# Total Synthesis of Nemorosone and Nemorosone II

**James David Taylor**

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**Department of Chemistry**
**College of Engineering and Physical Sciences**
**The University of Birmingham**
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# Abstract

The polycyclic polyprenylated acylphloroglucinols (PPAPs) are a class of naturally occurring compounds with a characteristic bicyclo core system (either [3.3.1]nonane or [3.2.1]octane). This class of compounds have been shown to have significant biological activity, including activity against cancer, HIV, bacterial infection and depression. The biological activity and the structural challenges that these compounds pose have led to significant interest from the synthetic chemists. A review of the previous synthetic work and the biological activity of the PPAPs is contained within Chapter 1.

A total synthesis of the type A PPAP nemorosone (**4**) is described, using a modified Effenberger cyclisation as the key bicyclo[3.3.1]nonane forming step. Further functionalisation was performed through a difficult bridgehead iodination, followed by halogen-metal exchange and benzoylation. Chapter 2 describes our total synthesis of nemorosone (**4**) in 13 synthetic steps and 6% overall yield.

Chapter 3 describes the first total synthesis of the type C PPAP nemorosone II (**150**) in 10 synthetic steps and 1.5% overall yield. Once more the key bicyclo[3.3.1]nonane core was formed using the modified Effenberger cyclisation.

Alongside the synthesis, biological studies have been performed on the final compounds and a range of intermediates (Chapter 4). Investigations into the difficult bridgehead substitution and resulting halogen-metal exchange reactions are discussed in Chapter 5. Preliminary studies were undertaken into a new shorter route to nemorosone (**4**) using a Dieckmann condensation as the key bicyclo[3.3.1]nonane forming step. (Chapter 6).

# Acknowledgements

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# Glossary of Abbreviations

| | |
|---|---|
| AIBN | azobisisobutyronitrile |
| BLC2 | B-cell lymphoma 2 (gene family) |
| Bn | benzyl |
| Bu | butyl |
| Cdc(x) | cell division cycle (protein number) |
| CDK | cyclin-dependent kinase |
| CSA | camphorsulfonic acid |
| DBU | 1,8-diazabicyclo[5.4.0]undecene-7 |
| DCM | dichloromethane |
| DDIT3 | DNA damage inducible transcript 3 (a gene) |
| DFT | density functional theory |
| DIBAL-H | diisobutylaluminiumhydride |
| DMAP | 4-dimethylaminopyridine |
| DMDO | dimethyl dioxirane |
| DMEM | Dulbecco's Modified Eagle Medium (culture medium) |
| DMF | dimethylformamide |
| DMP | Dess-Martin periodinane |
| DMS | dimethyl sulfide |
| DMSO | dimethyl sulfoxide |
| DNA | deoxyribonucleic acid |
| $EC_{50}$ | effective concentration (required to induce a 50% effect) |
| ESI | electrospray ionisation |
| Et | ethyl |

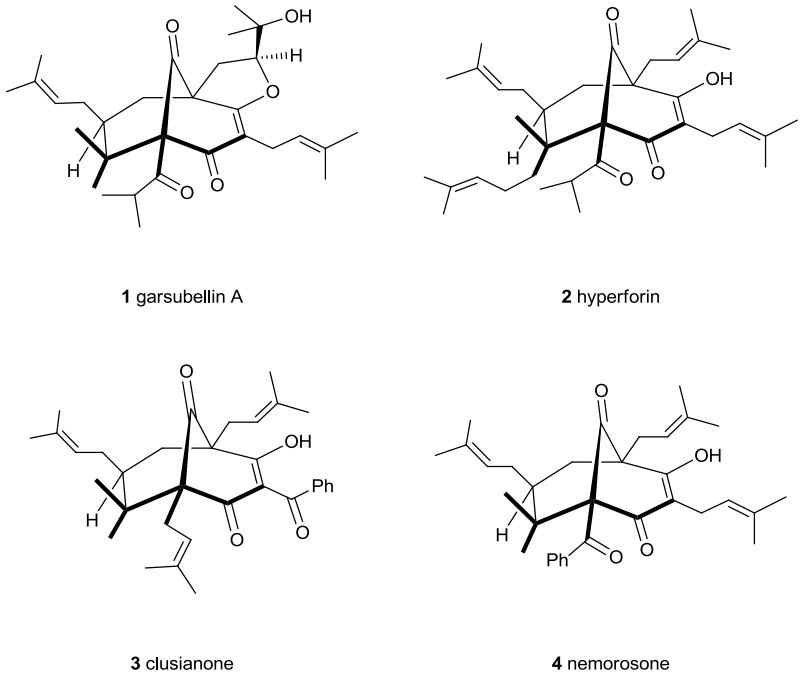
| | |
|---|---|
| ER | endoplasmic reticulum |
| FLICA | fluorochrome inhibitor of caspases |
| G0 phase | quiescent cells |
| G1 phase | gap 1 – growth phase |
| G2 phase | gap 2 – growth phase |
| GADD45α | growth arrest and DNA-damage-inducible protein |
| HMBC | heteronuclear multiple bond correlations |
| HMDS | hexamethyldisilazane |
| HMPA | hexamethylphosphoramide |
| HPLC | high performance liquid chromatography |
| HRMS | high resolution mass spectrometry |
| $IC_{50}$ | concentration of inhibitor required for 50% inhibition of target |
| IR | infra-red |
| $LC_{50}$ | (Lethal Concentration 50) is the concentration of a chemical which kills 50% of a sample population |
| LDA | lithium diisopropylamide |
| LiTMP | lithium tetramethylpiperidide |
| *m*-CPBA | *meta*-chloroperoxybenzoic acid |
| MDR | multi-drug resistant |
| MIC | minimum inhibitory concentration |
| MOM | methoxymethyl ether |
| mRNA | messenger ribonucleic acid |
| MS | mass spectrometry |
| Ms | mesyl |
| NBO | natural bond orbital |
| NBS | *N*-bromosuccinimide |

| | |
|---|---|
| NMR | nuclear magnetic resonance spectroscopy |
| nOe | nuclear Overhauser effect |
| NOESY | nuclear Overhauser effect spectroscopy |
| PCC | pyridinium chlorochromate |
| Ph | phenyl |
| p21 | cyclin-dependent kinase inhibitor 1 |
| PPAPs | polycyclic polyprenylated acylphloroglucinols |
| ppm | parts per million |
| Pr | propyl |
| PTSA | *para*-toluene sulphonic acid |
| Rb | retinoblastoma protein |
| $R_f$ | retention factor |
| RNA | ribonucleic acid |
| rt | room temperature |
| S phase | synthesis phase |
| SAR | structure-activity relationship |
| siRNA | silencing ribonucleic acid (double-stranded) |
| TBAF | tetrabutylammonium fluoride |
| TBS | *tert*-butyldimethylsilyl |
| TBDPS | *tert*-butyldiphenylsilyl |
| Tf | trifluoromethanesulfonyl |
| THF | tetrahydrofuran |
| TIPS | triisopropylsilyl |
| TLC | thin layer chromatography |
| TMS | trimethylsilyl |

# Chapter 1

# Polycyclic Polyprenylated Acylphloroglucinols

Recently, there has been much interest in the natural products from the plants and trees of the *Clusiaceae* (Guttiferae) family, particularly the polycyclic polyprenylated acylphloroglucinols (PPAPs), due to their varied and significant biological activity.[1] This activity, combined with their characteristic bicyclo[3.3.1]nonane-trione core, bearing additional acyl and prenyl substituents make PPAPs a challenging and interesting target for the synthetic chemist. Examples that have received recent attention include garsubellin A (**1**), hyperforin (**2**), clusianone (**3**) and nemorosone (**4**).



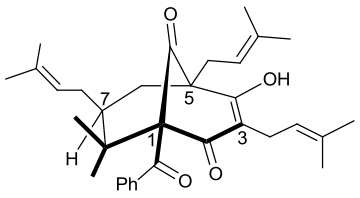

**1** garsubellin A **2** hyperforin

**3** clusianone **4** nemorosone

**Figure 1.0.1**

## 1.1 Introduction to PPAPs

The bridged bicyclic core is common to all the PPAPs, most have a bicyclo[3.3.1]nonane core although a few have a bicyclo[3.2.1]octane core. In some of these compounds further cyclisation takes place between the ß-diketone and the pendant olefinic groups, forming secondary ring systems on the bicyclic core, e.g. garsubellin A (**1**) (Figure 1.0.1). Numbering of the ring system has varied between publications but here the numbering will start with the bridgehead carbon next to the quaternary centre, which is commonly a *geminal* dimethyl, being denoted as C-1 (Figure 1.1.1).




**4** nemorosone

**Figure 1.1.1**

A recent review by Grossman collated all the isolations of PPAPs, which resulted in data on 119 individual species, although this number has grown considerably since the review was published.[1-5] Grossman organised them into three subgroups based on the position of the acyl group in relation to the quaternary C-8 centre (Figure 1.1.2).

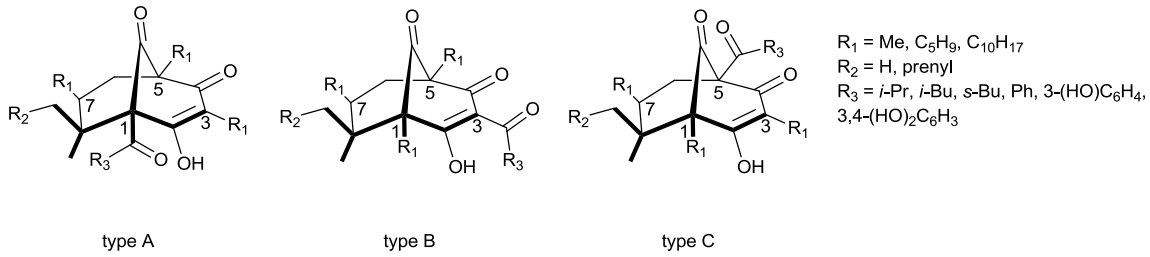



**Figure 1.1.2**

Type A structures, such as nemorosone (**4**), are the most common, with 80 known structures. These are characterised by having the acyl group at the C-1 bridgehead position adjacent to the C-8 quaternary centre. Type B structures, such as clusianone (**3**), are approximately half as prevalent as type A structures, and have the characteristic acyl group at the C-3 vinylic position. Type C is the least common of the sub groups with only three known compounds in the class. It is possible that these are mis-assignments of other products, as the corresponding type A analogues have already been isolated from the same plant source. Type C structures have the acyl group in the C-5 position, at the bridgehead position distal to the quaternary centre at C-8. Other variations in the groups of the PPAP family include extended alkyl chains at the C-8 *geminal* dimethyl position and compounds epimeric at the C-7 position.

PPAPs can often be found in propolis (a resinous substance) which is produced from plants and buds by honey bees.[6] Recent studies into Cuban propolis have divided them into three groups: brown, red and yellow. Brown propolis was found to be rich in PPAPs, particularly nemorosone (**4**). The wide ranging bioactivity of propolis, much of which is attributed to the PPAP content, led to its use as a health drink and

in various creams and gels. An extensive database on the toxicological and biological activity of propolis indicates that it may be effective against bacteria, fungal infections, viruses and tumours. More specific health effects attributed to the propolis drink include prevention of heart disease, inflammation, diabetes and cancer.[7]

## 1.2 Medicinal Properties of PPAPs

PPAPs have been used as a treatment of many ailments since 300 BC, as either an extract direct from the plant or as a propolis extract.[7] The most common use of a PPAP as a medicinal remedy is the use of St. John's Wort; a "herbal" remedy used in the treatment of mild depression and skin injuries, the active ingredient of which is hyperforin (**2**).[8-9] Initially it was the antibacterial effects that were of interest but hyperforin's (**2**) major anti-depressant effects in St. John's Wort have become a leading area of interest. Subsequent studies have been performed to discover if there is any activity in other areas, especially due to its low human toxicity and pharmacokinetic profile. So far only two biological targets of hyperforin (**2**) have been discovered, a neuronal $[Na^+]/[Ca^{2+}]$ exchanger and the pregnane X-receptors.[10-12] These activities appear to be involved in the anti-depressant effects as well as unfavourable drug interactions. Other interesting activity includes action against cancer cell lines,[13] viral strains, Alzheimer's disease,[14] HIV[15] and well documented antidepressant effects.[16] More recently hyperforin (**2**) has been discovered to have anti malarial properties.[17] In vitro studies have shown an $IC_{50}$ of 1.5 μM against antiplasmodial effects of *Plasmodium falciparum*. Further studies into

the SAR were also performed and the activity was shown not to be dependent on the phenol like part or the prenyl double bonds of hyperforin (**2**).

Clusianone (**3**) has been known since 1976 and recent screening has ascribed potent anti-HIV and cancer chemopreventive properties.[15,19] Against HIV infection in C8166, clusianone (**3**) showed a 0.02 μM $EC_{50}$ value.

Garsubellin A (**1**) has shown positive activity against Alzheimer's disease.[14] Treatment with a 10 μM concentration of garsubellin A (**1**) produced an increase of 154% of acetylcholine in P10 rat septal neuron cultures. Some anti-inflammatory properties have also been attributed to garsubellin A (**1**) including inhibition of β-glucuronidase and histamine at $IC_{50}$ = 15.6 μM.[19]

Nemorosone (**4**) displays a range of biological activities which are discussed in Chapters 2 and 4.

Various other PPAPs (Figure 1.2.1) have shown biological activity and are summarised in Table 1.2.1.

| PPAP | Disease | Cell Line | Activities |
|---|---|---|---|
| Propolone A (**5**) | HIV | C8166 human T lymphoblastoid cells | $EC_{50}$ = 0.32 μM[15] |
| 7-*epi*-clusianone (**6**) | HIV | C8166 human T lymphoblastoid cells | $EC_{50}$ = 2.0 μM[15] |
| 7-*epi*-clusianone (**6**) | Chagas' disease | Trypanosoma cruzi | $LC_{50}$ = 260 μM[20] |
| Hyperibone J (**7**) | Cancer | Breast and lung | $IC_{50}$ = 17.8 and >20 mM[21] |
| Papuaforins A-E (**8-12**) | Cervical cancer | KB cell line | $IC_{50}$ = 4.9-13.0 mM |
| Garcinol (**13**) | Antibiotic | Methicillin-resistant *S. Aureus* | MIC = 3-12 μg/ml[22] |
| Garcinol (**13**) | | Topoisomerases I and II | $IC_{50}$ = 43 and 55 mM[23] |
| Xanthochymol (**14**) | Antibiotic | Methicillin-resistant *S. Aureus* | MIC = 6-25 μg/mL[22] |
| Xanthochymol (**14**) | | Topoisomerases I and II | $IC_{50}$ = 33 and 40 mM[23] |
| Guttiferone F (**15**) | HIV-1 | HIV-1 | $EC_{50}$ 23 μg/mL[24] |

**Table 1.2.1**

As can be seen from the range of activities shown in the Table 1.2.1 above the PPAPs have demonstrated biological properties in many of the key areas of current pharmacological research. By looking at the compounds that are effective against the various diseases it becomes obvious that both type A and type B PPAPs are potential drug candidates. Even the more elaborate PPAPs show biological activity, suggesting that this class of compounds have a lot to offer in future drug development.

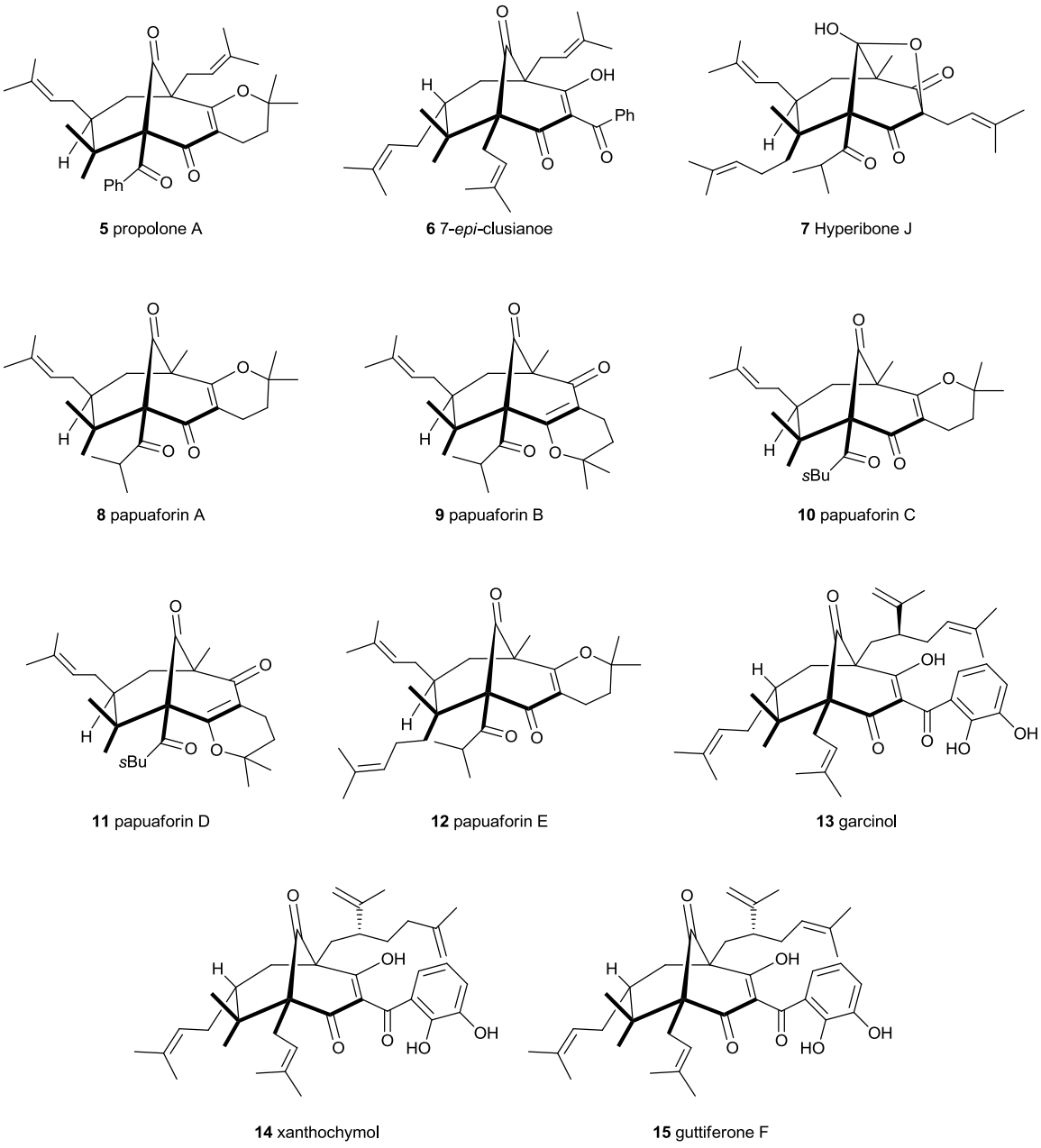
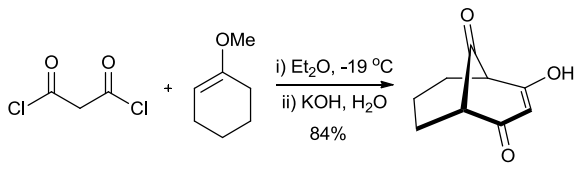

5 propolone A
6 7-epi-clusianoe
7 Hyperibone J
8 papuaforin A
9 papuaforin B
10 papuaforin C
11 papuaforin D
12 papuaforin E
13 garcinol
14 xanthochymol
15 guttiferone F

**Figure 1.2.1**

## 1.3 Review of Previous Synthetic Work Towards the Bicyclo[3.3.1]nonane Core

Although the PPAP class of compounds have attracted much synthetic and biological attention, very few total syntheses have been completed due to the complexity of forming the bicyclo[3.3.1]nonane-trione core with the correct positioning of the substituents. As a result, much of the synthetic work has been in constructing the bicyclo[3.3.1]nonane core of these molecules. Among the notable achievements in this area are the works of Spessard and Stoltz,[25] K. C. Nicolaou,[26] G. A. Kraus,[27-28] R. Grossman,[29] G. Mehta and M. K. Bera,[30-31, 34] R. Takagai,[32] and J.P. Sleeman.[13] In fact only clusianone (**3**), garsubellin A (**1**) and more recently nemorosone (**4**) have succumbed to synthesis (Sections 1.4 and 1.5).

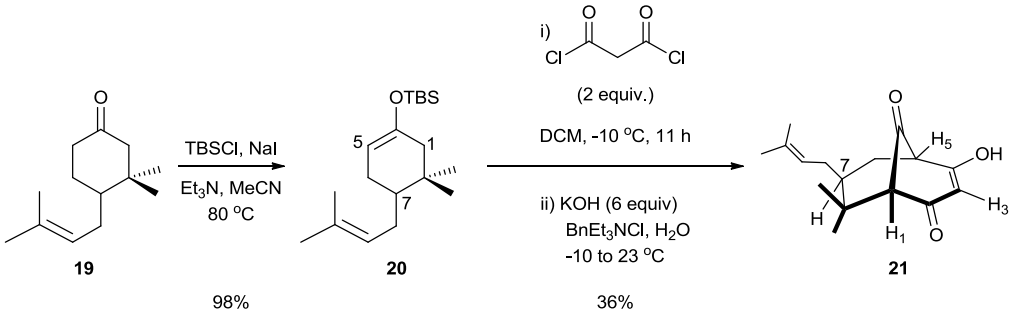
Early 2002 saw Spessard and Stoltz's diastereoselective synthesis of the bicyclo[3.3.1]nonane core (Scheme 1.3.1).[25] Their work used a modified version of the Effenberger cyclisation as the key bicyclo[3.3.1]nonane forming reaction, a reaction which would become widely applied to synthetic approaches in this field.[33] Effenberger had shown that it was possible to form the bicyclo system **18** in good yields (84%) using an excess of enol ether **17** with malonyl dichloride (**16**) (Scheme 1.3.1).




16 17 18

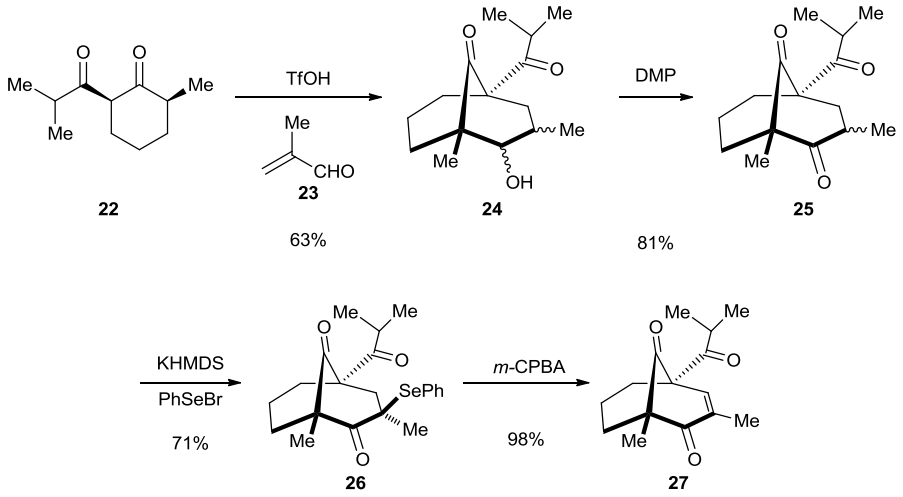
**Scheme 1.3.1**

For PPAP synthesis using an excess of valuable enol ether was not favourable, and therefore Spessard and Stoltz modified the procedure to use only one equivalent of enol ether **20** (Scheme 1.3.2). However, the isolated yields of bicyclo[3.3.1]nonane core **21** were modest, at only 36%. Fortunately, the ketone **19** was recovered alongside the bicyclo[3.3.1]nonane system **21**, which provided 95% yield based on recovered ketone **19**. By recycling the recovered ketone **19** a yield of 55% of the bicyclo[3.3.1]nonane-trione **21** was achieved over two cycles. Crucially the cyclisation had been completed diastereoselectively and only the anti-isomer at C-7 was formed, which would become advantageous for syntheses completed later by other groups.



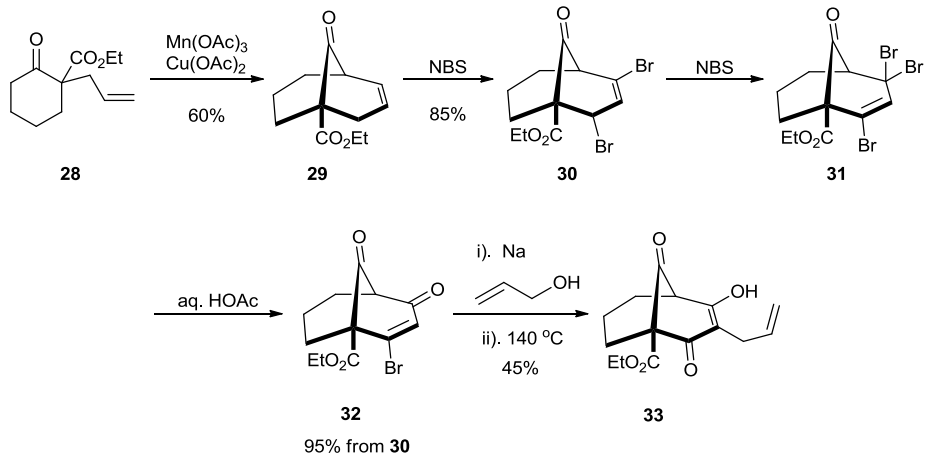

**Scheme 1.3.2**

Work by the Nicolaou group led to another approach to synthesising the bicyclo[3.3.1]nonane core.[26] This was performed via annulation of a diketone **22** with an α,β-unsaturated aldehyde **23**, followed by oxidation of the secondary alcohol **24**. Treatment with KHMDS and PhSeBr followed by oxidation with *m*-CPBA and subsequent elimination gave enone **27** (Scheme 1.3.3).



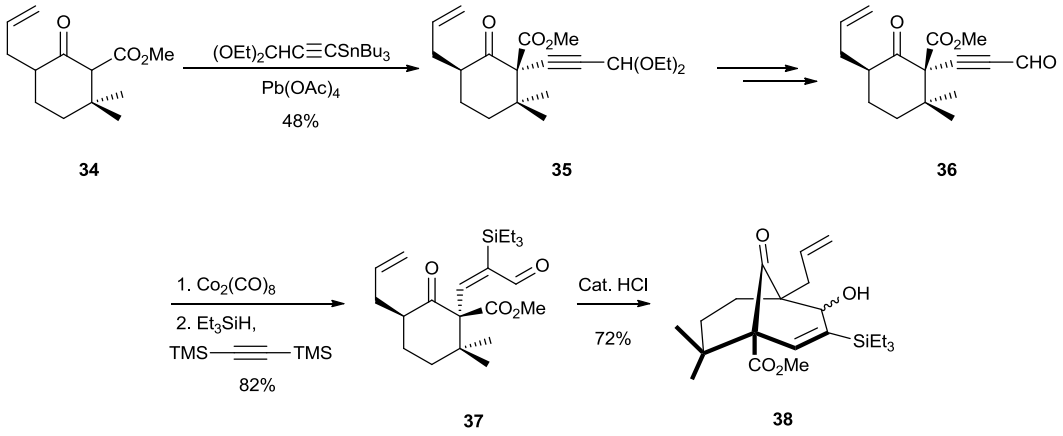

**Scheme 1.3.3**

George Kraus and his team have shown that it is possible to make the bicyclo[3.3.1]nonane core using a manganese mediated cyclisation (Scheme 1.3.4).[27-28] Starting with keto ester **28** which underwent intramolecular cyclisation using manganese acetate and copper acetate the bicyclo[3.3.1]nonane **29** was formed. Treatment of intermediate **29** with NBS led to the dibromide intermediate **30** (Scheme 1.3.4). Further addition of NBS gave the tribromide **31** which was transformed into **32** using aqueous acetic acid at 115 °C. The sodium salt of allyl alcohol underwent 1,4-addition followed by a Claisen rearrangement to provide C-3 allylated bicyclo[3.3.1]nonane core **33**.



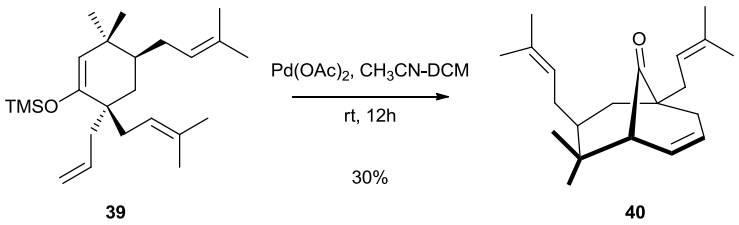

**Scheme 1.3.4**

Grossman produced his intermediate **38** through the use of a three carbon α,α'-annulation of a sterically hindered cyclic β-keto ester **34** with alkynylation using tributylstannylated 3,3-diethoxypropyne. This was followed by *syn* reduction with $Co_2(CO)_8$ and $Et_3SiH$ and an intramolecular aldol to give the bicyclo[3.3.1]nonane **38** (Scheme 1.3.5).[29]



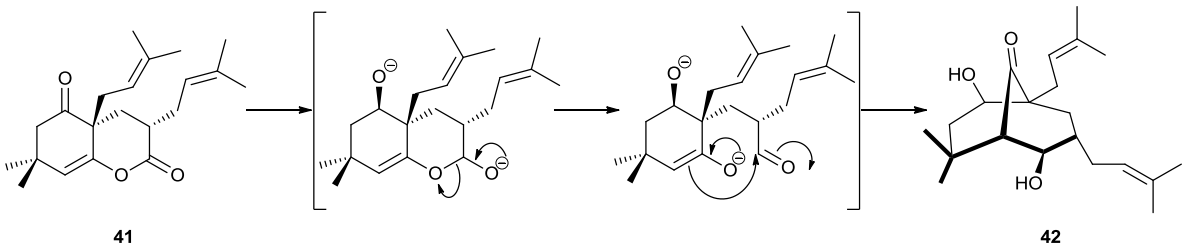

**Scheme 1.3.5**

Mehta and Bera have explored two different approaches to the bicyclo[3.3.1]nonane core structure, initially using a palladium catalysed Kende cyclisation (Scheme 1.3.6).[30-31,34]



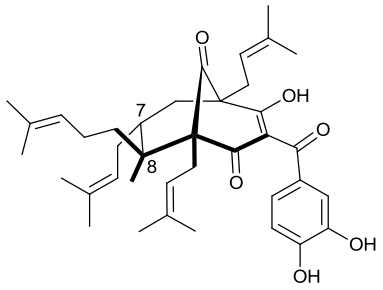

**Scheme 1.3.6**

In later work they have used a DIBAL-H mediated reduction of a ring fused lactone to generate a metallated lactol that can fragment and then undergo intramolecular aldol reaction, much as in the Nicolaou example (Scheme 1.3.7).

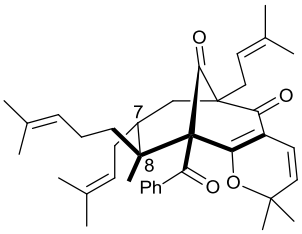



**Scheme 1.3.7**

Having performed a proof of concept in 2006, the Mehta group took their DIBAL-H reduction methodology and applied it to the syntheses of the bicyclo[3.3.1]nonane core of guttiferone A (**43**) and hypersampsone F (**44**) (Figure 1.3.1).[34] They were able to selectively construct products with the correct stereochemistry at the C-7 and C-8 positions.



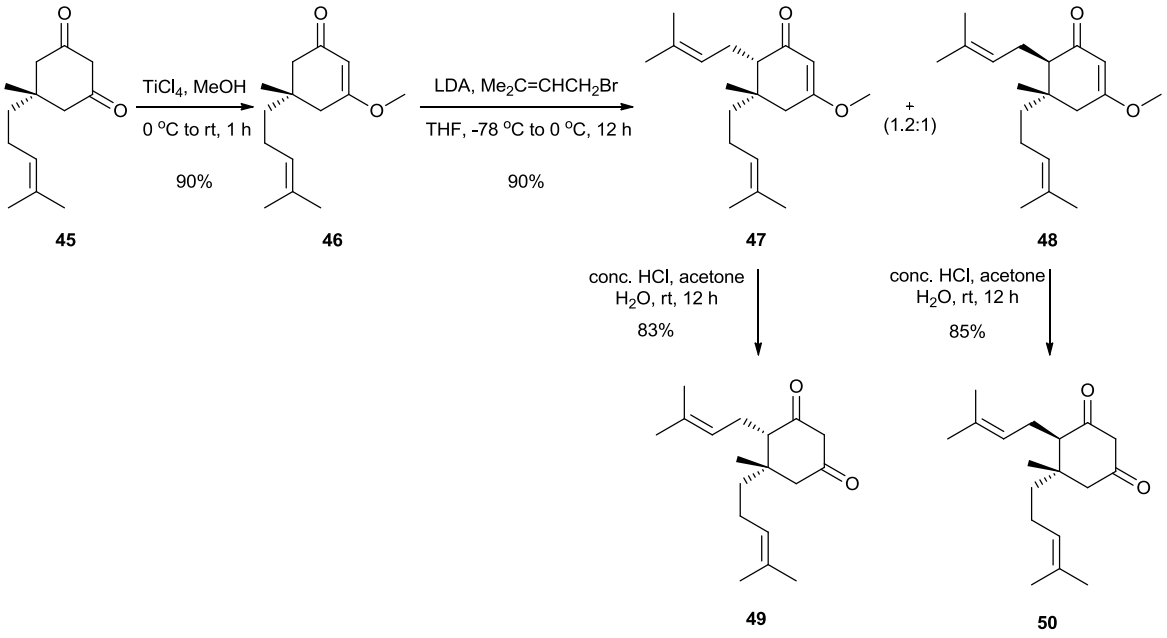
**43** guttiferone A



**44** hypersampsone F

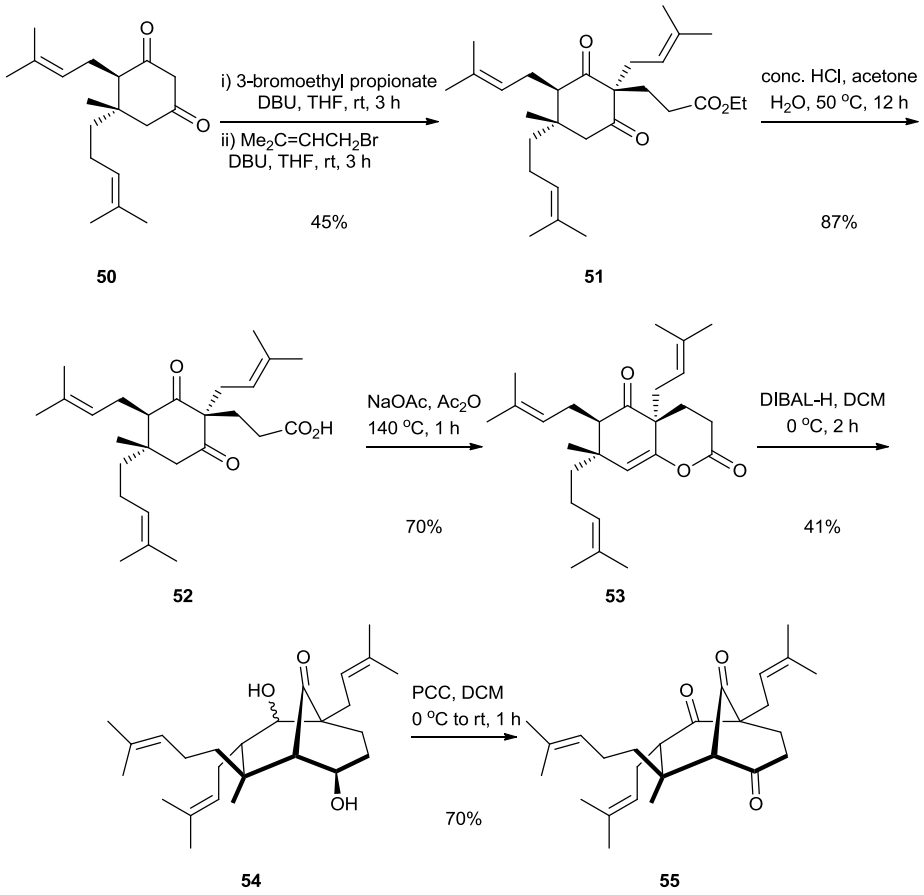
**Figure 1.3.1**

The natural product targets both contain an *endo* C-7 prenyl group and an *exo* homoprenyl C-8 group. Therefore, the Mehta group synthesised 1,3-diketone **52** with the correct substitution pattern that they could submit to their DIBAL-H rearrangement conditions (Scheme 1.3.8).




**Scheme 1.3.8**

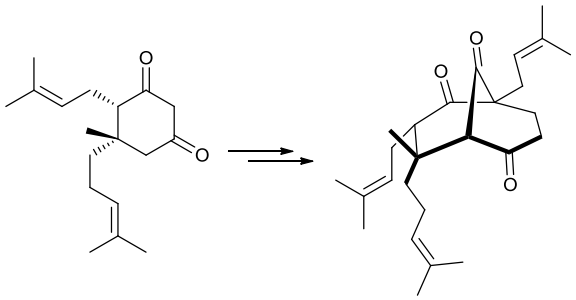
Readily available 1,3-diketone **45** was easily methylated to give the methyl enol ether **46**. Further prenylation was performed using kinetic enolate conditions to give a diastereomeric mixture of enol ethers **47** and **48** which were separated and hydrolysed to their respective 1,3-diketones **49** and **50** using concentrated HCl. Initially only the 1,3-diketone **50** was further functionalised before being submitted to the DIBAL-H reduction conditions (Scheme 1.3.9).




**Scheme 1.3.9**

Successive DBU promoted alkylations were carried out in one pot using 3-bromoethyl propionate and prenyl bromide to give the single diastereomer **51**. This pentalkylated diketone was hydrolysed using concentrated HCl to give the carboxylic

acid **52**. Lactonisation took place to form the enol lactone **53** which would be subjected to the rearrangement conditions the group had described in previous papers. The rearrangement took place as shown previously in Scheme 1.3.7 in the presence of DIBAL-H and gave the desired bicyclo[3.3.1]nonane **54** with concurrent reduction of the ketone carbonyl. PPC oxidation of the diol gave the bicyclo[3.3.1]nonane core structure **55** with the correct configurations at C-7 and C-8 for the natural products guttiferone A (**43**) and hypersampsone F (**44**). Attention then turned to the 1,3-diketone **49** and the bicyclo system it would form under these conditions (Scheme 1.3.10).

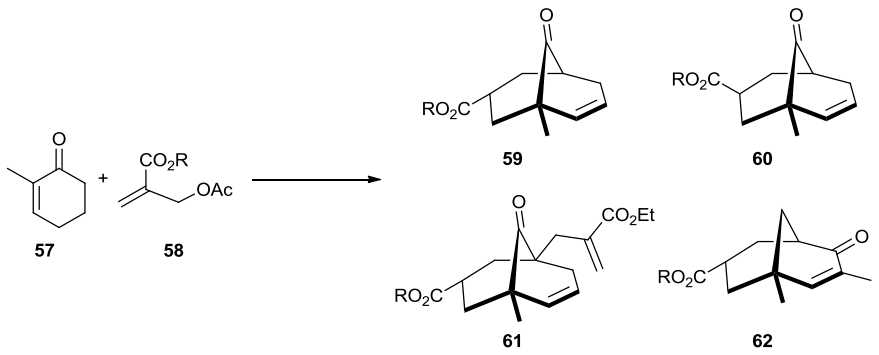


**49** **56**

**Scheme 1.3.10**

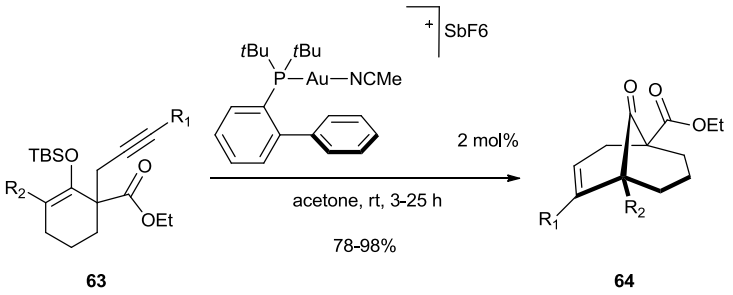
By applying the same conditions as above to 1,3-diketone **49** it was possible to form the bicyclo[3.3.1]nonane system **56** in which both C-7 and C-8 were *endo*. This stereochemical arrangement has yet to be encountered in the natural PPAPs.

Takagi showed that a mixture of bicyclo[3.3.1]nonane core structures can be made through annulation of 2-cyclohexanone (**57**) with acrylate **58** by successive Michael reactions (Scheme 1.3.11).[32]




**Scheme 1.3.11**

The Barriault group have published a gold catalysed method for producing bicyclo[3.3.1]nonane systems.[35] They envisaged a gold catalysed 6-*endo-dig* cyclisation of a triple bond containing cyclic silyl enol ether **63**. The gold complex of the silyl enol ether is available to both 6-*exo* and 6-*endo-dig* cyclisations but by adjusting the conditions, it was possible to make the reaction 6-*endo-dig* selective (Scheme 1.3.12).

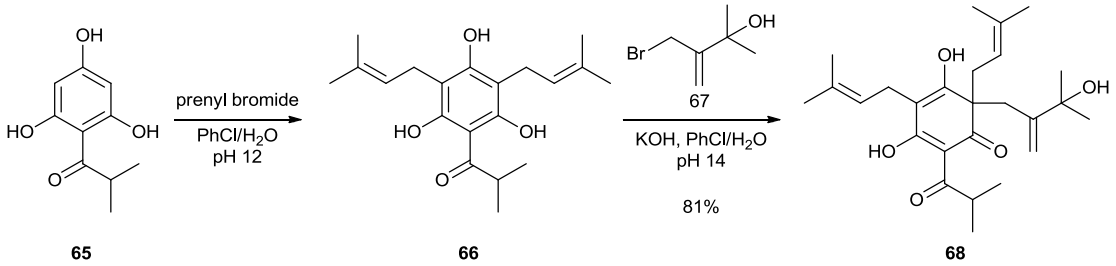



**Scheme 1.3.12**

By using this method, the Barriault group were able to access a range of different bicyclo[3.3.1]nonane systems, some with substituents applicable to natural product synthesis. This route offered quick access to the bicyclo[3.3.1]nonane core but

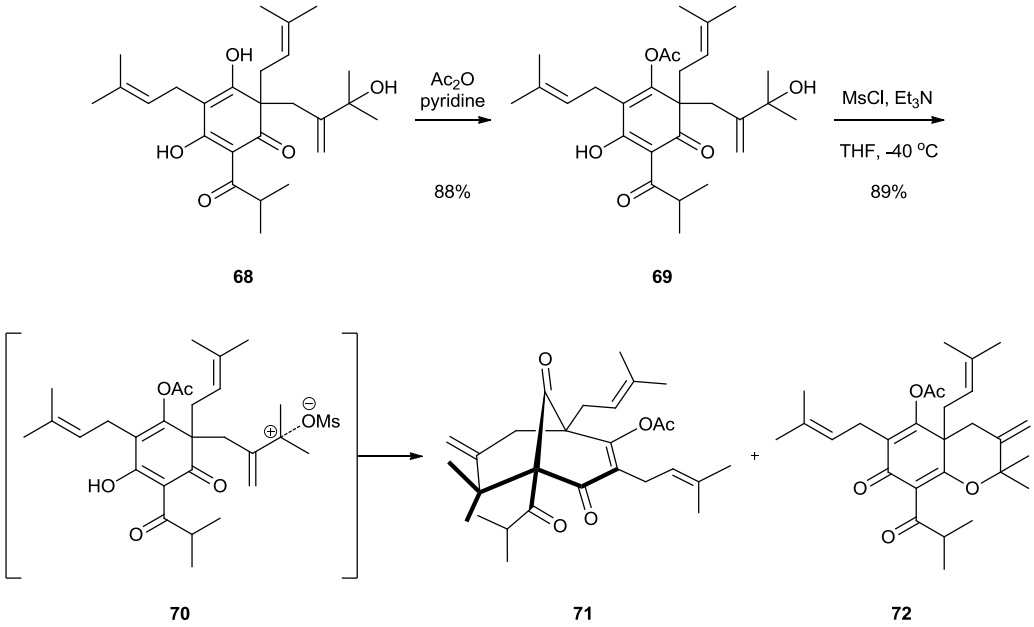
needs further introduction of the oxygen functionalities before a complete PPAP will be realised.

The Couladouros group in Greece have published a route to the type A PPAP bicyclo[3.3.1]nonane core using a *C*-alkylation, cation cyclisation method (Scheme 1.3.13).[36] Starting from an acylphloroglucinol **65**, a series of analogues of colupulone were formed by diprenylation of **65** and subsequent reaction with bromide **67**.




**Scheme 1.3.13**

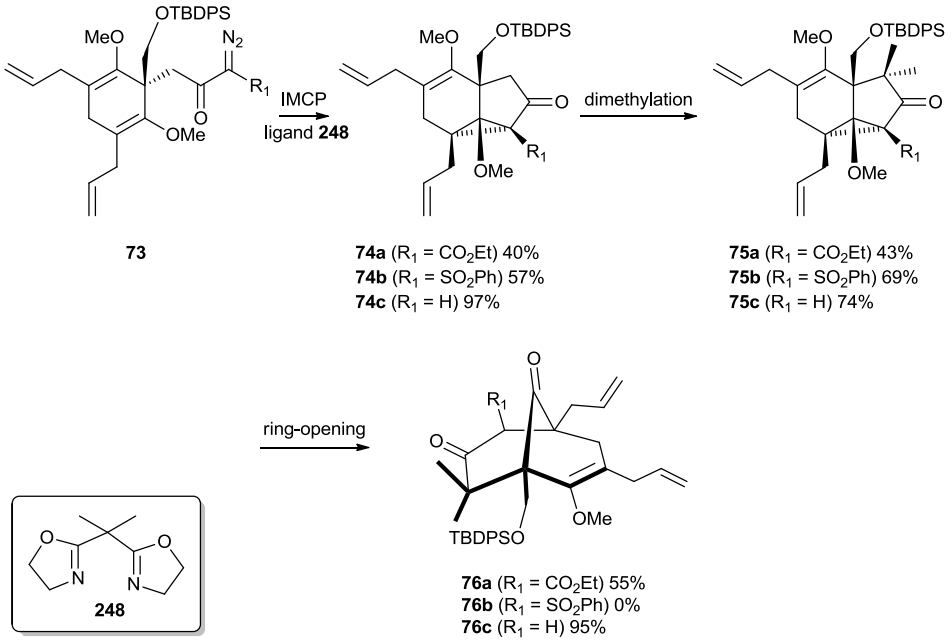
The colupulone analogue **68** was selectively acylated before being subjected to conditions for the cation cyclisation. The cation proved elusive under conditions of direct cation formation with the use of acids. However, it was possible to make the methanesulphonic ester **70** which underwent unexpected and direct transformation to the bicyclo[3.3.1]nonane **71** (Scheme 1.3.14).




**Scheme 1.3.14**

A by-product **72** resulting from *O*-cyclisation was also isolated but this route still offers a quick way of making PPAPs with all of the quaternary centres and oxygen functionalities.

The Nakada group have studied a route to nemorosone (**4**) using an enantioselective intramolecular cyclopropanation.[37] Starting with three different α-diazo-β-keto esters (**74a-74c**) with different substitution at $R_1$, they aimed to select the route with the highest yields of the desired bicyclo[3.3.1]nonane system (**76a-76c**) (Scheme 1.3.15).




**73**

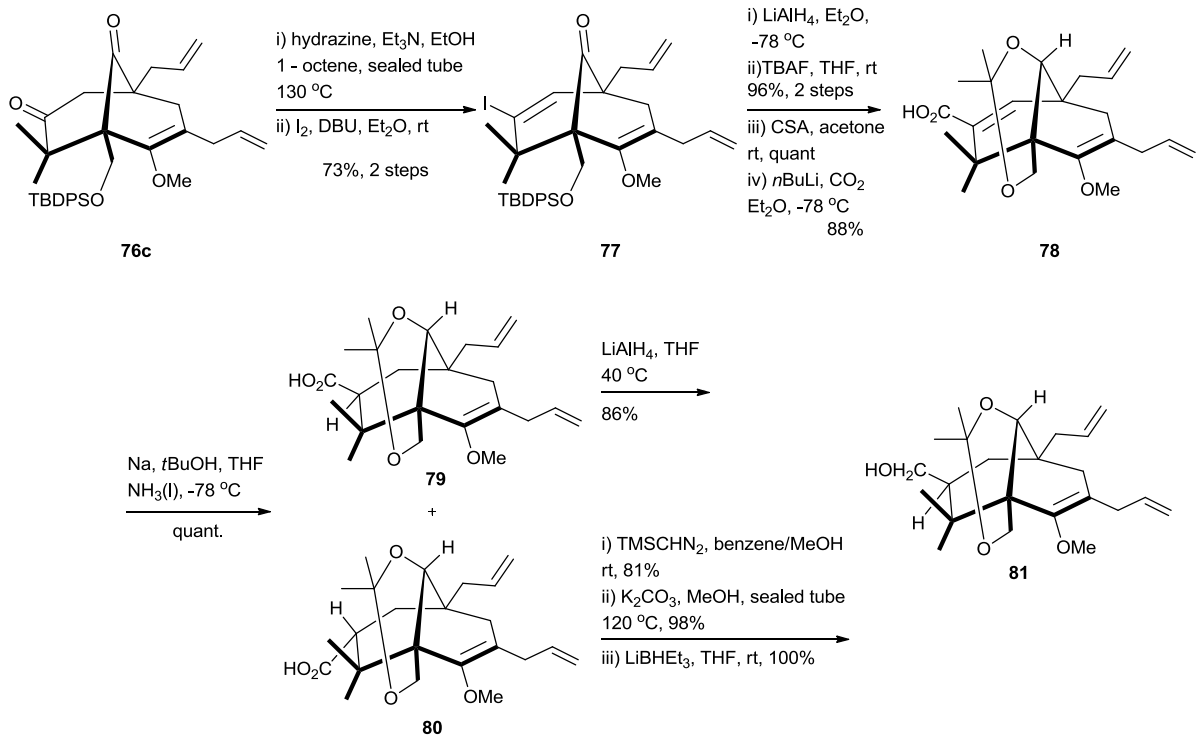
**74a** ($R_1$ = $CO_2Et$) 40%
**74b** ($R_1$ = $SO_2Ph$) 57%
**74c** ($R_1$ = H) 97%

**75a** ($R_1$ = $CO_2Et$) 43%
**75b** ($R_1$ = $SO_2Ph$) 69%
**75c** ($R_1$ = H) 74%

**76a** ($R_1$ = $CO_2Et$) 55%
**76b** ($R_1$ = $SO_2Ph$) 0%
**76c** ($R_1$ = H) 95%

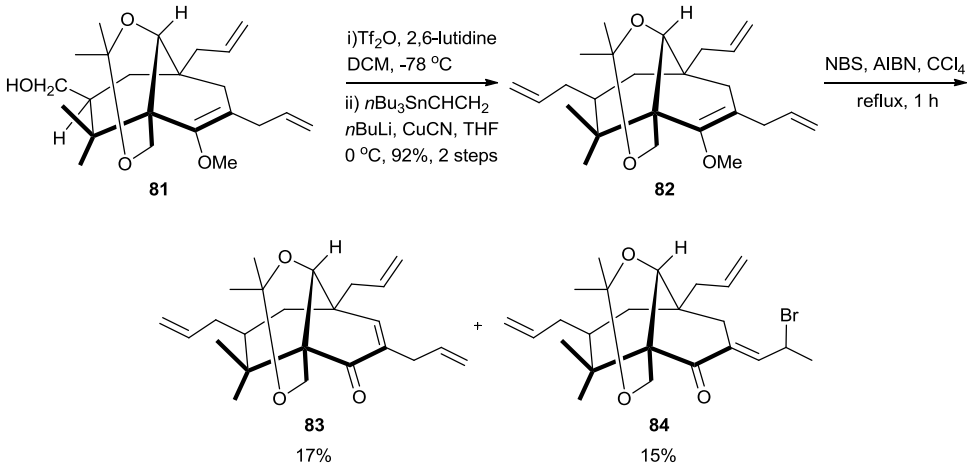
**Scheme 1.3.15**

By optimising the conditions for each of the reactions, they were able to access the bicyclo[3.3.1]nonane **76c** system in good yields for the successive intramolecular cyclopropanation, dimethylation and ring opening (Scheme 1.3.16). The bicyclo[3.3.1]nonane **76c** was successfully converted into the iodide **77** via a hydrazone under Barton's conditions. This was converted into the carboxylic acid **78** by reduction from the least hindered side with $LiAlH_4$, removal of the TBDPS group, acetonide formation of the resultant diol, lithiation via halogen-metal exchange and trapping with $CO_2$. Birch reduction gave the mixture of diastereomeric acids **79** and **80** which could be transformed into the alcohol **81**.




**Scheme 1.3.16**

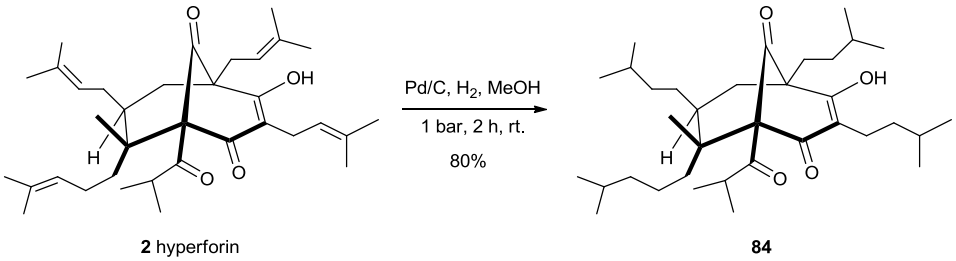
The resulting alcohol **81** was converted to the triflate and coupled with divinylcuprate to give the triallylated compound **82**. Although discovering further transformations were limited, a further reaction was performed with NBS under conditions of allylic bromination to give a mixture of compounds **83** and **84** (Scheme 1.3.17).




**Scheme 1.3.17**

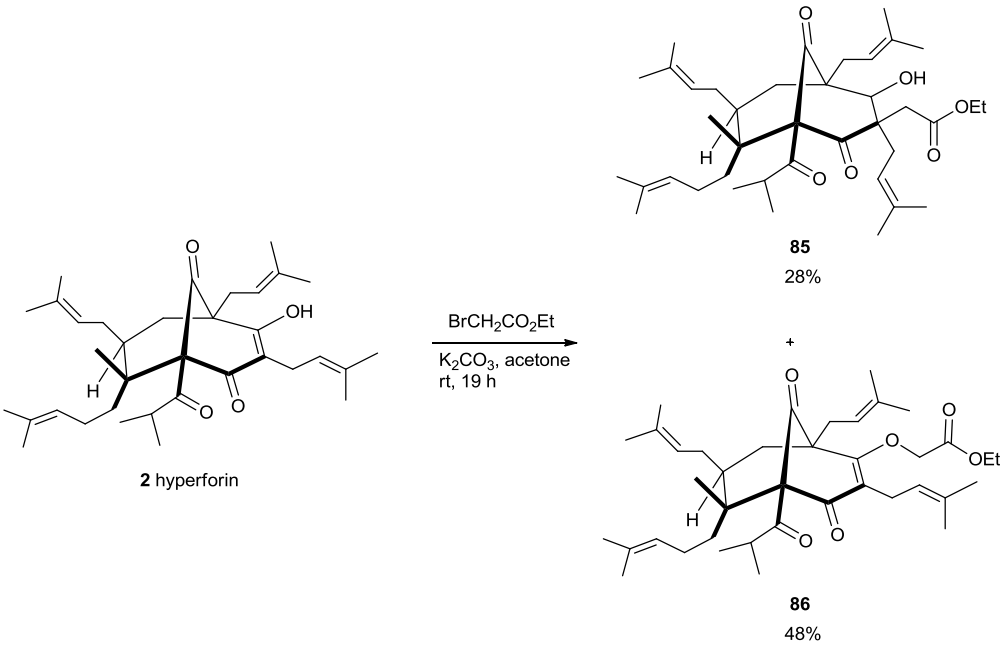
Intermediate **83** has the correct stereogenic substitution for conversion to nemorosone (**4**), however the route is overly long and contains many low yielding steps.

A final strategy that has been used for synthesis of analogues of the PPAP structure was developed by Sleeman, who performed various transformations directly on hyperforin (**2**) to make analogues.[13] By using $H_{2\ (g)}$ with a Pd/C catalyst they were able to hydrogenate the alkenes of the prenyl chains to make derivative **84** (Scheme 1.3.18).



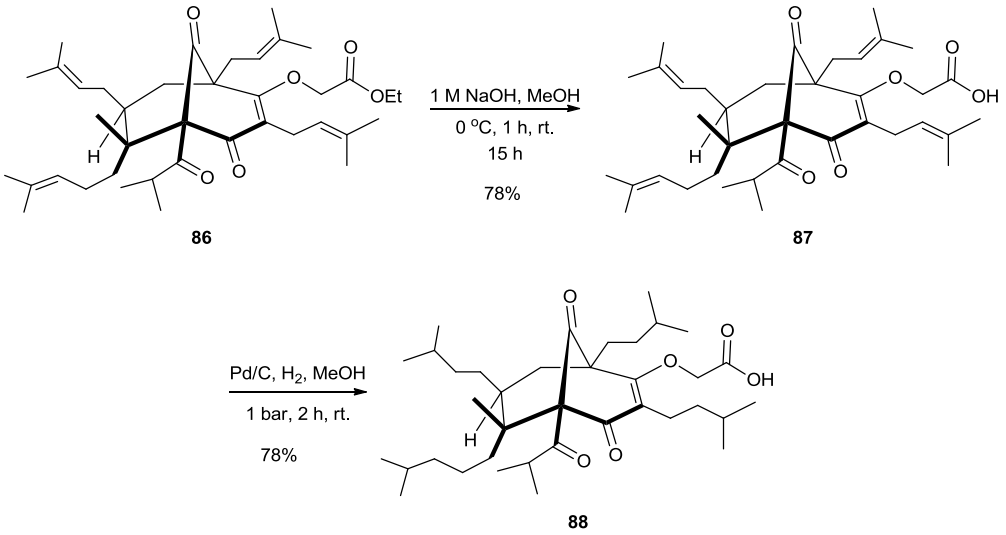

**Scheme 1.3.18**

In another approach to derivatisation, hyperforin (**2**) was alkylated using bromoacetate. In this way the *C*-alkylated **85** and the *O*-alkylated derivative **86** were synthesised. The *O*-alkylated derivative **86** was then taken on for further transformations (Scheme 1.3.19).




**Scheme 1.3.19**

The ester **86** was cleaved using basic conditions to give the synthetic hyperforin derivative aristoforin (**87**), which was further transformed by hydrogenating the double bonds to give fully saturated **88** (Scheme 1.3.20). The solubility and stability of the range of derivatives were then investigated as well as their activity as an anti cancer agent. The group discovered that aristoforin (**87**) was more stable and soluble than hyperforin (**2**) but offered the same anti tumour properties.

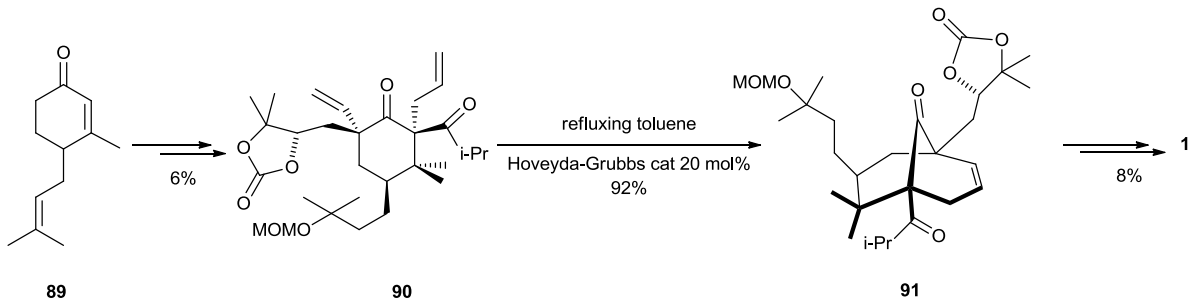



**Scheme 1.3.20**

## 1.4 Review of Total Syntheses

Very few of the 119 known PPAPs have been synthesised, due to the inherent complexity of the bicyclo[3.3.1]nonane core and the difficulty of carrying bridgehead components through key cyclisations. The appendage of C-1, C-3 and C-5 groups at a late stage of the synthesis, particularly at bridgehead positions, also poses a problem to the synthetic chemist. Late 2005 saw the first syntheses of garsubellin A (**1**) published, independently performed by the groups of Shibasaki and Danishefsky.[38-39] The Shibasaki group managed the first synthesis of garsubellin A (**1**) through a mechanism utilising ring closing metathesis as the key bicyclo[3.3.1]nonane forming cyclisation (Scheme 1.4.1). Through a series of enolate reactions, protections, deprotections and aldol reactions the intermediate **90** was constructed in 13 steps and 6% yield. Ring closing metathesis was affected with Hoveyda-Grubbs catalyst to give the heavily functionalised bicyclo[3.3.1]nonane

system **91**. The resulting alkene was oxidised at the allylic position with $(PhSe)_2$ and $PhIO_2$. Both protecting groups were removed and the alcohol and enone were condensed via an intramolecular Wacker oxidation. Iodination at the vinylic position, dehydration of the tertiary alcohol and Stille coupling with prenyltributyltin gave racemic garsubellin A (**1**) in 21 steps and a 4% overall yield.

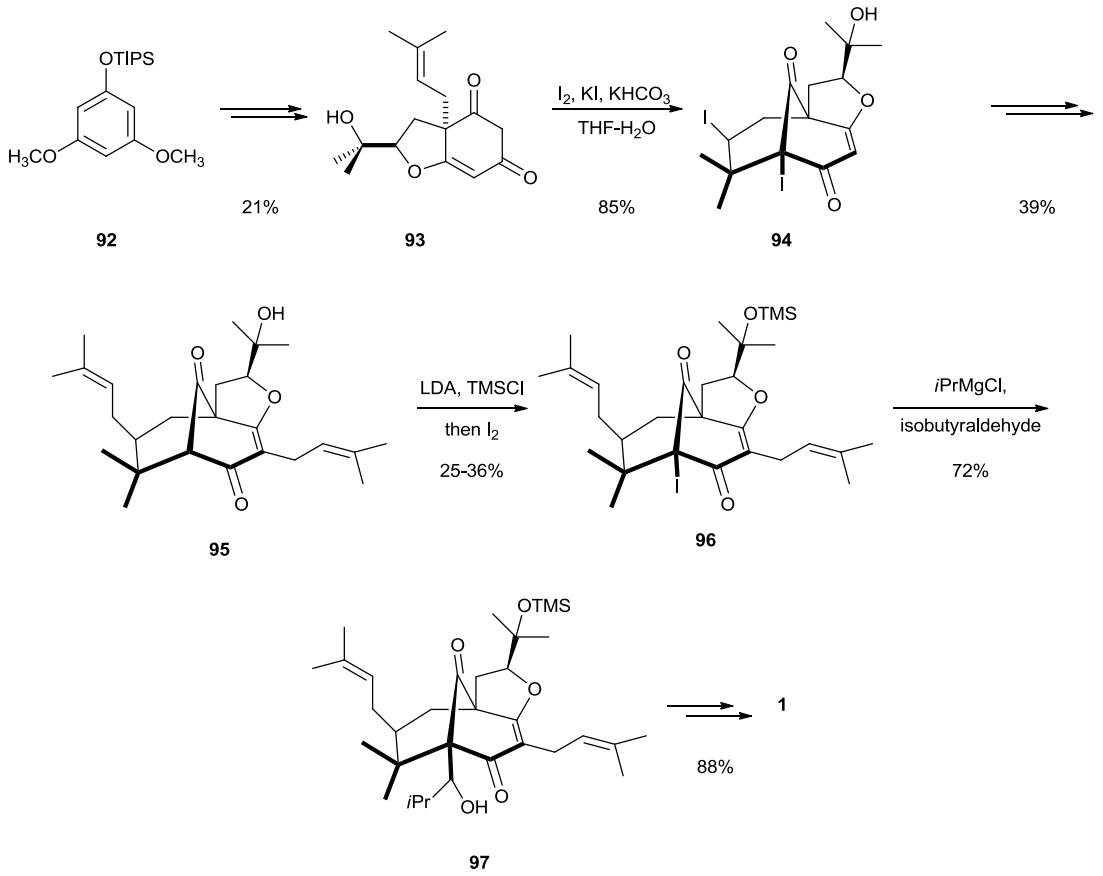



89 90 91

**Scheme 1.4.1**

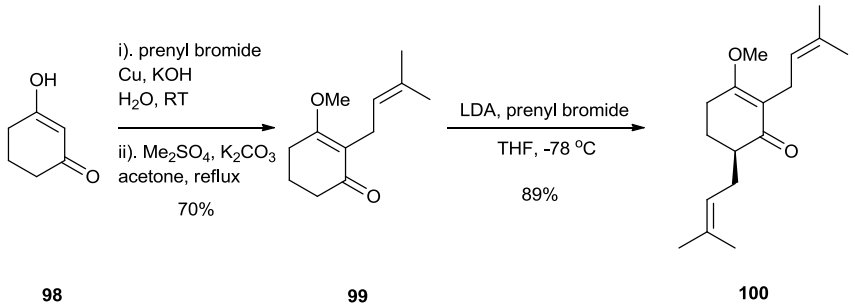
Danishefsky's approach used an iodocarbocyclisation in the key bicyclo[3.3.1]nonane forming reaction, from **93** to **94** (Scheme 1.4.2). The iodocarbocyclisation was used by Danishefsky later in the clusianone (**3**) and nemorosone (**4**) syntheses and will be discussed later in Chapter 2. With bicyclo[3.3.1]nonane **95** in hand an intriguing C-1 bridgehead iodination was performed. By using $I_2$ as the electrophile and performing a TMS protection of the alcohol concurrently it was possible to substitute at the sterically hindered C-1 position. Halogen-metal exchange was used to form the C-1 isopropyl alcohol **97**. Although, Danishefsky chooses not to discuss this transformation in this publication it provides a manner in which to substitute the C-1 position and would play a vital role in our nemorosone (**4**) synthesis (Chapters 2 and 5). Completion of the synthesis was performed using a DMP oxidation of the

isopropyl alcohol and a fluoride cleavage of the TMS ether to yield garsubellin A (**1**) in 15 steps and 1% overall yield.



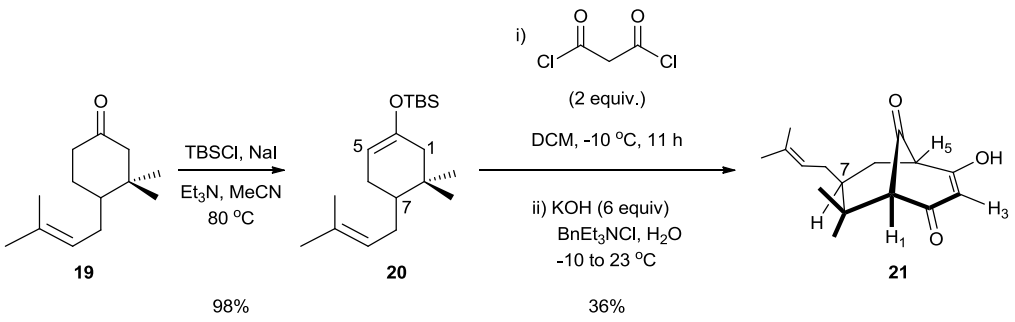

**Scheme 1.4.2**

The first syntheses of clusianone (**3**) have been performed by our group (see Section 1.5), using an Effenberger cyclisation,[40-41] as well as more recently, by the Marazano group in France using a similar pathway.[42] Both syntheses started from cyclohexane-1,3-dione (**98**), which undergoes a series of deprotonations and electrophilic quenches to install the substituents around the cyclohexanone ring (Scheme 1.4.3).




**Scheme 1.4.3**

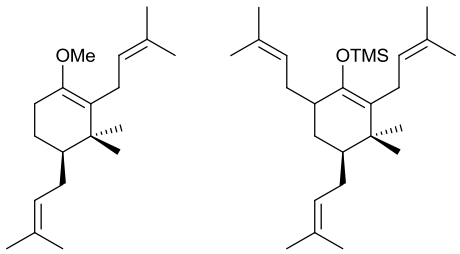
The key bicyclo[3.3.1]nonane forming step in both these syntheses was the cyclisation step following the Effenberger reaction. This is the reaction of an enol ether with malonyl dichloride as pioneered in PPAP synthesis by Spessard and Stoltz (Scheme 1.4.4). [25,33]




**Scheme 1.4.4**

The syntheses differ in the extent of the substitution of the enol ether intermediate that enters the Effenberger reaction. Our synthesis used an enol ether intermediate **101** that was not fully substituted and a series of reactions at the bridgehead C-5 and vinylic C-3 position were employed to complete the synthesis in nine steps with a 12% overall yield. The Marazano group used a fully substituted intermediate **102** in

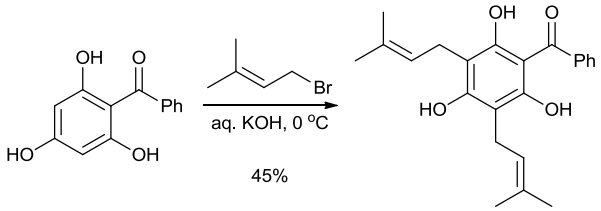
the Effenberger cyclisation which led to clusianone (**3**) as the product in 7 steps and a 14% overall yield (Figure 1.4.1)




**101** **102**

**Figure 1.4.1**

An alternative route to clusianone (**3**) was demonstrated by Porco using an alkylative dearomatisation–annulation.[43] The synthesis commenced with *C*-prenylation of acylphloroglucinol **103** with prenyl bromide under basic conditions to give clusiaphenone B (**104**) in modest yield (Scheme 1.4.5).

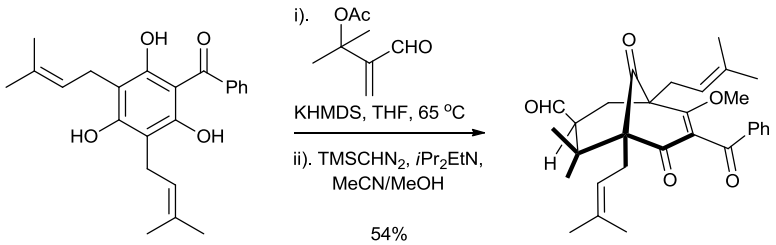



**103** **104**

**Scheme 1.4.5**

To install an aldehyde that could be further functionalised to form the C-7 prenyl group and invoke the annulation, α-acetoxy enal was used with KHMDS (Scheme 1.4.6). The methyl vinylogous ester **105** was synthesised so that the annulation product could be isolated and characterised. The methyl vinylogous ester

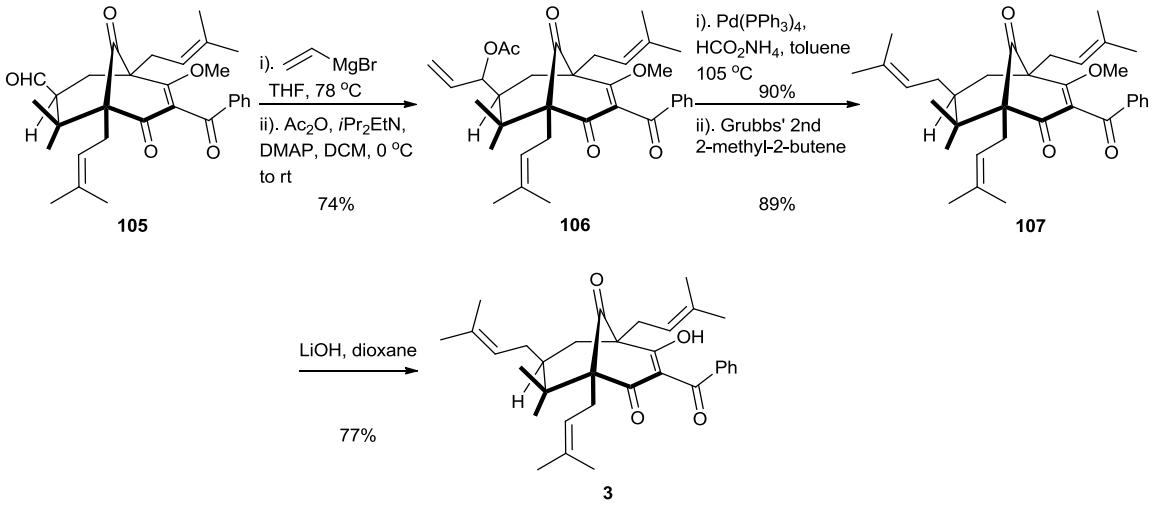
was isolated as a mixture of diastereoisomers, both of which were progressed through the synthesis.




104 105

**Scheme 1.4.6**

With the bicyclo[3.3.1]nonane system synthesised in good yield it remained to convert the C-7 position into the desired prenyl group and hydrolyse the vinylogous ester. Reaction of the aldehyde **105** with vinyl magnesium bromide gave the secondary alcohol that was converted to the acetate **106** (Scheme 1.4.7). The acetate was removed with a palladium catalysed formate reduction which was followed by olefin cross-metathesis with Grubbs' second generation catalyst which gave *O*-methylated clusianone (**107**).

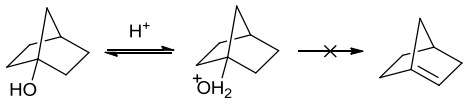



**Scheme 1.4.7**

Hydrolysis of the methyl vinylogous ester was performed using LiOH in dioxane and afforded clusianone (**3**) in good yield. The complete synthesis gave clusianone (**3**) in eight steps and 11% overall yield and offers an alternative to the Effenberger annulation for PPAP synthesis.

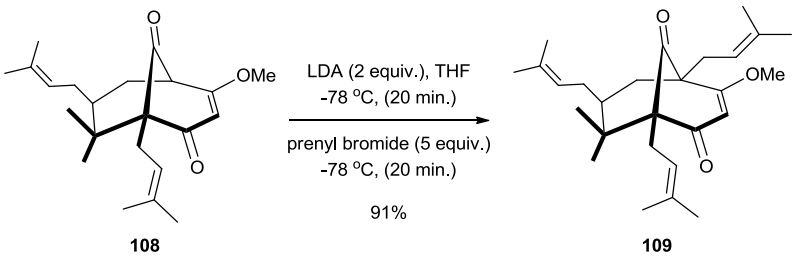
## 1.5 Previous Research by Our Group

Our group have worked in the area of bridgehead functionalisation for some time. Our work with forming bicyclo[3.3.1]nonane bridgehead enolates and effectively quenching them with a variety of electrophiles opened up work on PPAPs as a target driven test of this chemistry.[44] Bridgehead lithiation at this position should be forbidden due to Bredt's rule, "the high strain of planar bridgehead carbons means that double bonds can rarely be formed at the bridgehead carbons in bicyclic systems" (Scheme 1.5.1).[45]




**Scheme 1.5.1**

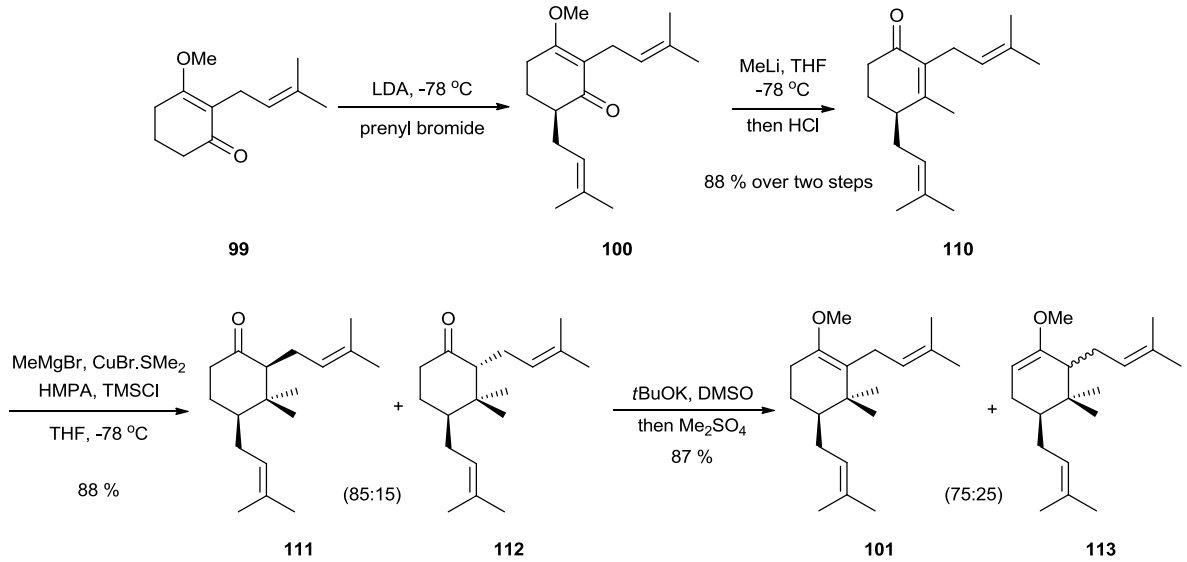
However, our group have previously shown that it is possible to deprotonate at the bridgehead and perform an electrophilic quench when there is a carbonyl functionality α- to the bridgehead.[44,46] This allows the formation of a "bridgehead enolate", with the use of a lithium amide base to deprotonate and an external quench with the desired electrophile enables substitution of the bridgehead position (Scheme 1.5.2).




**Scheme 1.5.2**

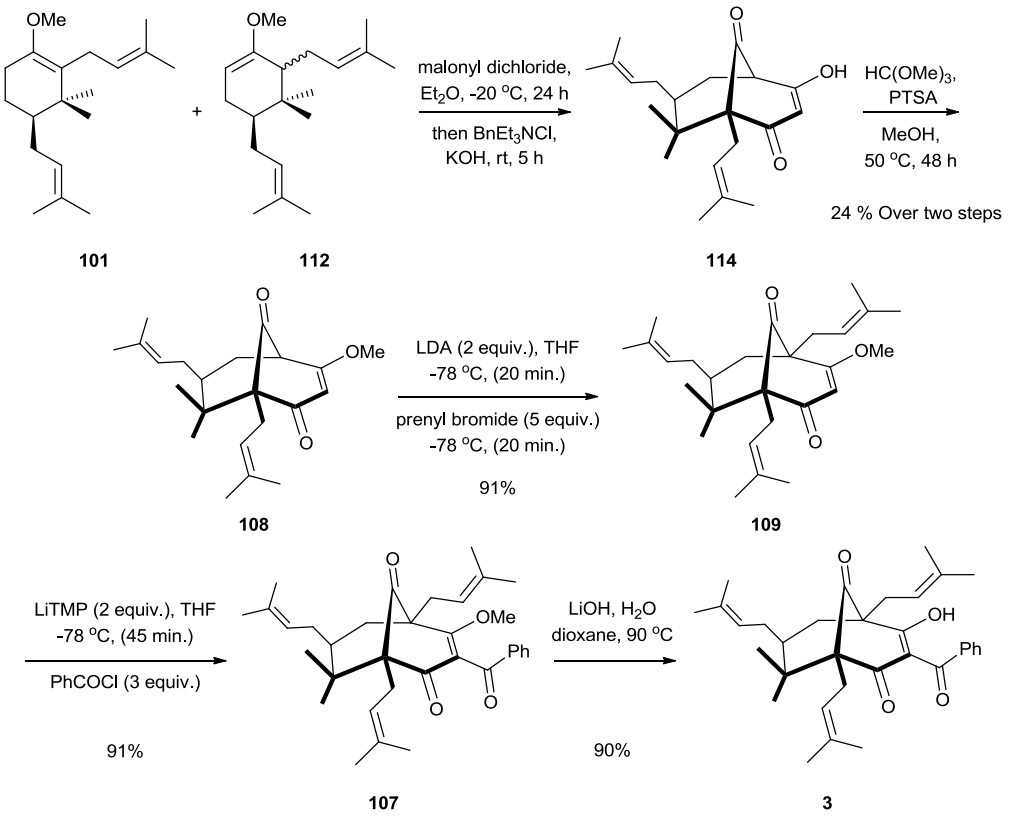
Our group published our first paper of specific PPAP synthetic work in 2006 with the total synthesis of clusianone (**3**).[40-41,47] Having observed the Spessard and Stoltz modification of the Effenberger annulations and its quick access to the core bicyclo[3.3.1]nonane, but also noting that it is limited further by addition of α or α' substituents on the starting enol ether which left the need for bridgehead substitution. However, having already shown that it was possible to append various groups via bridgehead lithiation-substitution, accessing PPAPs by an Effenberger route would

be a suitable showcase for these ideas. The idea of constructing unnatural derivatives or natural PPAPs from one core structure is an attractive one, as it opens the door to quick access to many compounds that could be used to probe SAR in the PPAP systems. Synthesis of the core by Effenberger cyclisation allows for this. With this in mind, suitable enol ethers **101** and **113** were synthesised from known methyl enol ether **99** in four steps (Scheme 1.5.3).




**Scheme 1.5.3**

Considering the substitution at the C-1 bridgehead as an extreme test of the bridgehead enolate methodology, an enol ether was formed with the prerequisite C-1 prenyl group. This allowed the C-1 bridgehead prenyl group to be carried through the formation of the bicyclo[3.3.1]nonane core. With the bicyclo[3.3.1]nonane system formed using the modified Effenberger annulations, the C-5 bridgehead prenyl and the C-3 benzoyl group were installed using deprotonation chemistry (Scheme 1.5.4).

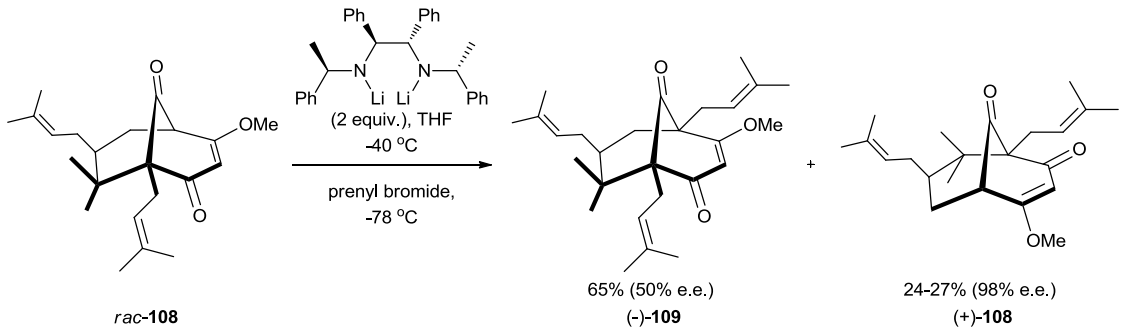



**Scheme 1.5.4**

The Effenberger cyclisation gave low yields as expected with 24% of the *O*-methylated bicyclo[3.3.1]nonane **108** being isolated. However, if the recovered ketones **111** and **112** are included then the process is actually 60% efficient. Bridgehead alkylation proved to be a highly effective reaction, yielding 91% of the C-5 prenylated product **109**. The acylation of C-3 position was equally efficient under LiTMP deprotonation and benzoyl chloride quench. The synthesis of racemic clusianone (**3**) achieved a 12% overall yield over nine steps, with spectroscopic data that matched the reported data of the natural material.

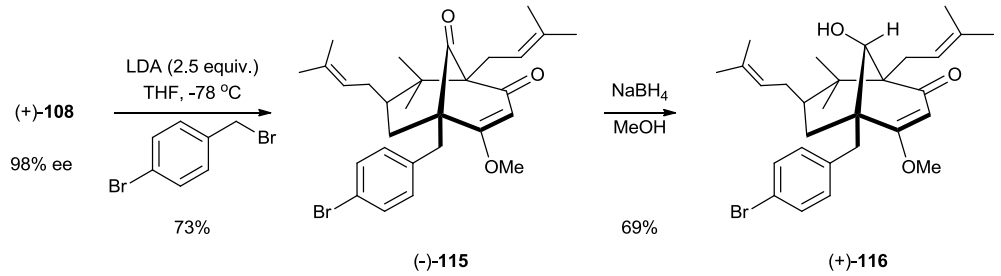
Having established an effective route to clusianone (**3**), a kinetic resolution was employed to separate the enantiomers of a late stage intermediate **108**. These could

then be taken on to synthesise clusianone's (**3**) enantiomers separately and therefore assign the absolute configuration of the natural material.[41] It was noted that only three members of the PPAP family have had their absolute configurations experimentally determined. Hyperforin (**2**) had been determined using X-ray crystallography on an enol ether derivative and it is this configuration in which PPAPs are most commonly presented. The kinetic resolution was possible by using a bis lithium amide base for metalation and quenching with prenyl bromide. This gave the prenylated product (-)-**109** in 65% yield and 50% e.e., starting material was recovered as (+)-**108** in 24-27% yield and in >98% e.e. (Scheme 1.5.5).



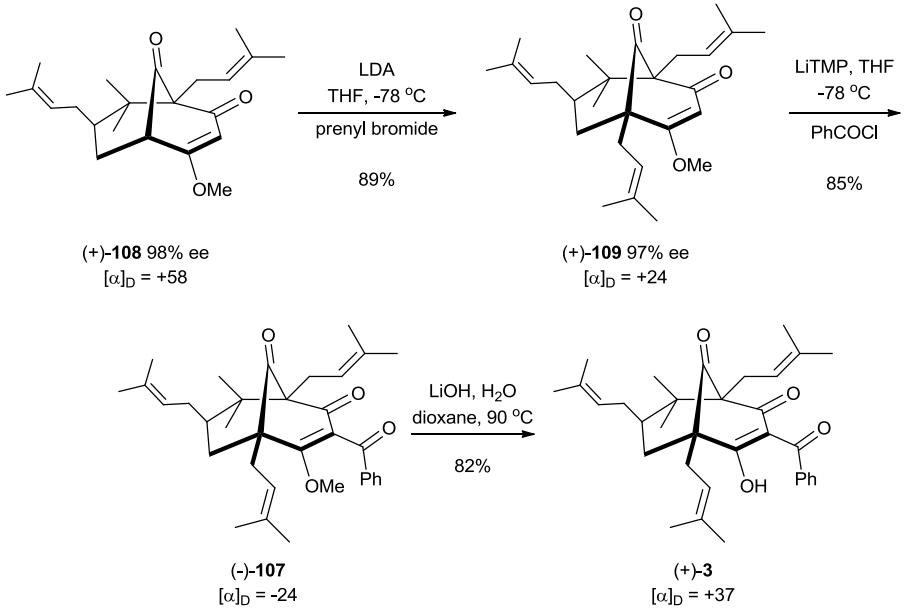

**Scheme 1.5.5**

With the enantiomerically enriched material it was possible to append a benzoyl bromide to the C-5 bridgehead using LDA and *p*-bromobenzyl bromide to give (-)-**115**. This was reduced using $NaBH_4$ to give (+)-**116** which was suitable for X-ray crystallography allowing the absolute configuration to be assigned (Scheme 1.5.6).




**Scheme 1.5.6**

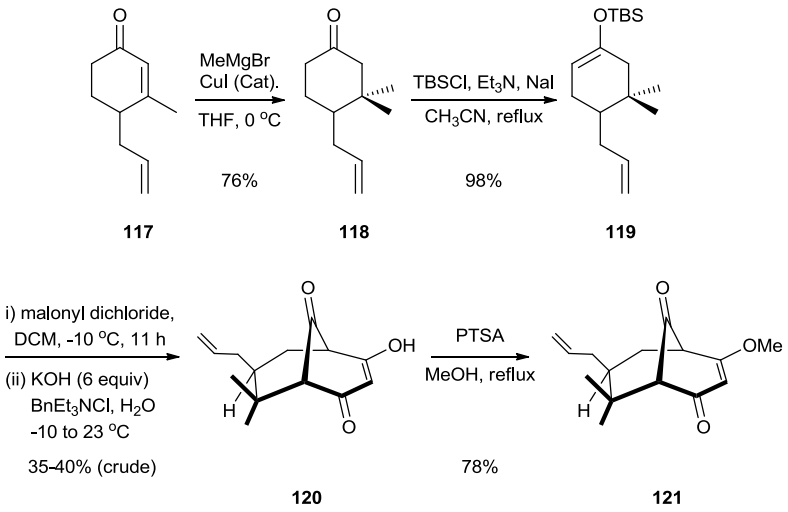
With the absolute configuration of (+)-**116** assigned, it was possible to assign the absolute configurations of **115** and **108**. To establish the absolute configuration of clusianone (**3**), intermediate (+)-**108** was converted into the natural product (Scheme 1.5.7). In this way it was possible to assign the absolute configuration of (+) isomer of clusianone (**3**).




**Scheme 1.5.7**

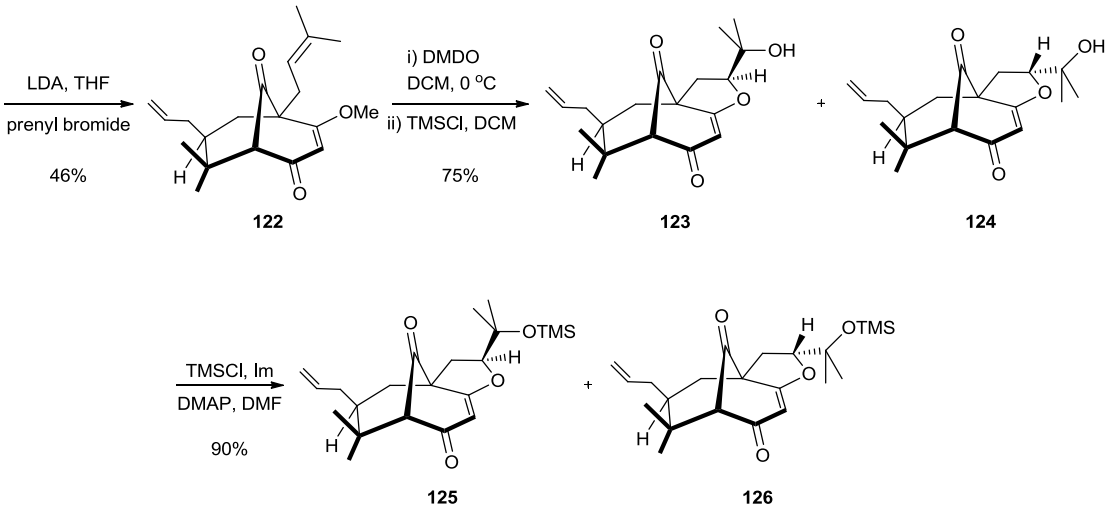
Comparing the optical rotations shows that the (+) configuration is that of the clusianone (**3**) isolated from *Clusia torresii*.[18] Data from another paper reports an extract from various sources was analysed as the *O*-alkylated derivative but showed (+) rotation, the opposite of which had been found in the *Clusia torresii* sample. To establish that the data was correct, the (-) derivative **109** was converted to the *O*-methylated clusianone **107** and the NMR spectra matched. By comparing the optical rotation of this and the deprotected compound, it was shown that the optical rotation is inverted between *O*-methylated derivative and the final compound. This suggests that clusianone (**3**) can be isolated from natural sources as either enantiomer.

Alongside the synthesis of clusianone (**3**) a formal synthesis of garsubellin A (**1**) was completed by the group.[47] It was thought that the use of the Effenberger cyclisation would give rapid access to the tricyclic intermediate **128** of Danishefsky's garsubellin A (**1**) synthesis.[39] Starting with known enone **117**, conversion to ketone **118** was possible by copper catalysed Michael addition of MeMgBr. The ketone **118** was then effectively converted into the silyl enol ether **119** that would be used in the Effenberger cyclisation. The Effenberger cyclisation was conducted under our standard conditions of malonyl dichloride at -20 °C for 24 h followed by basic workup to afford the bicyclo[3.3.1]nonane system that was immediately protected as the methyl enol ether **121** under acidic conditions (Scheme 1.5.8).



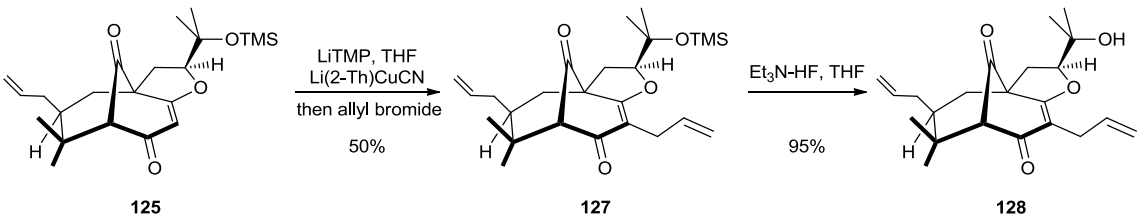

**Scheme 1.5.8**

Bridgehead prenylation of **121** proved to be less efficient than the bridgehead prenylations of the clusianone (**3**) synthesis. However, the difficulty in bridgehead substitution at the C-1 position and the relative sluggishness of C-3 prenylation when not in the presence of copper made it unlikely that competing reactions were taking place. No products were isolated with substitution at either the C-1 or C-3 positions. Stereocontrol of the oxidation of the bridgehead prenyl group for the THF ring had not been possible in this system. By using an epoxidation - epoxide opening strategy with a novel ring forming reaction using TMSCl, an efficient formation of a 1:1 mixture of diastereomers **123** and **124** was possible. These were then converted to the silyl enol ethers **125** and **126** under standard conditions (Scheme 1.5.9).




**Scheme 1.5.9**

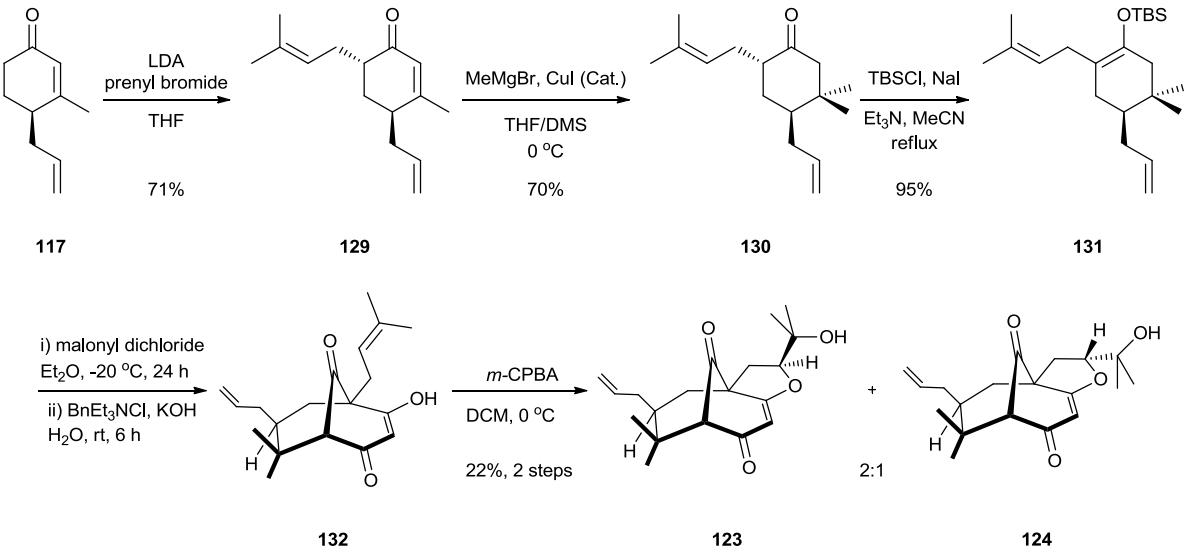
The correct isomer **125** was subjected to lithiation and allylation using the higher order cuprate conditions that had been described before giving a protected form of Danishefsky's intermediate **127**. Treatment with $Et_3N$-HF provided an identical compound to that of Danishefsky (**128**) (Scheme 1.5.10).




**Scheme 1.5.10**

Though it was step efficient, the above route produced poor yields at key steps and lacked stereoselectivity in the side chain oxidation. Therefore, an alternative route was devised. Starting from the same enone **117**, silyl enol ether **131** was prepared in a similar way to the previous enol ether **119**. Prenylation of enone **117** gave **129**,

which was methylated using the copper catalysed Grignard reaction to give ketone **130**. The ketone was efficiently converted to the silyl enol ether **131** and underwent cyclisation to the bicyclo[3.3.1]nonane **132** under the typical Effenberger conditions. Rather than purifying the unprotected 1,3-diketone at this stage, it was submitted to epoxidation with *m*-CPBA and led to the diastereomeric alcohols **123** and **124** but this time in a more favourable 2:1 ratio (Scheme 1.5.11).




**Scheme 1.5.11**

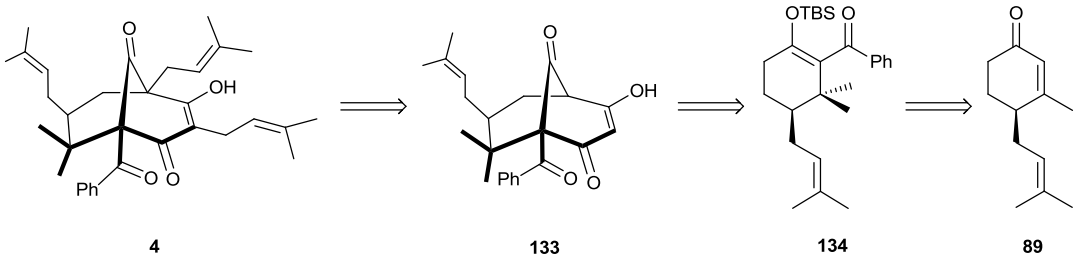
With this revised approach the modest bridgehead prenylation was avoided, as was methyl vinylogous ester formation. The epoxidation step also had an increase in its selectivity. Overall, this gave quite a step-efficient process, allowing rapid access to the advanced tricyclic structures **123** and **124**.

## 1.6 Nemorosone as a Target

Nemorosone (**4**) is the most prevalent PPAP found in the *Clusia rosea,* and a regioisomer of clusianone (**3**), with the acyl group at the C-1 bridgehead as opposed

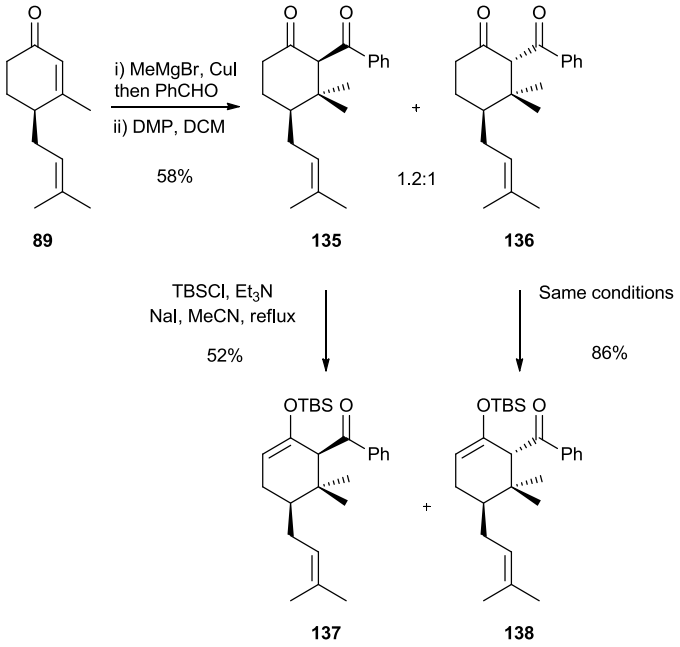
to the vinylic C-3 position. The first isolation was performed by C.M.A de Oliveira *et al.* in 1996 and they assigned nemorosone (**4**) with a type C structure. However, this was later revised by Cuesta-Rubio to the accepted type A structure (see Chapter 2 for more detail).[6,48-49] Nemorosone (**4**) has already shown some interesting biological effects, displaying antiseptic properties that have been known about since the time of Aristotle.[50] Studies into the antibacterial effects of nemorosone (**4**) and *O*-methylated nemorosone (**149)** have shown that the presence of the enone is important for the antibacterial effect, as is the prenylation pattern.[51] Alongside the antimicrobial activity, nemorosone (**4**) has been shown to have some cytotoxic and antioxidant effects. It showed 3.3-7.2 μM $IC_{50}$ values against four cancer cell lines, also targeting DNA topoisomerase and telomerase.[6] $EC_{50}$ values of 0.8 μM have been found against HIV infection of C8166 human T lymphoblastoid cells.[18] Alongside the activity against the HIV infection, nemorosone (**4**) was tested against uninfected cells to measure cytotoxicity, with a $TC_{50}$ of 5.0 μM being measured.

As our group had already synthesised clusianone (**3**), nemorosone (**4**) was to be synthesised using a similar procedure. Some preliminary studies had been performed by Dr. V. Rodeschini aiming to use the Effenberger cyclisation, **134** to **133**, for rapid access to the bicyclic core of nemorosone (**4**), followed by bridgehead enolate chemistry to substitute the C-5 position and more traditional deprotonation chemistry to substitute the C-3 (Scheme 1.6.1).



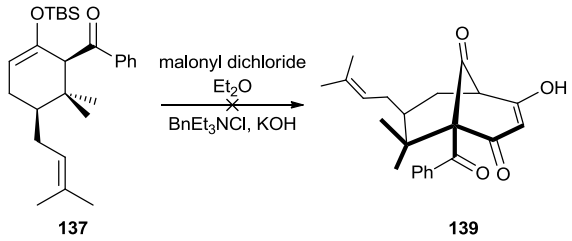

**Scheme 1.6.1**

The silyl enol ethers **137** and **138** could be made from known enone **134** through conjugate addition of methyl cuprate, quenching with benzaldehyde and DMP oxidation of the resulting alcohol. The silyl enol ethers **137** and **138** were formed using TBSCl under thermodynamic conditions and gave the nonconjugated enol silanes (Scheme 1.6.2). This was due to the steric interaction of the benzoyl group and the *geminal*-dimethyl and would result in noncoplanar carbonyl functions.



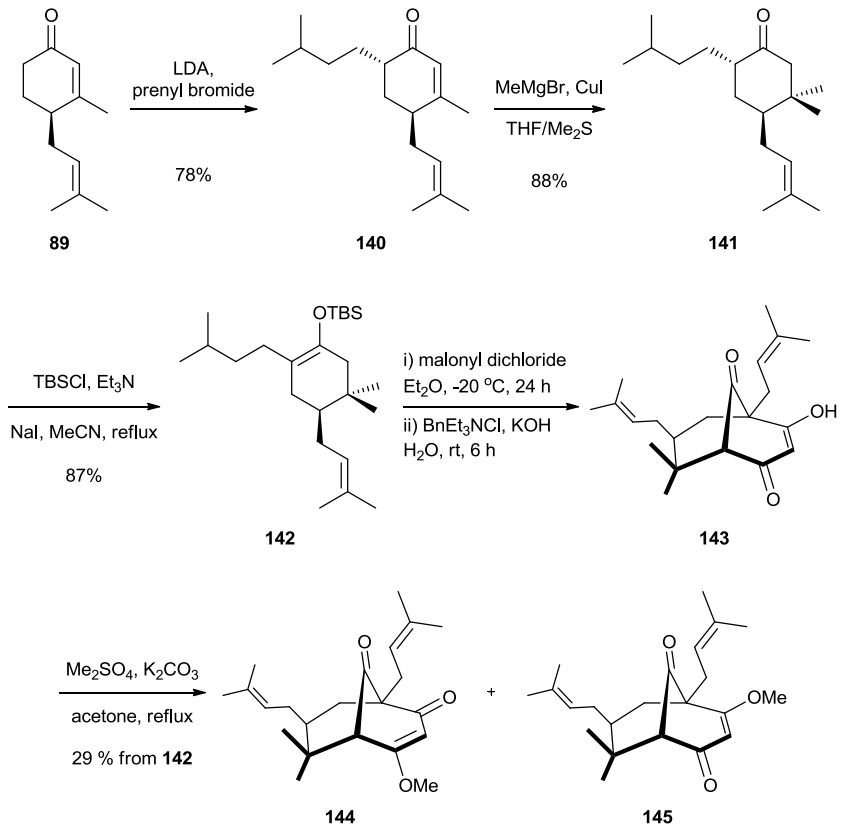

**Scheme 1.6.2**

Installation of the C-1 bridgehead acyl group would be a severe test of our bridgehead lithiation chemistry due in part to the steric hindrance of the *geminal* dimethyl group. Therefore, it was hoped that the acyl group could be taken through the Effenberger cyclisation. The inclusion of the benzoyl functionality before the cyclisation would be a complete change in the electronics of the starting ketone, but it was hoped that this change in electronics would promote the Effenberger annulation. However, when submitting the silyl enol ether **137** or **138** to the Effenberger conditions no reaction took place (Scheme 1.6.3).



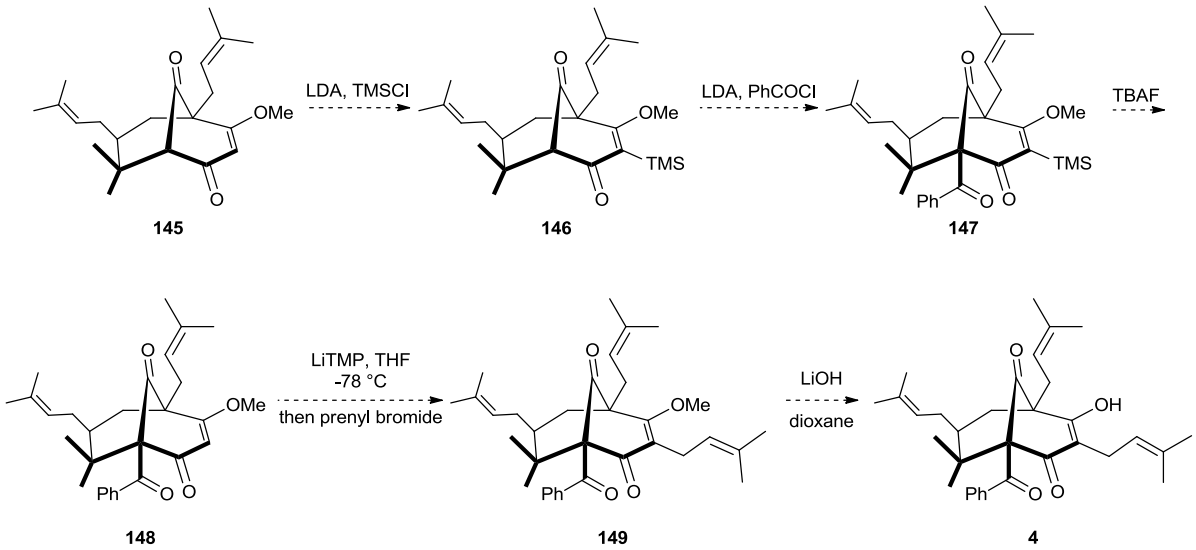

**Scheme 1.6.3**

Therefore, a route with C-1 bridgehead enolate chemistry was devised. Dr. Rodeschini synthesised a suitable silyl enol ether **142** for the cyclisation that would install the C-5 and C-7 prenyl groups (Scheme 1.6.4). He also showed that C-3 substitution was more favoured than C-1 substitution but trying to perform further bridgehead enolate substitutions would lead to alkene formation in the C-3 prenyl rather than C-1 substitution.




**Scheme 1.6.4**

With a route to the bicyclo[3.3.1]nonane core of nemorosone (**4**) established by Dr. Rodeschini it was intended to perform C-1 bridgehead acylation and subsequent C-3 prenylation to form *O*-methylated nemorosone (**149**). For this to be selective it would be necessary to protect the C-3 position with TMS first. After completing C-1 benzoylation the C-3 TMS group could be removed by the use of TBAF. C-3 prenylation would give *O*-methylated nemorosone (**149**) which would then be hydrolysed to nemorosone (**4**) using similar conditions to those of the clusianone (**3**) synthesis (Scheme 1.6.5).

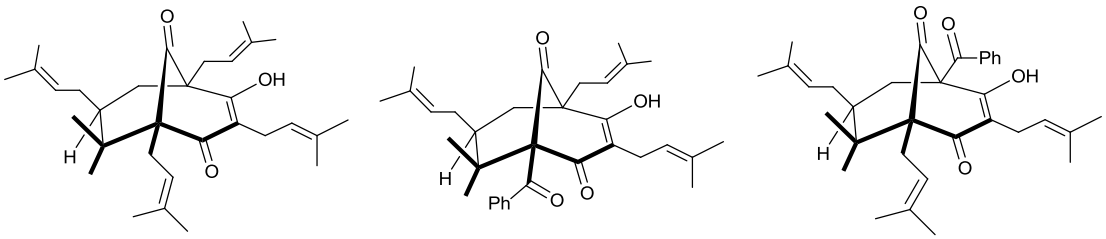



**Scheme 1.6.5**

This would give nemorosone (**4**) in ten steps from known enone **134**. This synthesis would also allow for different substitution at the C-3 and C-5 positions which would enable a range of derivatives to be synthesised. The results of this proposed synthesis can be found in Chapter 2 along with more information about nemorosone (**4**), its isolation, characterisation and biological activity.

## 1.7 Nemorosone II as a Target

Currently, there is no literature describing the synthesis of a type C PPAP or its bicyclo[3.3.1]nonane core. Nemorosone (**4**), clusianone (**3**) and nemorosone II (**150**) are all regioisomers of each other and make up a set of type A, B and C PPAPs with the same components (Figure 1.7.1). Nemorosone II (**150**) is a type C PPAP that was proposed as a possible structure for nemorosone (**4**) but was later rejected when the type A structure was confirmed by NMR experiments.

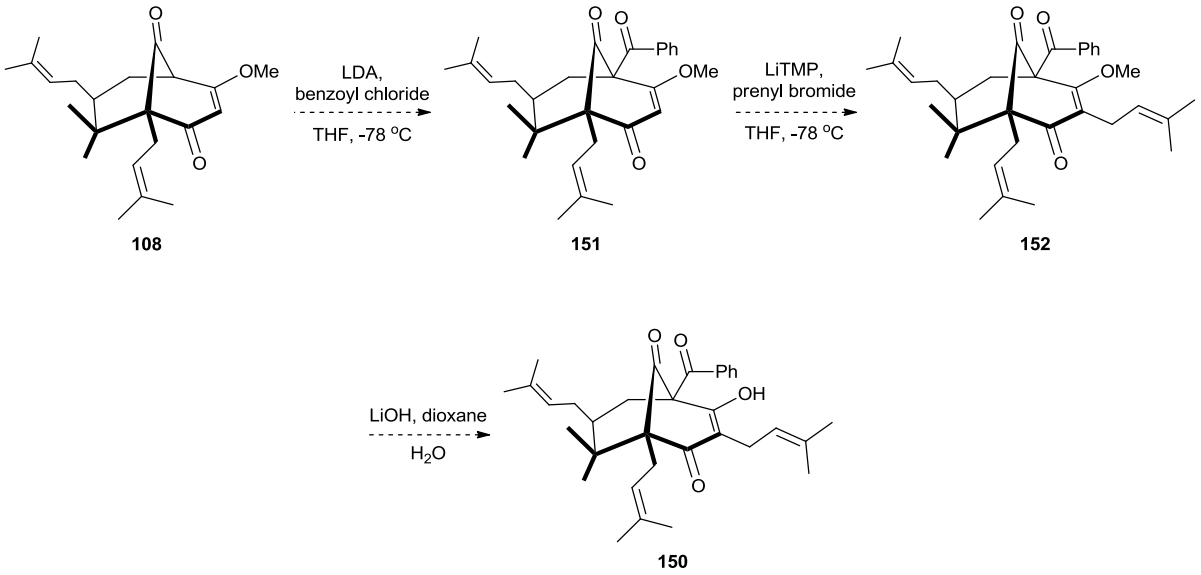



**3** clusianone **4** nemorosone **150** nemorosone II

**Figure 1.7.1**

As nemorosone II (**150**) is an unknown compound in nature, its synthesis would allow an insight into the structure-activity relationship of these compounds and may give an insight into why the type C structures are rarely isolated.

To synthesise nemorosone II (**150**) should be relatively straightforward as the bicyclo[3.3.1]nonane core has a C-1 prenyl group like clusianone (**3**). To complete the synthesis it is necessary to append the acyl group at C-5 bridgehead and prenylate the C-3 vinylic position (Scheme 1.7.1). This could be achieved using similar conditions to the clusianone (**3**) synthesis.




108 151 152 150

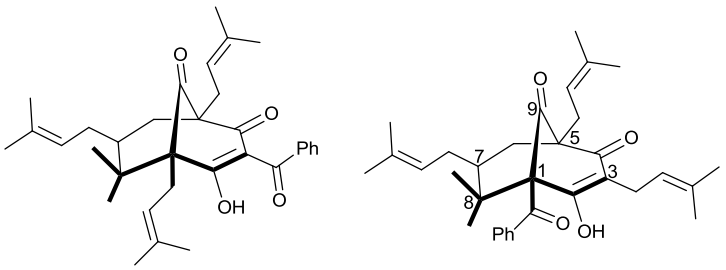
**Scheme 1.7.1**

Bridgehead enolate formation would be accessed using LDA, and a benzoyl chloride quench would give the correct benzoylation of the C-5 position. LiTMP, followed by prenyl bromide, would give *O*-methylated nemorosone II (**152**). The synthesis of nemorosone II (**150**) would be attempted alongside that of nemorosone (**4**), the results of which can be seen in Chapter 3. Preliminary studies into the synthesis of nemorosone II (**150**) had all ready been performed in our group by Peter Crick.

# Chapter 2

# Synthesis of Nemorosone

With a successful synthesis of the natural compound clusianone (**3**) completed by our group, it seemed appropriate to challenge our methodology and approach the synthesis of nemorosone (**4**), a regioisomer of clusianone (**3**) (Figure 2.0.1).[40] With an acyl group at the C-1 bridgehead position, adjacent to a *geminal* dimethyl group (C-8) and a bridgehead ketone (C-9) nemorosone (**4**) offered a significant challenge to our bridgehead enolate methodology.



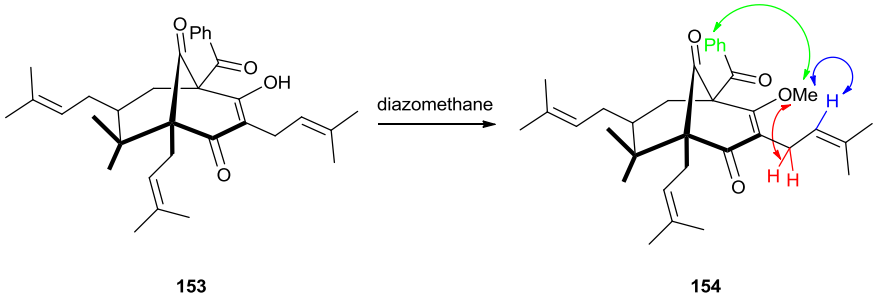

**3** clusianone **4** nemorosone

**Figure 2.0.1**

## 2.1 Isolation and Characterisation

Nemorosone (**4**) is a natural product that was isolated by C. M. A. de Oliveira *et al.* in 1996, through the extraction of floral resins of *Clusia rosea, grandiflora, insignis* and *nemorosa* which are a family of trees found in tropical and subtropical regions.[48] The resins were collected directly from the plants and extracted using diethyl ether or ethyl acetate and then treated with diazomethane to methylate the 1,3-diketone

which allowed for better separation on silica gel. Both the known compound, clusianone (**3**), and the new structure of nemorosone (**153**) were reported. Somewhat surprisingly only one regioisomeric enol ether derivative was mentioned for each of these compounds. In the case of nemorosone, the structure of the enol ether **154** was assigned as shown, with a benzoyl substituent at the C-5 position (Scheme 2.1.1). It was noted by the author that the methyl enol ether derivatives were more stable to acidic conditions than the unprotected tetra ketones which were susceptible to polymerisation and decomposition.

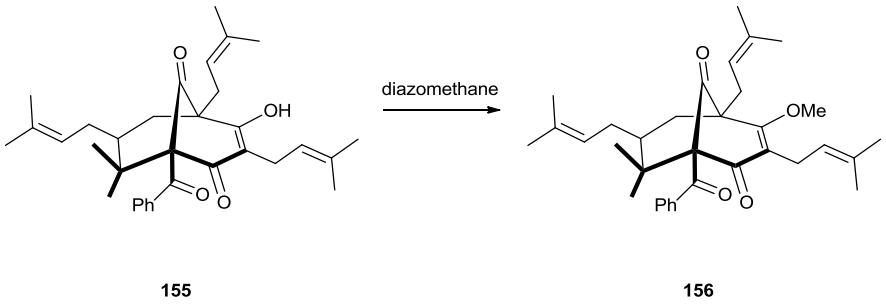



153 154

**Scheme 2.1.1** *O*-methylated nemorosone configuration as described by C. M. A. de Oliveira in 1996.

The evidence for the proposed structure came from nOe NMR experiments. Thus irradiation of the methoxy group led to the observation of positive nOe at the allylic methylene at δ 3.25 and 3.34 (1.3%), vinyl at δ 5.00 (2.1%) and aromatic protons at δ 7.62 (1.4%). These combined with long range correlations, led the de Oliveira group to assign the configuration shown in Scheme 2.1.1. In a subsequent paper in 1999, the same research group described further *O*-methylated derivatives from the same floral resins. Evidence was provided for a compound called nemorosone II (**155**), based on the NMR data of the *O*-methylated derivative **156**. This compound has the

bridgehead substituents exchanged with respect to the proposed structure of nemorosone (**153**), and so is a type A PPAP (Scheme 2.1.2).

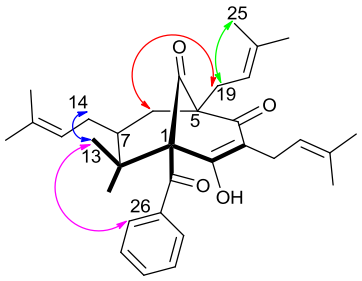



**155** **156**

**Scheme 2.1.2** *O*-methylated nemorosone II configuration as described by C. M. A. de Oliveira in 1996.

Further study in 2001 led to revision of the perceived configuration of nemorosone (**4**) and nemorosone II (**150**). Cuesta-Rubio *et al*. studied the constituents of the floral resin of *Clusia rosea* without previous treatment of the resin with diazomethane, therefore isolating nemorosone (**4**) as the tetra ketone rather than the methyl enol ether. They discovered that nemorosone (**4**) exists as a pair of keto-enol tautomers in its natural state.[54] In-depth NMR analysis showed that the structures of the keto-enol tautomers were not in accordance with the structure of the methylated derivative that de Oliveira *et al*. reported. By performing nOe difference experiments, it was possible to determine the position of the benzoyl group and the vinylic prenyl group. Saturation at the frequency of the *geminal* dimethyl (C-13) led to positive nOe at the aromatic protons (C-26a and C-26b) and methylene (C-14). Further to this, saturation at the methylene proton frequency (C-19) saw positive nOe of the methylene at C-6 and methyl at C-25, unequivocally qualifying the structure of

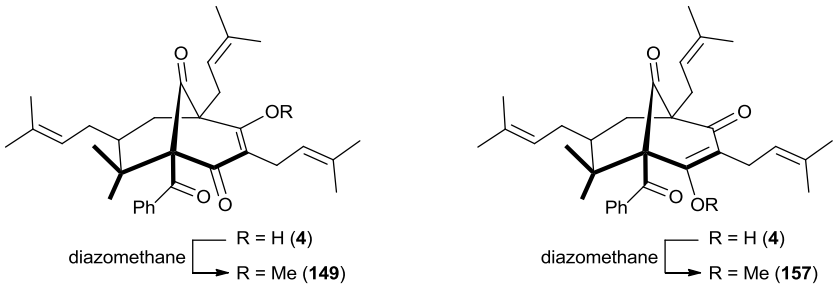
nemorosone (**4**) with the benzoyl at C-1 and a prenyl in the C-5 position (Figure 2.1.1).




**4** nemorosone

**Figure 2.1.1**

They proceeded to methylate using diazomethane to confirm whether the NMR spectrum of the methylated derivative would match that of de Oliveira (Scheme 2.1.1).[48-49] They found that both regioisomers could be isolated, in a 3:1 ratio favouring the compound **157** with the methoxy adjacent to the C-1 bridgehead acyl group. From these results it appears that de Oliveira mis-assigned the position of the methoxy group (C-10) and therefore, the nOe experiments led them to believe the C-1 and C-5 groups were in the opposite regiochemical arrangement.

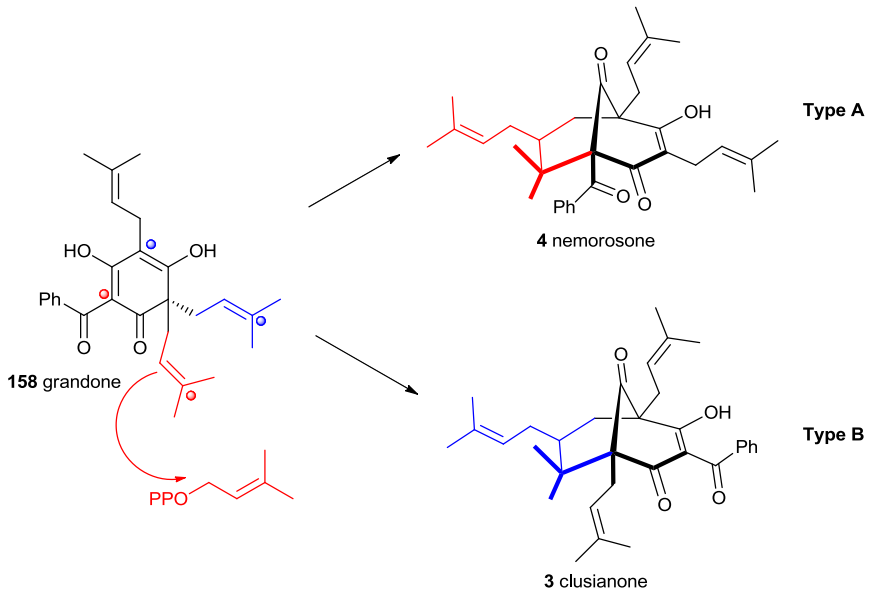



The methylated form of this isomer corresponds to de Oliveria's nemorosone II

**Scheme 2.1.3**

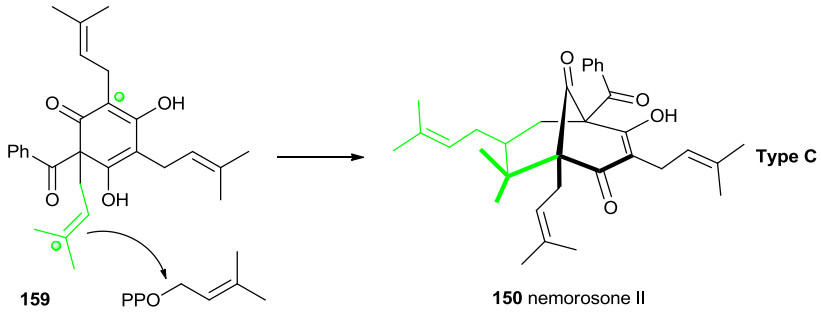
By comparison of the NMR spectra from the methylated products with that of de Oliveira it was possible to establish that nemorosone had the structure **4**, a structure that fits the data of both groups. Further to confirming the structure of nemorosone (**4**) Cuesta-Rubio compared the NMR data of the minor isomer from the methylation **149** with that of de Oliveira's "*O*-methylated nemorosone II" (**156**). The data matched perfectly. This established that nemorosone (**4**) has a type A PPAP structure and exists as two tautomers that correspond to two regioisomeric methyl enol ethers **157** and **149**, rather than the originally proposed type C structure **153**.

Helping to explain the lack of type C structures in nature is the proposed biogenetic pathway. The pathway shows that cyclisation of grandone (**158**) by addition across the double bond of a prenyl group and further reaction with a phosphate can lead to either type A or type B PPAPs depending on direction of attack (Scheme 2.1.4).




**Scheme 2.1.4** Biogenetic pathway for nemorosone and clusianone formation.

It also demonstrates that type C structures are unable to be formed by this mechanism from grandone (**158**). To form the type C structure the attacking prenyl group and the benzoyl group would have to be appended on the same carbon (on a grandone analogue **159**) and then cyclise forming the *geminal* dimethyl adjacent to the C-1 prenyl (Scheme 2.1.5).

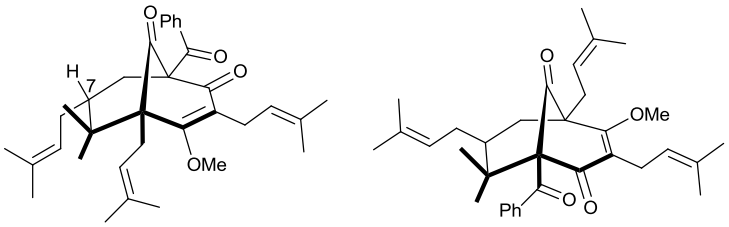



**Scheme 2.1.5**

Looking at the isolation papers of natural products an analogue of grandone with the acyl substituent and a prenyl group on the same carbon has yet to be discovered. This implies that the necessary precursor to the type C PPAPs is not available in nature and would explain the lack of type C PPAPs.

In a follow up paper the de Oliveira group isolated more derivatives of nemorosone, which were referred to as "*O*-methylated 7-*epi*-nemorosone" (**160**) and "*O*-methylated nemorosone II" (**156**) and assigned them the structures shown in Figure 2.1.2.[49] As mentioned above de Oliveira's "*O*-methylated nemorosone II" (**156**) corresponds to the minor product of methylation of nemorosone (**149**), the structure proposed is correct, as confirmed by Cuesta-Rubio but the naming is not correct.[54] Cuesta-Rubio also speculates that "*O*-methylated 7-*epi*-nemorosone (**160**) has also been mis-assigned because of the confusion over the identity of nemorosone (**4**), it is most

likely that the true structure would fit Cuesta-Rubio's assignment for nemorosone (**4**) (Figure 2.1.2) but with an epimeric prenyl substituent at the C-7 position.

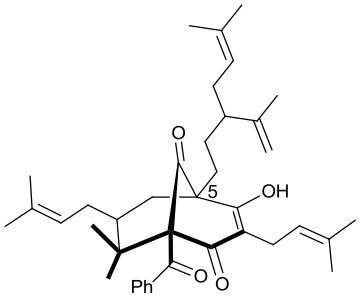



**160** **156**

**Figure 2.1.2** Nemorosone II and 7-*epi*-nemorosone as described by de Oliveira in 1999.

The second isolation of de Oliveira's "nemorosone II" (**155**) took place in 2000 by Lokvam *et al.* The change in assignment proposed by Cuesta-Rubio was yet to take place so the Lokvam group reported their data using the nemorosone II title rather than the corrected methyl enol ether of nemorosone (**149**).[53] Their isolation was performed as the de Oliveira group had done, through extraction with ethyl acetate followed by extensive chromatography, with the NMR spectra matching that of minor methyl enol ether of nemorosone (**149**) as reported by de Oliveira and Cuesta-Rubio. Lokvam studied the antibacterial activity of their isolated "*O*-methylated nemorosone II" (**156**) (actually the methyl enol ether of nemorosone (**4**)) and as the tetra ketone against honeybee pathogens *P. larvae* and *P. alvei*. The antibacterial effects were compared using a disc diffusion assay technique to those of chamone I (**161**) a structurally similar compound to nemorosone (**4**) but with further modification of the C-5 bridgehead prenyl substituent.[51] The results showed that the methyl enol ethers of both nemorosone (**4**) and chamone I (**161**) had no antibacterial activity, however

by working with the natural tetra ketones it was shown that the prenylation pattern has a large effect on the antibacterial activity of these molecules, with nemorosone (**4**) proving much more active than chamone I (**161**) (Figure 2.1.3).




**161** chamone I

**Figure 2.1.3**

## 2.2 Natural Sources and Degradation Studies

During the last decade interest in the PPAP family of compounds has risen considerably with new members frequently being isolated and many groups involved in the pursuit of synthetic routes to the bicyclo[3.3.1]nonane core. This has led to a great deal of associated information about the characteristics and properties of these molecules. For example, 2007 saw work by Cuesta-Rubio elucidating the content of PPAPs in propolis, a natural resinous substance produced by honey bees that has an antibacterial function in the bee's nests.[6,65] Interest in these substances has been generated by the use of propolis as a health drink which is thought to be effective against heart disease, diabetes and cancer.[72] The proposed biological activity has spawned an interest in the chemical composition of these resins and their active ingredients. HPLC study of propolis resins from Cuba has shown the major constituent in the resins are PPAPs and in particular nemorosone (**4**).

Having discovered the abundance of nemorosone (**4**) in propolis, Cuesta-Rubio was involved in a mass spectrometry study to determine the stability of nemorosone (**4**) and the nature of its degradation products.[67] Previous studies of other PPAPs, and Danishefsky's discovery that synthetic nemorosone (**4**) was unstable, suggested that PPAPs undergo further cyclisation and possibly polymerisation.[62-63,66] Exposure to heat, light and air, as well as inorganic and organic acids, have been shown to produce oxidised derivatives. Cuesta-Rubio's previous studies had isolated the oxidised and cyclised products garcinielliptone I (**162**), hyperibone B (**163**), and propolones A-D (**5 and 164-168**) (Figure 2.2.1).[60,73]

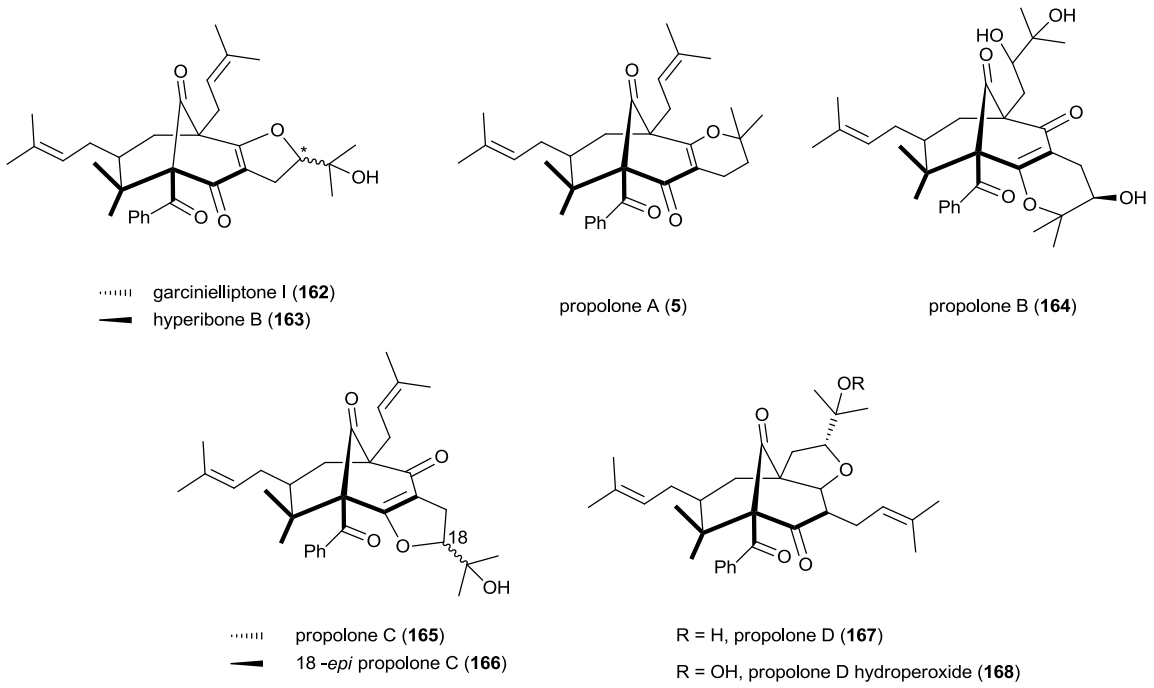



**Figure 2.2.1**

Studies of the stability of nemorosone (**4**) were performed in *n*-hexane, chloroform, ethanol or methanol and as a solid at 40 °C. HPLC-MS revealed that nemorosone (**4**) degraded to mainly garcinielliptone I (**162**) and hyperibone B (**163**) in less than 24

hours when dissolved in *n*-hexane. Chloroform solutions mainly led to propolone D hydroperoxide (**168**) after 72 hours. Ethanol solutions showed only 13% degradation after 60 days, and less than 1% degradation was observed in methanol. No degradation of the solid sample was seen after 7 days. From this study it is possible to say that many of the products that are structurally similar to nemorosone (**4**) are not in fact natural products but products of degradation during the extraction process. X-ray and quantum mechanical studies have also been performed by Pagano with the help of Cuesta-Rubio.[64] Using X-ray crystallography they were able to confirm the molecular structure of nemorosone (**4**) matched that reported by Cuesta-Rubio. DFT calculations allowed insight into the chair boat equilibrium and showed that the chair conformation was 16 Kcal/mol more stable than the boat conformation (Figure 2.2.2).

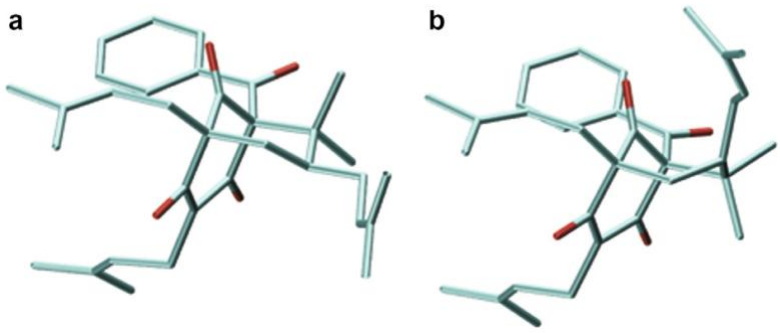



**Figure 2.2.2** Chair conformation (a), boat conformation (b) of nemorosone (**4**) taken from B. Pagano *et al*., Chemical Physics Letters, 462, **2008**, 158.[64]

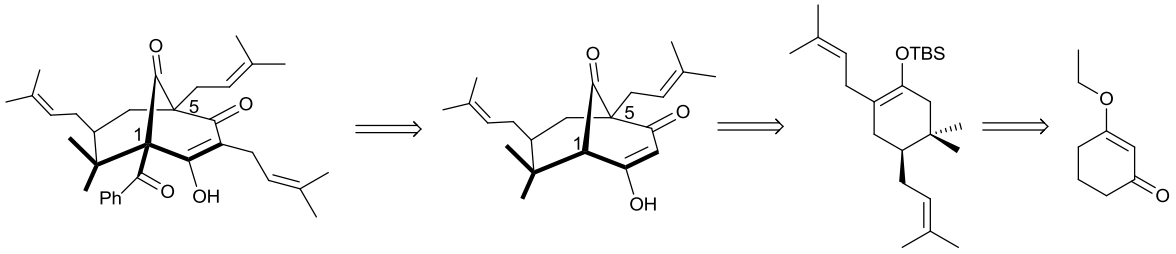
## 2.3 Biological Activity of Nemorosone

The Cuesta-Rubio group have studied propolis, its constituent parts and its biological activity. As part of this work they studied the biological activity of nemorosone (**4**) and its methyl enol ether derivatives.[69] This, coupled with the reports of bactericide activity of nemorosone (**4**) and chamone I (**161**) by Lokvam,[53] led them to study the antioxidant and cytotoxic properties of nemorosone (**4**) and its methyl enol ethers **157** and **149**.[6] They tested the compounds against four cancer cell lines, human cervix carcinoma (HeLa), human larynx carcinoma (HEp-2), prostate carcinoma (PC-3) and central nervous system carcinoma (U251). The assays showed that nemorosone (**4**) was effective in the μM concentrations against each of these cell lines. The methyl enol ether derivatives **157** and **149**, however, were at least an order of magnitude less active and are therefore outside of what would be considered a biologically active $IC_{50}$ value. The antioxidant properties of the compounds were also tested by observing the scavenging effect of the compounds on the stable free radical of 1,1-diphenyl-picryhydrazyl. It was observed that nemorosone (**4**) had an $IC_{50}$ of 44 μM for scavenging 1,1,-diphenyl-picryhydrazyl free radicals, where as the methyl enol ethers **157** and **149** showed no antioxidant activity.

## 2.4 Results and Discussion

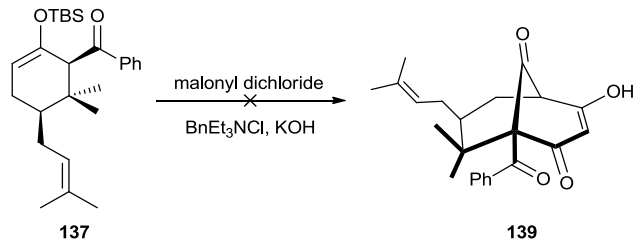
To synthesise nemorosone (**4**) we envisaged a series of disconnections that employed bridgehead enolate chemistry to append the groups in the C-1 and C-5 positions. Formation of the bicyclo[3.3.1]nonane core **143** would be performed by the Effenberger cyclisation, as our group had already successfully used the

Effenberger cyclisation to synthesise clusianone (**3**).[33,40] This led to the following retrosynthesis which would allow the application of our bridgehead enolate chemistry (Scheme 2.4.1).



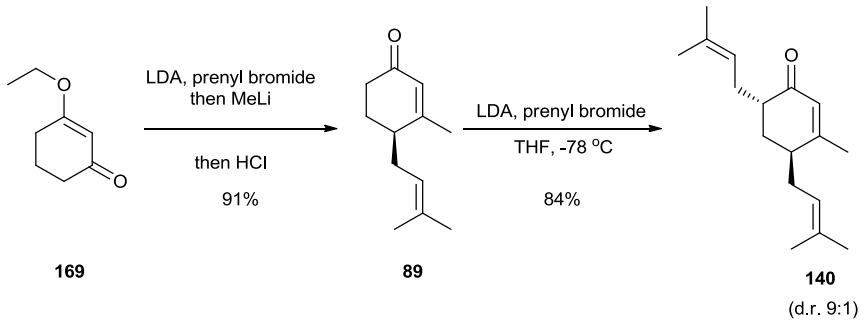

**Scheme 2.4.1**

Our group had previously established that it was impossible to perform the Effenberger cyclisation and obtain a bicyclo[3.3.1]nonane **139** with an acyl group at the C-1 bridgehead position. These conditions led to only starting material being recovered (Scheme 2.4.2).[40] With this in mind we planned to install the acyl group after the cyclisation had been completed, using a bridgehead enolate. Therefore, the acyl group would have to be installed at a bridgehead (C-1) adjacent to a *geminal* dimethyl group which would be an extreme test of bridgehead enolate methodology.



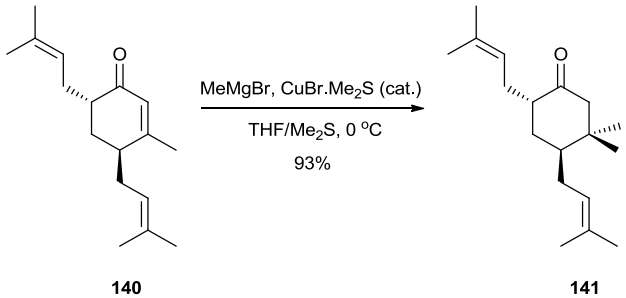

**Scheme 2.4.2**

With these limitations in mind, synthesis of the required silyl enol ether **142** was embarked upon. Starting with 3-ethoxy cyclohexenone (**169**), prenylation was achieved by enolate formation with lithium diisopropylamide (LDA) followed by electrophilic quenching with prenyl bromide (Scheme 2.4.3). Addition of methyl lithium to this reaction mixture with a hydrochloric acid work up afforded enone product **89**. Further deprotonation with LDA led to selective lithium enolate formation and an electrophilic quench using prenyl bromide gave diprenylated enone **140** as a 9:1 diasteromeric mixture.



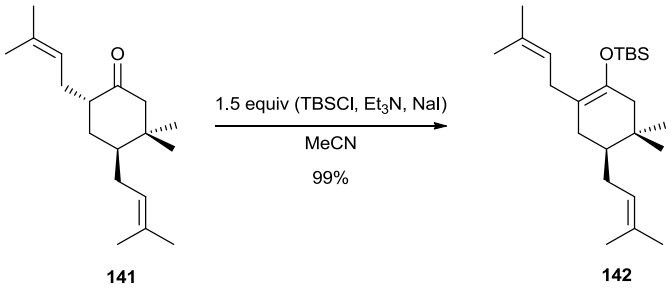

**Scheme 2.4.3**

Installation of the second methyl of the *geminal* dimethyl group was affected through copper mediated 1,4-Grignard addition (Scheme 2.4.4).



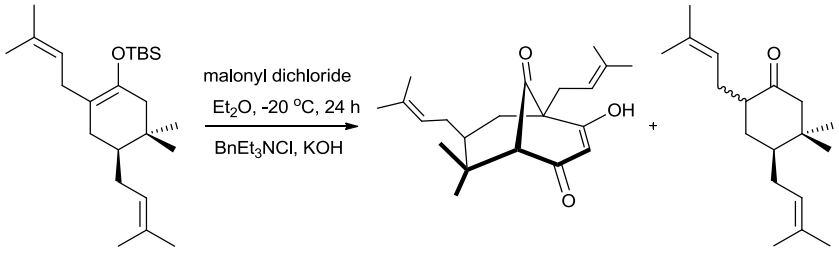

**Scheme 2.4.4**

To perform the Effenberger annulation it would be necessary to form either a silyl or methyl enol ether of ketone **141**. Both can be formed and are effective in the subsequent reaction but the silyl enol ether forming reaction proved to be robust and high yielding. Silyl enol ether **142** was accessed quickly under these thermodynamic conditions in near quantitative yield (Scheme 2.4.5).




**Scheme 2.4.5**

With silyl enol ether **142** in hand the Effenberger cyclisation was undertaken. This cyclisation enables a rapid increase in molecular complexity which makes it an attractive route to the bicyclo[3.31]nonane system. However, the yields are modest at best. Ketone **141** was recovered as a side product of the reaction in 83% yield and this helps to ease the burden of the inefficiency of the step as it can be easily recycled (Scheme 2.4.6).

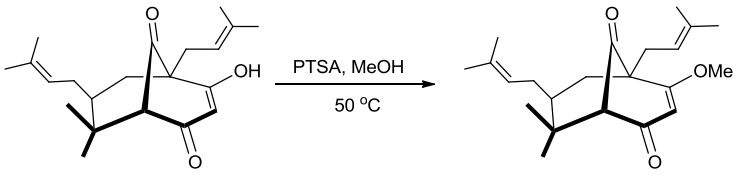



not isolated at this stage 83%

**142** **143** **141**

**Scheme 2.4.6**

The triketone **143** product of the Effenberger reaction was unstable and highly polar which made its isolation difficult and therefore the triketone was protected as methyl enol ethers **144** and **145**. Initial attempts to form the methyl enol ether **145** under acidic conditions failed, recovering mainly decomposition products and very small quantities of the desired bicyclo[3.3.1]nonane **145** (Scheme 2.4.7).

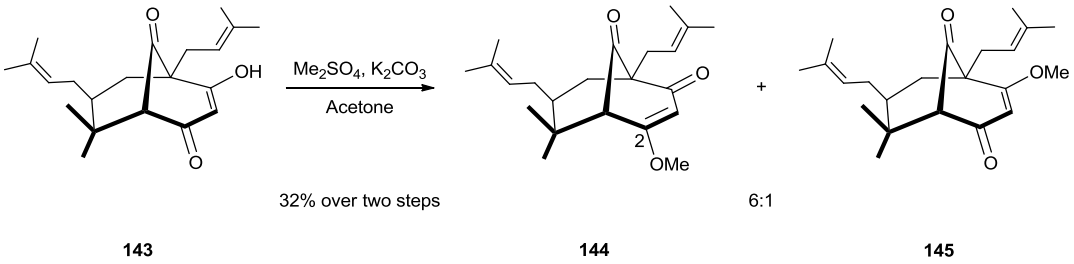



11% over two steps

**143** **145**

**Scheme 2.4.7**

Basic conditions were therefore employed to methylate the enol **143**, giving a 6:1 mixture of regioisomers **144** and **145** favouring **144** the enol ether with methylation at C-2 (Scheme 2.4.8).




**Scheme 2.4.8**

At this stage the two regioisomers **144** and **145** were separated by column chromatography and the major isomer taken on in the synthesis. X-ray crystallography had been performed upon the major isomer (**144**) by us, which had confirmed the regio- and stereochemistry of this compound.[40] $^1$H NMR analysis of the methyl enol ether **144** shows characteristic signals for the protons at the C-1 bridgehead and the C-3 vinyl position (Figure 2.4.1). These signals could be used throughout the rest of the synthesis to confirm that transformations at these positions had been successful.

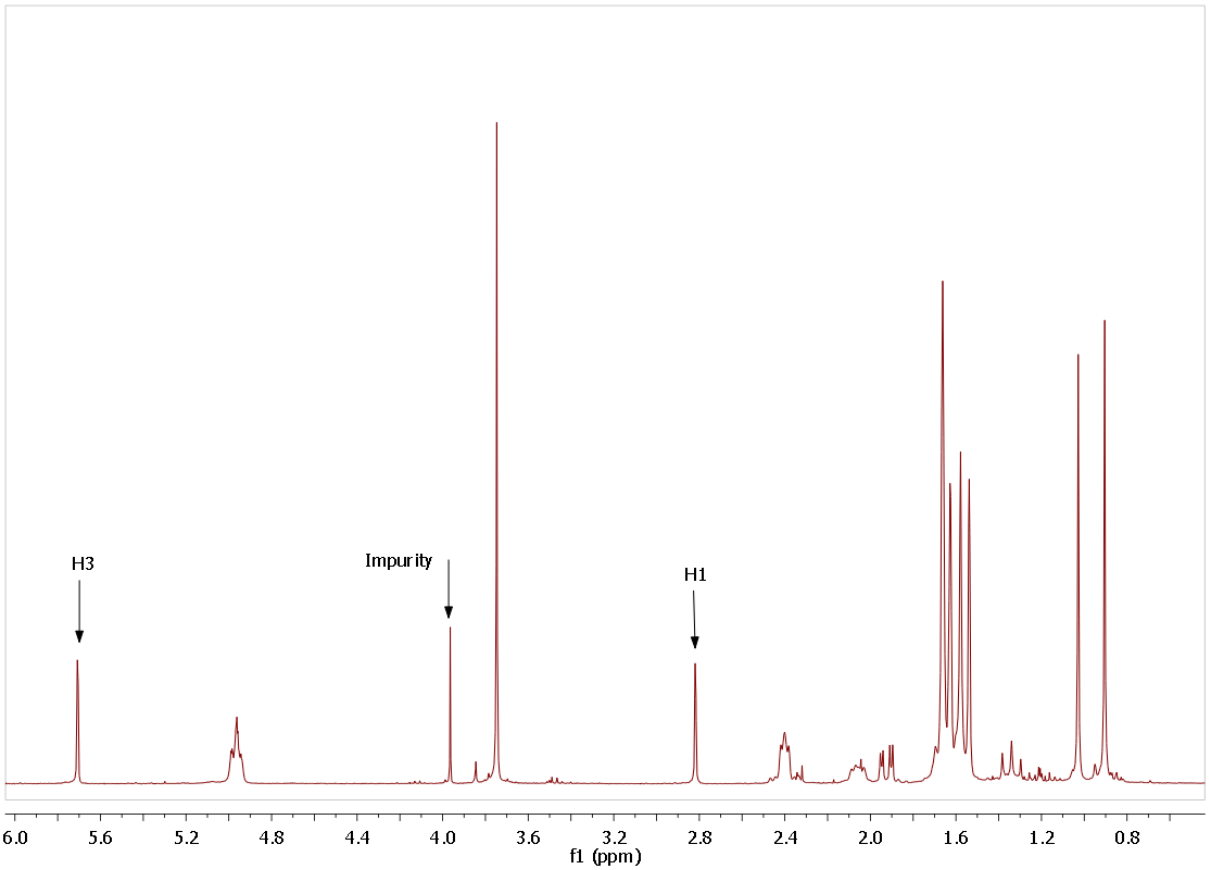
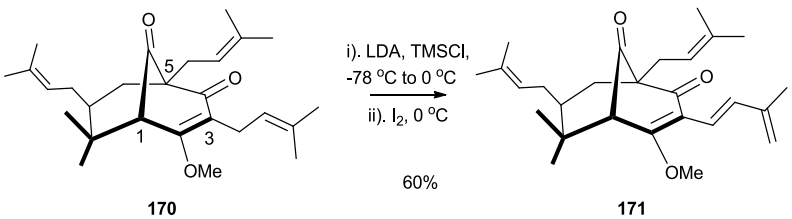


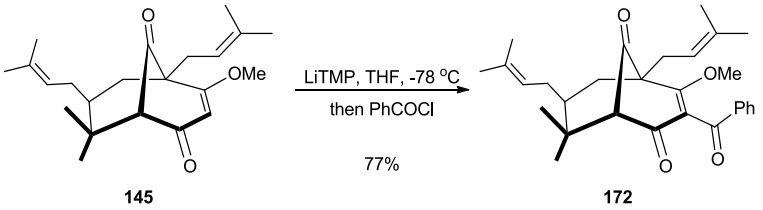

**Figure 2.4.1** $^{1}$H NMR of **144** (500 MHz, $CDCl_3$)

Further progress required installation of the bridgehead acyl group (C-1) and the vinylic prenyl group (C-5). During some earlier preliminary studies in the group we discovered that the C-3 prenyl group would undergo deprotonation to form the conjugated diene **171** on treatment with reagents intended to iodinate the C-1 bridgehead (Scheme 2.4.9).[47]




**Scheme 2.4.9**

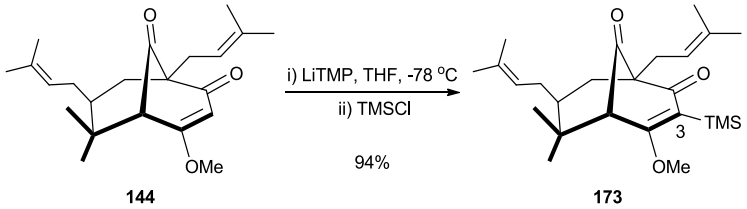
Therefore the acyl substituent at the C-1 bridgehead would have to be installed first using the previously established bridgehead enolate chemistry and the prenyl group at C-3 installed afterwards. Previous attempts in the group to directly acylate at the C-1 bridgehead were unsuccessful as the C-3 vinylic position was acylated and no C-1 acylation was observed (Scheme 2.4.10).




145 172

**Scheme 2.4.10**

Based on the previous studies we had to selectively acylate at the bridgehead, therefore protection of the C-3 position had to be performed. Deprotonation with LiTMP followed by electrophilic quench with TMSCl gave vinylsilane **173** in excellent yield (Scheme 2.4.11).




144 173

**Scheme 2.4.11**

The $^{1}$H NMR spectra for the silyl protected compound showed the C-3 proton signal at δ 5.71 had been replaced by a singlet at δ 0.16 corresponding to nine protons. This confirmed quickly that installation of the vinylic TMS group had been successful (Figure 2.4.2).

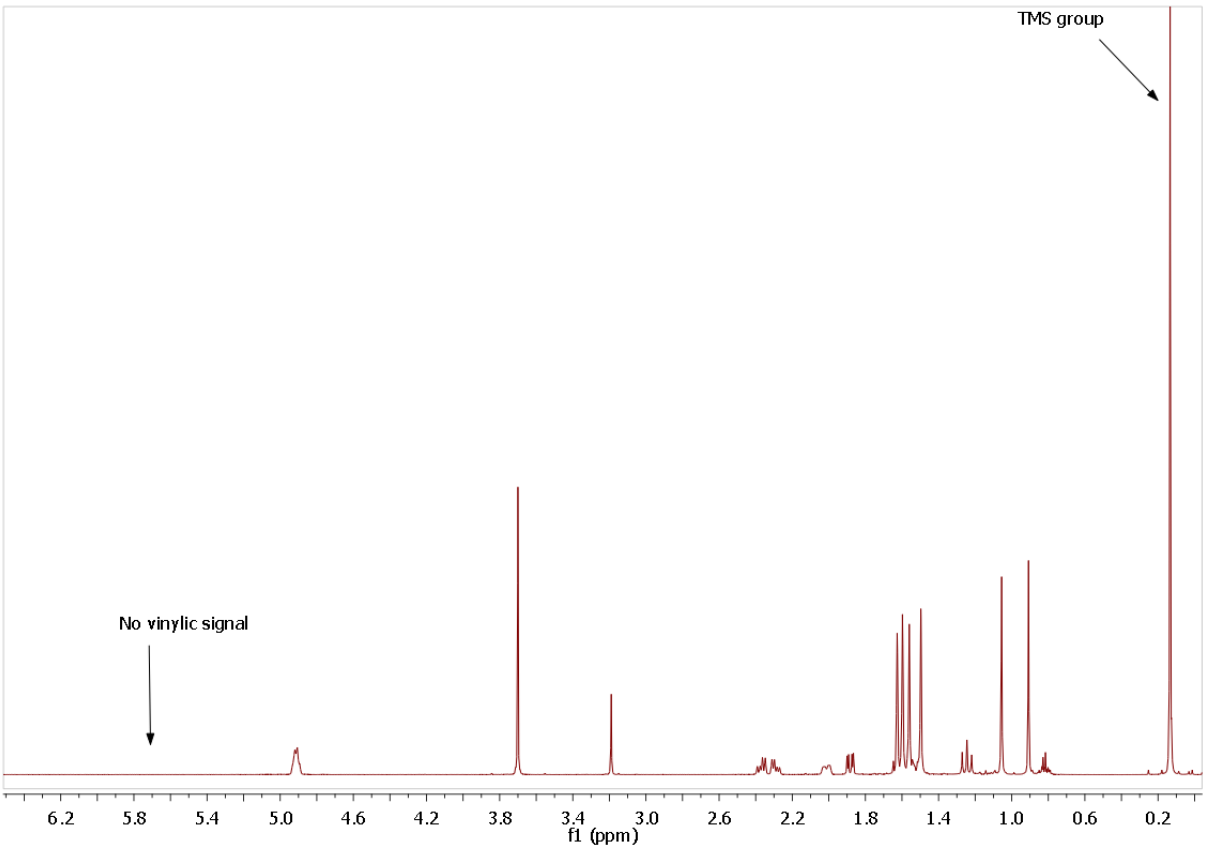
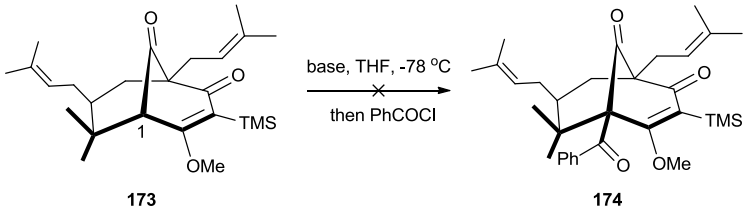



**Figure 2.4.2** $^{1}$H NMR of **173** (500 MHz, $CDCl_3$)

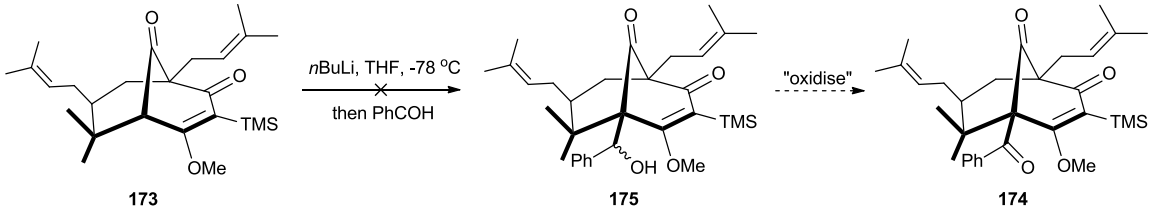
Initial attempts to acylate the bridgehead enolate under standard conditions of LDA at -78 °C in tetrahydrofuran with a benzoyl chloride quench proved unsuccessful, giving nothing but unreacted starting material. Different bases (LDA, LiTMP, and NaOMe) and additives (LiCl, HMPA) were tried to activate the C-1 position towards acylation. Unfortunately, we were unable to install the necessary benzoyl group directly at the C-1 position (Scheme 2.4.12).




173 174

**Scheme 2.4.12**

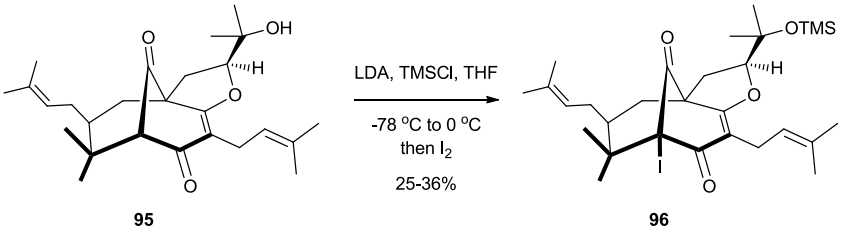
Having been unsuccessful in direct bridgehead acylation, we planned instead to install the C-1 benzoyl group using benzaldehyde as electrophilic quench for the bridgehead enolate, followed by oxidation of the so-formed secondary alcohol (Scheme 2.4.13). Once more the C-1 position was not substituted, with only unreacted starting material being recovered.




173 175 174

**Scheme 2.4.13**

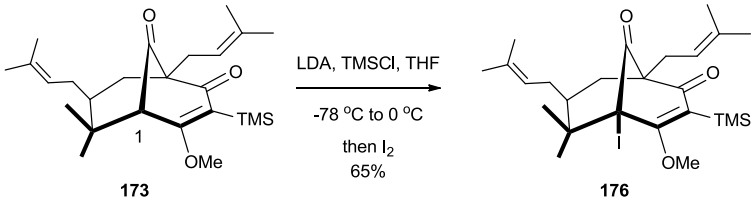
Having discovered the apparent lack of reactivity at the C-1 bridgehead a deeper study was conducted into the reactivity of this position the results of which are presented in Chapter 5. Danishefsky had shown in his synthesis of garsubellin A (**1**) that it was possible to form a bridgehead iodide **96** in a similar system to ours (Scheme 2.4.14).[39]




95 96

**Scheme 2.4.14**

This transformation used the somewhat odd reaction of chlorotrimethylsilane addition to the lithium enolate forming conditions followed by warming to 0 °C, for half an hour before cooling to -78 °C and adding $I_2$. By applying these conditions to our system, iodination of the C-1 bridgehead of **173** was achieved in reasonable yield (Scheme 2.4.15).




173 176

**Scheme 2.4.15**

Once more drawing attention to the $^1$H NMR spectra allows clarification that iodination was possible at the C-1 position, as the signal for the bridgehead proton is no longer visible (Figure 2.4.3). The $^1$H NMR spectra is not definitive about the iodine incorporation however the mass spectrometry shows a m/z of 543.18 which corresponds to the $[M + H]^+$ ion of the iodide **176**.

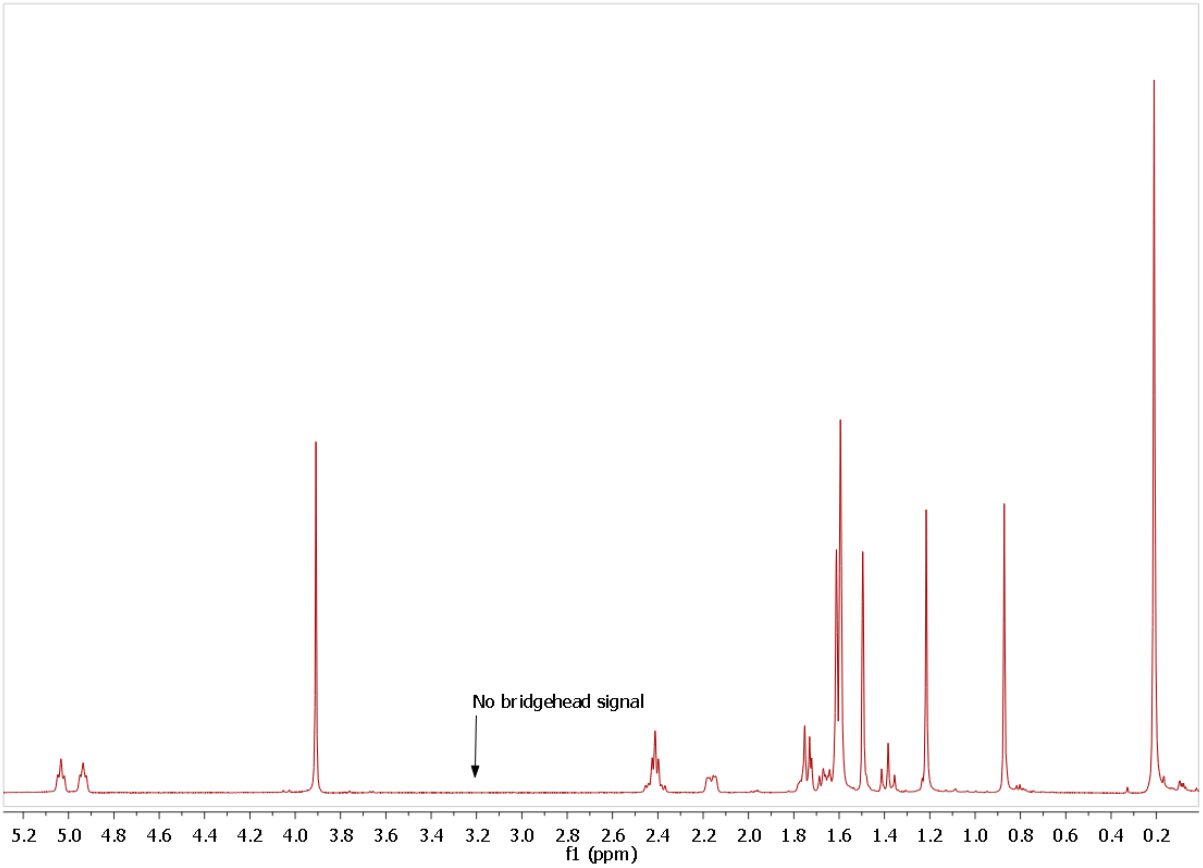
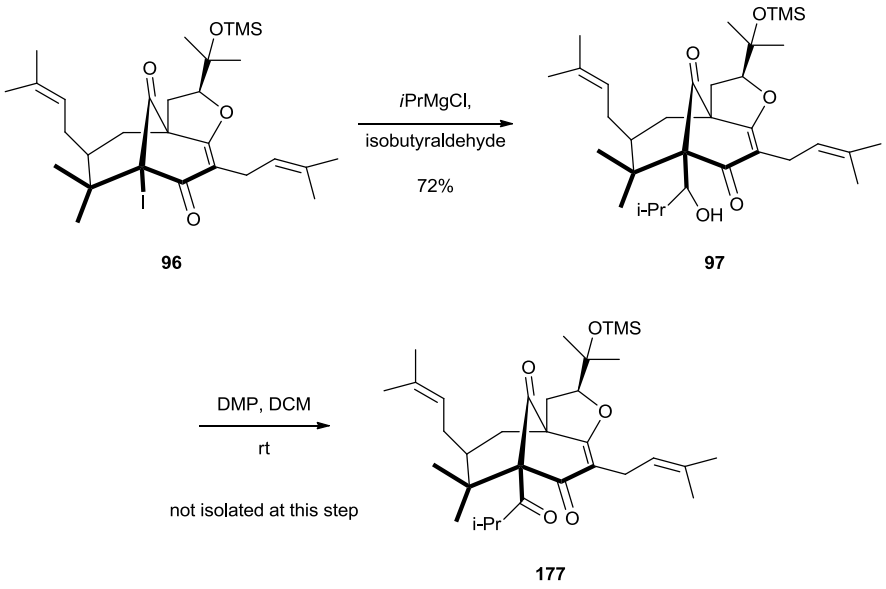



**Figure 2.4.3** $^{1}$H NMR of **176** (500 MHz, $CDCl_3$)

To our surprise, the Danishefsky conditions appeared uniquely suitable for the preparation of the bridgehead iodide, and none of the lower temperature protocols, employed with great success by our research group in the past, gave any sign of the desired product. Attempts to form the bridgehead iodide **176** with the omission of TMSCl from the reaction mixture were unsuccessful with only unreacted starting material (**173**) being obtained. It was unclear at this stage what role the TMSCl played in the reaction so, further investigation was performed and is discussed in Chapter 5.

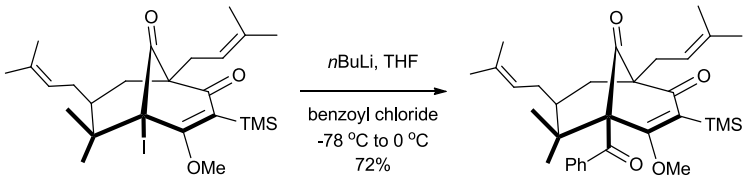
In his synthesis of garsubellin A (**1**) Danishefsky used *i*PrMgBr with LiCl to effect iodine-magnesium exchange on the bridgehead iodide **96**, quenching with benzaldehyde.[39] Oxidation of the resulting alcohol **97** gave the C-1 benzoylated product **177** (Scheme 2.4.16).




**96** **97** **177**

**Scheme 2.4.16**

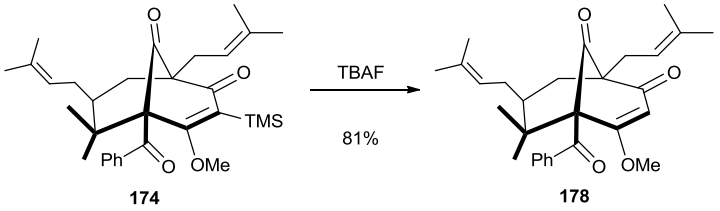
However, these conditions were unsuccessful when applied to our nemorosone (**4**) system. To our satisfaction, we found that *direct* bridgehead benzoylation could be performed by a more conventional iodine–lithium exchange, using *n*BuLi in THF, followed by addition of benzoyl chloride (Scheme 2.4.17).




**176** **174**

**Scheme 2.4.17**

With the C-1 bridgehead acylation completed, attention turned to prenylation of the C-3 position. This required desilylation, to deprotect the vinylic position followed by prenylation. Deprotection of the vinylic position proved trivial using tetrabutylammonium fluoride to give **178** (Scheme 2.4.18).




**Scheme 2.4.18**

Proton NMR spectra once more gives clear information proving the transformation has been successful as the C-3 proton returns at its characteristic δ 5.80 shift (Figure 2.4.4).

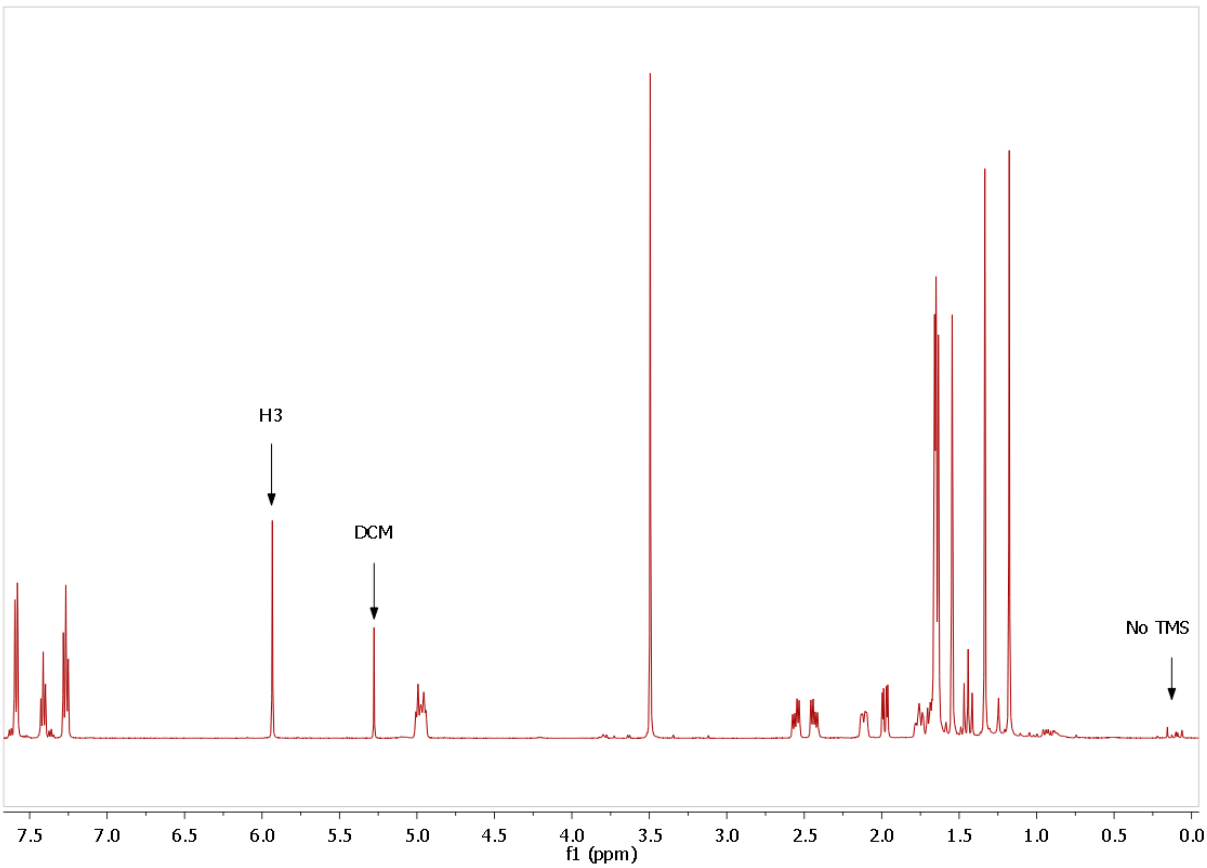
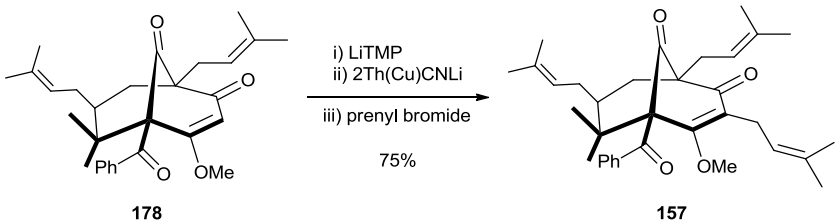



**Figure 2.4.4** $^{1}$H NMR of **178** (500 MHz, $CDCl_3$)

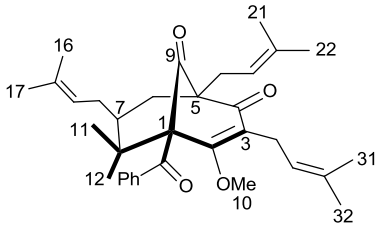
Deprotonation and an electrophilic quench with prenyl bromide were employed to complete the architecture of nemorosone (**4**). A mixed order cuprate was employed for this addition, using conditions that had been developed previously within our research group (Scheme 2.4.19).[40,47]




**178** **157**

**Scheme 2.4.19**

The prenylation afforded *O*-methylated nemorosone (**157**) in 75% yield. In this form nemorosone was stable and easy to manipulate. At this stage it was compared with the data from natural sources and from the Danishefsky publication. Below are our $^{1}H$ and $^{13}C$ NMR spectra, with the proton spectra showing the corresponding compound numbering (Figure 2.4.5 and Figure 2.4.6).




**157**

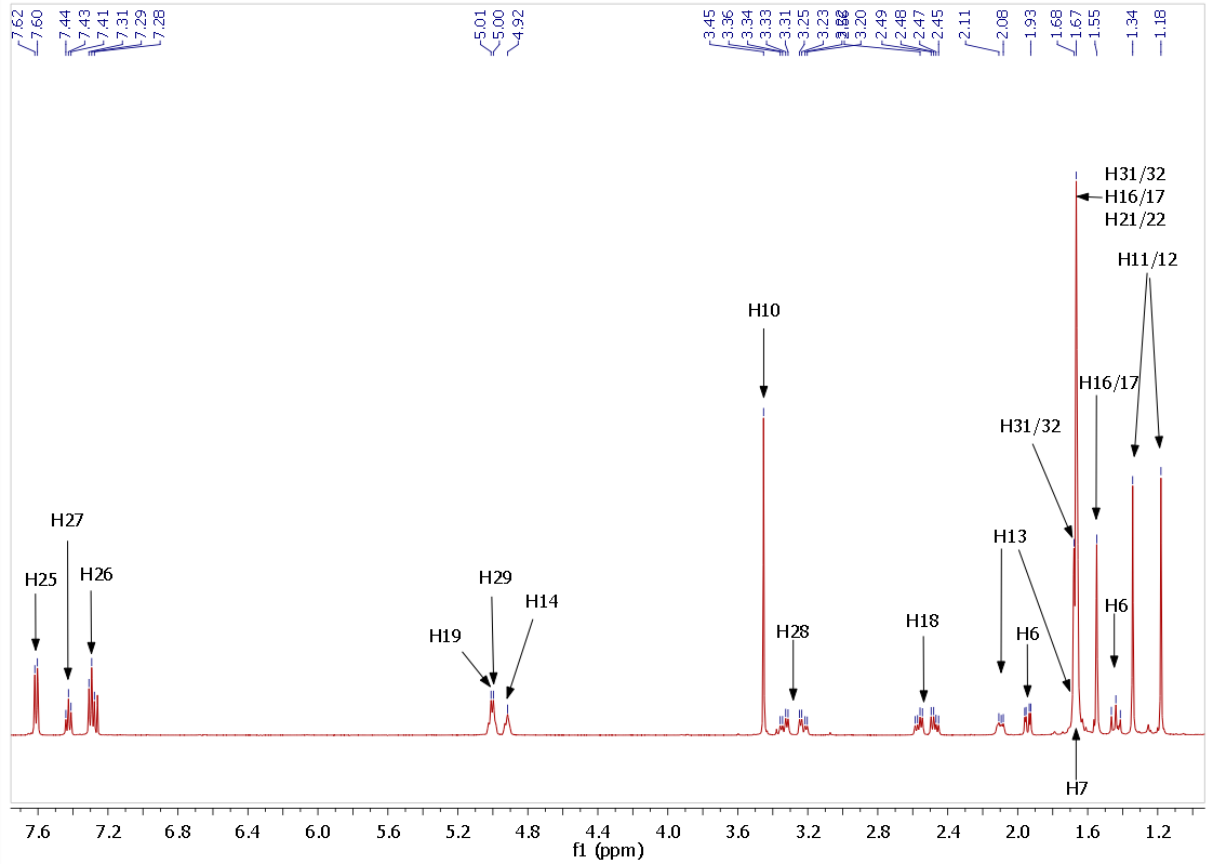



**Figure 2.4.5** $^{1}H$ NMR of **157** (500 MHz, $CDCl_3$)

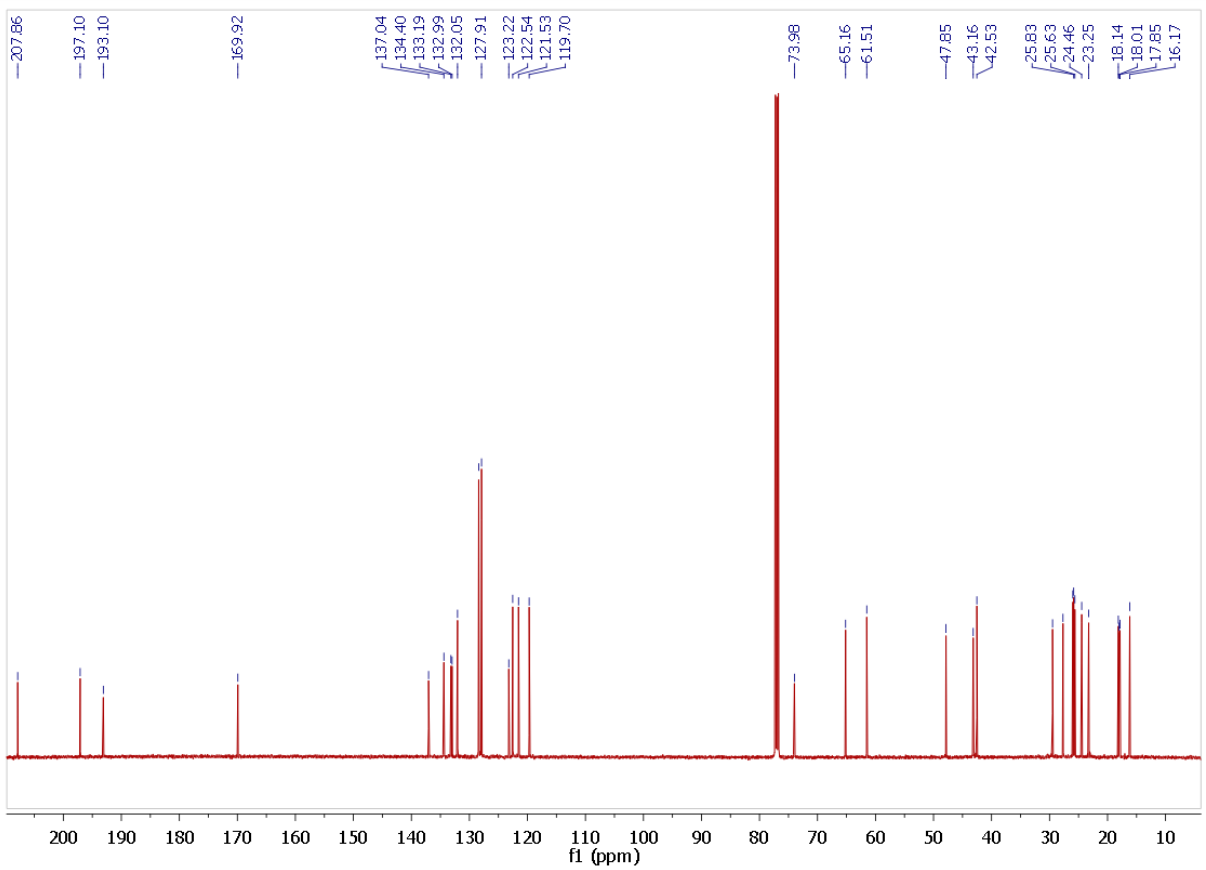



**Figure 2.4.6** $^{13}C$ NMR of **157** (125 MHz, $CDCl_3$)

To compare the data with the original isolation paper the Tables 2.4.1 and 2.4.2 have been made for direct easy comparison of the NMR data. As can be seen the data match closely except for the assignment of the benzoyl (C-23) and the enone (C-4) carbonyls in the $^{13}C$ NMR which have been interchanged. Also the fully substituted carbons (C-20 and C-30) of the double bond in the vinylic (C-3) and bridgehead (C-5) prenyls were originally mis-assigned. The assignments were clear from the HMBC and HMQC experiments that were performed on our sample and which are shown in appendix 11 and 12.

| Carbon number | de Oliveira's *O*-methylated nemorosone (154) | Our *O*-methylated nemorosone (157) |
|---|---|---|
| 1 | 74.4 | 74 |
| 2 | 169.9 | 169.9 |
| 3 | 123.2 | 123.2 |
| 4 | 193.1 | 197.1 |
| 5 | 65.1 | 65.1 |
| 6 | 43.1 | 43.1 |
| 7 | 42.5 | 42.5 |
| 8 | 47.8 | 47.8 |
| 9 | 207.9 | 207.9 |
| 10 | 61.6 | 61.5 |
| 11 | 24.4 | 24.4 |
| 12 | 16.1 | 16.2 |
| 13 | 27.6 | 27.7 |
| 14 | 122.5 | 122.5 |
| 15 | 133.2 | 133.2 |
| 16 | 26 | 26 |
| 17 | 17.8 | 17.8 |
| 18 | 29.5 | 29.5 |
| 19 | 119.6 | 119.7 |
| 20 | 133 | 134.4 |
| 21 | 25.6 | 25.6 |
| 22 | 18.1 | 18.1 |
| 23 | 197.1 | 193.1 |
| 24 | 137 | 137 |
| 25 | 128.4 | 128.4 |
| 26 | 127.9 | 127.9 |
| 27 | 132.1 | 132 |
| 28 | 23.2 | 23.2 |
| 29 | 121.5 | 121.5 |
| 30 | 134.4 | 133 |
| 31 | 25.8 | 25.8 |
| 32 | 18 | 18 |

**Table 2.4.1**

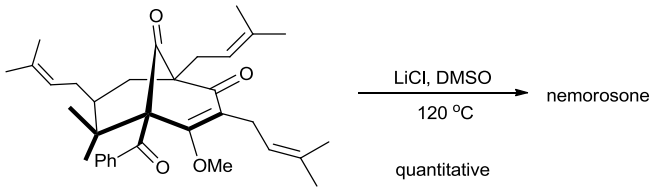
The proton NMR spectra of our *O*-methylated nemorosone (**157**) and de Oliveira's match very well. Unfortunately, Danishefsky worked with a different regioisomer of *O*-methylated nemorosone (**149**) to our synthesis and the original isolations and it is therefore not easy to compare the data of each group.

| Carbon number | de Oliveira's *O*-methylated nemorosone (154) | Our *O*-methylated nemorosone (157) |
|---|---|---|
| 6 | 1.94 (1H, dd, *J* = 12.6, 3.4)<br>1.42 (1H, t, *J* = 12.6) | 1.95 (1H, dd, *J* = 13.0, 3.9)<br>1.45 (1H, t, *J* = 12.8) |
| 7 | 1.66 (1H, overlap) | 1.72 -1.64 (1H, m) |
| 10 | 3.45 (3H, s) | 3.46 (3H, s) |
| 11 | 1.34 (3H, s) | 1.35 (3H, s) |
| 12 | 1.19 (3H, s) | 1.19 (3H, s) |
| 13 | 2.10 (1H, m) 1.66 (1H, overlap) | 2.14 - 2.08 (1H, m) 1.72 -1.64 (1H, m) |
| 14 | 4.97 (1H, m) | 4.95 - 4.90 (1H, m) |
| 16 | 1.66 (3H, s) | 1.68 (3H, s) |
| 17 | 1.55 (3H, s) | 1.56 (3H, s) |
| 18 | 2.56 (1H, dd, *J* = 13.7, 7)<br>2.50 (1H, dd, *J* = 13.7, 7.1) | 2.58 (1H, dd, *J* = 13.9, 6.6)<br>2.48 (1H, dd, *J* = 13.9, 7.3) |
| 19 | 5.00 (1H, m) | 5.02 (1H, t, *J* = 6.5) |
| 21 | 1.66 (3H, s) | 1.68 (3H, s) |
| 22 | 1.66 (3H, s) | 1.68 (3H, s) |
| 25 | 7.62 (1H, dd, *J* = 8, 1) | 7.62 (2H, d, *J* = 7.3) |
| 26 | 7.32 (1H, t, *J* = 8) | 7.30 (2H, t, *J* = 7.9) |
| 27 | 7.44 (1H, tt, *J* = 8, 1) | 7.44 (1H, t, *J* = 7.3) |
| 28 | 3.34 (1H, dd, *J* = 16, 6.8)<br>3.25 (1H, dd, *J* = 16, 6.8) | 3.34 (1H, dd, *J* = 15.8, 6.6)<br>3.23 (1H, dd, *J* = 15.8, 6.2) |
| 29 | 5.00 (1H, m) | 5.01 (1H, t, *J* = 6.5) |
| 31 | 1.66 (3H, s) | 1.68 (3H, s) |
| 32 | 1.66 (3H, s) | 1.69 (3H, s) |

**Table 2.4.2**

With the methyl enol ether of nemorosone **157** in hand all that remained was to deprotect the enol and obtain the tetra ketone. A modification of the Krapcho type

conditions was employed for this transformation.[58] Heating the methyl protected compound **157** to 120 °C in $d_6$-DMSO with LiCl for 4 hours gave quantitative yields of nemorosone (**4**) without the need for extraction and purification by column chromatography (Scheme 2.4.20). Isolation of the free compound proved non-trivial, since, as noted by other research groups nemorosone (**4**) is unstable to light and particularly acids.[48,66] This meant that NMR spectroscopy using $CDCl_3$ would decompose the compound. Also $^1$H NMR spectra in $CDCl_3$ show two separate tautomers, making NMR hard to interpret. However, by recording the NMR spectra in $d_6$-DMSO both of these problems were alleviated and clear NMR of only a single tautomer could be achieved straight from the reaction without the need for column chromatography purification.




**157** **4**

**Scheme 2.4.20**

However some of the signals were obscured by the $d_6$-DMSO in the $^1$H NMR spectra. To obtain full characterisation nemorosone (**4**) was extracted from the crude reaction mixture and the NMR performed in $d_4$-MeOD which gave unobscured spectra showing only one tautomer. With our synthetic nemorosone (**4**) in hand we obtained a sample of natural nemorosone (**4**) from Frank Holtrup at The German Cancer Research Centre. This enabled us to perform NMR experiments in $d_4$-MeOD on

natural nemorosone (**4**) and compare them to our synthetic sample (Figure 2.4.7 and Figure 2.4.8).

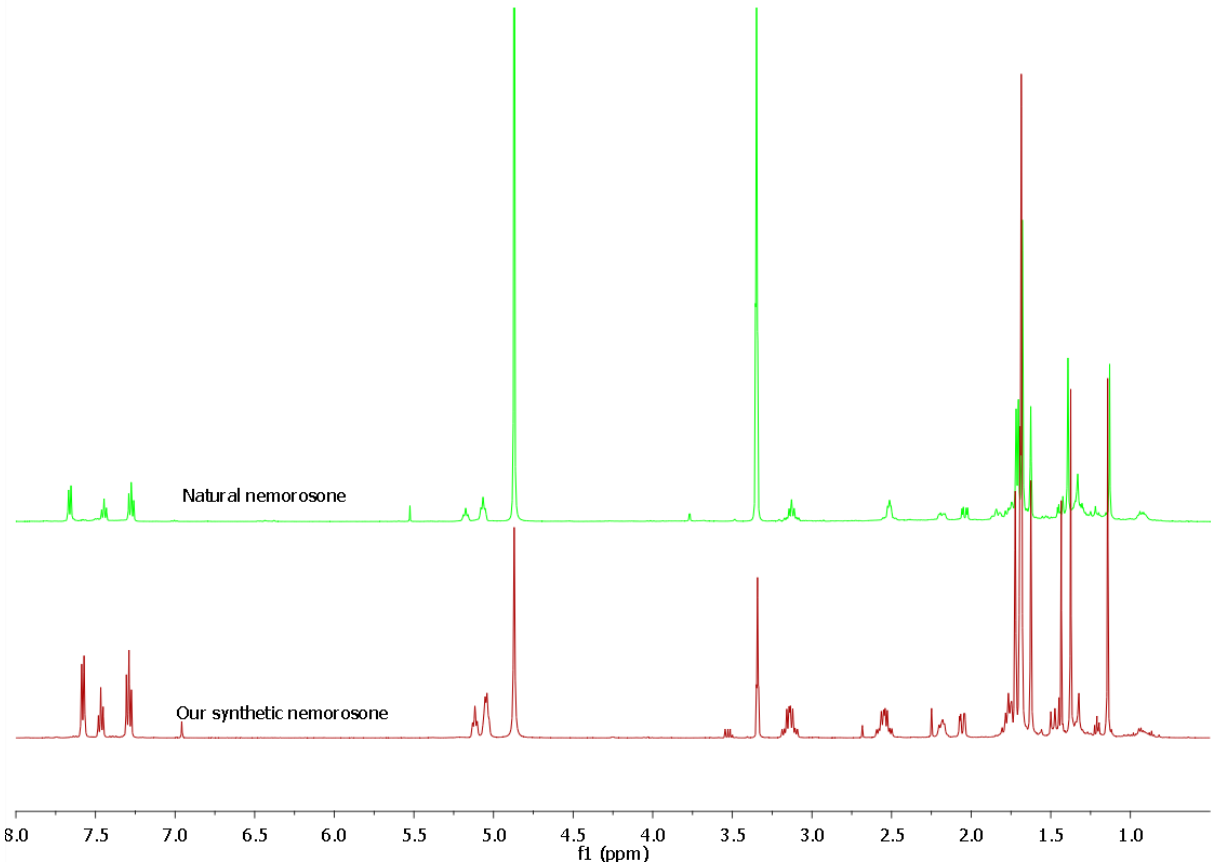



**Figure 2.4.7** Comparison of $^{1}$H NMR of natural and synthetic nemorosone (**4**) (500 MHz, MeOD)

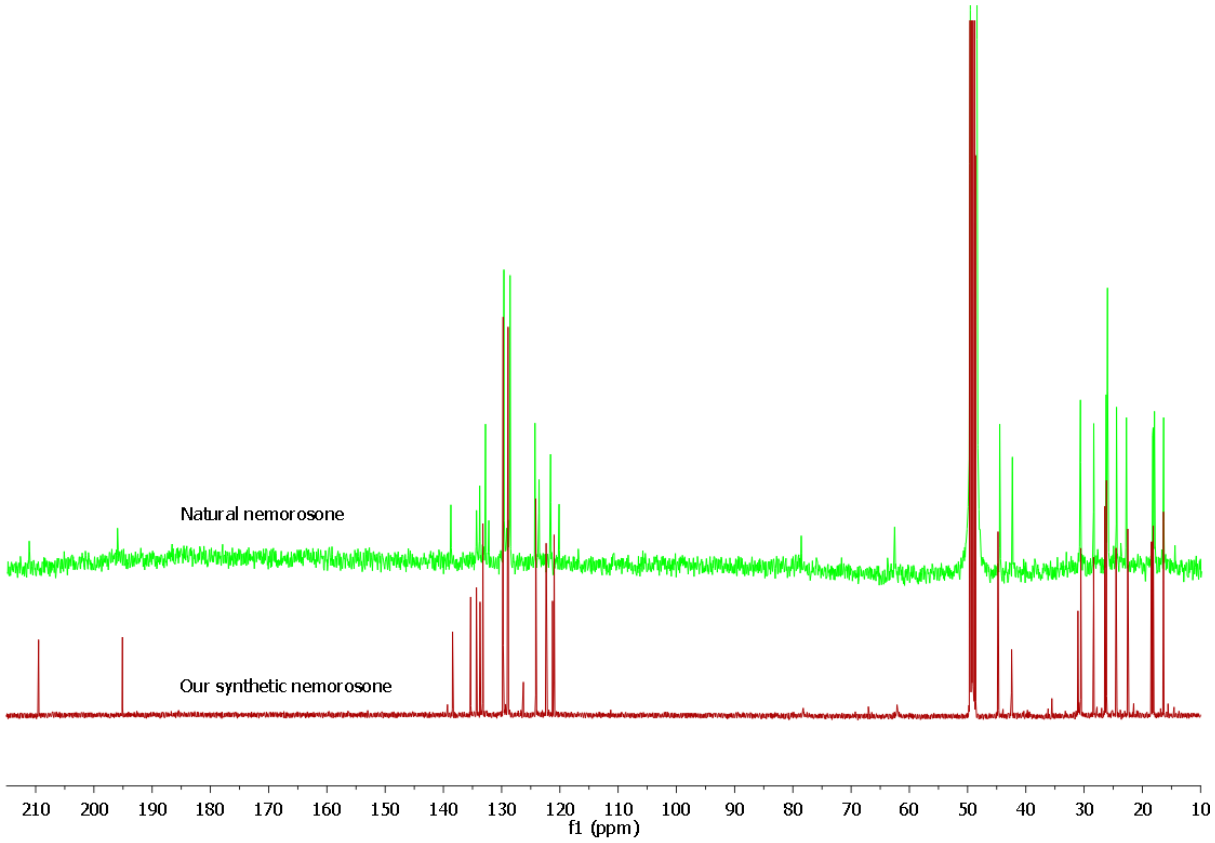


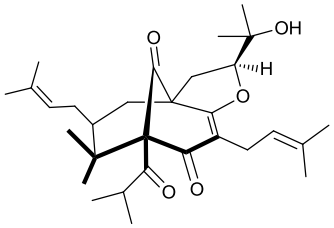

**Figure 2.4.8** Comparison of $^{13}C$ NMR of natural and synthetic nemorosone (**4**) (125 MHz, MeOD)

The NMR spectra of natural nemorosone (**4**) and our synthetic sample match well, confirming that we have synthesised the type A PPAP nemorosone (**4**). Nemorosone (**4**) had been synthesised in 13 steps and 6% overall yield (one step less and 5.5% improvement over Danishefsky) and was submitted for biological testing (Chapter 4).

## 2.5 Danishefsky's Synthesis

Late 2007 saw the first published synthesis of nemorosone (**4**) by the Danishefsky group, building on from their earlier synthesis of garsubellin A (**1**) (Figure 2.5.1).[39,66]

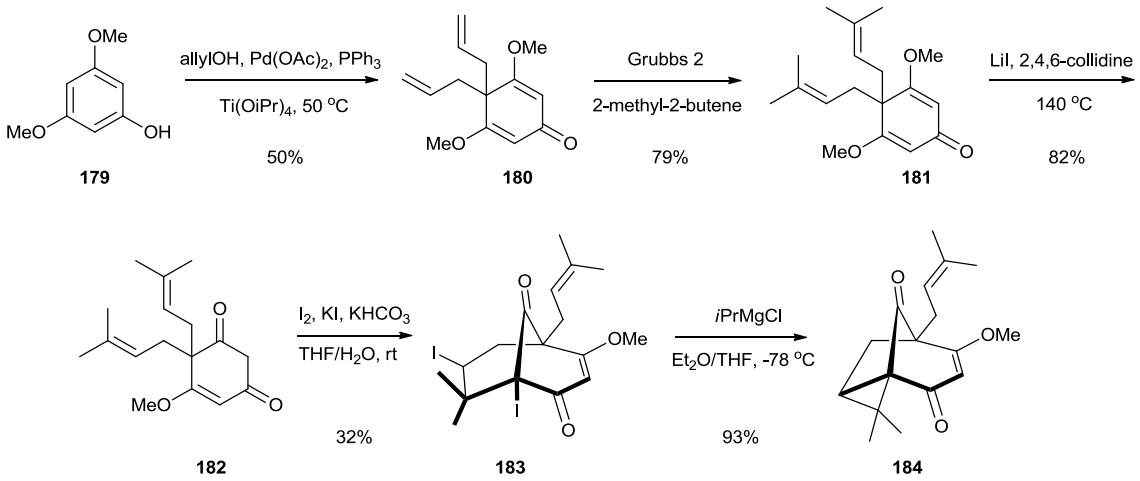
The Danishefsky synthesis appeared after ours had been completed in the laboratory but had not been published. Although conducted completely independently the two syntheses have some common features, particularly in the use of reactive bridgehead carbanions to install the crucial C-1 carbonyl function.




**1** garsubellin A

**Figure 2.5.1**

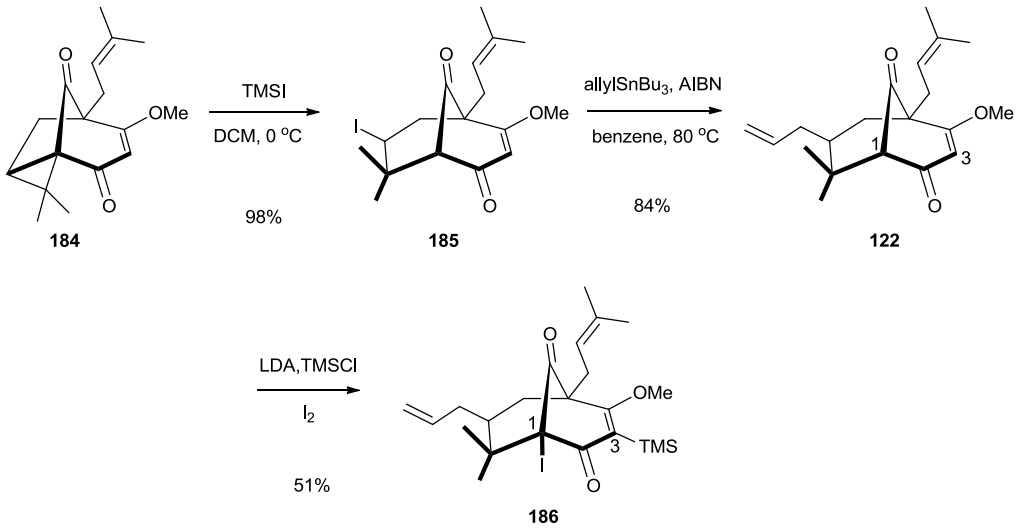
Danishefsky's route was devised to make different PPAPs from the same core bicyclic system and had already been used to successfully synthesise garsubellin A (**1**) and clusianone (**3**). Starting from commercially available 3,5-dimethoxy phenol (**179**), diallylation was performed using allylpalladium chemistry to give **180**. Twofold cross metathesis led to the diprenylated derivative **181**, and subsequent mono-demethylation was achieved using lithium iodide in 2,4,6-*sym*-collidine, to give diketone **182** (Scheme 2.5.1).




**Scheme 2.5.1**

Cyclisation to the bicyclo[3.3.1]nonane **183** was performed using an iodonium induced carbocyclisation. Although the overall yield for these first five steps is poor, accessing the bicyclo[3.3.1]nonane core in so few steps is a notable achievement. However, it would appear that Danishefsky (although he chooses not to comment) was unable to perform any selective chemistry upon this system and resorted to reductive elimination which gave the bridgehead fused cyclopropane **184**.

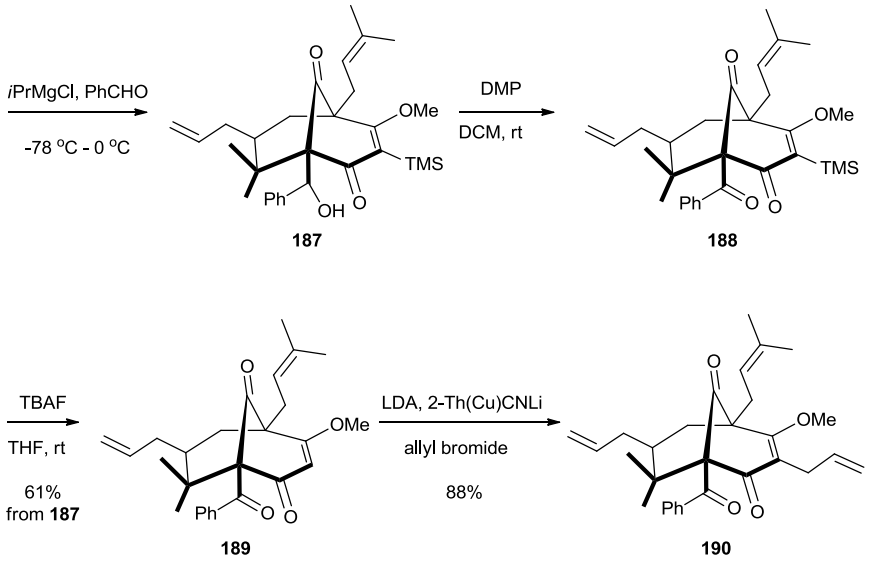
Nucleophilic ring opening of cyclopropane **184** with TMSI gave the iodinated product **185**. Allylation was performed using a protocol inspired by Keck and Yates.[57] This gave intermediate **122** which can be used to make both nemorosone (**4**) and clusianone (**3**). Advancing the nemorosone (**4**) synthesis required the installation of the bridgehead acyl group (C-1) and the vinylic prenyl group (C-3). Silylation of the vinylic position (C-3) was performed in one pot along with iodination of the bridgehead position (C-1) (Scheme 2.5.2).




**Scheme 2.5.2**

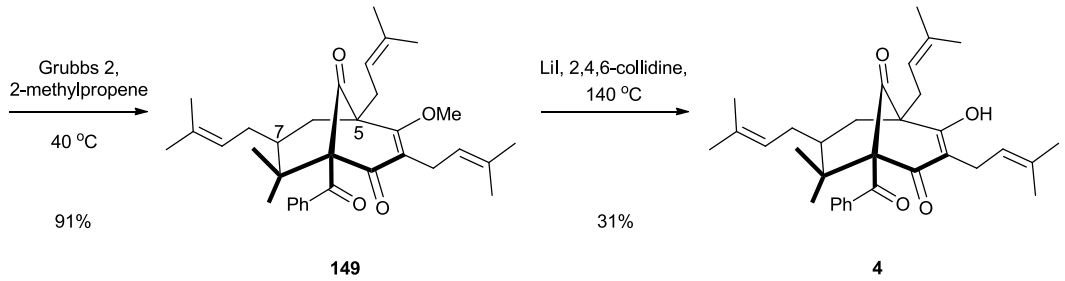
For the key bridgehead iodination, deuterium incorporation studies were performed upon **122** to determine whether C-1 and C-3 could be differentiated during deprotonation and electrophilic quenching reactions. The results of this study fit with those previously reported by ourselves.[47] It was discovered that with one equivalent of LDA and a $d_4$-MeOD quench that both positions were deuterated in 30% yield. Use of excess base yielded 70% of the monodeuterated products, which led to the conclusion that C-1 and C-3 could be lithiated in approximately equal amounts. Therefore, differential quenching reactions were exploited to distinguish the two positions. The use of an in situ quench with TMSCl gave 74% of the C-3 silylated product. Functionalisation of C-1 was attempted with LDA and an $I_2$ quench but gave a low yield. However, repeating the reaction with TMSCl in situ gave a 45% yield of the desired product. Danishefsky then attempted to perform the silylation and iodination in one pot relying on the differential in quenching to afford a single product and was successful in 51% yield.

With the C-1 position now iodinated, installation of the benzoyl group at C-1 could be investigated. The TMS group at the C-3 position would act as protecting group so that halogen metal exchange could be performed upon the C-1 position. Installation of the acyl group was executed using *iso*-propyl magnesium chloride followed by addition of benzaldehyde which gave the secondary alcohol at the C-1 position and subsequent oxidation of this using Dess-Martin periodinane gave the ketone **188** (Scheme 2.5.3).




**Scheme 2.5.3**

Desilylation of the C-3 position was performed successfully, using TBAF to give triketone **189**. Allylation of the vinylic (C-3) position was performed using LDA followed by addition of a mixed order cuprate and quenching with allyl bromide as we had demonstrated in our clusianone (**3**) synthesis.[47] The allyl groups at C-5 and C-7 were converted to the desired prenyl group by cross metathesis using Grubbs' second generation catalyst to give *O*-methylated nemorosone (**149**) (Scheme 2.5.4).




**Scheme 2.5.4**

Demethylation to give nemorosone (**4**) was performed upon *O*-methylated nemorosone (**149**) by hydrolysis with lithium iodide in 2,4,6-collidine. When trying to characterise the tetra ketone it was discovered that nemorosone (**4**) is particularly unstable to chloroform used in NMR analysis. This 14 step synthesis gave Danishefsky nemorosone (**4**) in 0.6% overall yield.

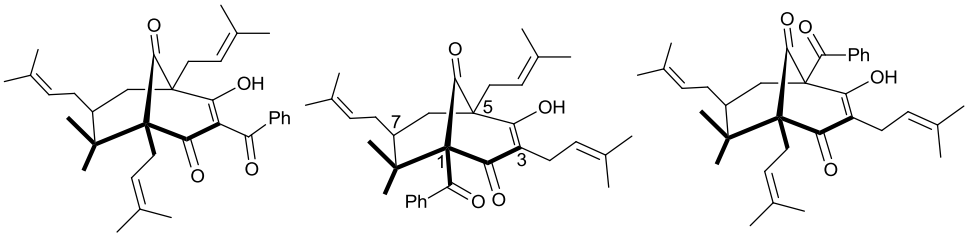
## 2.6 Conclusion

The natural product nemorosone (**4**) has been synthesised in 13 steps and a 6% overall yield. This was possible through a series of bridgehead functional group interconversions and enolate chemistry. The data for the synthesised material matched that of the natural isolation data and Danishefsky's synthetic sample.

# Chapter 3

# Synthesis of Nemorosone II

With the synthesis of clusianone (**3**) and nemorosone (**4**) successfully completed, we decided to synthesise nemorosone II (**150**), the unknown third regioisomer of this series of compounds. It was hoped that a study of the three regioisomers would give some insight into the structure-activity relationship of this class of compounds. Nemorosone II (**150**) is the name we have assigned to the regioisomer that has the benzoyl group at the C-5 position and a prenyl at the C-1, which is the reverse of nemorosone (**4**) (Figure 3.0.1). Although some reports of nemorosone II (**150**) exist in the literature, it is believed that they are mis-assignments caused by the confusion in the initial nemorosone (**4**) isolation and characterisation.[48] The work described within this chapter aims to clarify these mis-assignments of nemorosone (**4**) and constitutes the first synthesis of a type C PPAP.

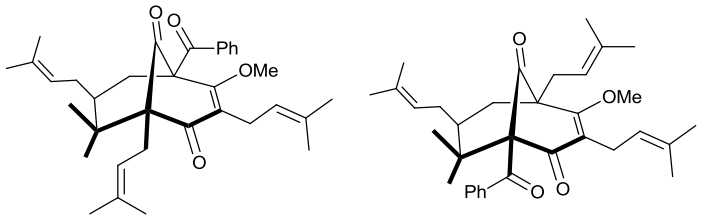



**3** clusianone **4** nemorosone **150** nemorosone II

**Figure 3.0.1**

## 3.1 Reported Isolation

The first claim was made of the isolation of "*O*-methylated nemorosone II" (**156**) by de Oliveira *et al*. in a follow-up paper to their isolation of "*O*-methylated nemorosone" (**154**) (Figure 3.1.1).[49] Using the same procedure as before (collection of the resin by scraping it off the flowers with a glass rod and dissolving it in diethyl ether or ethyl acetate, followed by treatment with diazomethane to make the methyl enol ethers), de Oliveira isolated a methyl enol ether protected PPAP that had the same mass as "*O*-methylated nemorosone" (**154**) but different NMR spectra. Analysis of the NMR spectra led de Oliveira to assign the structure as a type A PPAP, complementary to nemorosone (**4**) which was believed to have a type C structure at this point.

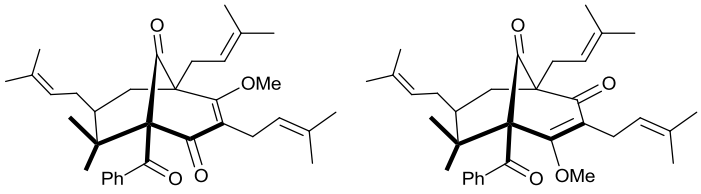



**154** "*O*-methylated nemorosone" **156** "*O*-methylated nemorosone II"

**Figure 3.1.1** de Oliveira's assignment of "*O*-methylated nemorosone" (**154**) and "*O*-methylated nemorosone II" (**156**)

However, it was later shown by Cuesta-Rubio, using more in-depth NMR experimentation, that the assignment of "*O*-methylated nemorosone" (**154**) was incorrect and nemorosone (**4**) really had the type A structure.[73] This obviously left a question over the true structure of "nemorosone II" (**155**). In their isolation the Cuesta-Rubio group had isolated two methyl enol ether regioisomers of nemorosone **149** and **157**, with the spectra of one already being shown to correspond to de

Oliveira's "*O*-methylated nemorosone" (**154**). By comparison of the NMR spectra for the other regioisomer of *O*-methylated nemorosone (**149**) to that of de Oliveira's "*O*-methylated nemorosone II" (**156**), Cuesta-Rubio showed that they were in fact the same compound (Figure 3.1.2). The result of this was that nemorosone II (**150**) as the type C regioisomer (with C-1 prenyl group and C-5 benzoyl group) of nemorosone (**4**) had not in fact been isolated at all.

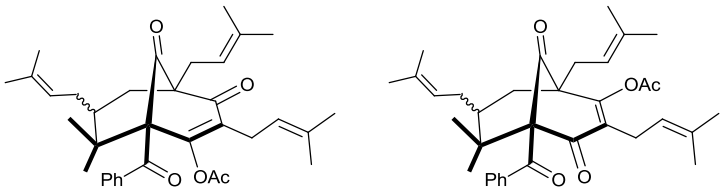



**149** **157**

**Figure 3.1.2**

Lokvam *et al.* studied what they believed to be nemorosone II (**150**) in 2000 and observed the biological effects on bacteria.[51] Unfortunately, this compound was clarified by Cuesta-Rubio as the minor methyl enol ether derivative of nemorosone (**149**) during the nemorosone (**4**) re-assignment.

Also around the time of the reassignment of nemorosone (**4**) to the type A structure, Henry *et al*. isolated a pair of tautomers which were regioisomeric with nemorosone (**4**).[55] The tautomeric pair were named plukenetione D and E (**191** and **192**), isolated as the acetates and assigned a type A structure although the stereochemistry at C-7 was left undefined (Figure 3.1.3).




**191** plukenetione D **192** plukenetione E

**Figure 3.1.3**

It would appear that Henry isolated the acetates of the tautomers of 7-*epi*-nemorosone (**195** and **196)** and assigned them to a new compound as de Oliveira had not yet published the nemorosone II (**150**) assignment and Cuesta-Rubio had not highlighted the mistaken identity of these compounds.

In 2000, Grossman and Jacobs tried to clarify the identity of plukenetione D and E (**191** and **192**) with regards to nemorosone (**4**) and nemorosone II (**150**).[52] In this paper they attempted to compare the NMR spectra for the methyl enol ethers of nemorosone (**149** and **157**) and one of 7-*epi*-nemorosone (**160**) with the acetates of plukenetione D and E (**191** and **192**). As direct comparison could not be carried out, characteristic signals in the NMR spectra were compared, in particular the Δδ of the ring $CH_2$ ($^1H$) and *geminal* methyls ($^{13}C$) and the C-7 ($^{13}C$) shifts of *O*-methylated nemorosone (**149** and **157**), and one methyl enol ether of 7-*epi*-nemorosone (**160**) (Figure 3.1.4).

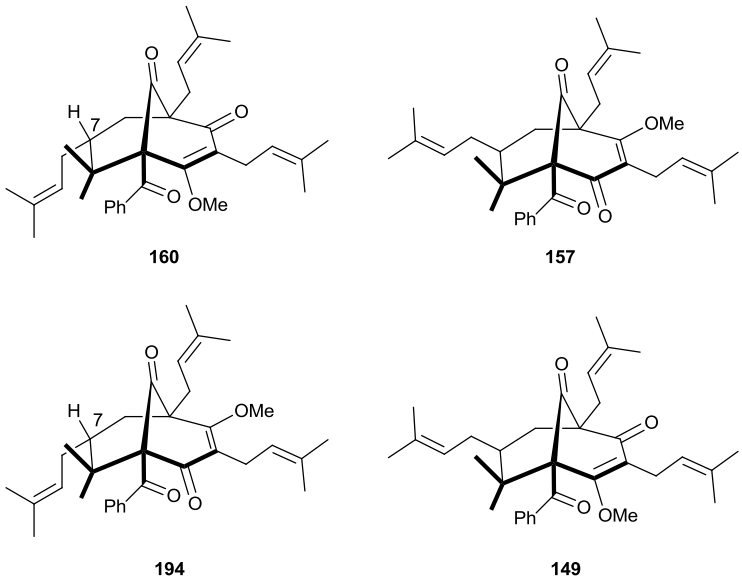



**Figure 3.1.4**

Through these comparisons they concluded that plukenetione D and E (**191** and **192**) related to *O*-methylated 7-*epi*-nemorosone (**194**). Since the structure of nemorosone (**4**) and *O*-methylated 7-*epi*-nemorosone (**160**) have been corrected Grossman's assignment of plukenetione D and E actually shows that plukenetione D and E are the tautomers of 7-*epi*-nemorosone (**195** and **196**) (Figure 3.1.5).

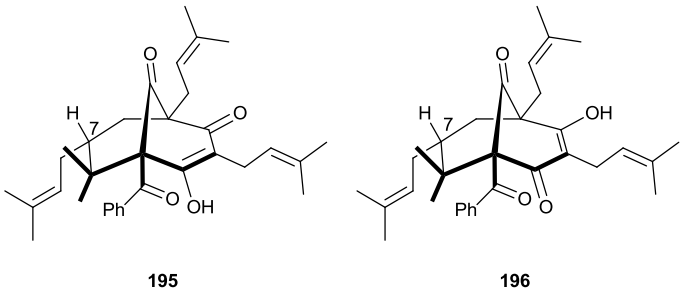
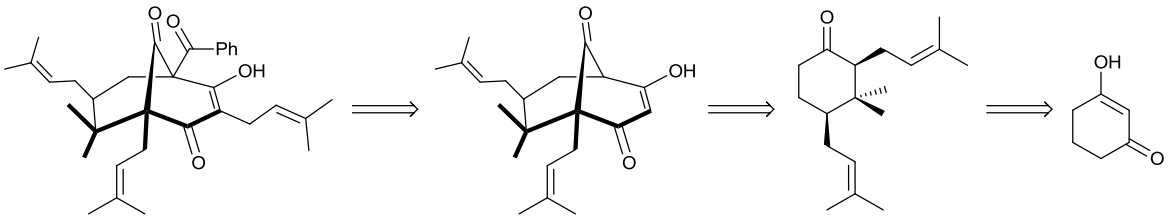



**Figure 3.1.5**

After thorough analysis of the literature it would appear that no type C regioisomer of nemorosone (**4**) has yet been isolated from natural sources. Therefore, the challenge of making a novel unknown type C PPAP was undertaken.

## 3.2 Retrosynthesis

Much of the ground work for this synthesis was already in place from our synthesis of clusianone (**3**).[40] During the synthesis of clusianone (**3**) the bicyclo[3.3.1]nonane-trione core was formed by the Stoltz modification of the Effenberger cyclisation, giving the bicyclo[3.3.1]nonane system **114** with a prenyl group appended to the C-1 position (Chapter 1.5).[25,33] Acylation of the C-5 position could be envisaged by direct deprotonation of the bridgehead and an electrophilic quench using benzoyl chloride. The final C-3 prenyl group would be added using a cuprate mediated deprotonation and reaction of the anion with prenyl bromide (Scheme 3.2.1).



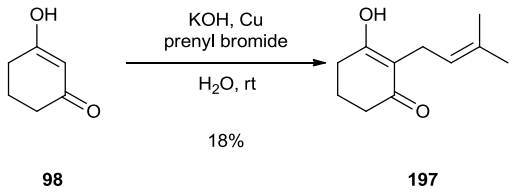

**150** **114** **111** **98**

**Scheme 3.2.1**

## 3.3 Synthesis of Nemorosone II

Using chemistry described previously during the synthesis of clusianone (**3**) the bicyclo[3.3.1]nonane-trione core was built up.[40] Starting with 1,3-cyclohexanone

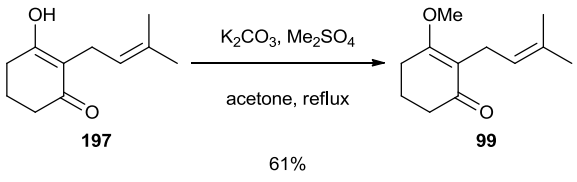
(**98**), prenylation was affected by copper catalysed deprotonation and enolate formation. The enolate underwent an electrophilic quench with prenyl bromide, to give the desired product 3-hydroxy-2-(3-methyl-but-2-enyl)-cyclohex-2-enone (**197**) in a disappointing 18% yield (Scheme 3.3.1).




**98** **197**

**Scheme 3.3.1**

Prenylated enone **197** was protected as the methyl enol ether **99**. This was formed by refluxing the substrate in acetone with dimethyl sulphate and potassium carbonate to give a 61% conversion to the desired product 3-methoxy-2-(3-methyl-but-2-enyl)-cyclohex-2-enone (**99**) (Scheme 3.3.2). The yield for these reactions was low compared to those performed by Dr. Rodeschini but produced enough material for them not to need repeating.



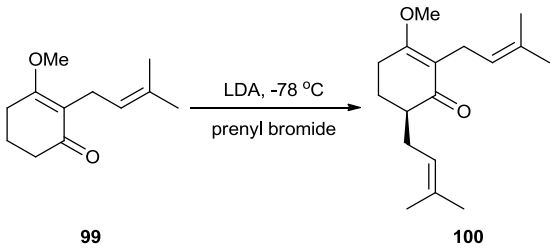

**197** **99**

61%

**Scheme 3.3.2**

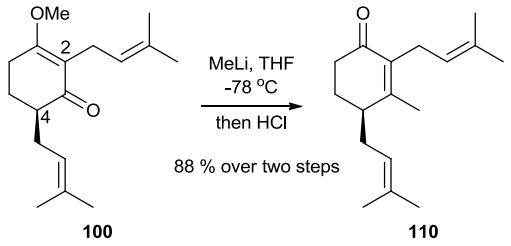
Selective prenylation of the C-4 position was carried out by enolate formation and quenching the anion by addition of prenyl bromide. This gave a diprenylated product

**100** which was sufficiently pure for use in subsequent reactions without further purification (Scheme 3.3.3).



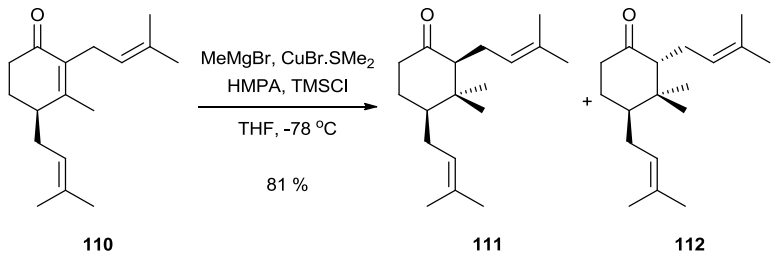

**Scheme 3.3.3**

Methyl vinylogous ester **100** with prenyl substituents at C-2 and C-4 (that would become the C-1 and C-7 prenyl groups in nemorosone II (**150**)) possessed almost all the functionality required prior to the Effenberger cyclisation. The only further elaboration required was the installation of the methyl groups that would become the C-8 *geminal* dimethyls in the bicyclo[3.3.1]nonane system. Treatment of the crude methyl vinylogous ester **100** with MeLi followed by an HCl workup installed the first methyl at the C-3 position in excellent overall yield to give enone **110** (Scheme 3.3.4).



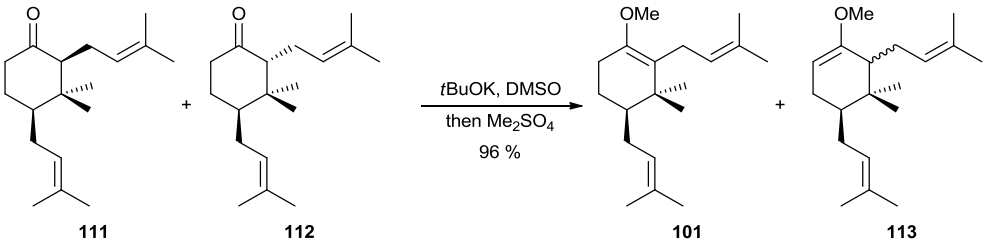

**Scheme 3.3.4**

The second of the methyl groups was then introduced using a copper mediated reaction with a Grignard reagent to give selective addition at the C-3 position. The reaction afforded the ketones **111** and **112** as two stereoisomers in a ratio of 85:15 in 81% yield (Scheme 3.3.5).




**Scheme 3.3.5**

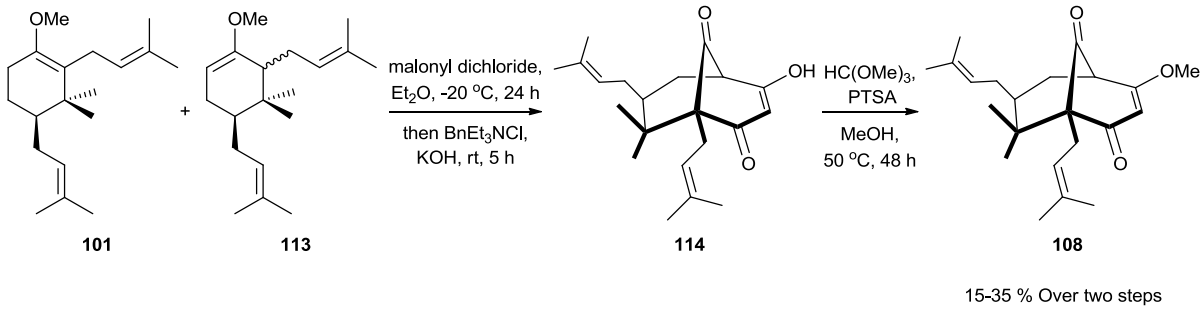
The ketones **110** and **111** were transformed into methyl enol ethers **101** and **113** using potassium butoxide and dimethyl sulphate in DMSO. The transformation proceeded in 96% yield forming two regioisomers which were separable by chromatography (Scheme 3.3.6).




**Scheme 3.3.6**

For the Effenberger annulations it had been demonstrated that it was not necessary to separate the two isomers **101** and **113**. Hence, the mixture of isomers was treated under our standard Effenberger-type conditions (one equivalent of malonyl dichloride

for 24 h at -20 °C followed by KOH solution for 5 h at rt) to form the C-1, C-7 diprenylated bicyclo[3.3.1]nonane core **114**. The bicyclo[3.3.1]nonane-trione **114** was not purified at this stage due to its high polarity and relative instability, but was instead methylated directly. Methylation of the bicyclo[3.3.1]nonane-trione **114** was performed under acidic conditions, which gave just one regioisomer **108** with the methyl group installed at the C-4 oxygen, distal to the bulk of the prenyl substituent at C-1. Although the Effenberger cyclisation was low yielding we continued to use it as it enables rapid access to a well developed bicyclo[3.3.1]nonane system. Furthermore ketones **111** and **112** are recovered alongside the desired product **108** and can be recycled (Scheme 3.3.7).

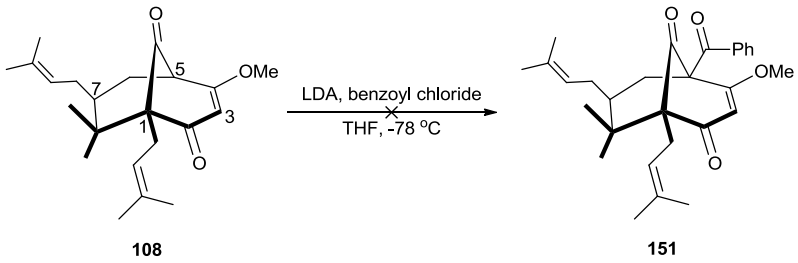



**Scheme 3.3.7**

At this stage the synthesis diverges from that of the clusianone (**3**) synthesis. The yields were comparable to those of Dr. Rodeschini for each of the above steps.

Clusianone (**3**) has a prenyl at C-5 and a benzoyl group in the C-3 position whereas nemorosone II (**150**) has these groups interchanged. At this point a project student in our lab, Peter Crick, took over the synthesis and established the steps necessary to synthesise nemorosone II (**150**) but was unable to perform the final deprotection.

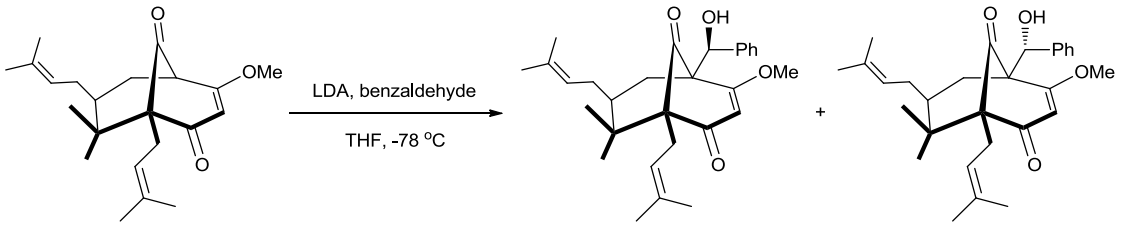
Direct acylation at C-5 was initially attempted using a benzoyl chloride quench of the bridgehead enolate, however only starting material was recovered (Scheme 3.3.8).




**108** **151**

**Scheme 3.3.8**

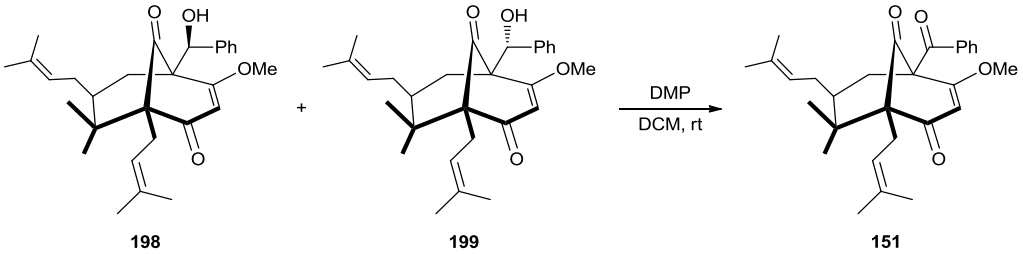
Therefore, it was decided to treat the bicyclo[3.3.1]nonane **108** with benzaldehyde to make the secondary alcohol products **198** and **199** that could be oxidised to the ketone **151**. The secondary alcohol was formed successfully using LDA and benzaldehyde, giving the product as two diastereoisomers **195** and **196**. Selectivity was not an issue as the stereocentre would be lost during the oxidation to the desired ketone (Scheme 3.3.9).




**108** **198** **199**

**Scheme 3.3.9**

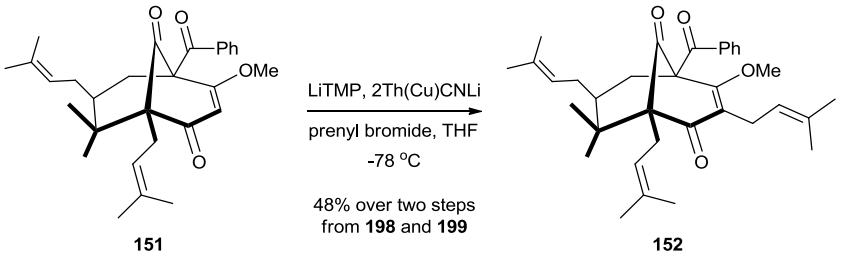
The oxidation of the mixture of secondary alcohols **198** and **199** was achieved using Dess-Martin periodinane in DCM at rt. The reaction proceeded smoothly and the product was sufficiently pure to be used without further purification (Scheme 3.3.10).




198 199 151

**Scheme 3.3.10**

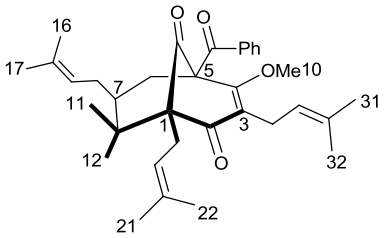
The final prenyl group was added at the vinylic position (C-3) using a mixed higher order cuprate reaction. Selective deprotonation of the C-3 position was performed with LiTMP. Transmetallation to the cuprate gave the organometallic which underwent successful reaction with prenyl bromide to give *O*-methylated nemorosone II (**152**) (Scheme 3.3.11).




151 152

**Scheme 3.3.11**

As its methyl vinylogous ester **152**, nemorosone II was stable and could be easily handled and purified. The $^{1}$H NMR spectra at this stage shows no signal around δ 6.00 which would correspond to the C-3 proton. Furthermore the δ 5.00 region shows a multiplet with the integration of one proton that relates to vinylic proton of a prenyl group which corresponds with the desired product (Figure 3.3.1).




**152**

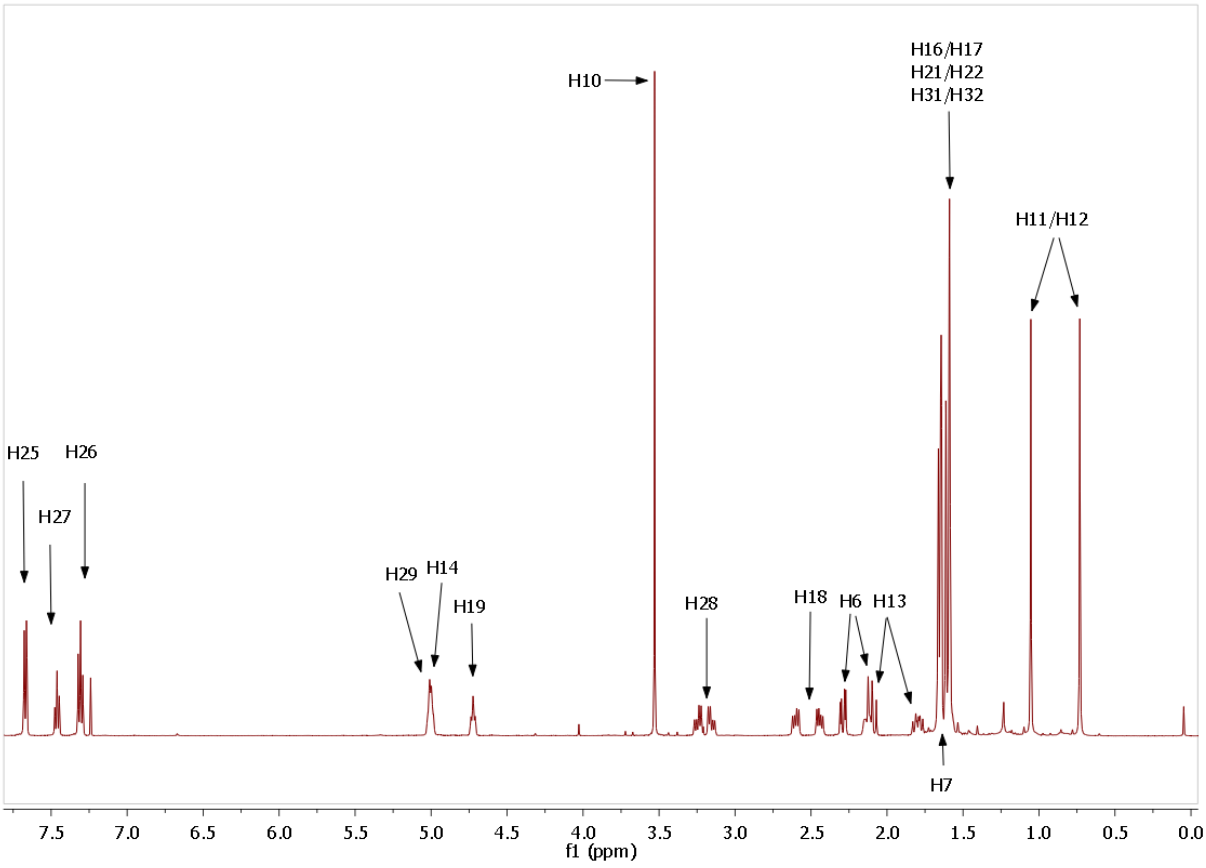



**Figure 3.3.1** $^{1}$H NMR of *O*-methylated nemorosone II (**152**) (500 MHz, $CDCl_3$)

At the methyl vinylogous ester stage, the $^{1}$H and $^{13}$C NMR spectra could easily be compared to those of nemorosone (**4**); as expected they show distinct differences. Looking at the proton NMR spectra it would be hard to confuse the two compounds. Although both spectra show similar signals from the protons, the δ values are not coincident (Figure 3.3.2).

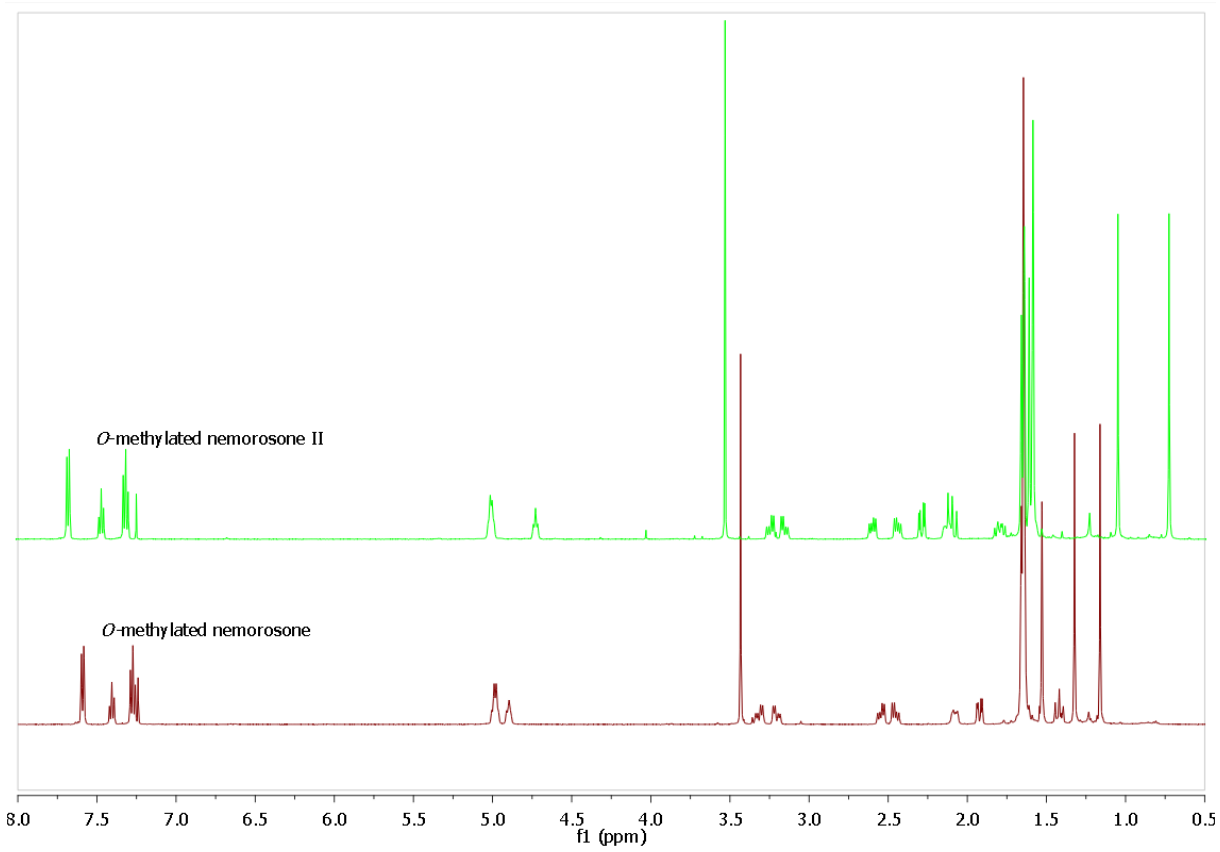



**Figure 3.3.2** Comparison of $^{1}H$ NMR of *O*-methylated nemorosone (**157**) and *O*-methylated nemorosone II (**152**) (500 MHz, $CDCl_3$)

The same is true of the $^{13}C$ NMR spectra. Again these shows similar signals for the various carbon environments in the areas that one would expect them but the shifts are different between the two compounds (Figure 3.3.3). It is clear from these NMR spectra that nemorosone (**4**) and nemorosone II (**150**) are spectroscopically very different molecules and may be differentiated by NMR analysis.

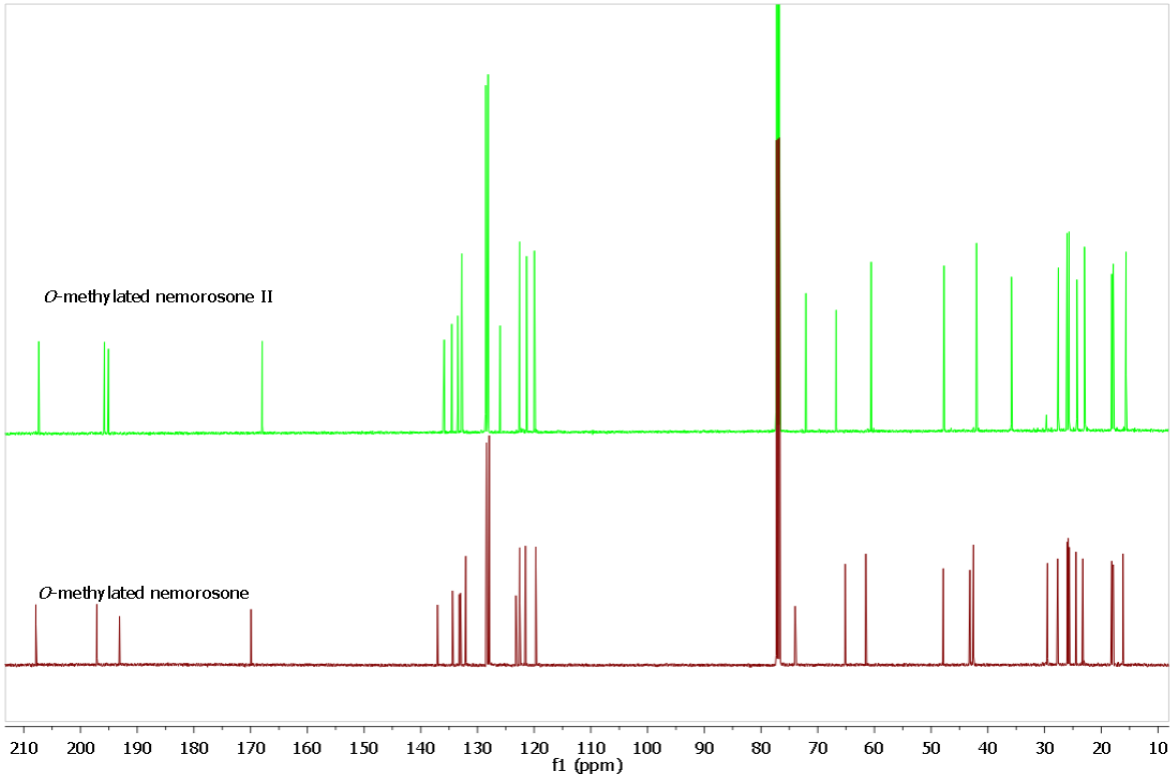
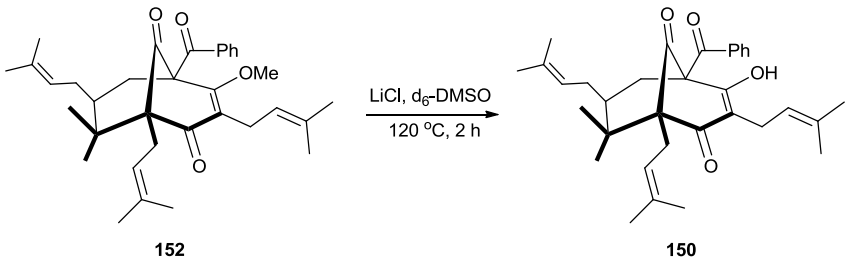



**Figure 3.3.3** Comparison of $^{13}C$ NMR of *O*-methylated nemorosone (**157**) and *O*-methylated nemorosone II (**152**) (125 MHz, $CDCl_3$)

The final step in the synthesis required *O*-methylated nemorosone II (**152**) to be deprotected, leading to the first synthetically produced type C PPAP, nemorosone II (**150**). Based on our previous work on this class of compounds the Krapcho conditions were favoured once more (Scheme 3.3.12).[58]




**152** **150**

**Scheme 3.3.12**

Our previous work with nemorosone (**4**) had shown that the tetra ketone products could be very sensitive to acidic conditions, light and air. This difficulty had been overcome by performing the final deprotection in $d_6$-DMSO, which allowed NMR experiments to be performed upon the crude reaction mixture without the need for work up or exposure to air. In this way it was possible to obtain NMR spectra for nemorosone II (**150**), which appears to exist as a single tautomer as evidenced by the presence of just one compound in the NMR spectra. As with the nemorosone (**4**) synthesis the final compound was extracted from the $d_6$-DMSO and NMR spectra obtained in $d_4$-MeOD (Figure 3.3.4 and Figure 3.3.5).

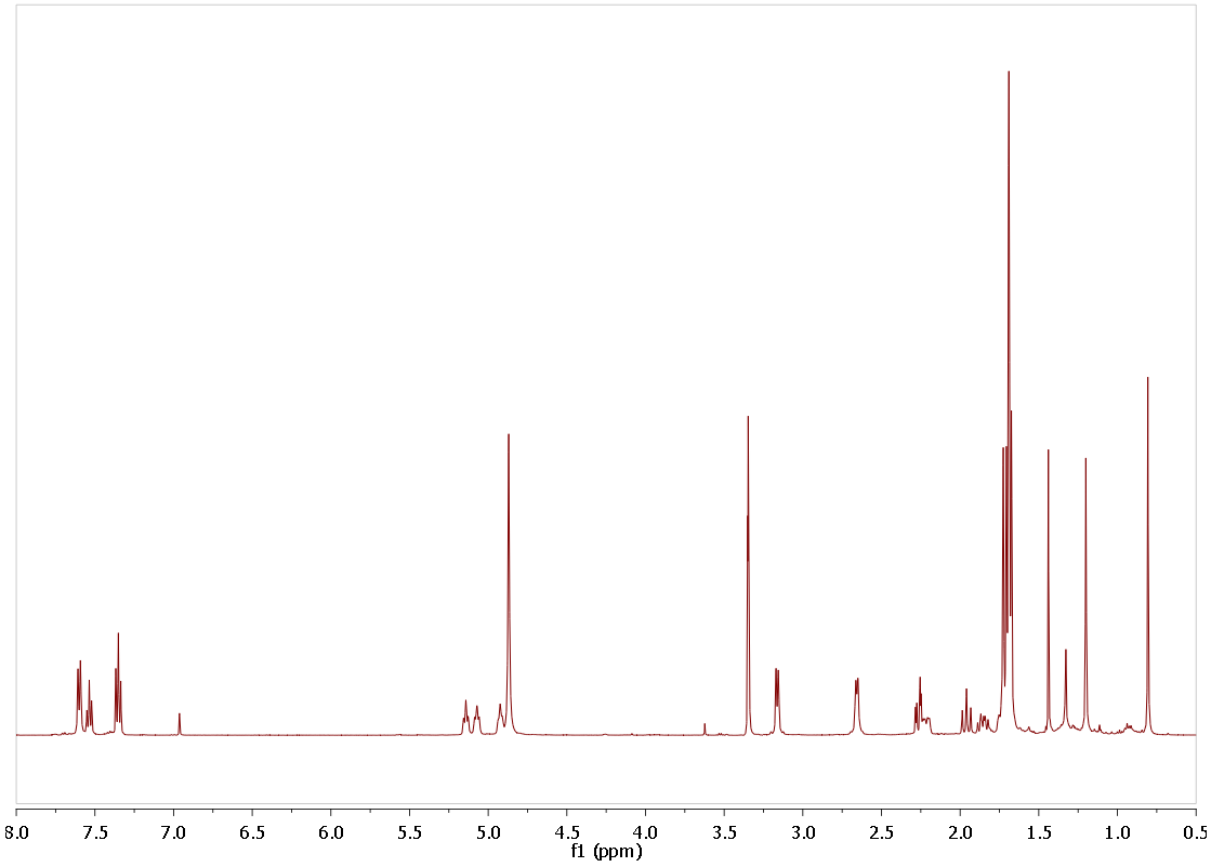



**Figure 3.3.4** $^1$H NMR of nemorosone II (**150**) (500 MHz, MeOD)

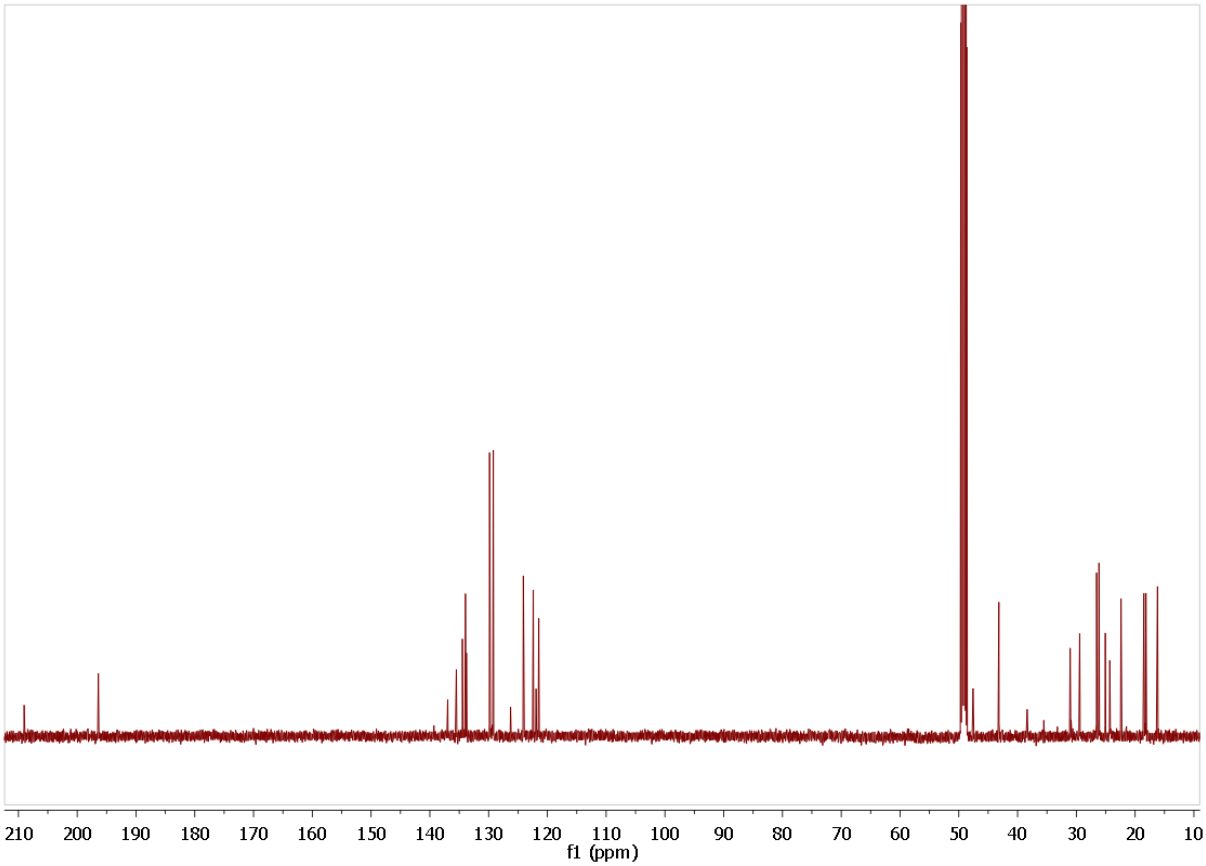



**Figure 3.3.5** $^{13}$C NMR of nemorosone II (**150**) (125 MHz, MeOD)

## 3.4 Conclusion

The first successful synthesis of nemorosone II (**150**), a type C PPAP, as yet unknown in nature, was completed in 10 steps from commercially available 1,3-cyclohexanone (**98**) in a 1.5% yield. This compound completes the set of type A, B and C regioisomers. Nemorosone II (**150**) was sent for biological screening along with nemorosone (**4**) and clusianone (**3**) in the hope that some insight into the structure-activity relationship of these compounds could be gained. The results of these studies can be found in Chapter 4.

# Chapter 4

# Biological Studies

Part of the reason for synthesising nemorosone (**4**) and nemorosone II (**150**) was to study the structure-activity relationship for PPAPs. As clusianone (**3**) had already been separated into its enantiomers by kinetic resolution, these could be used alongside the separated enantiomers of nemorosone (**4**) and nemorosone II (**150**) to study the relative activity of the type A, B and C PPAPs. Biological studies were performed by Frank Holtrup at The German Cancer Research Centre.

## 4.1 Previous Biological Studies on Nemorosone

The Diaz-Carballo group have investigated the activity of nemorosone (**4**) against various cancers including breast, colon, ovary, liver and lung cancer.[61] More recently, they have investigated the effect of nemorosone (**4**) on neuroblastoma cancer cell lines (LAN-1 and NB69).[70] It was shown that nemorosone (**4**) was active in parental neuroblastoma cells (LAN-1) as well as clones (NB69) that were resistant to adriamycin, cisplatin, etoposide or 5-fluorouracil. The cell studies showed that nemorosone (**4**) treatment at an 8 μM concentration induced an accumulation of cells in the G0/G1 phase and subsequent reduction in cells in the S phase of the cell cycle after 24 h incubation. It was proposed that this effect was due to the dose dependent (seen from 4 μM upwards) up-regulation of p21, an important protein in the control of cell cycle progression. Their cytotoxicity studies also showed that nemorosone (**4**) treatment caused a reduction in cell numbers ($IC_{50}$ value in the region of 6.5 μM) due

to the initiation of apoptosis, producing DNA fragmentation and caspase up-regulation and activation. It was also discovered that simultaneously treated fibroblasts were more resistant to nemorosone (**4**) than the neuroblastoma cells, making nemorosone a suitable selective agent for treatment of neuroblastoma tumours.

In a recently published work, Frank Holtrup has extensively studied the activity of nemorosone (**4**) on a selection of pancreatic cancer cell lines.[59] The studies were carried out on MIA-PaCa-2 (an early stage pancreatic cancer cell line) alongside the later stage cancer cell lines AsPC-1 (gut lining) and Capan-1 (liver). Studies were performed to demonstrate the selective action of nemorosone (**4**) against cancer cell lines, while leaving non-cancerous fibroblasts relatively untouched. Nemorosone (**4**) shows cytotoxicity in a dose and time dependent manner with a much greater effect on the pancreatic cancer cells than on the fibroblasts. There was an 80% reduction in the cancer cell number after 10 μM treatment, with only a negligible effect seen on fibroblast numbers (Figure 4.1.1.A). Further flow cytometry and assays provided some insight into the mode of action of nemorosone (**4**) against these cancer cells. Nemorosone (**4**) targets the cell cycle checkpoints that exert control over the progression of cells through the cell cycle required for them to grow and divide. At low concentrations, nemorosone (**4**) exhibits a G1 blocking effect, but a G2 blocking effect is seen at higher concentrations (Figure 4.1.1.B). The G1 block is controlled by the p21 (protein) which normally binds to the cyclin D1,2,3/CDK4,6 complex and inactivates it. If the cell is ready for progression into S phase, the p21 dissociates, allowing the complex to activate and phosphorylate a downstream protein (Rb). The

phosphorylated Rb then activates E2F, a gene transcription factor (DNA synthesis initiator), to start DNA synthesis. Studies demonstrated that nemorosone (**4**) up-regulates p21 and down-regulates E2F, decreasing the progression into DNA synthesis (S phase). The G2 block is controlled by the GADD45α protein which normally binds to the cyclin B/cdc-2 complex. If the cell is allowed to progress into G2 the GADD45α dissociates from the complex allowing cdc-25 to activate it. Nemorosone (**4**) up-regulates the production of GADD45α causing the proportion of cyclin B/cdc-2 bound to increase, inactivating it and halting the cell cycle. Targeting cell replication is important in cancer treatment due to the different replication rates of cancerous and non-cancerous cells. Cancer cells replicate at a much higher rate than normal cells, and so will be affected to a greater degree by agents that regulate replication.

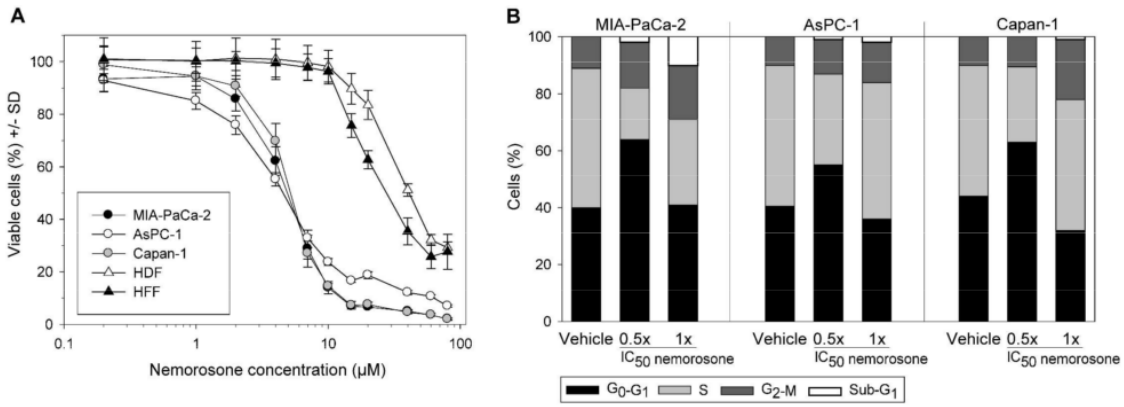



**Figure 4.1.1 with permission of Frank Holtrup.**[59] Effects of nemorosone (**4**) on the viability and cell cycle of pancreatic cancer cells and fibroblasts. A: Cell viability assay. B: Cell cycle analysis.

Apoptosis is programmed cell death which is initiated if a cell is unable to repair damage to its DNA or organelles. It is a highly controlled process involving regulated

signalling pathways and the activation of a cascade of specific enzymes called caspases. DNA staining using propidium iodide was used to examine the DNA fragmentation in the cells treated with nemorosone (**4**). This was seen to be dose dependent, with higher concentrations producing more fragmentation. The largest amount of fragmentation was seen in the MIA-PaCa-2 cell line (early stage cancer in the pancreas) but the fibroblasts were spared any DNA fragmentation even at 50 μM doses.

Activation of caspases was investigated using fluorescent probes (FLICA assay) that bind covalently to only the activated forms of the enzymes offering great sensitivity (can detect normal levels of apoptosis in untreated cells). The addition of nemorosone (**4**) to the different cancer cell lines showed that caspase activation is dose dependent, but selective in different cell types. The early stage cancer cells (MIA-PaCa-2) showed only caspase 3/7 activation while the later stage cancer cells (AsPC-1 and Capan-1) showed the presence of caspase 8 as well as 3/7 (Figure 4.1.2). This difference is explained since caspase 3/7 is activated as part of the intrinsic apoptotic pathway while caspase 8 is part of a different, extrinsic, apoptotic pathway.

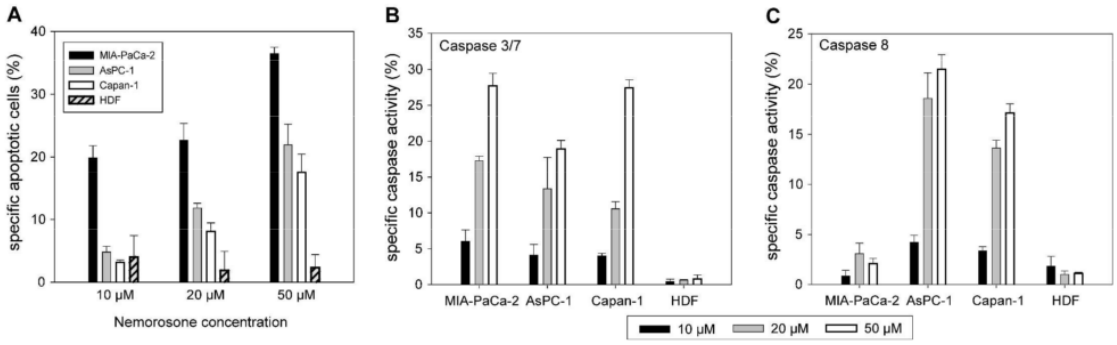



**Figure 4.1.2 with permission of Frank Holtrup.**[59] Analysis of nemorosone (**4**)-induced apoptosis in pancreatic cancer cells and fibroblasts by flow-cytometric assessment of apoptotic DNA fragmentation and caspase acitivation. A: Percentage of cells demonstrating nemorosone (**4**) specific apoptotic DNA fragmentation. B: Percentage of cells demonstrating nemorosone (**4**) specific activation of caspase 3/7. C: Percentage of cells demonstrating nemorosone (**4**) specific activation of caspase 8.

Collapse of the electrochemical gradient across the mitochondrial wall is one of the first events to occur when apoptosis is initiated in a cell. JC-1 is a fluorescent cationic dye used to show this collapse. In healthy cells, the dye localises to the mitochondria and aggregates causing it to fluoresce red. Once the mitochondrial gradient collapses, the JC-1 cannot localise in the mitochondria and thus remains in the cytoplasm as monomers which fluoresce green. Nemorosone (**4**) shows a shift of JC-1 into the cytoplasm starting at 5 µM in the cancer cell lines with full collapse at 20 µM, but no shift from the mitochondrial localisation is seen in fibroblasts until 20 µM with full collapse not observed until 160 µM (Figure 4.1.3). A time study in which prestained cells were treated with 20 µM nemorosone (**4**) was set up and the fluorescence measured every ten seconds after treatment. The response was very

rapid with the membrane potential reducing within seconds and the red/green ratio was inverted within two minutes.

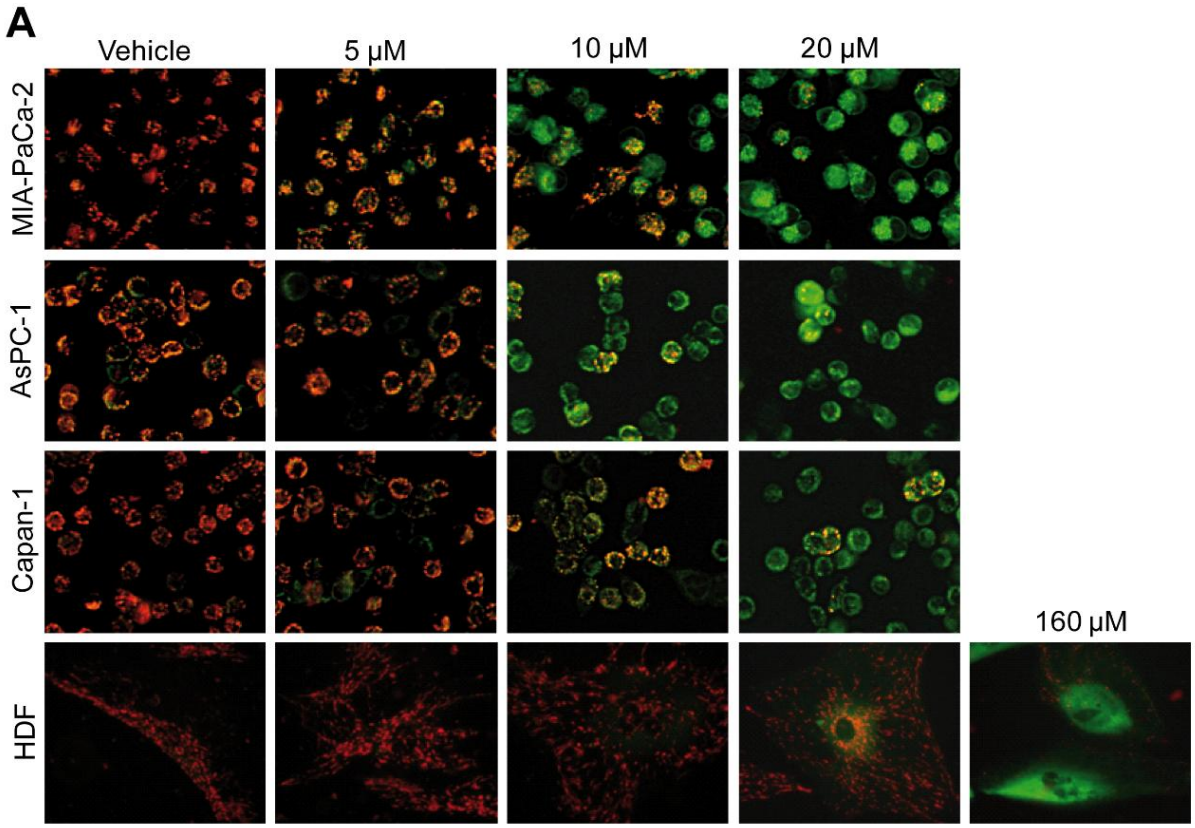



**Figure 4.1.3 with permission of Frank Holtrup.**[59] Analysis of effects of nemorosone (**4**) on mitochondrial membrane potential ΔΨm and cytosolic calcium concentration $[Ca^{2+}]c$ in pancreatic cancer cells and fibroblasts. A: Cells were treated with the indicated concentrations of nemorosone (**4**) or vehicle for 4 h and stained with voltage-dependent dye JC-1 prior to fluorescence microscopy. Intact mitochondria fluoresce red while cells with aborated mitochondrial membrane potential ΔΨm exhibit cytosolic green fluorescence.

MIA-PaCa-2 cells were treated with 100 µM and 200 µM nemorosone (**4**) and assayed for changes in intracellular $Ca^{2+}$ concentration. $Ca^{2+}$ plays an essential role in the control of many intracellular pathways and altering $Ca^{2+}$ homeostasis could

explain the action of nemorosone (**4**) on the apoptosis initiation. Again, a lower effect is observed on fibroblasts compared with cancer cells but much higher concentrations of nemorosone (**4**) are required for an effect to be seen on $Ca^{2+}$ concentrations in any of the cancerous cell lines. Previous research has shown that mitochondria take up $Ca^{2+}$ to preserve intracellular concentration, but that this can lead to $Ca^{2+}$ overload and collapse of the membrane potential possibly linking to the activity of nemorosone (**4**).[74-75] It is believed that the increase in intracellular $Ca^{2+}$ is caused by release from the endoplasmic reticulum (ER).

Genetic analysis of the three cell lines, using RNA collected from treated cells, shows which genes are affected by the treatment with nemorosone (**4**); either being up-regulated or down-regulated. Modelling was used to find out how the affected genes expressed in all three cell lines were linked. They all appeared in the signal pathways of the ER stress response or further downstream. These responses are initiated by the occurrence of a build-up of unfolded or misfolded proteins in the ER caused by alterations in the $Ca^{2+}$ homeostasis. After a prolonged period of stress, the cell ceases repair and enters apoptosis instead.

DDIT3 is one gene strongly induced as a result of nemorosone (**4**) treatment and produces a transcription factor controlling the expression of the BCL2 family of genes involved in apoptosis. Accumulation of DDIT3 in a cell initiates the ER stress response to cause cells to enter apoptosis. This occurs due to an alteration in the balance of anti- and pro-apoptotic BCL2 family members in favour of apoptosis, eventually leading to the activation of the caspase enzyme cascade. If the mRNA were prevented from being translated into proteins, silencing of DDIT3 would be

observed. In studies, silencing was produced by incorporating siRNA (section of silencing double stranded RNA) into the mRNA causing it to be cleaved before translation took place. When the production of DDIT3 in the MIA-PaCa-2 cells was stopped the effect of nemorosone (**4**) was reduced, demonstrated by a 75% decrease in apoptotic cells after 48 h incubation with 20 μM nemorosone (**4**). The levels of active caspase 9 and 3/7 after 24 h were also reduced, supporting the link between DDIT3 and apoptosis initiation. Capan-1 cells did not show any reduction in DNA fragmentation or in the activity of caspase 3/7 and 8, but decreased activity of caspase 9. This is probably due to the differential activation of caspase 8, normally as a result of the intrinsic and extrinsic pathways for apoptosis. None of the genes with altered expression in the MIA-PaCa-2 cell line were found to be significantly altered in fibroblasts, again supporting the preferential targeting of cancer cells.

ER stress is very likely to occur due to the high metabolic stress (lack of oxygen and nutrients) that occurs within tumours, so control of the ER stress response signalling within tumour cells could be useful in preventing their repair and targeting them for induction into the apoptotic pathway. Fibroblasts have a much lower energy requirement and are therefore less likely to suffer ER stress; meaning that they would be less affected by treatment with nemorosone (**4**).

## 4.2 Proposed Biological Study

The biological activities of nemorosone (**4**) and clusianone (**3**) have been noted already, but comparing the compounds' activity against the MIA-PaCa-2 cancer cell line will allow more about the SAR to be determined, along with some insights into

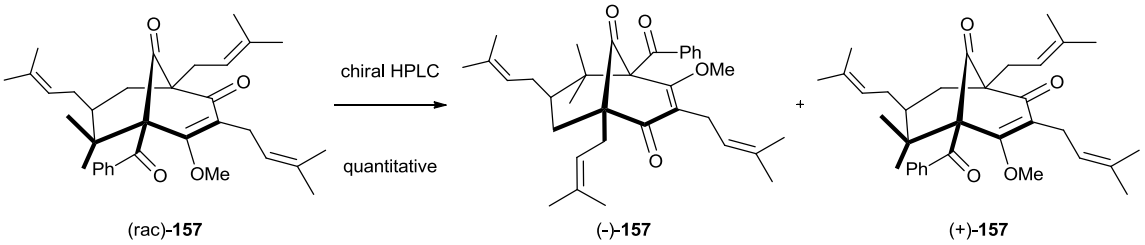
the mode of action. To accurately study the activity of a compound it is necessary to separate the enantiomers, as each enantiomer can exhibit widely different bio-activity. With clusianone (**3**) we had separated the enantiomers using a kinetic resolution.[41] Although the same could be tried with the nemorosone (**4**) and nemorosone II (**150**) systems, they were instead separated by chiral HPLC to access the enantiomers. The benefit of HPLC is that relatively large quantities of material can be separated in a short period of time without fear of incomplete enantioselectivity, and with no need to introduce further synthetic steps. With the enantiomers separated, it would be possible to accurately compare the cytotoxicity of the type A, B and C PPAPs.

Alongside the study of the final compounds, some of the intermediates also presented interesting candidates for study, due to their different substitution around the bicyclo[3.3.1]nonane core. This afforded an insight into the SAR of these molecules. The intermediates were studied as their racemate and indicate which parts of the PPAP structure are necessary for activity against MIA-PaCa-2 cancer cell lines.

## 4.3 Synthesis of Compounds

Application of HPLC on the final compounds was not viable as they were unstable, particularly in the presence of acidic column media. As a result, the compounds were separated into their enantiomers while protected as the methyl vinylogous esters **157** and **152** (Scheme 4.3.1 and Scheme 4.3.3). By doing this, the compounds were stable and easy to manipulate, but it meant separating larger

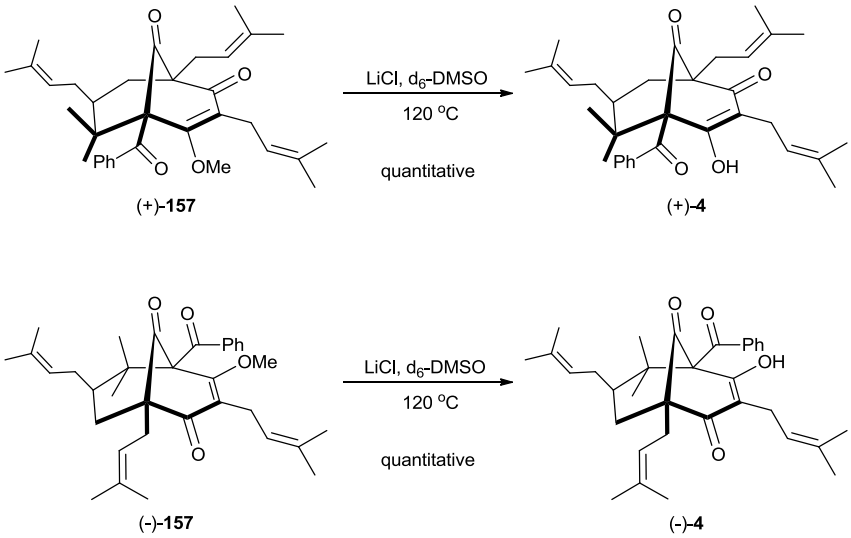
quantities of material as the final hydrolysis step was performed on each individual enantiomer.




**Scheme 4.3.1**

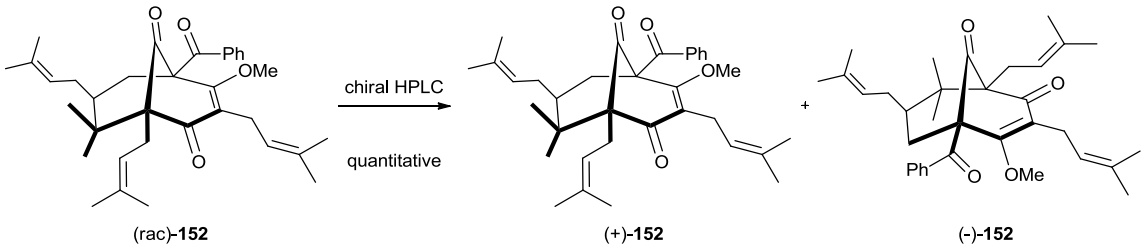
The structures of the separate enantiomers are shown as in the hyperforin (**2**) system, in which the (+) isomer is the one displayed throughout this work. However, nemorosone (**4**) has not had its absolute configuration assigned and the identities of the individual enantiomers have yet to be determined. Nemorosone (**4**) has only been isolated from natural sources as the (+) enantiomer.

A study of various chiral HPLC columns with different solvent systems showed that a Lux cellulose II column and a mobile phase of 1.5% MeOH in hexane offered a baseline separation of the two enantiomers of *O*-methylated nemorosone (**157**). The hydrolysis was performed separately on each methyl vinylogous ester enantiomer and the enantiomers of nemorosone (**4**) were isolated in quantitative yields (Scheme 4.3.2).




**Scheme 4.3.2**

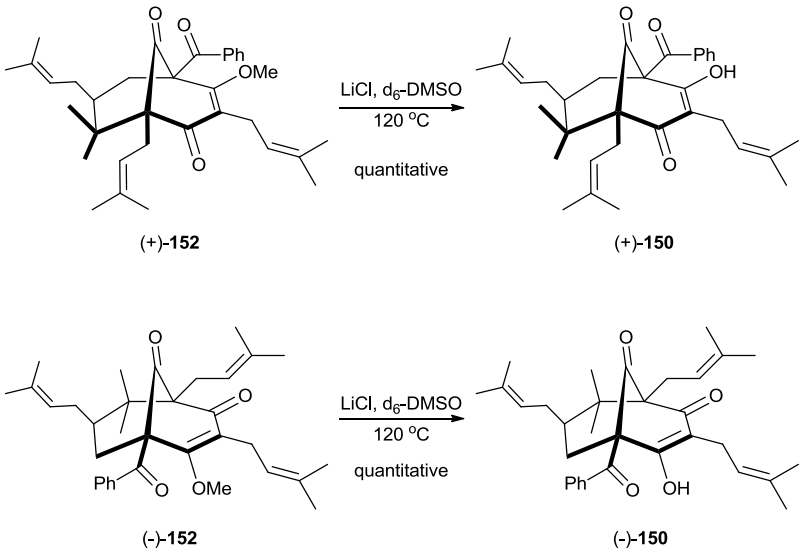
In the clusianone (**3**) synthesis, we saw an inversion of the optical rotation between the methyl vinylogous ester **108** and the final compound (**3**). However, nemorosone (**4**) does not display this trait. The *O*-methylated nemorosone II (**152**) enantiomers were also separated by HPLC on the Lux cellulose II column using 1% IPA in hexane as the mobile phase (Scheme 4.3.3).




**Scheme 4.3.3**

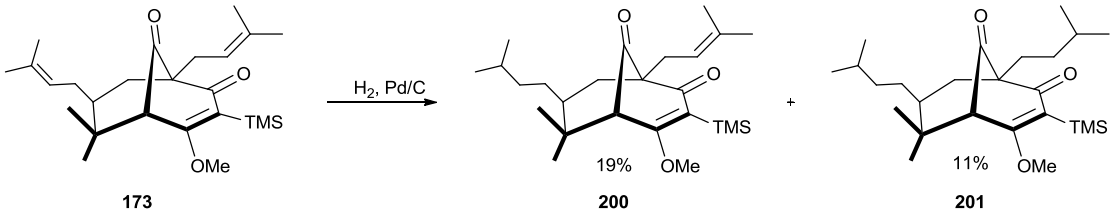
The final hydrolysis step was performed on the *O*-methylated enantiomers of nemorosone II (**152**) separately (Scheme 4.3.4). In this way the enantiomers of

nemorosone (**4**), nemorosone II (**150**) and clusianone (**3**) were separated and tested by Frank Holtrup against the MIA-PaCa-2 cancer cell lines.




**Scheme 4.3.4**

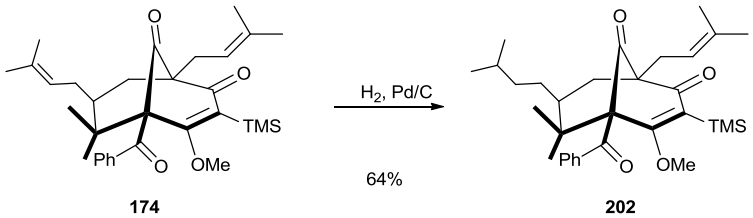
Having studied various intermediates (**144**, **157** and **173-176**) from the synthesis, the effect of prenyl groups and bridgehead ketones on the activity of the molecules was investigated. Thus, the double bonds in the prenyl side chains were hydrogenated. As *O*-methylated nemorosone (**157**) was a valuable product and in short supply, more abundant derivatives were considered. Intermediate **173** possesses most of the functionality of nemorosone (**4**) and was in abundant supply so made a good candidate for further derivitisation. The double bonds were reduced using $H_{2\,(g)}$ over Pd/C catalyst (Scheme 4.3.5).




**173** **200** **201**

**Scheme 4.3.5**

The hydrogenation was successful, although recovery of products was disappointing. The reaction gave a mixture of products; some with total hydrogenation (**201**) and others with only partial hydrogenation (**200**). Analysis of the $^{1}$H NMR spectra allowed us to determine which of the prenyl groups is reduced. The characteristic proton signals of the C-7 prenyl group, which do not coalesce with those of C-5 prenyl group, had been replaced by signals further upfield, including a pair of doublets relating to the C-16 and C-17 methyls. Submission of both compounds for biological analysis allowed the relevance of each prenyl group to be assessed. The same hydrogenation conditions were applied to intermediate **174** (Scheme 4.3.6).

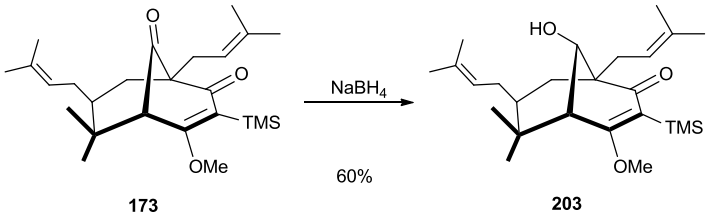



**174** **202**

**Scheme 4.3.6**

Intermediate **174** gave only one product (**202**) when hydrogenated, where the C-7 prenyl group was fully saturated. As before this was confirmed by a change in the characteristic signals in the $^{1}$H NMR spectra.

To investigate the importance of the bridgehead C-9 ketone, the ketone **173** was reduced and the resulting hydroxyl was subjected to biological testing. Reduction was performed using an excess of sodium borohydride and gave intermediate **203** (Scheme 4.3.7).




173 203

**Scheme 4.3.7**

It is assumed that the reduction takes place from the least hindered side of the molecule (across the methyl vinylogous ester rather than across the bulky prenyl group). This would be consistent with what was found in the kinetic resolution of clusianone (**3**) (Scheme 1.5.6).[41]

### 4.4 Results of Biological Study

The samples were sent to Frank Holtrup at the German Cancer Research Centre for testing against MIA-PaCa-2 pancreatic cancer cells. Using a Resazurin cell viability assay it was possible to compare the activity of the PPAPs. The standard procedure for this assay was seeding the cells at 2000 cells/well in 100 µL of Dulbecco's Modified Eagle Medium (DMEM) and incubating them for 24 h at 37 °C with 5% $CO_2$. The cells were then treated with different PPAP concentrations but the DMSO content was kept at 0.25%. The cells were incubated for a further 72 h followed by medium exchange with 50 µL of assay medium (1 mg/mL resazurin 1:50 cell culture

medium). The plates were read with a fluorescence mode plate reader (excited 544 nm, emitted 590 nm) to give dose-response plots.

Investigations of clusianone (**3**), nemorosone (**4**) and nemorosone II (**150**) showed that the position of the acyl group- and therefore the prenyl groups- had a small effect on the bio-activity of these molecules (Figure 4.4.1). Clusianone (**3**) and nemorosone (**4**) showed the greatest activity, and nemorosone II (**150**) was approximately a factor of ten less active. It also showed that neither the (+) nor the (-) enantiomers of the particular compounds were any more effective against the cancer cell line.

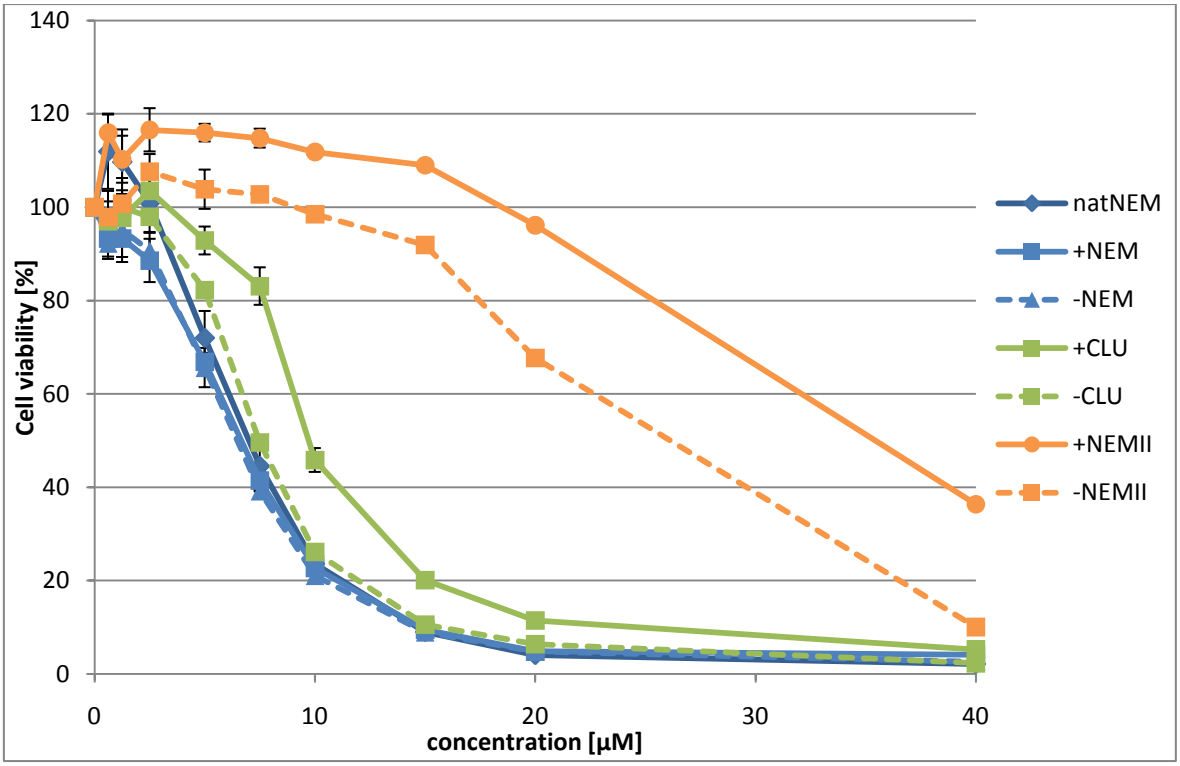



**Figure 4.4.1** Dose-response curves for the enantiomers of nemorosone (**4**), nemorosone II (**150**) and clusianone (**3**).

Figure 4.4.1 shows that the cell viability of the cancer cell line is decreased to less than 50% when treated with 7 μM of (+)-nemorosone (**4**), (-)-nemorosone (**4**) or (-)-clusianone (**3**). (+)-Clusianone (**3**) is slightly less active with an $IC_{50}$ of 10 μM. The (-) enantiomer of nemorosone II (**150**) has an $IC_{50}$ of 25 μM and the (+) enantiomer (**150**) an $IC_{50}$ 35μM.

Some of the derivatives that were produced during the synthesis of nemorosone (**4**) were studied to discover the effect on the molecules bio-activity with different functional groups appended to the bicyclo[3.3.1]nonane system (Figure 4.4.2).

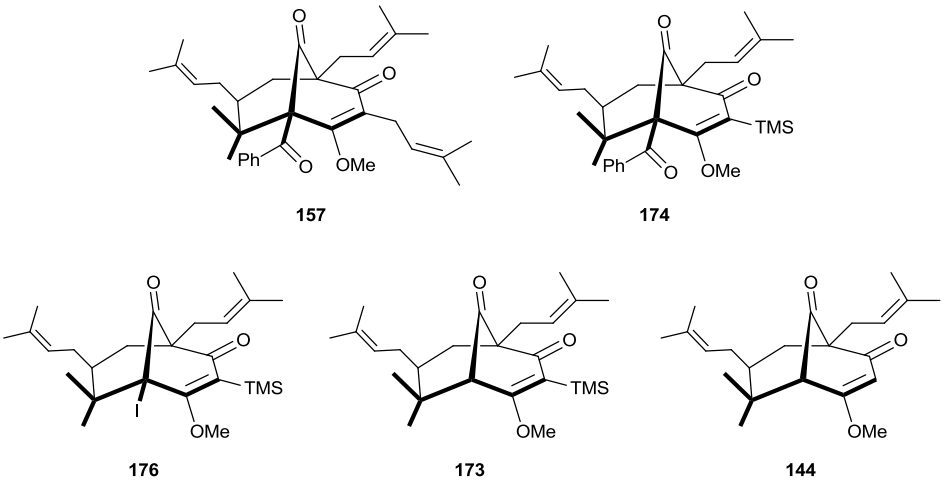



**Figure 4.4.2**

Figure 4.4.3 shows that *O*-methylated nemorosone (**157**) is inactive against the MIA-PaCa-2 cancer cell line. It also shows that by stripping the molecule of much of its functionality, as in the Effenberger product **144**, activity is drastically reduced to an $IC_{50}$ of 40 μM. The compounds **173, 174** and **176** that have functionality at the C-3 vinylic position show activity that was comparable to that of natural nemorosone (**4**).

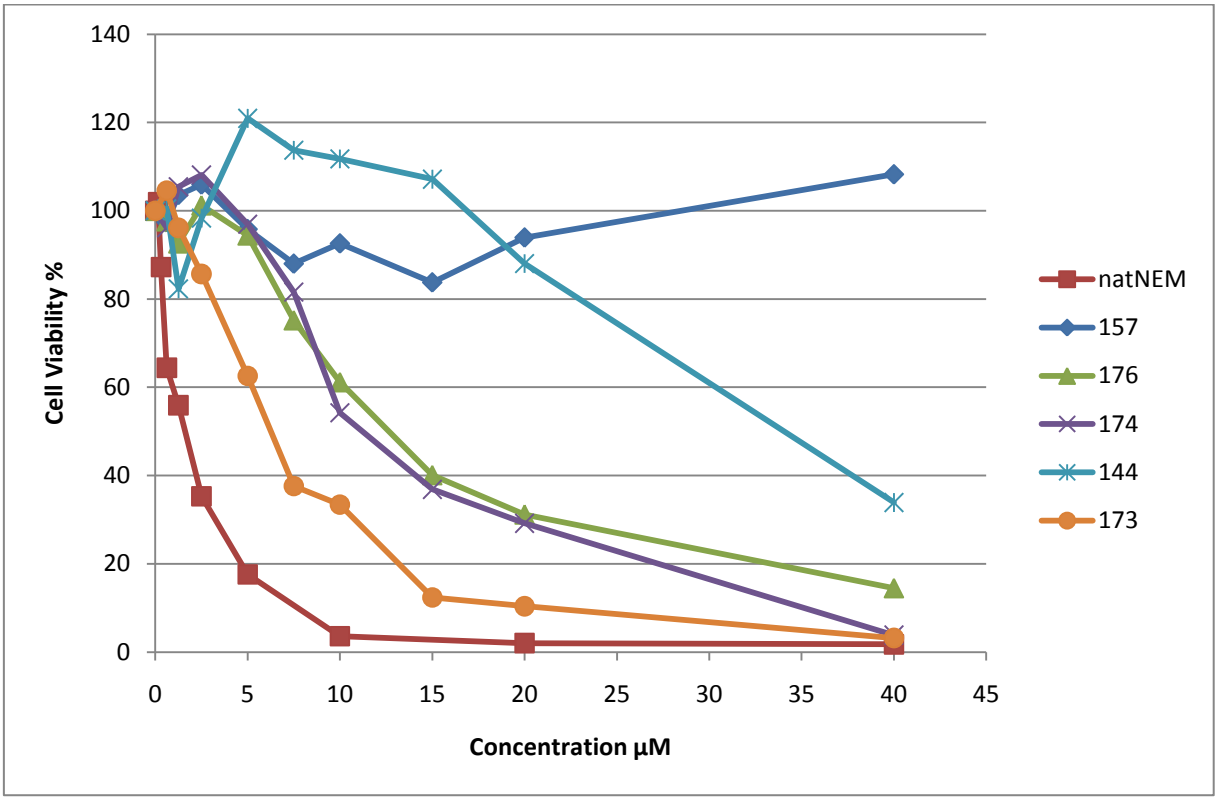
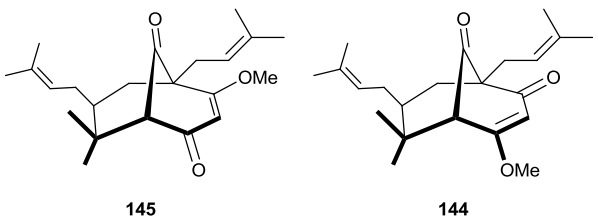



**Figure 4.4.3** Dose-response curves for **144**, **157**, **173**, **174** and **176**.

Having observed the effect that changing the groups appended to the bicyclo[3.3.1]nonane system has on the bio-activity, and the effect of the methylation of the 1,3-diketone system, it was possible to look at the position of *O*-methylation (C-2 or C-4) of the 1,3-diketone part of the bicyclo[3.3.1]nonane. This was done by comparing the activity of the *O*-methylated bicyclo[3.3.1]nonanes **144** and **145** from the Effenberger reaction (Figure 4.4.4).




**145** **144**

**Figure 4.4.4**

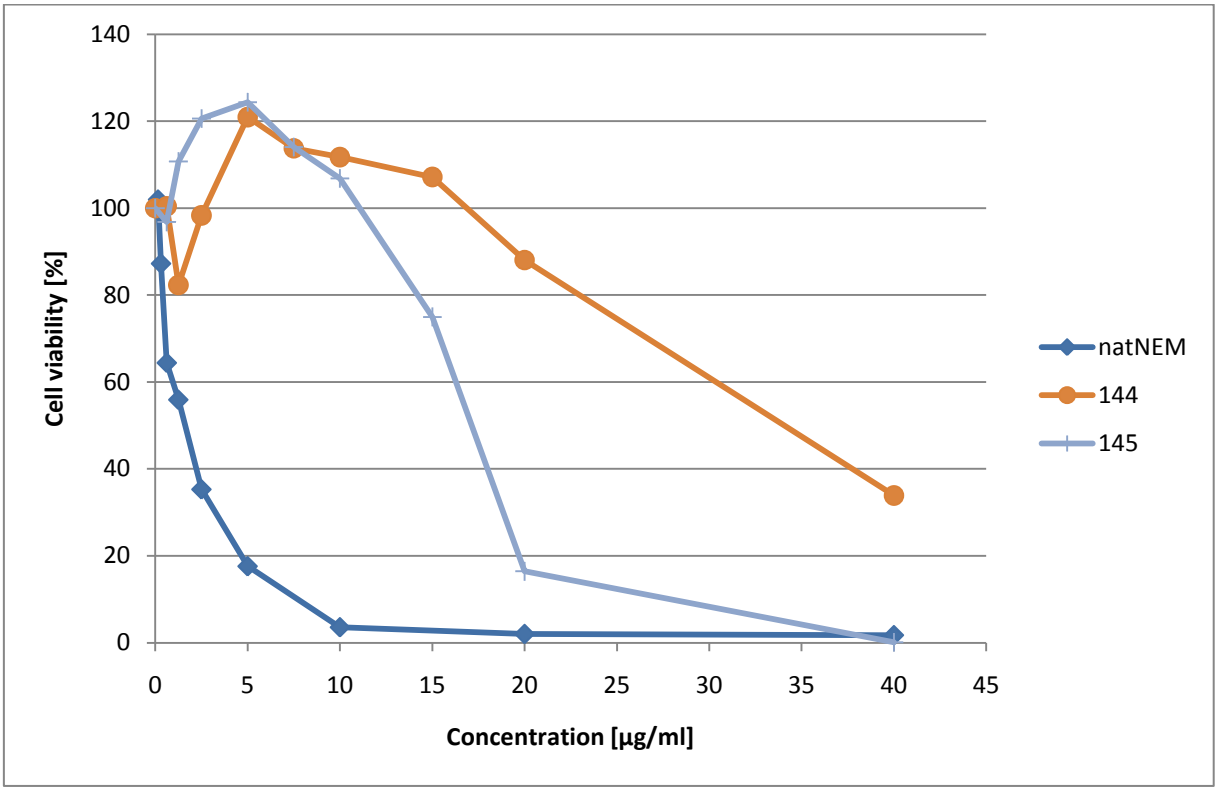



**Figure 4.4.5** Dose-response curves for **144** and **145**.

There is a clear difference in the response to treatment with the two different isomers. The C-2 *O*-methylated derivative **144** shows an $IC_{50}$ of 35 µM and the C-4 *O*-methylated derivative **145** has an $IC_{50}$ of 19 µM (Figure 4.4.5). This suggests that the site of methylation on the bicyclo[3.3.1]nonane is important to the anti-cancer activity of this class of compound. The difference in activity of these enol ethers in cell tests could be due to their difference in susceptibility to hydrolysis.

A final set of compounds were tested to investigate the effect of changes to the saturation of the PPAP prenyl groups and the change of oxidation level of the bridgehead ketone (Figure 4.4.6 and 4.4.7).

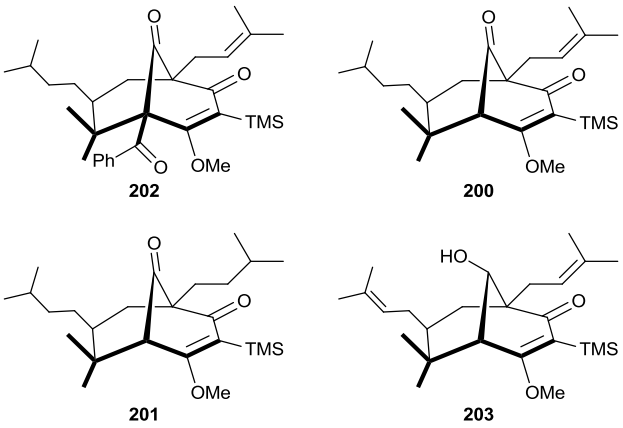



**Figure 4.4.6**

By using the compounds **173** and **174**, it was possible to compare the relationship between these compounds, their unsaturated/oxidised derivatives **200-203** and nemorosone (**4**).

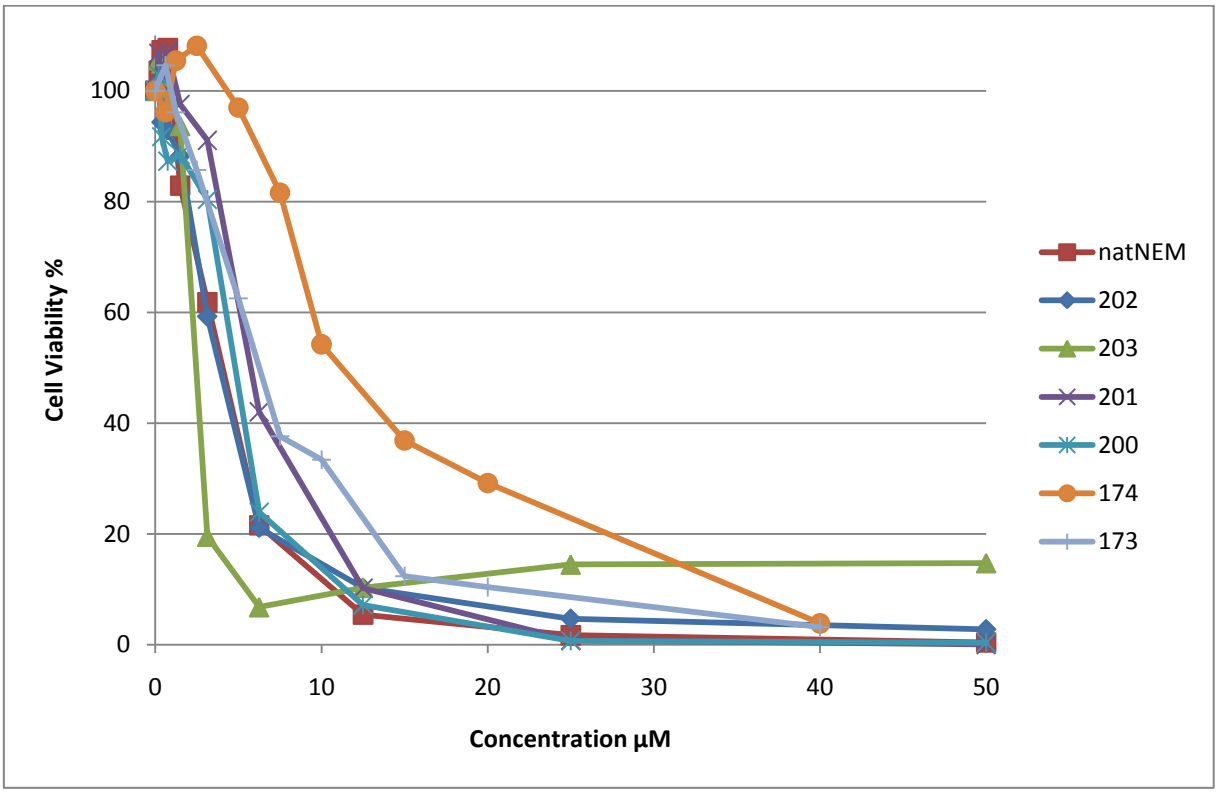



**Figure 4.4.7** Dose-response curves for **200-203**.

Figure 4.4.7 shows that a change in the saturation level of the prenyl chains in these molecules (**200-202**) has little effect on the $IC_{50}$ values. However, a greater effect was seen when using the hydroxyl compound **203**, the $IC_{50}$ value of 2 μM for **203** is less than that for the nemorosone (**4**) control.

## 4.5 Conclusion

This study has shown that the enantiomers of clusianone (**3**) and nemorosone (**4**) are of similar activity. Nemorosone II (**150**) is less active against the (MIA-PaCa-2) cancer cell line. By looking at the different derivatives, it is possible to gain some insight into the SAR of this class of compounds. It has been shown that the presence of the 1,3-diketone system is important and that reduction of the bridgehead ketone can lead to lower $IC_{50}$ values.

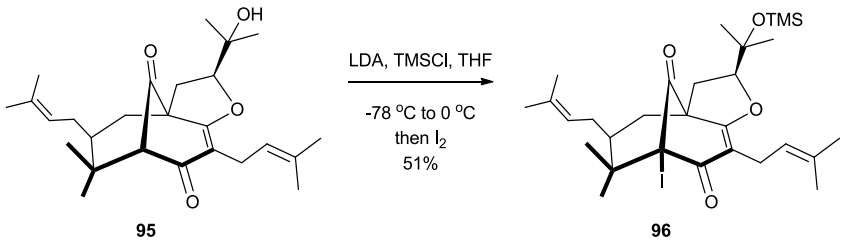
# Chapter 5

# Investigation into C-1 Bridgehead Functionalisation

During the synthesis of nemorosone (**4**) in Chapter 2 it was discovered that the C-1 bridgehead of the bicyclo[3.3.1]nonane system would not undergo substitution using our previously established protocols. However, using iodination followed by a halogen-metal exchange, substitution of the C-1 bridgehead could be achieved. This chapter will discuss the chemistry that is available at the C-1 position and explain the limitations.

## 5.1 Attempted C-1 Enolate Formation

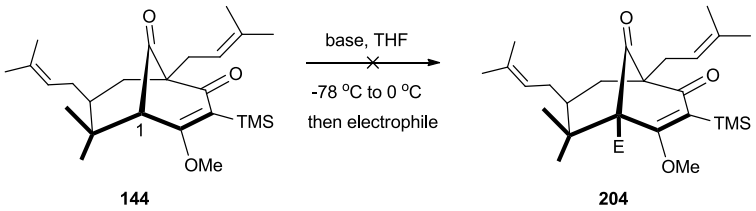
In the nemorosone (**4**) synthesis, the C-1 bridgehead was acylated using iodination followed by halogen-metal exchange and quenching with benzoyl chloride, since direct C-1 enolate acylation proved ineffective. These conditions were adapted from Danishefsky's synthesis of garsubellin A (**1**), when traditional enolate forming methods failed to enable access to further functionalised molecules (Scheme 5.1.1).[39]




**95** **96**

**Scheme 5.1.1**

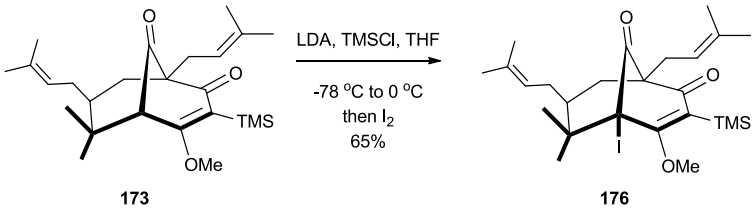
To fully test the reactivity of the C-1 bridgehead position of **144**, a range of bases, electrophiles and conditions were tried. Bases that were tested included *t*BuLi, *n*BuLi, LDA, LiTMP, KOH and NaOMe, and the reactions quenched with MeI, benzoyl chloride or TMSCl. In all cases the tell-tale colour change from a colourless solution to a yellow one occurred on addition of the base but the desired product was not observed after electrophile addition (Scheme 5.1.2). This suggested that the bridgehead enolate was being formed although it could not be reacted successfully.




**144** **204**

**Scheme 5.1.2**

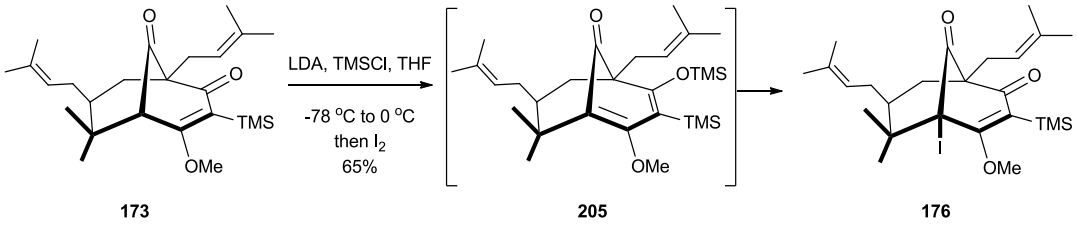
One possible explanation for this would be that the enolates were forming aggregates that prevented the electrophile from attacking the C-1 position. To test this hypothesis, LiCl was added to the LDA solution to make mixed aggregates before electrophile addition.[76] Unfortunately, this failed to provide an isolatable product. At this point we were unable to explain the lack of reactivity of the C-1 bridgehead. The final attempt, using a modification of Danishefsky's intriguing bridgehead iodination reaction, gave the lead needed to complete the nemorosone (**4**) synthesis (Scheme 5.1.3).




173 176

**Scheme 5.1.3**

With C-1 bridgehead iodination established we wanted to test the application of these unusual conditions with alternative electrophiles. A range of different electrophiles were tried including acylating and alkylating agents (benzoyl chloride, benzaldehyde, prenyl bromide, methyl iodide and TMSCl). In all cases none of the desired product was observed and starting material was recovered. Thinking that TMSCl may be acting as a Lewis acid it was changed for $BF_3.Et_2O$ and iodine was used as the electrophile. This resulted in no reaction and only starting material was recovered. Isolation of intermediates for this reaction proved impossible but it is hypothesised that a silyl enol ether was formed that underwent quenching during the work up of the reaction (Scheme 5.1.4).




173 205 176

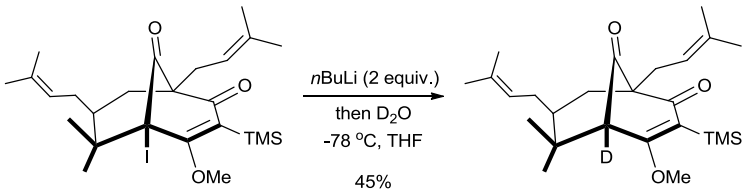
**Scheme 5.1.4**

These experiments have shown that the C-1 bridgehead can be iodinated but only under very specific conditions which we have been unable to adapt to other

electrophiles. At this stage it is hard to explain these results although it may be due to the steric encumberment around the C-1 position allowing only small electrophiles close enough to react.

## 5.2 Bridgehead Iodide Chemistry

Having established that the only chemistry possible at the C-1 bridgehead of methyl enol ether **173** was iodination, the available chemistry of the resulting iodide **176** was investigated. During the synthesis of nemorosone (**4**) it was shown that halogen-lithium exchange could be performed and the lithium anion quenched with deuterium (Scheme 5.2.1).

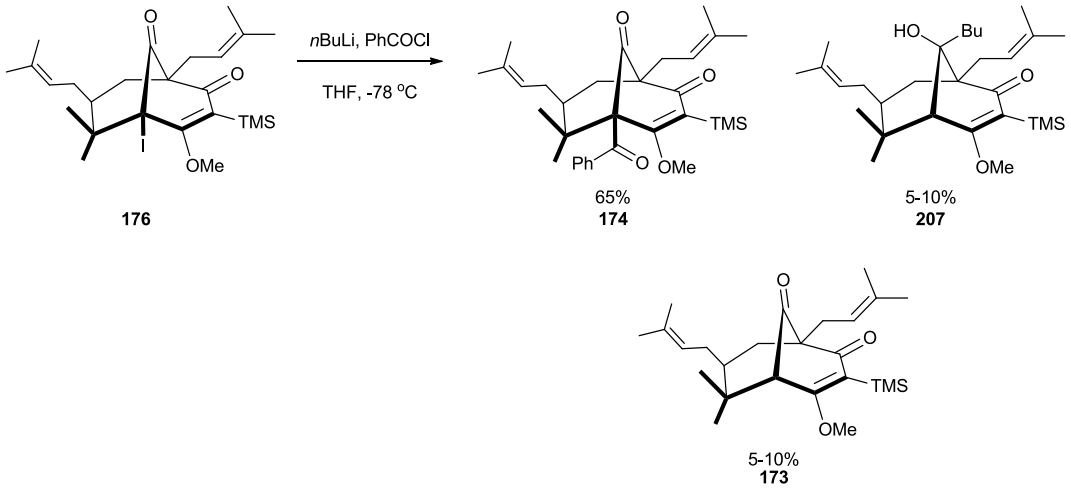



**176** **206**

**Scheme 5.2.1**

The reaction produced a low recovery due to butyl addition to the C-9 bridging carbonyl but complete conversion to the deuterated product **206** was achieved. This confirmed that the lithium anion was being formed at the C-1 bridgehead and could be quenched with an electrophile, which led to the successful benzoylation during the nemorosone (**4**) synthesis.

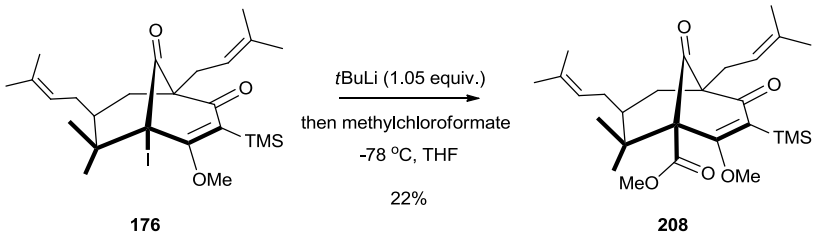
When performing halogen-metal exchange with *n*BuLi for the nemorosone (**4**) synthesis, moderate yields alongside some butyl addition to the bridgehead ketone **207** and the protonation product **173** were observed (Scheme 5.2.2).




**Scheme 5.2.2**

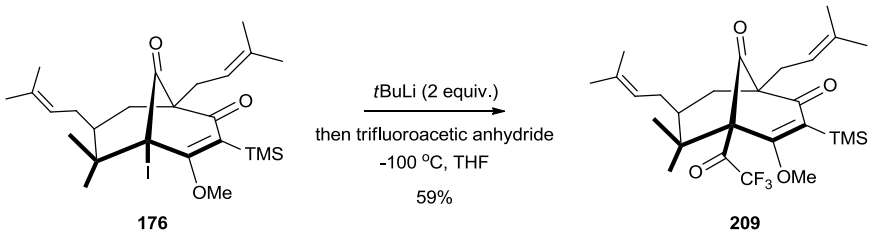
It was therefore necessary to perform some optimisation of the conditions before screening a selection of electrophiles. *t*BuLi was used to limit the amount of butyl addition to the bridging carbonyl since it is less nucleophilic. Reactions were also performed at a lower temperature (-100 °C) to try and limit any undesired reactions taking place before the electrophile was added.

Having shown that acylation was possible with benzoyl chloride to make **174** in the nemorosone (**4**) synthesis, a series of other acylating agents were tested. Using methyl chloroformate as the electrophile it was possible to form the methyl formate intermediate **208** (Scheme 5.2.3).




**Scheme 5.2.3**

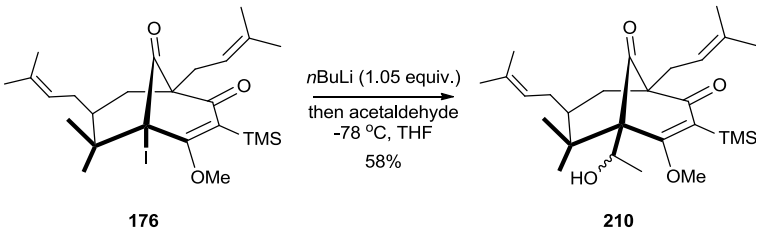
Unfortunately, the reaction was low yielding, with the remainder of the material being isolated as the C-1 protonated product **173**. This suggested that the anion was either particularly basic and being protonated by solvents or the glass, or that it was very stable and was only quenched upon work up. The trifluoroacetyl derivative **209** was made using trifluoroacetic anhydride as the electrophile (Scheme 5.2.4).




**Scheme 5.2.4**

The yield was good for this quench although some of the C-1 protonated product was isolated alongside the desired product.

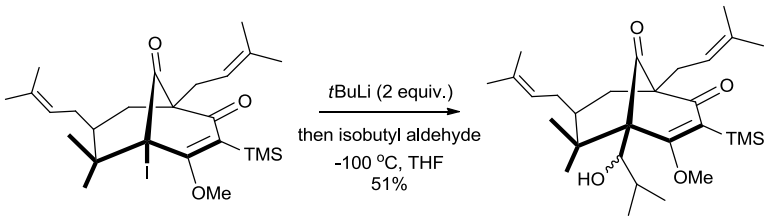
To investigate the reactivity of the C-1 anion further it was tested with a series of aldehydes to make the C-1 aldols. Reaction with acetaldehyde afforded the secondary alcohol **210** in good yield (Scheme 5.2.5).




176 210

**Scheme 5.2.5**

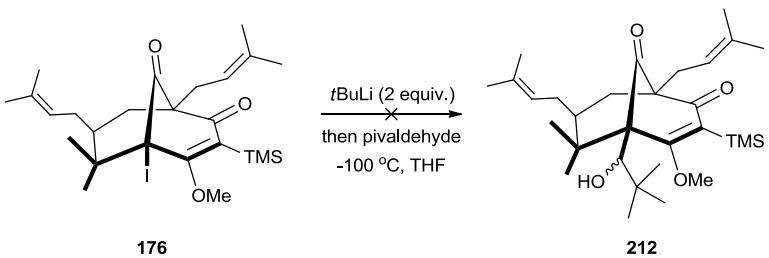
To discover something about the size of electrophile that could be reacted at the C-1 position, a reaction with isobutyl aldehyde was performed. The isobutyl aldehyde offered a more hindered electrophile, indicating whether larger electrophiles would substitute at the C-1 position of the iodide **176** (Scheme 5.2.6).




176 211

**Scheme 5.2.6**

The reaction produced a moderate yield of aldol product **211**. The natural progression of this series was reaction with pivaldehyde (Scheme 5.2.7).

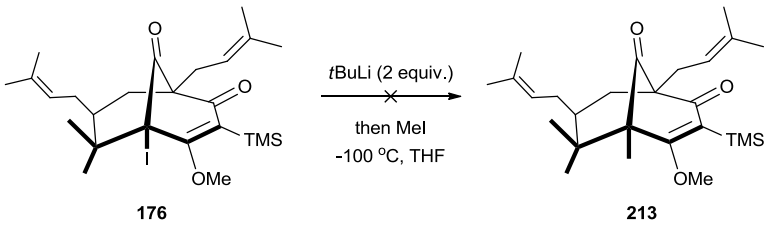



176 212

**Scheme 5.2.7**

On this occasion no reaction was observed. The *tert*butyl group proved too great a steric bulk for reactions to occur. This series of reactions showed that for a reaction to be successful at the C-1 position the electrophile must be relatively small. This is not unexpected due to the steric impingement caused by the C-8 *geminal* dimethyl group and the *O*-methylation at the C-2 position.

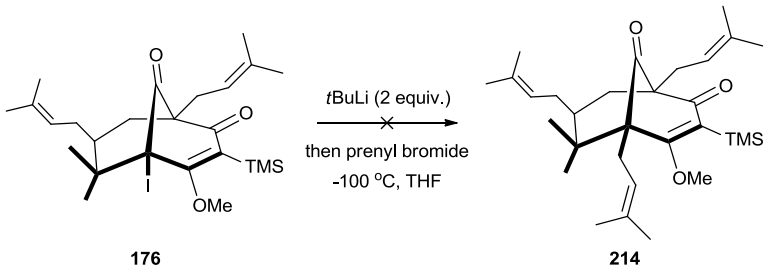
Having shown that reactions with carbonyls were possible, producing the expected acylated products or alcohols, we also wanted to test the corresponding reactions using alkylating agents. Thus, using our standard conditions for halogen-metal exchange at the C-1 position, MeI was tested as the reactive electrophile (Scheme 5.2.8).




**176** **213**

**Scheme 5.2.8**

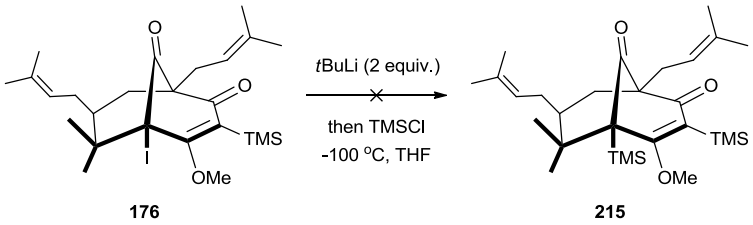
No reaction was observed under our standard conditions and only the protonated product **173** was isolated. Upon increasing the concentration of electrophile to ten equivalents no desired product was obtained. The reaction was also tried using prenyl bromide as the alkylating agent (Scheme 5.2.9).




176 214

**Scheme 5.2.9**

Again no reaction was seen and only the protonated product **173** was recovered. In conclusion, the alkylation of the C-1 position was not possible. Functionalisation of the C-1 position was further studied with attempts at silylation. To test this TMSCl was used as the electrophile (Scheme 5.2.10).

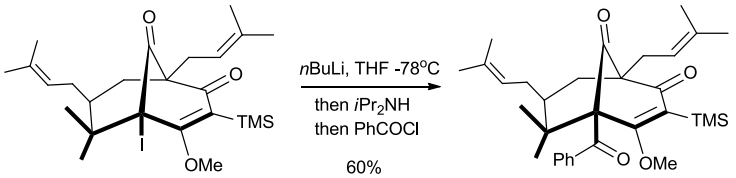



176 215

**Scheme 5.2.10**

This showed no favourable reaction returning only the C-1 protonated product **173** so different silylating agents (TBSCl, TIPSCl) were tested and gave no improvement. Although the yields for this series of products did not match that of the benzoylation reaction they gave an insight into some of the chemistry possible at the C-1 position. The above results indicate that an effective electrophile needs to be small, highly reactive and acyl in character. It was preferable that the electrophile be non-enolisiable since the lithium anion could abstract a proton from the electrophile, protonating the C-1 position in the process.

These experiments showed that using typical enolate forming conditions it was not possible to substitute the C-1 bridgehead. However, under very specific conditions C-1 bridgehead iodination could be performed. The bridgehead iodide could then be substituted by a range of electrophiles using a halogen-metal exchange. This was somewhat surprising as both sets of conditions would form the same lithium enolate. This suggested that something in the direct enolate forming conditions was interrupting the reaction. A possible cause was complexation of the enolate with diisopropylamine, blocking the enolate from the electrophile. To test this diisopropylamine was added before the electrophilic quench of the mixture, produced by bridgehead iodination and the halogen-metal exchange (Scheme 5.2.11).



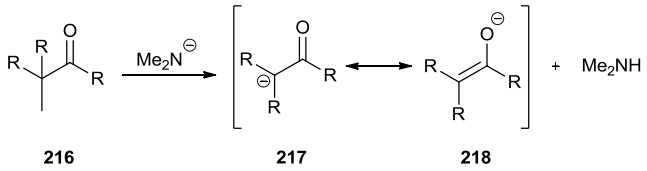

176 174

**Scheme 5.2.11**

It was expected that if complexation was taking place then the addition of the benzoyl group would be restricted. However, this was not the case and the reaction went to completion as normal. Therefore, a stable complex with diisopropylamine was unlikely to be the reason substitution by direct enolate formation and quenching failed.

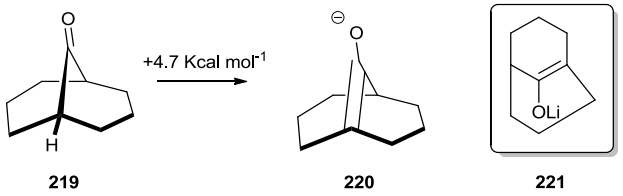
## 5.3 Computational Studies

Although the C-1 bridgehead position could be substituted, it was desirable to understand how substitution was only possible via the bridgehead iodide intermediate. Thus, Dr. Chris Hayes at the University of Nottingham performed some computational modelling of these systems.[68,71] Using B3LYP calculations it was possible to predict which systems would undergo bridgehead deprotonation; viable reactions gave a negative $\Delta E_{RXN}$ and not viable reactions gave a positive $\Delta E_{RXN}$. $\Delta E_{RXN}$ is the energy of reaction for the simplified deprotonation reaction using dimethyl amide, shown in Scheme 5.3.1. The results of these calculations fit with what has been experimentally determined.



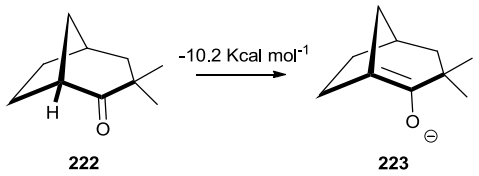

**Scheme 5.3.1**

Through computer modelling it was possible to look at simplified systems and have some insight into the actual system in question. These calculations show that the anion of **219** would be unfavourable with a $\Delta E_{RXN}$ of +4.7 Kcal $mol^{-1}$. This was due to the [3.3.1] system not offering enough flexibility to incorporate the double bond of an enolate (Scheme 5.3.2).




**Scheme 5.3.2**

By studying systems that contain the ketone in a larger ring it was possible to show that bridgehead deprotonation can be a highly favoured reaction. Calculation based on the [3.2.1] system **222** show a $\Delta E_{RXN}$ – 10.2 Kcal $mol^{-1}$. The lower the value of $\Delta E_{RXN}$ the more stable the enolate produced (Scheme 5.3.3).




**Scheme 5.3.3**

By applying these results to the experimental system it can be shown that the bridgehead enolate cannot form at the C-9 oxygen. In the experimental system **173** the methyl vinylogous ester section offers a way of forming a dienolate from deprotonation of the C-1 position (Figure 5.3.1).

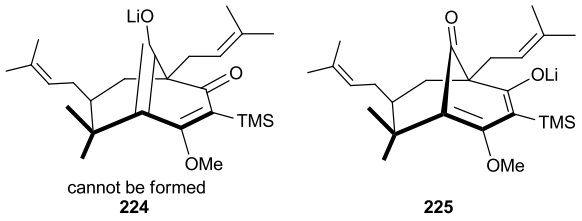
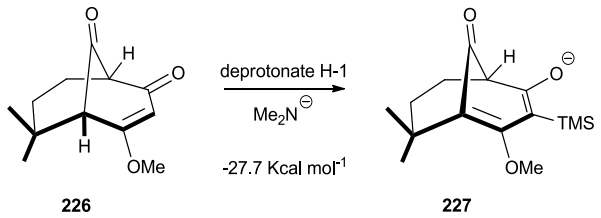


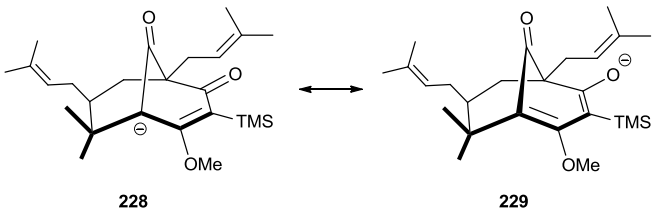

**Figure 5.3.1**

Having performed calculations on model systems to demonstrate that bridgehead deprotonation can be a favourable reaction and that the resulting anion can be stable, calculations were performed on an *O*-methylated bicyclo[3.3.1]nonane system **226**. The calculations show that the dienolate made from the deprotonation of **226** is particularly stable $\Delta E_{RXN}$ -27.7 Kcal $mol^{-1}$ and therefore may explain why the experimental system undergoes limited reaction (Scheme 5.3.4).




**Scheme 5.3.4**

NBO calculations were also performed upon the anionic deprotonated species. It was discovered that these could be described as either bridgehead enolates or α-keto carbanions depending upon the system. Our bicyclo[3.3.1]nonane model system was best described as the dienolate form. With this in mind the experimental system could be described as two quite different resonance structures, with **229** being thermodynamically more stable (Scheme 5.3.5).




**Scheme 5.3.5**

Having made this definition, it was possible that different lithiated species were being produced in the two different sets of conditions. It is hypothesised that the different lithiated species have different stability and therefore reactivity with the electrophiles, although more computational studies would have to be completed to prove this. Under LDA conditions the particularly stable lithium dienolate **225** was formed which undergoes very limited further reaction due to its stability and the steric bulk of the neighbouring substituents. However, under halogen-metal exchange the bridgehead lithium **230** was formed instead which may be less stable and therefore more reactive (Figure 5.3.2).

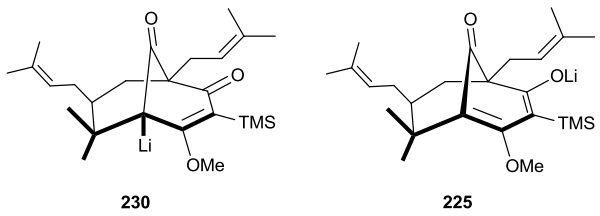



**Figure 5.3.2**

It is also possible that the energy barrier between the two different lithiated species may be unexpectedly high therefore making the two resonance structures act as two independent systems, leading to the results we have seen experimentally.

## 5.4 Conclusion

During the synthesis of nemorosone (**4**) it was discovered that direct C-1 deprotonation and substitution was only possible under the very specific conditions of iodination. However, by using a halogen-metal exchange it was possible to functionalise this position. Computational studies have also shown that the anion is

stable and a dienolate. With this in mind it is proposed that the dienolate, through direct deprotonation, is stable and sterically hindered against electrophilic quenching. Whereas under the conditions of halogen-metal exchange a bridgehead lithiated species is formed, which is less stable and therefore more reactive.

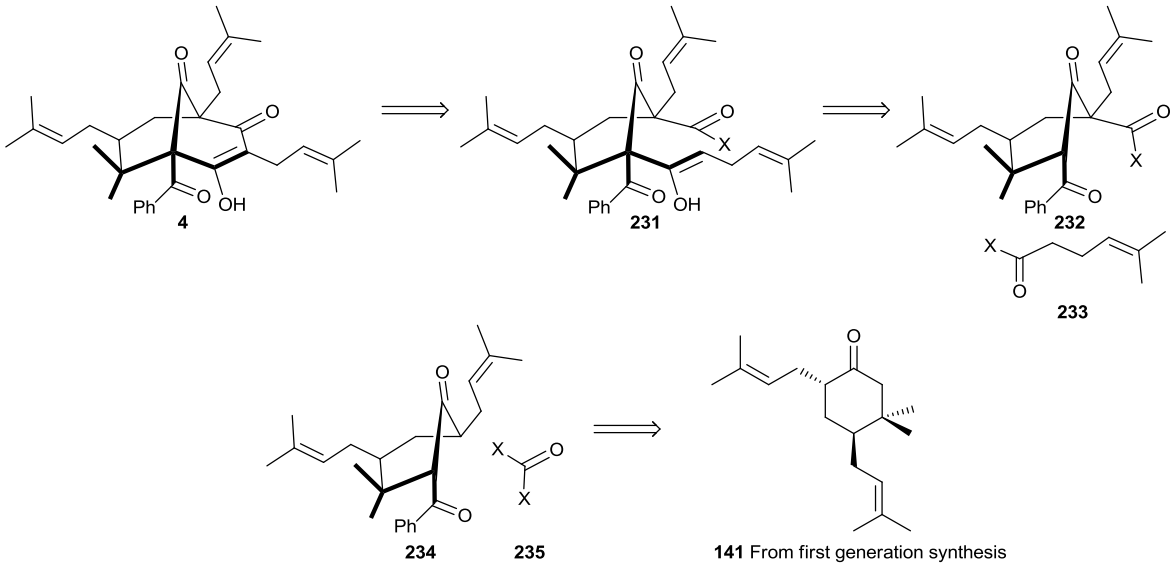
# Chapter 6

# Toward a Second Generation Synthesis of Nemorosone

Having synthesised nemorosone (**4**) using the Effenberger annulation as the key bicyclo[3.3.1]nonane core forming reaction, we sought to improve the overall yield and decrease the number of steps in the synthesis. Although only 13 steps in length our initial synthesis gave an overall yield of 6%. In practical terms this would never be applicable to scale up and potential industrial use, therefore a second generation synthesis was devised.

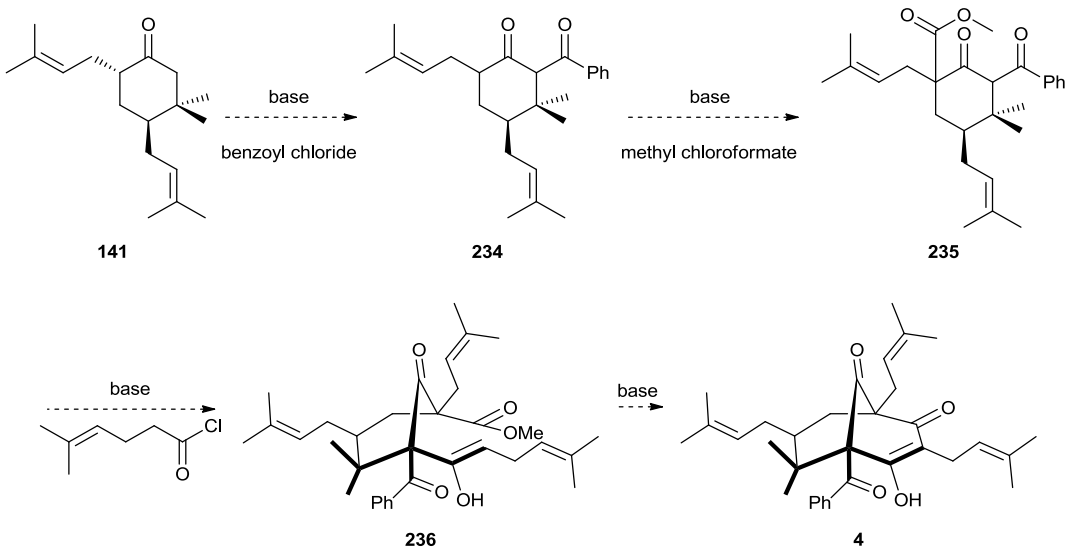
## 6.1 Retrosynthesis

We envisaged a new strategy that would be distinct to those already established, shorten the route to nemorosone (**4**) and would improve the overall yield of the synthesis. The following disconnections would lead to nemorosone (**4**) in just seven steps, and should improve the overall yield (Scheme 6.1.1).




**Scheme 6.1.1**

The first three steps of the synthesis would be those of the first generation synthesis and would give diprenylated ketone **141** in circa 70% yield. The next step would be to form the 1,3-diketone **234**, through enolate formation followed by acylation with benzoyl chloride to afford the diketone system **234** expediently. Further deprotonation and electrophilic quenches with acyl groups could be used to build up the architecture that would later form the diketone part of the ring in nemorosone (**4**). By installing an enolisable acyl group and one with a leaving group it was hoped that this would drive a Dieckmann cyclisation between them forward (Scheme 6.1.2).




**Scheme 6.1.2**

Using this methodology would be a departure from our bridgehead enolate chemistry, but may offer benefits in terms of improved yield and more reliable reactions.

Acylation of dicarbonyl compounds is not well known although a few procedures have been developed.[77-80] Many of these procedures require stoichometric amounts of base and catalysis with d-block metal derivatives and are not applicable to cyclic systems. We feel that it may be possible in this situation as the dicarbonyl **234** is not a traditional dicarbonyl. The bulk of the phenyl group and the *geminal* dimethyl groups mean that the benzoyl group is forced out of the plane of the cyclohexanone carbonyl (Figure 6.1.1).

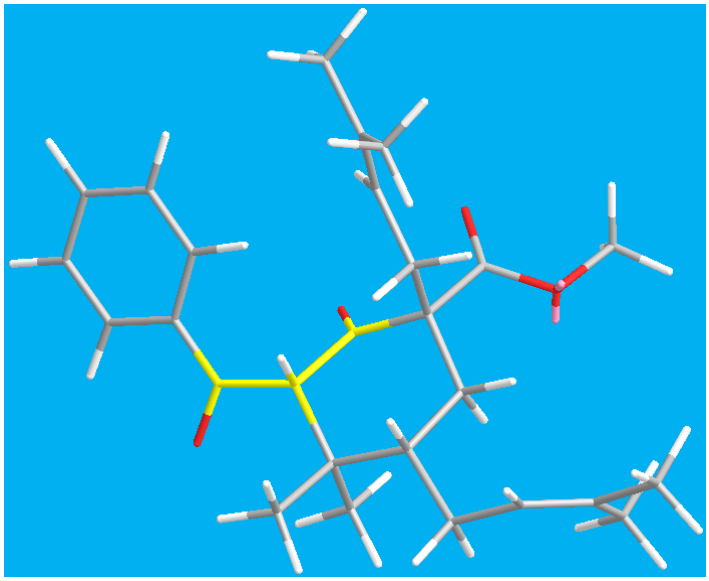
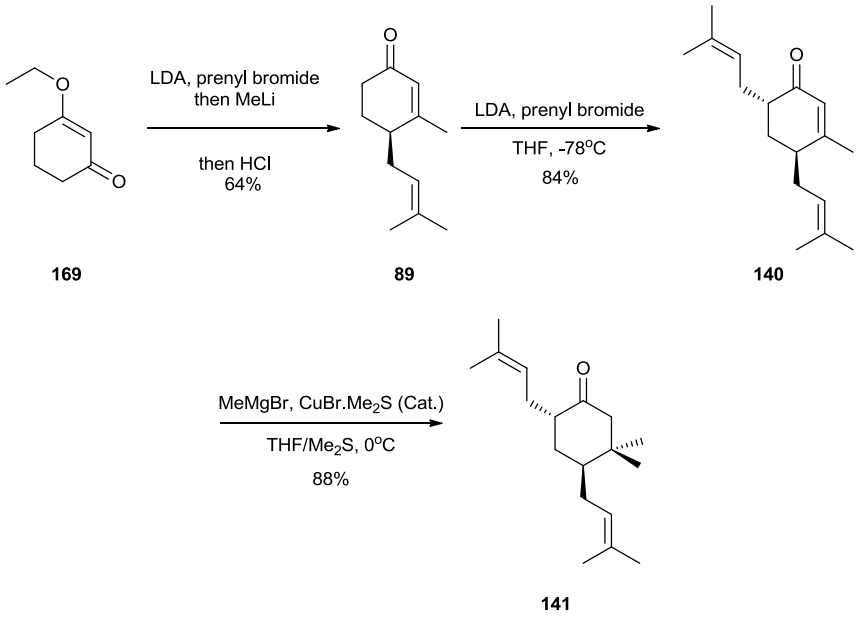


**Figure 6.1.1** Chem 3D molecular model of **235** showing non-coplanar

This results in poor orbital overlap and the system reacting more like a ketone than a dicarbonyl. We hope to exploit this for our double acylation of the C-2 position.

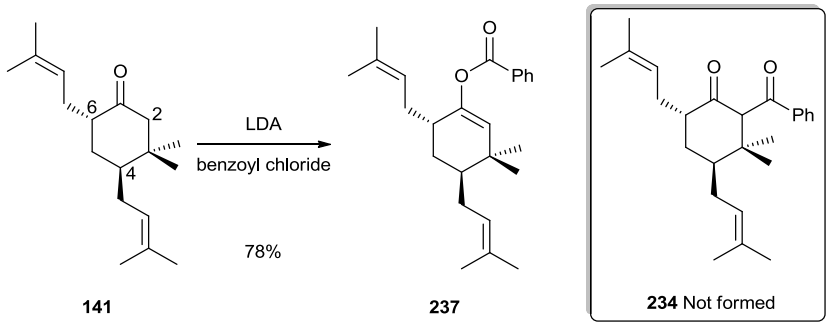
## 6.2 Synthesis of Nemorosone

Formation of the 4,6-diprenylated ketone **141** followed the chemistry established in Chapter 2 and gave the product in multi gram quantities (Scheme 6.2.1).



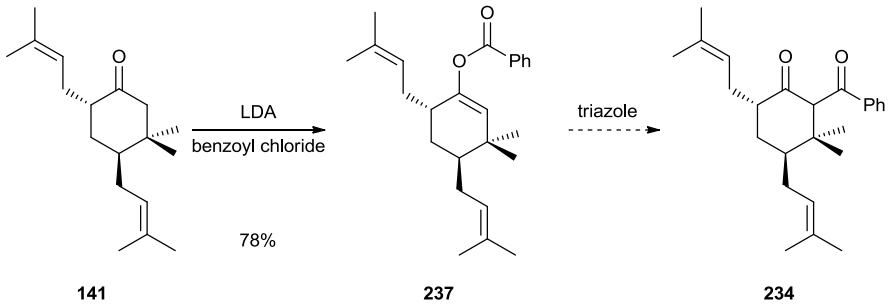

**Scheme 6.2.1**

To continue towards nemorosone (**4**) the C-2 acyl group which would become the C-1 bridgehead benzoyl group in nemorosone (**4**) would need to be installed. Our initial aim was to deprotonate the less hindered position (C-2) and acylate the resulting enolate using benzoyl chloride. Performing the deprotonation with LDA gave 78% conversion to a product with the correct mass, and a proton NMR with a singlet signal at δ = 5.26 that could correspond to the acidic C-2 proton between the two carbonyls. However, $^{13}C$ NMR spectra showed only one peak corresponding to the enol benzoate carbon, and IR spectroscopy confirmed this with only one stretch in the carbonyl region. Rather than the desired product the reaction lead selectively to *O*-acylation, giving enol benzoate **237** as the only product (6.2.2).



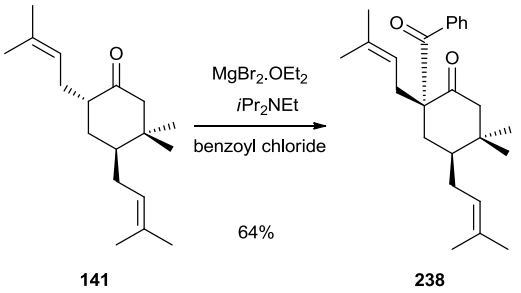

**Scheme 6.2.2**

It may be possible to perform a rearrangement of the enol benzoate **237** to a 1,3-diketone **234** using triazole and potassium carbonate. Attempts have been made using a protocol described by Bentley *et al*. but have as yet been unsuccessful and only starting material was recovered (6.2.3).[81]




**Scheme 6.2.3**

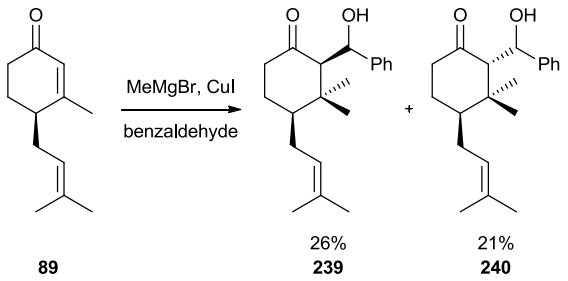
In an attempt to obtain the *C*-acylated product, thermodynamic conditions were investigated to perform the deprotonation, followed by an electrophilic quench with benzoyl chloride giving a 1,3-diketone **238**. Not unexpectedly this gave the product with the acyl group at the C-6 position along with the prenyl group (Scheme 6.2.4).




**Scheme 6.2.4**

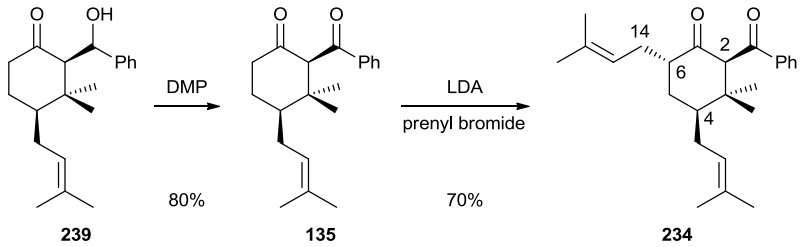
The $^{1}$H NMR spectra did not show a singlet signal in the 4-5 ppm region that confirmed that acylation was C-6 rather than C-2. The $^{13}$C NMR shows two signals in the 200 ppm region that corresponds to the two carbonyl carbons, confirming that *C*-acylation has taken place rather than *O*-acylation.

To continue the synthesis we retraced our steps and investigated the use of an indirect acylating approach previously developed in our group.[47] It has been shown that it is possible to make a suitable aldol product, albeit in modest yield, through quenching an enolate with benzaldehyde. Starting from enone **89** (Chapter 2.4), methylation of the C-3 position was performed by a 1,4-Michael addition and this was followed by immediate addition of benzaldehyde to give the keto-alcohols **239** and **240** as a 1:1 diastereomeric mixture (Scheme 6.2.5).




**Scheme 6.2.5**

Keto-alcohol **239** was then oxidised using Dess-Martin periodinane to give the 1,3-diketone **135**. Dr. Rodeschini had reported a 58% yield over the two steps from **89** to **135** and **136**, however only a 21% yield for the two steps was possible on this occasion.[47] Prenylation at the C-6 position to install the bridgehead C-5 prenyl of nemorosone (**4**) was performed by deprotonation with LDA and electrophilic quench with prenyl bromide, to give the diprenylated-1,3-diketone **234** (Scheme 6.2.6).

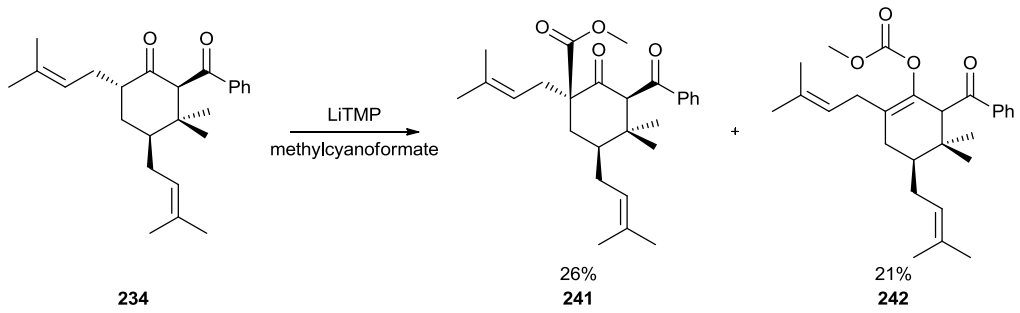



**Scheme 6.2.6**

Analysis by NOESY NMR allowed the stereochemistry to be defined. The spectra showed correlation between the protons C-2 and C-4 confirming that the benzoyl group and C-4 prenyl group are on the same side. No correlation was seen between the proton of C-2 and C-6 or C-4 and C-6, suggesting that the C-6 prenyl group was on the opposite side to the C-4 prenyl and benzoyl. Further confirming this was the correlation between the C-14 protons and the C-2 proton.

The next phase in our planned synthesis involved appending the required acyl groups at C-2 and C-6 that would be used to cyclise and form the 1,3-diketone ring system of nemorosone (**4**). The question of which group to install first, the methyl formate or the pentenoyl chain, was answered by considering the reactivity of these groups. Installation of the formate group first would give a product in which there

would be no competing centre for deprotonation and therefore no potential side reaction with the acid chloride when installing the C-2 acyl group. The pentenoyl group however, can be easily enolised, which could lead to problems when using the intended deprotonation and enolate quench to install the final group. For this reason reaction with LiTMP and quench with methyl cyanoformate was conducted (Scheme 6.2.7).



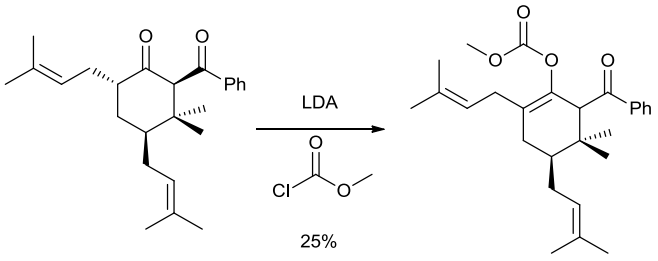

**234** **241** **242**

**Scheme 6.2.7**

Unfortunately, these conditions gave a mixture of the *O*-acylated **242** and *C*-acylated **241** products, which proved co-polar in various solvent systems and were inseparable by normal column chromatography. HPLC (C18 column) offered a solution to this problem. This would not be suitable for our improved synthesis so conditions were adjusted and reagents changed to try to favour *C*-acylation. Different bases (LDA and dilithiated N,N'-diphenylethylenediamine) were tested but gave no change in product ratio and no improvement in yield. The identity of the *C*-acylated compound was confirmed by the $^{13}C$ NMR spectra containing three signals in the low field which correspond to the two ketones and the ester. The IR confirmed this with three stretches in the carbonyl region. NOSEY NMR spectra were used to confirm the stereochemistry, once more there was correlation between

the C-2 and C-4 protons establishing that they were on the same side. Correlation between the C-4 and C-14 signals confirmed that the C-6 prenyl group was on the same side as the C-2 and C-4 protons.

By changing to methyl chloroformate as the electrophile, selective *O*-acylation was achieved to give **242** in 25% yield (Scheme 6.2.8).

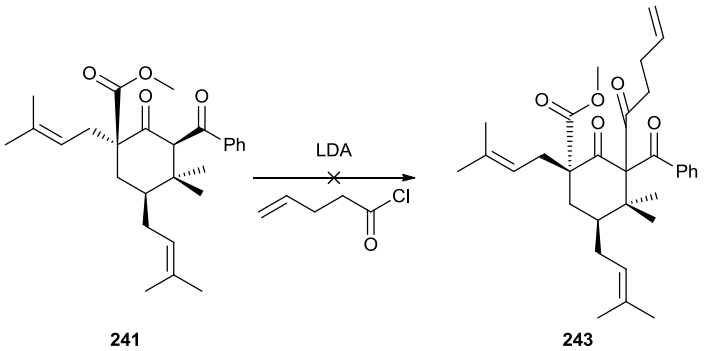



**234** **242**

**Scheme 6.2.8**

Under softer thermodynamic conditions (*i*$Pr_2$NEt, $MgBr_2$.$EtO_2$, heat) no reaction was observed. Again it may be possible to carry out *O*-acyl to *C*-acyl rearrangement using triazole, although test reactions have been unsuccessful in achieving this conversion to date. This would at least allow for recovery of the desired product if selective *C*-acylation conditions could not be found.

With the triketone **241** in hand installation of the final group, which would become the vinylic prenyl group, was attempted (Scheme 6.2.9). The intention once more was to deprotonate and quench at the acidic proton between the ketones. However attempts with LDA or *t*BuLi as the base gave no reaction and only starting material was recovered.

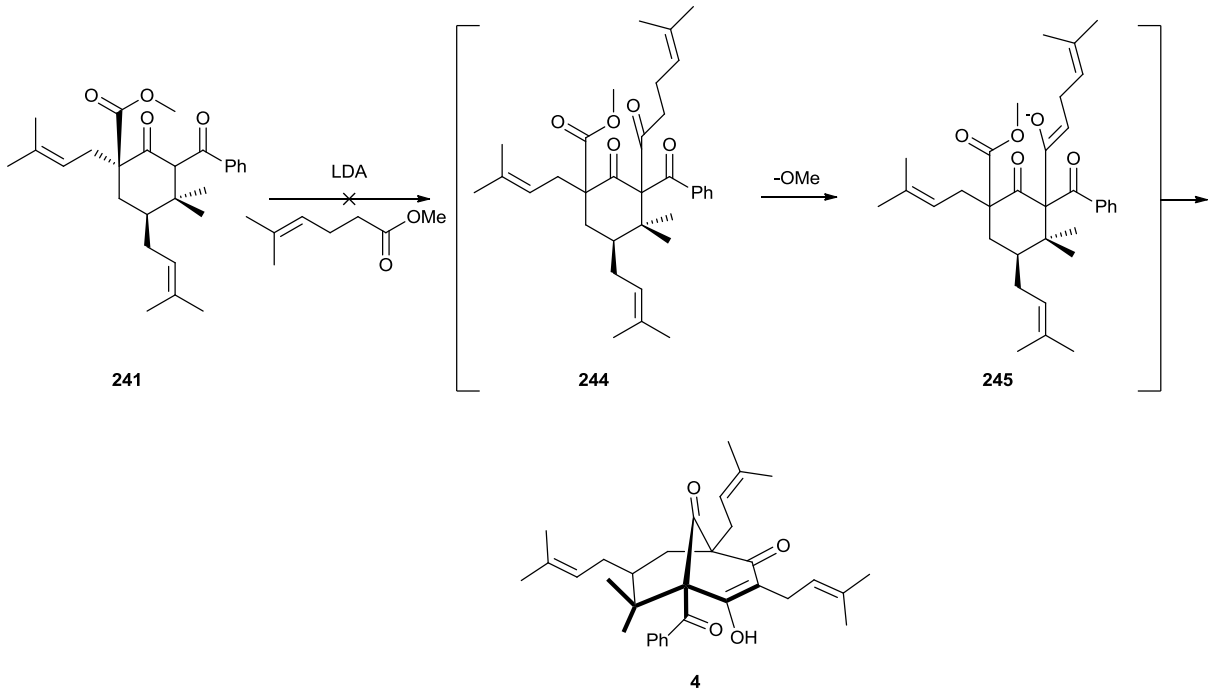



241 243

**Scheme 6.2.9**

To try to confirm that the anion was being formed the deprotonations were performed with LDA and quenched with $d_4$-MeOD or TMSCl. It was hoped that this would lead to deuterium incorporation or *O*-silylation. However, neither of these products was observed. The C-2 proton is extremely hindered by the C-2 benzoyl group as well as the C-4 prenyl and formate groups. It is likely that this was preventing deprotonation and therefore further substitution of the C-2 position has so far proved elusive.

If acylation of the C-2 position can be affected successfully it would be desirable to perform the acylation with a prenyl ester. By using the ester, the Dieckmann condensation could also be performed sequentially. The released alkoxide from the ester addition would then deprotonate the α-proton of the prenyl group and affect cyclisation to nemorosone (**4**) (Scheme 6.2.10).




**Scheme 6.2.10**

## 6.3 Conclusions

It has been shown that there is potential for a new and more expedient route to nemorosone (**4**). However, at this stage the yields of several steps are modest and the final acylation process, which we hoped would lead to the 1,3-diketone bridge, has not been accomplished to date. Although the route has not come to a satisfactory completion it is thought that further work could overcome these issues and afford a significantly more efficient route to nemorosone (**4**).

Most of the reactions discussed in this chapter are not optimised, therefore the yields should improve. There is much scope for adjusting additives and the bases used in the initial steps. The benzoylation could be carried out via *O*-acylation followed by rearrangement to the *C*-acylated product all in one pot. This hopefully would improve the yield of 1,3-diketone **234** greatly. Having established that the 1,3-diketone can

be made quickly and in large quantities, testing different conditions to favour *C*-acylation with the formate group would become possible. Even if the reaction cannot be made completely *C*-selective there is the possibility to *O*-acylate and then rearrange to the *C*-acylated product. Hopefully using these procedures the triketone **241** could be made in gram quantities. If this were the case then testing various electrophiles could take place to see if any reactions are possible, and conditions could then be optimised to allow installation of the desired acyl group. It is hoped that the resulting tetra ketone would undergo Dieckmann condensation and give a new short route to nemorosone (**4**).

# Chapter 7

# Experimental

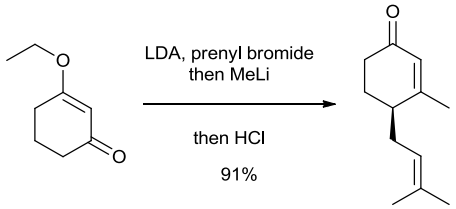
**General Procedures.** All glassware was flame dried and all reactions were carried out under an atmosphere of dry $N_2$ unless otherwise stated. All reagents were used as received from the supplier with the exception of DCM (distilled from calcium hydride), THF (distilled from sodium and benzophenone), diisopropylamine (distilled from calcium hydride), prenyl bromide (distilled under reduced pressure), benzoyl chloride (distilled from quinoline), tetramethylpiperidine (distilled from calcium hydride), triethylamine (distilled from calcium hydride).

$^{1}H$ and $^{13}C$ NMR were performed on Bruker AC270, AC300, AV300, AV400 and DMX500 spectrometers. Chemical shifts (δ) are quoted in ppm, coupling constants (*J*) in Hz. All spectra are referenced to the $CDCl_3$ peaks at 7.26 ppm for proton and 77.0 ppm for carbon unless otherwise stated. The following abbreviations apply: (br) broad, (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet, etc. IR was performed either as a neat oil or as a solid using a Perkin-Elmer Spectrum 100 FTIR spectrometer. Wavelengths (v) are reported in $cm^{-1}$. Optical rotations were recorded as dilute solutions in the indicated solvent in 25 mm glass cell using a JASCO DIP370 digital polarimeter at 294 nm. Mass spectra were obtained using a VG Micromass 70E or VG Micron Autospec spectrometer. Melting points were performed using a Gallenkamp melting point apparatus and are uncorrected.

Reactions were monitored by thin layer chromatography on aluminium plates with Keiselgel $F_{254}$, 0.2mm thick plates. Visualisation was achieved through UV light (254nm) and anisaldehyde. Flash column chromatography was performed using silica gel 60 (230-400 mesh, Merck and Co.). HPLC was performed using a Dionex P580 pump with a Walters 996 photo diode array detector. Columns and mobile phase are as stated. All reaction temperatures refer to values recorded for an external bath and room temperature (rt) implies temperatures in the range 18-25 °C. Where it is stated that petrol or petroleum ether was used this refers to 40-60 petroleum ether.

## 7.1 Experimental for Chapter 2

### (±)-(4*R*)-3-Methyl-4-(3'-methylbut-2'-enyl)cyclohex-2-enone (89)[47]



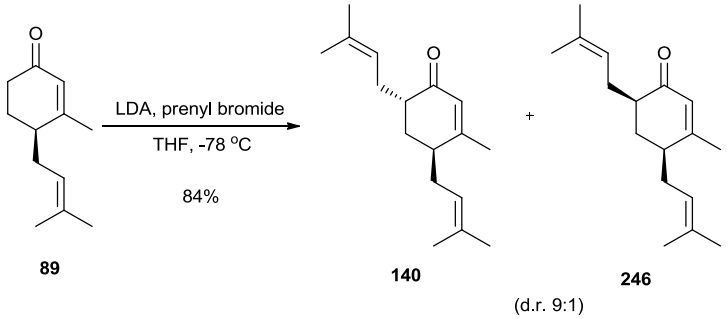

169 89

*n*BuLi (33 mL of a 2.5 M solution in hexane, 78 mmol, 1.1 equiv.) was added to a solution of diisopropylamine (11 mL, 78 mmol, 1.1 equiv.) in THF (100 mL) at -78 °C and the solution stirred for 10 min. To this 3-ethoxy-2-cyclohexane (9.6 mL, 71 mmol) was added and the resulting solution stirred at -78 °C for 10 min. After this time prenyl bromide (10 mL, 86 mmol, 1.2 equiv.) was added dropwise and the solution was stirred and allowed to warm to 0 °C over 2 h. This solution was cooled to -40 °C then treated with MeLi (90 mL of a 1.6 M solution in $Et_2O$, 144 mmol, 2 equiv.) which was added dropwise. The solution was stirred and allowed to warm to rt over 3 h and then quenched with 2 M HCl (100 mL) and the mixture stirred for 30 min. This was extracted with $Et_2O$ (3 x 100 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (gradient of 9:1 to 8:2 petroleum ether:EtOAc) to afford **89** (11.5 g, 91%) as a pale yellow oil. $R_f$ = 0.32 (20% EtOAc in petroleum ether); $^1$H NMR (300 MHz, $CDCl_3$): δ = 5.82 (s, 1H, O=CC*H*=C), 5.09 (t, *J* = 7, 1H, C=C*H*$CH_2$), 2.47-1.96 (m, 6H, $CH_2$, CH), 1.95 (s, 3H, C*H*$_3$C(CH)=CH), 1.90-1.76 (m, 1H, $CH_2$), 1.69 (s, 3H, C*H*$_3$C=CH), 1.60 (s, 3H, C*H*$_3$C=CH); $^{13}$C NMR (68 MHz, $CDCl_3$): δ = 199.6, 165.7, 134.0, 127.0, 121.9, 40.1,

34.1, 29.8, 26.6, 25.9, 23.1, 17.9; ESI-HRMS (TOF): *m/z* calculated for $C_{12}H_{18}O$ $[M+Na]^+$: 201.1255; found 201.1243.

Data were in accord with that of Dr. Rodeschini.[47]

**(±)-(4*S*,6*S*)-4,6-Bis(3'-methyl-but-2'-enyl)3-methyl-cyclohex-2-enone (140)**[47]

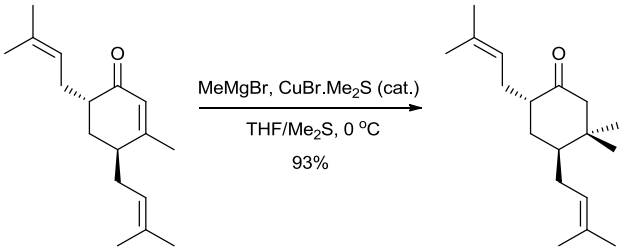



*n*BuLi (24.7 mL of a 2.5 M solution in hexane, 68 mmol, 1.1 equiv.) was added to a solution of diisopropylamine (8.6 mL 68 mmol, 1.1 equiv.) in THF (50 mL) at -78 °C and the solution stirred for 10min. To this a solution of enone **89** (11.0 g, 62 mmol) was added dropwise. The solution was stirred at -78 °C for 30 min., after which time prenyl bromide (7.2 mL, 68 mmol, 1.1 equiv.) was added dropwise. The mixture was stirred and allowed to warm to rt over 3.5 h. The reaction was quenched with saturated aqueous $NH_4Cl$ (20 mL) and stirred for a further 10 min. The mixture was extracted with $Et_2O$ (3 x 25 mL), the combined organic layers dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (gradient of 9:1 to 8:2 petrol ether:EtOAc) to afford **140** and **246** (12.8 g, 84%) as an inseparable yellow oil. $R_f$ = 0.38 (20% EtOAc in petroleum ether). Data for major compound **140**, minor compound was not isolated separately. $^1$H NMR (270 MHz, $CDCl_3$): δ = 5.78 (s, 1H, C=C*H*C=O), 5.09 (m, 1H, C=C*H*$CH_2$),

5.03 (m, 1H, C=C*H*$CH_2$), 2.52 (m, 1H, CHC*H*$_2$CH), 2.32 (m, 2H, CHC*H*$_2$CH), 2.27-2.10 (m, 3H, CHC*H*$_2$CH, $CH_2$C*H*($CH_2$)(C)), 2.01 (m, 1H, CHC*H*$_2$CH), 1.95-1.90 (m, 1H, CHC*H*$_2$CH), 1.93 (s, 3H, C*H*$_3$C(CH)=CH), 1.70 (s, 3H, C*H*$_3$C=CH), 1.67 (s, 3H, C*H*$_3$C=CH), 1.59 (s, 3H, C*H*$_3$C=CH), 1.57 (s, 3H, C*H*$_3$C=CH); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 201.0, 164.5, 133.6, 133.1, 126.3, 122.2, 121.9, 41.7, 39.7, 31.2, 29.6, 27.8, 25.7 (2 C), 22.8, 17.7 (2 C); ESI-HRMS (TOF): *m/z* calculated for $C_{17}H_{26}O$ $[M+Na]^+$: 269.1881; found 269.1882.

Data were in accord with that of Dr. Rodeschini.[47]

**(±)-(4*S*,6*S*)-4,6-Bis(3'-methyl-but-2'-enyl)-3,3-dimethyl-cyclohexanone (141)**[47]



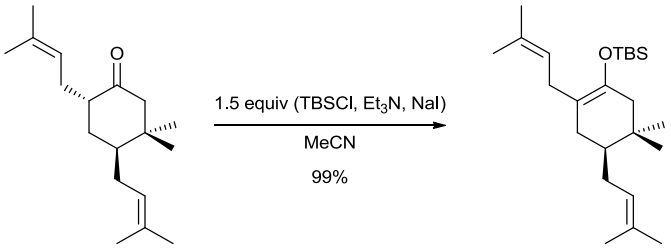

**140** **141**

To a solution of $Me_2S$ (11.25 mL, 152 mmol, 5.6 equiv.) in THF (150 mL), CuI (0.515 g, 2.7 mmol, 10 mol%) was added. This solution was cooled to 0 °C and a solution of enone **140** (6.74 g, 27 mmol) in THF (20 mL) was added and the solution stirred. To this MeMgBr (11 mL of a 3.1 M in $Et_2O$, 33 mmol, 1.2 equiv.) was added dropwise and the solution stirred for a further 3 h and allowed to warm to rt. The reaction was quenched with saturated aqueous $NH_4Cl$ (50 mL), and stirred for 10 min. This was extracted with $Et_2O$ (3 x 100 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (9:1 petrol ether:EtOAC) to afford **141**

(6.55 g, 93%) as a pale yellow oil. $R_f$ = 0.65 (10% EtOAc in petroleum ether); $^1$H NMR (270 MHz, $CDCl_3$): δ = 5.08 (t, *J* = 8, 1H, C=C*H*$CH_2$), 4.98, (t, *J* = 8, 1H, C=C*H*$CH_2$), 2.33-1.79 (m, 7H, CC$H_2$C, $CH_2$C*H*($CH_2$)(C), CHC$H_2$CH), 1.70 (s, 3H, C$H_3$C=CH), 1.66 (s, 3H, C$H_3$C=CH), 1.72-1.64 (m, 2H, CHC$H_2$CH), 1.59 (s, 3H, C$H_3$C=CH), 1.57 (s, 3H, C$H_3$C=CH), 1.57-1.55 (m, 1H, $CH_2$C*H*($CH_2$)(C)), 0.98 (s, 3H, CC$H_3$), 0.87 (s, 3H, CC$H_3$); $^{13}$C NMR (68 MHz, $CDCl_3$): δ = 214.2, 133.3, 132.4, 123.6, 121.6, 52.3, 48.0, 42.5, 39.1, 31.5, 29.4, 29.0, 27.3, 25.8, 25.8, 23.9, 17.9 (2C); ESI-HRMS (TOF): *m/z* calculated for $C_{18}H_{30}O$ $[M+H]^+$: 263.2369; found 263.2368.

Data were in accord with that of Dr. Rodeschini.[47]

**(±)-(4*S*)-4,6-Bis(3'-methyl-but-2'-enyl)-1-(*tert*-butyl-dimethyl-silyloxy)-3,3-dimethyl-cyclohexene (142)**[47]



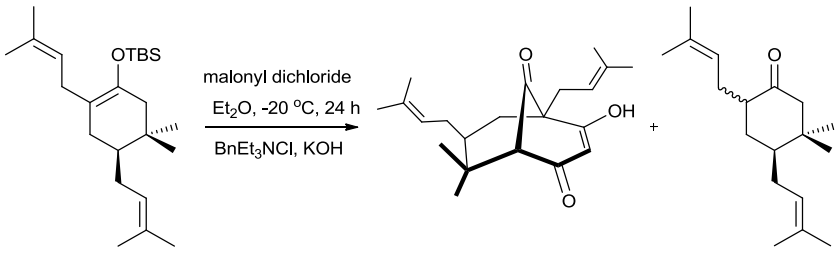

**141** **142**

Ketone **141** (3.79 g, 14.5 mmol) was dissolved in dry MeCN (30 mL). Freshly distilled $Et_3N$ (3 mL, 21 mmol, 1.5 equiv.) was added and the solution stirred. NaI (3.25 g, 22 mmol, 1.5 equiv.) and TBSCl (3.18 g, 21 mmol, 1.5 equiv.) were added and the mixture was heated to reflux for 2 h. After this time the solvent was removed under reduced pressure. The resulting brown residue was triturated using petroleum ether and concentrated to a pale yellow oil, which was purified by flash column

chromatography (99:1 petrol:$Et_2O$), to afford **142** (5.42 g, 99%) as a colourless oil. $R_f$ = 0.22 (1% $Et_2O$ in petroleum ether); $^1H$ NMR (300 MHz, $CDCl_3$) δ: = 5.09 (t, *J* = 6.6, 1H, C=C*H*$CH_2$), 5.03 (t, *J* = 7.0, 1H, C=C*H*$CH_2$), 2.78 (dd, *J* = 14.5, 7.0, 1H, CHC*H*$_2$C=C), 2.68 (dd, *J* = 14.5, 7.0, 1H, CHC*H*$_2$C=C), 2.10 (d, *J* = 13.5, 1H, CHC*H*$_2$CH), 1.99 (dd, *J* = 17.0, 5.5, 1H, CHC*H*$_2$CH), 1.89 (d, *J* = 16.5, 1H, CC*H*$_2$C), 1.77 (d, *J* = 16.5, 1H, CC*H*$_2$C), 1.70 (s, 6H, C*H*$_3$C=CH), 1.70-1.58 (m, 2H, CHC*H*$_2$CH), 1.62 (s, 3H, C*H*$_3$C=CH), 1.59 (s, 3H, C*H*$_3$C=CH), 1.22 (m, 1H, $CH_2$C*H*($CH_2$)(C)), 0.95 (s, 9H, (C*H*$_3$)$_3$CSi), 0.93 (s, 3H,C*H*$_3$C), 0.85 (s, 3H, C*H*$_3$C), 0.12 (s, 6H, (C*H*$_3$)$_2$Si); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ: = 141.4, 132.1, 131.7, 131.6, 124.5, 123.0, 114.4, 113.1, 55.6, 45.0, 43.4, 33.6, 30.6, 28.9, 28.6, 28.0, 26.0, 25.9, 25.8, 22.5, 18.3, 17.9, -3.6 (2C); ESI-HRMS (TOF): *m/z* calculated for $C_{24}H_{44}OSi$ $[M+Na]^+$: 399.3059; found 399.3042.

Data were in accord with that of Dr. Rodeschini.[47]

**(±)-(5*R*,7*S*)-5,7-Bis(3'-methyl-but-2'-enyl)-8,8-dimethyl-4-hydroxy-bicyclo[3.3.1]non-3-ene-2,9-dione (143)**[47]

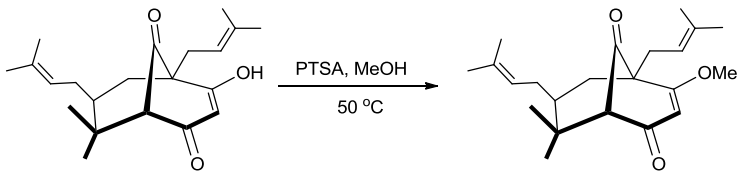



**142** not isolated at this stage **143** 83% **141**

A solution of the silyl enol ether **142** (0.954 g, 2.6 mmol) in $Et_2O$ (15 mL) was cooled to -20 °C (dry ice/$CCl_4$ bath). To this solution malonyl dichloride (246 µL, 2.6 mmol, 1 equiv.) was added dropwise. The mixture was stirred for 24 h at -20 °C. After this time benzyltriethylammonium chloride (33.8 mg, 0.15 mmol, 0.06 equiv.) was added, followed by a solution of KOH (0.654 g, 11.7 mmol, 4.5 equiv.) in $H_2O$ (1 mL). The mixture was stirred and allowed to warm to rt then stirred for a further 5 h. This solution was then diluted with $H_2O$ (25 mL) and petroleum ether (25 mL). The pH was adjusted to ~10 with 2 M NaOH. This was extracted with petroleum ether (2 x 25 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure to afford impure ketone **141** (0.580 g, 83%).

The aqueous layer was cooled to 0 °C and carefully acidified with 2 M HCl. This was extracted with DCM (4 x 25 mL), combined organic layers were dried ($MgSO_4$) and solvent removed under reduced pressure, to afford **143** (301 mg) of crude yellow oil which was used without further purification.

**(±)-(5*R*,7*S*)-5,7-Bis(3'-methyl-but-2'-enyl)-8,8-dimethyl-4-methoxy-bicyclo[3.3.1]non-3-ene-2,9-dione (145)**[47]




11% over two steps

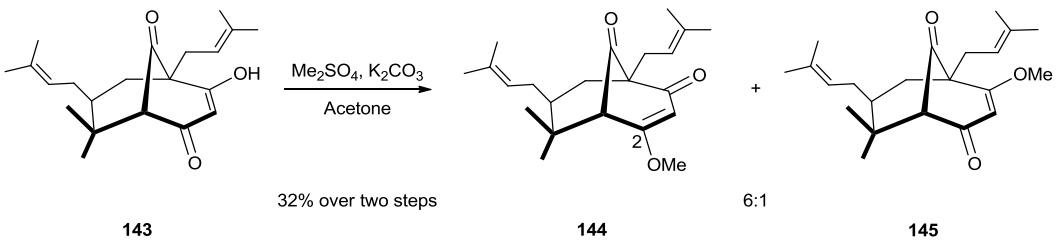
**143** **145**

To a solution of bicyclo[3.3.1]nonane **143** (300 mg, 1 mmol) in MeOH (7 mL), PTSA (17.4 mg, 0.1 mmol, 0.1 equiv.) in trimethyl orthoformate (3 mL, 27 mmol, 27 equiv.)

was added. The mixture was heated to 50 °C for 38 h then cooled to rt and two drops of $Et_3N$ were added. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography (8:2 petrol:EtOAc) to afford **145** (95.2 mg 11% yield over two steps) as a pale yellow solid. m.p. = 71-74 °C; $R_f$ = 0.35 (20% EtOAc in petroleum ether); $^1$H NMR (270 MHz, $CDCl_3$): δ = 5.73 (s, 1H, C=C*H*COMe), 4.98 (m, 2H, C=C*H*-$CH_2$), 3.76 (s, 3H, C$H_3$O), 2.84 (s, 1H, $C_3$C*H*), 2.42 (m, 2H, CHC$H_2$C), 2.10 (m, 1H, CHC$H_2$CH), 1.93 (d, *J* = 13.7, 1H, CC$H_2$CH), 1.72-1.61 (m, 2H, CHC$H_2$CH, $(CH_2)_2$C*H*C), 1.70 (s, 3H, C$H_3$C=C), 1.66 (s, 3H, C$H_3$C=C), 1.65 (s, 3H, C$H_3$C=C), 1.57 (s, 3H, C$H_3$C=C), 1.37 (t, 1H, CC$H_2$CH), 1.10 (s, 3H, C$H_3$C), 0.86 (s, 3H, C$H_3$C); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 206.6, 193.8, 177.8, 133.7, 133.1, 122.5, 119.3, 106.1, 74.6, 57.1, 56.8, 42.9, 40.7, 39.3, 29.6, 27.6, 26.6, 25.9, 25.8, 20.7, 17.9, 17.8; ESI-HRMS (TOF): *m/z* calculated for $C_{22}H_{32}O_3$ $[M+H]^+$: 345.2424; found 345.2420.

Data were in accord with that of Dr. Rodeschini.[47]

**(±)-(5*R*,7*S*)-5,7-Bis(3'-methyl-but-2'-enyl)-8,8-dimethyl-2-methoxy-bicyclo[3.3.1]non-2-ene-4,9-dione (144) and (±)-(5*R*,7*S*)-5,7-Bis(3'-methyl-but-2'-enyl)-8,8-dimethyl-4-methoxy-bicyclo[3.3.1]non-3-ene-2,9-dione (145)**[47]




Crude cyclic triketone **143** (450 mg, 1.3 mmol) was dissolved in acetone (25 mL). To this $K_2CO_3$ (0.965 g, 6.5 mmol, 5 equiv.) was added followed by $Me_2SO_4$ (129 µL,

1.3 mmol, 1 equiv.). This mixture was refluxed for 2 h and allowed to cool to rt. The solid was removed by filtration and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (gradient of 9:1 to 8:2 petrol:EtOAC) to afford **144** and **145** (219 mg, 32% over two steps) as a white solid of the mixed regioisomers in a 6:1 ratio.

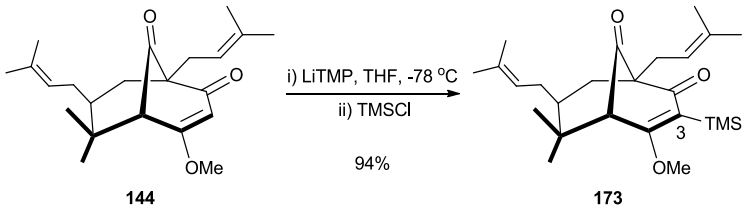
Major isomer (**144**)

m.p. = 94-96 °C; $R_f$ = 0.53 (20% EtOAc in petroleum ether); $^1$H NMR (270 MHz, $CDCl_3$): δ = 5.72 (s, 1H, C=C*H*-COMe), 4.98 (m, 2H, C=C*H*-$CH_2$), 3.76 (s, 3H, C$H_3$O), 2.83 (s, 1H, $C_3$C*H*), 2.42 (m, 2H, CHC$H_2$C), 2.06 (m, 1H, CHC$H_2$CH), 1.93 (dd, *J* = 13.0, 4.0, 1H, CC$H_2$CH), 1.68 (s, 6H C$H_3$C=CH), 1.64 (s, 3H C$H_3$C=CH), 1.72-1.59 (m, 2H, CHC$H_2$CH, $(CH_2)_2$C*H*C), 1.58 (s, 3H, C$H_3$C=CH), 1.35 (m, 1H, CC$H_2$CH), 1.04 (s, 3H, C$H_3$C), 0.92 (s, 3H C$H_3$C); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 207.5, 197.7, 174.4, 133.7, 133.1, 122.4, 119.8, 105.9, 65.9, 63.9, 56.4, 41.8, 40.8, 39.7, 29.4, 28.1, 27.1, 25.9, 25.8, 20.5, 18.0, 17.9; ESI-HRMS (TOF): *m/z* calculated for $C_{22}H_{32}O_3$ $[M+H]^+$: 345.2424; found 345.2425.

Minor isomer as **145** above.

Data were in accord with that of Dr. Rodeschini.[47]

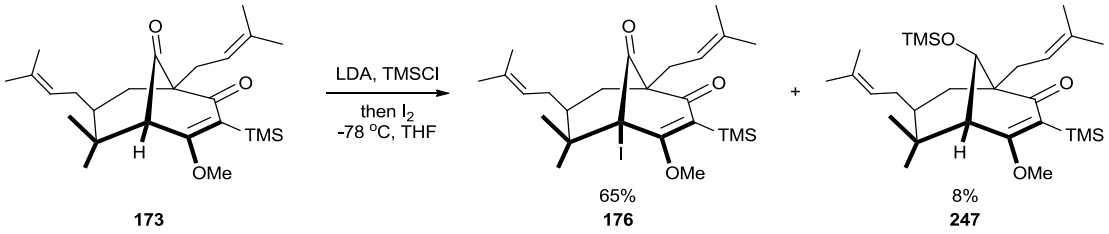
**(±)-(5*S*,7*S*)-5,7-Bis(3'-methyl-but-2'-enyl)-8,8-dimethyl-2-methoxy-3-trimethylsilyl-bicyclo[3.3.1]non-2-ene-4,9-dione (173)**




144 173

LiTMP (22.6 mL of a 0.5 M solution in THF, 11.3 mmol, 2 equiv.) was added dropwise to a solution of *O*-methylated trione **144** (1.95 g, 5.7 mmol) in THF (60 mL) at -78 °C. The reaction mixture was stirred for 5 min. before addition of chlorotrimethylsilane (2.87 mL, 22.6 mmol, 4 equiv.). The reaction mixture was stirred and allowed to warm to -40 °C over 2 h before quenching with saturated aqueous $NH_4Cl$ (30 mL). This was extracted with $Et_2O$ (3 × 40 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (9:1 petrol:$Et_2O$) to afford vinylsilane **173** (2.21 g, 94%) as a white solid. m.p. = 46-48 °C; $R_f$ = 0.34 (10% EtOAc in petroleum ether); FTIR (neat) 2975, 2911, 1731, 1641, 1560, 1447, 1219, 1057, 842 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ = 4.96-4.92 (m, 2H, C=C*H*$CH_2$), 3.73 (s, 3H, OC*H*$_3$), 3.22 (s, 1H, *H*C-1), 2.40 (dd, *J* = 14.3, 7.7, 1H, CHC*H*$_2$C), 2.32 (dd, *J* = 14.3, 7.7, 1H, CHC*H*$_2$C), 2.04 (dd, *J* = 13.2, 5.1, 1H, CHC*H*$_2$C), 1.91 (dd, *J* = 13.2, 4.0, 1H, CC*H*$_2$CH), 1.66 (s, 3H, C*H*$_3$C=CH), 1.63 (s, 3H, C*H*$_3$C=CH), 1.60 (t, *J* = 13.2, 1H, CHC*H*$_2$C), 1.59 (s, 3H, C*H*$_3$C=CH), 1.53 (s, 3H, C*H*$_3$C=CH), 1.54-1.51 (m, 1H, $CH_2$C*H*($CH_2$)(C)), 1.28 (app. t, *J* = 12.8, 1H, CC*H*$_2$CH), 1.09 (s, 3H, C*H*$_3$C), 0.94 (s, 3H, C*H*$_3$C), 0.16 (s, 9H, C*H*$_3$Si); $^{13}C$ NMR (125 MHz, $CDCl_3$): δ = 208.4, 201.9, 177.9, 133.6, 133.2, 123.5, 122.3, 119.6, 63.7, 61.1, 55.8, 42.8, 41.0, 39.4, 29.6, 27.9, 25.8, 25.73, 25.72, 20.9, 18.0, 17.8, 0.33 (3C); ESI-HRMS (TOF): *m/z* calculated for $C_{25}H_{40}O_3Si$ $[M+H]^+$: 417.2820; found 417.2823.

**(±)-(1*S*,5*R*,7*S*)-5,7-Bis(3'-methyl-but-2'-enyl)-8,8-dimethyl-1-iodo-2-methoxy-3-trimethylsilyl-bicyclo[3.3.1]non-2-ene-4,9-dione (176) and (±)-(5*S*,7*S*,9*S*)-5,7-Bis(3'-methylbut-2'-en-1'-yl)-8,8-dimethyl-2-methoxy-3-(trimethylsilyl)-9-((trimethylsilyl)oxy)bicyclo[3.3.1]non-2-en-4-one (247)**




173 65% 176 8% 247

To a solution of TMS protected enone **173** (253 mg, 0.61 mmol,) and chlorotrimethylsilane (0.31 mL, 2.44 mmol, 4 equiv.) in THF (2 mL) at -78 °C, LDA (4.9 mL of a 0.5 M solution in THF, 2.44 mmol, 4 equiv.) was added dropwise. The reaction mixture was stirred at -78 °C for 10 min. before warming to 0 °C over 30 min. The reaction mixture was then cooled to -78 °C and a solution of iodine (0.709 g, 5.5 mmol, 9 equiv.) in THF (2 mL) was added. The reaction mixture was stirred at -78 °C for 10 min. before stirring at 0 °C for 30 min. The reaction mixture was diluted with EtOAc (20 mL), washed with saturated aqueous $Na_2SO_3$ (3 × 10 mL) and brine (3 × 10 mL). The combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (95:5 petrol:EtOAc) to afford bridgehead iodide **176** (214 mg, 65%) as a white solid and by-product **247** (177 mg, 8%) as a white solid.

Major (**176**)

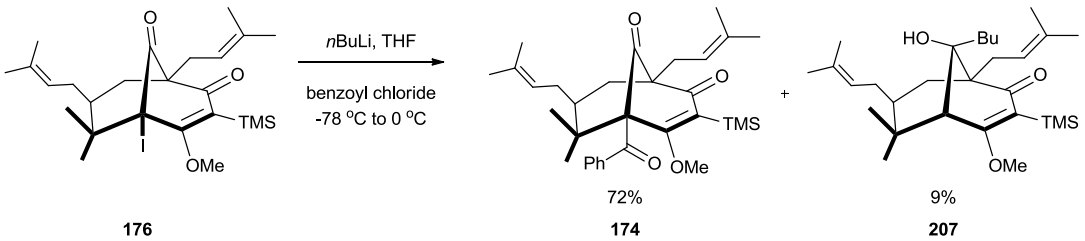
m.p. = 59-61 °C; $R_f$ = 0.5 (10% EtOAc in petroleum ether); FTIR (neat) 2976, 1734, 1654, 1548, 1437, 1250, 1034, 848, 758 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ = 5.04 (t, *J* = 7.2, 1H, C=C*H*$CH_2$), 4.95 (t, *J* = 7.2, 1H, C=C*H*$CH_2$), 3.92 (s, 3H, C$H_3$O), 2.46-

2.38 (m, 2H, CHC$H_2$C), 2.17 (dd, $J$ = 13.4, 5.3, 1H, CHC$H_2$CH), 1.80-1.73 (m, 2H, $CH_2CH(CH_2)$(C), CC$H_2$CH), 1.69-1.63 (m, 1H, CHC$H_2$CH), 1.62 (s, 3H, C$H_3$C=CH), 1.60 (s, 6H, C$H_3$C=CH), 1.51 (s, 3H, C$H_3$C=CH), 1.39 (app. t, $J$ = 14.3, 1H, CC$H_2$CH), 1.23 (s, 3H, C$H_3$C), 0.88 (s, 3H, C$H_3$C), 0.22 (s, 9H, C$H_3$Si); $^{13}$C NMR (125 MHz, $CDCl_3$): δ = 200.0, 199.9, 178.9, 134.0, 133.4, 125.2, 122.2, 119.6, 86.0, 65.6, 65.5, 47.7, 40.9, 38.9, 30.4, 30.2, 29.0, 25.8, 25.6, 20.6, 17.9, 17.8, 0.3 (3C); ESI-HRMS (TOF): *m/z* calculated for $C_{25}H_{39}IO_3Si$ $[M+H]^+$: 543.1791; found 543.1779.

By-product (**247**)

m.p. = 159-160 °C; $R_f$ = 0.66 (20% EtOAc in petroleum ether); FTIR (neat) 2954, 1632, 1564, 1465, 1447, 1385, 1332, 1249, 1089, 952, 835 $cm^{-1}$; $^1$H (300 MHz, $CDCl_3$) δ = 4.95 (dd, $J$ = 6.3, 1H, C=C$H$C$H_2$), 4.81 (dd, $J$ = 6.5, 1H, C=C$H$C$H_2$), 4.01 (d, $J$ = 2.3, 1H, OC$H$(C)(CH)), 3.70 (s, 3H, C$H_3$O), 2.65 (d, $J$ = 3.0, 1H, $H$C-1), 2.43 (dd, $J$ = 14.3, 6.4, 1H, CHC$H_2$C), 2.07-1.94 (m, 2H, CHC$H_2$C), 1-70-1.50 (m, 3H, CHC$H_2$C, CC$H_2$CH, $CH_2CH(CH_2)$(C)), 1.65 (s, 3H, C$H_3$C=CH), 1.61 (s, 3H, C$H_3$C=CH), 1.59 (s, 3H, C$H_3$C=CH), 1.55 (s, 3H, C$H_3$C=CH), 1.40 (dd, $J$ = 12.9, 1H, CC$H_2$CH), 1.13 (s, 3H, C$H_3$C), 0.92 (s, 3H, C$H_3$C), 0.15 (s, 9H, C$H_3$Si), 0.14 (s, 9H, C$H_3$Si); $^{13}$C (100 MHz, $CDCl_3$) δ = 206.2, 181.8, 132.4, 131.5, 123.1, 120.4, 119.2, 70.8, 54.6, 51.8, 48.9, 39.3, 34.7, 32.0, 30.9, 28.3, 27.5, 25.3, 25.2, 23.7, 17.6, 17.3, 0.0 (3C), -0.3 (3C); ESI-HRMS (TOF): *m/z* calculated for $C_{28}H_{50}O_3Si_2$ $[M+Na]^+$: 513.3196; found 513.3199.

**(±)-(1*R*,5*S*,7*S*)-1-Benzoyl-5,7-bis(3'-methyl-but-2'-enyl)-8,8-dimethyl-2-methoxy-3-trimethylsilyl-bicyclo[3.3.1]non-2-ene-4,9-dione (174)**

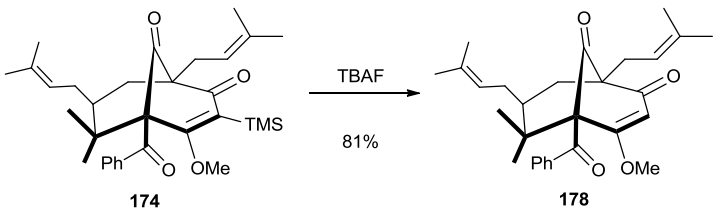



*n*BuLi (0.57 mL of a 2.5 M solution in hexane, 1.42 mmol, 1.2 equiv.) was added dropwise to a solution of bridgehead iodide **176** (0.643 g, 1.19 mmol) in THF (9 mL) at -78 °C. The reaction mixture was stirred at -78 °C for 5 min. before addition of benzoyl chloride (159 µL, 1.42 mmol, 1.2 equiv.) dropwise. The reaction mixture was stirred for 1 h at -78 °C and at rt for 30 min. before quenching with saturated aqueous $NH_4Cl$ (10 mL). This was extracted into $Et_2O$ (3 × 15 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (9:1 petrol:$Et_2O$) to afford the bridgehead benzoylated product **174** (446 mg, 72%) as a white solid. m.p. = 104-106 °C; $R_f$ = 0.42 (20% $Et_2O$ in petroleum ether); FTIR (neat) 2915, 1721, 1703, 1650, 1559 (s), 1446, 1391, 1247, 1063, 848 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ = 7.58 (d, *J* = 7.4, 2H, $H^{ortho\text{-ar}}$), 7.41 (t, *J* = 7.4, 1H, $H^{para\text{-ar}}$), 7.29 (t, *J* = 7.8, 2H, $H^{meta\text{-ar}}$), 5.07 (t, *J* = 7.1, 1H, C=C*H*$CH_2$), 4.99 (t, *J* = 7.8, 1H, C=C*H*$CH_2$) 3.46 (s, 3H, C*H*$_3$O), 2.52 (dd, *J* = 14.5, 7.45, 1H, CC*H*$_2$C(H)=), 2.44 (dd, *J* = 14.0, 7.2, 1H, CC*H*$_2$C(H)=), 2.13 (dd, *J* = 11.3, 5.1, 1H, CHC*H*$_2$CH), 1.94 (dd, *J* = 13.4, 3.9, 1H, CC*H*$_2$CH), 1.67 (s, 3H,C*H*$_3$C=CH), 1.66 (s, 6H, C*H*$_3$C=CH), 1.65 (m, 2H, CHC*H*$_2$CH, $CH_2$C*H*(C)($CH_2$)) 1.55 (s, 3H, C*H*$_3$C=CH), 1.45 (dd, *J* = 12.6, 1H, CC*H*$_2$CH), 1.36 (s, 3H, C*H*$_3$C=C), 1.18 (s, 3H, C*H*$_3$C=C), 0.23 (s, 9H, $(CH_3)_3$Si); $^{13}C$ NMR (125 MHz,

$CDCl_3$): δ = 208.2, 200.7, 193.1, 181.2, 137.0, 134.3, 133.4, 132.1, 128.5 (2C), 127.9 (2C), 122.9, 122.4, 119.7, 75.0, 66.0, 64.1, 48.2, 43.4, 42.5, 29.4, 27.5, 26.0, 25.7, 24.6, 18.1, 17.8, 16.3, 0.81 (3C); ESI-HRMS (TOF): *m/z* calculated for $C_{32}H_{44}O_4$ $[M+H]^+$: 521.3082; found 521.3104.

By-product (**207**) was isolated (53 mg, 9%) as a clear oil. $R_f$ = 0.83 (20% EtOAc in petroleum ether); $^1$H NMR (400 MHz, $CDCl_3$): δ = 5.43 (t, *J* = 7.8, 1H, C=C*H*$CH_2$), 4.95 (t, *J* = 7.0, 1H, C=C*H*$CH_2$), 3.76 (s, 3H, C*H*$_3$O), 2.53 (s, 1H, *H*C-1), 2.40-2.35 (m, 2H, CHC*H*$_2$C), 2.10-2.02 (m, 1H, CHC*H*$_2$CH), 1.86 (dd, *J* = 13.1, 1H, CC*H*$_2$CH), 1.86 (s, 1H, *H*O), 1.78-1.58 (m, 3H, $CH_2$C*H*(C)($CH_2$), CHC*H*$_2$CH, CC*H*$_2$CH), 1.70 (s, 3H, C*H*$_3$C=CH), 1.63 (s, 6H, C*H*$_3$C=CH), 1.54 (s, 3H, C*H*$_3$C=CH), 1.52-1.20 (m, 6H, $Bu^{chain}$), 1.17 (s, 3H, C*H*$_3$C), 0.90 (s, 3H, C*H*$_3$C), 0.86 (t, *J* = 7.3, 3H, C*H*$_3$$CH_2$), 0.14 (s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 205.7, 182.0, 133.1, 131.6, 123.0, 121.1, 118.2, 78.4, 54.9, 54.3, 49.1, 38.9, 37.9, 35.4, 32.1, 30.3, 28.0, 27.0, 25.6, 25.1, 24.2 (2C), 22.7, 17.4, 17.2, 13.2, 0.0 (3C); ESI-HRMS (TOF): *m/z* calculated for $C_{29}H_{50}O_3Si$ $[M+Na]^+$: 497.3427; found 497.3434.

**(±)-(1*R*,5*R*,7*S*)-1-Benzoyl-5,7-bis(3'-methyl-but-2'-enyl)-8,8-dimethyl-2-methoxy-bicyclo[3.3.1]non-2-ene-4,9-dione (178)**

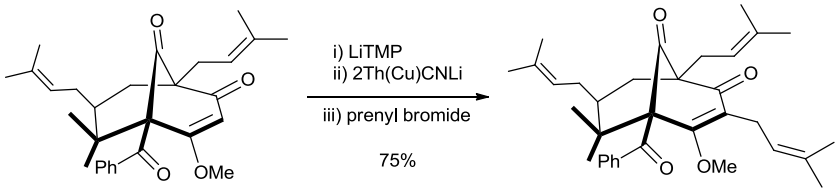



**174** **178**

A solution of TBAF (0.33 mL of a 1 M solution in THF, 0.33 mmol, 1.5 equiv.) was added to a solution of **174** (117 mg, 0.22 mmol) in THF (3 mL) at -78 °C. The

reaction mixture was allowed to warm to rt over 1.5 h before quenching with saturated aqueous $NH_4Cl$ (10 mL). The product was extracted with $Et_2O$ (3 × 15 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography ($CH_2Cl_2$) to afford the benzoylated product **178** (80 mg, 81%) as a white solid. m.p. = 141-142 °C (sublim.); $R_f$ = 0.40 (8:2 petrol:EtOAc); FTIR (neat) 2931, 1720, 1655, 1606, 1445, 1344, 1237, 1218 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ = 7.59 (d, *J* = 7.3, 2H, $H^{ortho\text{-ar}}$), 7.42 (t, *J* = 7.3, 1H, $H^{para\text{-ar}}$), 7.27 (app. t, *J* = 7.9, 2H, $H^{meta\text{-ar}}$), 5.94 (s, 1H, C=C*H*C(=O)), 5.00 (t, *J* = 7.0, 1H, C=C*H*$CH_2$), 4.96 (t, *J* = 7.0, 1H, C=C*H*$CH_2$), 3.50 (s, 3H, C*H*$_3$O), 2.56 (dd, *J* = 14.3, 7.0, 1H, CC*H*$_2$CH), 2.44 (dd, *J* = 14.2, 7.2, 1H, CC*H*$_2$CH), 2.12 (dd, *J* = 13.2, 5.1, 1H, CHC*H*$_2$CH), 1.98 (dd, *J* = 13.2, 4.4, 1H, CC*H*$_2$CH), 1.79-1.72 (m, 1H, $CH_2$C*H*(C)($CH_2$)), 1.71-1.62 (m, 1H, CHC*H*$_2$CH), 1.66 (s, 3H, C*H*$_3$C=CH), 1.65 (s, 3H, C*H*$_3$C=CH), 1.64 (s, 3H, C*H*$_3$C=CH), 1.55 (s, 3H, C*H*$_3$C=CH), 1.45 (app. t, *J* = 13.0, 1H, CC*H*$_2$CH), 1.34 (s, 3H, C*H*$_3$C), 1.18 (s, 3H, C*H*$_3$C); $^{13}C$ NMR (125 MHz, $CDCl_3$): δ = 207.3, 196.1, 192.5, 173.8, 137.0, 134.4, 133.3, 132.1, 127.92 (2C), 127.91 (2C), 122.3, 119.5, 106.5, 72.4, 65.6, 56.5, 47.5, 42.6, 42.5, 29.0, 27.7, 25.9, 25.8, 24.3, 18.1, 17.8, 15.8; ESI-HRMS (TOF): *m/z* calculated for $C_{29}H_{36}O_4$ $[M+H]^+$: 449.2692; found 449.2691.

**(±)-(1*R*,5*R*,7*S*)-1-Benzoyl-8,8-dimethyl-2-methoxy-3,5,7-tris(3'-methyl-but-2'-enyl)-bicyclo[3.3.1]non-2-ene-4,9-dione (157)**




178 157

LiTMP (2.6 mL of a 0.1 M solution in THF, 0.26 mmol, 2 equiv.) was added to a solution of enone **178** (58.9 mg, 0.13 mmol) in THF (2 mL) at -78 °C and stirred for 10 min. The reaction mixture was stirred for a further 30 min. at -40 °C before addition of Li(2-Th)CuCN (2.6 mL of a 0.1 M solution in THF, 0.26 mmol, 2 equiv.). The reaction mixture was stirred at -78 °C for 10 min. and at -40 °C for 30 min. The reaction mixture was then cooled to -78 °C and prenyl bromide (0.077 mL, 0.66 mmol, 5 equiv.) was added. The reaction mixture was warmed to 0 °C over 3 h before quenching with $NH_4Cl/NH_4OH_{(aq)}$ (10% *w/w*, 20 mL). This was extracted with $Et_2O$ (3 × 20 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (1:1 DCM:petrol) to afford *O*-methylated nemorosone **157** (50 mg, 75%) as a colourless oil. $R_f$ = 0.35 (20% $Et_2O$ in petroleum ether); FTIR (neat) 2914, 1721, 1702, 1655, 1598, 1446, 1240, 690 $cm^{-1}$; $^1H$ (500 MHz, $CDCl_3$): δ = 7.62 (d, *J* = 7.3, 2H, $H^{ortho\text{-ar}}$), 7.44 (t, *J* = 7.3, 1H, $H^{para\text{-ar}}$), 7.30 (t, *J* = 7.9, 2H, $H^{meta\text{-ar}}$), 5.02 (t, *J* = 6.5, 1H, C=C*H*$CH_2$), 5.01 (t, *J* = 6.5, 1H, C=C*H*$CH_2$), 4.95-4.90 (m, 1H, C=C*H*$CH_2$), 3.46 (s, 3H, C*H*$_3$O), 3.34 (dd, *J* = 15.8, 6.6, 1H, CHC*H*$_2$C), 3.23 (dd, *J* = 15.8, 6.2, 1H, CHC*H*$_2$C), 2.58 (dd, *J* = 13.9, 6.6, 1H, CHC*H*$_2$C), 2.48 (dd, *J* = 13.9, 7.3, 1H, CHC*H*$_2$C), 2.14-2.08 (m, 1H, CHC*H*$_2$CH), 1.95 (dd, *J* = 13.0, 3.9, 1H, CC*H*$_2$CH),

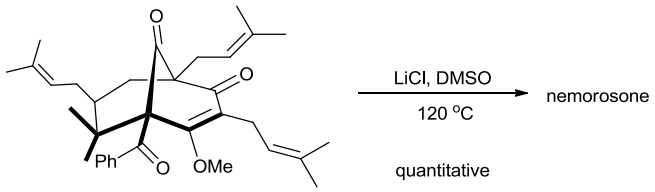
1.72-1.64 (m, 2H, CHC*H*$_2$CH, CH$_2$C*H*(CH$_2$)(C)), 1.69 (s, 3H, C*H*$_3$C=CH) 1.68 (s, 12H, C*H*$_3$C=CH), 1.56 (s, 3H, C*H*$_3$C=CH), 1.45 (t, *J* = 12.8, 1H, CC*H*$_2$CH), 1.35 (s, 3H, C*H*$_3$C), 1.19 (s, 3H, C*H*$_3$C); $^{13}$C (125 MHz, $CDCl_3$): δ = 207.9, 197.1, 193.1, 169.9, 137.0, 134.4, 133.2, 133.0, 132.0, 128.4 (2C), 127.9 (2C), 123.2, 122.5, 121.5, 119.7, 74.0, 65.1, 61.5, 47.8, 43.1, 42.5, 29.5, 27.7, 26.0, 25.8, 25.6, 24.4, 23.2, 18.1, 18.0, 17.8, 16.2; ESI-HRMS (TOF): *m/z* calculated for $C_{34}H_{44}O_4$ $[M+H]^+$: 517.3312; found 517.3305.

Enantiomers separated by chiral HPLC: 1.5% MeOH in hexane on Lux cellulose II column 14.11, 18.13 min.

$[\alpha]_D^{22}$ = -133 (*c* 0.018, MeOH) (component 1 from HPLC).

$[\alpha]_D^{22}$ = +139 (*c* 0.018, MeOH) (component 2 from HPLC).

**Nemorosone (4)**




**157** **4**

*O*-Methylated nemorosone (**157**) (41 mg, 79 μmol) and lithium chloride (29 mg, 0.68 mmol) were dissolved in $d_6$-DMSO (0.75 mL) and heated to 120 °C for 2 h. The reaction mixture was then cooled to rt diluted with $H_2O$ (5 mL) and extracted with $Et_2O$ (3 × 5 mL). The combined organic layers were washed with brine (5 mL) and dried ($MgSO_4$) and the solvent was removed under reduced pressure to afford nemorosone **4** (39 mg, quant.) as a colourless oil which did not require further purification. $R_f$ = 0.7 (DCM) ; FTIR (neat) 3400, 2923, 1722, 1699, 1626, 1597, 1447,

1373, 1223 $cm^{-1}$; $^1H$ NMR (500 MHz, $d_4$-methanol): δ = 7.58 (d, *J* = 8.4, 2H, $H^{ortho\text{-ar}}$), 7.47 (t, *J* = 7.3, 1H, $H^{para\text{-ar}}$), 7.29 (t, *J* = 7.7, 2H, $H^{meta\text{-ar}}$), 5.12 (t, *J* = 7.3, 1H, C=C*H*$CH_2$), 5.06-5.02 (m, 2H, C=C*H*$CH_2$), 3.17 (dd, *J* = 14.7, 7.3, 1H, CHC*H*$_2$C), 3.12 (dd, *J* = 14.7, 7.3, 1H, CHC*H*$_2$C), 2.57 (dd, *J* = 14.3, 7.0, 1H, CHC*H*$_2$C), 2.52 (dd, *J* = 14.3, 7.0, 1H, CHC*H*$_2$C), 2.21-2.17 (m, 1H, CHC*H*$_2$CH), 2.06 (dd, *J* = 13.6, 3.7, 1H, CC*H*$_2$CH), 1.82-1.74 (m, 2H, CHC*H*$_2$CH, $CH_2$C*H*($CH_2$)(C)), 1.72 (s, 3H, C*H*$_3$C=C), 1.693 (s, 3H, C*H*$_3$C=C), 1.685 (s, 9H, C*H*$_3$C=C), 1.63 (s, 3H, C*H*$_3$C=C), 1.47 (app. t, *J* = 13.2, 1H, CC*H*$_2$CH), 1.37 (s, 3H, C*H*$_3$C), 1.14 (s, 3H, C*H*$_3$C). The resonance for the hydroxy proton could not be identified in the $^1H$ NMR spectrum. $^{13}C$ NMR (125 MHz, $d_4$-methanol): δ = 209.5, 195.1, 138.4, 135.3, 134.3, 133.7, 133.2, 129.7 (2C), 128.9 (2C), 124.1, 122.4, 121.3, 121.0, 78.2, 62.1, 49.1, 44.8, 42.4, 30.6, 28.4, 26.4, 26.18, 26.16, 24.5, 22.5, 18.5, 18.3, 18.1, 16.4. The resonances for C-2 and C-4 could not be identified in the $^{13}C$ NMR spectrum due to tautomeric exchange. ESI-HRMS (TOF): *m/z* calculated for $C_{33}H_{42}O_4$ $[M+H]^+$: 503.3161; found 503.3159.

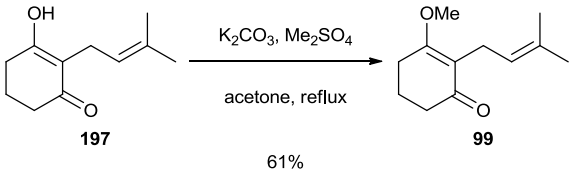
$[\alpha]_D^{22}$ = -77 (*c* 0.024, MeOH) (component 1 from *O*-Meth Nem (**157**) HPLC).

$[\alpha]_D^{22}$ = +86 (*c* 0.022, MeOH) (component 2 from *O*-Meth Nem (**157**) HPLC).

Lit $[\alpha]_D^{23}$ = +113 (*c* 0.1, $CHCl_3$)[54]

## 7.2 Experimental for Chapter 3

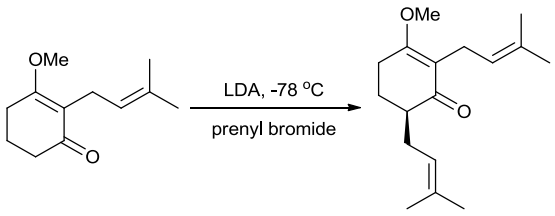
### (±)-3-Methoxy-2-(3'-methylbut-2'-enyl)cyclohex-2-enone (99)[47]




Known enol ether **197** (5.69 g, 32 mmol) was dissolved in acetone (180 mL) and to this was added potassium carbonate (21.83 g, 158 mmol, 5 equiv.) followed by dimethyl sulphate (3.6 mL, 38 mmol, 1.2 equiv). The mixture was heated to reflux for 3 h, allowed to cool and the solid removed by filtration. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography (8:2 petrol:EtOAc) to afford **99** (3.78 g, 61%) as a pale yellow oil. $R_f$ = 0.11 (20% EtOAc in petroleum ether); $^1$H NMR (300 MHz, $CDCl_3$): δ = 5.05 (t, *J* = 7.2, 1H, C=C*H*$CH_2$), 3.81 (s, 3H, C*H*$_3$O), 2.96 (d, *J* = 7.0, 2H, CHC*H*$_2$C), 2.57 (t, *J* = 6.3, 2H, CC*H*$_2$$CH_2$), 2.33 (t, *J* = 7.0, 2H, $CH_2$C*H*$_2$C), 2.05-1.94 (m, 2H, $CH_2$C*H*$_2$$CH_2$), 1.70 (s, 3H, C*H*$_3$C=C), 1.65 (s, 3H, C*H*$_3$C=C); $^{13}$C NMR (68 MHz, $CDCl_3$): δ = 198.0, 171.6, 131.1, 122.7, 119.1, 55.1, 36.4, 25.7, 24.8, 21.2, 20.8, 17.7; ESI-HRMS (TOF): *m/z* calculated for $C_{12}H_{18}O_2$ $[M+Na]^+$: 217.1199; found 217.1203.

Data were in accord with that of Dr. Rodeschini.[47]

**(±)-(6*R*)-2,6-Bis(3'-methylbut-2'-enyl)-3-methoxycyclohex-2-enone (100)**[47]

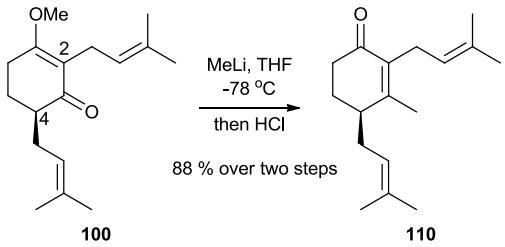



**99** **100**

*n*BuLi (8.6 mL of a 2.5 M solution in hexane, 21.4 mmol, 1.1 equiv.) was added to a solution of diisopropylamine (3 mL, 21.4 mmol, 1.1 equiv.) in THF (100 mL) at -78 °C and the solution stirred for 10 min. To this methyl enol ether **99** (3.78 g, 19.4 mmol) was added and the resulting solution was stirred at this temperature for 30 min. After this time prenyl bromide (2.7 mL, 23.3 mmol, 1.2 equiv.) was added dropwise and the solution was stirred and allowed to warm to rt over 3 h before quenching with saturated aqueous $NH_4Cl$. This was extracted with $Et_2O$ (3 x 75 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure to afford **100** (5.48 g) of crude yellow oil which was used without further purification. $R_f$ = 0.36 (20% EtOAc in petroleum ether); $^1$H NMR (300 MHz, $CDCl_3$): δ = 5.11 (t, *J* = 7.35, 1H, C=C*H*$CH_2$), 5.04 (t, *J* = 7.17, 1H, C=C*H*$CH_2$), 3.80 (s, 3H, C*H*$_3$O), 3.03-2.88 (m, 2H, CHC*H*$_2$C), 2.69-2.42 (m, 3H, CHC*H*$_2$CH, CC*H*$(CH_2)_2$), 2.22-1.99 (m, 3H, $CH_2$C*H*$_2$CH, CC*H*$_2$$CH_2$), 1.71 (s, 6H, C*H*$_3$C=C), 1.67 (m, 1H, $CH_2$C*H*$_2$CH), 1.65 (s, 3H, C*H*$_3$C=C), 1.62 (s, 3H, C*H*$_3$C=C); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 199.5, 170.7, 133.0, 130.8, 122.8, 122.1, 118.4, 54.9, 44.9, 28.2, 25.7, 25.6, 25.3, 23.8, 21.5, 17.7, 17.6; ESI-HRMS (TOF): *m/z* calculated for $C_{17}H_{26}O_2$ $[M+H]^+$: 263.2006; found 263.2012.

Data were in accord with that of Dr. Rodeschini.[47]

**(±)-(4*R*)-2,4-Bis(3'-methylbut-2'-enyl)-3-methylcyclohex-2-enone (110)**[47]

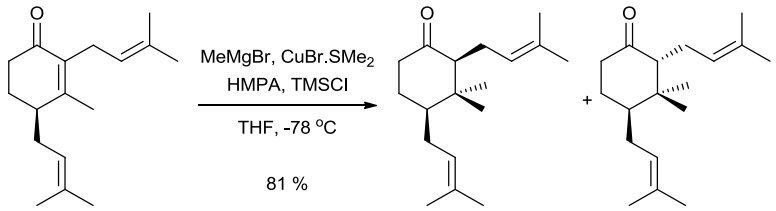



**100** **110**

**100** (5.48 g, ~21 mmol) was dissolved in THF (50 mL). The solution was cooled to -78 °C and treated with MeLi (19.6 mL of a 1.6 M solution, 31.3 mmol, ~1.5 equiv.). The reaction was stirred and allowed to warm to rt over 3 h, after which time 1 M HCl (40 mL) was added and the mixture stirred for 10 min. This was extracted with $Et_2O$ (3 x 50 mL), combined organic layers were dried ($MgSO_4$) and solvent removed under reduced pressure. The mixture was purified by flash column chromatography (9:1 petrol:$Et_2O$) to afford **110** (4.22 g, 88% over two steps) as a yellow oil. $R_f$ = 0.62 (20% EtOAC in petroleum ether); $^1$H NMR (300 MHz, $CDCl_3$): δ = 5.17-5.09 (m, 1H, C=C*H*$CH_2$), 4.95-4.86 (m, 1H, C=C*H*$CH_2$), 3.08-2.90 (m, 2H, CHC*H*$_2$C), 2.56-2.41 (m, 1H, CC*H*$(CH_2)_2$), 2.38-2.12 (m, 4H, CHC*H*$_2$CH, CC*H*$_2$$CH_2$), 2.07-1.97 (m, 1H, $CH_2$C*H*$_2$CH), 1.95 (s, 3H, C*H*$_3$C=C), 1.89-1.78 (m, 1H, $CH_2$C*H*$_2$CH), 1.73 (s, 3H, C*H*$_3$C=C), 1.71 (s, 3H, C*H*$_3$C=C), 1.67 (s, 3H, C*H*$_3$C=C), 1.63 (s, 3H, C*H*$_3$C=C); $^{13}$C NMR (68 MHz, $CDCl_3$): δ = 198.2, 158.8, 134.9, 133.6, 131.4, 122.4, 122.1, 41.7, 33.8, 29.7, 25.8, 25.7, 25.5, 24.5, 20.0, 17.8 (2C); ESI-HRMS (TOF): *m/z* calculated for $C_{17}H_{26}O$ $[M+Na]^+$: 269.1881; found 269.1860.

Data were in accord with that of Dr. Rodeschini.[47]

**(±)-(2*S*,4*R*)-Bis(3'-methylbut-2'-enyl)-3,3-dimethylcyclohexanone (111) and (±)-(2*R*,4*R*)-Bis(3'-methylbut-2'-enyl)-3,3-dimethylcyclohexanone (112)**




**110** **111** **112**

A suspension of $CuBr.Me_2S$ (181 mg, 0.88 mmol, 0.5 equiv.) in THF (100 mL) was cooled to -78 °C. This solution was treated with MeMgBr (14.2 mL of a 3 M solution, 43 mmol, 2.5 equiv.) and HMPA (6 mL, 34 mmol, 2 equiv.) and was stirred for 10 min. After which time a solution of enone **110** (4.22 g, 17 mmol) and TMSCl (4.4 mL, 34 mmol, 2 equiv.) in THF (17 mL) was added to the reaction mixture. The solution was stirred and allowed to warm to rt over 2.5 h before quenching with 2 M HCl (~40 mL). This was extracted with $Et_2O$ (3 x 50 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The mixture was purified by flash column chromatography (9:1 petrol:$Et_2O$) to afford **111** and **112** (3.61 g, 81%) as a yellow oil which were not separated. $R_f$ = 0.5 (10% EtOAc in petroleum ether).

Major (**111**)

$^1$H NMR (300 MHz, $CDCl_3$): δ = 5.13 (m, 1H, C=C*H*$CH_2$), 5.04 (m, 1H, C=C*H*$CH_2$), 2.39 (m, 1H, CHC*H*$_2$CH), 2.32 (m, 2H, CC*H*$_2$C$H_2$), 2.23 (m, 1H, CHC*H*$_2$CH), 2.17 (dd, *J* = 9.5, 2.0, 1H, $CH_2$C*H*$(C)_2$), 2.08-1.99 (m, 2H, CHC*H*$_2$CH, $CH_2$C*H*$_2$CH), 1.71 (s, 3H, C*H*$_3$C=C), 1.69 (m, 1H, CHC*H*$_2$CH), 1.63 (s, 6H, C*H*$_3$C=C), 1.60 (s, 3H, C*H*$_3$C=C), 1.55 (m, 1H, CC*H*$(CH_2)_2$), 1.41 (m, 1H, CHC*H*$_2$C$H_2$), 1.15 (s, 3H, C*H*$_3$C), 0.63 (s, 3H,

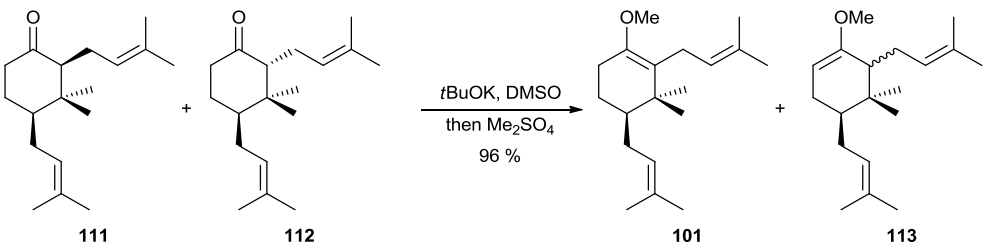
C*H*$_3$C); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 212.4, 132.5, 131.5, 124.3, 123.7, 62.0, 48.2, 43.4, 42.5, 29.2, 28.6, 26.8, 25.8, 25.7, 21.9, 17.8, 17.7, 15.4; ESI-HRMS (TOF): *m/z* calculated for $C_{18}H_{30}O$ $[M+H]^+$: 263.2369; found 263.2368.

Minor (**112**)

$^1$H NMR (300 MHz, $CDCl_3$): δ = 5.14 (m, 1H, C=C*H*$CH_2$), 4.96 (m, 1H, C=C*H*$CH_2$), 2.34 (m, 2H, $CH_2CH_2C$, $CHCH_2CH$), 2.21 (m, 3H, $CCH_2CH$, $CH_2CH_2C$, $CHCH_2CH$), 2.05 (dd, *J* = 11.0, 4.0, 1H, CC*H*$(CH_2)(C)$), 1.97 (m, 1H, $CHCH_2CH_2$), 1.83 (m, 1H, $CHCH_2CH$), 1.72 (s, 3H, C*H*$_3$C=C), 1.67 (m, 1H, CC*H*$(CH_2)_2$), 1.65 (s, 3H, C*H*$_3$C=C), 1.62 (s, 3H, C*H*$_3$C=C), 1.60 (s, 3H, C*H*$_3$C=C), 1.51 (m, 1H, $CHCH_2CH_2$), 0.98 (s, 3H, C*H*$_3$C), 0.89 (s, 3H, C*H*$_3$C); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 214.8, 132.6, 132.5, 123.7, 121.8, 62.0, 42.1, 40.3, 37.9, 27.5, 27.3, 26.0, 25.9, 25.8, 25.1, 23.4, 17.9, 17.8; ESI-HRMS (TOF): *m/z* calculated for $C_{18}H_{30}O$ $[M+H]^+$: 263.2369; found 263.2370.

Data were in accord with that of Dr. Rodeschini.[47]

**(±)-(4*R*)-Bis(3-methylbut-2-enyl)-3,3-dimethyl-1-methoxycyclohex-1-ene (101) and (±)-(4*S*)-Bis(3-methylbut-2-enyl)-5,5-dimethyl-1-methoxycyclohex-6-ene (113)**[47]



*t*BuOK (2.33 g, 21 mmol, 1.5 equiv.) was dissolved in DMSO (30 mL). A solution of **111** and **112** (3.61 g, 13.8 mmol) in DMSO (30 mL) were added. The solution was stirred for 1 h. After this time $Me_2SO_4$ (2 mL, 20.7 mmol, 1.5 equiv.) was added and the mixture stirred for a further 15 min. The reaction mixture was extracted with 2 portions of petrol (2 x 20 mL) and petrol:$Et_2O$ (8:2) (20 mL). The combined organic layers were washed with water and brine, dried ($MgSO_4$) and the solvent was removed under reduced pressure. The mixture was purified by flash column chromatography (8:2 petrol:EtOAc) to afford **101** and **113** (3.67 g, 96%) as a yellow oil. $R_f$ = 0.77 and 0.67 (20% $Et_2O$ in petroleum ether).

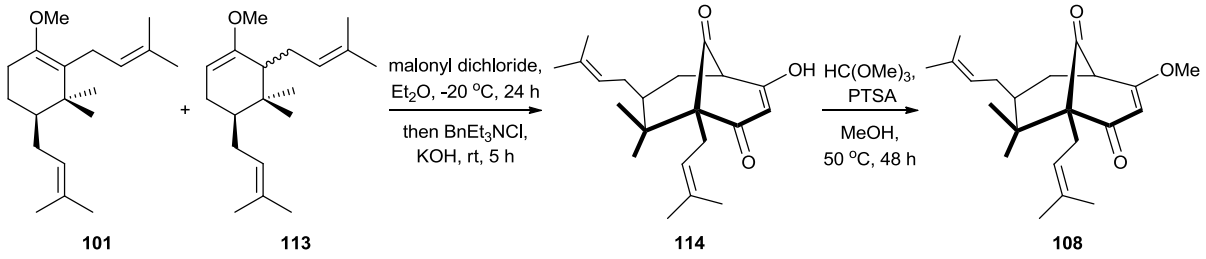
Major (**101**)

$^1$H NMR (300 MHz, $CDCl_3$): δ = 5.12 (m, 1H, C=C*H*$CH_2$), 5.03, (m, 1H, C=C*H*$CH_2$), 3.45 (s, 3H, C*H*$_3$O), 2.81 (m, 1H, CHC*H*$_2$C), 2.62 (m, 1H, CHC*H*$_2$C), 2.15-2.08 (m, 3H, $CH_2$C*H*$_2$C, CHC*H*$_2$CH), 1.76-1.69 (m, 2H, CHC*H*$_2$CH, CHC*H*$_2$$CH_2$), 1.71 (s, 3H, C=CC*H*$_3$), 1.67 (s, 6H, C=CC*H*$_3$), 1.61 (s, 3H, C=CC*H*$_3$), 1.39-1.31 (m, 1H, CC*H*$(CH_2)_2$), 1.22-1.15 (m, 1H, CHC*H*$_2$$CH_2$), 1.03 (s, 3H, CC*H*$_3$), 0.86 (s, 3H, CC*H*$_3$); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 148.5, 131.9, 129.0, 126.1, 124.9, 124.5, 55.3, 45.5, 37.6, 28.5, 26.6, 25.9, 25.8, 25.0, 24.3, 23.2, 21.9, 17.8, 17.7; ESI-HRMS (TOF): *m/z* calculated for $C_{19}H_{32}O$ $[M+H]^+$: 277.2526; found 277.2523.

Minor not isolated separately.

Data were in accord with that of Dr. Rodeschini.[47]

**(±)-(1*R*,7*S*)-1,7-Bis(3'-methyl-but-2'-enyl)-8,8-dimethyl-4-methoxybicyclo[3.3.1]non-3-ene-2,9-dione (108)**[47]




15-35 % Over two steps

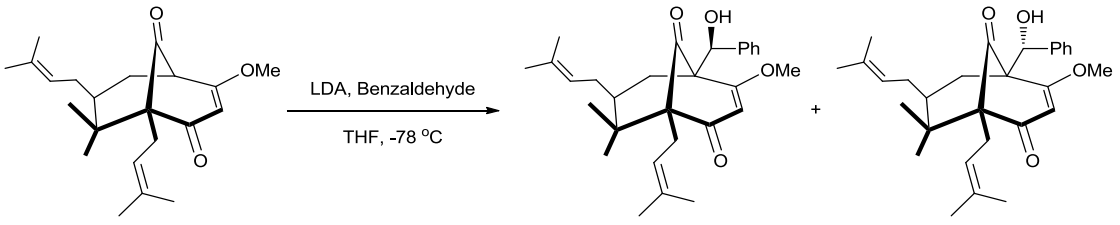
A mixture of **101** and **113** (3.67 g, 13.2 mmol) was dissolved in $Et_2O$ (7.5 mL), cooled to -20 °C and treated with malonyl dichloride (1.3 mL, 13.2 mmol, 1 equiv.). This solution was stirred at -20 °C for 24 h and then treated with $BnEt_3NCl$ (150 mg, 0.6 mmol, 0.05 equiv.) and KOH (2.96 g, 53 mmol, 4 equiv.) in $H_2O$ (7.5 mL). The solution was stirred for 3.5 h and allowed to warm to rt. The mixture was diluted with $H_2O$ (50 mL) and petrol (50 mL), the solution was basified (~12 pH) with 2 M NaOH and extracted with petrol. The combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure to afford the ketones **111** and **112**.

The aqueous layer was cooled to 0 °C and carefully acidified using 2 M HCl. The solution was extracted with DCM, the combined organic layers dried ($MgSO_4$) and the solvent was removed under reduced pressure to afford 1.25 g of crude bicyclo[3.3.1]nonane **114** which was used directly without further purification. Bicyclo[3.3.1]nonane **114** (1.25 g, max 3.79 mmol) was dissolved in MeOH (30 mL) and treated with PTSA (76 mg, 10 mol%, 0.379 mmol) and trimethyl orthoformate (4.1 mL, 37.9 mmol, 10 equiv.). The solution was heated to 50 °C for 2 days and then cooled to rt. Four drops of $Et_3N$ were added and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography

(9:1 petrol:EtOAC) to afford **108** (0.68 g, 15% over two steps) as a colourless oil. $R_f$ = 0.4 (20% EtOAc in petroleum ether); $^1$H NMR (300 MHz, $CDCl_3$): δ = 5.73 (s, 1H, *H*C-3), 4.96 (t, *J* = 8.0, 1H, C=C*H*$CH_2$), 4.66 (t, *J* = 7.1, 1H, C=C*H*$CH_2$), 3.76 (s, 3H, C$H_3$O), 3.18 (s, 1H, *H*C-5), 2.59 (dd, *J* = 13.8, 7.2, 1H, CHC$H_2$C), 2.41 (dd, *J* = 13.9, 6.0, 1H, CHC$H_2$C), 2.18-1.98 (m, 2H, CHC$H_2$CH, CHC$H_2$CH), 1.75-1.65 (m, 3H, CHC$H_2$CH, CC*H*$(CH_2)_2$, CHC$H_2$CH), 1.67 (s, 6H, C$H_3$C=C), 1.58 (s, 3H, C$H_3$C=C), 1.55 (s, 3H, C$H_3$C=C), 1.08 (s, 3H, C$H_3$C), 0.77 (s, 3H, C$H_3$C); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 207.4, 195.2, 177.6, 133.5, 133.3, 122.5, 119.7, 107.7, 64.4, 61.7, 56.3, 45.2, 41.3, 34.2, 28.6, 25.9, 25.8, 23.9, 23.8, 18.0, 17.9, 16.1; ESI-HRMS (TOF): *m/z* calculated for $C_{22}H_{32}O_3$ $[M+Na]^+$: 367.2244; found 367.2239.

Data were in accord with that of Dr. Rodeschini.[47]

**(±)-(1*R*,5*S*,7*S*)-1,7-Bis(3'-methyl-but-2'-enyl)-8,8-dimethyl-5-((*R*)-hydroxyphenyl-methyl)-4-methoxybicyclo[3.3.1]non-3-ene-2,9-dione (199) and (±)-(1*R*,5*S*,7*S*)-1,7-Bis(3'-methyl-but-2'-enyl)-8,8-dimethyl-5-((*S*)-hydroxyphenyl-methyl)-4-methoxybicyclo[3.3.1]non-3-ene-2,9-dione (198)**




**108** **198** **199**

*n*BuLi (0.3 mL of a 1.6 M solution, 0.48 mmol, 1.5 equiv.) was added to a solution of diisopropylamine (67 µL, 0.48 mmol, 1.5 equiv.) in THF (1.5 mL) at -78 °C and the solution was stirred for 10 min. To this bicyclo[3.3.1]-nonane **108** (110 mg,

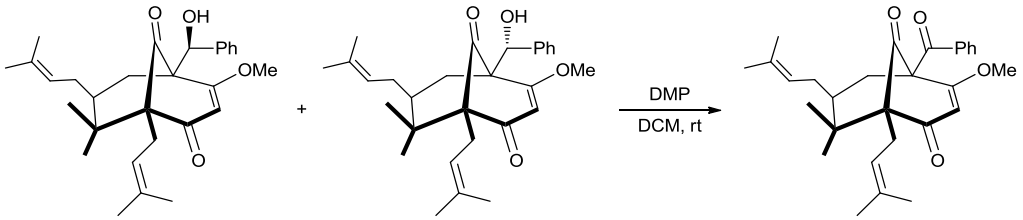
0.32 mmol) was added and the resulting solution was stirred at -78 °C for 30 min. After which time benzaldehyde (65 µL, 0.64 mmol, 2 equiv.) was added and the solution stirred for 2 h and allowed to warm to rt before quenching with saturated aqueous $NH_4Cl$ (5 mL). This was extracted with $Et_2O$ (3 x 5 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The mixture was purified by flash column chromatography (9:1 to 7:3 petrol:EtOAc) to afford two isomers **198** and **199** (1:3 ratio, combined 166 mg, 81%) as a colourless viscous oil. $R_f$ = 0.74 and 0.66 (40% EtOAc in petroleum ether).

Major (**199**)

FTIR (neat) 3500, 2913, 1719, 1643, 1601, 1375, 1235, 1043, 728 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ = 7.30 (d, *J* = 7.6, 2H, $H^{ortho\text{-ar}}$), 7.22 (t, *J* = 7.0, 2H, $H^{meta\text{-ar}}$), 7.17 (t, *J* = 7.1, 1H, $H^{para\text{-ar}}$), 5.78 (s, 1H, CC*H*=C), 5.32 (s, 1H, CC*H*(C)(OH)), 4.94-4.88 (m, 1H, C=C*H*$CH_2$), 4.68-4.62 (m, 1H, C=C*H*$CH_2$), 3.61 (s, 3H, C*H*$_3$O), 3.31 (br s, 1H, *H*O), 2.55 (dd, *J* = 13.9, 6.5, 1H, CHC*H*$_2$C), 2.42 (dd, *J* = 13.9, 6.1, 1H, CHC*H*$_2$C), 2.12 (dd, *J* = 14.1, 3.5, 1H, CC*H*$_2$CH), 2.08-2.02 (m, 1H, CHC*H*$_2$CH), 1.64 (s, 6H, C*H*$_3$C=C), 1.62-1.58 (m, 2H, CHC*H*$_2$CH, $CH_2$C*H*($CH_2$)(C)), 1.57 (s, 3H, C*H*$_3$C=C), 1.48 (s, 3H, C*H*$_3$C=C), 1.37 (t, *J* = 12.5, 1H, CHC*H*$_2$CH), 1.01 (s, 3H, C*H*$_3$C), 0.61 (s, 3H, C*H*$_3$C); $^{13}C$ NMR (125 MHz, $CDCl_3$): δ = 209.5, 195.3, 174.9, 141.0, 133.7, 133.2, 127.8 (2C), 127.5 (2C), 127.2, 122.5, 120.1, 108.4, 74.2, 71.7, 59.9, 56.8, 46.4, 41.8, 37.3, 27.9, 25.9, 25.8, 24.3, 22.6, 18.1, 17.6, 15.8; ESI-HRMS (TOF): *m/z* calculated for $C_{29}H_{38}O_4$ $[M+Na]^+$: 473.2662; found 473.2673.

Minor (**198**) was not isolated separately.

**(±)-(1*R*,5*R*,7*S*)-5-Benzoyl-1,7-bis(3'-methyl-but-2'-enyl)-8,8-dimethyl-4-methoxybicyclo[3.3.1]non-3-ene-2,9-dione (151)**

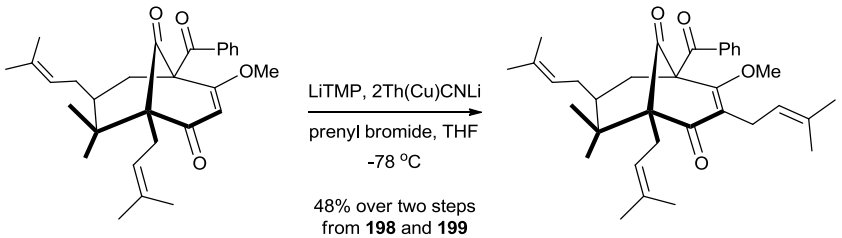



198 199 151

A mixture of stereoisomers **198** and **199** (255 mg, 0.57 mmol) was dissolved in DCM (5 mL) and stirred at rt. To this was added Dess-Martin periodinane (2.4 mL of a 15 % wt in DCM solution, 1.14 mmol, 2 equiv.). The mixture was stirred at room temperature for 1.5 h before quenching with saturated aqueous $NaHCO_3$ (5 mL) and saturated aqueous $Na_2S_2O_3$ (5 mL) and stirred for 1 h. The mixture was extracted with DCM (3 x 10mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure to afford **151** (299 mg) of crude material as a white solid which was used without further purification. m.p. = 128-130 °C; $R_f$ = 0.43 (20% EtOAc in petroleum ether); FTIR (neat) 2980, 1717, 1699, 1650, 1609, 1450, 1355, 1345, 1251, 1236, 1177 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): δ = 7.64 (d, *J* = 7.2, 2H, $H^{ortho\text{-ar}}$), 7.47 (t, *J* = 7.4, 1H, $H^{para\text{-ar}}$), 7.31 (t, *J* = 7.7, 2H, $H^{meta\text{-ar}}$), 5.91 (s, 1H, CC*H*=C), 4.97 (t, *J* = 7.1, 1H, C=C*H*$CH_2$), 4.79 (t, *J* = 6.8, 1H, C=C*H*$CH_2$), 3.52 (s, 3H, C$H_3$O), 2.60 (dd, *J* = 13.6, 6.6, 1H, CC$H_2$C), 2.46 (dd, *J* = 13.5, 6.9, 1H, CC$H_2$C), 2.25 (dd, *J* = 14.2, 4.4, 1H, CC$H_2$CH), 2.20-2.10 (m, 1H, CHC$H_2$CH), 2.08 (t, *J* = 13.4,1H, CC$H_2$CH), 1.89- 1.75 (m, 1H, CHC$H_2$CH), 1.72-1.67 (m, 1H, $CH_2$C*H*C), 1.66 (s, 3H, C$H_3$C=C), 1.62 (s, 3H, C$H_3$C=C), 1.61 (s, 3H, C$H_3$C=C), 1.59 (s, 3H, C$H_3$C=C), 1.08 (s, 3H, C$H_3$C), 0.75 (s, 3H, C$H_3$C); $^{13}C$ NMR

(100 MHz, $CDCl_3$): δ = 207.0, 195.4, 193.9, 173.4, 135.8, 134.7, 133.5, 132.8, 128.2 (2C), 128.1 (2C), 122.4, 119.8, 106.3, 72.7, 65.7, 56.8, 47.6, 41.8, 35.9, 27.9, 26.1, 25.8, 23.9, 23.0, 18.2, 18.0, 15.7; ESI-HRMS (TOF): *m/z* calculated for $C_{29}H_{36}O_4$ $[M+Na]^+$: 471.2506; found 471.2496.

**(±)-(1*R*,5*R*,7*S*)-5-Benzoyl-8,8-dimethyl-4-methoxy-1,3,7-tris(3'-methyl-but-2'-enyl)-bicyclo[3.3.1]non-3ene-2,9-dione (151)**




151 152

TMP (0.12 mL, 0.73 mmol, 2 equiv.) in THF (4 mL) was cooled to 0 °C and treated with *n*BuLi (0.29 mL of a 2.5 M solution, 0.73 mmol, 2 equiv.). The solution was cooled to -78 °C and **151** (162 mg, 0.36 mmol) in THF (12 mL) was added via cannula. After stirring for 30 min. 2-Th(Cu)CNLi (7.25 mL of a 0.1 M solution, 0.73 mmol, 2 equiv.) was added and the mixture warmed to -40 °C and stirred for a further 30 min. After this time the solution was cooled once more to -78 °C and prenyl bromide (0.21 mL, 1.8 mmol, 5 equiv.) was added. The mixture was stirred and allowed to warm to rt over 2 h before quenching with $NH_4Cl/NH_4OH_{(aq)}$ (10% w/w, 20 mL). This was extracted with $Et_2O$ (3 x 20 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (gradient of 98:2-9:1, petrol:EtOAc) to afford **152** (89.5 mg, 48% over 2 steps) as a white solid. m.p. 92-

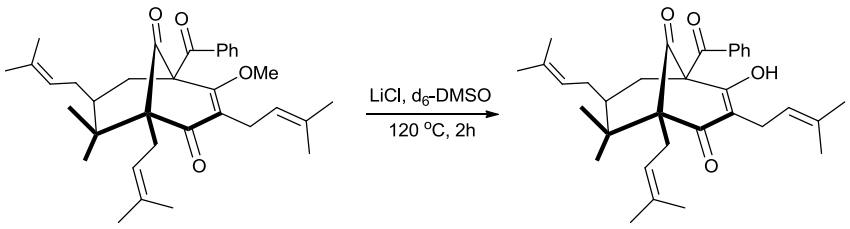
93 °C; $R_f$ = 0.65 (20% EtOAc in petroleum ether); FTIR (neat) 2925, 1722, 1692, 1651, 1613, 1448, 1328, 1253, 1045 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ = 7.70 (dd, *J* = 8.6, 1.3, 2H, $H^{ortho\text{-ar}}$), 7.49 (t, *J* 7.5, 1H, $H^{para\text{-ar}}$), 7.34 (t, *J* 7.9, 2H, $H^{meta\text{-ar}}$), 5.05-5.01 (m, 2H, C=C*H*$CH_2$), 4.75 (t, *J* = 6.8, 1H, C=C*H*$CH_2$), 3.56 (s, 3H, C*H*$_3$O), 3.27 (dd, *J* = 14.7, 7.0, 1H, CHC*H*$_2$C), 3.18 (dd, *J* = 14.7, 7.0, 1H, CHC*H*$_2$C), 2.63 (dd, *J* = 13.6, 7.0, 1H, CHC*H*$_2$C), 2.47 (dd, *J* = 13.4, 6.8, 1H, CHC*H*$_2$C), 2.32 (dd, *J* = 14.3, 4.4, 1H, CC*H*$_2$CH), 2.19-2.09 (m, 2H, CC*H*$_2$CH, CHC*H*$_2$CH), 1.87-1.78 (m, 1H, CHC*H*$_2$CH), 1.69 (s, 3H, C*H*$_3$C=C), 1.67 (s, 6H, C*H*$_3$C=C), 1.64 (s, 3H, C*H*$_3$C=C), 1.62 (s, 6H, C*H*$_3$C=C), 1.63-1.59 (m, 1H, CH$_2$C*H*C), 1.08 (s, 3H, C*H*$_3$C), 0.76 (s, 3H, C*H*$_3$C); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ = 207.4, 195.8, 195.1, 168.0, 135.9, 134.5, 133.5, 132.8, 132.7, 128.6 (2C), 128.1 (2C), 126.0, 122.6, 121.3, 120.0, 72.1, 66.7, 60.6, 47.7, 42.0, 35.8, 27.6, 26.0, 25.74, 25.68, 24.3, 23.0, 22.9, 18.16, 17.92, 17.89, 15.6; ESI-HRMS (TOF): *m/z* calculated for $C_{34}H_{44}O_4$ $[M+H]^+$: 539.3137; found 539.3011.

Enantiomers separated by chiral HPLC: 1% IPA in hexane on Lux cellulose II column. 8.11 and 10.17 min.

$[\alpha]_D^{22}$ = -56 (0.001 c, $CDCl_3$) component 1 from HPLC

$[\alpha]_D^{22}$ = +36 (0.001 c, $CDCl_3$) component 2 from HPLC

**Nemorosone II** (**150**)




**152** **150**

*O*-methylated nemorosone II (**152**) (22.6 mg, 43.7 µmol) was dissolved in $d_6$-DMSO (0.75 mL), and LiCl (20 mg, 0.47 mmol, 1.1 equiv.) added. The solution was stirred and heated to 120 °C for 2 h. The reaction was cooled to rt and diluted with $Et_2O$ (2 mL) and $H_2O$ (2 mL). The solution was extracted with $Et_2O$ (3 x 10mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure to afford crude nemorosone II **150** (15 mg, 68%) as an unstable solid. $R_f$ = 0.34 (20% EtOAc in petroleum ether) ; FTIR (neat) 3334, 2919, 1724, 1681, 1626, 1449, 1376, 1258, 1046 $cm^{-1}$; $^1$H NMR (500 MHz, $d_4$-Methanol): δ = 7.60 (d, *J* = 7.7, 2H, $H^{ortho\text{-ar}}$), 7.54 (t, *J* =7.4, 1H, $H^{para\text{-ar}}$), 7.35 (t, J = 7.8, 2H, $H^{meta\text{-ar}}$), 5.14 (t, *J* = 7.3, 2H, C=C*H*$CH_2$), 5.07 (t, *J* = 7.2, 1H, C=C*H*$CH_2$), 4.92 (t, *J* = 6.0, 1H, C=C*H*$CH_2$), 3.16 (d, *J* = 7.1, 2H, CHC*H*$_2$C), 2.66 (d, *J* =6.6, 2H, CHC*H*$_2$C), 2.27 (dd, *J* = 13.7, 4.3, 1H, CC*H*$_2$CH), 2.22 (dd, *J* = 14.4, 5.3, 1H, CHC*H*$_2$CH), 1.96 (t, *J* = 13.5, 1H, CHC*H*$_2$CH), 1.89-1.82 (m, 1H, CC*H*$_2$CH), 1.77-1.73 (m, 1H, $CH_2$C*H*C), 1.73 (s, 3H, C*H*$_3$C=C), 1.71 (s, 3H, C*H*$_3$C=C), 1.69 (s, 9H, C*H*$_3$C=C), 1.67 (s, 3H, C*H*$_3$C=C), 1.20 (s, 3H, C*H*$_3$C), 0.81 (s, 3H, C*H*$_3$C); $^{13}$C NMR (125 MHz, $d_4$-Methanol): δ = 208.9, 196.3, 136.8, 135.4, 134.3, 133.8, 133.6, 129.7 (2C), 129.1 (2C), 124.0, 122.3, 121.8, 121.3, 71.4, 69.0, 47.4, 43.0, 38.2, 29.3, 26.4, 26.04, 26.00, 24.9, 24.2, 22.3,

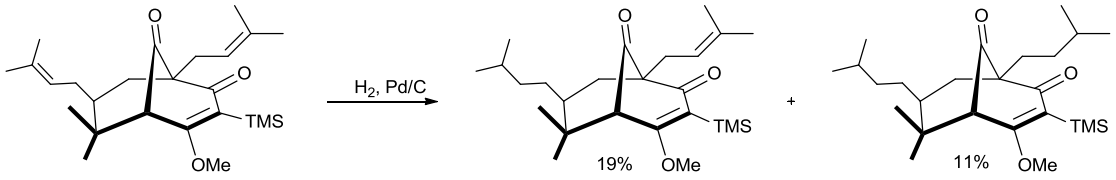
18.4, 18.1, 18.0, 16.0; ESI-HRMS (TOF): *m/z* calculated for $C_{33}H_{42}O_4$ $[M+H]^+$: 503.3156; found 503.3158.

$[\alpha]_D^{22}$ = -28 (0.003 c, MeOH) component 1 from HPLC of **151**

$[\alpha]_D^{22}$ = +14 (0.01 c, MeOH) component 2 from HPLC of **151**

### 7.3 Experimental for Chapter 4

**(±)-(5*S*,7S)-8,8-Dimethyl-7-isopentyl-2-methoxy-5-(3'-methylbut-2'-en-1'-yl)-3-(trimethylsilyl)bicyclo[3.3.1]non-2-ene-4,9-dione (200) and (±)-(5*S*,7*S*)-5,7-Diisopentyl-8,8-dimethyl-2-methoxy-3-(trimethylsilyl)bicyclo[3.3.1]non-2-ene-4,9-dione (201)**




**173** **200** **201**

Bicyclo[3.3.1]nonane **173** (62 mg, 0.15 mmol) and Pd/C (17 mg, 10% w/w, 0.02 mmol) were placed in a round bottom flask and the atmosphere exchanged for $H_2$. To this EtOH (5 mL) was added and the suspension was stirred for 24 h under $H_2$ atmosphere. The solution was filtered through a celite plug and washed with EtOAc. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography (9:1 petrol:$Et_2O$) to afford two products, **200** (12 mg, 19%) and **201** (7 mg, 11%) as colourless oils.

Major (**200**)

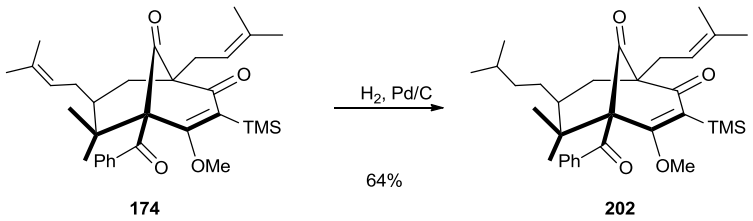
$R_f$ = 0.46 (20% EtOAc in petroleum ether); FTIR (neat) 2955, 1729, 1645, 1565, 1466, 1334, 1245, 1215, 844 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): δ = 4.91 (t, *J* = 7.2,

1H, C=C*H*$CH_2$), 3.71 (s, 3H, C$H_3$O), 3.21 (s, 1H, *H*C-1), 2.40 (dd, *J* = 14.1, 7.7, 1H, CHC$H_2$C), 2.31 (dd, *J* = 14.1, 6.6, 1H, CHC$H_2$C), 1.93 (dd, *J* = 12.8, 4.1, 1H, CC$H_2$CH), 1.62 (s, 3H, C$H_3$C=C), 1.58 (s, 3H, C$H_3$C=C), 1.52-1.32 (m, 7H,$CH_2$C*H*(C)($CH_2$), $(CH_3)_2$C*H*$CH_2$, CC$H_2$CH, CHC$H_2CH_2$, $CH_2$C$H_2$CH), 1.03 (s, 3H, CC$H_3$), 0.89 (s, 3H, CC$H_3$), 0.83 (d, *J* = 6.6, 3H, $CHCH_3$), 0.80 (d, *J* = 6.6, 3H, $CHCH_3$, 0.15 (s, 9H, C$H_3$Si); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 208.5, 202.1, 177.9, 133.7, 123.5, 119.6, 63.6, 61.2, 55.8, 43.2, 41.2, 38.8, 37.1, 29.7, 28.0, 26.9, 25.7, 23.0, 22.4, 22.1, 20.9, 18.0, 0.34 (3C); ESI-HRMS (TOF): *m/z* calculated for $C_{25}H_{42}O_{3Si}$ $[M+H]^+$: 441.2801; found 441.2800.

Minor (**201**)

$R_f$ = 0.5 (20% EtOAc in petroleum ether); FTIR (neat) 2954, 1729, 1647, 1567, 1465, 1245, 1214, 844 $cm^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$): δ = 3.71 (s, 3H, C$H_3$O), 3.20 (s, 1H, *H*C-1), 1.92 (dd, *J* = 12.9, 4.1, 1H, CC$H_2$CH), 1.68-1.00 (m, 12H, CC$H_2$CH, $(CH_2)_2$C*H*C, $CH_2$C$H_2$CH, CHC$H_2CH_2$, $(CH_3)_2$C*H*$CH_2$, $CH_2$C$H_2$C), 1.03 (s, 3H, $CH_3$), 0.88 (s, 3H, $CH_3$), 0.84 (d, *J* = 4.6, 3H, C$H_3$CH), 0.82 (d, *J* = 6.0, 3H, C$H_3$CH), 0.81 (d, *J* = 5.1, 3H, C$H_3$CH), 0.79 (d, *J* = 6.7, 3H, C$H_3$CH), 0.17 (s, 9H, C$H_3$Si); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 208.7, 202.5, 178.1, 123.6, 63.5, 61.2, 55.9, 43.3, 42.3, 38.6, 37.0, 33.4, 29.3, 28.7, 27.9, 26.8, 25.7, 23.0, 22.4 (2C), 22.0, 20.8, 0.4 (3C); ESI-HRMS (TOF): *m/z* calculated for $C_{25}H_{44}O_3Si$ $[M+Na]^+$: 443.2957; found 443.2961.

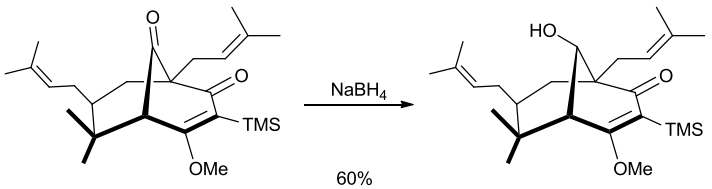
**(±)-(1*S*,5*S*,7*S*)-1-Benzoyl-8,8-dimethyl-7-isopentyl-2-methoxy-5-(3'-methylbut-2'-en-1'-yl)-3-(trimethylsilyl)bicyclo[3.3.1]non-2-ene-4,9-dione (202)**




174 202

Bicyclo[3.3.1]nonane **174** (25.8 mg, 0.05 mmol) and Pd/C (7 mg, 10% w/w, 0.005 mmol) were placed in a round bottom flask and the atmosphere exchanged for $H_2$. To this EtOH (5 mL) was added and the suspension was stirred for 24 h under $H_2$ atmosphere. The solution was filtered through a celite plug and washed with EtOAc. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography (9:1 petrol:$Et_2O$) to afford **202** (16.8 mg, 64%) as a colourless oil. $R_f$ = 0.61 (20% EtOAc in petroleum ether); FTIR (neat) 2954, 1721, 1703, 1650, 1558, 1446, 1251, 849 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ = 7.58 (d, *J* = 7.3, 2H, $H^{ortho\text{-ar}}$), 7.41 (t, *J* = 7.4, 1H, $H^{para\text{-ar}}$), 7.28 (t, *J* = 7.8, 2H, $H^{meta\text{-ar}}$), 5.04 (t, *J* = 7.1, 1H, C=C*H*$CH_2$), 3.48 (s, 3H, C$H_3$O), 2.53 (dd, *J* = 14.1, 7.4, 1H, CHC$H_2$C), 2.44 (dd, *J* = 14.0, 7.0, 1H, CHC$H_2$C), 1.97 (dd, *J* = 12.8, 4.2, 1H, CC$H_2$CH), 1.64 (s, 6H, C$H_3$C=C), 1.60-1.35 (m, 4H, CC$H_2$CH, $(CH_2)_2$C*H*C, $(CH_3)_2$C*H*$CH_2$, CHC$H_2CH_2$), 1.31 (s, 3H, C$H_3$C),1.25-1.15 (m, 1H, CHC$H_2CH_2$) 1.14 (s, 3H C$H_3$C), 1.05-0.90 (m, 2H, $CH_2$C$H_2$CH), 0.85 (d, *J* = 6.6, 3H, C$H_3$CH), 0.83 (d, *J* = 6.6, 3H, C$H_3$CH) 0.21 (s, 9H, C$H_3$Si); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ = 208.4, 201.0, 193.2, 181.2, 137.1, 134.4, 132.1, 128.6 (2C), 127.9 (2C), 122.9, 119.7, 75.1, 65.8, 64.0, 48.6, 43.0, 42.7, 37.5, 29.5, 28.2, 26.8, 26.0, 24.6, 22.9, 22.2, 18.1, 16.3, 0.93 (3C); ESI-HRMS (TOF): *m/z* calculated for $C_{32}H_{46}O_4Si$ $[M+Na]^+$: 545.3063; found 545.3054.

**(±)-(5*S*,7*S*,9*S*)-5,7-Bis(3'-methylbut-2'-en-1'-yl)-8,8-dimethyl-9-hydroxy-2-methoxy-3-(trimethylsilyl)bicyclo[3.3.1]non-2-en-4-one (203)**



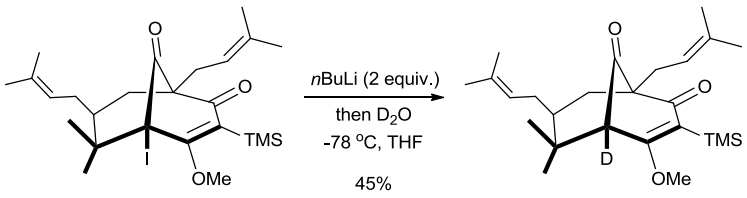

**173** **203**

Bicyclo[3.3.1]nonane **173** (43.8 mg, 0.11 mmol) was dissolved in 2:1 DCM:MeOH (1.9 mL) and cooled to -78 °C. The solution was treated with $NaBH_4$ (6 mg, 0.16 mmol) in MeOH (0.54 mL) and the reaction stirred at -78 °C for 2 h. After this time further $NaBH_4$ (10 mg) was added and the solution stirred and allowed to warm to rt overnight before quenching with saturated aqueous $NH_4Cl$ (5 mL). This was extracted with $Et_2O$ (3 x 5 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (9:1 petrol:$Et_2O$) to afford **203** (27.7 mg, 60%) as a colourless oil. $R_f$ = 0.38 (20% EtOAc in petroleum ether); FTIR (neat) 3401, 2927, 1617, 1556, 1332, 1218, 842 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): δ = 4.96-4.83 (m, 2H, C=C*H*$CH_2$), 4.01 (t, *J* = 3.1, 1H, *OCH*(C)(CH)), 3.70 (s, 3H, C*H*$_3$O), 2.75 (d, *J* = 3.1, 1H, *H*C-1), 2.50 (dd, *J* = 14.3, 6.1, 1H, CHC*H*$_2$C), 2.09 (dd, *J* = 14.3, 8.9, 1H, CHC*H*$_2$C), 2.06-1.96 (m, 1H, CHC*H*$_2$CH), 1.75 (d, *J* = 3.9, 1H, *H*O), 1.70-1.50 (m, 2H, CC*H*$_2$CH, CHC*H*$_2$CH), 1.63 (s, 3H, C*H*$_3$C=C), 1.61 (s, 6H, C*H*$_3$C=C), 1.52 (s, 3H, C*H*$_3$C=C), 1.41-1.17 (m ,2H, CC*H*$_2$CH, $CH_2$C*H*C), 1.15 (s, 3H, C*H*$_3$C), 0.92 (s, 3H, C*H*$_3$C), 0.12 (s, 9H, C*H*$_3$Si); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ = 206.2, 182.7, 133.7, 132.4, 123.4, 120.7, 119.9, 71.3, 55.4, 52.1, 49.0, 39.8, 34.9, 32.2, 32.0, 28.8, 27.4,

26.0, 25.8, 23.9, 17.8 (2C), 0.6 (3C); ESI-HRMS (TOF): *m/z* calculated for $C_{25}H_{42}O_3Si$ $[M+Na]^+$: 441.2801; found 441.2806.

### 7.4 Experimental for Chapter 5

### (±)-(1*S*,5*R*,7*S*)-5,7-Bis(3'-methyl-but-2'-enyl)-1-deteruio-8,8-dimethyl-2-methoxy-3-trimethylsilanyl-bicyclo[3.3.1]non-2-ene-4,9-dione (206)

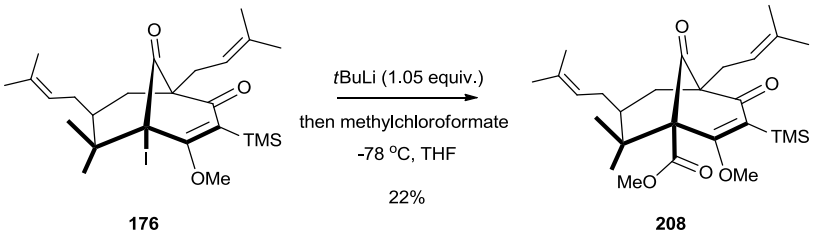



**176** **206**

Iodide **176** (28.9 mg, 53 μmol) was dissolved in THF (0.5 mL) and cooled to -78 °C. To this solution *n*BuLi (7 μL of a 1.6 M solution, 107 μmol, 2 equiv.) was added dropwise. The solution was stirred for 10 min. after which time $D_2O$ (11 μL, 533 μmol, 10 equiv.) was added dropwise. The solution was stirred and allowed to warm to rt over 2 h, then stirred at room temperature for 1 h before quenching with saturated aqueous $NH_4Cl$ (~2.5 mL). This was extracted with EtOAc (3 x 10 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (9:1 petrol:EtOAc) to afford **203** (10 mg, 45%) as a pale yellow oil. $R_f$ = 0.34 (10% EtOAc in petroleum ether); FTIR (neat) 2964, 2920, 1723, 1642, 1559, 1455, 1372, 1268, 1242, 1220, 1162, 1066, 925, 838 $cm^{-1}$; $^1H$ (500 MHz, $CDCl_3$): δ = 4.96-4.93 (m, 2H, C=C*H*$CH_2$), 3.73 (s, 3H, C$H_3$O), 2.40 (dd, *J* = 14.1, 7.8, 1H, CHC$H_2$C), 2.33 (dd, *J* = 14.1, 6.6, 1H, CHC$H_2$C), 2.06-2.03 (m, 1H, CHC$H_2$CH), 1.92 (dd, *J* = 13.3, 4.2, 1H, CC$H_2$CH), 1.66 (s, 3H, C$H_3$C=C), 1.63 (s, 3H, C$H_3$C=C), 1.60 (t, *J* = 13.2, 1H,

CHC$H_2$C), 1.60 (s, 3H, C$H_3$C=C), 1.53 (m, 1H, $(CH_2)_2$C$H$C), 1.53 (s, 3H, C$H_3$C=C), 1.28 (dd, *J* = 12.9, 1H, CC$H_2$CH), 1.09 (s, 3H, C$H_3$C), 0.94 (s, 3H, C$H_3$C), 0.17 (s, 9H, C$H_3$Si); $^{13}$C (125 MHz, $CDCl_3$): δ = 208.5, 202.0, 177.9, 133.7, 133.3, 123.5, 122.3, 119.6, 63.7, 60.73 (t, *J* = 20.3), 55.8, 42.8, 41.0, 39.4, 29.6, 27.9, 25.83, 25.77 (2C), 20.9, 18.0, 17.8, 0.4 (3C); ESI-HRMS (TOF): *m/z* calculated for $C_{25}H_{39}DO_3Si$ $[M+2H]^+$: 440.2707; found 440.2690.

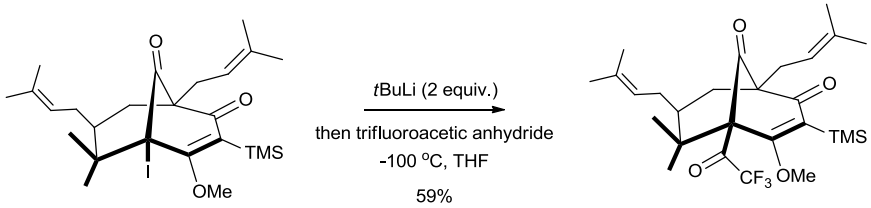
**(±)-(1*S*,5*S*,7*S*)-5,7-Bis(3'-methylbut-2'-en-1'-yl)-8,8-dimethyl-4,9-dioxo-2-methoxy-methyl-3-(trimethylsilyl)bicyclo[3.3.1]non-2-ene-1-carboxylate (208)**




Iodide **176** (117.4 mg, 0.22 mmol) was dissolved in THF (1 mL) and cooled to -78 °C. To this solution *t*BuLi (0.13 mL of a 1.7 M solution, 0.23 mmol, 1.05 equiv.) was added dropwise. The solution was stirred for 15 min. upon which methyl chloroformate (0.17 mL, 2.17 mmol, 10 equiv.) was added dropwise. The solution was stirred and allowed to warm slowly over 3 h, then stirred at rt for 30 min. before quenching with saturated aqueous $NH_4Cl$ (~2.5 mL). This was extracted with $Et_2O$, the combined organic layers were dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (9:1 petrol:$Et_2O$) to afford **208** (22.6mg, 22% yield, 54% brsm) as a colourless oil. $R_f$ = 0.51 (20% EtOAc in petroleum ether); FTIR (neat) 2951, 1755, 1728, 1651, 1565, 1437, 1247, 1223, 844, 752 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$): δ = 5.06 (t,

*J* = 6.9, 1H, C=C*H*$CH_2$), 4.97 (t, *J* = 7.9, 1H, C=C*H*$CH_2$), 3.86 (s, 3H, C$H_3$O), 3.74 (s, 3H, C$H_3$O), 2.39 (d, *J* = 6.8, 2H, CHC$H_2$C), 2.12 (dd, *J* = 11.2, 5.6, 1H CHC$H_2$CH), 1.76 (dd, *J* = 13.0, 4.0, 1H, CC$H_2$CH), 1.68-1.58 (m, 2H, CHC$H_2$CH, $(CH_2)_2$C*H*C), 1.65 (s, 6H, C$H_3$C=C), 1.63 (s, 3H, C$H_3$C=C), 1.54 (s, 3H, C$H_3$C=C), 1.43 (t, *J* = 12.8, 1H, CC$H_2$CH), 1.27 (s, 3H, C$H_3$C), 1.10 (s, 3H, C$H_3$C), 0.27 (s, 9H, C$H_3$Si); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 204.4, 199.9, 179.5, 168.0, 133.7, 133.4, 125.7, 122.3, 119.9, 74.9, 64.9, 64.8, 51.8, 45.1, 42.8, 40.0, 29.3, 27.6, 25.9, 25.7, 24.1, 18.0, 17.8, 16.0, 0.5 (3C); ESI-HRMS (TOF): *m/z* calculated for $C_{27}H_{42}O_5Si$ $[M+Na]^+$: 497.2699; found 497.2694.

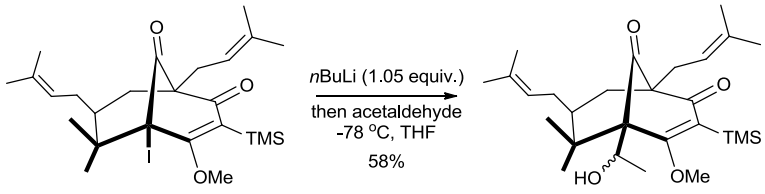
**(±)-(1*S*,5*S*,7*S*)-5,7-Bis(3'-methylbut-2'-en-1'-yl)-8,8-dimethyl-2-methoxy-1-(2',2',2'-trifluoroacetyl)-3-(trimethylsilyl)bicyclo[3.3.1]non-2-ene-4,9-dione (209)**




Iodide **176** (44.8 mg, 0.08 mmol) was dissolved in THF (0.5 mL) and cooled to -100 °C (liquid $N_2$ $Et_2O$ bath). To this solution *t*BuLi (97 µL of a 1.7 M solution, 0.165 mmol, 2 equiv.) was added dropwise. The solution was stirred for 30 min. after which time TLC showed no remaining starting material. Trifluoroacetic anhydride (117 µL, 0.826 mmol, 10 equiv.) was added dropwise. The solution was stirred and allowed to warm slowly over 2 h to -40 °C before quenching with saturated aqueous $NH_4Cl$ (~2.5 mL). This was extracted with $Et_2O$ (3 x 10 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure.

The residue was purified by flash column chromatography (9:1 petrol:$Et_2O$) to afford **209** (25 mg, 59%) as a colourless oil. $R_f$ = 0.32 (5% EtOAc in petroleum ether); FTIR (neat) 2917, 1768, 1723, 1655, 1561, 1233, 1194, 1163, 845, 720 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz): δ = 5.02-4.88 (m, 2H, C=C*H*$CH_2$), 3.96 (s, 3H, C*H*$_3$O), 2.54-2.31 (m, 2H, CHC*H*$_2$C), 2.19-2.04 (m, 1H, CHC*H*$_2$CH), 1.86 (dd, *J* = 13.2, 3.9, 1H, CC*H*$_2$CH), 1.70-1.52 (m, 2H, CHC*H*$_2$CH, $(CH_2)_2$C*H*C), 1.67 (s, 3H, C*H*$_3$C=C), 1.63 (s, 6H, C*H*$_3$C=C), 1.55 (s, 3H, C*H*$_3$C=C), 1.41 (dd, *J* = 12.9, 1H, CC*H*$_2$CH), 1.25 (s, 3H, C*H*$_3$C), 1.07 (s, 3H, C*H*$_3$C), 0.30 (s, 9H, C*H*$_3$Si); $^{19}F$ NMR ($CDCl_3$, 282 MHz): δ = -74.7 (s); $^{13}C$ NMR ($CDCl_3$, 100 MHz): δ = 205.8, 199.4, 188.9 (q, $^2J_{C\text{-}F}$ = 33.8), 176.3, 134.2, 133.9, 122.2, 121.9, 119.2, 115.0 (q, $J_{C\text{-}F}$ = 294.8), 75.5, 64.9, 64.1, 46.6, 42.7, 40.9, 29.1, 27.4, 25.8, 25.7, 23.9, 18.0, 17.8, 15.8, 1.1 (3C); ESI-HRMS (TOF): *m/z* calculated for $C_{27}H_{39}O_4F_3Si$ $[M+Na]^+$: 535.2467; found 535.2473.

**(±)-(1*S*,5*S*,7*S*)5,7-Bis(3'-methylbut-2'-en-1'-yl)-8,8-dimethyl-1-(1'-hydroxyethyl)-2-methoxy-3-(trimethylsilyl)bicyclo[3.3.1]non-2-ene-4,9-dione** (**210**)

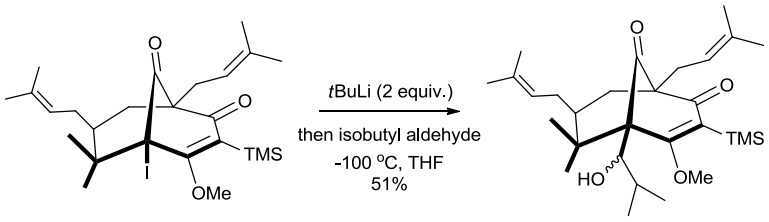



**176** **210**

Iodide **176** (95.7 mg, 0.18 mmol) was dissolved in THF (1.5 mL) and cooled to -78 °C. To this solution *n*BuLi (0.12 mL of a 1.6 M solution, 0.19 mmol, 1.05 equiv.) was added dropwise. The solution was stirred for 10 min. after which time acetaldehyde (99 μL, 1.8 mmol, 10 equiv.) was added dropwise. The solution was stirred and allowed to warm to rt over 2 h before quenching with saturated aqueous

$NH_4Cl$ (~ 2.5 mL). This was extracted with $Et_2O$ (2 x 5 mL) and EtOAc (5 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (9:1 petrol:$Et_2O$) to afford **210** (48.4 mg, 58%) as a colourless oil. $R_f$ = 0.47 (20% EtOAc in petroleum ether); FTIR (neat) 3529, 2969, 1710, 1649, 1547, 1228, 1107, 843, 754 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ = 5.00 (t, *J* = 6.6, 1H, C=C*H*$CH_2$), 4.95 (t, *J* = 5.9, 1H, C=C*H*$CH_2$), 4.50 (sextet, *J* = 6.3, 1H, $CH_3$C*H*(OH)C), 3.95 (s, 3H, C$H_3$O), 3.65 (d, *J* = 12.0, 1H, *H*O), 2.42 (dd, *J* = 14.5, 7.7, 1H, CHC$H_2$C), 2.34 (dd, *J* = 14.5, 6.4, 1H, CHC$H_2$C), 2.10-2.07 (m, 1H, CHC$H_2$CH), 1.82 (dd, *J* = 13.2, 4.2, 1H, CC$H_2$CH), 1.70-1.58 (m, 1H, CHC$H_2$CH), 1.66 (s, 3H, C$H_3$C=C), 1.64 (s, 3H, C$H_3$C=C), 1.62 (s, 3H, C$H_3$C=C), 1.54 (s, 3H, C$H_3$C=C), 1.52-1.48 (m, 1H, $(CH_2)_2$C*H*C), 1.40 (dd, *J* = 13.0, 1H, CC$H_2$CH), 1.28 (d, *J* = 6.3, 3H, C$H_3$CH), 1.23 (s, 3H, C$H_3$C), 1.11 (s, 3H, C$H_3$C), 0.22 (s, 9H, C$H_3$Si); $^{13}C$ NMR (125 MHz, $CDCl_3$): δ = 214.6, 200.2, 185.0, 133.9, 133.1, 124.8, 122.5, 119.8, 69.0, 67.5, 65.2, 63.3, 46.2, 44.1, 41.2, 29.9, 27.7, 25.9, 25.8, 23.9, 21.8, 18.4, 18.0, 17.9, 0.8 (3C); ESI-HRMS (TOF): *m/z* calculated for $C_{27}H_{44}O_4Si$ $[M+Na]^+$: 483.2907; found 483.2902.

**(±)-(1*S*,5*S*,7*S*)-5,7-Bis(3'-methylbut-2'-en-1'-yl)-8,8-dimethyl-1-(1'-hydroxy-2'-methylpropyl)-2-methoxy-3-(trimethylsilyl)bicyclo[3.3.1]non-2-ene-4,9-dione (211)**

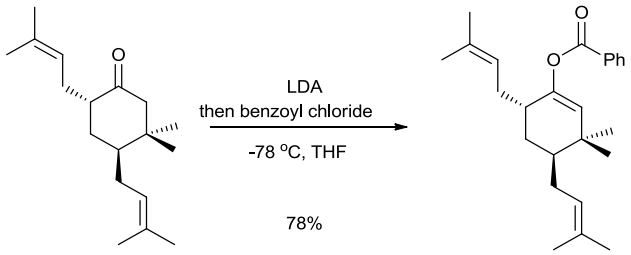



Iodide **176** (40.6 mg, 0.075 mmol) was dissolved in THF (0.5 mL) and cooled to -100 °C (liquid $N_2$, $Et_2O$ bath). To this solution *t*BuLi (88 µL of a 1.7 M solution, 0.15 mmol, 2 equiv.) was added dropwise. The solution was stirred for 30 min. after which time TLC showed no remaining starting material. Isobutyl aldehyde (71 µL, 0.75 mmol, 10 equiv.) was added dropwise. The solution was stirred and allowed to warm slowly over 2 h to -40 °C before quenching with saturated aqueous $NH_4Cl$ (~ 2.5 mL). This was extracted with $Et_2O$ (3 x 10 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (9:1 petrol:$Et_2O$) to afford **211** (18.8 mg, 51%) as a colourless oil. $R_f$ = 0.52 (20% EtOAc in petroleum ether); FTIR (neat) 3484, 2958, 1708, 1649, 1548, 1421, 1229, 1017, 844, 756 $cm^{-1}$; $^1H$ (300 MHz, $CDCl_3$): δ = 4.92 (m, 2H, C=C*H*$CH_2$), 4.56 (d, *J* = 11.8, 1H, (CH)(OH)C*H*C), 4.26 (dd, *J* = 11.8, 2.0, 1H, $(CH_3)_2$C*H*CH), 3.95 (s, 3H, C$H_3$O), 2.45 (dd, *J* = 14.0, 8.3, 1H, CHC$H_2$C), 2.31 (dd, *J* = 14.0, 5.8, 1H, CHC$H_2$C), 2.07 (d, *J* = 12.3, 1H, CHC$H_2$CH), 1.92-1.85 (m, 1H, CHC$H_2$CH), 1.88 (dd, *J* = 12.2, 3.4, 1H, CC$H_2$CH), 1.73-1.51 (m, 1H, $(CH_2)_2$C*H*C), 1.66 (s, 6H, C$H_3$C=C), 1.58 (s, 3H, C$H_3$C=C), 1.53 (s, 3H,

C$H_3$C=C), 1.34 (dd, *J* = 12.5, 1H, CC$H_2$CH), 1.25 (s, 3H, C$H_3$C), 1.15 (s, 3H, C$H_3$C), 1.02 (d, *J* = 6.8, 3H, C$H_3$CH), 0.65 (d, *J* = 6.9 Hz, 3H, C$H_3$CH), 0.22 (s, 9H, C$H_3$Si); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 216.2, 199.9, 185.0, 134.0, 133.1, 124.0, 122.5, 119.7, 65.7, 65.6, 63.0, 48.0, 44.3, 42.7, 32.0, 30.0, 29.7, 27.8, 25.9, 25.8, 24.3, 22.2, 18.7, 18.0, 17.9, 17.3, 0.7 (3C); ESI-HRMS (TOF): *m/z* calculated for $C_{29}H_{48}O_4Si$ $[M+Na]^+$: 511.3220; found 511.3228.

## 7.5 Experimental for Chapter 6

### (±)-(4*S*,6*S*)-4,6-Bis(3'-methylbut-2'-en-1'-yl)-3,3-dimethyl-cyclohex-1-en-1-yl-benzoate (237)

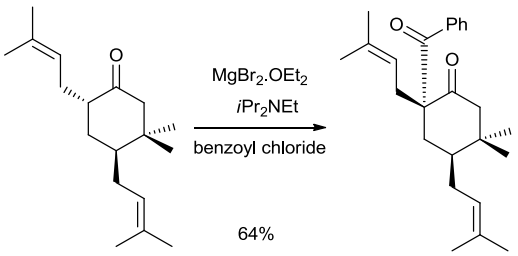



**141** **237**

*n*BuLi (2.79 mL of a 1.6 M, 4.46 mmol, 1.1 equiv.) was added to a solution of diisopropylamine (0.63 mL, 4.46 mmol, 1.1 equiv.) in THF (20 mL) at -78 °C and the solution stirred for 15 min. To this ketone **141** (1.063 g, 4.06 mmol) was added and the resulting solution stirred at -78 °C for 15 min. After which time benzoyl chloride (0.68 mL, 6.08 mmol, 1.5 equiv.) was added. The solution was stirred and allowed to warm over 2.5 h followed by 30 min. at rt before quenching with saturated aqueous $NH_4Cl$ (~25 mL). This was extracted with $Et_2O$ (3 x 25 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (9:1 petrol:EtOAc) to

afford **237** (1.14 g, 78%) as a colourless oil. $R_f$ = 0.58 (20% EtOAc in petroleum ether); FTIR (neat) 2914, 1732, 1451, 1267, 1112, 1026, 705 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ = 8.08 (d, *J* = 7.1, 2H, $H^{ortho\text{-ar}}$), 7.57 (t, *J* = 7.4, 1H, $H^{para\text{-ar}}$), 7.45 (t, *J* = 7.7, 2H, $H^{meta\text{-ar}}$), 5.26 (s, 1H, C=C*H*C), 5.13 (dd, *J* = 7.7, 1H, C=C*H*$CH_2$), 5.07 (dd, *J* = 7.4, 1H, C=C*H*$CH_2$), 2.43-2.38 (m, 1H, $(CH_2)_2$C*H*C), 2.26-2.05 (m, 3H, CHC$H_2$CH), 1.79-1.53 (m, 3H, CHC$H_2$CH, CHC$H_2$CH), 1.73 (s, 3H, C$H_3$C=C), 1.65 (s, 3H, C$H_3$C=C), 1.64 (s, 3H, C$H_3$C=C), 1.59 (s, 3H, C$H_3$C=C), 1.46 (tt, *J* = 11.2, 2.5, 1H, CC*H*$(CH_2)_2$), 1.13 (s, 3H, C$H_3$C), 0.97 (s, 3H, C$H_3$C); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ = 165.1, 149.5, 133.0, 132.6, 132.0, 130.3, 129.8 (2C), 128.3 (2C), 126.1, 124.1, 123.0, 40.2, 36.5, 35.3, 30.9, 29.2, 28.0, 27.9, 25.8, 25.7, 23.2, 17.81, 17.79; ESI-HRMS (TOF): *m/z* calculated for $C_{25}H_{34}O_2$ $[M+H]^+$: 367.2634; found 367.2632.

**(±)-(4*S*,6*S*)-6-Benzoyl-4,6-bis(3'-methylbut-2'-en-1'-yl)-3,3-dimethyl-cyclohexanone (238)**

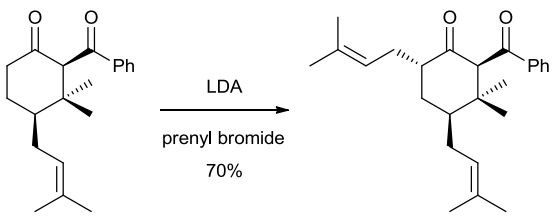



141 238

Cyclohexanone **141** (258.1 mg, 0.99 mmol) and $MgBr.Et_2O$ (639.0 mg, 2.46 mmol, 2.5 equiv.) were suspended in DCM (10 mL) and stirred for 5 min. To which *i*$Pr_2$NEt (0.52 mL, 3.0mmol, 3 equiv.) was added and the suspension stirred for 30 min. The suspension changed from pale yellow to deep orange in this time. Benzoyl chloride (0.17 mL, 1.48 mmol, 1.5 equiv.) was added and the solution was heated to 50 °C for

20 h before quenching with 2 M HCl (10 mL). This was extracted with DCM (3 x 10 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (8:2 petrol:$Et_2O$) to afford **238** (234 mg, 64%) as a yellow oil. $R_f$ = 0.57 (20% EtOAc in petroleum ether); FTIR (neat) 2914, 1711, 1671, 1597, 1581, 1447, 1376, 1114, 706 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ = 7.85 (d, *J* = , 2H, $H^{ortho\text{-ar}}$), 7.50 (t, *J* = , 1H, $H^{para\text{-ar}}$), 7.39 (t, *J* = , 2H, $H^{meta\text{-ar}}$), 5.24 (t, *J* = 7.0, 1H, C=C*H*$CH_2$), 4.92 (t, *J* = 8.1, 1H, C=C*H*$CH_2$), 2.87-2.79 (m, 2H, CHC$H_2$C, CHC$H_2$CH), 2.56-2.49 (dd, *J* = 15.1, 7.6, 1H, CHC$H_2$C), 2.19-2.03 (m, 3H, CHC$H_2$CH, CC$H_2$C), 1.76 (s, 3H, C$H_3$C=C), 1.80-1.50 (m, 3H, CC*H*$(CH_2)_2$, CHC$H_2CH_2$), 1.60 (s, 6H, C$H_3$C=C), 1.37 (s, 3H, C$H_3$C=C), 0.96 (s, 3H, C$H_3$C), 0.78 (s, 3H, C$H_3$C); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ = 210.8, 197.2, 136.9, 134.9, 132.8, 132.3, 128.7 (2C), 128.6 (2C), 123.3, 117.9, 67.1, 57.1, 43.1, 41.0, 38.8, 34.0, 29.9, 28.5, 25.9 (2C), 19.8, 17.9, 17.7; ESI-HRMS (TOF): *m/z* calculated for $C_{25}H_{34}O_2$ $[M+Na]^+$: 389.2451; found 389.2451.

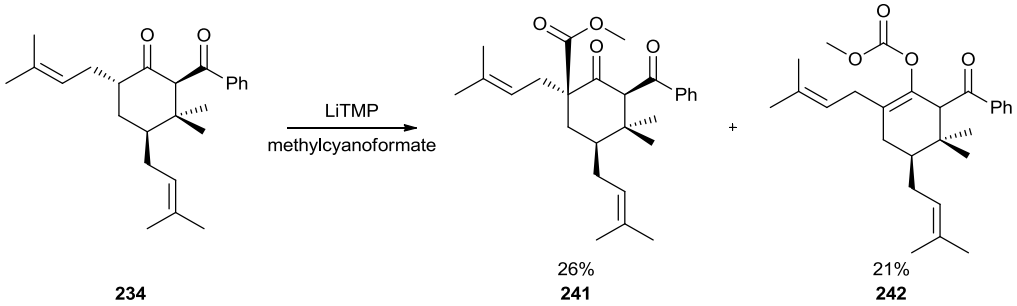
**(±)-(2S,4S,6*S*)-2-Benzoyl-4,6-bis(3'-methylbut-2'-en-1'-yl)-3,3-dimethyl-cyclohexanone (234)**




**135** **234**

Diketone **135** (247 mg, 0.83 mmol) was dissolved in THF (3 mL) and cooled to -78 °C and then treated with LDA (2 mL of a 0.5 M solution, 1.00 mmol, 1.2 equiv.) and stirred for 15 min. at -78 °C. To the resulting solution, prenyl bromide (0.24 mL, 2.08 mmol, 2.5 equiv.) was added, the solution was stirred and allowed to reach -20 °C over 2 h, then stirred at rt for 30 min. before quenching with saturated aqueous $NH_4Cl$ (5 mL). This was extracted with $Et_2O$ (3 x 10 mL), the combined organics were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (9:1 petrol:$Et_2O$) to afford **234** (0.211 g, 70%) as a colourless oil. $R_f$ = 0.59 (20% EtOAc in petroleum ether); FTIR (neat) 3402, 2926, 1709, 1687, 1597, 1448, 1369, 1231, 985, 765, 690 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ = 7.78 (d, *J* = 7.1, 2H, $H^{ortho\text{-ar}}$), 7.50 (t, *J* = 7.4, 1H, $H^{para\text{-ar}}$), 7.39 (t, *J* = 7.6, 2H, $H^{meta\text{-ar}}$), 5.08 (t, *J* = 7.1, 1H, C=C*H*$CH_2$), 5.02 (t, *J* = 7.1, 1H, C=C*H*$CH_2$), 4.50 (s, 1H, CC*H*$(C)_2$), 2.57-2.50 (m, 1H, CHC$H_2$CH), 2.45-2.38 (m, 2H, CHC$H_2$CH, $(CH_2)_2$C*H*C), 2.23 (dd, *J* = 12.1, 6.8, 1H, CHC$H_2$CH), 1.90-1.59 (m, 3H, CHC$H_2$CH, CHC$H_2$CH), 1.71 (s, 3H, C$H_3$C=C), 1.68 (s, 3H, C$H_3$C=C), 1.67 (s, 3H, C$H_3$C=C), 1.61 (s, 3H, C$H_3$C=C), 1.60-1.55 (m, 1H, CC*H*$(CH_2)_2$), 1.14 (s, 3H, C$H_3$C), 1.11 (s, 3H, C$H_3$C); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ = 210.8, 197.6, 139.0, 134.3, 132.8, 132.8, 128.5 (2C), 127.9 (2C), 123.3, 121.0, 64.5, 49.9, 44.2, 43.8, 31.6, 30.7, 27.6, 27.5, 25.9, 25.8, 18.1, 17.9, 17.2; ESI-HRMS (TOF): *m/z* calculated for $C_{25}H_{34}O_2$ $[M+Na]^+$: 389.2457; found 389.2447.

**(±)-(2*R*,4*S*,6*R*)-2-Benzoyl-4,6-bis(3'-methylbut-2'-en-1'-yl)-3,3-dimethyl-methyl-1-oxocyclohexanecarboxylate (241) + (±)-(4*S*)-2-Benzoyl-3,3-dimethyl-4S,6-bis(3'-methylbut-2'-en-1'-yl)cyclohex-6-en-6-yl methyl carbonate (242)**




**234** **241** **242**

1,3-diketone **234** (68.9 mg, 0.188 mmol) was dissolved in THF (0.5 mL) and cooled to -78 °C. This was treated with LiTMP (0.94 mL of a 0.5 M solution, 0.471 mmol, 2.5 equiv.) and stirred at -78 °C for 15 min. After which time methyl cyanoformate (0.15 mL, 1.88 mmol, 10 equiv.) was added, the resulting solution stirred for 2 h at -78 °C before quenching with saturated aqueous $NH_4Cl$ (5 mL). This was extracted with $Et_2O$ (3 x 5 mL), the combined organic layers dried ($MgSO_4$) and the solvent was removed under reduced pressure. The mixture was purified by flash column chromatography (9:1 petrol:$Et_2O$) to afford deacetylated product **141** (12.6 mg) and mixed *O*-acylated and *C*-acylated products **241** and **242** (36.2 mg, 43%). The mixed acylation products could be separated by HPLC (water:MeCN gradient) to afford *C*-acylated **241** (20.1 mg, 27%) and *O*-acylated **242** (16.1 mg, 21%). $R_f$ = 0.41 (20% EtOAc in petroleum ether).

Major (**241**)

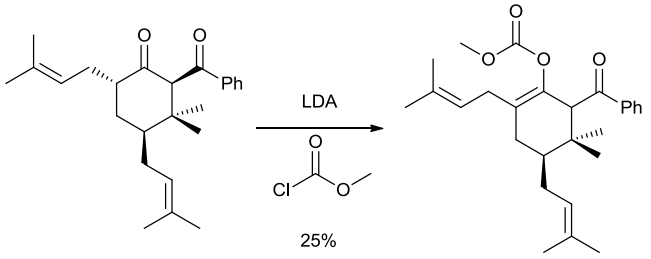
$^1$H NMR (300 MHz, $CDCl_3$): δ = 7.89 (d, *J* = 7.1, 2H, $H^{ortho\text{-ar}}$), 7.53 (t, *J* = 7.3, 1H, $H^{para\text{-ar}}$), 7.43 (t, *J* = 7.4, 2H, $H^{meta\text{-ar}}$), 5.16 (t, *J* = 6.7, 1H, C=C*H*$CH_2$), 5.06 (t, *J* = 8.3, 1H, C=C*H*$CH_2$), 4.49 (s, 1H, $(C)_2$C*H*C), 3.77 (s, 3H, C$H_3$O), 2.54-2.47 (m, 2H,

CHC*H*$_2$C), 2.35-2.15 (m, 2H, CHC*H*$_2$CH, CHC*H*$_2$C), 1.75-1.63 (m, 2H, CHC*H*$_2$CH, CHC*H*$_2$C), 1.76 (s, 3H, C*H*$_3$C=C), 1.67 (s, 3H, C*H*$_3$C=C), 1.62 (s, 3H, C*H*$_3$C=C), 1.57 (s, 3H, C*H*$_3$C=C), 1.45-1.35 (m, 1H, CC*H*(CH$_2$)$_2$), 1.12 (s, 3H, C*H*$_3$C), 1.05 (s, 3H, C*H*$_3$C); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 203.0, 196.8, 172.4, 139.0, 135.2, 133.0, 132.8, 128.5 (2C), 127.9 (2C), 122.8, 118.2, 66.5, 61.7, 52.3, 46.1, 44.4, 36.9, 33.4, 27.4, 26.7, 25.9, 25.8, 18.0, 17.8, 16.1; ESI-HRMS (TOF): *m/z* calculated for $C_{27}H_{36}O_4$ $[M+Na]^+$: 447.2511; found 447.2512.

Minor (**242**)

FTIR (neat) 2967, 1751, 1683, 1440, 1259, 1218 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$): δ = 7.92 (d, *J* = 7.1, 2H, *H*$^{ortho\text{-ar}}$), 7.54 (t, *J* = 7.3, 1H, *H*$^{para\text{-ar}}$), 7.44 (t, *J* = 7.4, 2H, *H*$^{meta\text{-ar}}$), 5.15-5.02 (m, 2H, C=C*H*CH$_2$), 4.68 (s, 1H, CC*H*(C)$_2$), 3.56 (s, 3H, C*H*$_3$O), 2.81 (dd, *J* = 14.2, 7.7, 1H, CHC*H*$_2$C), 2.67 (dd, *J* = 14.0, 7.1, 1H, CHC*H*$_2$C), 2.17 (dd, *J* = 17.3, 3.8, 1H, CHCH$_2$CH), 1.85-1.65 (m, 4H, CC*H*(CH$_2$)$_2$, CHC*H*$_2$C, CHC*H*$_2$CH), 1.70 (s, 3H, C*H*$_3$C=C), 1.63 (s, 3H, C*H*$_3$C=C), 1.60 (s, 3H, C*H*$_3$C=C), 1.56 (s, 3H, C*H*$_3$C=C), 0.92 (s, 3H, C*H*$_3$C), 0.85 (s, 3H, C*H*$_3$C); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 199.9, 154.0, 140.0, 139.4, 133.2, 132.7, 132.5, 128.6 (2C), 128.5 (2C), 126.7, 123.3, 120.4, 55.7, 54.9, 44.6, 38.0, 31.1, 29.3, 28.7, 27.8, 27.4, 25.8, 17.8, 17.7, 16.6; ESI-HRMS (TOF): *m/z* calculated for $C_{27}H_{36}O_4$ $[M+Na]^+$: 447.2511; found 447.2512.

**(±)-(4*S*)-2-Benzoyl-3,3-dimethyl-4S,6-bis(3'-methylbut-2'-en-1'-yl)cyclohex-6-en-6-yl methyl carbonate (242)**

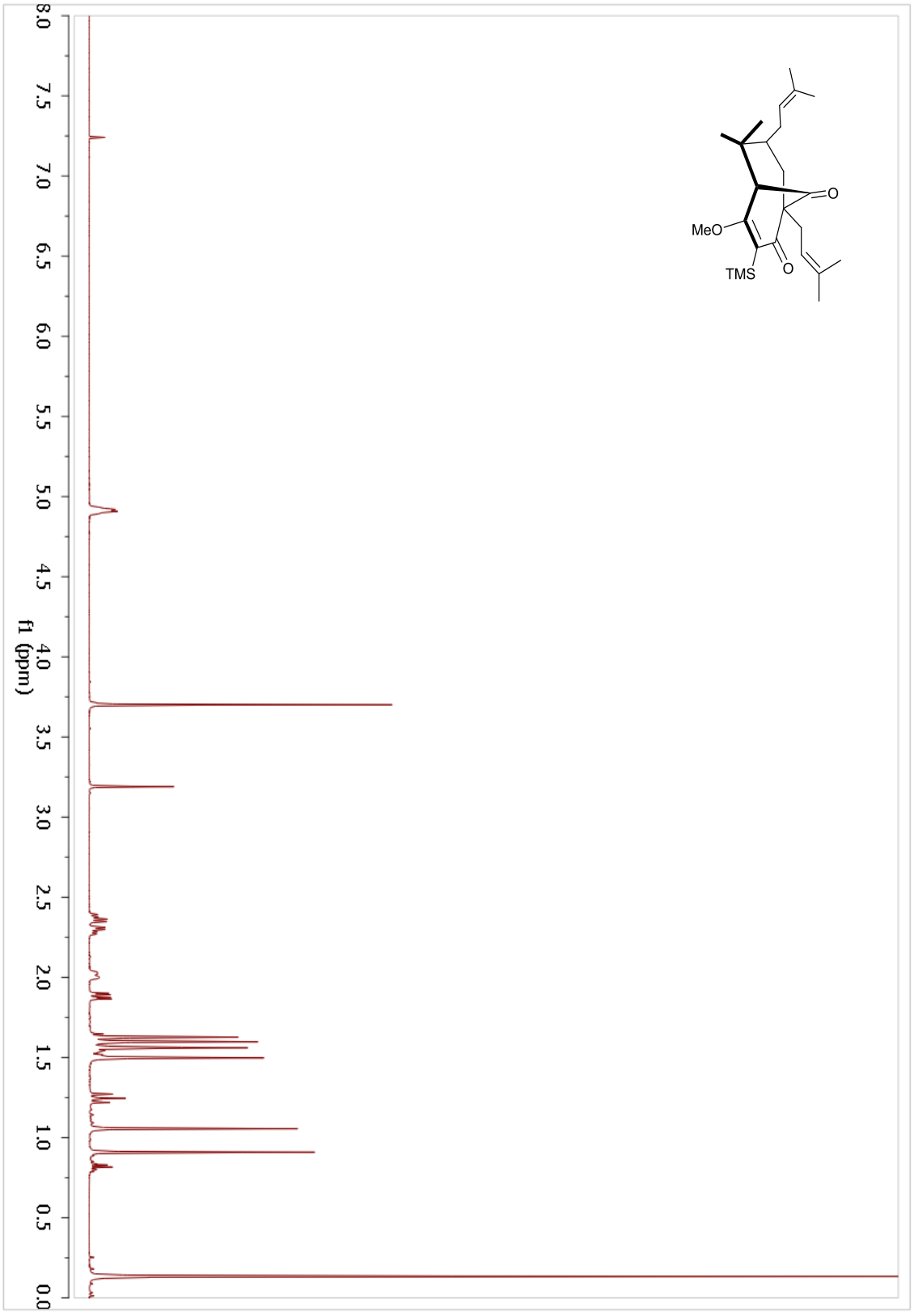



234 242

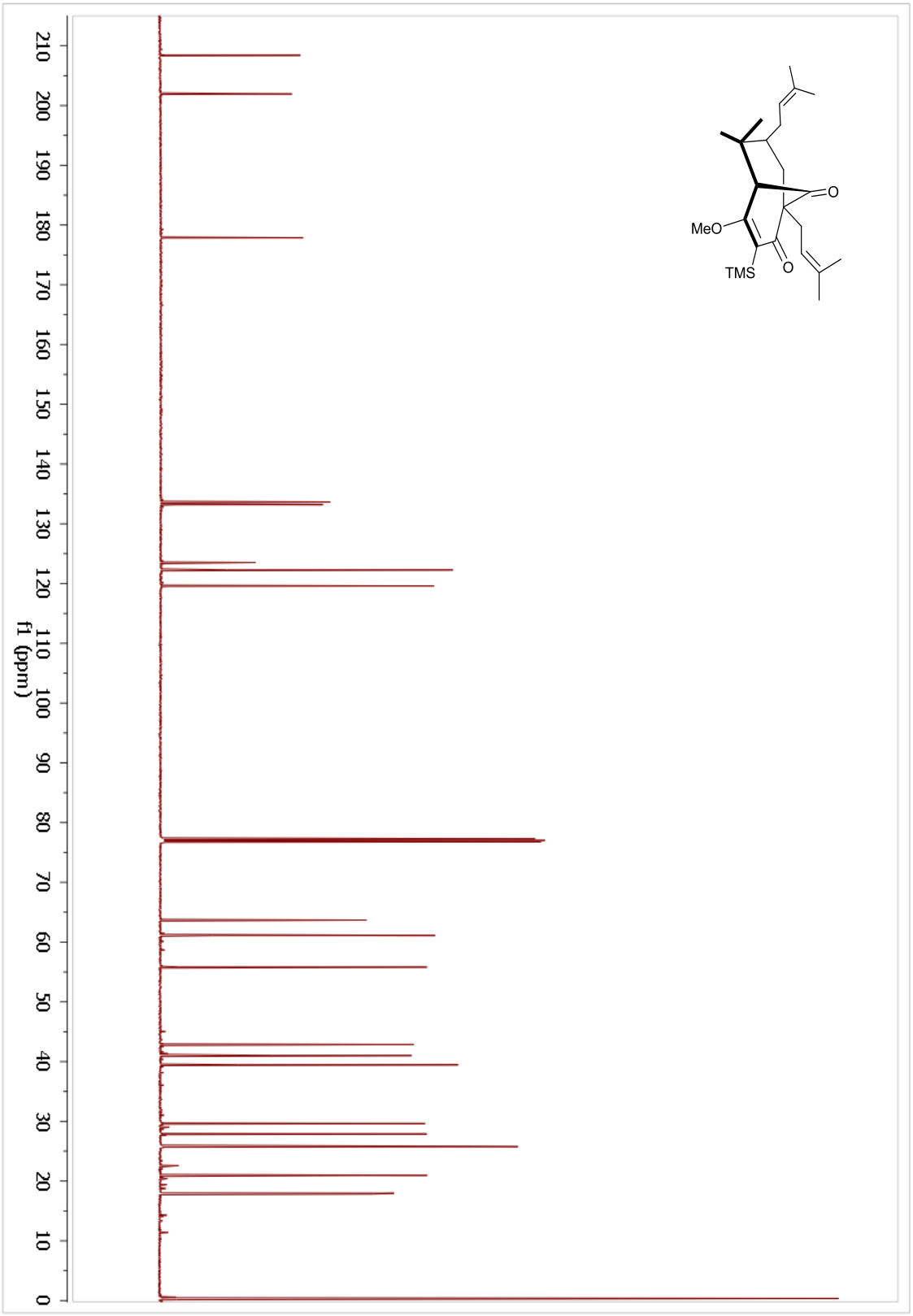
1,3-diketone **234** (34.1 mg, 0.09 mmol) was dissolved in THF (0.5 mL) and cooled to -78 °C and stirred for 15 min. The resulting solution was treated with LiTMP (0.47 mL of a 0.5 M solution, 0.23 mmol, 2.5 equiv.) and stirred for a further 5 min. After which time methyl chloroformate (72 µL, 0.93 mmol, 10 equiv.) was added. The solution was stirred and allowed to warm to -20 °C over 2 h before quenching with saturated aqueous $NH_4Cl$ (10 mL). This was extracted with $Et_2O$ (3 x 10 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by column chromatography (9:1 petol:$Et_2O$) to afford **242** (9.8 mg, 25%) as a colourless oil.

Data as quoted for *O*-acylation product **242** from above.

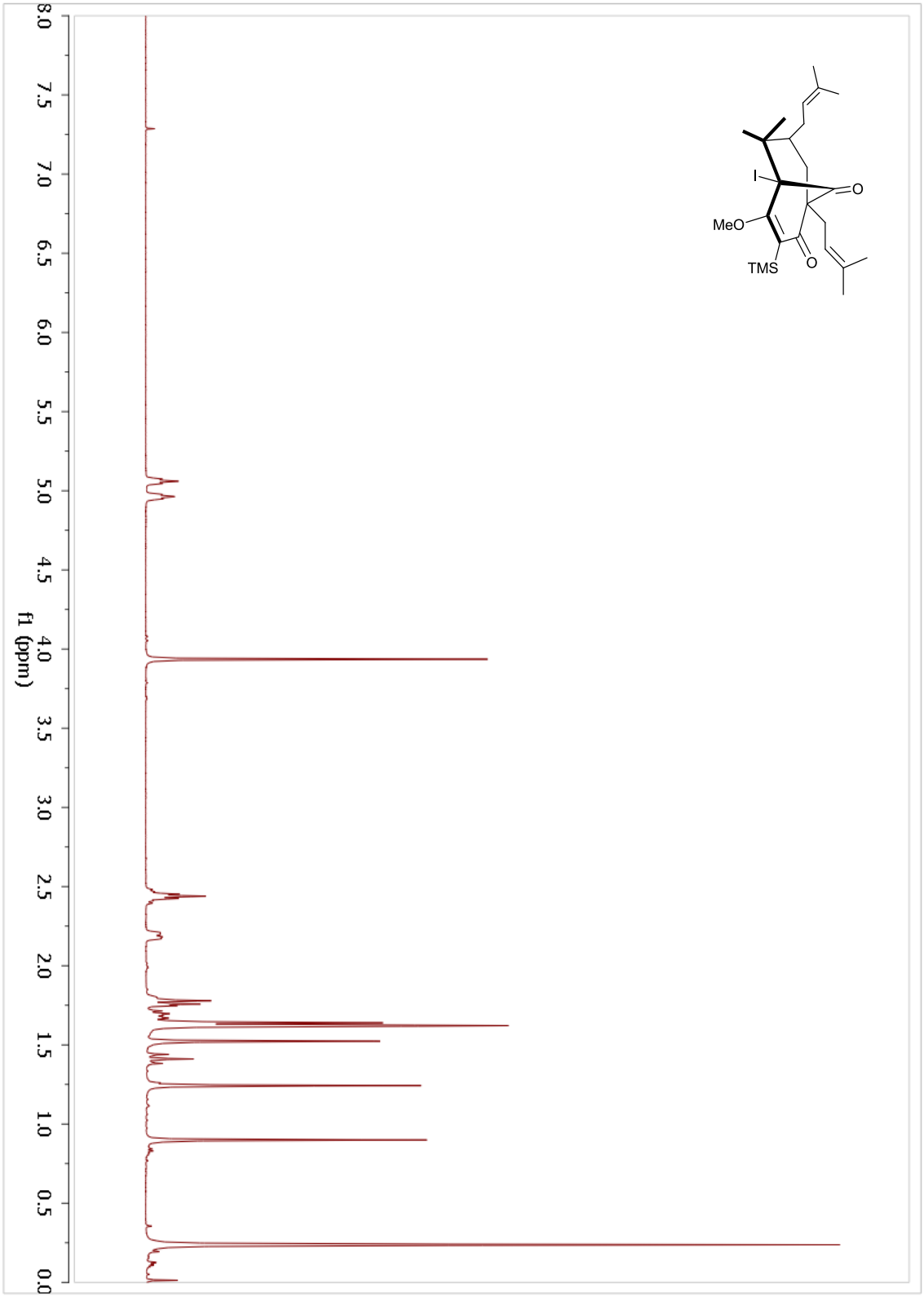
**Appendix 1:** $^{1}$H NMR of **173** (500 MHz, $CDCl_3$)



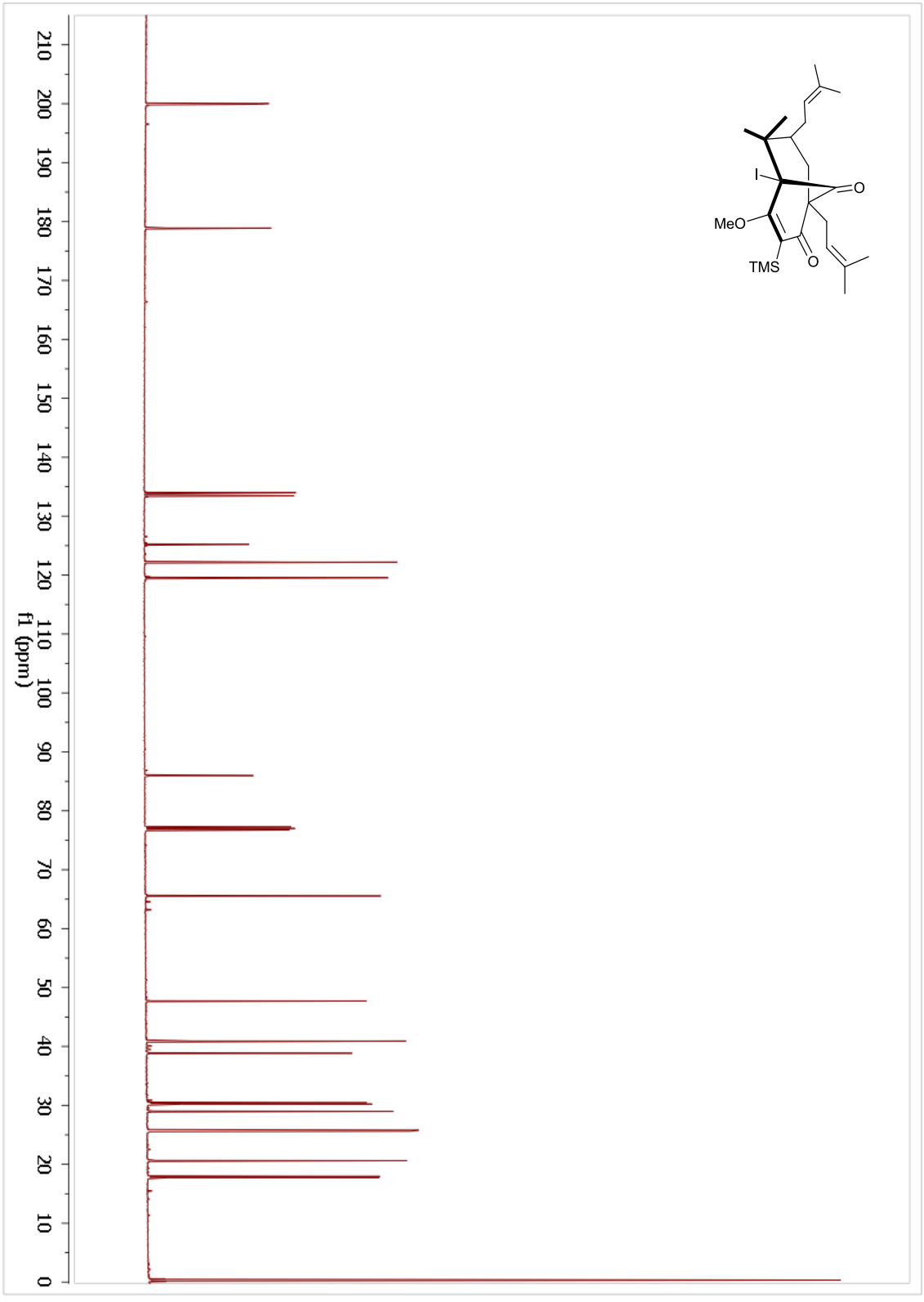
**Appendix 2:** $^{13}C$ NMR of **173** (125 MHz, $CDCl_3$)



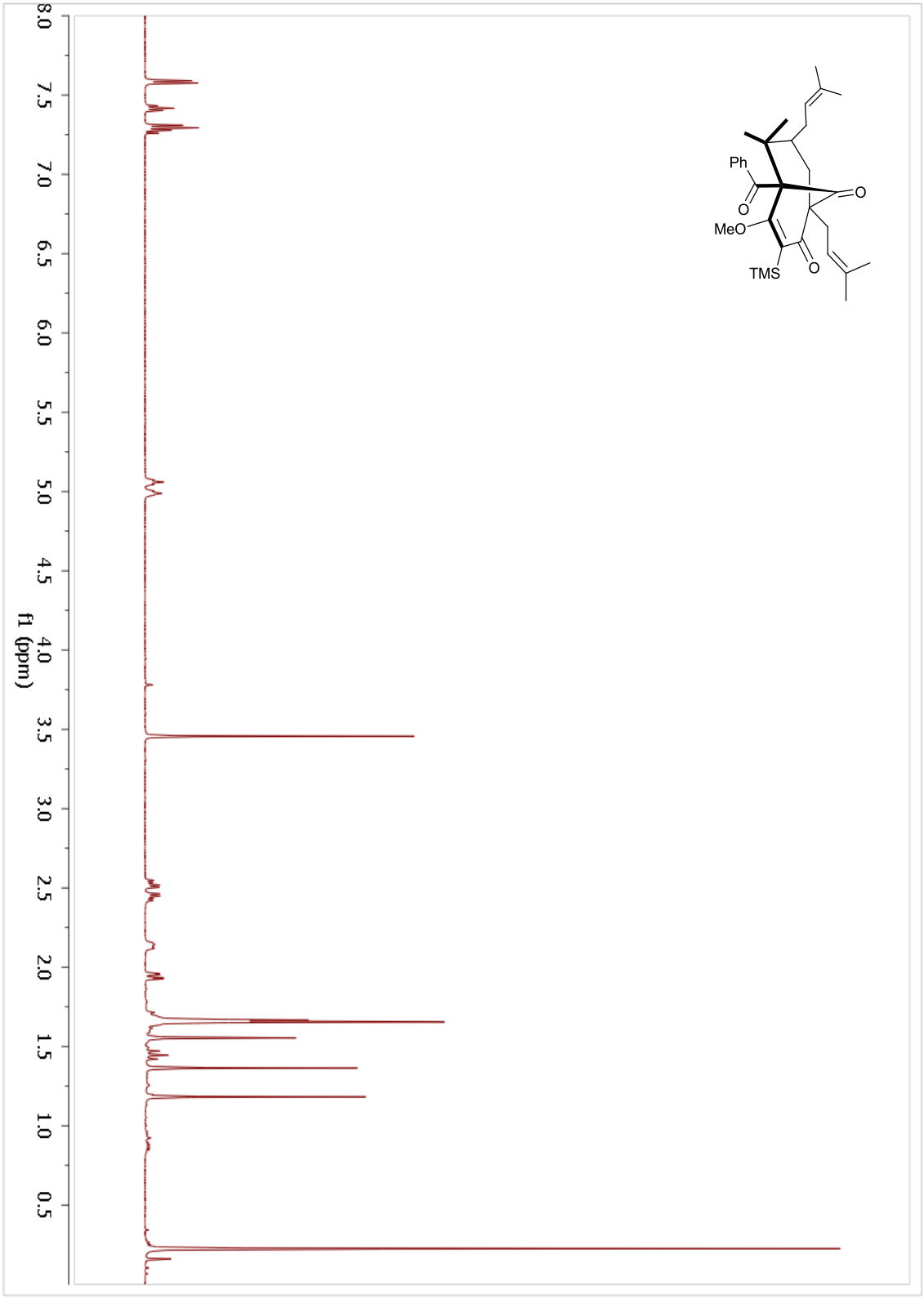
**Appendix 3:** $^{1}$H NMR of **176** (500MHz, $CDCl_3$)



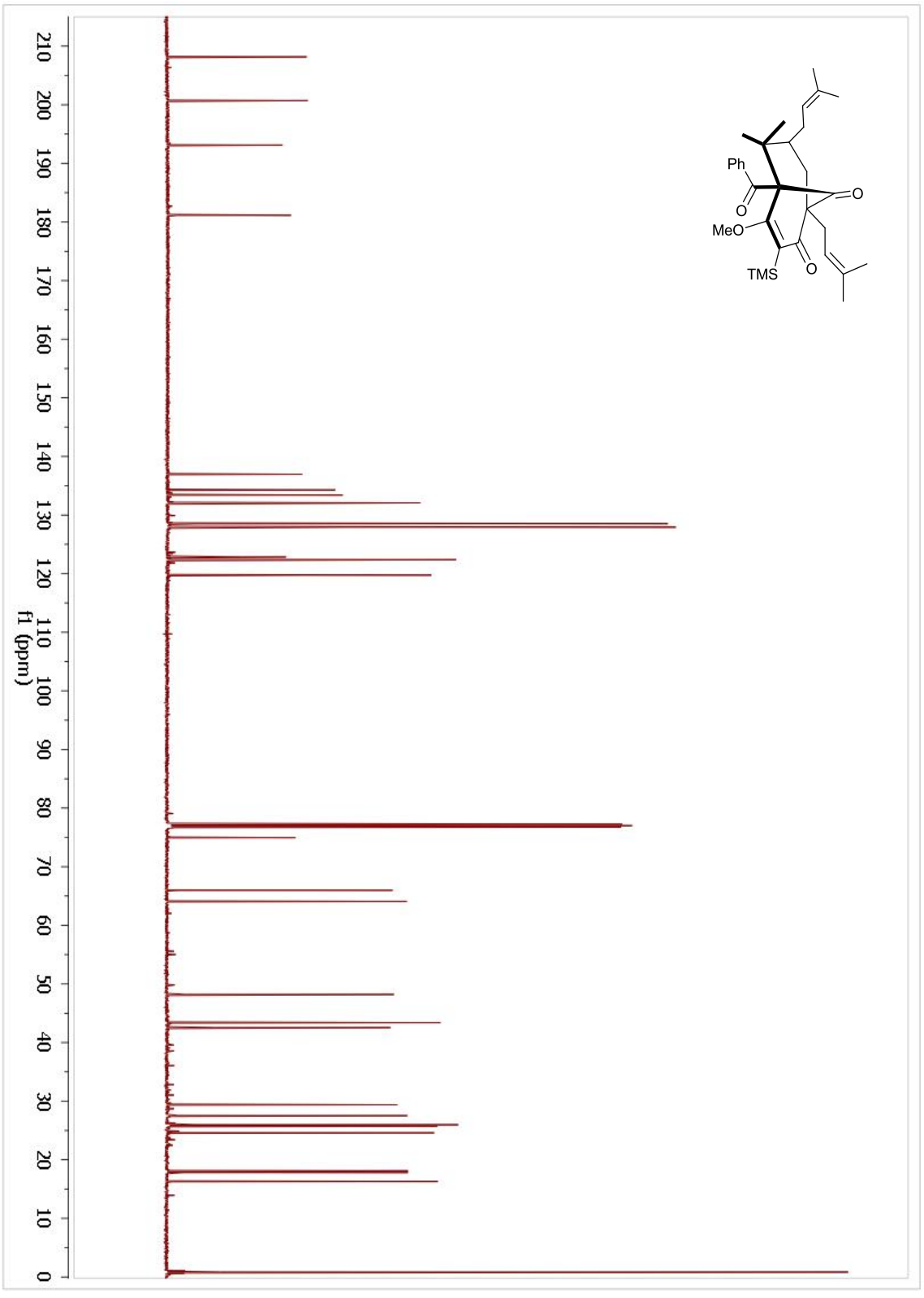
**Appendix 4:** $^{13}C$ NMR of **176** (125 MHz, $CDCl_3$)



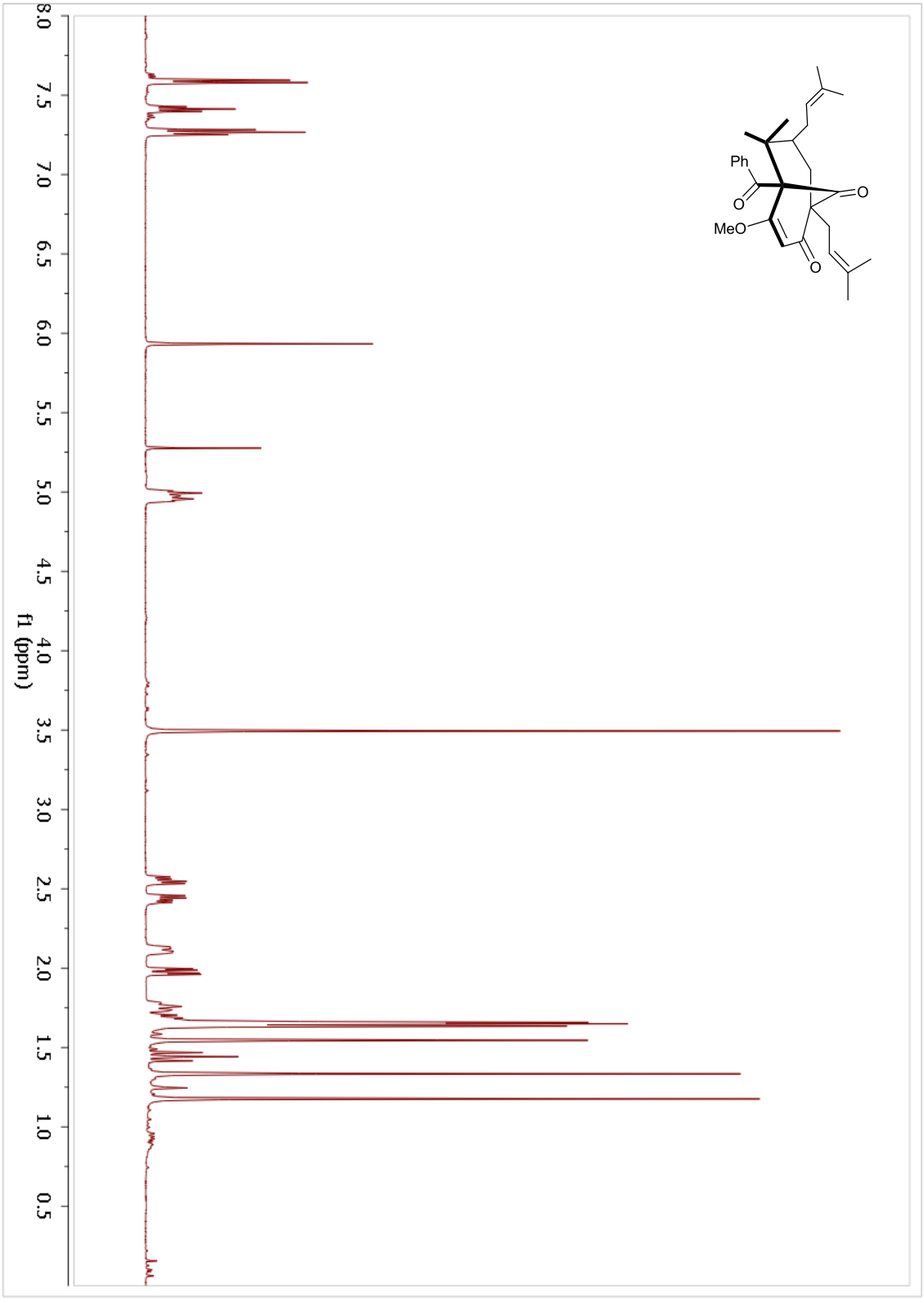
**Appendix 5:** $^{1}$H NMR of **174** (500 MHz, $CDCl_3$)



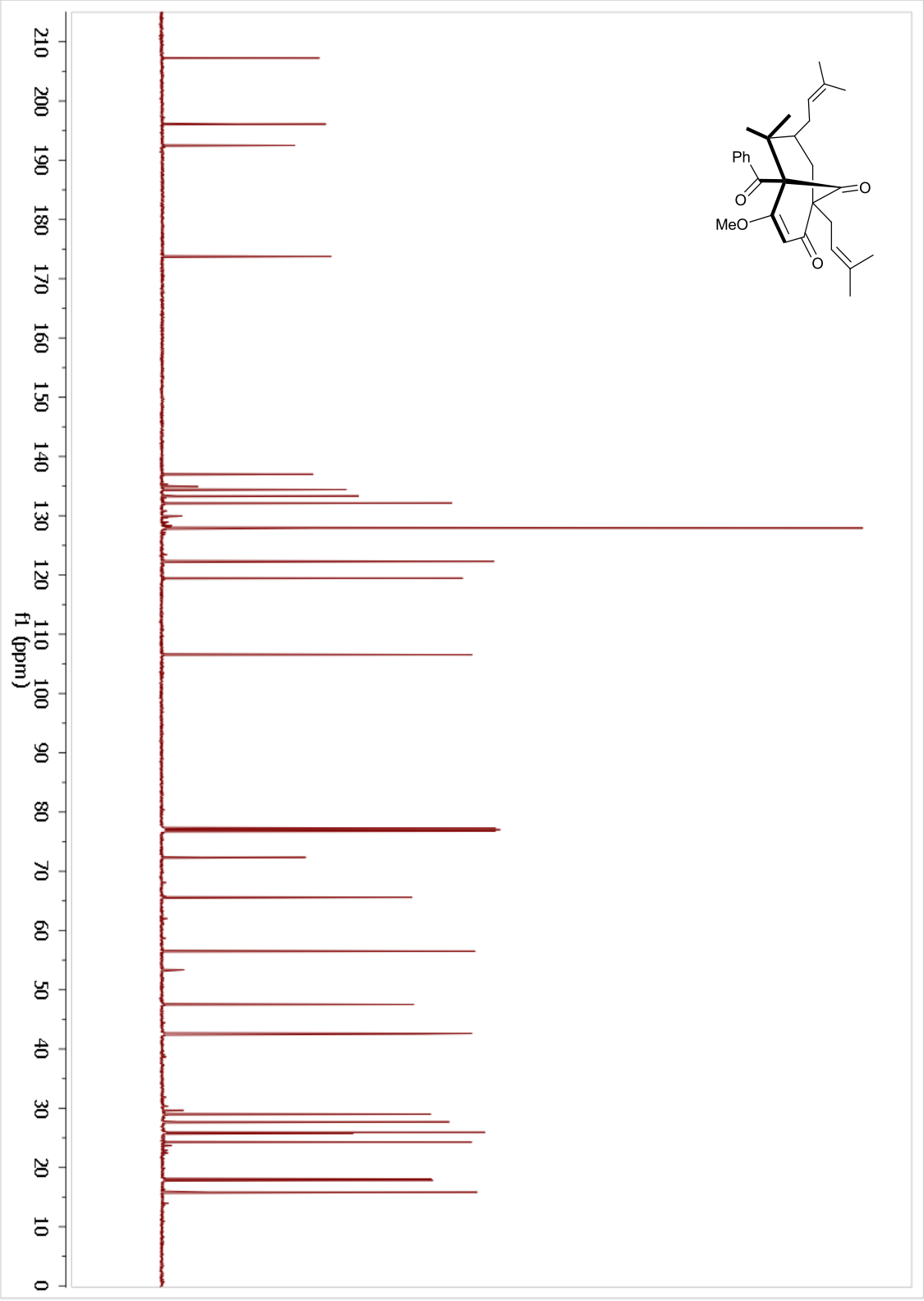
**Appendix 6:** $^{13}C$ NMR of **174** (125 MHz, $CDCl_3$)



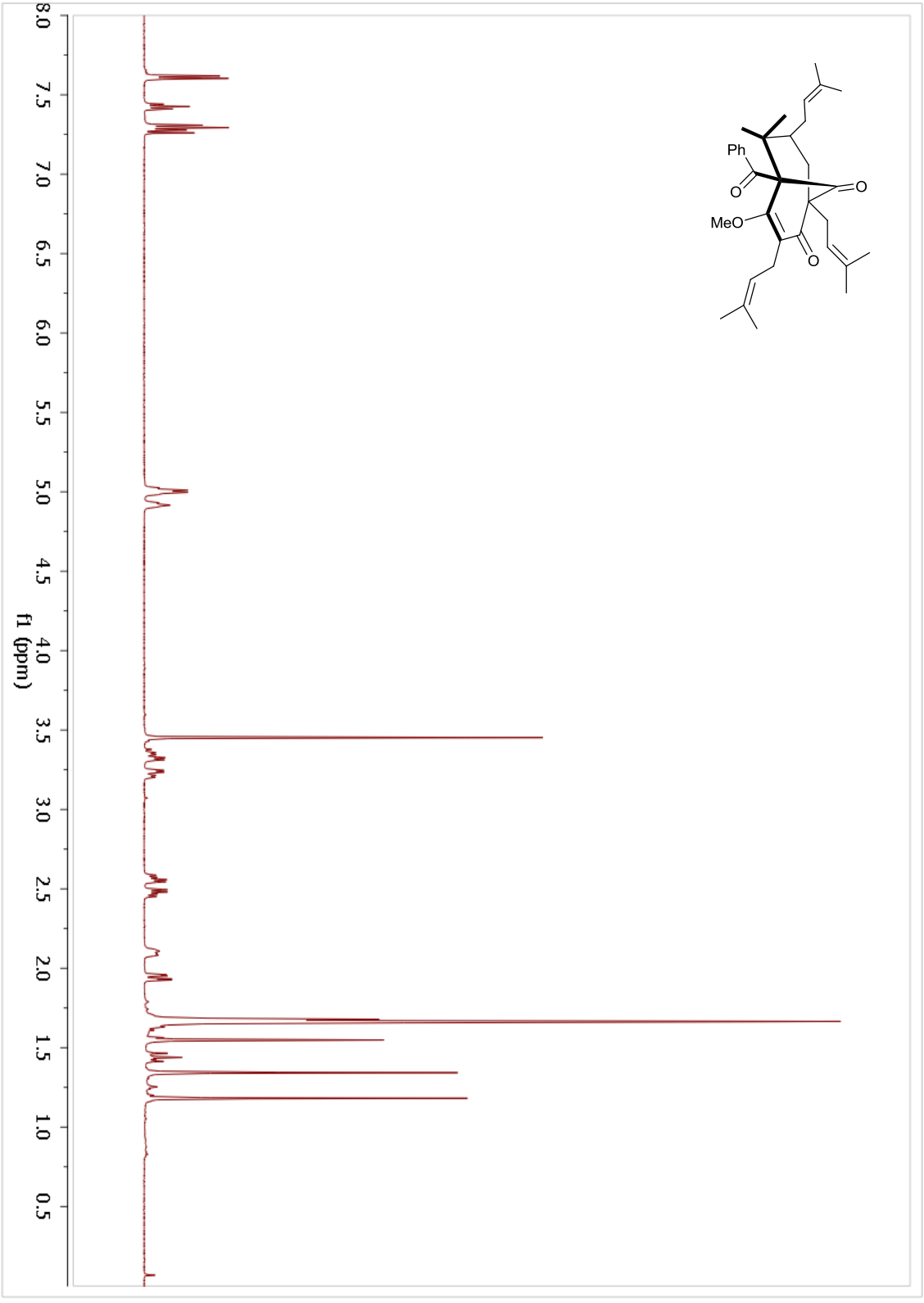
**Appendix 7:** $^{1}$H NMR of **178** (500 MHz, $CDCl_3$)



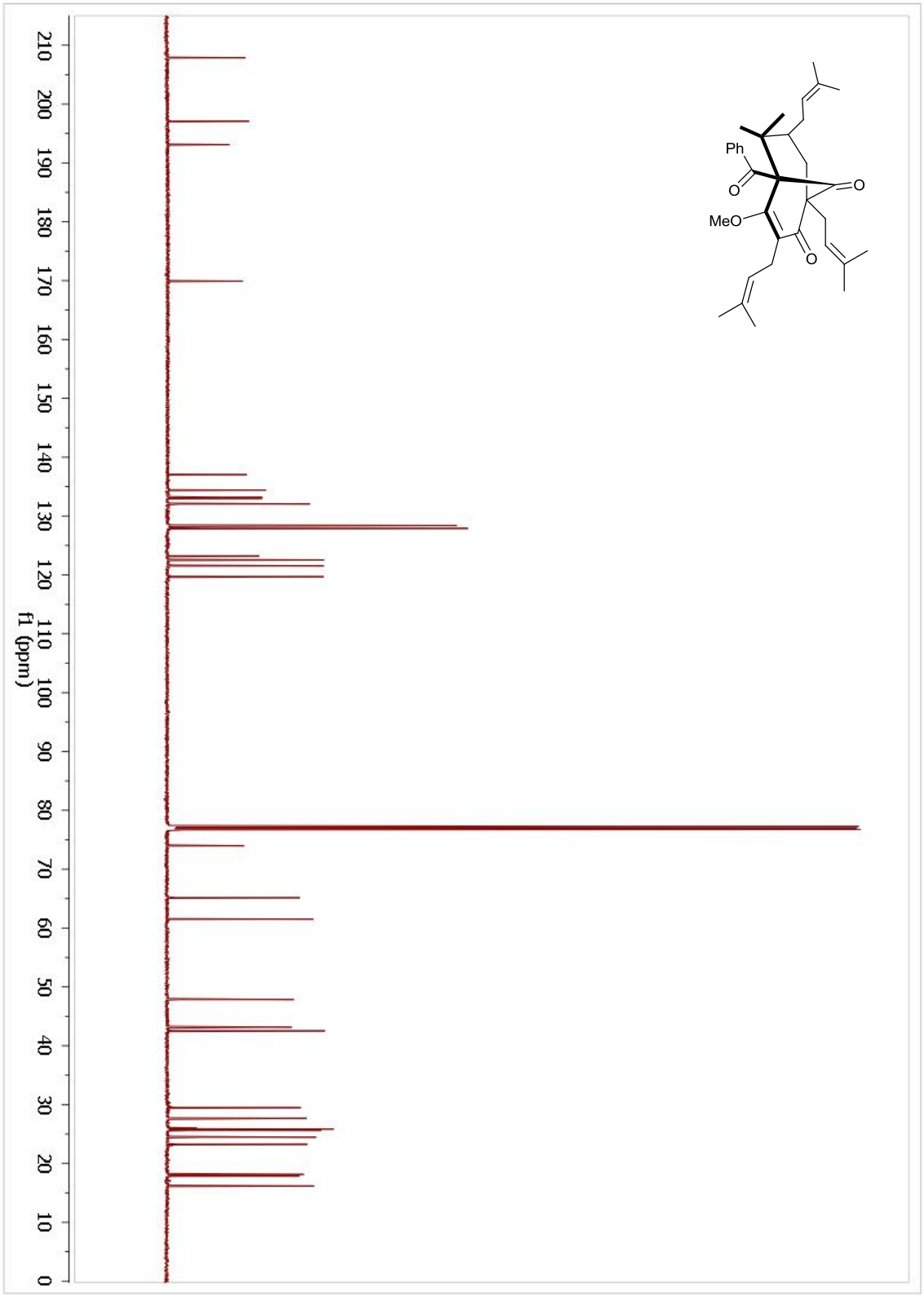
**Appendix 8:** $^{13}C$ NMR of **178** (125 MHz, $CDCl_3$)



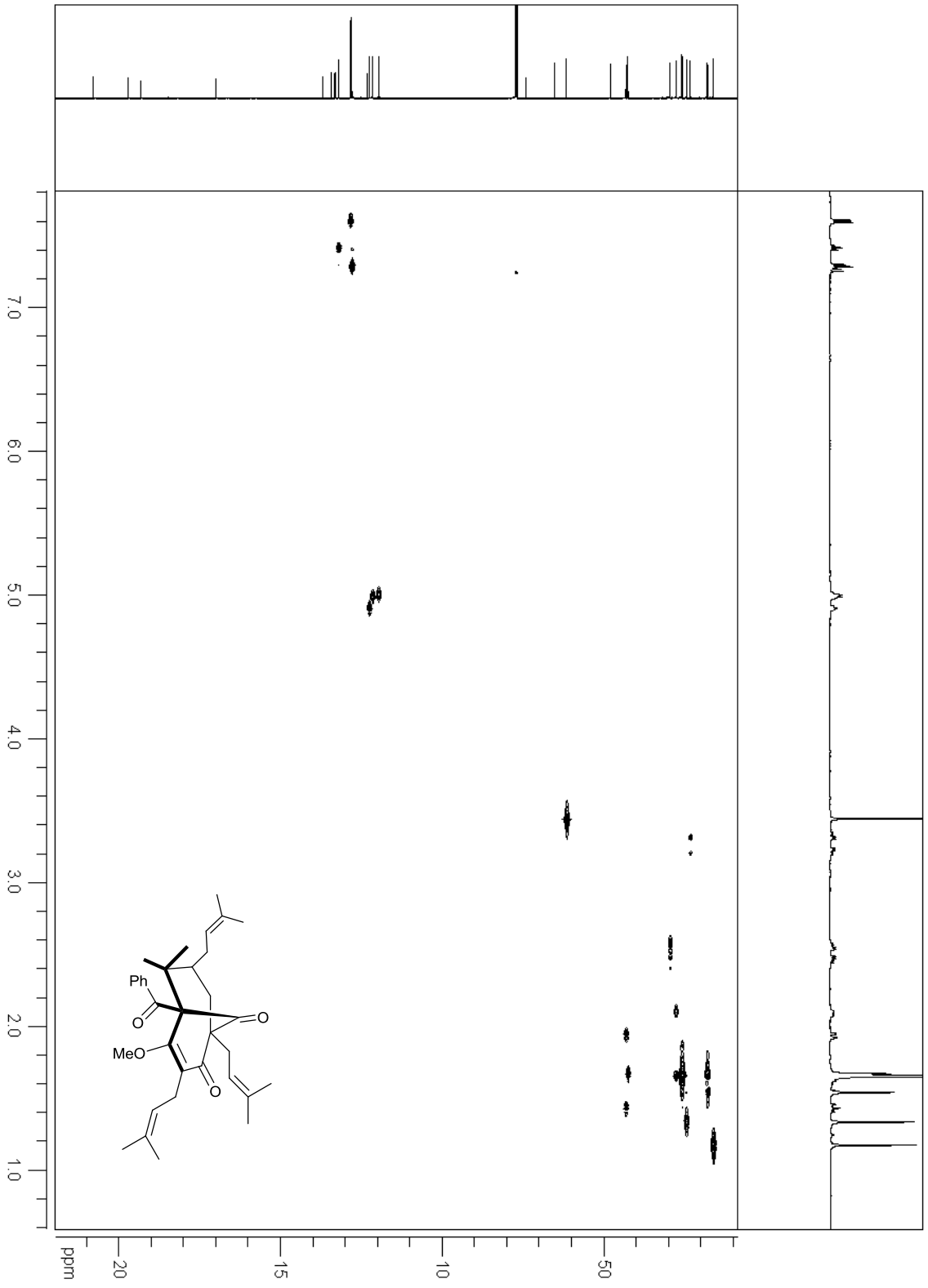
**Appendix 9:** $^{1}$H NMR of **157** (500 MHz, $CDCl_3$)



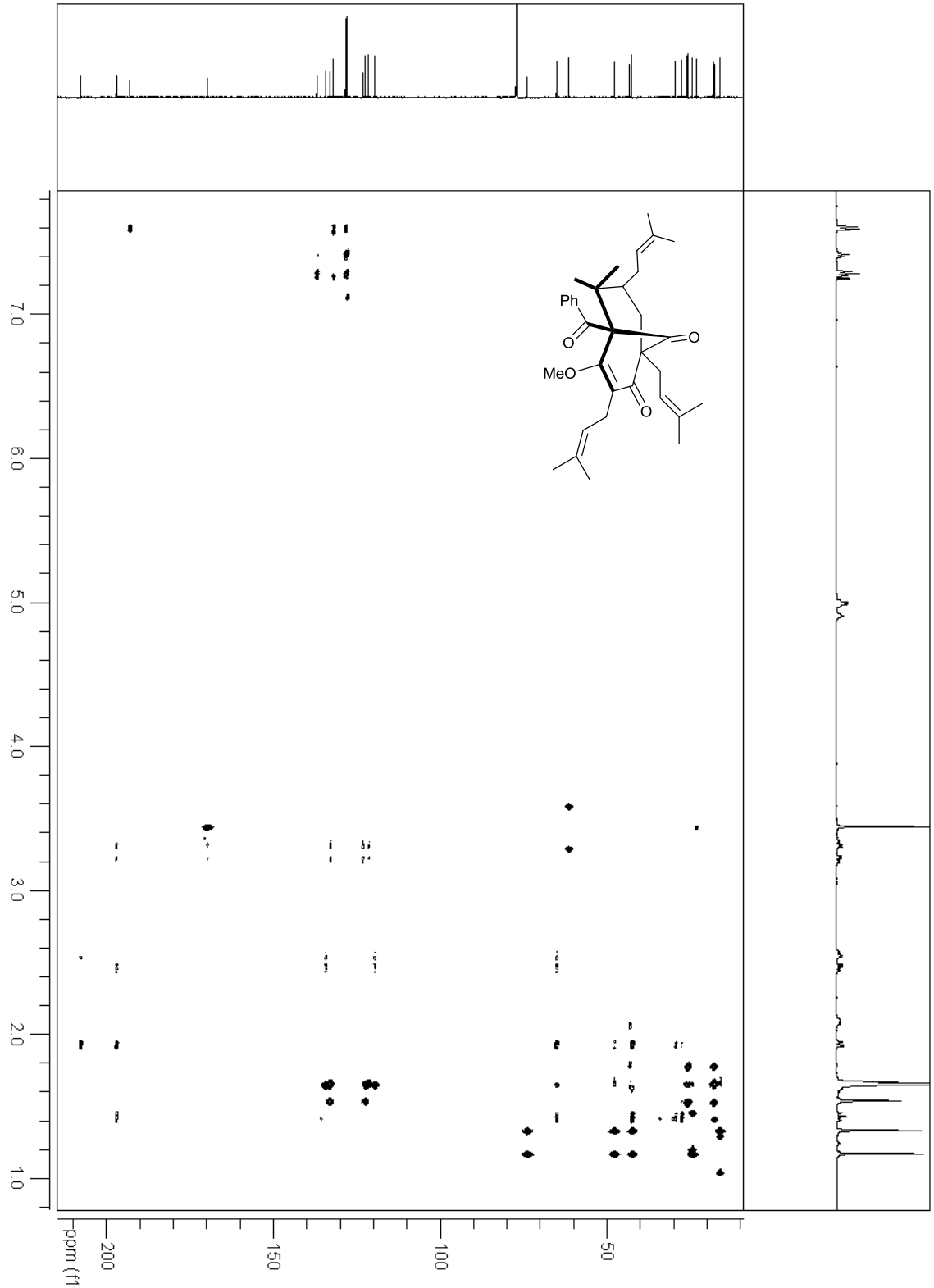
**Appendix 10:** $^{13}$ C NMR of **157** (125 MHz, $CDCl_3$)



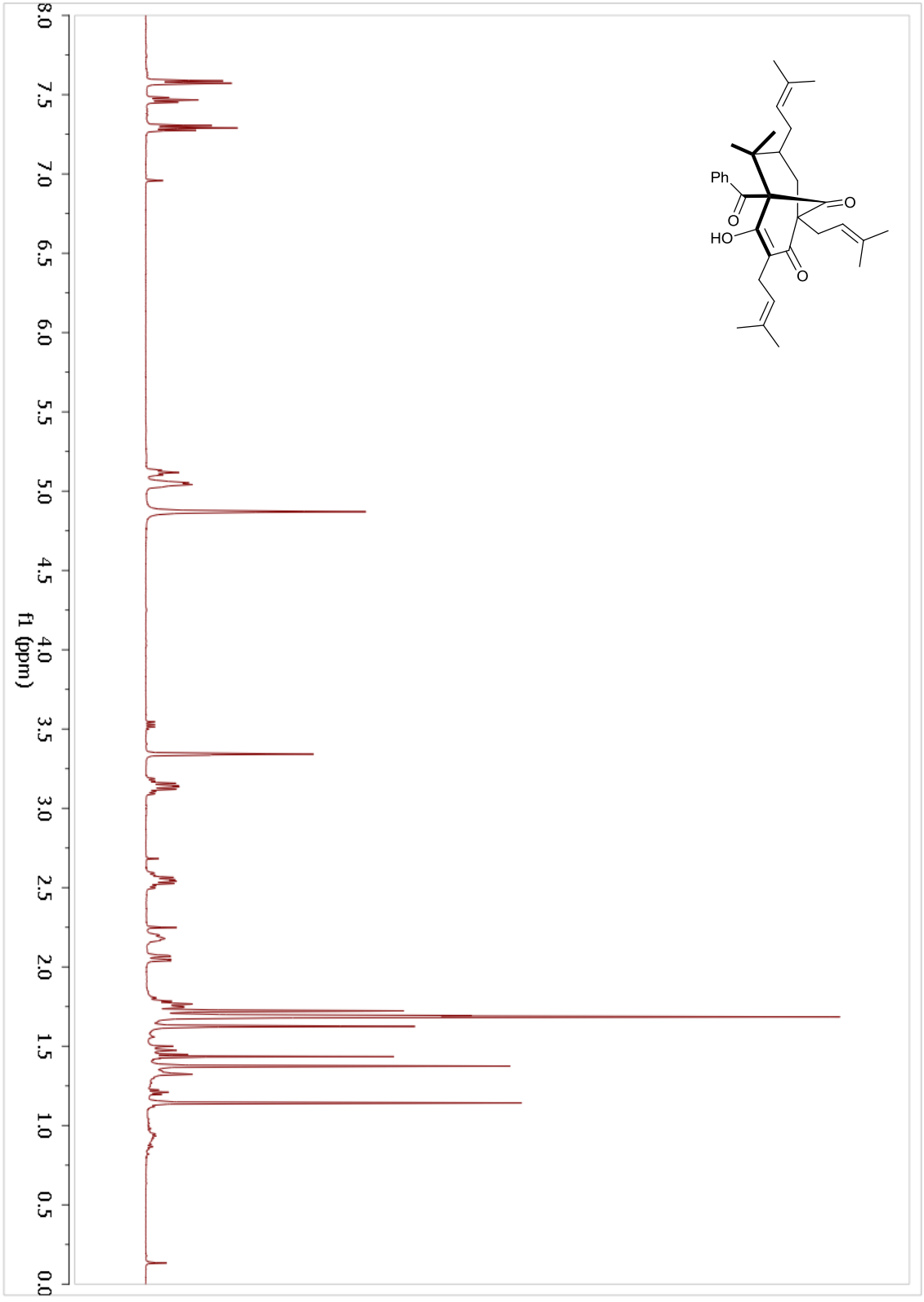
**Appendix 11:** HSQC NMR of **157** ($CDCl_3$)



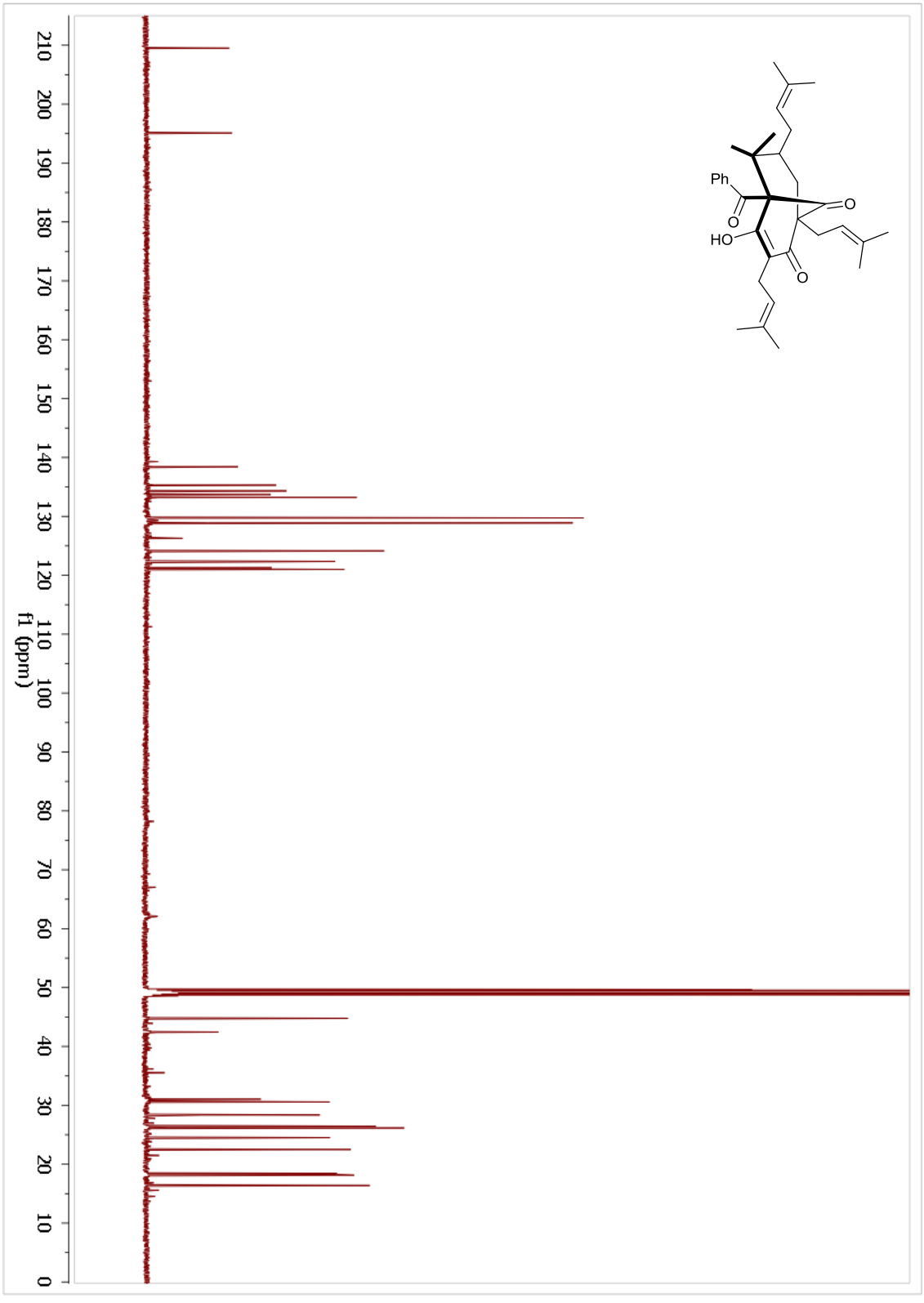
**Appendix 12:** HMBC NMR of **157** ($CDCl_3$)



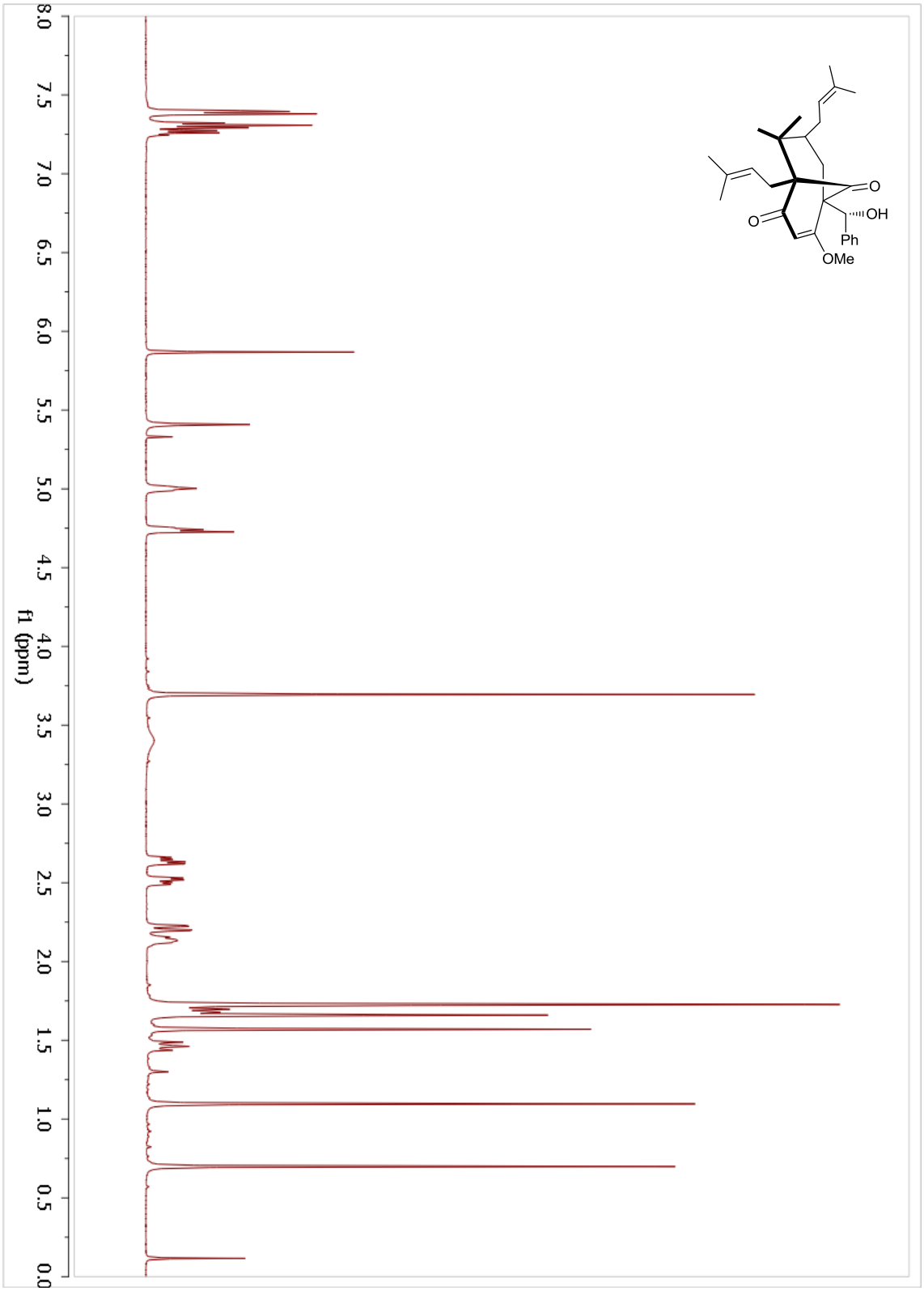
**Appendix 13:** $^{1}$H NMR of nemorosone (**4**) (500 MHz, MeOD)



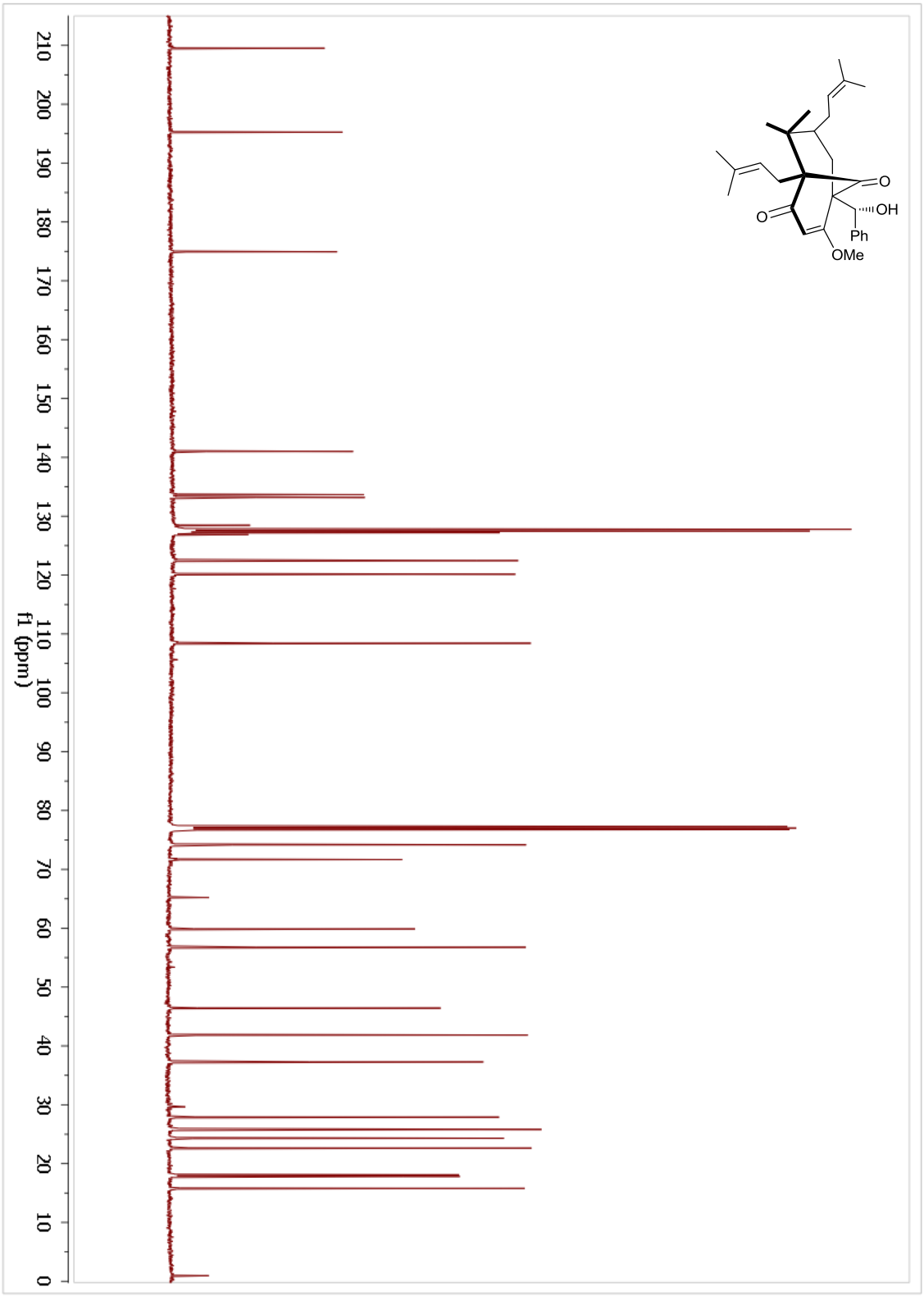
**Appendix 14:** $^{13}$C NMR of nemorosone (**4**) (125 MHZ, MeOD)



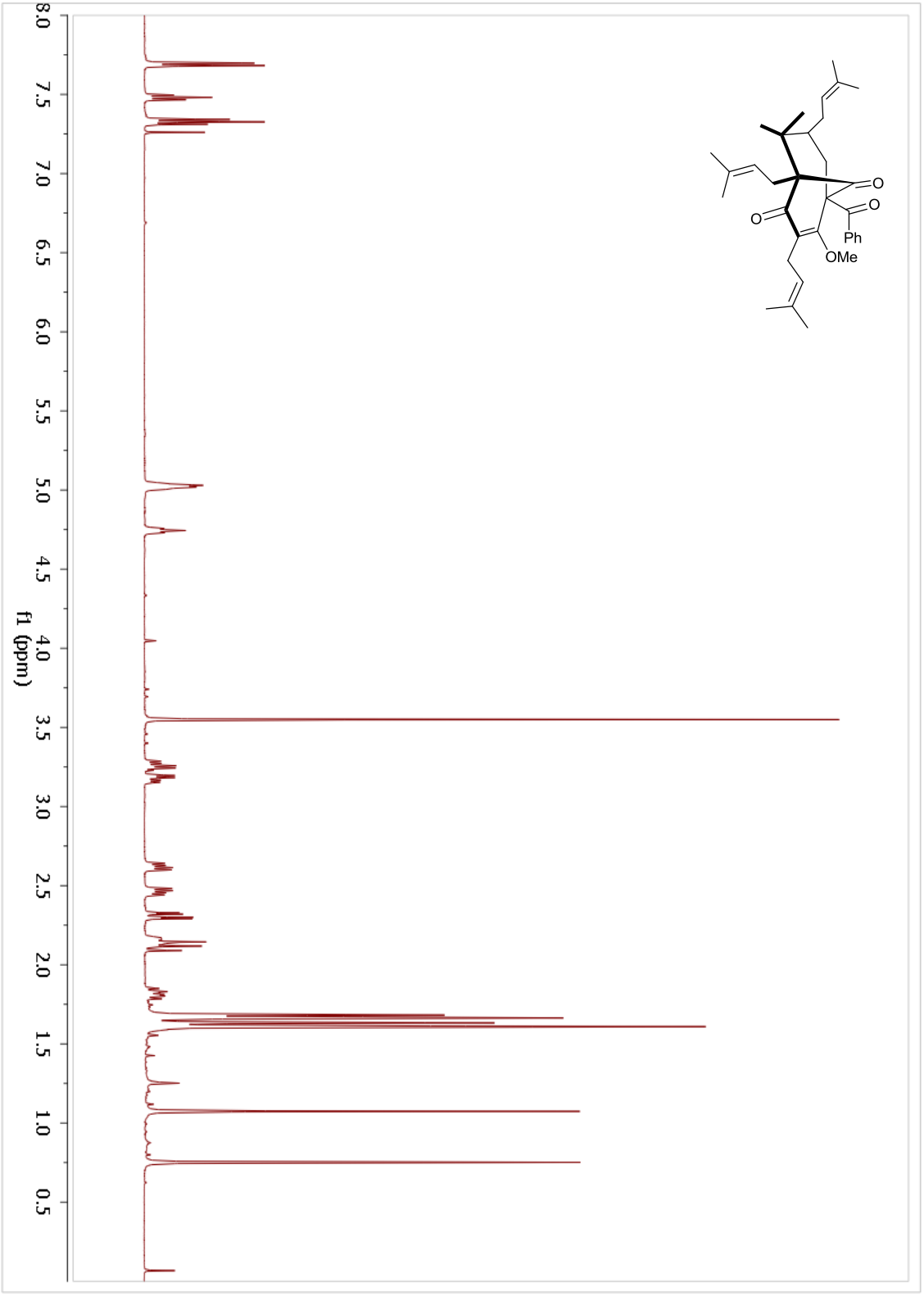
**Appendix 15:** $^{1}$H NMR of **199** (500 MHz, $CDCl_3$)



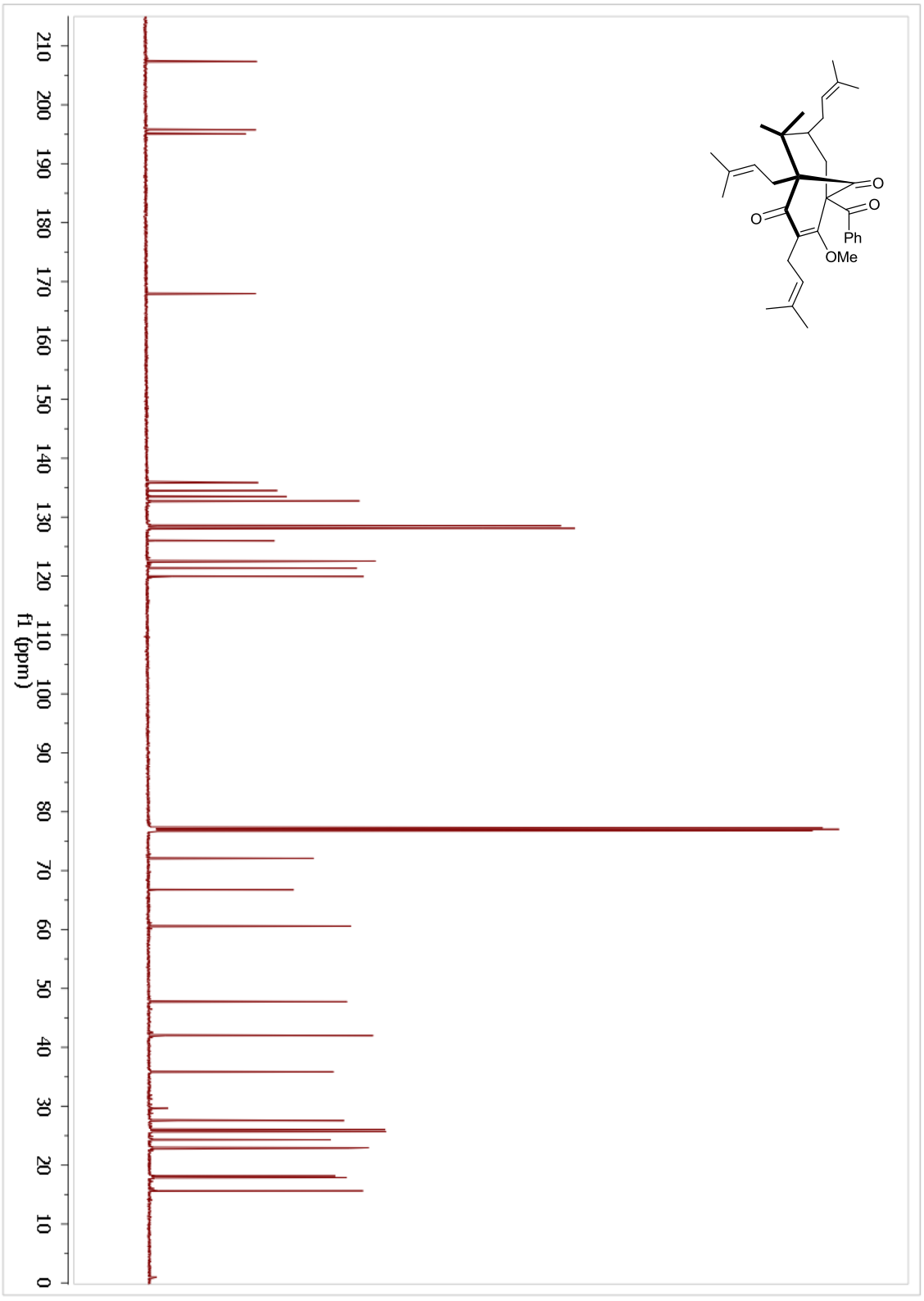
**Appendix 16:** $^{13}C$ NMR of **199** (125 MHz, $CDCl_3$)



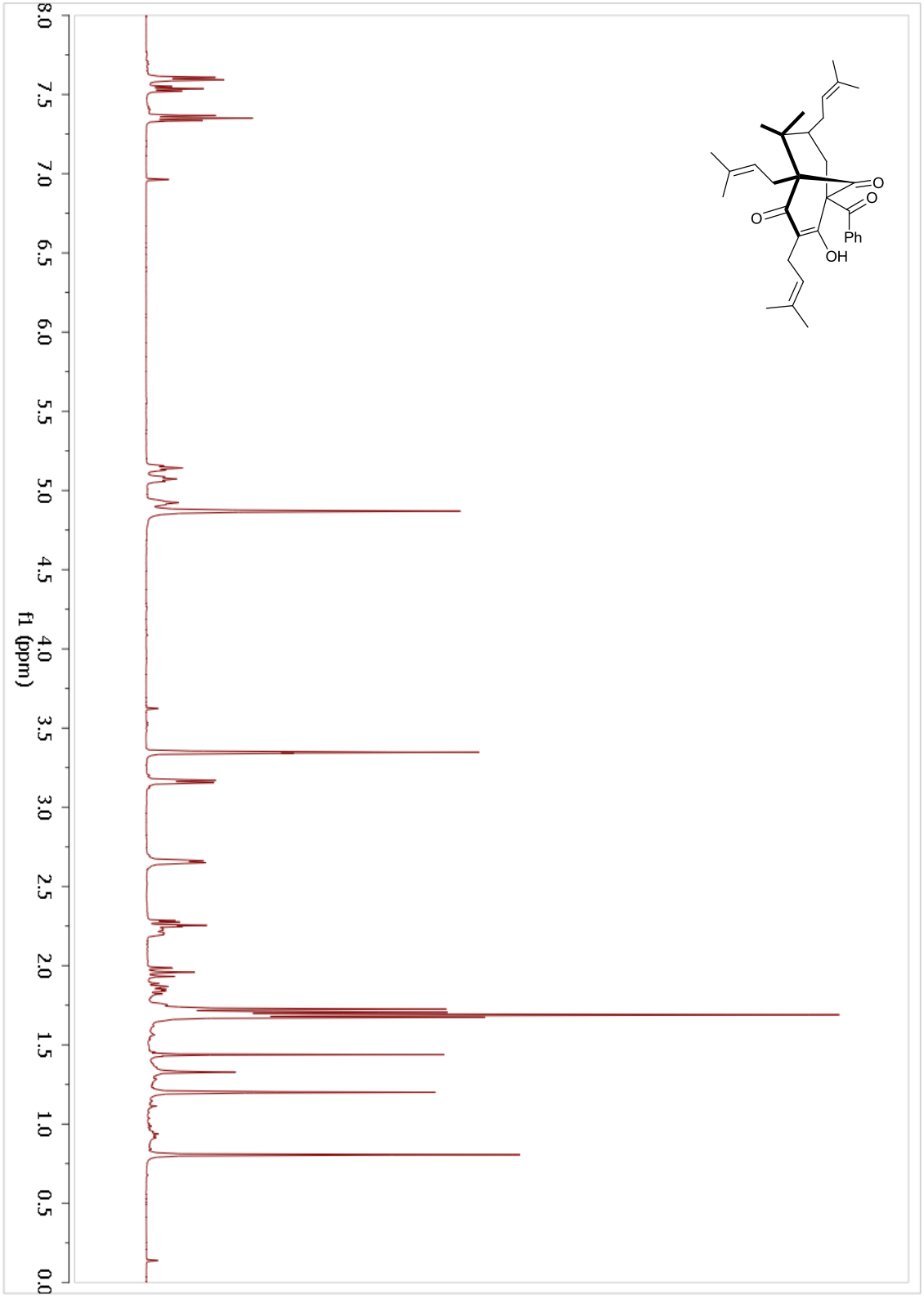
**Appendix 17:** $^{1}$H NMR of **152** (500 MHz, $CDCl_3$)



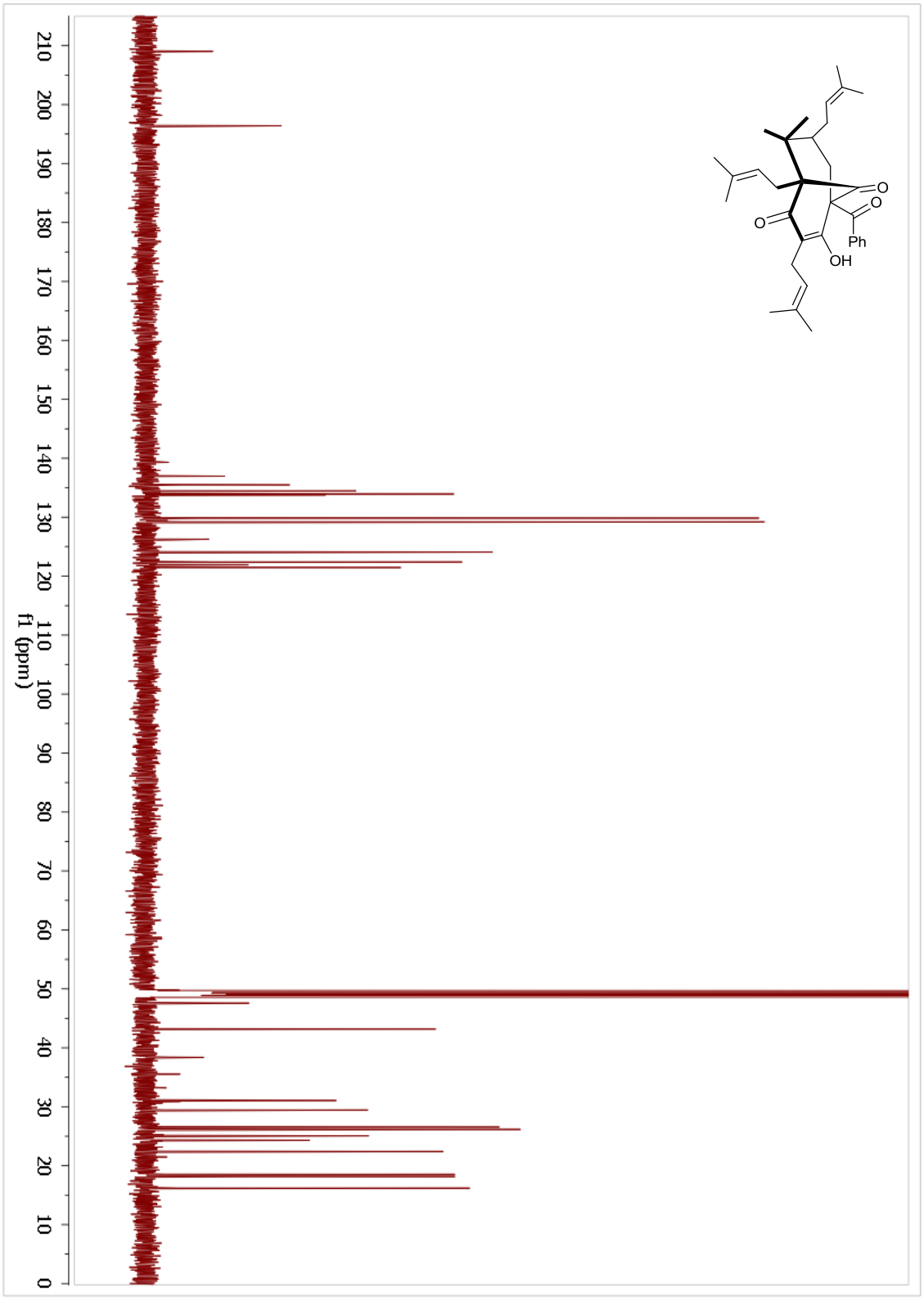
**Appendix 18:** $^{13}C$ NMR of **152** (125 MHz, $CDCl_3$)



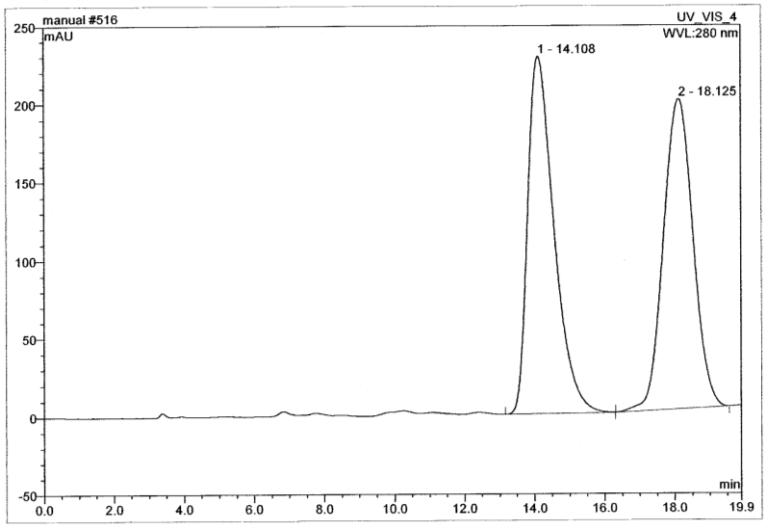
**Appendix 19:** $^{1}$H NMR of **150** (500 MHz, MeOD)



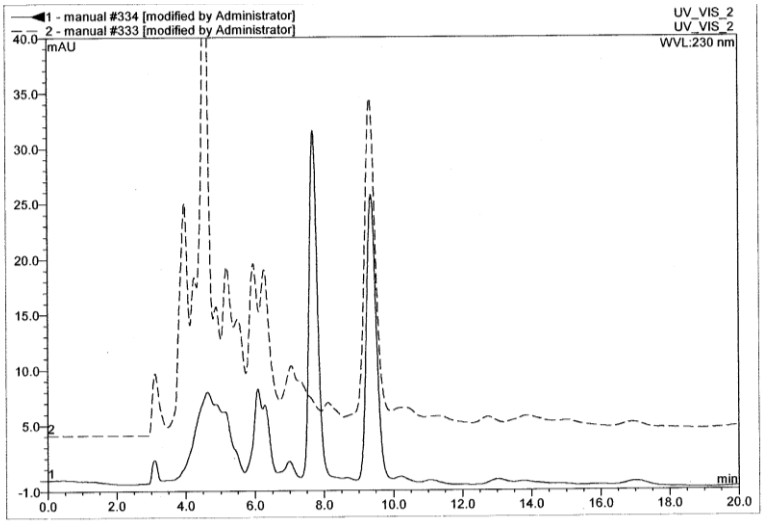
**Appendix 20:** $^{13}$C NMR of **150** (125 MHz, MeOD)



**Appendix 21:** HPLC trace for *O*-methylated nemorosone (**157**). Lux cellulose II column (1.5% MeOH in hexane)



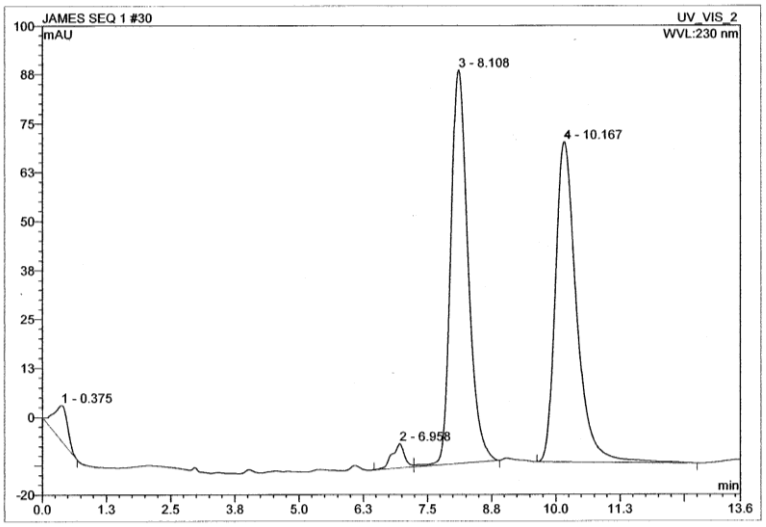

**Appendix 22:** HPLC trace of synthetic nemorosone (**4**) and natural nemorosone (**4**). AD column (10% IPA in hexane)




**----** = natural nemorosone

__ = synthetic nemorosone

**Appendix 23:** HPLC trace for *O*-methylated nemorosone II (**152**). Lux cellulose II column (1% IPA in hexane)



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