 309: R_f** = 0.16 (petrol/ether, 1:4); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 3515br (OH), 1722s (C=O), 1688w (C=O); **¹H NMR** (500 MHz, CDCl₃) δ 4.23 (dd, $J = 9.0, 3.5$, 1H, *H13*), 4.03 (s, 1H, *H3*), 2.88 (ddd, $J = 14.5, 11.5, 5.5$, 1H, *H10*), 2.63 (d, $J = 7.5$, 1H, *H1*), 2.44–2.36 (m, 1H, *H5*), 2.36–2.28 (m, 1H, *H10*), 2.26–2.20 (m, 1H, *H14*), 2.17 (ddd, $J = 16.0, 7.5, 3.5$, 1H, *H14*), 2.17–2.10 (m, 1H, *H5*), 1.98 (s, 3H, *H1^b*), 1.97–1.86 (m, 3H, *H6* and *H7*), 1.83–1.75 (m, 1H, *H9*), 1.49 (dt, $J = 15.5, 5.5$, 1H, *H9*), 1.41–1.37 (m, 1H, *H7*), 1.35 (s, 3H, *H1^c*), 1.22 (s, 3H, *H1^d*), 1.03 (s, 3H, *H1^e*); **¹³C NMR** (126 MHz, CDCl₃) δ 209.5 (C2), 206.7 (C4), 144.3 (C11), 132.3 (C12), 70.7 (C13), 66.6 (C1), 65.0 (C3), 43.9 (C8), 39.5 (C5), 39.1 (C9), 38.0 (C7), 37.6 (C15), 34.3 (C1^e), 30.1 (C14), 25.7 (C1^d), 25.6 (C10), 23.9 (C1^a), 20.8 (C6), 20.4 (C1^b); **MS** (ESI⁺): m/z 327.2 ([M+Na]⁺, 100%); **HRMS** (ESI⁺): m/z found 327.1941, calcd. for C₁₉H₂₈O₃²³Na [M+Na]⁺ 327.1936.

(1*R, 3*S**, 8*S**)-12-Hydroxymethyl-8,15,15-trimethyltricyclo[9.3.1.0^{3,8}]pentadec-11-ene-2,4-dione (310)**



Taxane **310** was synthesised following the *General Procedure B*, using diketone **179a** (20.8 mg, 72.2 mmol) and the P450_{BM3} mutant KSK19/I263A. The crude product was purified by flash column chromatography (petrol/ether, 1:1 to 1:4), affording taxane **310** (1.4 mg, 6%) as a colourless oil and taxane **309** (5.3 mg, 24%) as a white solid (data as above). **Data for 310:** $R_f = 0.31$ (ether); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 3488br (OH), 1721s (C=O), 1683w (C=O); **¹H NMR** (500 MHz, CDCl₃) δ 4.51 (s, 1H, *H*3), 4.46 (dd, $J = 11.5, 4.5$, 1H, *H*1^{*b*}), 4.23 (dd, $J = 11.5, 4.0$, 1H, *H*1^{*b*}), 3.02 (ddd, $J = 14.5, 12.0, 5.5$, 1H, *H*10), 2.89 (ddd, $J = 14.5, 11.5, 5.5$, 1H, *H*13), 2.52 (d, $J = 8.5$, 1H, *H*1), 2.50–2.47 (m, 1H, *H*13), 2.34–2.27 (m, 1H, *H*5), 2.27–2.20 (m, 3H, *H*5, *H*7 and *H*10), 2.11–2.05 (m, 1H, *H*14), 1.93–1.86 (m, 2H, *H*6), 1.80 (ddd, $J = 15.5, 12.0, 5.0$, 1H, *H*9), 1.71 (dd, $J = 16.0, 8.5$, 1H, *H*14), 1.42–1.38 (m, 1H, *H*9), 1.28 (s, 3H, *H*1^{*c*}), 1.27–1.23 (m, 1H, *H*7), 1.14 (s, 3H, *H*1^{*d*}), 1.01 (s, 3H, *H*1^{*a*}); **¹³C NMR** (126 MHz, CDCl₃) δ 209.9 (C2), 207.2 (C4), 141.9 (C11), 134.7 (C12), 64.0 (C3), 63.0 (C1^{*b*}), 62.4 (C1), 44.2 (C8), 39.7 (C5), 39.5 (C9), 38.9 (C15), 36.6 (C7), 29.2 (C1^{*d*}), 25.6 (C1^{*c*}), 24.9 (C13), 24.7 (C10), 24.4 (C1^{*a*}), 21.3 (C6), 18.0 (C14); **MS** (ESI⁺): m/z 327.2 ([M+Na]⁺, 100%); **HRMS** (ESI⁺): m/z found 327.1938, calcd. for C₁₉H₂₈O₃²³Na [M+Na]⁺ 327.1936.

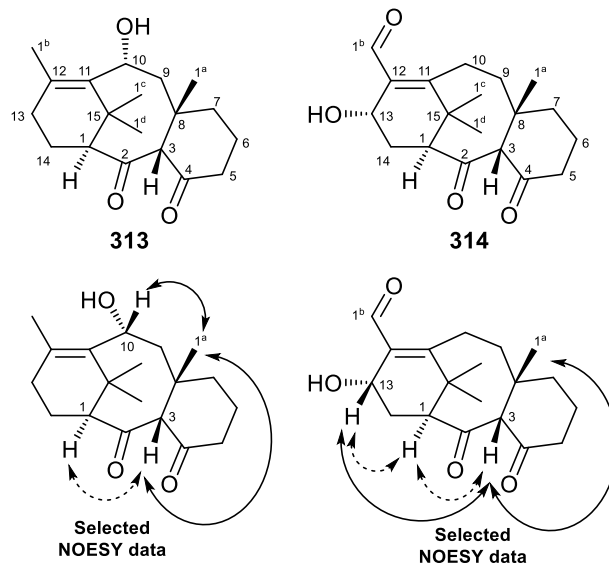
(1*R**, 3*R**, 8*S**, 14*R**)-14-hydroxy-8,12,15,15-tetramethyltricyclo[9.3.1.0^{3,8}]pentadec-11-ene-2,4-dione (**311**) and (1*S**, 3*R**, 8*S**, 13*S**)-13-hydroxy-8,12,15,15-tetramethyltricyclo[9.3.1.0^{3,8}]pentadec-11-ene-2,4-dione (**312**)



Taxanes **311** and **312** were synthesised following the *General Procedure B*, using diketone **179b** (20.0 mg, 69.4 μmol) and the P450_{BM3} mutant KSK19/AI/AI. The crude product was purified by flash column chromatography (petrol/ether, 2:1 to 1:4), affording taxane **311** (4.0 mg, 19%) as a colourless oil and taxane **312** (5.0 mg, 24%) as a colourless oil. **Data for 311:** $R_f = 0.57$ (petrol/ether, 1:2); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 3464br (OH), 1703m (C=O), 1680s (C=O); **¹H NMR** (500 MHz, CDCl₃) δ 4.45 (app. dt, $J = 9.0, 5.0$, 1H, *H*14), 3.44 (s, 1H, *H*3), 2.91–2.78 (m, 3H, *H*5, *H*10 and *H*13), 2.56 (app. dddd, $J = 18.0, 5.0, 3.5, 1.5$, 1H, *H*13), 2.48 (s, 1H, *H*1), 2.32–2.25 (m, 1H, *H*5), 2.20–2.15 (m, 1H, *H*10), 2.11 (td, $J = 13.5, 4.5$, 1H, *H*7), 1.99–1.93 (m, 1H, *H*9), 1.92 (s, 3H, *H*1^b), 1.92–1.88 (m, 1H, *H*6), 1.78 (qt, $J = 13.5, 4.0$, 1H, *H*6), 1.37–1.30 (m, 1H, *H*9), 1.34 (s, 3H, *H*1^c), 1.26 (s, 3H, *H*1^d), 1.20–1.12 (m, 1H, *H*7), 0.97 (s, 3H, *H*1^a); **¹³C NMR** (126 MHz, CDCl₃) δ 209.1 (C4), 208.4 (C2), 137.9 (C11), 130.5 (C12), 73.3 (C1), 70.6 (C3), 64.8 (C14), 43.9 (C8), 40.8 (C13), 39.9 (C9), 39.4 (C5), 38.9 (C15), 35.9 (C7), 29.8 (C1^d), 25.4 (C1^c), 24.7 (C10), 24.4 (C1^a), 22.0 (C1^b), 21.0 (C6); **MS** (ESI⁺): m/z 327.2 ([M+Na]⁺, 100%); **HRMS** (ESI⁺): m/z found 327.1943, calcd. for C₁₉H₂₈O₃²³Na [M+Na]⁺ 327.1936. **Data for 312:** $R_f = 0.41$ (petrol/ether, 1:1); **IR**

($\nu_{\max}/\text{cm}^{-1}$): 3455br (O–H), 1702m (C=O), 1677s (C=O); **¹H NMR** (500 MHz, CDCl₃) δ 4.42 (app. dt, $J = 9.5, 3.5$, 1H, $H1\beta$), 3.51 (s, 1H, $H3$), 2.85 (td, $J = 14.0, 7.0$, 1H, $H5$), 2.82 (td, $J = 13.5, 5.5$, 1H, $H10$), 2.60 (d, $J = 7.0$, 1H, $H1$), 2.44 (dd, $J = 16.0, 9.5$, 1H, $H14$), 2.31–2.25 (m, 1H, $H5$), 2.25–2.19 (m, 1H, $H10$), 2.14 (ddd, $J = 16.0, 7.0, 3.5$, 1H, $H14$), 2.09 (dd, $J = 14.0, 4.0$, 1H, $H7$), 1.98–1.95 (m, 1H, $H9$), 1.94 (s, 3H, $H1^b$), 1.93–1.87 (m, 1H, $H6$), 1.74 (qt, $J = 14.0, 4.0$, 1H, $H6$), 1.36 (s, 3H, $H1^c$), 1.33 (ddd, $J = 15.0, 5.5, 2.5$, 1H, $H9$), 1.26 (s, 3H, $H1^d$), 1.19–1.13 (m, 1H, $H7$), 0.96 (s, 3H, $H1^e$); **¹³C NMR** (126 MHz, CDCl₃) δ 210.5 (C2), 209.2 (C4), 143.1 (C11), 132.3 (C12), 70.6 (C13), 69.8 (C3), 67.0 (C1), 43.6 (C8), 39.3 (C5), 39.2 (C9), 37.4 (C15), 35.7 (C7), 34.3 (C1^c), 30.2 (C14), 25.5 (C10), 25.1 (C1^d), 24.5 (C1^a), 21.0 (C6), 20.2 (C1^b); **MS** (ESI⁺): m/z 327.2 ([M+Na]⁺, 54%), 305.2 ([M+H]⁺, 21%); **HRMS** (CI⁺): m/z found 305.2110, calcd. for C₁₉H₂₈O₃ [M+H]⁺ 305.2112.

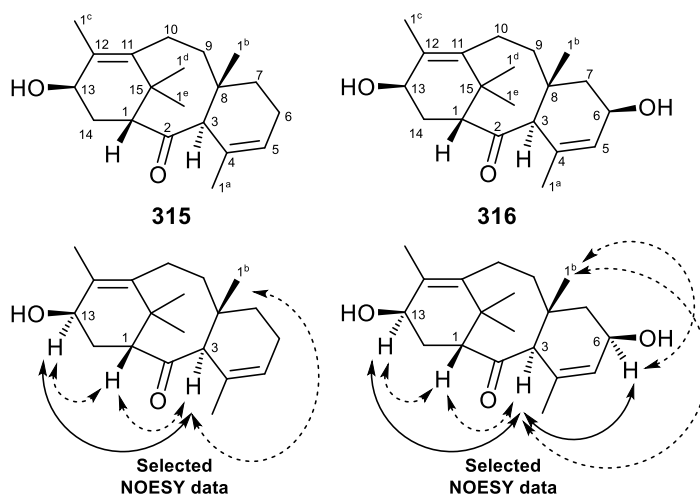
(1*S**, 3*R**, 8*S**, 10*R**)-10-Hydroxy-8,12,15,15-tetramethyltricyclo[9.3.1.0^{3,8}] pentadec-11-ene-2,4-dione (**313**) and (1*S**, 3*R**, 8*S**, 13*S**)-12-formyl-13-hydroxy-8,15,15-trimethyltricyclo[9.3.1.0^{3,8}]pentadec-11-ene-2,4-dione (**314**)



Taxanes **313** and **314** were synthesised following the *General Procedure B*, using diketone **179b** (20.8 mg, 72.2 mmol) and the P450_{BM3} mutant KSK19/I263A. The crude product was purified by flash column chromatography (petrol/ether, 1:1 to 1:4), affording taxane **313** (1.3 mg, 6%) as a colourless oil and taxane **314** (1.9 mg, 9%) as a colourless oil. **Data for 313:** $R_f = 0.61$ (ether); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 3460br (OH), 1705m (C=O), 16792 (C=O); **¹H NMR** (500 MHz, CDCl₃) δ 5.15 (dd, $J = 12.0, 6.0, 1\text{H}, H10$), 3.46 (s, 1H, $H3$), 2.90 (td, $J = 14.0, 7.5, 1\text{H}, H5$), 2.55 (dt, $J = 18.5, 9.0, 1\text{H}, H13$), 2.44 (d, $J = 3.5, 1\text{H}, H1$), 2.33–2.27 (m, 1H, $H5$), 2.26–2.21 (m, 1H, $H13$), 2.20–2.10 (m, 2H, $H7$ and $H9$), 2.03–1.95 (m, 2H, $H14$), 1.93 (d, $J = 1.5, 3\text{H}, H1^b$), 1.92–1.86 (m, 1H, $H6$), 1.82–1.73 (m, 1H, $H6$), 1.63 (dd, $J = 15.0, 6.0, 1\text{H}, H9$), 1.46 (s, 3H, $H1^c$), 1.23–1.19 (m, 1H, $H7$), 1.18 (s, 3H, $H1^d$), 0.96 (s, 3H, $H1^a$); **¹³C NMR** (126 MHz, CDCl₃) δ 209.9 (C2), 209.0 (C4), 137.8 (C11), 135.9 (C12), 68.1 (C3), 68.0 (C10), 63.3 (C1), 47.3 (C9), 41.5 (C8), 39.2 (C5), 37.8 (C15), 35.2 (C7), 31.1 (C1^d), 29.3 (C13), 25.2 (C1^c), 25.1 (C1^a), 22.0 (C1^b), 20.7 (C6), 17.8 (C14); **MS** (ESI⁺): m/z 327.2 ([M+Na]⁺, 56%); **HRMS** (ESI⁺): m/z found 327.1934, calcd. for C₁₉H₂₈O₃²³Na

$[M+Na]^+$ 327.1936. **Data for 314:** $R_f = 0.50$ (ether); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 3460br (OH), 1706m (C=O), 1581s (C=O); **$^1\text{H NMR}$** (500 MHz, CDCl_3) δ 10.15 (s, 1H, $H1^b$), 5.24 (dt, $J = 9.5, 3.5$, 1H, $H13$), 3.54 (td, $J = 13.0, 6.0$, 1H, $H10$), 3.31 (s, 1H, $H3$), 2.79 (dd, $J = 14.0, 7.0$, 1H, $H5$), 2.74 (s, 1H, $H1$), 2.58 (dd, $J = 16.0, 9.5$, 1H, $H14$), 2.49 (ddd, $J = 13.0, 5.5, 3.0$, 1H, $H10$), 2.32–2.28 (m, 1H, $H5$), 2.18 (ddd, $J = 16.0, 7.5, 3.5$, 1H, $H14$), 2.12–2.00 (m, 2H, $H7$ and $H9$), 1.94–1.88 (m, 1H, $H6$), 1.72 (ddd, $J = 17.0, 7.0, 4.0$, 1H, $H6$), 1.52 (dd, $J = 6.0, 3.0$, 1H, $H9$), 1.48 (s, 3H, $H1^c$), 1.35 (s, 3H, $H1^d$), 1.22–1.19 (m, 1H, $H7$), 0.87 (s, 3H, $H1^a$); **$^{13}\text{C NMR}$** (126 MHz, CDCl_3) δ 208.6 (C2), 207.3 (C4), 188.8 (C1^b), 172.2 (C11), 142.1 (C12), 69.5 (C3), 67.0 (C1), 64.3 (C13), 43.1 (C8), 39.9 (C15), 39.2 (C5), 37.6 (C9), 35.9 (C7), 32.9 (C1^c), 27.7 (C14), 24.9 (C10), 24.0 (C1^d), 23.9 (C1^a), 20.6 (C6); **MS** (ESI⁺): m/z 341.2 ($[M+Na]^+$, 100%); **HRMS** (ESI⁺): m/z found 341.1732, calcd. for $\text{C}_{19}\text{H}_{26}\text{O}_4^{23}\text{Na}$ $[M+Na]^+$ 341.1729.

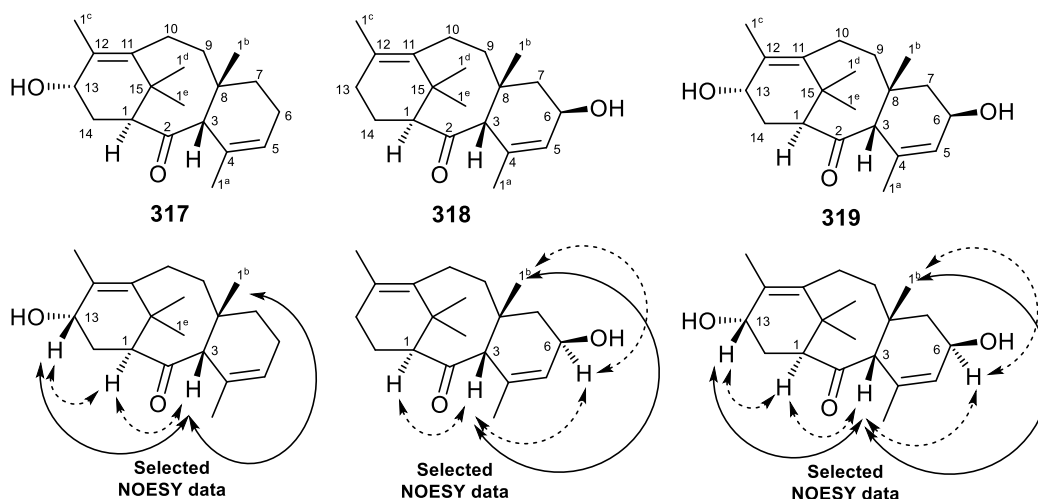
(1*R**,3*S**,8*S**,13*R**)-13-Hydroxy-4,8,12,15,15-pentamethyltricyclo[9.3.1.0^{3,8}]pentadec-4,11-diene-2-one (**315**) and (1*R**,3*S**,6*R**,8*R**,13*R**)-6,13-dihydroxy-4,8,12,15,15-pentamethyltricyclo[9.3.1.0^{3,8}]pentadec-4,11-diene-2-one (**316**)



Taxanes **315** and **316** were synthesised following the *General Procedure B*, using taxadienone **16** (20.7 mg, 72.3 μmol) and the P450_{BM3} mutant KSK19/AI/AI. The crude product was purified by flash column chromatography (petrol/ether, 9:1 to 1:4), affording taxane **315** (4.8 mg, 22%) as a colourless oil, taxane **316** (7.4 mg, 32%) as a colourless oil and recovered unchanged taxadienone **16** (6.1 mg, 30%) as a white solid (data as above). **Data for 315:** R_f = 0.47 (petrol/ether, 1:1); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 3437br (OH), 1673m (C=O), 1456m (O-H); **¹H NMR** (500 MHz, CDCl₃) δ 5.57 (d, J = 6.5, 1H, *H*5), 4.43–4.36 (m, 1H, *H*13), 3.16 (s, 1H, *H*3), 2.75 (d, J = 7.5, 1H, *H*1), 2.61–2.54 (m, 1H, *H*6), 2.47 (dd, J = 16.0, 9.5, 1H, *H*14), 2.35 (ddd, J = 15.5, 12.5, 4.0, 1H, *H*6), 2.22 (ddd, J = 16.0, 7.5, 3.5, 1H, *H*14), 1.90 (d, J = 1.0, 3H, *H*1^c), 1.88–1.77 (m, 1H, *H*7), 1.49–1.47 (m, 3H, *H*1^e), 1.38 (dt, J = 14.5, 4.0, 1H, *H*7), 1.33 (s, 3H, *H*1^d), 1.31–1.23 (m, 4H, *H*9 and *H*19), 1.19 (s, 3H, *H*1^a), 0.92 (s, 3H, *H*1^b); **¹³C NMR** (126 MHz, CDCl₃) δ 217.3 (C2), 144.6 (C11), 133.0 (C4), 131.7 (C12), 125.0 (C5), 70.1 (C13), 69.4 (C1), 57.1 (C3), 43.0 (C7), 41.0 (C9), 37.8 (C15), 37.4 (C8), 33.9 (C1^d), 30.6 (C14), 29.9 (C10), 27.7 (C1^c), 24.2 (C6), 23.7 (C1^a), 20.9 (C1^b), 20.3 (C1^e); **MS** (ESI⁺): m/z 325.2 ([M+Na]⁺, 100%), 303.2 ([M+H]⁺, 14%); **HRMS** (ESI⁺): m/z found 325.2139,

calcd. for $C_{20}H_{30}O_2^{23}Na$ $[M+Na]^+$ 325.2138. **Data for 316:** $R_f = 0.42$ (petrol/ether, 1:4); **IR** (ν_{max}/cm^{-1}): 3404br (OH), 1676s (C=O), 1453m (O-H); **1H NMR** (500 MHz, $CDCl_3$) δ 5.39 (dq, $J = 5.5, 1.5, 1H, H5$), 4.34 (d, $J = 9.0, 1H, H13$), 3.48–3.39 (m, 1H, $H6$), 3.31 (s, 1H, $H3$), 2.75 (d, $J = 7.0, 1H, H1$), 2.68 (dt, $J = 15.5, 5.5, 1H, H10$), 2.44 (dd, $J = 16.0, 9.0, 1H, H14$), 2.29 (ddd, $J = 15.5, 11.0, 4.0, 1H, H10$), 2.22 (ddd, $J = 16.0, 7.0, 4.0, 1H, H14$), 2.19–2.11 (m, 1H, $H7$), 2.10–2.01 (m, 1H, $H7$), 1.92 (ddd, $J = 15.0, 5.5, 4.0, 1H, H9$), 1.90 (s, 3H, $H1'$), 1.78 (ddd, $J = 15.0, 11.0, 5.5, 1H, H9$), 1.48 (app. dq, $J = 2.5, 1.5, 3H, H1''$), 1.34 (s, 3H, $H1^d$), 1.21 (s, 3H, $H1'$), 0.90 (s, 3H, $H1^b$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 216.1 (C2), 144.8 (C11), 133.3 (C4), 131.8 (C12), 122.0 (C5), 75.4 (C6), 70.2 (C13), 69.1 (C1), 57.1 (C3), 42.5 (C8), 37.8 (C15), 36.2 (C9), 34.0 (C1^d), 31.3 (C7), 30.6 (C14), 27.4 (C1^e), 24.2 (C10), 23.5 (C1^a), 20.3 (C1^c), 13.7 (C1^b); **MS** (ESI⁺): m/z 341.2 ($[M+Na]^+$, 44%); **HRMS** (ESI⁺): m/z found 319.2268, calcd. for $C_{20}H_{31}O_3$ $[M+H]^+$ 319.2267.

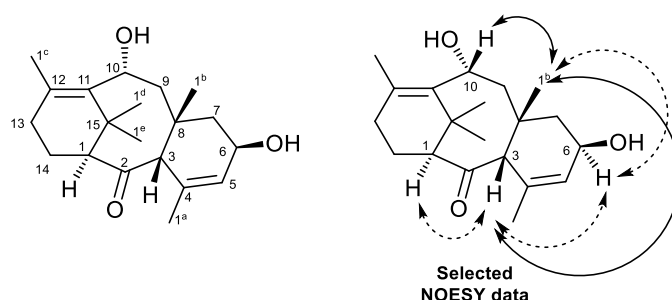
(1*S**,3*R**,8*S**,13*S**)-13-Hydroxy-4,8,12,15,15-pentamethyl-tricyclo[9.3.1.0^{3,8}]pentadec-4,11-diene-2-one (317), (1*S**,3*R**,6*R**,8*R**)-6-hydroxy-4,8,12,15,15-pentamethyl-tricyclo[9.3.1.0^{3,8}]pentadec-4,11-diene-2-one (318), and (1*S**,3*R**,6*R**,8*R**,13*S**)-6,13-dihydroxy-4,8,12,15,15-pentamethyltricyclo[9.3.1.0^{3,8}]pentadec-4,11-diene-2-one (319)



Taxanes **317**, **318** and **319** were synthesised following the *General Procedure B*, using di-*epi*-taxadienone **220** (20.0 mg, 69.9 μmol) and the P450_{BM3} mutant KSK19/AI/AI. The crude product was purified by flash column chromatography (petrol/ether, 2:1 to 1:4), affording taxane **317** (6.6 mg, 31%) as a colourless oil, taxane **318** (3.7 mg, 18%) as a colourless oil and taxane **319** (8.1 mg, 36%) as a white solid. **Data for 317**: $R_f = 0.50$ (petrol/ether, 1:1); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 3463br (OH), 1683s (C=O), 1453m (O-H), 1033m (C-O), 1009s (C-O); **¹H NMR** (500 MHz, CDCl₃) δ 5.55 (s, 1H, *H*5), 4.30 (dd, $J = 9.5, 4.0$, 1H, *H*13), 2.83 (s, 1H, *H*3), 2.83 (td, $J = 13.5, 5.5$, 1H, *H*10), 2.64 (d, $J = 7.0$, 1H, *H*1), 2.51 (dd, $J = 16.0, 9.5$, 1H, *H*14), 2.27–2.20 (m, 1H, *H*10), 2.23 (ddd, $J = 16.0, 7.0, 4.0$, 1H, *H*14), 2.04–1.96 (m, 2H, *H*6), 1.95 (s, 3H, *H*1^c), 1.84–1.75 (m, 2H, *H*7 and *H*9), 1.54 (dd, $J = 3.5, 1.5$, 3H, *H*1^a), 1.34 (s, 3H, *H*1^d), 1.26 (s, 3H, *H*1^e), 1.25–1.20 (m, 1H, *H*9), 0.95–0.92 (m, 1H, *H*7), 0.91 (s, 3H, *H*1^b); **¹³C NMR** (126 MHz, CDCl₃) δ 217.3 (C2), 144.1 (C11), 130.8 (C4), 130.7 (C12), 123.9 (C5), 70.9 (C13), 67.1 (C1), 58.1 (C3), 39.7 (C9), 37.9 (C8), 37.2 (C15), 34.6 (C1^d), 32.8 (C7), 30.7 (C14), 25.8 (C10), 25.2 (C1^e), 24.7 (C1^a), 23.7 (C1^b), 22.1 (C6), 20.0 (C1^c);

MS (ESI⁺): m/z 325.2 ([M+Na]⁺, 11%); **HRMS** (ESI⁺): m/z found 325.2140, calcd. for C₂₀H₃₀O₂²³Na [M+Na]⁺ 325.2144. **Data for 318:** R_f = 0.26 (petrol/ether, 1:1); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 3422br (OH), 1683s (C=O), 1455m (O-H); **¹H NMR** (500 MHz, CDCl₃) δ 5.70 (dq, J = 4.5, 1.5, 1H, *H5*), 4.28–4.23 (m, 1H, *H6*), 3.10 (s, 1H, *H3*), 2.84 (td, J = 13.5, 5.0, 1H, *H10*), 2.54–2.46 (m, 1H, *H14*), 2.45 (d, J = 7.0, 1H, *H1*), 2.17–2.09 (m, 2H, *H10* and *H14*), 2.09–2.06 (m, 1H, *H7*), 2.05–1.98 (m, 2H, *H13*), 1.84 (s, 3H, *H1'*), 1.74 (td, J = 14.0, 5.0, 1H, *H9*), 1.63 (app. td, J = 1.5, 0.5, 3H, *H1''*), 1.34–1.28 (m, 2H, *H7* and *H9*), 1.27 (s, 3H, *H1''*), 1.14 (s, 3H, *H1''*), 1.11 (s, 3H, *H1''*); **¹³C NMR** (126 MHz, CDCl₃) δ 216.1 (C2), 136.8 (C11), 135.3 (C4), 130.1 (C12), 125.5 (C5), 64.7 (C6), 62.5 (C1), 57.4 (C3), 42.4 (C7), 40.3 (C9), 37.9 (C15), 37.5 (C8), 30.1 (C1^e), 28.7 (C14), 26.3 (C1^b), 25.0 (C1^d), 24.8 (C1^a), 24.7 (C10), 22.1 (C1^c), 18.5 (C13); **MS** (ESI⁺): m/z 325.2 ([M+Na]⁺, 19%); **HRMS** (ESI⁺): m/z found 325.2140, calcd. for C₂₀H₃₀O₂²³Na [M+Na]⁺ 325.2144. **Data for 320:** R_f = 0.48 (ether); **IR** ($\nu_{\max}/\text{cm}^{-1}$): 3393br (OH), 1680s (C=O), 1452m (O-H), 1034m (C-O), 1008s (C-O); **¹H NMR** (500 MHz, CDCl₃) δ 5.70 (dq, J = 4.0, 1.5, 1H, *H5*), 4.30 (dd, J = 9.5, 4.0, 1H, *H13*), 4.28–4.24 (m, 1H, *H6*), 2.97 (s, 1H, *H3*), 2.84 (td, J = 13.5, 5.5, 1H, *H10*), 2.62 (d, J = 7.0, 1H, *H1*), 2.47 (dd, J = 16.0, 9.5, 1H, *H14*), 2.30–2.23 (m, 1H, *H14*), 2.23–2.21 (m, 1H, *H10*), 2.02 (dd, J = 14.5, 6.0, 1H, *H7*), 1.94 (s, 3H, *H1'*), 1.79 (td, J = 13.5, 5.5, 1H, *H9*), 1.62 (app. t, J = 1.5, 3H, *H1''*), 1.35 (s, 3H, *H1''*), 1.34–1.28 (m, 2H, *H7* and *H9*), 1.24 (s, 3H, *H1''*), 1.13 (s, 3H, *H1''*); **¹³C NMR** (126 MHz, CDCl₃) δ 215.4 (C2), 144.0 (C11), 134.8 (C4), 131.0 (C12), 125.4 (C5), 70.7 (C13), 66.9 (C1), 64.5 (C6), 58.4 (C3), 42.1 (C7), 39.5 (C9), 37.5 (C8), 37.2 (C15), 34.6 (C1^d), 30.7 (C14), 26.4 (C1^b), 25.4 (C10), 25.2 (C1^c), 24.6 (C1^a), 20.0 (C1^e); **MS** (ESI⁺): m/z 341.2 ([M+Na]⁺, 100%); **HRMS** (ESI⁺): m/z found 341.2080, calcd. for C₂₀H₃₀O₃²³Na [M+Na]⁺ 341.2093.

(1*S,3*R**,6*R**,8*S**,10*R**)-6,10-Dihydroxy-4,8,12,15,15-pentamethyltricyclo[9.3.1.0^{3,8}]pentadec-4,11-diene-2-one (320)**



Taxanes **319** and **320** was synthesised following the *General Procedure B*, using di-*epi*-taxadienone **22** (20.0 mg, 69.9 μmol) and the P450_{BM3} mutant KSK19/I263A. The crude product was purified by flash column chromatography (petrol/ether, 1:1 to 1:4), affording taxane **320** (6.5 mg, 30%) as a colourless oil and taxane **319** (4.5 mg, 20%) as a white solid (data as above). **Data for 320:** R_f = 0.27 (ether); **IR** ($\nu_{\text{max}}/\text{cm}^{-1}$): 3402br (OH), 1679s (C=O), 1443m (O-H), 1032m (C-O), 1006m (C-O); **¹H NMR** (500 MHz, CDCl₃) δ 5.70 (dq, J = 5.0, 1.5, 1H, H_5), 5.19 (ddd, J = 12.0, 5.5, 3.0, 1H, H_{10}), 4.32–4.27 (m, 1H, H_6), 2.91 (s, 1H, H_3), 2.60–2.51 (m, 1H, H_{13}), 2.46 (d, J = 8.0, 1H, H_1), 2.17 (td, J = 10.0, 3.5, 1H, H_{13}), 2.14–2.04 (m, 2H, H_7 and H_{14}), 2.04–1.96 (m, 2H, H_9 and H_{14}), 1.92 (d, J = 1.0, 3H, H_1^a), 1.62 (app. t, J = 1.5, 3H, H_1^c), 1.62–1.59 (m, 1H, H_9), 1.45 (s, 3H, H_1^d), 1.37–1.33 (m, 1H, H_7), 1.18 (s, 3H, H_1^e), 1.15 (s, 3H, H_1^b); **¹³C NMR** (126 MHz, CDCl₃) δ 214.8 (C2), 138.3 (C11), 134.8 (C4), 134.3 (C12), 125.6 (C5), 68.0 (C10), 64.6 (C6), 63.1 (C1), 56.5 (C3), 47.6 (C9), 41.9 (C7), 37.3 (C15), 35.8 (C8), 31.5 (C1^e), 29.2 (C13), 27.2 (C1^b), 25.2 (C1^d), 24.6 (C1^a), 22.0 (C1^c), 18.4 (C14); **MS** (ESI⁺): m/z 341.2 ([M+Na]⁺, 100%); **HRMS** (ESI⁺): m/z found 341.2092, calcd. for C₂₀H₃₀O₃²³Na [M+Na]⁺ 341.2093.

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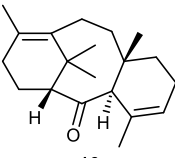
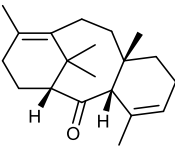
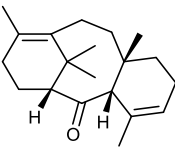
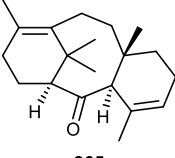
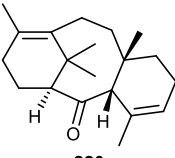
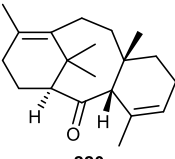
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APPENDIX A: Computational studies of the IMDA of enone **232**

Within Spartan '14 for Mac, four transition states were obtained for the DA reaction of 2,3,4-trimethylpenta-1,3-diene and but-3-en-2-one, for the *exo* and *endo* modes and for the *s-cis* and *s-trans* conformations of the dienophile. These were used as the starting point for the IMDA of *cis*- and *trans*- precursors **232a** and **232b**, respectively, and only reasonable structures were used in further calculations (all four from the *cis*- isomer, just two from the *trans*- isomer). Transition state calculations were then performed with a variety of basis sets: HF/STO-3G then HF/3-21G then HF/6-31G* then the DFT method EDF2/6-31G*. At all levels of theory tested, transition states were found with all or most of the six possibilities. The general conclusion from these being that the formation of taxadienone **16** (from the *trans*-precursor **232b**) proceeds by the lowest energy transition state and the undesired di-*epi*-taxadienone **220** (from the *cis*-precursor **232a**) is then only slightly less favourable. The minor mono-epimers **264** and **265** are accessible *via* the transition states marked “3rd or 4th most accessible” and the energies are similar but variable. These preliminary calculations were enthalpy-only (no entropic contribution), did not include a solvent model (gas phase), and did not include a bound Lewis acid.

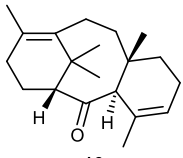
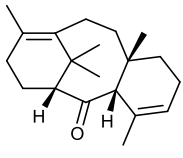
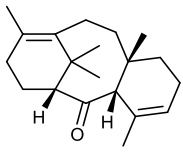
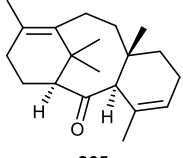
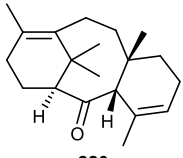
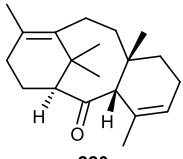
Appendix A

Table A.1. Calculations on the IMDA reaction to obtain taxadienone epimers **16**, **220**, **264** and **265**.

Entry	Compound	HF/STO-3G	HF/3-21G	HF/6-31G*	DFT/EDF2/6-31G*	DFT/EDF2/6-31G* (protonated C=O)
<i>gem-dimethyl 'up'</i>						
1	 16 <i>trans-B/C, s-trans</i>	-839.266957	-844.826886	-849.518594	-854.600923	-890.533361
2	 264 <i>cis-B/C, s-cis</i>	-839.253972	-844.820118	Failed	Failed	Failed
3	 264 <i>cis-B/C, s-trans</i>	-839.253153	-844.810593	-849.509479	-854.594389	-890.515614
<i>gem-dimethyl 'down'</i>						
4	 265 <i>trans-B/C, s-cis</i>	-839.257667	-844.819719	-849.510284	-854.592784	-890.526033
5	 220 <i>cis-B/C, s-cis</i>	-839.244743	-844.808725	Failed	-854.584972	-890.520990
6	 220 <i>cis-B/C, s-trans</i>	-839.263769	-844.820636	-849.563490	-854.598572	-890.531378

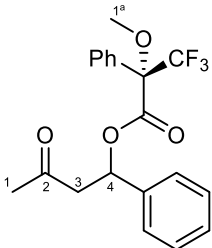
Numbers for calculated TSs of apparently accessible combinations of *cis*- and *trans*-B/C ring fusion with the *gem*-dimethyl substituent 'up' or 'down' and with *s-cis* and *s-trans* conformations of the dienophile. Values are in hartrees. Basis sets: HF = Hartree Fock, STO = Slater Type Orbitals, DFT = Density Functional Theory, and EDF2 = 2nd generation Empirical Density Functional; the 3G, 3-21G, 6-31G* then refer to shells of Gaussian approximations to the orbitals.

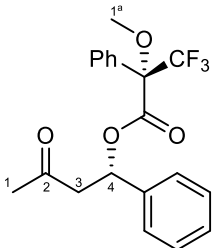
Table A.2. Calculations on the IMDA reaction to obtain taxadienone epimers **16**, **220**, **264** and **265** normalised to the lowest value.

Entry	Compound	HF/STO-3G	HF/3-21G	HF/6-31G*	DFT/EDF2/6-31G*	DFT/EDF2/6-31G* (protonated C=O)	Comment
<i>gem-dimethyl 'up'</i>							
1	 16 <i>trans-B/C, s-trans</i>	0	0	0	0	0	'Always' the lowest
2	 264 <i>cis-B/C, s-cis</i>	+0.012985 [+34.1]	+0.006768 [+17.8]	Failed	Failed	Failed	'Failed' may mean that this TS is inaccessible
3	 264 <i>cis-B/C, s-trans</i>	+0.013804 [+36.2]	+0.016293 [+42.8]	+0.009115 [+23.9]	+0.006534 [+17.2]	+0.017747 [+46.6]	3 rd or 4 th most accessible
<i>gem-dimethyl 'down'</i>							
4	 265 <i>trans-B/C, s-cis</i>	+0.009290 [+24.4]	+0.007167 [+18.8]	+0.008310 [+21.8]	+0.008139 [+21.4]	+0.007328 [19.2]	3 rd or 4 th most accessible
5	 220 <i>cis-B/C, s-cis</i>	+0.022214 [+58.3]	+0.018161 [+47.7]	Failed	+0.015951 [+41.9]	+0.012371 [+32.5]	Consistently high energy and unlikely to proceed by this TS
6	 220 <i>cis-B/C, s-trans</i>	+0.003188 [+8.4]	+0.006250 [+16.4]	-0.044896 ^a [-117.9]	+0.002351 [+6.2]	+0.001983 [+5.2]	Consistently the 2 nd lowest

^a This calculation was the lowest in energy but it seemed way out of step with the other calculations, so it was normalised against the 1st compound that came out the lowest in energy in all the other calculations. Values are in hartrees and kJ·mol⁻¹ in []. Basis sets: HF = Hartree Fock, STO = Slater Type Orbitals, DFT = Density Functional Theory, and EDF2 = 2nd generation Empirical Density Functional.

APPENDIX B: ¹H-NMR data for Mosher ester derivativesTable B.1. ¹H-NMR data for the Mosher ester derivatives of alcohol **276**.





Proton	Racemic Mosher ester ^a	Enantioenriched Mosher ester ^b (ee 67%)
<i>H1</i>	2.10 (s, 3H) 2.00 (s, 3H)	2.10 (s, 3H) 2.00 (s, 0.60H).
<i>H3</i>	3.17 (app. ddd, <i>J</i> = 17.0, 9.5, 3.5, 2H) 2.75 (app. ddd, <i>J</i> = 17.0, 4.0, 2.0, 2H)	3.17 (app. ddd, <i>J</i> = 17.0, 9.5, 3.5, 1.20H) 2.75 (app. ddd, <i>J</i> = 17.0, 4.0, 2.0, 1.20H)
<i>H4</i>	6.41 (dd, <i>J</i> = 9.5, 4.0, 1H) 6.31 (dd, <i>J</i> = 10.0, 3.5, 1H)	6.41 (dd, <i>J</i> = 9.5, 4.0, 1H) 6.31 (dd, <i>J</i> = 10.0, 3.5, 0.20H)
<i>H1^a</i>	3.45 (q, <i>J</i> = 1.5, 3H) 3.37–3.36 (m, 3H, overlap with ¹ H signals of (R)-MTPA-Cl derivatives)	3.45 (q, <i>J</i> = 1.5, 0.6H) 3.37–3.36 (m, 3H, overlap with ¹ H signals of (R)-MTPA-Cl derivatives)

^a The racemic Mosher's ester was prepared following the *General Procedure C* using a racemic mixture of alcohol **276** and enantiomerically pure (R)-MTPA-Cl; ^b The enantioenriched Mosher ester was prepared following the *General Procedure C* using an enantioenriched mixture of alcohol **276** and enantiomerically pure (R)-MTPA-Cl.

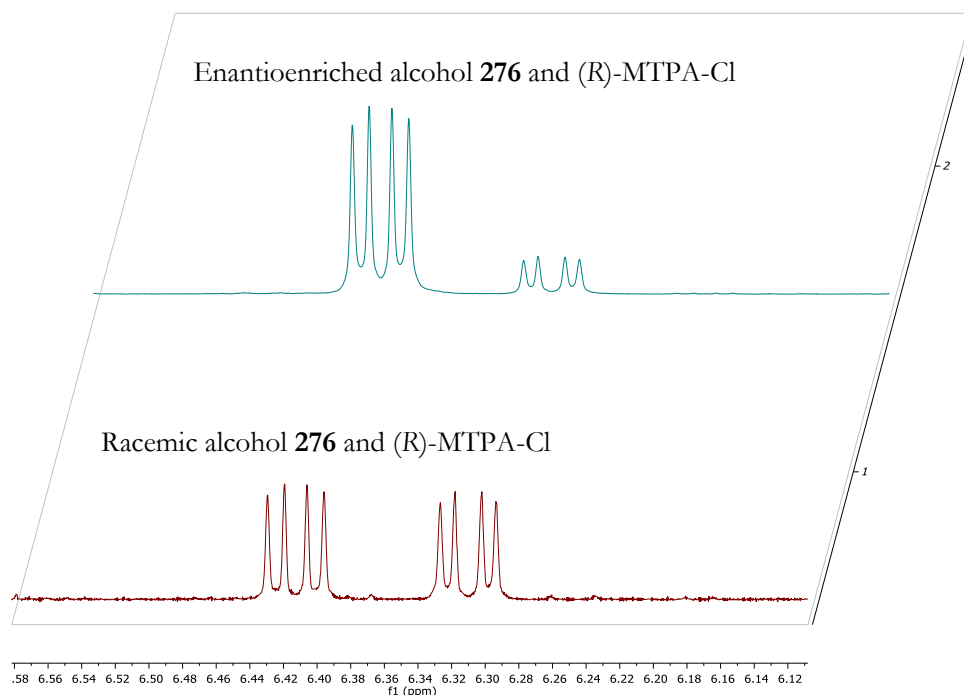
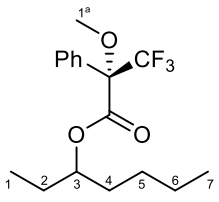
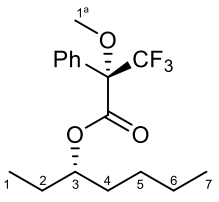
Figure B.1. Part of the ¹H-NMR spectra of the Mosher ester derivatives of alcohol **276** corresponding to *H4*.

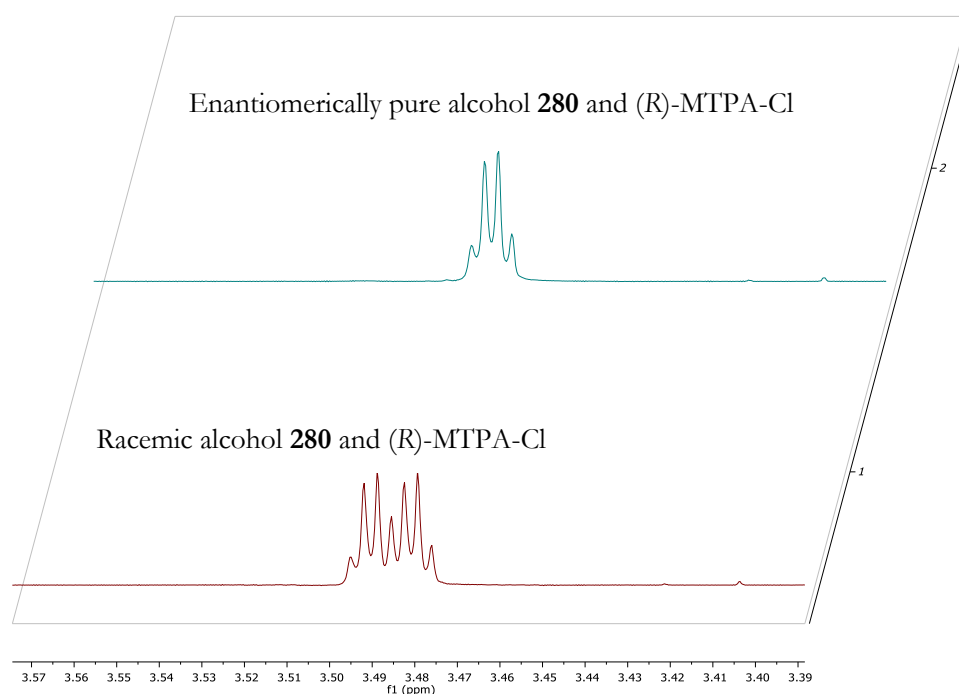
Table B.2. $^1\text{H-NMR}$ data for the Mosher ester derivatives of alcohol **280**.





Proton	Racemic Mosher ester ^a	Enantiopure Mosher ester ^b (ee >99%)
<i>H1</i>	0.84 (t, $J = 7.5$, 3H) 0.82–0.78 (m, 3H)	0.82–0.78 (m, 3H)
<i>H2</i>	1.66–1.45 (m, 8H, overlap with <i>H4</i>)	1.66–1.45 (m, 4H, overlap with <i>H4</i>)
<i>H3</i>	4.96 (tt, $J = 7.0, 5.5$, 2H)	4.96 (tt, $J = 7.0, 5.5$, 1H)
<i>H4</i>	1.66–1.45 (m, 8H, overlap with <i>H2</i>)	1.66–1.45 (m, 4H, overlap with <i>H4</i>)
<i>H5</i>	1.30–1.02 (m, 8H, overlap with <i>H6</i>)	1.30–1.15 (m, 4H, overlap with <i>H6</i>)
<i>H6</i>	1.30–1.02 (m, 8H, overlap with <i>H5</i>)	1.30–1.15 (m, 4H, overlap with <i>H5</i>)
<i>H7</i>	0.74 (t, $J = 7.0$, 3H) 0.73 (t, $J = 7.5$, 3H)	0.73 (t, $J = 7.5$, 3H)
<i>H1^a</i>	3.49 (q, $J = 1.5$, 3H) 3.48 (q, $J = 1.5$, 3H)	3.48 (q, $J = 1.5$, 3H)

^a The racemic Mosher ester was prepared following the *General Procedure C* using a racemic mixture of alcohol **280** and enantiomerically pure (R)-MTPA-Cl; ^b The enantioenriched Mosher ester was prepared following the *General Procedure C* using enantiomerically pure alcohol **280** and (R)-MTPA-Cl.

**Figure B.2.** Part of the $^1\text{H-NMR}$ spectra of the Mosher ester derivatives of alcohol **280** corresponding to *H1^a*.

APPENDIX C: Screening results of taxane compounds

Table C.1. Product distribution and substrate conversion of the screen with diketone 179a.



Entry	Enzyme	Retention time (min) ^a					
		4.45 (179a)	4.88	5.02 (308)	5.06 (309)	5.18	5.43 (310)
1	RLYF/KSK19/AI	83%	-	5%	11%	1%	-
2	KSK19/AM	48%	-	6%	46%	-	-
3	KSK19/AI/Y51L	85%	-	6%	8%	-	1%
4	KSK19/AI/AI	20%	-	22%	51%	5%	2%
5	RP/FV/EV/L437VL	89%	-	4%	6%	-	-
6	KSK19/I263A	33%	-	4%	50%	-	13%
7	RP	95%	-	2%	3%	-	-
8 ^b	RP/EV	-	-	-	-	-	-
9	RT2/F81W	98%	-	-	2%	-	-
10 ^b	RP/FV/EV	-	-	-	-	-	-
11	KSK19/AI/F87V	95%	-	2%	3%	-	-
12	RP/FV/EV/L437LV	77%	20%	-	3%	-	-
13	KSK19/AI/V78I	17%	-	44%	37%	-	2%
14	RT2/AP/I401M	99%	-	-	1%	-	-
15	RT2/AP/AM	87%	2%	4%	7%	-	-
16	RT2/IP	69%	-	1%	30%	-	-
17	RT2/AP	97%	-	1%	2%	-	-
18	RT2/AP/A184I	94%	3%	-	3%	-	-
19	RT2/AP/V78I	100%	-	-	-	-	-
20	RT2	100%	-	-	-	-	-
21	RP/IA/EV	82%	-	-	17%	-	1%
22	WT	100%	-	-	-	-	-
23	RP/AP	75%	-	11%	12%	-	2%
24	A330P	92%	-	3%	5%	-	-
25	RP/FW	100%	-	-	-	-	-
26	RT2/AP/AI/SA	93%	-	-	7%	-	-
27	RP/HL/IG	89%	1%	-	10%	-	-
28	RT2/AP/PV	93%	-	-	7%	-	-
29	GVQ	100%	-	-	-	-	-
30	RT2/AW	81%	-	4%	9%	-	6%

Appendix C

31	F87A	100%	-	-	-	-	-
32	RLYF/KSK19	15%	-	44%	39%	-	2%
33	RP/AM/IA	18%	-	38%	43%	-	1%
34	RT2/VF	99%	-	-	1%	-	-
35	RLYF	45%	-	-	52%	-	3%
36	KSK19/AM/EF	84%	-	-	16%	-	-
37	RP/HL	45%	16%	6%	32%	-	1%
38	RP/EG	96%	-	-	4%	-	-
39	KSK48	100%	-	-	-	-	-
40	KSK19/QP/FV	100%	-	-	-	-	-
41	RT2/AP/VI/AI	92%	-	3%	5%	-	-
42	RP/FV/EV/LQ	100%	-	-	-	-	-
43	RP/FV/EV/VF	85%	-	-	13%	-	2%
44	RT2/FW/AI	94%	-	-	6%	-	-
45	RP/EM	98%	-	-	2%	-	-
46	KSK19/AI/IA	90%	-	-	10%	-	-
47	RT2/SW/AW	44%	-	2%	53%	-	1%
48	A330P	100%	-	-	-	-	-

^a The screening of diketone **179a** was performed following the *General Procedure A*. The oxidised products were identified using GC and the product distributions were determined from the integration of peak areas. The GC retention times represent the following products:

4.45 min product: diketone **179a** (starting material recovered unchanged)

4.88 min product: unidentified

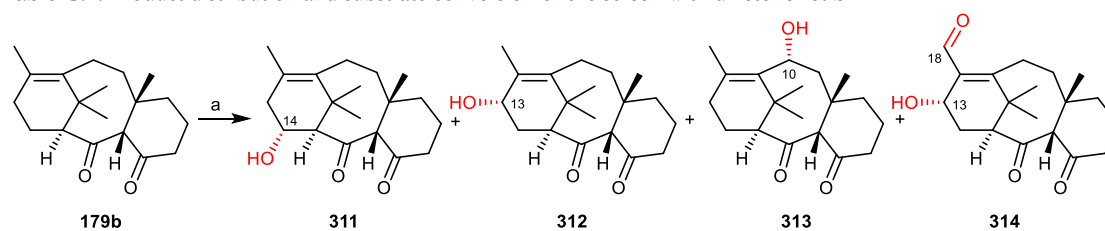
5.02 min product: taxane **308** (characterised by NMR and MS after scaling up using P450 mutant KSK19/AI/AI)

5.06 min product: taxane **309** (characterised by NMR and MS after scaling up using P450 mutant KSK19/AI/AI)

5.18 min product: unidentified

5.42 min product: taxane **310** (characterised by NMR and MS after scaling up using P450 mutant KSK19/I263A)

^b Results were not obtained due to problems during the GC analysis.

Table C.2. Product distribution and substrate conversion of the screen with diketone **179b**.

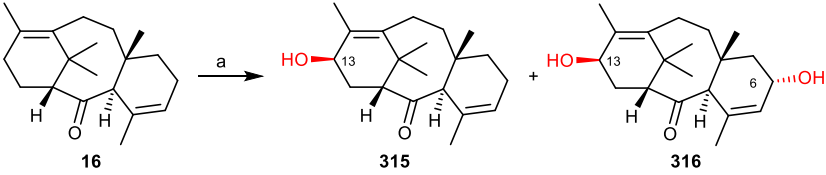
Entry	Enzyme	Retention time (min) ^a		
		4.81	4.92 (179b)	5.70 (311 , 312 , 313 and 314)
1	RLYF/KSK19/AI	3%	13%	84%
2	KSK19/AM	-	-	100%
3	KSK19/AI/Y51L	4%	40%	56%
4	KSK19/AI/AI	-	-	100%
5	RP/FV/EV/L437VL	5%	84%	11%
6	KSK19/I263A	-	-	100%
7	RP	6%	94%	-
8	RP/EV	6%	94%	-
9	RT2/F81W	5%	95%	-
10	RP/FV/EV	5%	95%	-
11	KSK19/AI/F87V	5%	95%	-
12	RP/FV/EV/L437LV	6%	94%	-
13	KSK19/AI/V78I	5%	83%	12%
14	RT2/AP/I401M	6%	94%	-
15	RT2/AP/AM	5%	89%	6%
16	RT2/IP	5%	86%	9%
17	RT2/AP	6%	94%	-
18	RT2/AP/A184I	6%	94%	-
19	RT2/AP/V78I	6%	94%	-
20	RT2	5%	95%	-
21	RP/IA/EV	6%	89%	5%
22	WT	6%	94%	-
23	RP/AP	6%	94%	-
24	RP/FV	3%	80%	17%

^a The screening of diketone **179b** was performed following the *General Procedure A*. The oxidised products were identified using GC and the product distributions were determined from the integration of peak areas. The GC retention times represent the following products:

4.81 min product: unidentified impurity present in the starting material

4.91 min product: diketone **179b** (starting material recovered unchanged)

5.70 min product: taxane **311**, **312**, **313** and **314** (characterised by NMR and MS after scaling up using P450 mutant KSK19/AI/AI and KSK19/I263A)

Table C.3. Product distribution and substrate conversion of the screen with taxadienone **220**.


NA	Enzyme	Retention time (min) ^a						
		3.86	4.07 (16)	4.45	4.65 (315)	4.70	5.31 (316)	5.37
1	RLYF/KSK19/AI	4%	8%	10%	21%	2%	37%	19%
2	KSK19/AM	6%	63%	-	9%	-	17%	5%
3	KSK19/AI/Y51L	7%	41%	9%	22%	1%	18%	2%
4	KSK19/AI/AI	5%	18%	4%	15%	-	44%	15%
5	RP/FV/EV/L437VL	5%	80%	-	15%	-	-	-
6	KSK19/I263A	6%	49%	-	22%	-	17%	6%
7	RP	13%	87%	-	-	-	-	-
8	RP/EV	14%	74%	-	12%	-	-	-
9	RT2/F81W	8%	87%	-	3%	2%	-	-
10	RP/FV/EV	11%	60%	-	22%	6%	-	-
11	KSK19/AI/F87V	7%	93%	-	-	-	-	-
12	RP/FV/EV/L437LV	14%	86%	-	-	-	-	-
13	KSK19/AI/V78I	9%	69%	9%	13%	-	-	-
14	RT2/AP/I401M	13%	87%	-	-	-	-	-
15	RT2/AP/AM	8%	81%	-	8%	3%	-	-
16	RT2/IP	11%	71%	-	11%	7%	-	-
17	RT2/AP	14%	77%	-	6%	3%	-	-
18	RT2/AP/A184I	12%	68%	-	10%	10%	-	-
19	RT2/AP/V78I	8%	92%	-	-	-	-	-
20	RT2	12%	88%	-	-	-	-	-
21	RP/IA/EV	15%	56%	-	21%	8%	-	-
22	WT	16%	84%	-	-	-	-	-
23	RP/AP	14%	86%	-	-	-	-	-
24	RP/FV	10%	52%	12%	16%	10%	-	-

^a The screening of taxadienone **16** was performed following the *General Procedure A*. The oxidised products were identified using GC and the product distributions were determined from the integration of peak areas. The GC retention times represent the following products:

3.86 min product: unidentified impurity present in the starting material

4.07 min product: taxadienone **16** (starting material recovered unchanged)

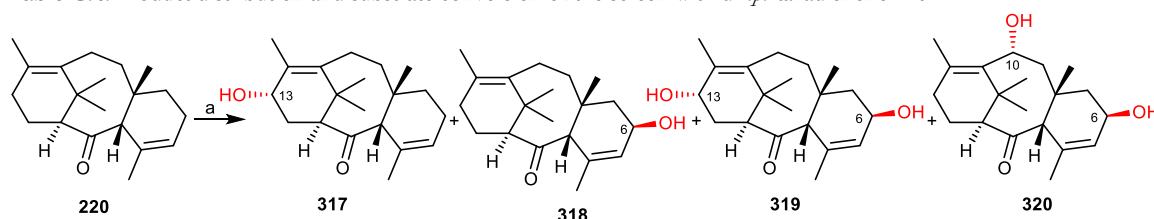
4.45 min product: unidentified

4.65 min product: taxane **315** (characterised by NMR and MS after scaling up using P450 mutant KSK19/AI/AI)

4.70 min product: unidentified

5.31 min product: taxane **316** (characterised by NMR and MS after scaling up using P450 mutant KSK19/AI/AI)

5.37 min product: unidentified

Table C.4. Product distribution and substrate conversion of the screen with di-*epi*-taxadienone **220**.

Entry	Enzyme	Retention time (min) ^a				
		3.90	4.06 (220)	4.64 (317)	4.68 (318)	5.33 (319 and 320)
1	RLYF/KSK19/AI	3%	11%	19%	44%	23%
2	KSK19/AM	11%	78%	6%	2%	3%
3	KSK19/AI/Y51L	11%	70%	4%	15%	-
4	KSK19/AI/AI	8%	40%	13%	33%	6%
5	RP/FV/EV/L437VL	8%	82%	-	10%	-
6	KSK19/I263A	3%	27%	13%	25%	32%
7	RP	12%	88%	-	-	-
8 ^b	RP/EV	12%	77%	4%	7%	-
9	RT2/F81W	12%	81%	-	7%	-
10 ^b	RP/FV/EV	11%	83%	3%	3%	-
11	KSK19/AI/F87V	13%	87%	-	-	-
12	RP/FV/EV/L437LV	11%	89%	-	-	-
13	KSK19/AI/V78I	12%	85%	1%	2%	-
14	RT2/AP/I401M	14%	85%	-	1%	-
15	RT2/AP/AM	9%	76%	-	15%	-
16	RT2/IP	10%	85%	5%	-	-
17	RT2/AP	11%	86%	1%	2%	-
18	RT2/AP/A184I	11%	87%	-	2%	-
19	RT2/AP/V78I	11%	89%	-	-	-
20	RT2	13%	87%	-	-	-
21	RP/IA/EV	13%	79%	3%	5%	-
22	WT	12%	88%	-	-	-
23	RP/AP	12%	88%	-	-	-
24	A330P	13%	85%	-	2%	-

^a The screening of di-*epi*-taxadienone **220** was performed following the *General Procedure A*. The oxidised products were identified using GC and the product distributions were determined from the integration of peak areas. The GC retention times represent the following products:

3.90 min product: unidentified impurity present in the starting material

4.06 min product: taxadienone **220** (substrate recovered unchanged)

4.64 min product: taxane **317** (characterised by NMR and MS after scaling up using P450 mutant KSK19/AI/AI)

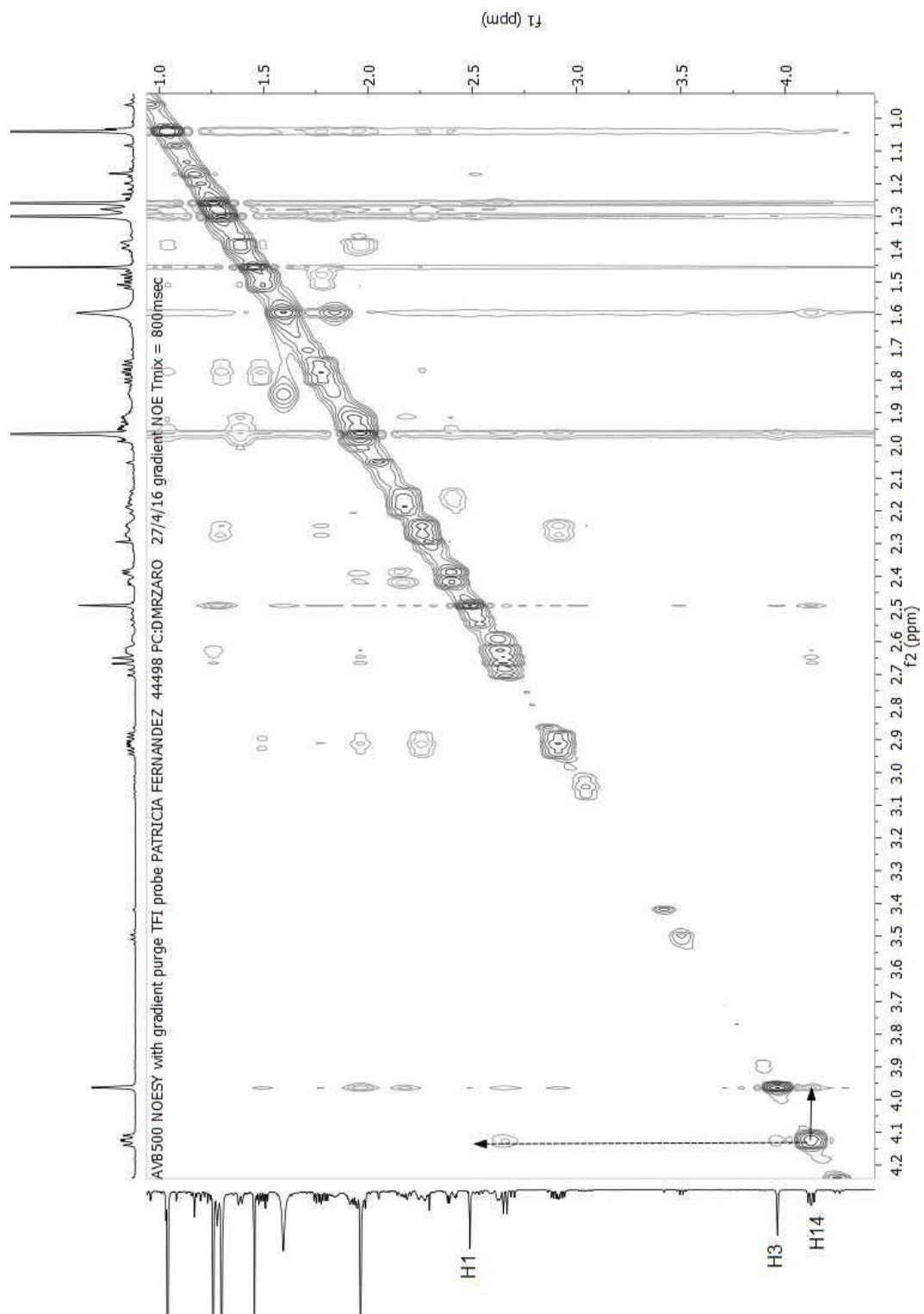
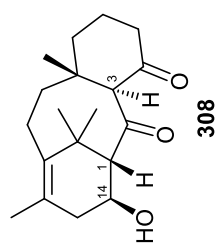
4.68 min product: taxane **318** (characterised by NMR and MS after scaling up using P450 mutant KSK19/AI/AI)

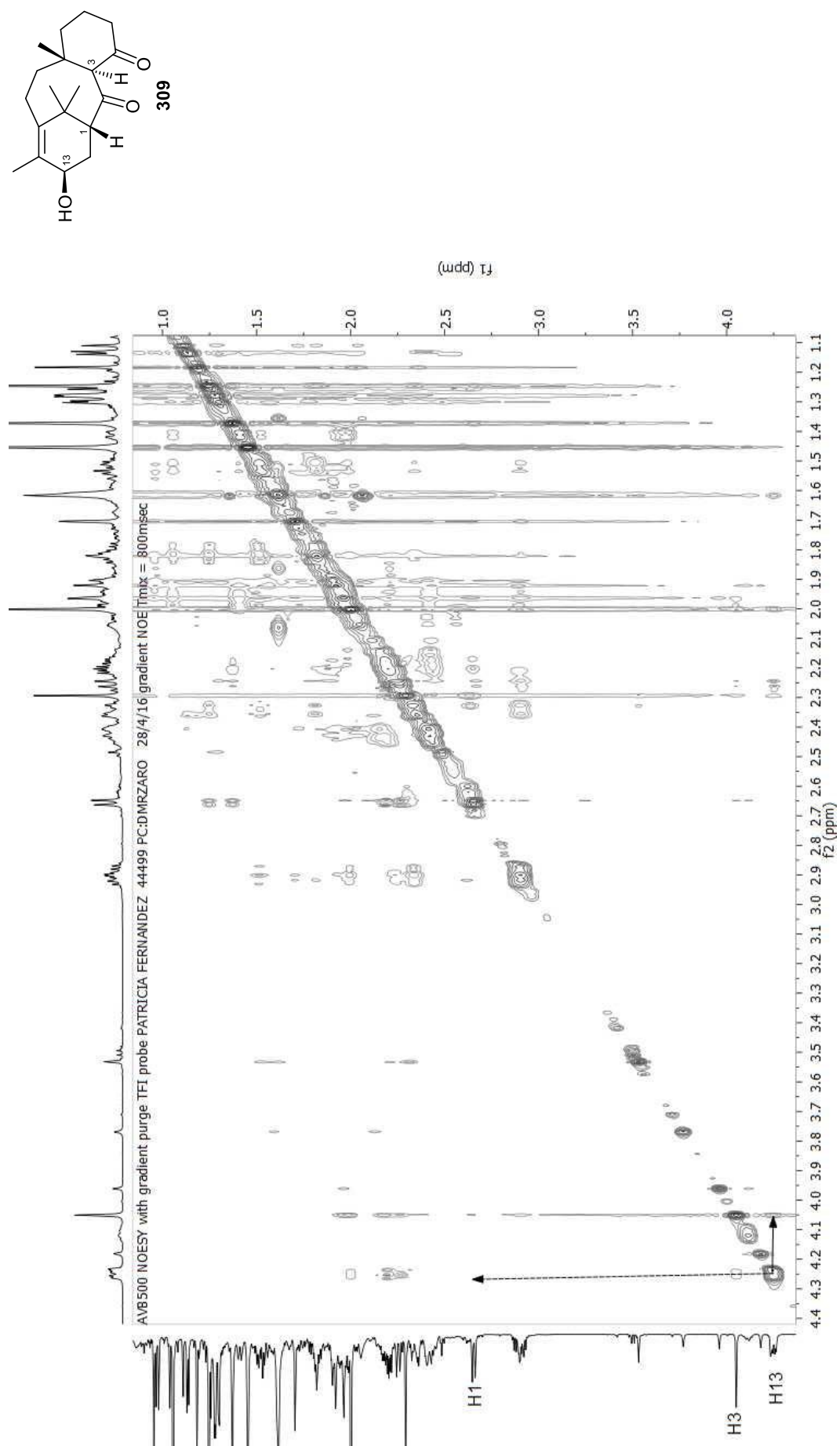
5.33 min product: taxane **319** and **310** (characterised by NMR and MS after scaling up using mp450 KSK19/AI/AI and KSK19/I263A)

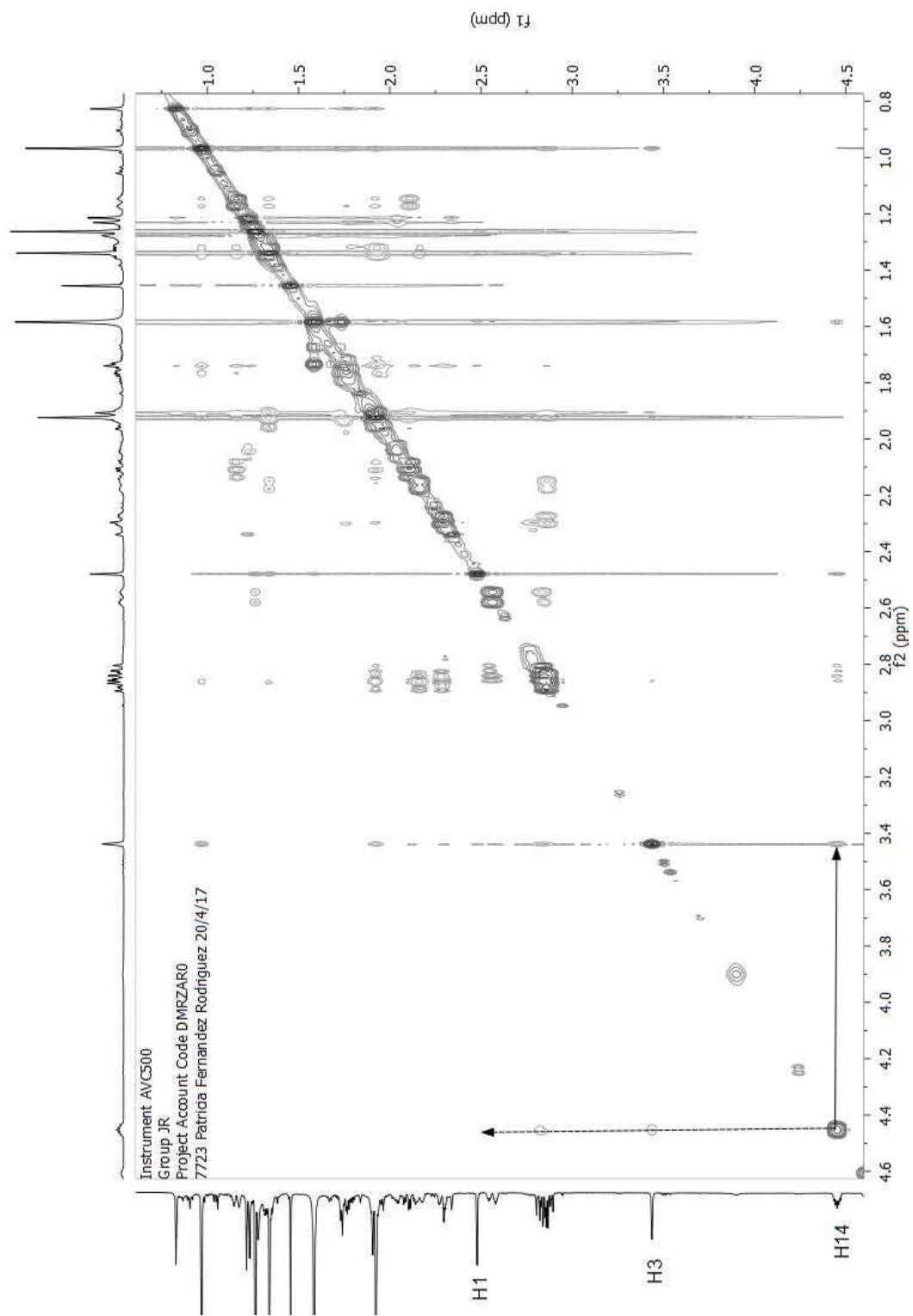
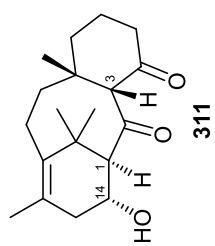
APPENDIX D: Structure elucidation of the oxidised taxanes

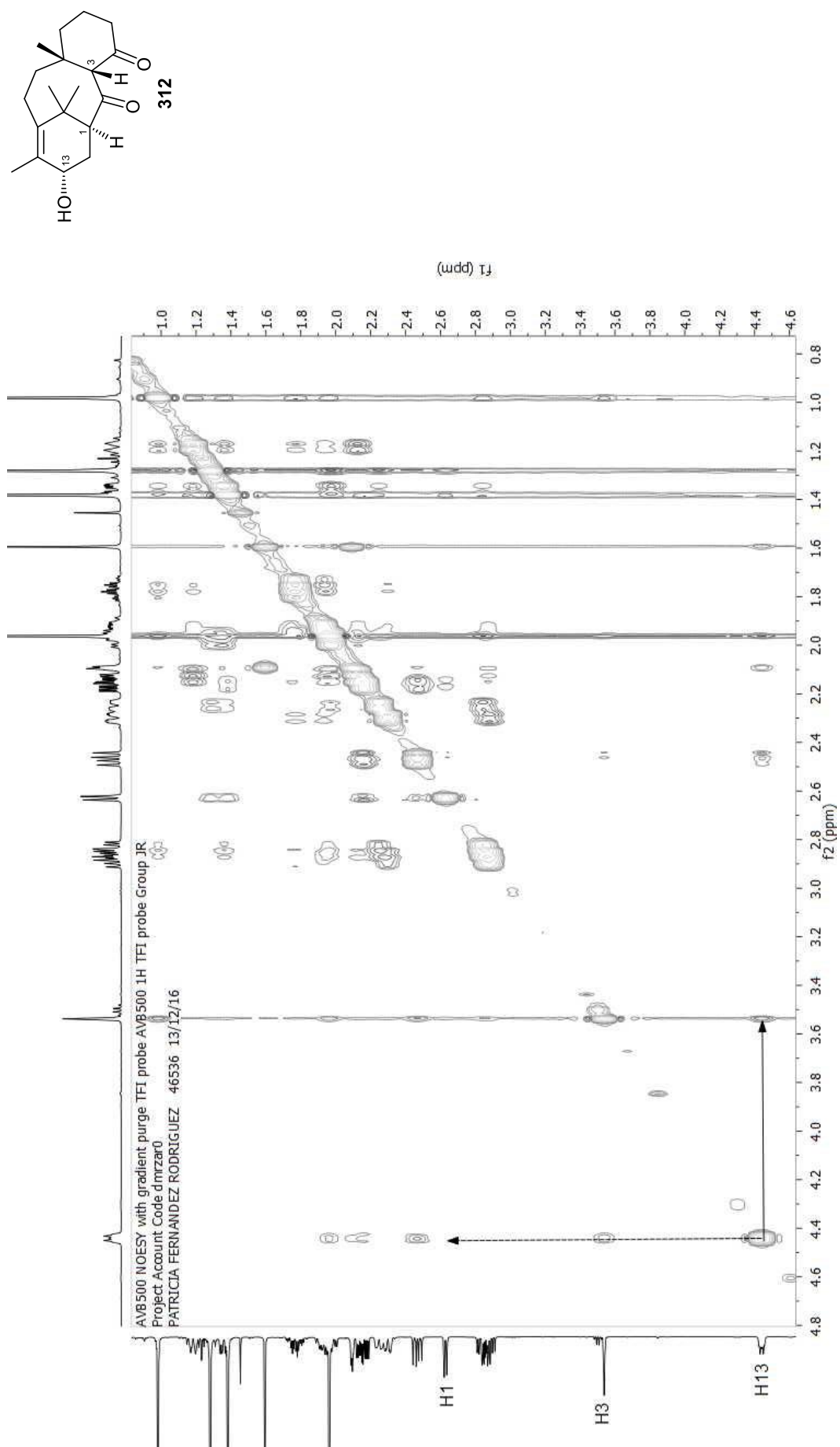
The molecular structure of the oxidised products was identified by NMR experiments, including ^1H , ^{13}C , COSY, HSQC, HMBC, and NOESY. Firstly, the number of carbons and their multiplicities (methyl, methylene, methane, or quaternary carbon) were identified by analysis of ^1H , ^{13}C and HSQC spectra. In particular, the HSQC spectrum was used to correlate ^1H and ^{13}C signals; hence, identifying to which carbon were the protons directly attached. Additionally, the chemical shift of both ^1H and ^{13}C signals provided information related to their chemical environment.

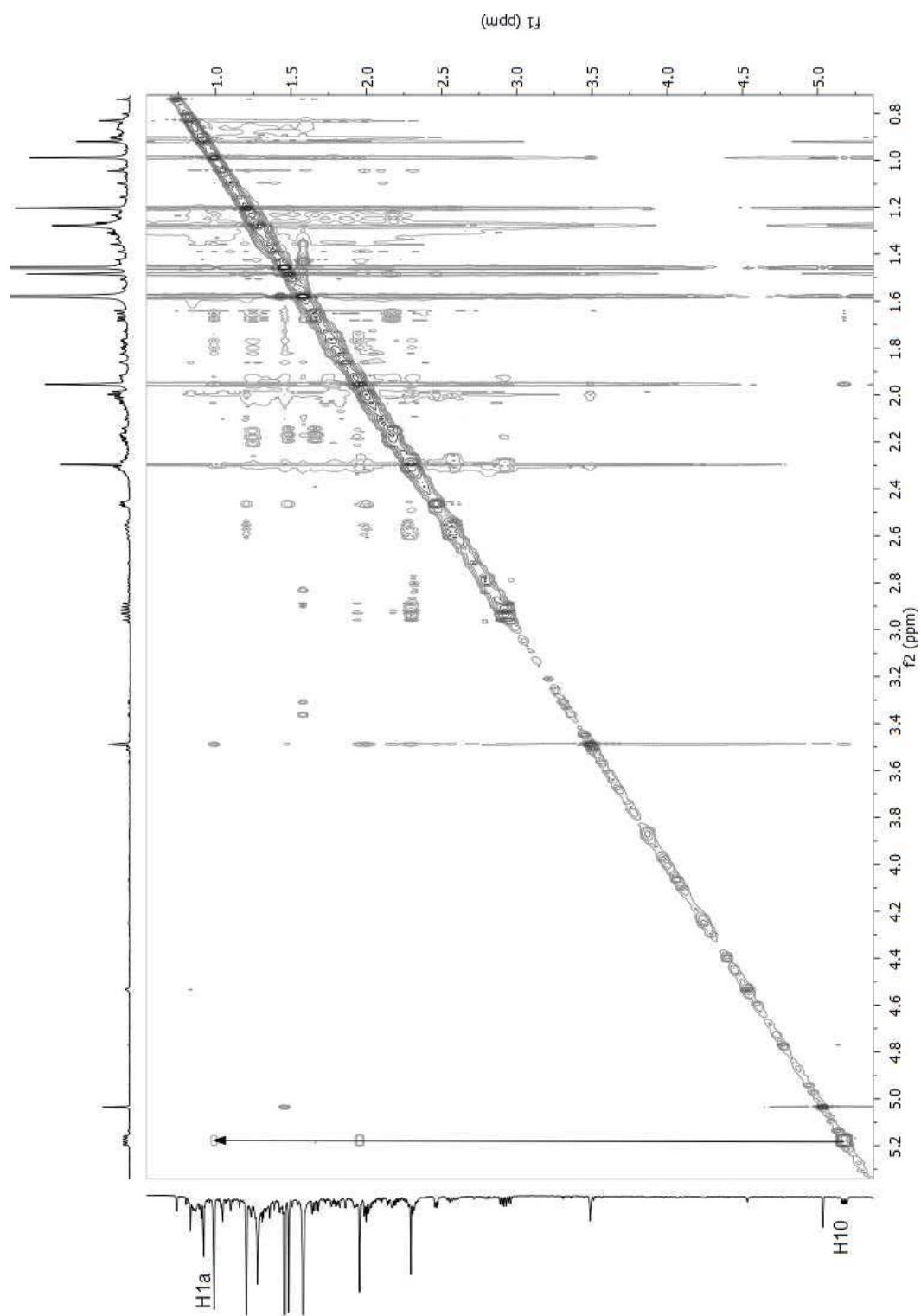
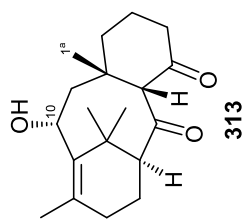
Then, HMBC and COSY spectra were used to establish the connectivity between carbons, thus identifying the oxidised positions. Finally, the relative stereochemistry was identified by analysis of the NOESY spectrum. Considering that the taxane core is rigid and thus, it cannot interconvert conformations at RT, the analysis of the NOESY spectrum, which show correlation between ^1H signals that are close in the space, permitted to establish the relative stereochemistry of the molecule. In this section the NOESY spectrum of the oxidised compounds are enclosed, along with the analysis used to determine the relative stereochemistry: correlation between ^1H signals is indicated by a black arrow and the lack of correlation between ^1H signals is shown by a dashed arrow.

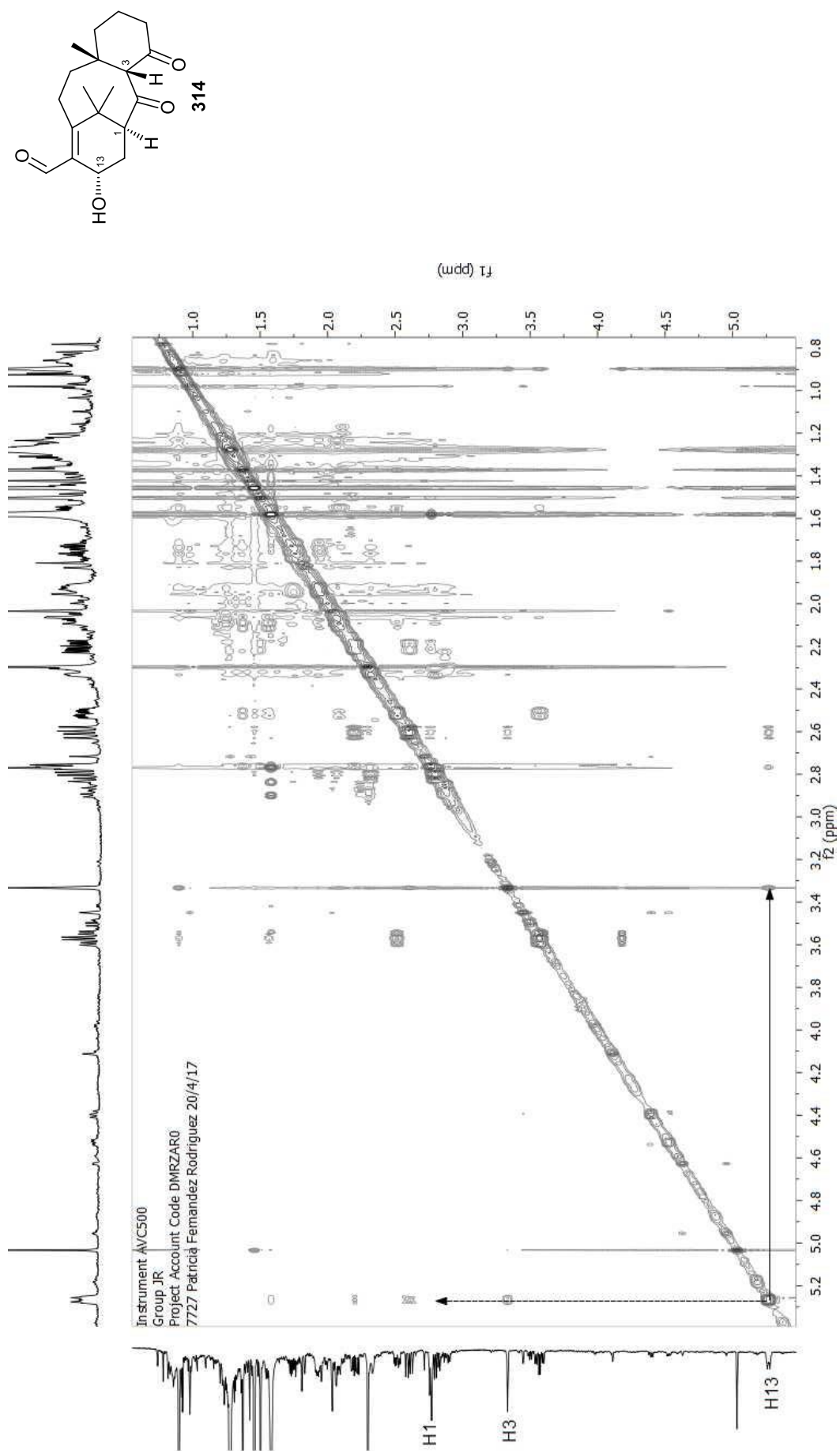


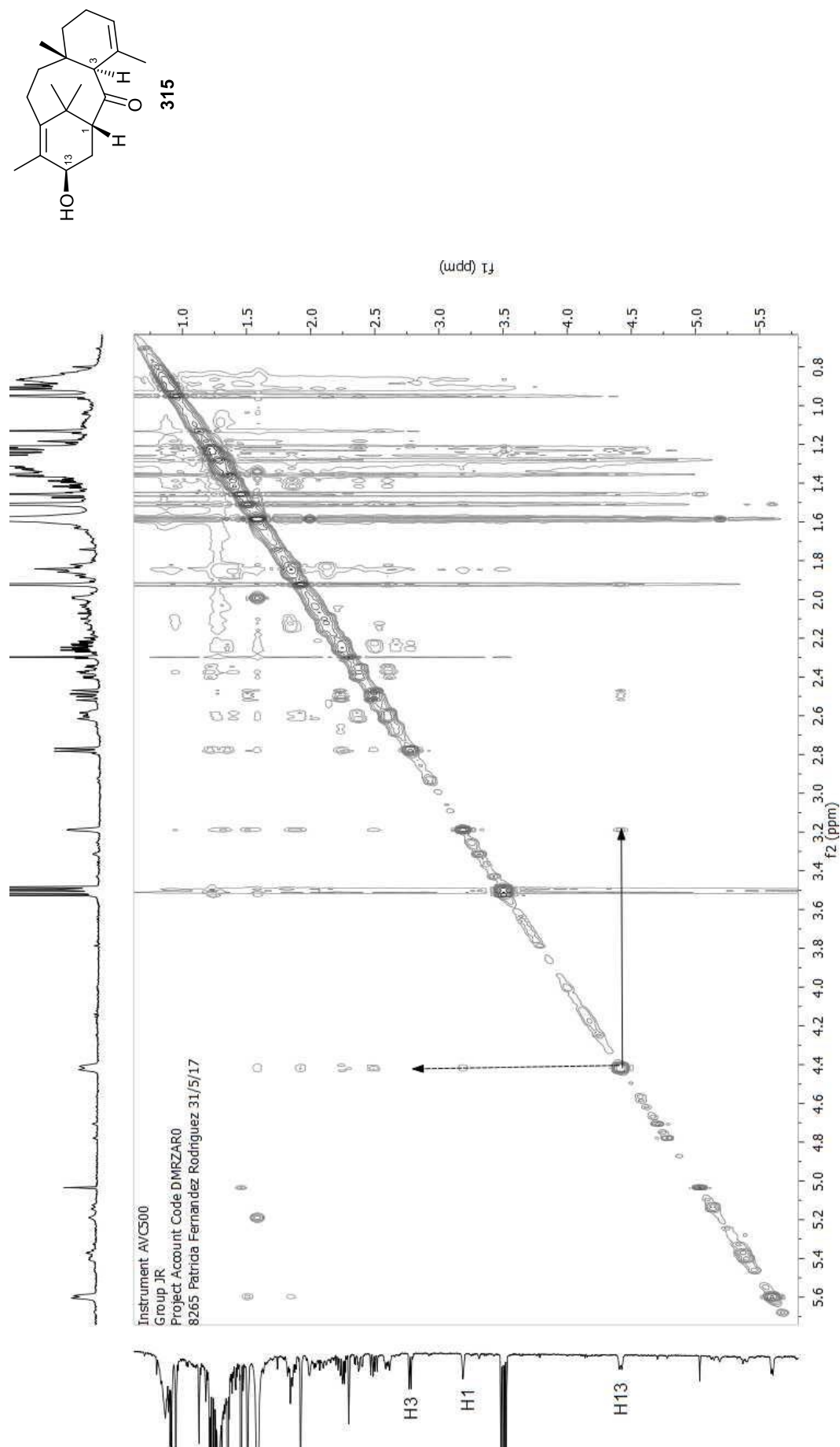


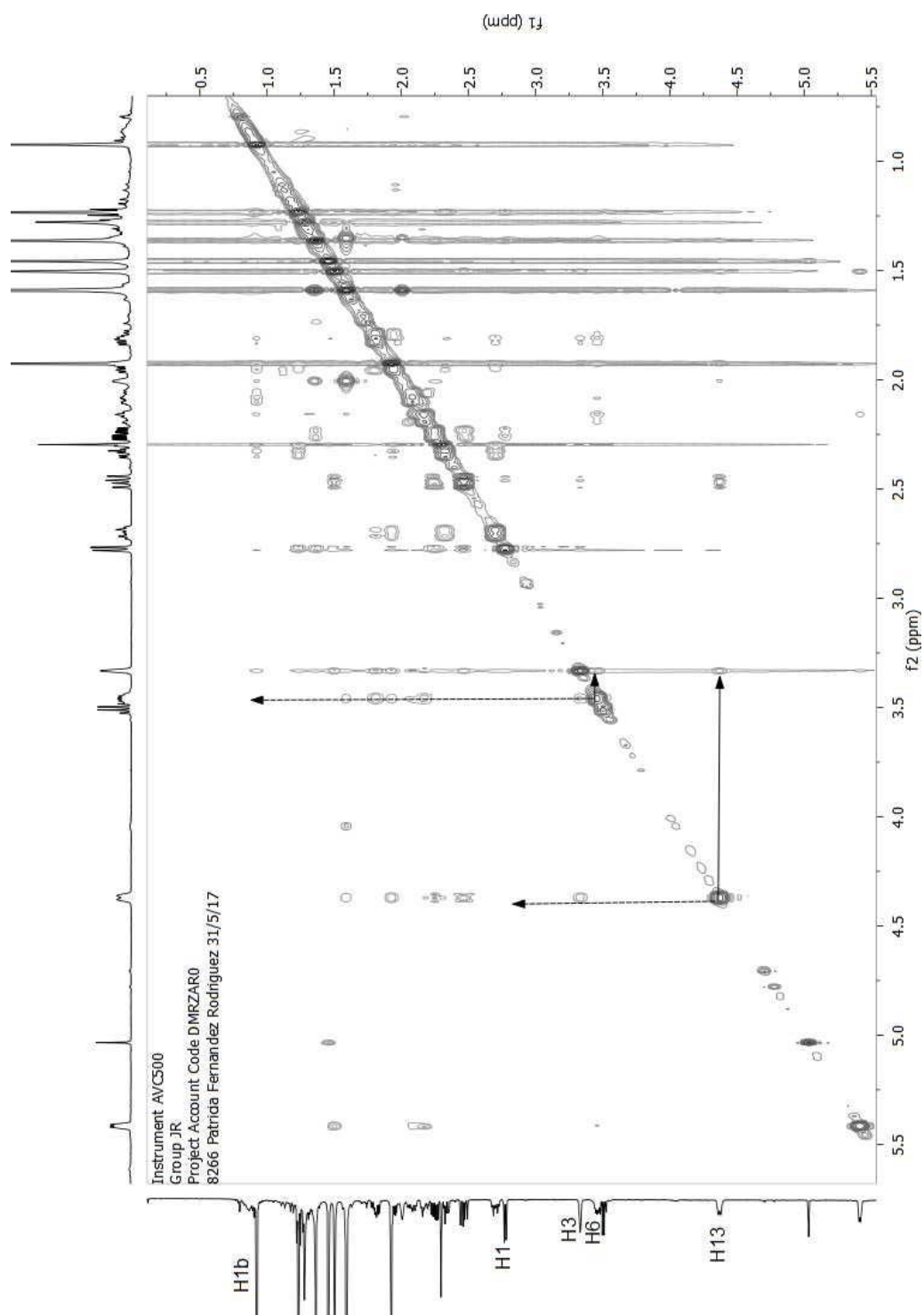
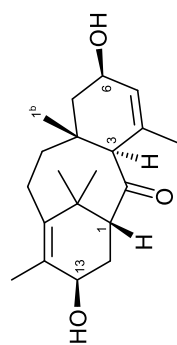


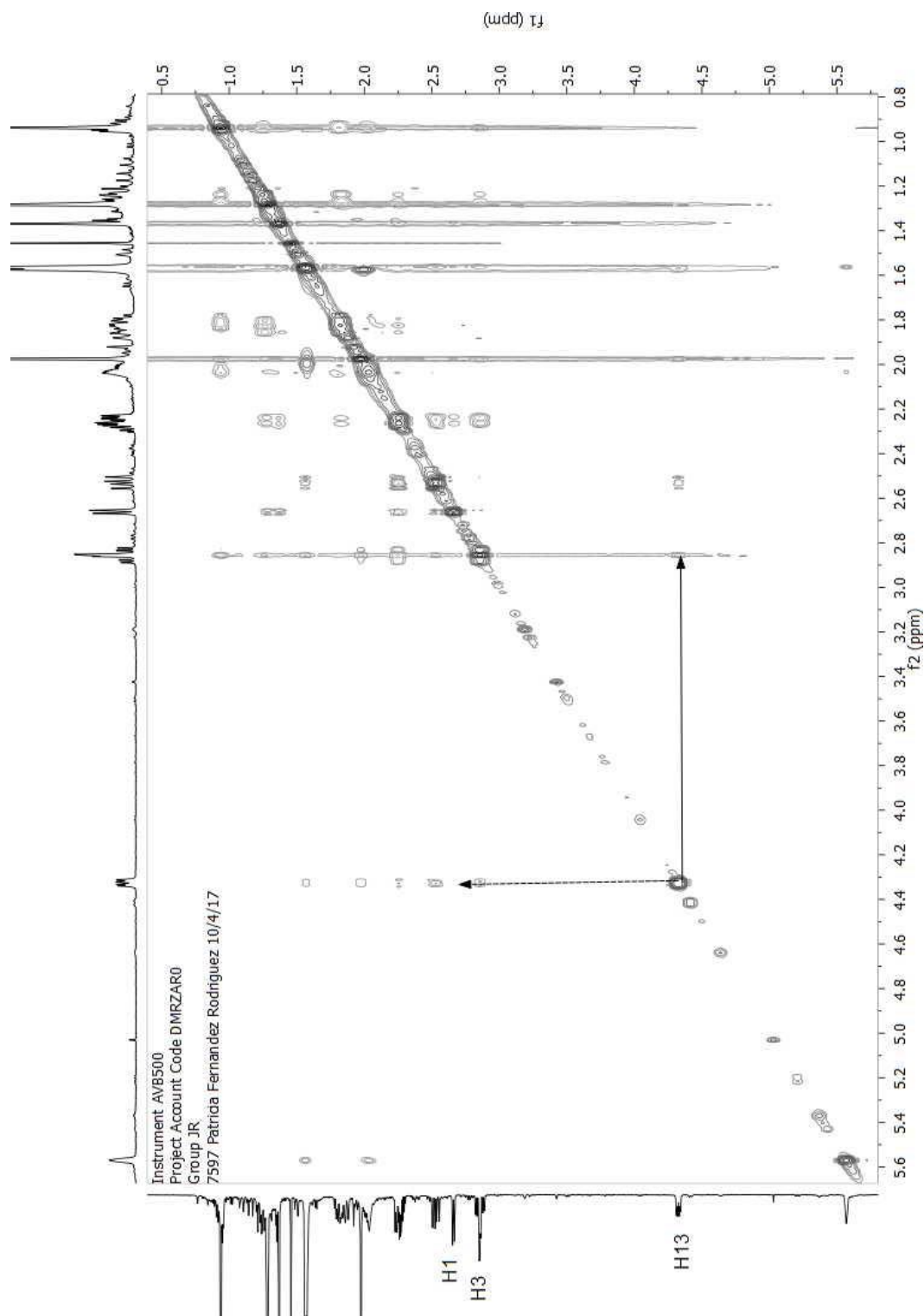
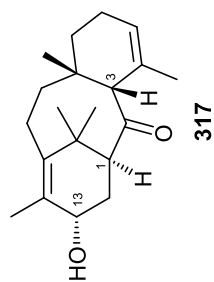


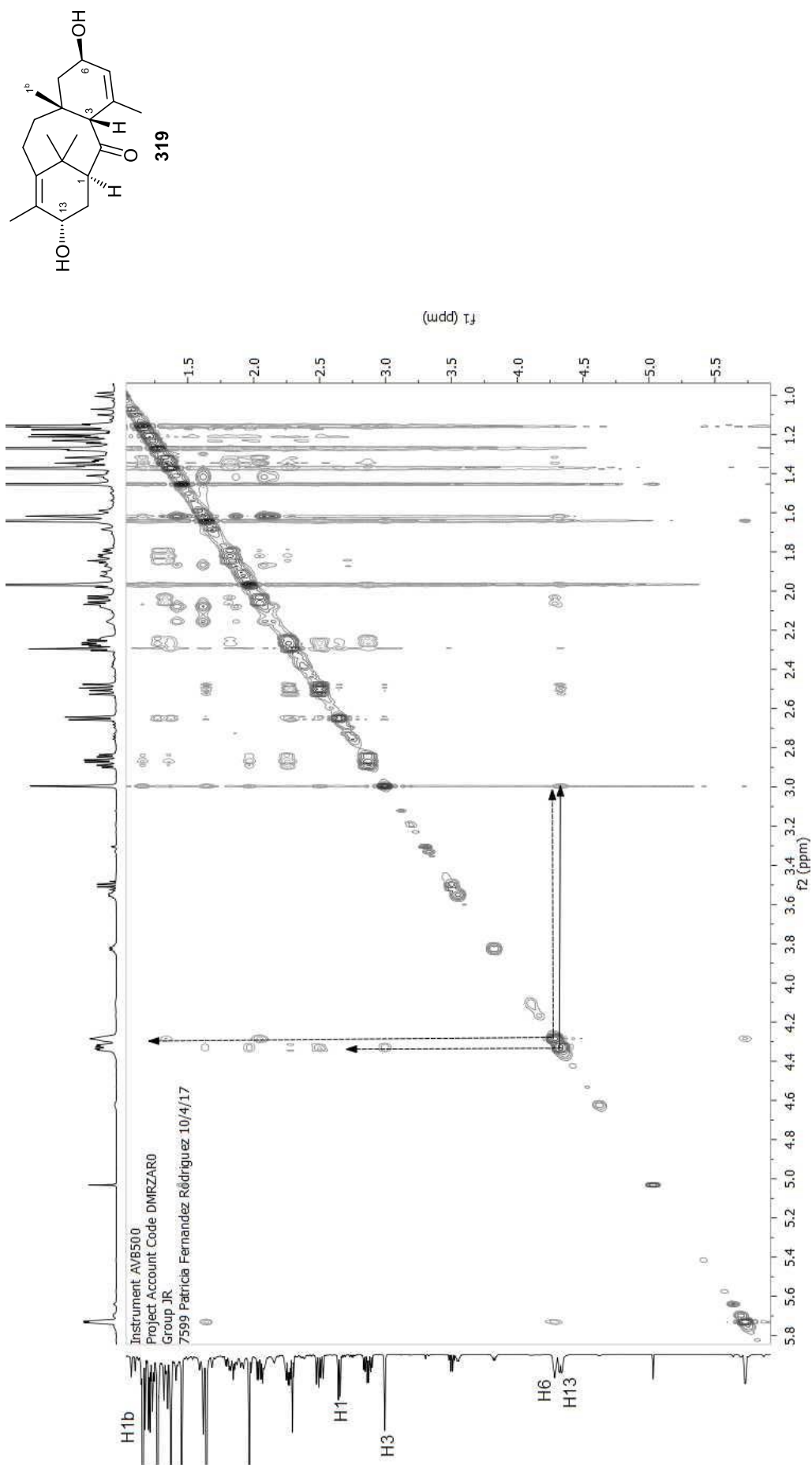


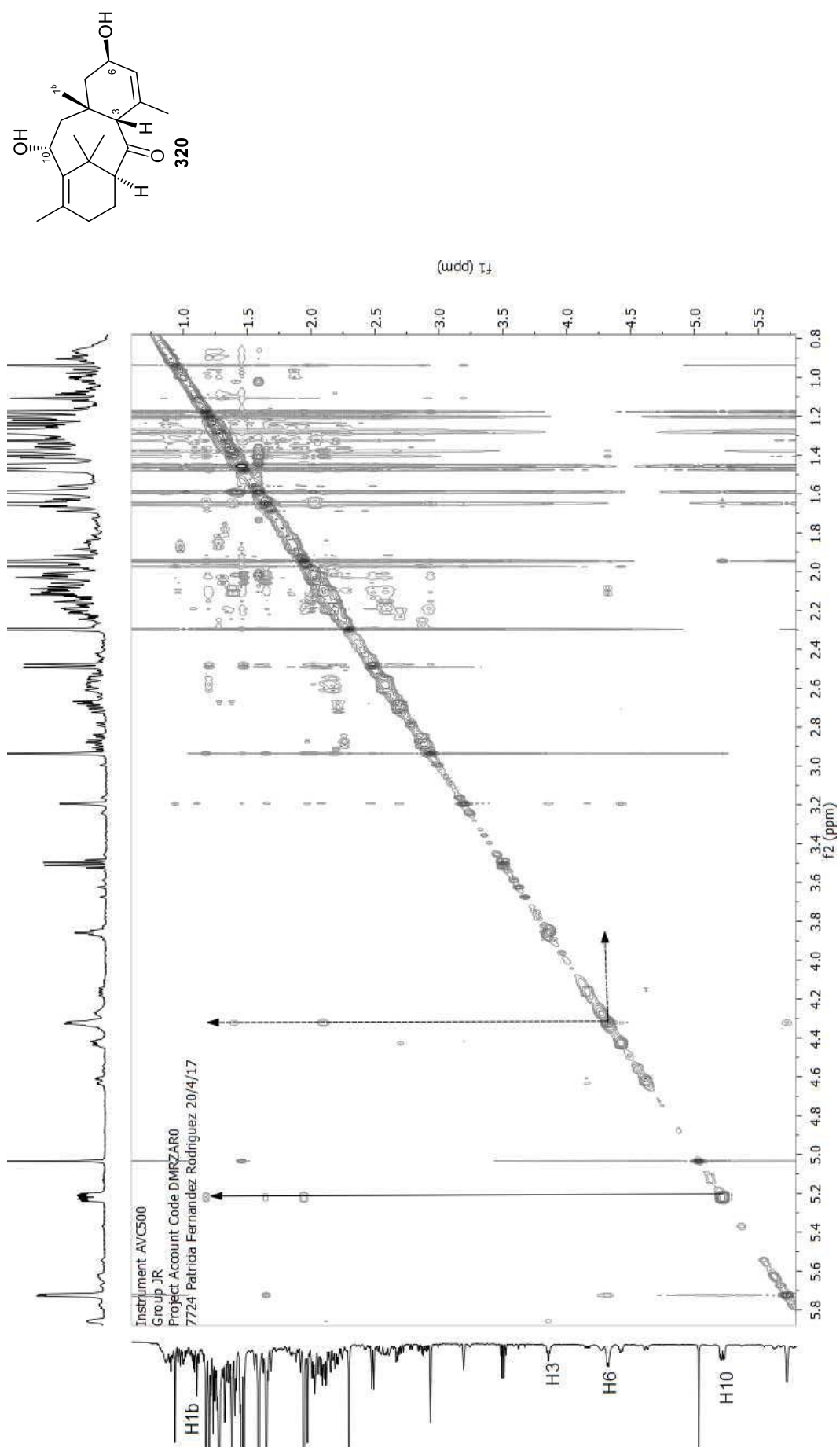










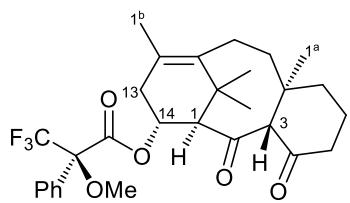


APPENDIX E: Absolute configuration of the oxidised taxanes

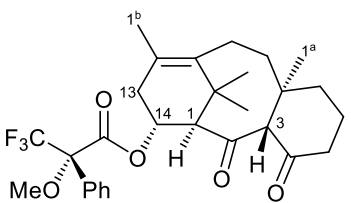
The absolute configuration of the oxidised products **308** and **309** was determined *via* the Mosher ester analysis. The two diastereomeric MTPA esters were prepared following the *General Procedure C*. In particular, the (*S*)-MTPA ester was prepared using (*R*)-MTPA-Cl and the data for the (*R*)-MTPA ester was inferred from the ¹H-NMR spectral data when using (±)-MTPA-Cl. Subsequently, the ¹H-NMR spectral data of each of the two diastereomeric MTPA esters was analysed and the difference in chemical shifts ($\Delta\delta^{SR}$) for each of the assignable analogous pairs of protons for the (*S*)- and (*R*)-MTPA esters was determined following the convention: $\Delta\delta^{SR} = \delta[(S)\text{-MTPA ester}] - \delta[(R)\text{-MTPA ester}]$. Applying the method reported by Hoye,^[216] the absolute configuration of the secondary alcohol was determined depending on which protons had positive or negative $\Delta\delta^{SR}$. The absolute configuration of the molecule was then assigned *via* correlation between ¹H signals in the NOESY spectrum.

When enantiopure taxane **308** was analysed via the Mosher ester method, the absolute configuration of C14 was inferred from the difference in chemical shift of analogous pair of protons (**Table E.1**), being 14*R*. Therefore, it was the enantiomer **308b**, which had the opposite configuration to the natural taxanes. The same protocol was applied to enantioenriched taxane **309** (**Table E.2**). In this case, the absolute configuration of C13 was 13*S*. Thus, the major enantiomer was **309b**, which also had the opposite configuration to the natural taxanes.

Table E.1. Analysis of the ¹H-NMR data for the Mosher ester derivatives of taxane **308**.



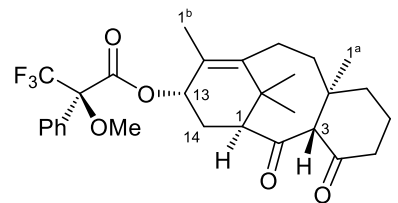
(S)-MTPA ester 325b



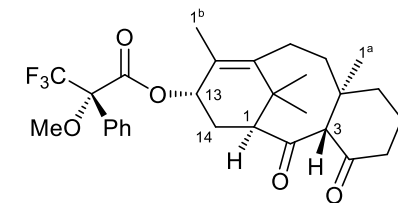
(R)-MTPA ester 325b

Entry	Proton	$\delta[(S)\text{-MTPA ester}]$	$\delta[(R)\text{-MTPA ester}]$	$\Delta\delta^{SR}$	
		(ppm)	(ppm)	ppm	Hz (500MHz)
1	<i>H</i> 13	2.62	2.50	0.12	+60
2	<i>H</i> 14	5.23	5.19	0.04	+20
3	<i>H</i> 1 ^a	1.03	1.02	0.01	+5
4	<i>H</i> 1 ^b	1.96	1.95	0.01	+5
5	<i>H</i> 13	2.91	2.91	0	0
6	<i>H</i> 3	3.94	3.95	-0.01	-5
7	<i>H</i> 1	2.46	2.55	-0.09	-45

N.B. ¹H-NMR data recorded in CDCl₃.**Table E.2.** Analysis of the ¹H-NMR data for the Mosher ester derivatives of taxane **309**.



(S)-MTPA ester 324b



(R)-MTPA ester 324b

Entry	Proton	$\delta[(S)\text{-MTPA ester}]$	$\delta[(R)\text{-MTPA ester}]$	$\Delta\delta^{SR}$	
		(ppm)	(ppm)	ppm	Hz (500MHz)
1	<i>H</i> 1 ^b	1.93	1.82	0.11	+55
2	<i>H</i> 1 ^a	1.09	1.04	0.05	+25
3	<i>H</i> 3	4.01	4.01	0	0
4	<i>H</i> 13	5.49	5.49	0	0
5	<i>H</i> 14	2.49	2.52	-0.03	-15
6	<i>H</i> 1	2.57	2.61	-0.04	-20
7	<i>H</i> 14	2.00	2.09	-0.09	-45

N.B. ¹H-NMR data recorded in CDCl₃.

