Synthesis of Some Marine Natural Product Fatty Acid Derivatives

Syntese av noen fettsyreavledede marine naturprodukter

Philosophiae Doctor (PhD) Thesis

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Dedicated to my son Olav Victor and my good friend Johan Pecoraro.

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Harrison C. Gallantree-Smith

Ås, August 2016

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I. *Total synthesis based on the originally claimed structure of mucosin*

Harrison C. Gallantree-Smith, Simen G. Antonsen, Carl H. Görbitz, Trond V. Hansen, Jens M. J. Nolsøe and Yngve H. Stenstrøm, *Org. Biomol. Chem.*, **2016**, Accepted; 09/08/2016

II. *The first synthesis of Crucigasterin 277 - a polyunsaturated C-18 amino alcohol from the Mediterranean tunicate Pseudodistoma crucigaster*

Solveig Flock, Simen Antonsen, Harrison Gallantree-Smith, Anne Marie Langseter, Lars Skattebøl, Yngve Stenstrøm, *Tetrahedron* **2016**, *72*, 4518-4522

Abstract

The research presented in this thesis puts forth total syntheses of two marine natural products: (-)-mucosin and (+)-crucigasterin 277. Both syntheses focus on producing enantiopure natural products derived from commercially available starting materials. One of the main aims for the manufacture of these two marine natural products is to make them both available for biological studies.

The first total synthesis focused on producing (-)-mucosin, a C_{20} fatty acid, isolated and elucidated as its methyl ester from a Mediterranean sponge, *Reniera mucosa*. Utilization of commercially available 1,4-cyclohexadiene and its subsequent conversion to a literature known *meso*-ketone in three steps proceeded with effective results. An ensuing enantioselective protocol with a chiral base furnished an optically active β-keto ester in excellent enantiomeric excess of ≥99%. Elaboration of this β-keto ester in a thirteen-step synthesis, involving a copper mediated cross-coupling reaction, two consecutive homologations to form a terminal alkyne and a novel stereospecific one-pot Negishi cross-coupling protocol to form an *E*-olefin, gave the literature stated structure of (-)-mucosin. On analysis of the relative stereochemistry of a late stage intermediate by X-ray crystallography and comparison of the literature NMR and optical rotation data of the (-)-mucosin methyl ester with the synthesized methyl ester it was found that the relative stereochemistry of the natural product methyl ester had been incorrectly elucidated, as well as its previously synthesized enantiomer (+)-mucosin.

The second total synthesis focused on producing (+)-crucigasterin 277, a C_{18} amino alcohol, isolated and elucidated as a diacetyl from a Mediterranean tunicate, *Pseudodistoma crucigaster*. Employment of commercially available eicosapentaenoic acid ethyl ester was reacted in a literature known seven-step synthesis, involving an iodolactonization, hydrolysis, oxidative cleavage and base catalyzed isomerization to form a C_{15} allylic bromide. This bromide was then converted to a sulfone, which underwent a nucleophilic addition with enantiopure *N*-Boc-D-alaninal (Boc = *tert*-butyloxycarbonyl) to form a mixture of four diastereoisomers. After subsequent purification, desulfonation and deprotection, (+)-crucigasterin 277 was synthesised in excellent optical purity on comparison with its diacetyl derivative. This is the first reported synthesis of this optically active marine amino alcohol.

Sammendrag

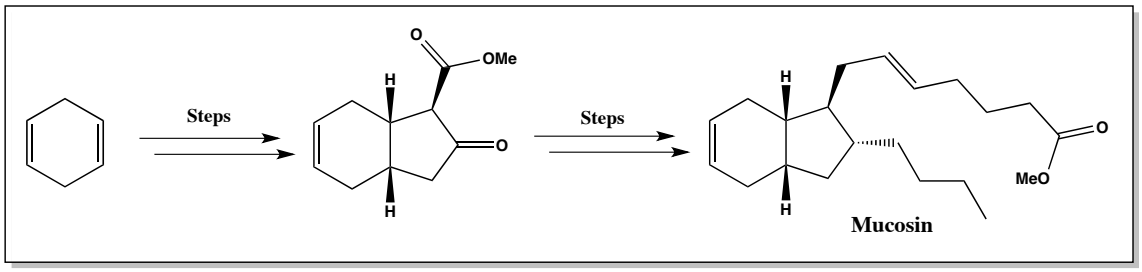
Avhandlingen beskriver totalsyntesene av to marine naturprodukter: (-)-mucosin og (+)-crucigasterin 277. Begge synteser fokuserer på å fremstille naturproduktene av høy enantiomerisk renhet fra kommersielt tilgjengelige startmaterialer. Et av hovedmålene for syntesene var også at de skulle ha en kjemisk og optisk renhet tilstrekkelig for fremtidig biologisk testing.

Den første totalsyntesen beskriver fremstillingen av (-)-mucosin, et C_{20} fettsyre-derivat, isolert fra en svamp, *Reniera mucosa*, som finnes i Middelhavet. Kommersielt tilgjengelig 1,4-cycloheksadien ble gjennom tre trinn omdannet til et *meso*-keton kjent fra litteraturen. Ved å benytte en kiral base ble optisk aktiv β-ketoester fremstilt med enantiomerisk overskudd over 99%. Gjennom 13 reaksjonstrinn, som blant annet involverer Michael-type addisjon, to påfølgende homologeringer for å danne et terminalt alkyn, samt en stereospesifikk ”one-pot” Negishi krysskobling for å danne *E*- alkenet, ble den litteratur-foreslåtte strukturen for (-)-mucosin syntetisert. Våre data viser klart at naturproduktet ikke har den strukturen som er foreslått i litteraturen. Røntgen-analyse av et av de siste intermediatene i syntesen av mucosin ga den relative stereokjemien til alle fire stereogene sentra på den bisykliske forbindelsen. Sammenligning av litteratur-NMR data og optisk rotasjon for (-)-mucosin metylesteren med den syntetiserte metylesteren viste at den relative stereokjemien til naturprodukt metylesteren er feil. Dette gjelder også enatiomeren som er syntetsert og publisert av andre.

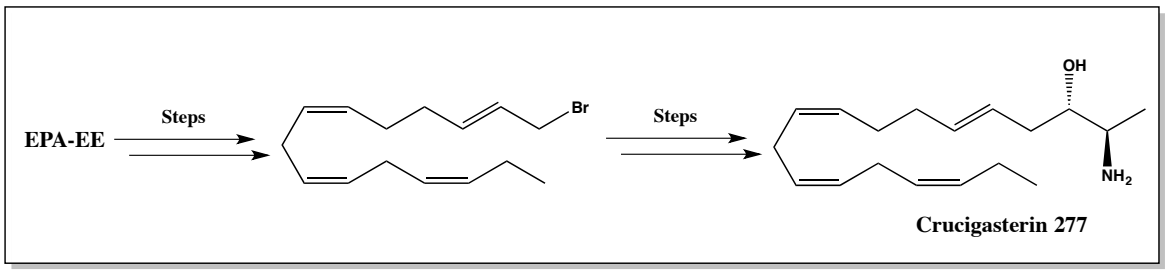
Den andre totalsyntesen fokuserer på (+)-crucigasterin 277, en C_{18} aminoalkohol, isolert fra et kappedyr, *Pseudodistoma crucigaster*, fra Middelhavet. Etylesteren av eicosapentaenoic syre av ble benyttet som startmateriale, og gjennom en reaksjonsrekke på syv trinn, som blant annet inkluderer jodlaktonisering, hydrolyse, oksidativ kløyving og basekatalysert isomerisering, ble C_{15} allylbromidet fremstilt. Dette bromidet ble omdannet til tilsvarende sulfon, og reagert mot *N*-Boc-D-alaninal (Boc = *tert*-butyloksykarbonyl) for å gi blandingen av fire diastereomerer. Etter opprensning ble sulfongruppen fjernet, etterfulgt av hydrolyse av Boc-beskyttelsesgruppen, for å gi (+)-crucigasterin 277. For å sammenligne med litteraturdata ble (+)-crucigasterin 277 omdannet til diacetatet, som er den forbindelsen som er beskrevet i litteraturen. Dataene stemte, og bekreftet da at vi har den første rapporterte syntesen av denne marine aminoalkoholen.

Graphical Abstracts

Paper I:



Paper II:



Abbreviations

4(*S*)-IPTT	4(*S*)-iso-propyl-1-3-thiozolidinone-2-thione
AA	Arachidonic Acid ((5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoic acid)
AcOH	Acetic acid
Acyl-CoA	Acyl-coenzyme A
AdA	Adrenic acid ((7*Z*,10*Z*,13*Z*,16*Z*)-docosatetraenoic acid)
COX-1	Prostaglandin H synthase
Cp_2ZrCl_2	*bis*(Cyclopentadienyl)zirconium(IV) dichloride
DHA	Docosahexaenoic acid ((4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosahexaenoic acid)
DIBAL-H	Diisobutyl aluminium hydride
DIC	*N*,*N*'-Diisopropylcarbodiimide
DMC	Dimethyl carbonate
DME	1,2-Dimethoxyethane
DMF	*N*,*N*-Dimethylformamide
DMP	Dess-Martin periodinane
DMSO	Dimethyl sulfoxide
EPA	Eicosapentaenoic acid ((5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosapentaenoic acid)
EPA-EE	Eicosapentaenoic acid ethyl ester
HETE	Hydroxyeicosatetraenioc acid
HPLC	High-Performance Liquid Chromatography
IsoPs	Isoprostanes
LDA	Lithium diisopropylamide
LiHMDS	Lithium *bis*(trimethylsilyl)amide
*m*CPBA	*meta*-Chloroperbenzoic acid
NaHMDS	Sodium *bis*(trimethylsilyl)amide
NBS	*N*-Bromosuccinimide
NCS	*N*-Chlorosuccinimide
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect Spectroscopy
PDC	Pyridinium dichromate
$[Pd_2(dba)_3]$	Tris(dibenzylideneacetone)dipalladium(0)

PGs	Prostaglandins
PMNs	Polymorphonuclear Cells
PUFA	Polyunsaturated Fatty Acid
RAMP	(*R*)-(+)-1-Amino-2-(methoxymethyl)pyrrolidine
ROESY	Rotating Frame Nuclear Overhauser Effect Spectroscopy
SAMP	(*S*)-(-)-1-Amino-2-(methoxymethyl)pyrrolidine
TBAF	Tetrabutylammonium fluoride
TEMPO	(2,2,6,6-Tetramethyl-piperidin-1-yl)oxyl
TES	Triethylsilyl
THF	Tetrahydrofuran
TMS	Trimethylsilyl
TMSCl	Timethylchlorosilane
WSC.HCl	*N*-Ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride

1. Introduction

1.1 Natural Products

In nature, many bioactive compounds have been isolated that have helped humanity overcome diseases and infections. As early as 4000 years ago, plants such as mandrake were prescribed for pain relief; turmeric for blood clotting and wild garlic for circulatory problems.[1]

During the nineteenth century great strides were made to actually isolate the bioactive medicinal compounds in these plants. Friedrich Sertürner was the first scientist to successfully acquire and characterize chemically pure morphine (**1**) in 1806 from the opium poppy *Papaver somniferum*.[2] Since then, many thousands of natural products, such as strychnine (**2**) and pacliataxel (**3**), have been isolated and many have been successfully incorporated into the pharmaceutical industry. All three can be seen in Figure 1.1 below. In the USA today about 121 certified drugs come from nature and about forty-seven percent of anti-cancer medicines come from natural products or natural product mimics.[3]

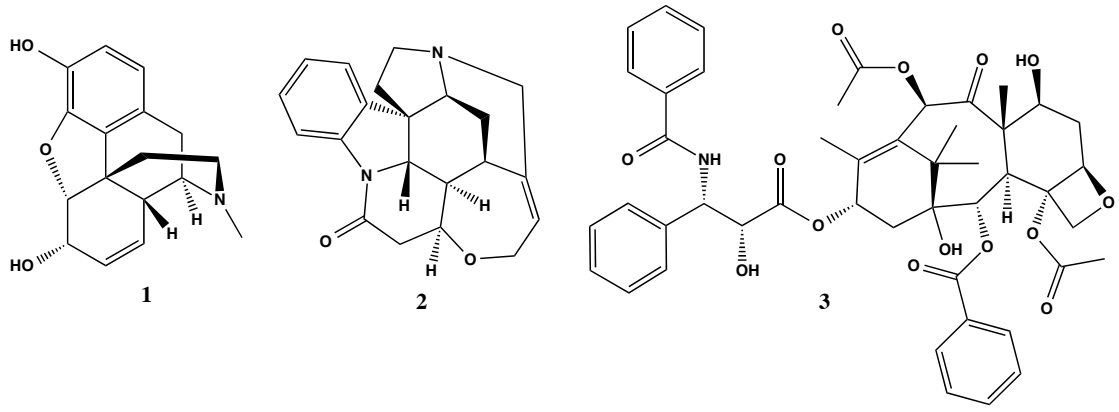


Figure 1.1 Structures of the natural products morphine (**1**), strychnine (**2**) and pacliataxel (**3**).

1.2 Marine Bioprospecting

During the last half of the 20th century, marine natural products entered the pharmaceutical radar as a possible alternative for extracting bioactive compounds. One potential reason for this is that the marine environment may contain over 80% of the world's plant and animal

species and has been quoted as the earth's "second rainforest."[4] This is now seen as a rich breeding ground for harvesting novel and curative natural products. One way to obtain these is by marine bioprospecting, which involves the collection of aquatic organisms and subsequent screening for a specific molecule or activity of interest.[5] Several potential therapeutic compounds from marine bioprospecting are given below in Figure 1.2. All have anti-cancer properties and are isolated from various aquatic fauna. Discodermolide (**4**), a polyhydroxylated polyketide lactone from the deep-sea sponge *Discodermia sp.*, is an immunosuppressive and cytotoxic agent that stabilizes microtubules.[6,7] Luffarin I (**5**), a sesterterpene isolated from an Australian marine sponge *Luffariella geometrica*, is an antiproliferative towards cancer cells.[8] Both can be seen in Figure 1.2 below.

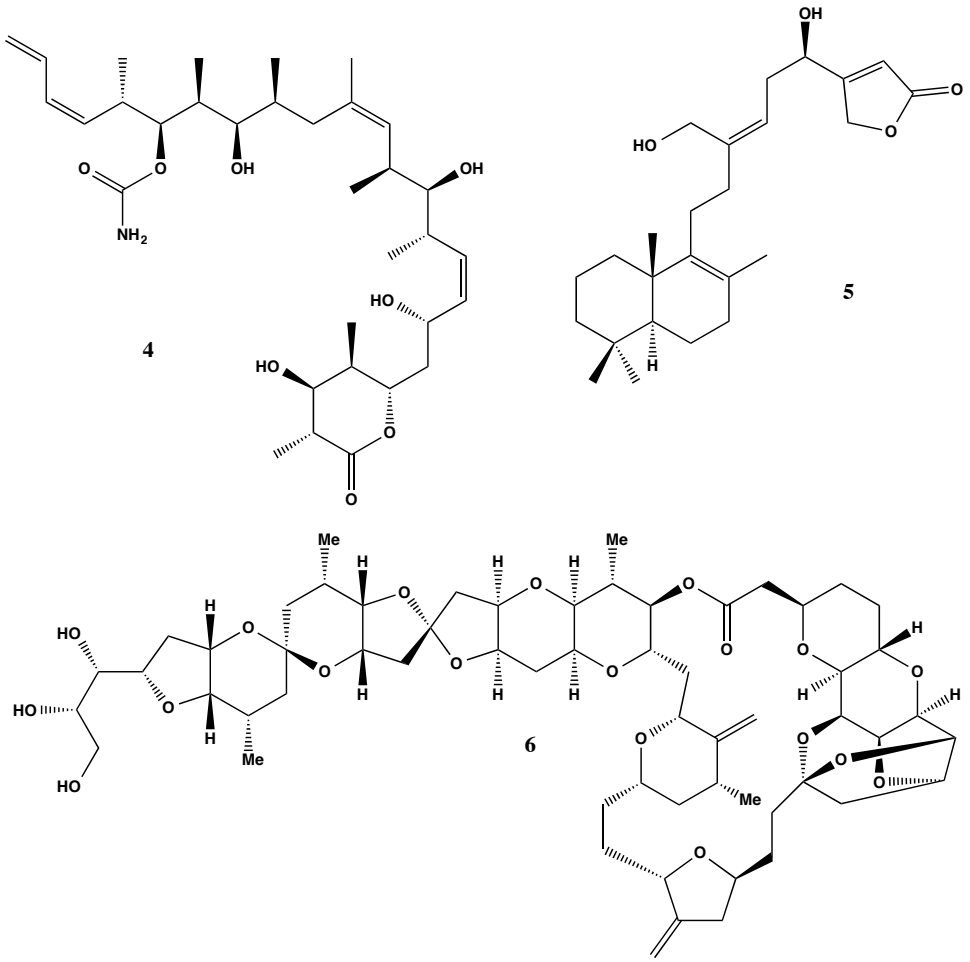


Figure 1.2 Structures of the marine natural products discodermolide (**4**), luffarin I (**5**) and halichondrin B (**6**).

One of the major problems with isolating and employing these marine natural products is sustainability. Simply removing hundreds of kilograms of sponges, sea worms and other marine flora and fauna for necessary analysis and biological testing has a huge ecological impact. Halichondrin B (**6**) (Figure 1.2) is a commercially available anticancer sponge

metabolite isolated from *Lissodendoryx sp*.[9,10] Distribution of the sponge globally is around five km^2 and only has an estimated total biomass of 289 tons. Employing clinical trials would involve the removal of >30 tons of the sponge to yield 1-5 Kg of halichondrin B and around 3000-17000 tons would be needed annually for commercial drug use.[11] This is obviously unsustainable and devastating to the marine environment. A synthetic methodology must be implemented to garner sufficient volume of the metabolite. The benefits of making this possible is seen with the drug discovery effort to synthesize eribulin (**7**) (Figure 1.3),[10,12] which is an anticancer macrocyclic analogue of halichondrin B. Eribulin has been used to treat patients with metastatic breast cancer and inoperable liposarcoma due to its suppression of microtubule growth.[13] Without synthetically producing halichondrin B it would be very difficult to envisage the manufacture of compounds like eribulin.

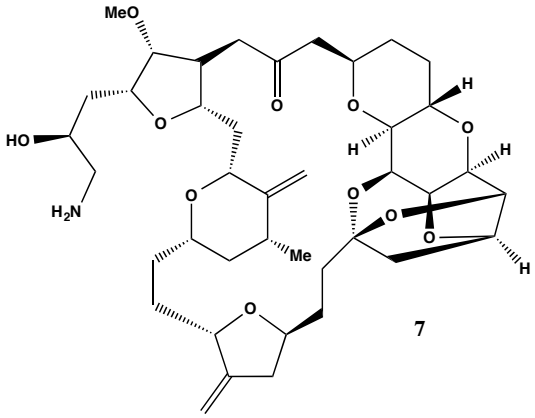


Figure 1.3 Structure of the anti-cancer macrocycle eribulin (**7**).

1.3 Polyunsaturated Fatty Acids

In Norway, polyunsaturated fatty acids (PUFAs), which are also marine natural products, have been studied for a long time and have some interesting bioactivities. PUFAs such as docosahexaenoic acid (DHA) (**8**) and eicosapentaenoic acid (EPA) (**9**) are ω-3 fatty acids and are abundantly available from various ocean fish, most notably from cod liver oil.[14] The notoriety of the benefits of cod liver oil, even as far back as the Viking era, has allowed a huge industry to evolve in the manufacturing and marketing of these valuable compounds. Reduction in coronary heart disease, blood pressure, arthritis, and cancer in humans has meant other structurally associated adducts related to PUFAs have been tested for their bioactivity.[15-19] Another PUFA is arachidonic acid (AA) (**10**), which differs from EPA and DHA due to it comprising of a ω-6 chain and reduced chain saturation. AA is a major component of membrane phospholipids throughout the animal kingdom, but very little is

found in the diet.[20] However, in the marine environment AA has been shown to be present in many metabolic pathways, especially in finfish and echinoderms.[21-23] This PUFA, along with DHA and EPA, as shown in Figure 1.4, is the parent molecule in the biosynthesis of many varying natural products such as prostaglandins and amino alcohols, which can also be isolated from the ocean.

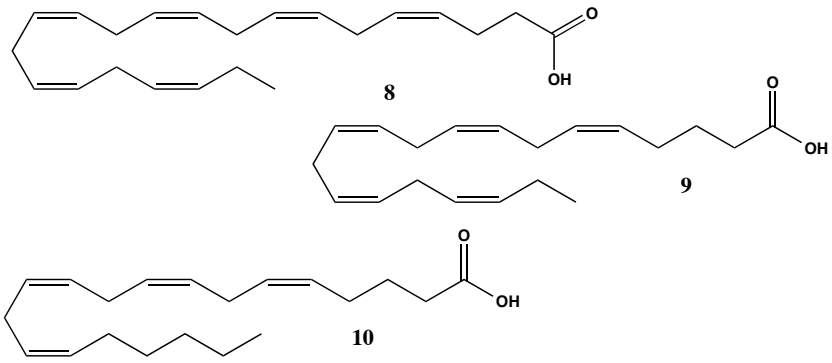


Figure 1.4 Structures of the PUFAs DHA (**8**), EPA (**9**) and AA (**10**).

1.4 Prostaglandins

Prostaglandins (PGs) are a class of physiologically active compounds that have diverse hormone-like effects in animals and are a subclass of eicosanoids. The first scientific proof of these hormone-like effects was reported in the 1930s with journals, by von Euler[24] and Goldblatt,[25] describing the effect of human semen on the uterus and the reduction of blood pressure via injection into animals. However, it was not until the 1960s that great strides were taken in understanding the structure of these compounds and how they were employed physiologically. Professors Sune Bergström and Bengt Samuelsson successfully elucidated four different PGs, PGE_1 (**11**), PGE_2 (**12**), PGE_3 (**13**) and $PGF_{2\alpha}$ (**14**) (Figure 1.5), from sheep semen.[26] All four displayed significant bioactivity especially towards muscle stimulation and as vasodepressors.[26] Professors E. J. Corey and Bengt Samuelsson were able to successfully determine and prove the cyclooxygenase (COX-1) pathway forms various PGs from AA.[27] Following the biological testing of a variety of isolated PGs they were shown to lower inflammation, pain and asthma.[27]

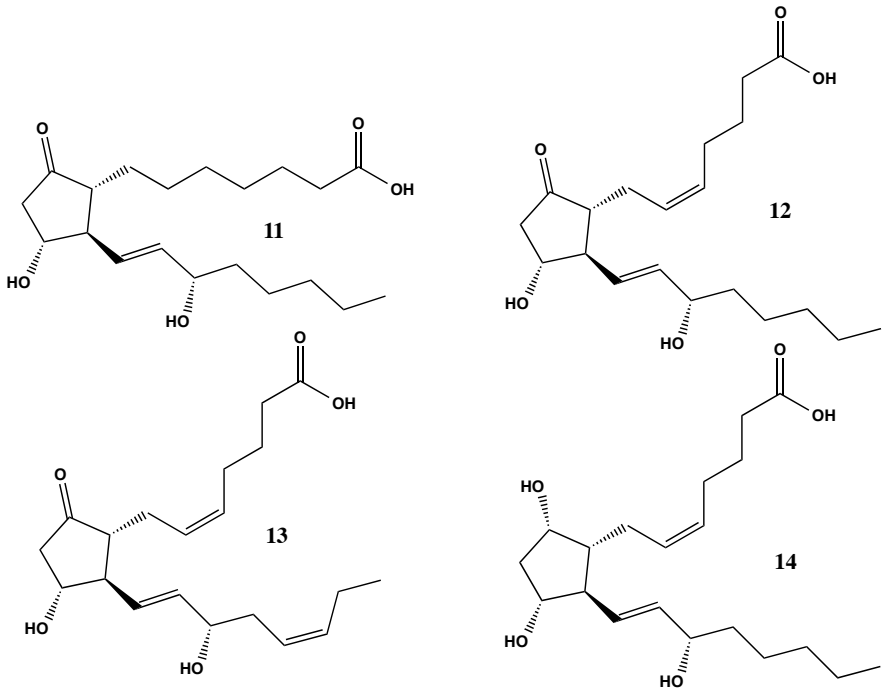
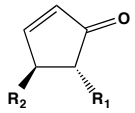
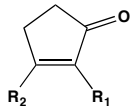
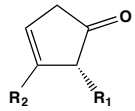
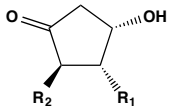
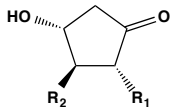
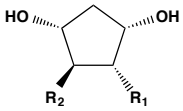
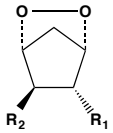
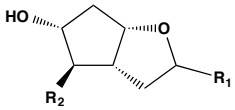
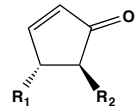
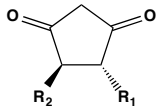


Figure 1.5 Structures of the PGs PGE_1 (**11**), PGE_2 (**12**), PGE_3 (**13**) and $PGF_{2\alpha}$ (**14**).

The following fifty years up, until the present-day, saw a huge explosion in isolating and producing various PGs along with other eicosanoids such as thromboxanes, leukotrienes and lipoxins. All have very acute bioactive effects on human and mammalian physiological activities varying from anticancer to vasomodulation. Table 1.1 displays a brief outline of the diverse cyclic skeletons of the subclass of PGs and their bioactivity.[28-52]

Type	Cyclic Structure*	Mammalian and Human Physiological Effects
PGA		Reduces: Ovarian Cancer, Vesicular Stomatitis Virus, Sendai Virus, Replication of HIV-1.
PGB		Improves CNS Ischemia
PGC		Reduction in Cardiovascular Activity
PGD		Anti-Tumour, Anti-Inflammatory
PGE		Anti-Inflammatory, Anti-Asthmatic, Renal Regulation, Induces Labour,
PGF		Induce Miscarriage, Reduction of Glaucoma
PGG/PGH		Increase Inflammation, Stimulate Platelet Aggregation and Muscle Contraction
PGI		Inhibition of Gastric Acid Secretion, Anti-Aggregation of Platelets and Increased Vasodilation
PGJ		Anti-Inflammatory, Leukaemia Inhibitor, Block Poliovirus Replication
PGK		N/A

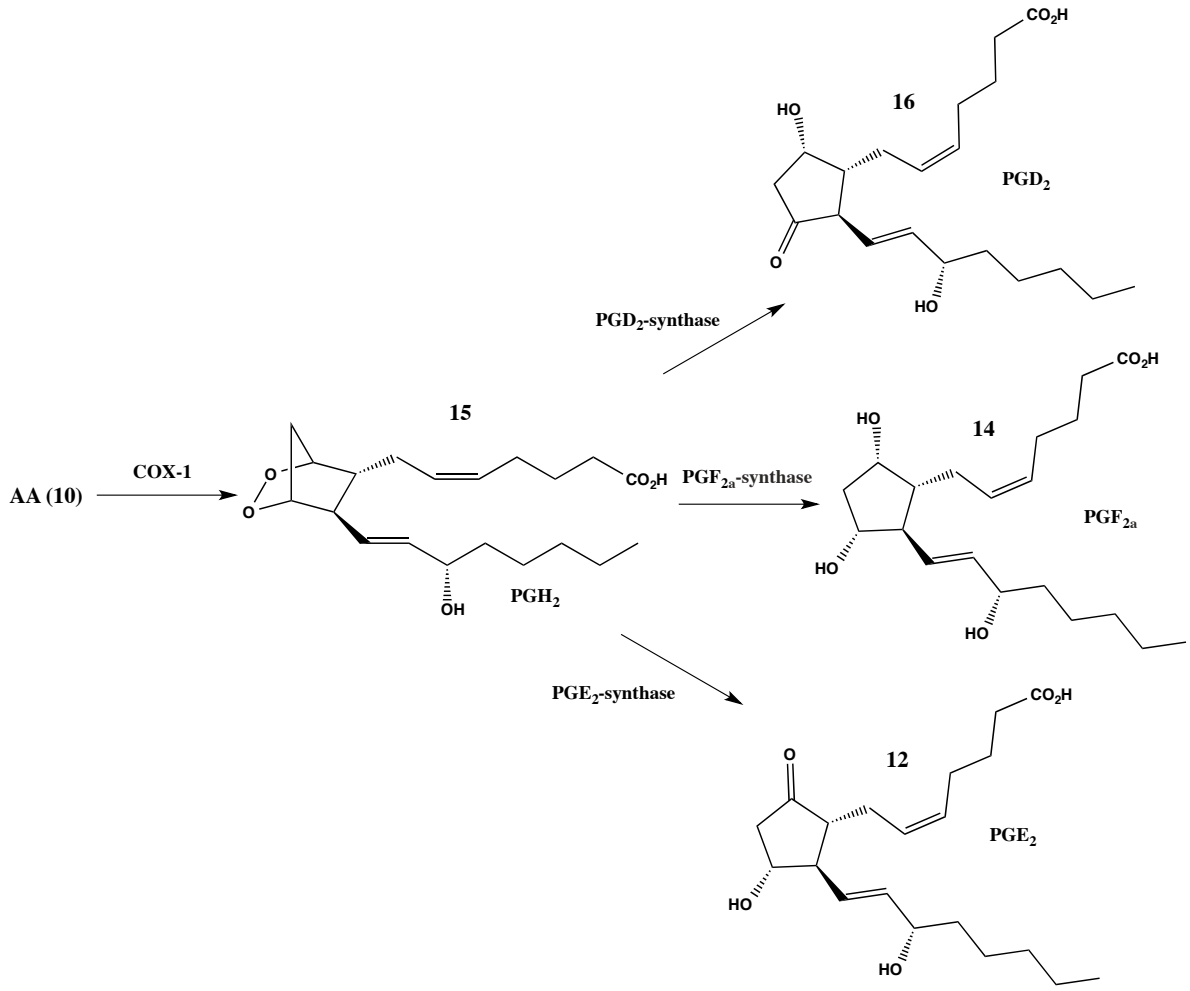
*** R_1 and R_2 are not repeating units and vary in each cyclic structure.**

Table 1.1 Outline of the cyclic skeletons of PGs.

1.4.1 Biosynthesis of Prostaglandins

A note must be made with regards to the biosynthesis of PGs, an outline of which is shown in Scheme 1.1. PGs are formed by most cells in the human body and in most cases are synthesized from the membrane-released prostanoid precursor AA (**10**).[53] This release of AA occurs when the surrounding cells have undergone trauma or other external stimuli. Once AA

is released by the cell membranes it undergoes oxygenation to intermediate prostaglandin endoperoxide H_2 (PGH_2) (**15**) by an enzyme called prostaglandin H synthase (PGHS or COX-1).[53] Following on from this, the unstable intermediate PGH_2 is then converted to various PGs inside the cell, such as PGD_2 (**16**), which is mainly found in Mast cells, $PGF_{2\alpha}$ (**14**), which is mainly found in the uterus and PGE_2 (**12**), which is found in most cells in the body, via specific synthases.[54] Once biosynthesized these PGs leave the cell membranes via a carrier mediated process, which in turn activates prostanoid receptors in other parts of the body.[54]



Scheme 1.1 Biosynthesis of PGD_2 (**16**), $PGF_{2\alpha}$ (**14**) and PGE_2 (**12**) via PGH_2 (**15**) from AA.

1.5 Marine Prostaglandins

PGs and their derivatives are present in earth's marine environment, especially in their fauna. Many biochemical studies have been focused on how pharmacologically active marine autocoids effect the organisms that produce them and subsequently their effect on the mammalian physiology. The first prostaglandin isolated from the marine environment, (15*R*)-

PGA_2 (**17**), was derived from a coral species *Plexaura homomalla* in 1969 and contains a non-mammalian (*R*)-configuration at C_{15}.[55] However, this compound possessed no bioactivity, whilst the mammalian (*S*)-configured diastereoisomer of **17**, PGA_2 (**18**), influences vasodilation.[55] Today, studies are still being carried out on **17** and investigations into comparable adducts of this compound, such as (15*R*)-PGE_2 (**19**), have shown *in vivo* anti-inflammatory effects as well as *in vitro* reduction of elastase in human polymorphonuclear cells (PMNs).[56] All three are shown in Figure 1.6 below.

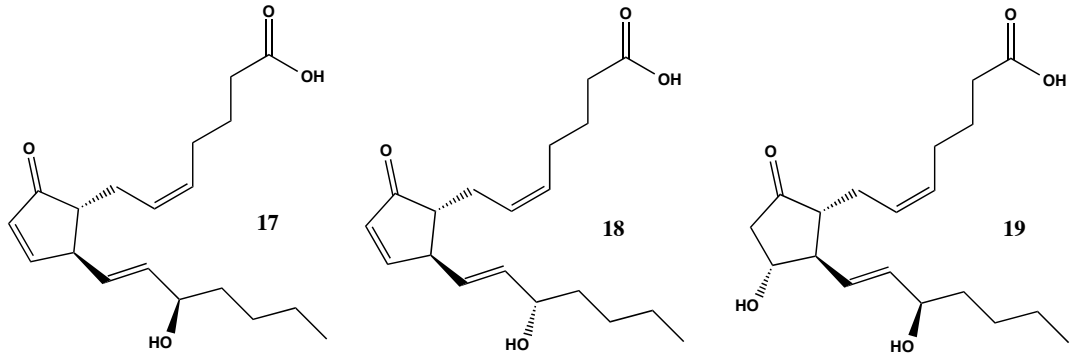


Figure 1.6 Structures of the PGs (15*R*)-PGA_2 (**17**), (15*S*)-PGA_2 (**18**) and (15*R*)-PGE_2 (**19**).

Additional bioactive marine metabolites have shown anti-inflammatory and anti-cancer pharmacological propensities. *Clavularia viridis*, a shore reef soft coral, produces a group of novel prostanoids called claviridic acids or clavulones.[57] These acids, including compounds **20** and **21** (Figure 1.7), were tested for their inhibitory effects on inflammation and cancer. Slight modifications were also undertaken on their structure with an additional alcohol/acetic moiety and stereochemical alteration on C_4 (**22**). These clavulones were especially effective against human gastric cancer cells and as an immunomodulator with very few *in vivo* side effects.[57]

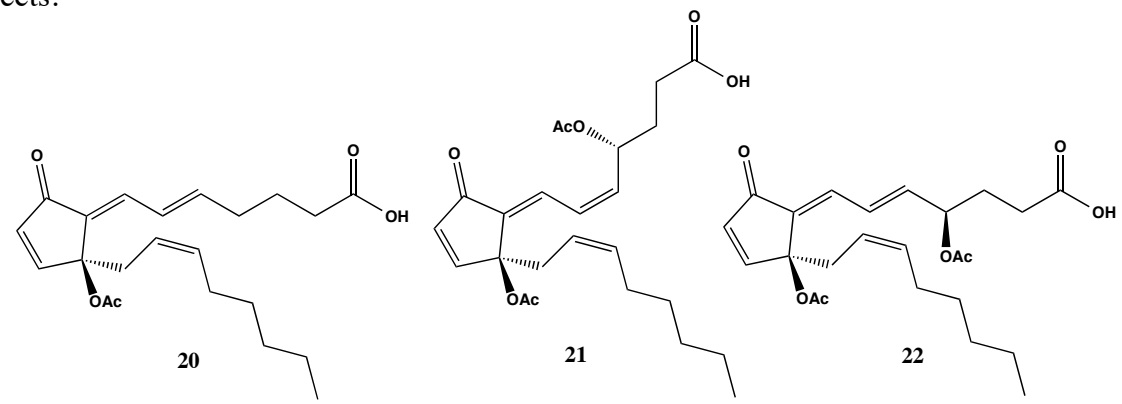


Figure 1.7 Bioactive marine prostanoids **20**, **21**, and **22** isolated from the coral *Clavularia viridis*.

1.6 Target Marine Prostanoid; (-)-mucosin[58]

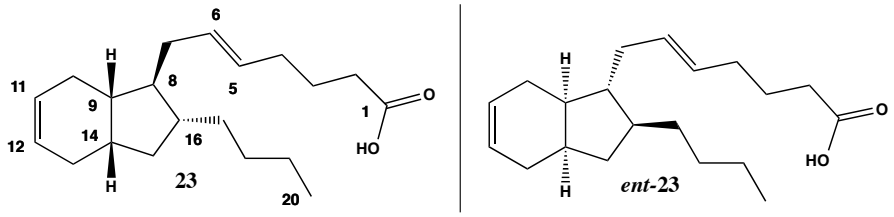


Figure 1.8 Target molecule (-)-mucosin (**23**) and synthesised enantiomer (+)-mucosin (***ent*-23**).

In 1995, a new eicosanoid metabolite was isolated by Casapullo *et al*.,[58] from the sponge *Reniera mucosa,* which is found in the Mediterranean Sea. After treatment with acetone and diazomethane the structure of the optically active methyl ester of (-)-mucosin (**23**) (Figure 1.8) was elucidated by ^{1}H and ^{13}C-NMR. A futher detailed analysis of the structure was carried out by a NOESY analysis so as to resolve the stereochemistry at the bridgeheads. However, the absolute configuration was not determined, which was possibly due to an overlap of the H-8 and H-9 environments on analysis by NOESY.

1.6.1 Elucidated Structure and Comparison with Other Bicyclic Natural Products

The metabolite **23** was elucidated to contain an unusual bicyclo[4.3.0]nonene skeleton including ring unsaturation ($\Delta^{11,12}$), *cis*-fused bridgeheads (C-9 and C-14), *trans*-related side-chains (C-8 and C-16) with respective lengths of C_7 and C_4 and an *E*-olefin ($\Delta^{5,6}$). The synthesis of the enantiomer of (-)-mucosin (**23**), (+)-mucosin (***ent*-23**) (Figure 1.8) has also been completed by Whitby *et al*.,[59] employing a zirconium induced co-cyclisation to yield the bicyclo[4.3.0]nonene core and install the correct stereochemistry at the four contiguous stereocenters. The structure reported for (+)-mucosin shows a direct comparison with that of (-)-mucosin from the natural product literature.[58] Hypothetically, (-)-mucosin (**23**) may be biosynthesized from AA through an intramolecular cycloaddition, or with Diels-Alder enzymes, involving C-8/C-9, C-14/C-15 and isomerization of the $\Delta^{5,6}$ olefin.[58]

The bicyclic carbon skeleton of (-)-mucosin is not unique by itself, as other natural products contain the bicyclo[4.3.0]nonene ring. Three bioactive sesquiterpenoids, shown in Figure 1.9, acutifolone A (**24**),[60] bisacutifolone C (**25**)[60] and pinguisenol (**26**)[61], all produced from liverworts, contain the *cis*-fused ring system.[62] The structures of these sequiterpenes have various anticancer and antimicrobial bioactivities.[62]

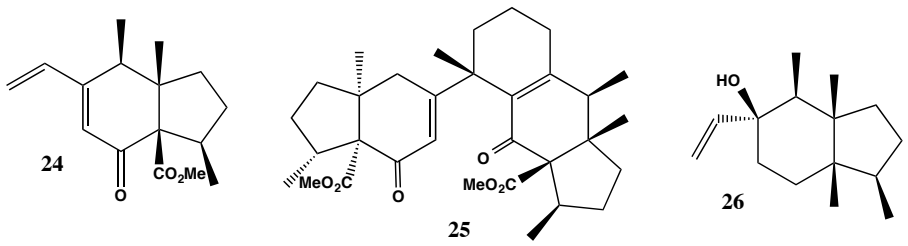


Figure 1.9 Structures of the *cis*-fused bioactive sesquiterpenoids acutifolone (**24**), bisacutifolone (**25**) and pinguisenol and (**26**).

With respect to marine metabolites such as (-)-mucosin (**23**), plakotenin (**27**), manzamenones A (**28**) and (+)-spiculoic acid A (**29**), all shown in Figure 1.10, are sequestered from the genera of *Plaktortis* sponges, consist of this bicyclo[4.3.0]nonene ring formation.[63,64] However, only the manzamenones A variant **28** contains the similar *cis*-fused hydrogens and none comprise of the prostanoidal side-chains.

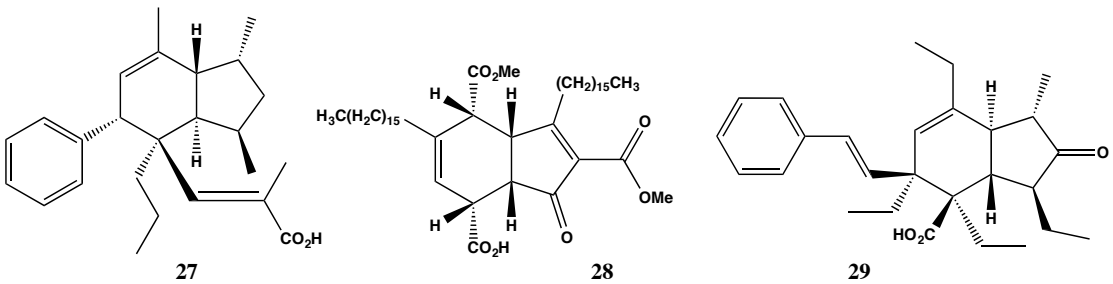


Figure 1.10 Structures of the bioactive marine metabolites plakotenin (**27**), manzamenones A (**28**) and (+)-spiculoic acid A (**29**).

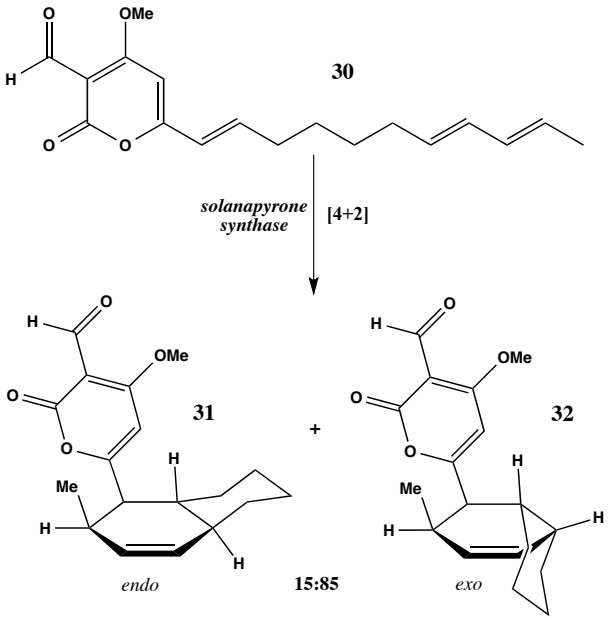
1.6.2 Postulated Bioactivity

(-)-Mucosins' bioactivity should be discussed with regard to the structural similarity between itself and PGs. No pharmacological testing has been carried out on **23** or its enantiomer (***ent*-23**). This might be possibly due to a deficiency in biomass of the required sponge (1.35 Kg of sponge gave only 8.5 mg of (-)-mucosin methyl ester), or because the isolation of the acid **23** has proved problematic in extraction, which is why the methyl ester was synthesised.[58] The arrangement of the *trans*-related side-chain bears many similarities with the aforementioned PG structures, as well as the cyclopentane moiety. The hope is that some of the bioactivity present in the PGs as well as metabolite **27**, which is anti-arthritic[65] metabolite **28**, which inhibits T-cell protein tyrosine phosphatases[66] and metabolite **29**, which is cytotoxic[64], as well as the *cis*-fused sesquiterpenoids in Figure 1.9, will also be present in **23**.

1.6.3 Postulated Biosynthesis

The possible biosynthesis of (-)-mucosin (**23**) raises some interesting questions regarding

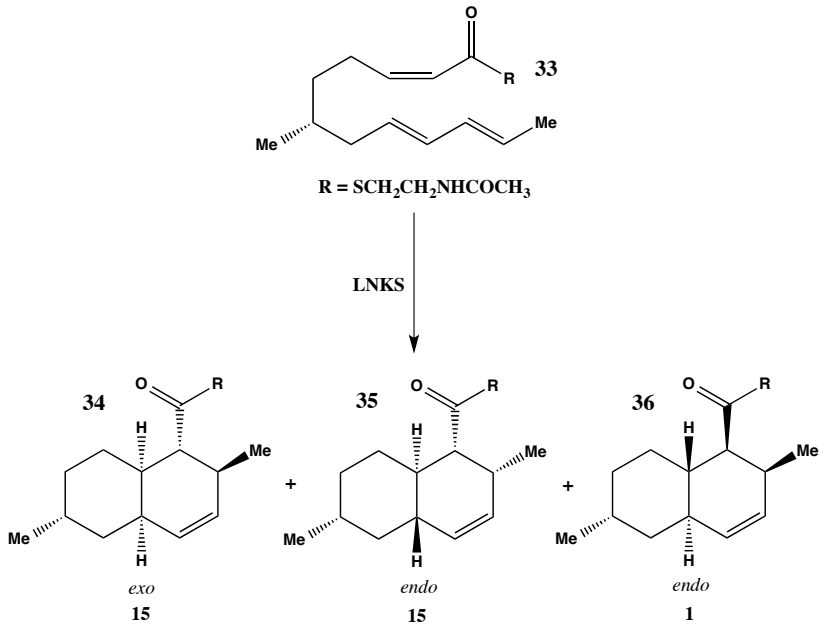
natures' use of enzymes in the formation of the cyclic structures. Since the late 1970s discoveries in the use of enzymes to catalyze the [4+2] cycloaddition reaction to create natural products have interested many organic chemists. This has given rise to the naming of these enzymes as Diels-Alderases.[67] One of the first identified naturally occurring Diels-Alderases was found by Ichihara and co-workers[67,68] and is shown in Scheme 1.2. Whilst performing feeding studies on the fungal pathogen *Alternaria solani* there was evidence of an oxidation, followed by a subsequent [4+2] cycloaddition on prosolanapyrone (**30**) to biosynthesize solanapyrones A (**31**) and B (**32**).[69] Ichihara managed to not only isolate the enzyme responsible (*solanapyrone synthase*), but also to monitor the enzymatic and non-enzymatic pathways by HPLC. The *exo* product is favored in a 85:15 ratio using the enzyme while employing the non-enzymatic pathway yields mainly the *endo* product in a 97:3 ratio.[68] Not only does the *exo/endo* selectivity change with respect to which pathway is used, but employing *solanapyrone synthase* increases the rate of reaction 4.1 times compared to the non-enzymatic pathway.[68]



Scheme 1.2 Biosynthesis of solanapyrone A (**31**) and B (**32**) via *solanapyrone synthase* from prosolanapyrone (**30**).

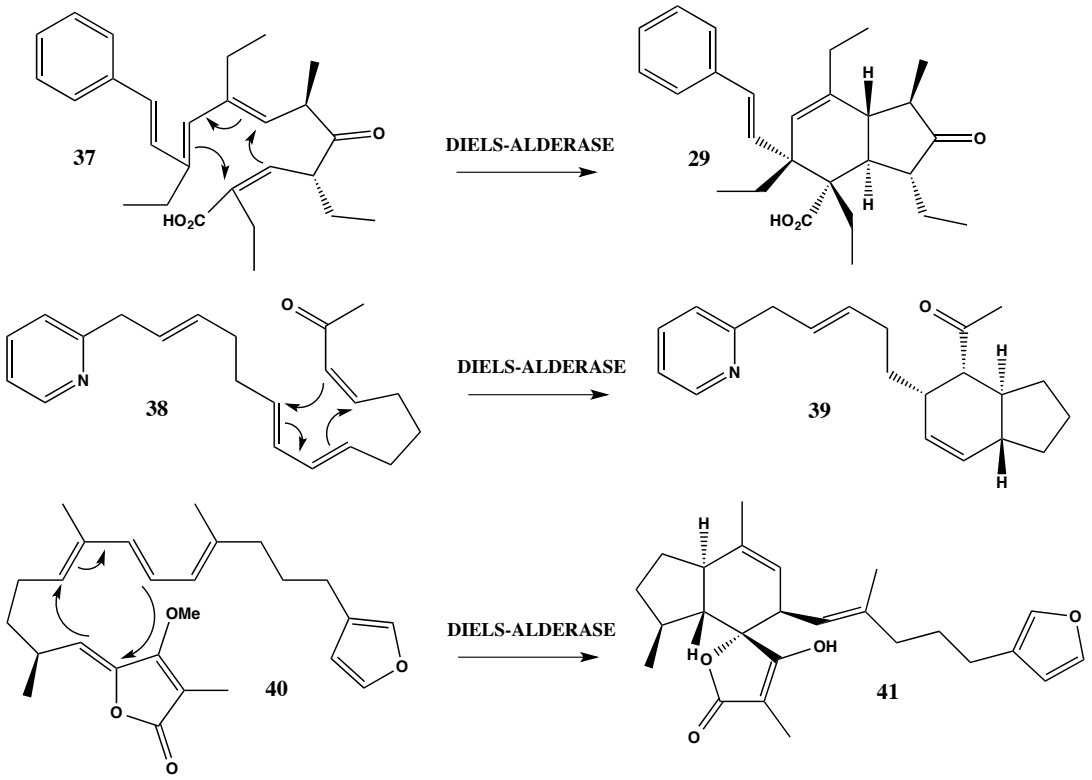
Another biosynthesis system that incorporates a Diels-Alderase is the formation of a decalin ring system that uses *lovastatin nonaketide synthase* (LNKS), as shown in Scheme 1.3. The decalin structure is similar to that of (-)-mucosin with the presence of a bicyclic system and a cyclohexene moiety. The LNKS catalyzes an internal [4+2] cycloaddition reaction to occur with a model thioester **33**. This leads to the formation of three different stereoisomers **34**:**35**:**36** in a 15:15:1 ratio,[70] as shown in Scheme 1.3. What is interesting to note is that one

of the main products is the *cis*-fused *exo* analogue (**34**), which is analogous to (-)-mucosin, with respect to the bridgehead carbons.



Scheme 1.3 Biosynthesis of a *cis*-fused decalin ring system (**34**) by LNKS on a model thioester (**33**).

With respect to marine metabolites, no formal Diels-Alderase enzymes have been isolated from the marine environment, which enzymatically catalyzes the formation of the bicyclic skeleton, present in many marine natural products, by a [4+2] cycloaddition reaction. However, many compounds found from ocean flora and fauna have been postulated to undergo one of these Diels-Alder type reactions facilitated by enzymes. The aforementioned (+)-spiculoic acid A (**29**)[71,72] is one such compound as is pulo'upone (**39**),[73,74] an antimicrobial marine metabolite produced from the mollusk *Philinopsis speciosa* and (-)-ircinianin (**41**),[74] a sesterterpene from the marine sponge *Ircinia wistarii.* Scheme 1.4 below details the presupposed enzymatic [4+2] cycloaddition reaction between a Diels-Alderase enzyme and hypothesized intermediates (**37**, **38** and **40**) of natural products **29**, **39** and **41**.



Scheme 1.4 Postulated synthesis of (+)-spiculoic acid (**29**), pulo'upone (**39**) and ircinianin (**41**) by Diels-Alderase enzymes.

1.7 Target Marine Prostanoid; (-)-dictyosphaerin[75]

In 1996 Rochfort and co-workers[75] isolated a unique bicyclic lipid, (-)-dictyosphaerin (**42**) (Figure 1.11), from a southern Australian marine green algae *Dictyosphaeria sericea*.[75]

1.7.1 Structural Elucidation and Comparison with (-)-mucosin

This novel fatty acid is analogous to (-)-mucosin due to its bicyclo[4.3.0]nonene skeleton, cyclohexene moiety ($\Delta^{13,14}$), and structurally comparable side-chains containing an *E*-olefin. Conversely, there are some notable differences. Firstly, (-)-dictyosphaerin (**42**) is a C_{22} compound with C_8 and C_5 side-chains with (-)-mucosin (**23**), being a C_{20} compound with C_7 and C_5 side-chains. Secondly, there is additional ring unsaturation ($\Delta^{9,17}$), contrasting chain unsaturation ($\Delta^{7,8}$) and three undistinguished chiral centers, the alcohol moiety (C-6) and the bridgehead carbons (C-11 and C-16).

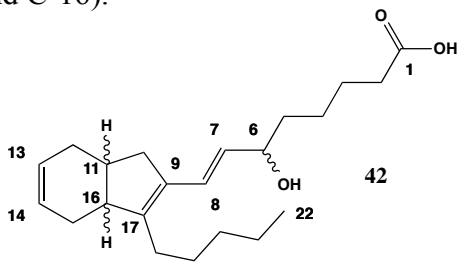


Figure 1.11 Target molecule (-)-dictyosphaerin (**42**).

1.7.2 Postulated Biosynthesis and Bioactivity

Biosynthetically, (-)-dictyosphaerin (**42**) will most probably relate to (-)-mucosin (**23**) with an intramolecular cycloaddition, possibly incorporating the Diels-Alderase reaction mentioned previously, but employing most likely adrenic acid (AdA) rather than AA, due to (-)-dictyosphaerin being a C_{22} acid. Interestingly, yet conversely, some similar pentacyclic eicosanoids with comparable side-chains, called isoprostanes (IsoPs) present in the myelin of human brains, are also generated from *in vitro* phospholipid bound DHA or AdA, by a free-radical-catalyzed mechanism.[76,77] These novel compounds are used as biomarkers in the detection of oxidative neurological stress by Alzheimer and Rett's disease in humans.[76,78] The most abundantly detected of these IsoPs are *ent*-7-*epi*-7-F_{2t}-dihomo-IsoP (**43**) and 5-F_{3t}-IsoP (**44**).[76] These IsoPs, as well as the bicyclic natural products in Figures 1.9 and 1.10, could determine the bioactivity with respect to (-)-dictyosphaerin, as no formal bioactivity studies have been started on this bicycle akin to (-)-mucosin.

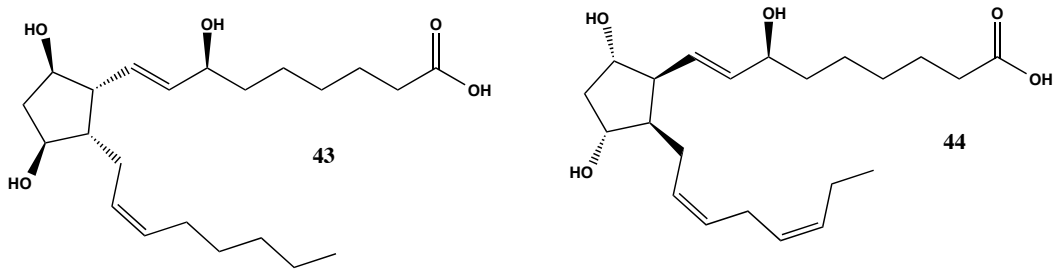


Figure 1.12 Structures of IsoP biomarkers *ent*-7-*epi*-7-F_{2t}-dihomo-IsoP (**43**) and 5-F_{3t}-IsoP (**44**).

1.8 Naturally Occurring Vicinal Amino Alcohols

Vicinal amino alcohols are a common unclassifiable component of bioactive natural products, which is in contrast to PGs.[79] This is mainly due to the spread of these particular structural components over a wide range of classifiable natural products. Some of the most basic structures of amino alcohols can be found in hydroxy amino acids like L-threonine and D-alanine. Adversely, they can also be quite complex, as in the structures of bestatin (**45**), an immunodilator, and the structurally unique AI-77-B (**46**), which possesses gastroprotective activity.[80-82] Cyclic amino alcohols also represent another important group containing the aforementioned moiety, but this time they are positioned on the ring, as shown with the antimalarial agent febrifugine (**47**) and antibiotic anisomycin (**48**).[83-86] Lipids and lipid-like molecules also exemplify the cross-class nature of these vicinal amino alcohols. Bioactive natural products sulfobacin B (**49**), an antithrombotic agent and myriocin (**50**), a strong

immunostimulatory agent, are potent examples of the utility of these lipid-like amino alcohols.[87-89] All these bioactive vicinal amino alcohols are shown below in Figure 1.13.

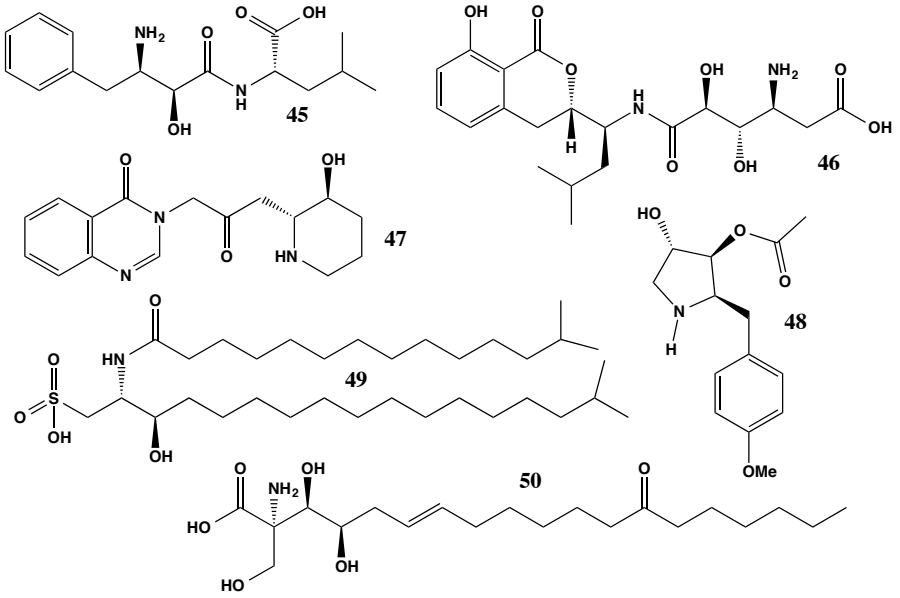


Figure 1.13 Structures of bioactive vicinal amino alcohols bestatin (**45**), AI-77-B (**46**), febrifugine (**47**), anisomycin (**48**), sulfobacin B (**49**) and myriocin (**50**).

1.9 Naturally Occurring Marine Vicinal Amino Alcohols

Vicinal amino alcohols are present in many marine species especially as lipids. These lipid compounds typically contain stereochemically defined amine and alcohol groups on neighboring carbons. They have a long alkyl chain with varying degrees of saturation and are usually highly pharmacologically active. In 1988, Gulavita and Scheuer[90] isolated the first marine amino alcohol 2(*S*)-aminotetradeca-5,7-dien-3(*R*)-ol from the sponge *Xestospongia sp*., found in the sea off Papua New Guinea. Over the past two decades the quantity of marine amino alcohols isolated, synthesised and bio-analysed has grown enormously. Three examples are shown in Figure 1.14. Bicycles amaminol A (**51**) and obscuraminol A (**52**), are cytotoxic against leukemia and lung cancer respectively, whilst pseudoaminol G (**53**) exhibits antimicrobial activity.[91-94] Both amaminol A[95] and obscuraminol A[96] have been synthesised.

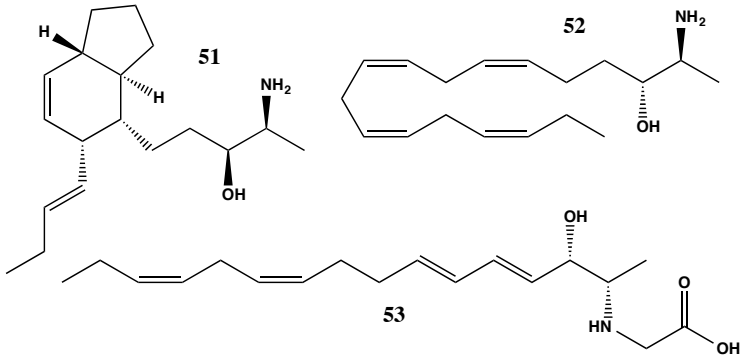


Figure 1.14 Structures of amaminol A (**51**), obscuraminol A (**52**) and pseudoaminol G (**53**).

Structures range from simple, long chain fatty acid type moieties, to polycyclic structures. There is no formal nomenclature system, as in PGs, but they are known under their IUPAC names and/or trivial names, the latter being derived from the tunicate they were isolated from, such as clavaminols (**54**),[97] cruciagasterins (**55**)[98] and obscuraminols (**56**).[92] An example of each is given in Figure 1.15. Biosynthetically, many of the amino alcohols are derived from either amino acids, like serine or alanine, and the appropriate fatty acyl-CoA, depending on their final structure.[99]

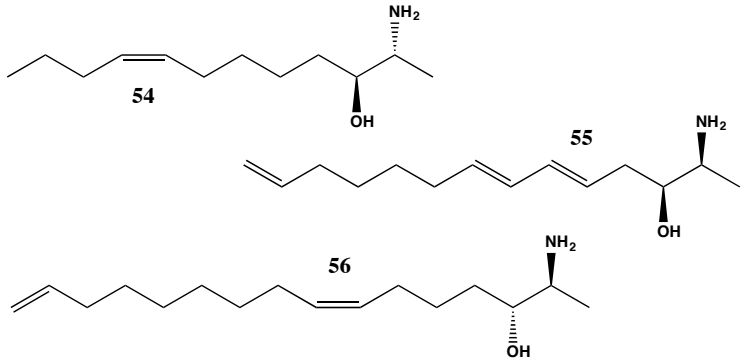


Figure 1.15 Examples of marine vicinal amino alcohols isolated from their tunicates.

1.10 Target Marine Vicinal Amino Alcohol; (+)-crucigasterin 277[100]

In 1993, Rinehart *et al*.,[100] isolated this novel vicinal amino alcohol (**57**), as shown in Figure 1.16, from a Mediterranean tunicate *Pseudodistoma crucigaster*. These alcoholic extracts of the tunicate exhibited cytotoxicity against L1210 murine leukaemia cells.[100]

1.10.1 Structural Elucidation

The number 277 refers to the molecular mass of the compound. The framework of this C_{18} compound is interesting, due to the three skipped *Z*-olefins ($\Delta^{9,10}$, $\Delta^{12,13}$, $\Delta^{15,16}$), the *E*-olefin ($\Delta^{5,6}$), the (*R*)-amino moiety (C-2) and (*S*)-alcohol moiety (C-3). Interestingly, with respect to the biosynthesis, the C-2(*R*) stereochemistry suggests that this compound is biosynthesized from D-alanine, unlike the usual plant and mammalian sphingosines, which are derived from L-serine.[100]

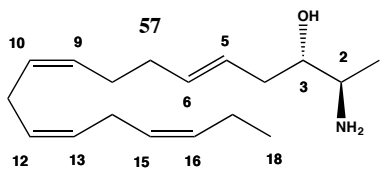


Figure 1.16 Target molecule (+)-crucigasterin 277 (**57**).

1.10.2 Postulated Bioactivity and Biosynthesis

A number of similar bioactive antimicrobial structures (**58**, **59**, **60**), known as 2-amino-3-tetradecanols, have been independently identified from the sponge *Xestospongia sp*.[90,101] They have many similar features to (+)-crucigasterin 277, such as chirality on the amino and alcohol substituents and chain unsaturation. However, none of the C_{14} amino alcohols (**58**, **59**, **60**) possesses the *cis*-skipped olefin bonds or isolated *trans*-olefin at $\Delta^{5,6}$ and the stereochemically defined (*R*)-amino group at C-2.

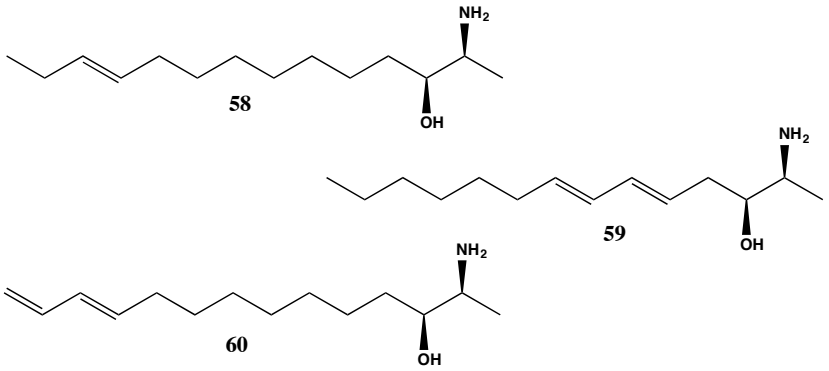
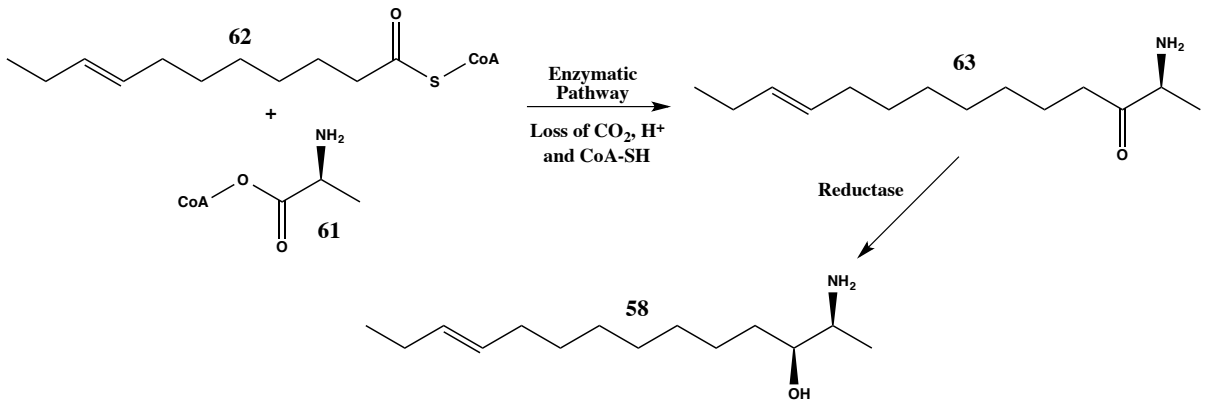


Figure 1.17 Structures of three bioactive 2-amino-3-tetradecanols (**58**,**59**,**60**) isolated from *Xestospongia sp.*

The biosynthesis of the C_{14} Pacific amino alcohols is also related to (+)-crucigasterin 277 but employs L-alanine (**61**), with loss of the carboxyl group, and a fatty acid CoA derivative **62**. An example of the biosynthesis, using compound **58**, is shown in Scheme 1.3 below.[90,101] An initial reaction between a specific enzyme, **62** and activated L-alanine **61** gives the intermediate keto-amine **63**, which is then selectively reduced at the ketone by a reductase enzyme to form the amino alcohol **58**.

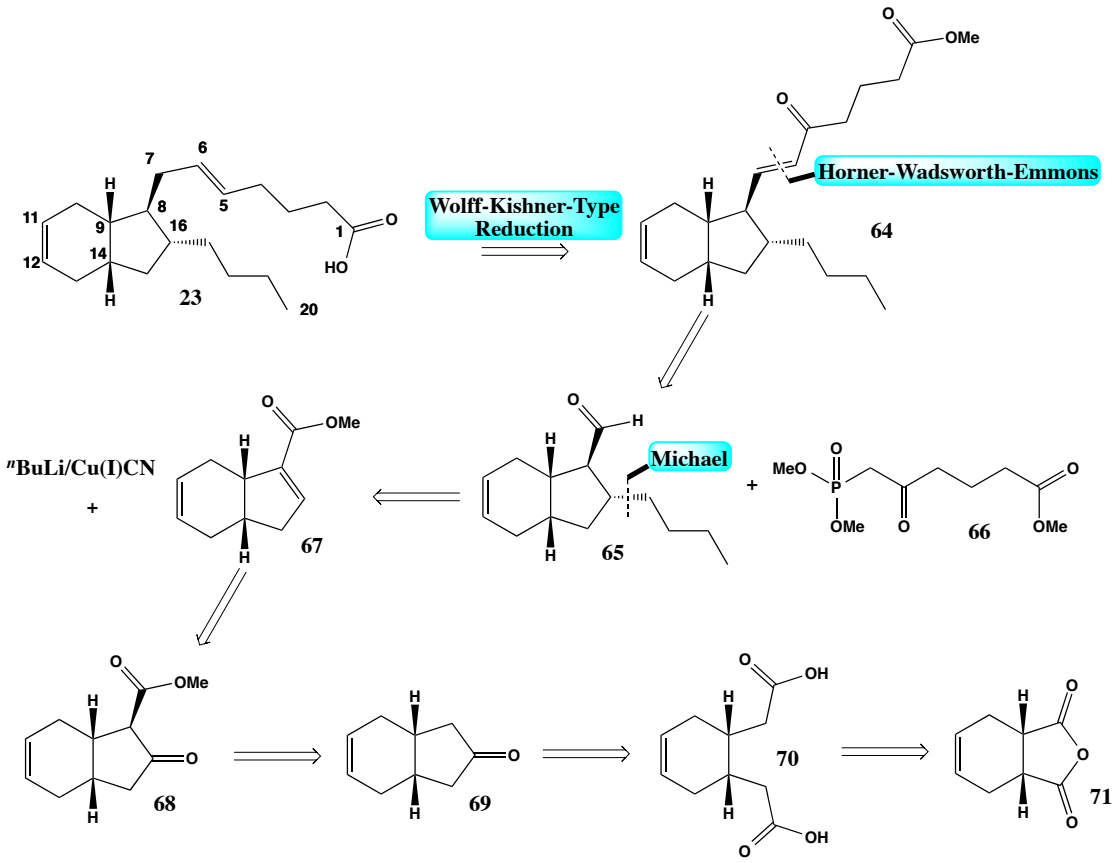


Scheme 1.3 Biosynthesis of compound **58** employing a fatty acid CoA starting material (**62**) with L-alanine (**61**) via an enzymatic pathway.

2. Retrosynthetic Analyses

2.1 Retrosynthetic Analysis of (-)-mucosin[58]

The target molecule (-)-mucosin (**23**), as shown in Scheme 2.1, a C_{20} natural product, reveals four stereogenic centres and two olefins. The two side-chains are attached to the bicyclo[4.3.0]nonene skeleton in *anti*-fashion at C-8 and C-16. The longest side-chain, bonded via an *S*-configuration (C-8), contains an *E*-olefin ($\Delta^{5,6}$) and a carboxylic acid terminus at C-1. The shorter side-chain, bonded via an *R*-configuration (C-16), is a butyl group with a methyl terminus at C-20. The bicyclo[4.3.0]nonene skeleton comprises of two *cis*-fused hydrogens on C-9 and C-14 and a cyclohexene moiety ($\Delta^{11,12}$). Proceeding from this analysis, building the correct stereochemistry at the side-chains and formation of the *E*-olefin moiety must be the main priority. Determination of the appropriate stereogenic reactions and reagents must be taken into careful consideration when formulating the retrosynthetic analysis. All this is attempted with the retrosynthetic analysis shown in Scheme 2.1.



Scheme 2.1 Retrosynthetic analysis of (-)-mucosin (**23**).

The most complex aspects to synthesize the correct structure of (-)-mucosin are an *E*-olefin at $\Delta^{5,6}$ and *anti*-configuration at C-8 and C-16. Formation of the *E*-olefin at $\Delta^{5,6}$ can be performed in many ways but one of the simplest would be to perform a Wolff-Kishner-type reduction[102,103] on an α,β-unsaturated hydrazone. This reduction, employing $Na(CN)BH_3$, would allow the olefin at $\Delta^{6,7}$ to undergo a hydride shift and move to $\Delta^{5,6}$ and solely furnish the *E*-olefin. The α,β-unsaturated hydrazone can be made directly from an α,β-unsaturated ketone **64**, which in turn can be synthesised via a Horner-Wadsworth-Emmons reaction,[104] with an optically active aldehyde **65** and a preformed C_6 phosphonate-ester **66**. This phosphonate-ester can easily be produced from a commercially available glutamic acid analogue.[104] These two main steps would allow for the necessary C_7 side-chain to be built.

The next stage would be to insert the butyl side-chain at C-16 with the correct stereochemistry. One method would be to undertake a stereoselective Michael addition reaction with an α,β-unsaturated ester **67**, ^{n}BuLi and a suitable cuprate additive, such as Cu(I)CN.[105] Due to the 3D-structure of the bicyclo[4.3.0]nonene skeleton the postulated Michael addition should form an *anti*-configuration at the bridgehead carbons. At this stage in the analysis it could be difficult to resolve in which direction (*syn* or *anti*) the butyl and ester side-chains could occupy. One possible method to help with this resolution of side-chain configuration would be the use of a chiral imine to replace the ester moiety before the Michael addition step.[106] This would help in giving some directional uniformity with respect to the butyl side-chain addition.

Following from this the α,β-unsaturated ester can be fashioned from the optically active β-keto-ester **68**. This optically active β-keto-ester can be produced in a number of ways in literature but these are quite lengthy.[107,108] One simpler method would be to employ the literature known *meso*-ketone[109] **69** and create an enolate using a chiral base[110], such as (*R,R*)-*bis*(phenylethyl)amine hydrochloride, and inserting the ester with use of a simple nucleophile, such as Mander's reagent (methyl cyanoformate).[110] The *meso*-ketone itself can easily be synthesised from the cheap and commercially available *cis*-1,2,3,6-tetrahydrophthalic anhydride **70** via the *bis*-acid intermediate **71** with a literature known five-step synthesis.[111]

2.2 Retrosynthetic Analysis of (-)-dictyosphaerin[75]

The second target molecule (-)-dictyosphaerin (**42**), in Figure 2.1, presents us with a C_{22} natural product containing a bicyclo[4.3.0]nonene skeleton with two side-chains that are C_8 and C_5 in length. At a glance, the structure of (-)-dictyosphaerin seems very similar to that of (-)-mucosin, especially as the identical cyclohexene moiety, here $\Delta^{13,14}$, and the bicyclo[4.3.0]nonene skeleton are present. Discernible similarities with the side-chains of (-)-dictyosphaerin and (-)-mucosin can also be seen as the longer side-chains, here C_8, in both natural products contain an *E*-olefin, here at $\Delta^{7,8}$, with an acid terminus, here C-1 and the shorter side-chains, here C-5 are simple alkyl moieties with a methyl terminus, here C-22.

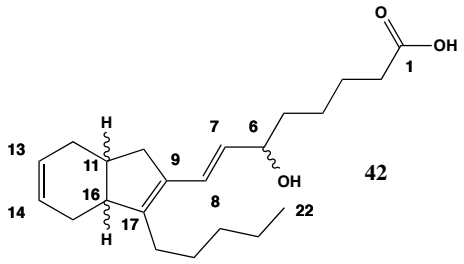
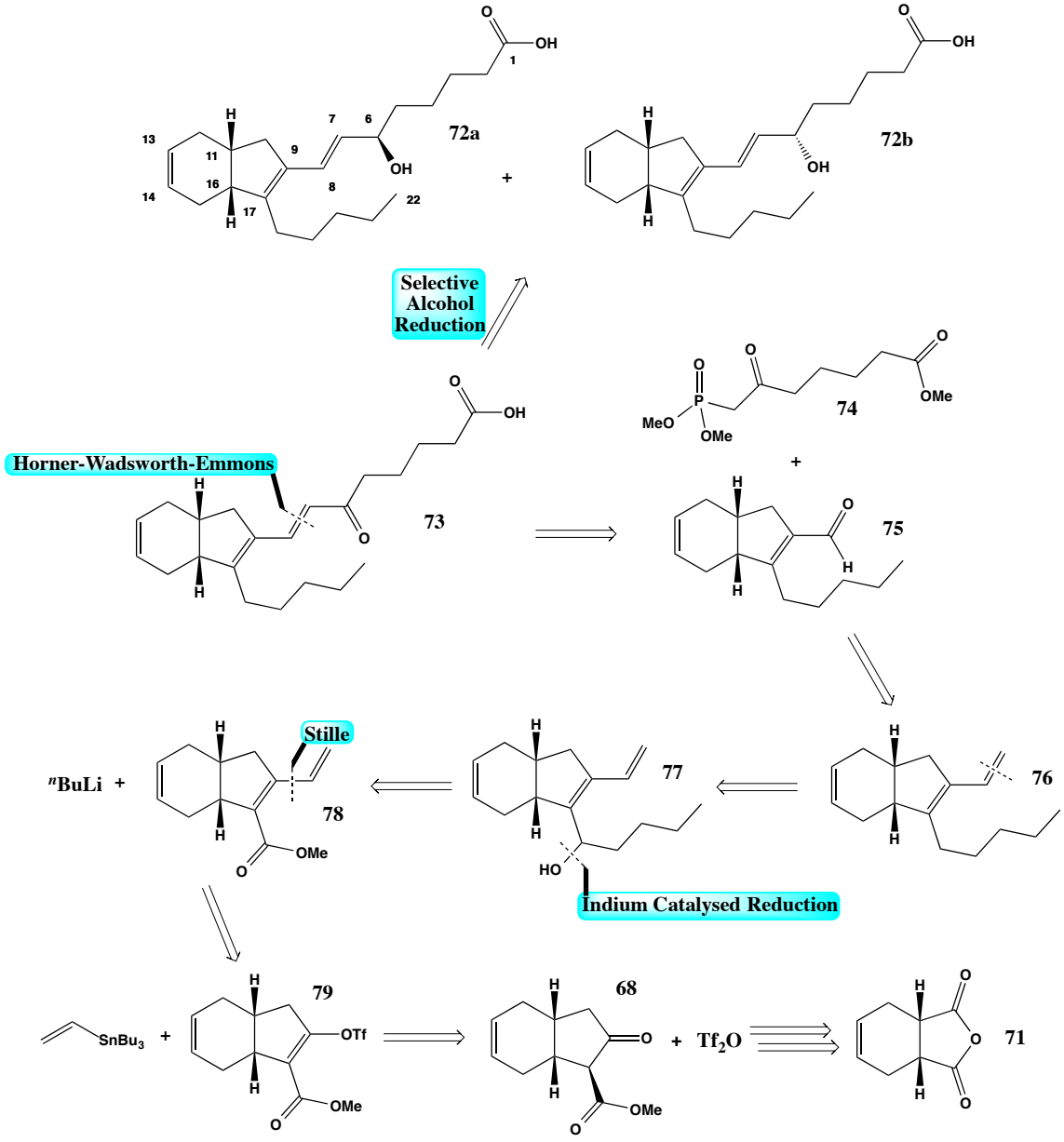


Figure 2.1 Target molecule (-)-dictyosphaerin (**42**).

However, on closer inspection of (-)-dictyosphaerin it has distinct structural differences to (-)-mucosin. (-)-dictyosphaerin is a C_{22} natural product as both its side-chains are one carbon longer in length (C_8 and C_5 respectively) when compared with (-)-mucosin, which is a C_{20} natural product. The bridgehead stereochemistry at C-11 and C-16 in (-)-dictyosphaerin has not been possible to characterize from the natural product analysis, even with NOESY experiments and Mosher's ester analysis.[75] This is not the case with (-)-mucosin, as the natural product analysis shows the suggested respective bridgehead stereochemistry being *cis*-fused.[58]

The bridgehead carbons for the side-chains at $\Delta^{9,17}$ have an olefin moiety unlike the (-)-mucosin bridgehead carbons, which are saturated, and *anti* in configuration. The C_8 side-chain in (-)-dictyosphaerin also contains an alcohol moiety at C-6, which also has an undetermined stereochemistry. This is not present in (-)-mucosin. Finally, the *E*-olefin in (-)-dictyosphaerin at $\Delta^{7,8}$ is directly bonded to the bridgehead at C-9. This is not the case with the *E*-olefin in (-)-mucosin, as the bridgehead and olefin moiety, are separated by a methylene group.

Synthesis of (-)-dictyosphaerin must take into careful consideration the choice of stereochemistry at C-11 and C-16, the ring unsaturation at $\Delta^{9,17}$, the *E*-olefin at $\Delta^{7,8}$ and the choice of stereochemistry for the alcohol moiety at C-6. Careful selection of stereogenic reactions and preformed reagents must be considered, especially when trying to form separate diastereoisomers for the alcohol at C-6, as this chiral center is undetermined. This will be the main focus of the retrosynthetic analysis.



Scheme 2.2 Retrosynthetic analysis of (-)-dictyosphaerin (**42**), incorporating the *cis*-fused bridgehead system **72a** and **72b**.

Due to the unascertained alcohol chiral center at C-6 in the structure of (-)-dictyosphaerin (**42**), it would be necessary to obtain both diastereoisomers **72a** and **72b** for spectral comparison with the natural product data. One of the easiest methods to do this would be a

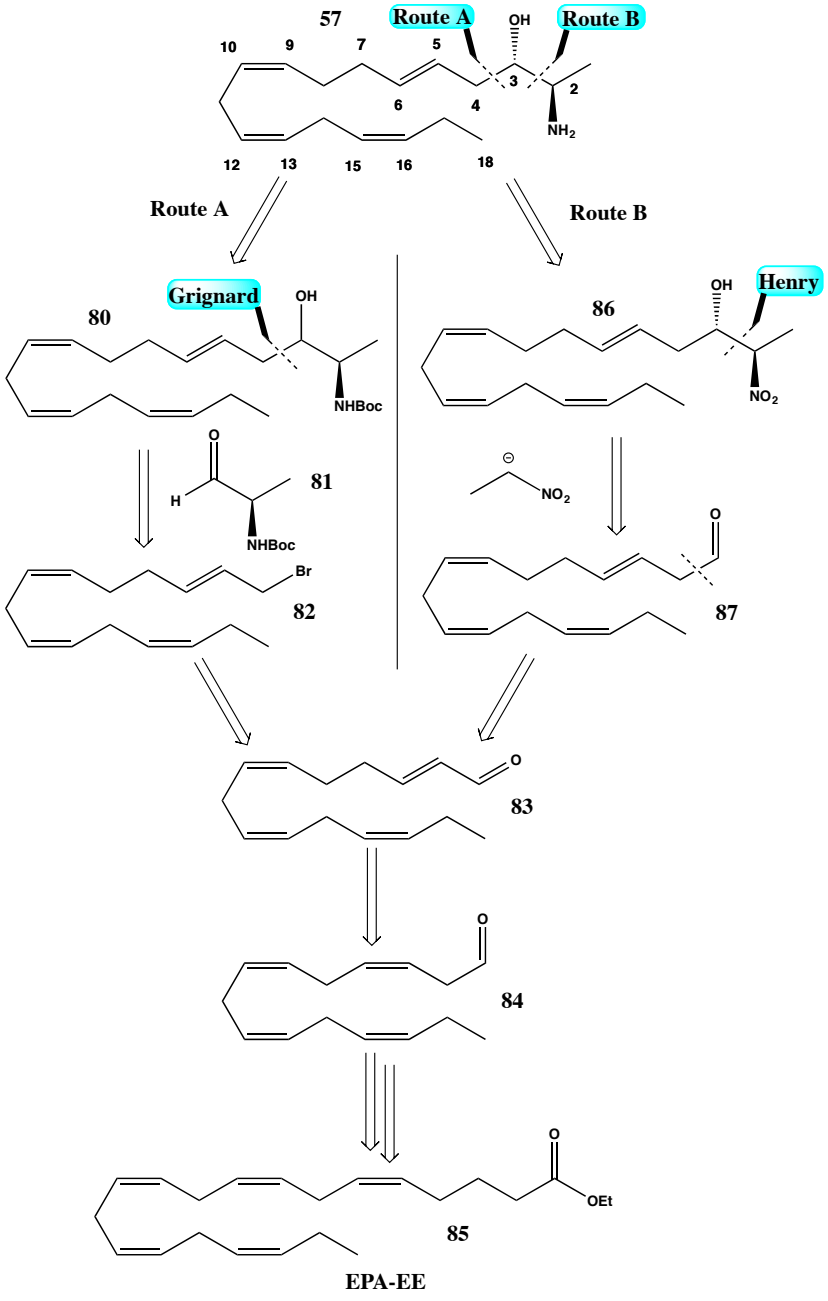
selective reduction of a ketone moiety at C-6 on compound **73** with a chiral hydride reagent[112] or chiral catalyst[113] to form a diastereospecific alcohol. The epimer of which, can then be made via employment of the opposite chiral hydride or chiral catalyst adopting the starting ketone **73**. This ketone, which contains α,β-unsaturation at $\Delta^{7,8}$, can easily be synthesised analogously by the Horner-Wadsworth-Emmons method in the retrosynthesis of (-)-mucosin.[104] The main difference is the synthesis of a preformed C_7 phosphonate-ester **74**, rather than a C_6, from a suitable starting material.

This scission at $\Delta^{7,8}$ would then leave us with an α,β-unsaturated aldehyde **75**. One method of making this would be a cleavage of a terminal olefin with a Pd(0) catalyst.[114] This is necessary, as ozonolysis would also cleave the cyclohexene ring. This would then leave a molecule **76** containing an α,β-unsaturated olefin with a pentyl group attached at C-17. One method to insert the pentyl chain would be via a controlled nucleophilic attack,[115] with ^{n}BuLi on an ester moiety to form a ketone at C-18. This ketone could then be reduced with $NaBH_4$ to an alcohol **77**, which in turn can be reduced by a protocol,[116] employing chlorodiphenylsilane and a catalytic amount of indium trichloride to form the pentyl moiety. This alcohol reduction method was chosen over the Wolff-Kishner and Barton-McCombie protocols so as to prevent movement of the $\Delta^{9,17}$ olefin.

This would then leave us with an α,β-unsaturated ester **78**, containing the terminal olefin bonded to C-9. This α,β-unsaturated ester now looks remarkably similar to the enantiomeric keto-ester **68** in the retrosynthesis of (-)-mucosin. A simple Stille cross-coupling protocol[117] with an α,β-unsaturated triflic-ester **79** and vinyl tributylstannane will afford **78**. This α,β-unsaturated triflic ester can then easily be formed from the postulated β-keto-ester **68**, seen previously, using triflic anhydride (Tf_2O) .[118] The synthesis of this β-keto-ester would then be identical to the one discussed from the commercially available *cis*-1,2,3,6-tetrahydrophthalic anhydride (**71**) in the retrosynthesis of (-)-mucosin.[111] With respect to the choice of stereochemistry at C-11 and C-16, it was decided to obtain the identical *cis*-fused partnership that is present in (-)-mucosin, due to the structural similarities between the two compounds. Once synthesised, we can directly compare the natural product spectral data to the synthetic spectral data and elucidate the results accordingly.

2.3 Retrosynthetic Analysis of (+)-crucigasterin 277[100]

Assessing the structure of (+)-crucigasterin 277 (**57**) presents a C_{18} vicinal amino alcohol with two stereo-centers at C-2 and C-3 and four olefins, one *E*-olefin at $\Delta^{5,6}$ and three skipped *Z*-olefins at $\Delta^{9,10}$, $\Delta^{12,13}$ and $\Delta^{15,16}$. The amino group on C-2 has an *R*-configuration while the alcohol group on C-3 has an *S*-configuration. (+)-crucigasterin 277 also begins and terminates with methyl groups. The preliminary retrosynthesis must focus on generating the optically active vicinal amino alcohol substituents on C-2 and C-3 in their respective *R* and *S* formats. Attention must also be concentrated towards the formation of the *E*-olefin between $\Delta^{5,6}$ and the three skipped *Z*-olefins at $\Delta^{9,10}$, $\Delta^{12,13}$ and $\Delta^{15,16}$.



Scheme 2.3 Retrosynthetic analysis of (+)-crucigasterin 277 (**57**).

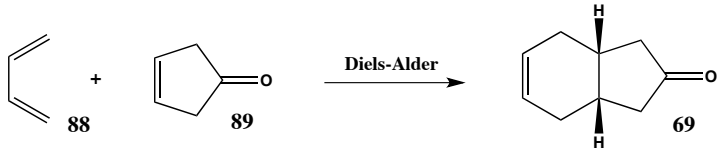
Route A: One way of inserting the optically active amino alcohol substituent onto the molecule would be scission of $\Delta^{3,4}$ to give a nucleophilic attack via Grignard reaction with a C_{15} allylic bromide **82** with the necessary *E*,*Z*,*Z*,*Z*-skeleton and *N*-Boc-D-alaninal (**81**).[119] This would produce a mixture of two diastereoisomers **80** at C-3, which could be separated by column chromatography and the preferred (*S*)-diastereoisomer at C-3 could be isolated and the Boc-protecting group can then be removed with use of TFA to form (+)-crucigasterin 277 **57**.[120] Another option to obtain the (*S*)-diastereoisomer at C-3 would be to oxidise the alcohol moieties of both diastereoisomers of **80** to a single ketone and selectively reduce it to the necessary (*S*)-diastereoisomer by a chiral hydride[112] or chiral base,[113] as mentioned with the (-)-dictyosphaerin retrosynthesis. Following on from this, the focus must be towards the formation of the C_{15} allylic bromide **82**. This can be easily synthesised by a reduction of a C_{15} allylic aldehyde **83** to an alcohol by $NaBH_4$ and a nucleophilic substitution to a bromide with Ph_3P and Br_2 under Appel conditions.[119] Formation of *N*-Boc-D-alaninal (**81**) can either be made by careful reduction by DIBAL-H[100] on *N*-Boc-D-alanine methyl ester or careful TEMPO or Parikh-Doering oxidation[121-124] on *N*-Boc-D-alaninol, both of which are commercially available. Creation of the C_{15} allylic aldehyde **83** precursor needs to be motivated towards the *E*-olefin ($\Delta^{5,6}$), which will be needed in the final structure. This *E*-olefin can be formed from a *Z*-olefin at $\Delta^{6,7}$ of a C_{15} aldehyde **84** via a reaction with DBU.[125] Production of this aldehyde can be undertaken from the commercially available EPA-EE **85** in a four-step literature synthesis.[126] Conversion of EPA-EE to EPA and then to an iodolactone, subsequent reduction with $LiOH{\cdot}H_2O$ to form a dihydroxyacid and treatment with $NaIO_4$ should lead to the desired aldehyde **84** in good yields.

Route B: Another method of inserting the optically active amino alcohol substituent onto the molecule would be scission of $\Delta^{2,3}$. This would give the possibility of an *anti*-selective Henry reaction between an aldehyde **87** and nitroethane with employment of a heterobimetallic Cu/Sm/aminophenol sulfonamide complex.[127] Of note, our group has used an asymmetric Henry-reaction in the synthesis of obscuraminol A.[96] Once this had been completed to form nitro alcohol **86**, reduction of the nitro moiety could be carried out with use of SmI_2 to form (+)-crucigasterin 277.[128] Synthesis of the aldehyde precursor **87** for the Henry reaction could be carried out from the common aldehyde intermediate **83** with a simple, literature known, homologation via a mesylation, cyanation and reduction.[129] The synthesis of **83** has already been discussed.

3. Results & Discussion of the Synthesis of (-)-mucosin

3.1 Synthesis Towards the *cis*-Fused Bicyclo[4.3.0]nonene Skeleton

The first key component of the synthesis of (-)-mucosin is the *cis*-fused bicyclo[4.3.0]nonene skeleton. Based on the retrosynthetic strategy in Scheme 2.1, an attempted synthesis of the *meso*-ketone **69** should be tackled first. The formation of this structure can be performed in a number of methods. On paper, the simplest method would be a Diels-Alder reaction via commercially available 1,3-butadiene (**88**) and 3-cyclopenten-1-one (**89**) to form a *cis*-fused *meso*-ketone **69**. This bicyclic *meso*-ketone, although optically inactive, is open to many facets of chemical modification.



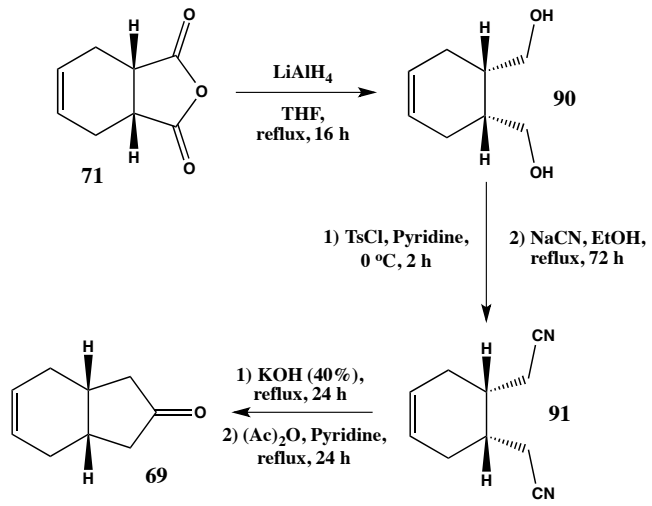
88 89 69

Scheme 3.1 Postulated Diels-Alder methodology to form *meso*-ketone (**69**).

Even though the cost of purchasing 1,3-butadiene is quite affordable there are several drawbacks with this strategy. Firstly, although the electron density on the diene is correct the electron density of the dienophile is reversed. The diene is electron-rich while the dienophile should be electron poor and this simply is not the case with this starting material and so leads to a mismatched pairing for a Diels-Alder reaction. This, ultimately, will give large amounts of 4-vinylcyclohexene in addition to the desired bicyclo[4.3.0]nonene compound. Secondly, commercially available, 3-cyclopenten-1-one is very expensive and for these reasons alone, a more reliable and economical method is required.

3.1.1 Literature Synthesis of *meso*-Ketone 69

Mundy and Theodore[111] reported one method to form this *cis*-fused *meso*-ketone **69** using a commercially available acetic anhydride precursor *cis*-1,2,3,6-tetrahydrophthalic anhydride (**71**) in a five-step synthesis. Initial reduction of the anhydride with $LiAlH_4$, under reflux conditions, generates the diol **90**. This diol undergoes tosylation at 0 °C to give a *bis*-tosylate, which is then substituted to the *bis*-nitrile **91** under reflux. Subsequent hydrolysis of **91** under reflux forms a *bis*-acid, which can then undergo a Ruzicka-type cyclisation with basic reflux to give the *meso*-ketone **69** in around 14% total yield over five-steps.

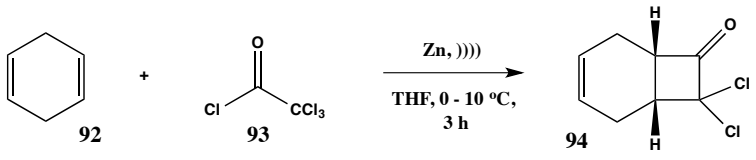


Scheme 3.2 Mundy and Theodore protocol to synthesize *meso*-ketone **69**.

This method, although lengthy, succeeds in retaining the necessary *cis*-fused hydrogens and provides the springboard to further manipulation towards (-)-mucosin. However, employing this method is laborious, contains multiple steps, involves high carbon expenditure and results in a poor total yield of 14% over five steps.

3.1.2 Advances Towards A More Effective Synthesis of *meso*-Ketone 69

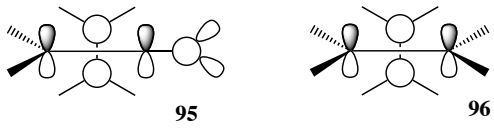
For the above-mentioned reasons we wanted a more simple and carbon economic procedure for the production of **69**. The known dichlorobicyclo[4.2.0]octene racemic adduct **94** can be easily made in a reasonable yield adding two equivalents of commercially available trichloroacetyl chloride (**93**) to one equivalent of 1,4-cyclohexadiene (**92**).[130] Sonication is highly effective at activating the zinc surface so it becomes more reactive to dehalogenation of trichloroacetyl chloride. The added benefit of this reaction is the establishment of the *cis*-fused hydrogens, which are necessary for the relative configuration of the bicyclo[4.3.0]nonene skeleton.



Scheme 3.3 Sonication reaction to form the racemic dichlorobicyclo[4.2.0]octene compound **94**.

The theory behind this stereospecific [2+2] cycloaddition reaction, although at times contradictory, is well documented. This [2+2] cycloaddition is arguably both a concerted and a charged process. Initially, the concerted process was the accepted theory due to the

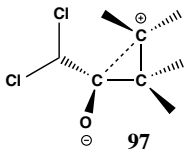
stereospecific addition to the olefins. The readiness of the ketene to react with an olefin is seen with the lack of steric hindrance from the planar ketene molecule **95** itself when compared with normal olefins **96** and the favorable bonding interactions between both the carbons of the olefin and the electron deficient *p*-orbitals of the carbonyl carbon.[131]



95 **96**

Figure 3.1 Orbital interactions between a planar ketene and olefin **95** and two olefins **96**.

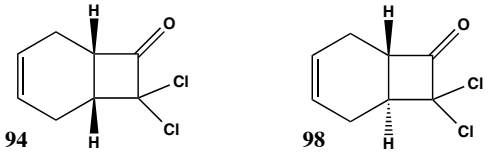
However, later mechanistic studies in the 1970s, by Woodward and Hoffmann, also indicated a stepwise reaction involving a dipolar intermediate.[132] The intermediate was proposed to be involved in an interaction between the cationic carbon and the enolate system of the dipolar intermediate **97**.



97

Figure 3.2 The dipolar intermediate **97** postulated to be involved in [2+2] cycloadditions.

Contemporary evidence supports both these hypotheses, as certain [2+2] cycloadditions are concerted processes while others seem to form dipolar intermediates.[131] The strongest evidence for a dipolar mechanism is the regioselectivity on non-symmetric olefins, which is possible due to the stability of carbocations. As regards the aforementioned cycloaddition reaction, in Scheme 3.3, to form **94** a concerted process seems more plausible. Actually, the reaction is completely stereoselective as seen by the absence of a *trans*-fused diastereoisomer **98** in the final product analysis. This implies that the reaction is carried out in a concerted process rather than a dipolar intermediate. If a dipolar intermediate were formed between the ketene and diene then both *cis* and *trans*-fused diastereoisomers would be detected. This is not the case as observed in the comparison of the NMR data for **94**.[130]

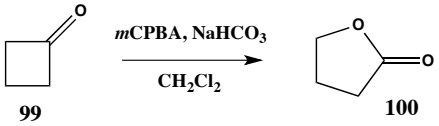


94 **98**

Figure 4.3 Structures of the *cis*-fused **94** and *trans*-fused **98** diastereoisomers of the dichlorobicyclo[4.2.0]octene adduct.

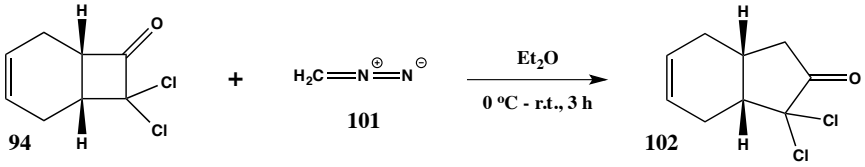
Regarding stereochemistry, the [2+2] cycloaddition reaction is stereospecific, depending on which type of olefin is applied. The *Z*-isomer of the substrate will lead only to *cis*-bicyclic compounds, as seen above in Scheme 3.3. The *E*-isomer of the same substrate will lead only to *trans*-bicyclic compounds with only a partial loss of stereochemistry.[133] Concerning the racemic bicyclo[4.2.0]octene compound, this sonicated [2+2] cycloaddition reaction is a very convenient method of forming a stereospecific *cis*-fused bicyclic ring in a 47% yield.

Subsequently, the second step considered a ring expansion of the cyclobutanone to a cyclopentanone via a carbon insertion reaction via diazomethane, which is analogous to the Bayer-Villiger reaction with ketone **99** to form lactone **100**, as shown below in Scheme 3.4.[134] This was needed to achieve the bicyclo[4.3.0]nonene skeleton present in (-)-mucosin.



Scheme 3.4 Reaction of a typical Bayer-Villiger reaction using *m*CPBA.

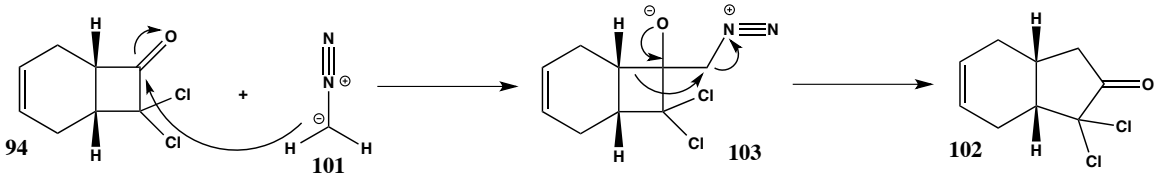
The carbon insertion reaction itself was initiated by utilizing diazomethane (**101**), formed from *N*-Nitroso-*N*-methylurea, dissolved in Et_2O and adding directly to **94** as shown in Scheme 3.5.[135-137] This reaction successfully formed the dichlorobicyclo[4.3.0]nonene compound **102** in 75% yield.



Scheme 3.5 Diazomethane insertion reaction with **94** to form dichlorobicyclo[4.3.0]nonene compound **102**.

The mechanism of this reaction is analogous to the Büchner–Curtius–Schlotterbeck reaction[138,139] between a ketone and an aliphatic diazoalkane, here diazomethane (**101**), as can be seen below in Scheme 3.6. The diazomethane molecule is highly polarized with both a partly positive and partly negative charge (as shown in Scheme 3.6) and this dielectric charge allows diazomethane to become very susceptible to nucleophilic additions. With the cyclobutanone precursor a nucleophilic attack occurs directly between the negatively charged diazomethane carbon and the carbonyl carbon. This produces a tetrahedral intermediate **103**, which immediately decomposes to a tertiary carbocation with evolution of nitrogen.[140,141] The

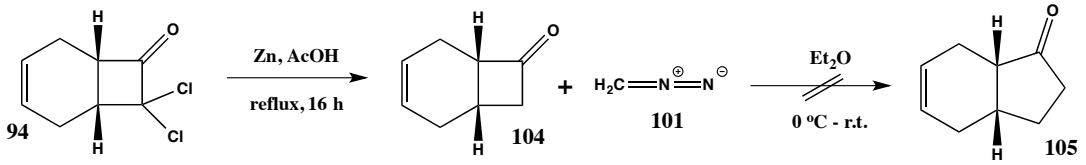
next stage involves the reformation of the carbonyl by a 1,2-alkyl migration. This occurs by migration of the bridgehead carbon containing the *cis*-fused hydrogen onto the newly bonded diazo-carbon to form the cyclopentanone ring and yield compound **102**.[140]



Scheme 3.6 Postulated mechanism of the Büchner–Curtius–Schlotterbeck reaction with **94**.

The alternative option for migration would occur from the dichlorinated carbon atom. Due to the electron-withdrawing propensity of the chlorine atoms with respect to the carbon, the low electron density formed on the dichlorinated carbon atom itself makes the migration seem unlikely. The first choice seems the more appropriate as the alkyl substituents present on the bridgehead carbon gives this atom more propensity towards this reaction. This gives this reaction a high degree of regioselectivity, usually being found to be regiospecific, which is also exhibited in analogous synthons attempted by Greene and Deprés.[137]

A note of interest is the implementation of the diazomethane insertion reaction using the dechlorinated bicyclo[4.2.0]cyclobutanone **104**, as shown in Scheme 3.7. This was attempted due to the suitability of the resulting ketone **105** in the synthesis of (-)-dictyosphaerin (**42**).

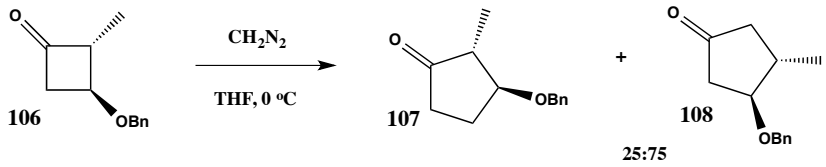


Scheme 3.7 Attempted diazomethane insertion reaction with dechlorinated ketone **104**.

Ketone **105** would appear to be susceptible towards esterification at the α-methylene position. This would allow elaboration towards (-)-dictyosphaerin (**42**) in a similar way to the proposed synthesis of (-)-mucosin, as given in the retrosynthesis in Scheme 2.1. The reaction with diazomethane from compound **105** was ineffective and so this line of thought was discontinued.

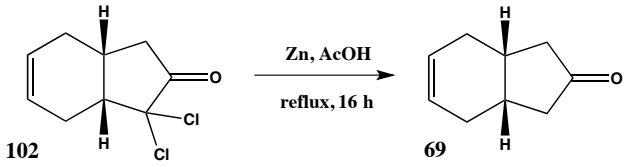
The retention of stereochemistry of the *cis*-fused bridgehead hydrogens during the rearrangement of the diazo intermediate **103** should also be remarked upon. This could be

converted to a mixture of *cis* and *trans* diastereoisomers at the ring-fusion. Analysis of the spectral data of the ensuing *meso*-ketone **69** reveals no trace of such a transformation.[109] This is also seen in similar cyclobutanone ring expansion reactions where there is retention of stereochemistry at the adjacent carbon atom to the inserted methylene carbon atom as shown in Scheme 3.8.[142] Another interesting observation seen in Scheme 3.8 is that the major isomer formed from racemic ketone **106** is the one where migration occurs on the more substituted α-carbon to form **107** and **108** in a 25:75 ratio. This observation in reduction of reactivity at the less substituted α-carbon would also tie in with the lack of reactivity of the dechlorinated cyclobutanone **104** towards this insertion reaction.



Scheme 3.8 An example of the observed migratory preference for the diazomethane insertion reaction.

The final stage of this three-step *meso*-ketone synthesis involves the reduction of the dichlorobicyclo[4.3.0]nonene **102** via AcOH and Zn under reflux, as shown in Scheme 3.9, to form the *meso*-ketone **69**.



Scheme 3.9 Dechlorination of dichlorobicyclo[4.3.0]nonene **102** to form *meso*-ketone **69**.

This step is highly efficient and yields are around 72% after silica-gel column purification. The total yield for this three-step synthesis is 26%, which is a significant improvement to the aforementioned Mundy and Theodore synthesis.[111] The reduction in time, lower carbon expenditure and more economical starting materials also means this unique three-step synthesis is much more effective.

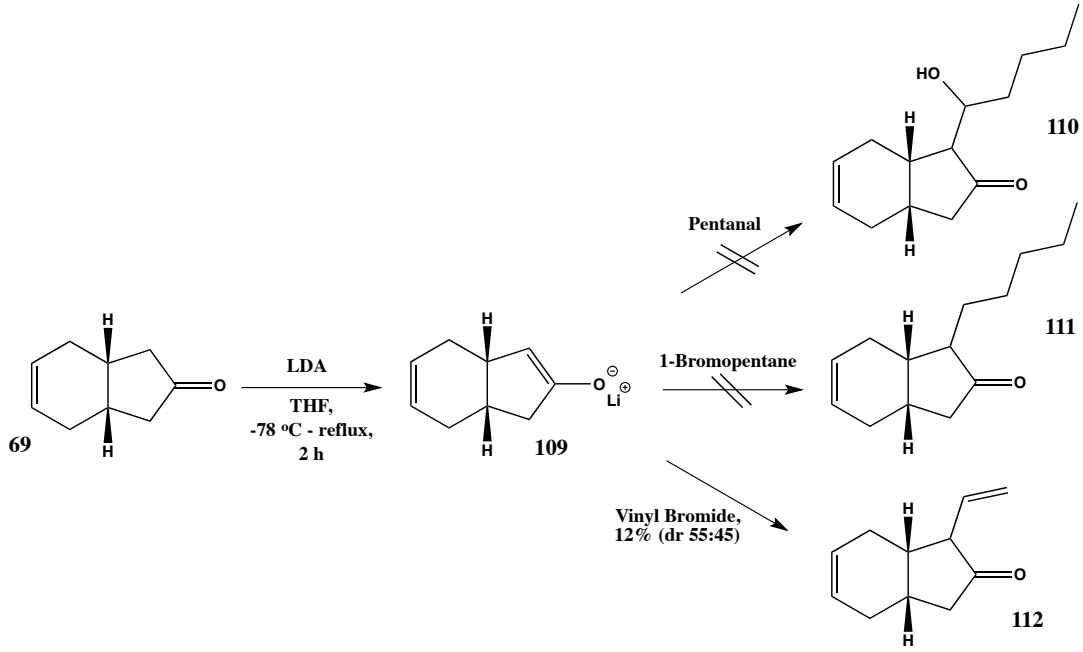
3.2 Methods Towards Enantiomeric Desymmetrisation of *meso*-Ketone 69

The next stage of the synthesis, towards (-)-mucosin, focused on enantiomeric desymmetrisation of the *meso*-ketone **69** by insertion of a substituent at the α-position to the

ketone via manipulation with a chiral reagent. This would allow the elaboration of the necessary *trans*-related C_7 and C_4 side-chains at a later stage.

3.2.1 Initial Attempts Towards Racemic Desymmetrisation Incorporating 69

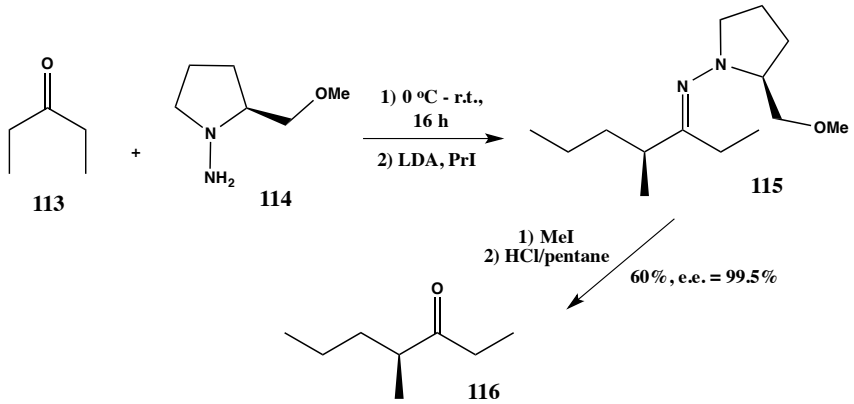
Many options were postulated, but initial attempts at desymmetrisation focused on racemic model reactions. One of the simplest was to form the lithium enolate **109** of *meso*-ketone **69**, via LDA, and react it with nucleophiles such as allyl bromide, pentanal and 1-bromopentane, as shown in Scheme 3.10. After numerous attempts with differing conditions, such as temperature and solvents, only a mixture of vinylic diastereoisomers **112** was formed in a poor yield (12%). This methodology was then abandoned and a new approach was chosen.



Scheme 3.10 Attempted racemic desymmetrisation reactions using **69**.

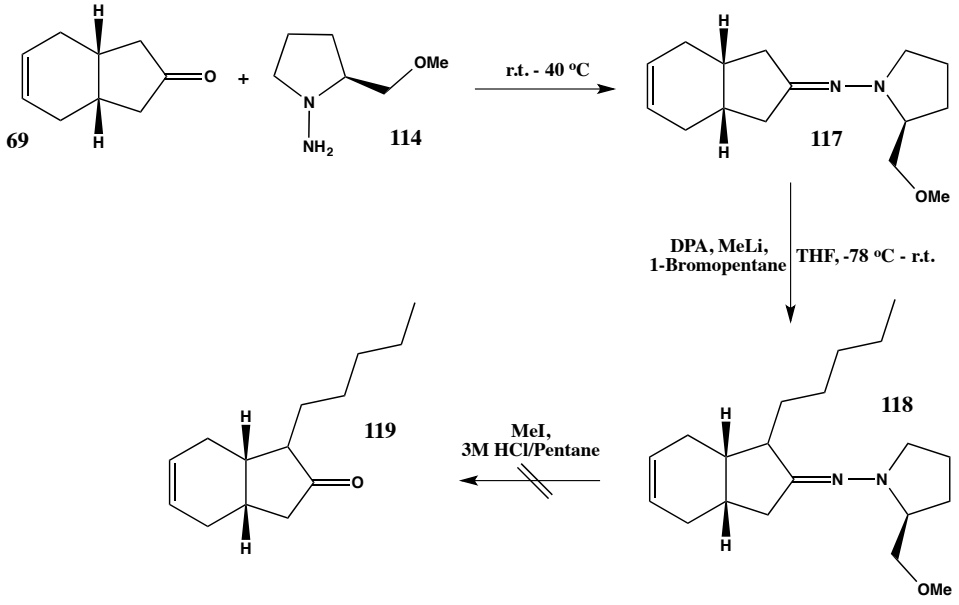
3.2.2 SAMP and RAMP Desymmetrisation Strategy Incorporating 69

An alternative desymmetrisation strategy was then attempted via employment of a hydrazine. This avenue was adopted due to the work carried out by Dieter Enders *et al.*,[143,144] who focused on the SAMP/RAMP hydrazone methodology, which can give enantio- and diastereoselectivity in alkylation asymmetry reactions, as shown by the example below in Scheme 3.11. Formation of the optically active ketone **116** from 3-pentanone (**113**) via the use of SAMP (**114**) and a subsequent hydrolysis of the hydrazine **115**, shows that the yield and enantiomeric excess can be sufficient enough to attempt this approach.[144]



Scheme 3.11 Enantioselective desymmetrisation reaction of **113** employing the SAMP methodology.

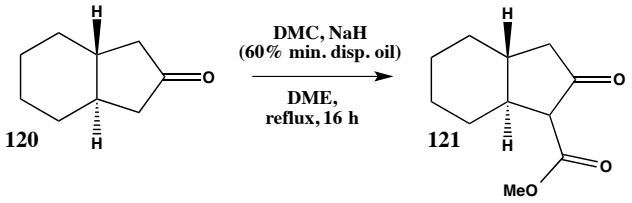
The SAMP auxiliary (**114**) is commercially available and formed the hydrazone **117** in good yield with **69**. This would allow the α-protons of the hydrazone **115** to become more acidic compared to the lithium enolate **109** and therefore asymmetric induction should occur more readily. As shown in Scheme 3.12, the formation of the hydrazone from the *meso*-ketone **69** was straightforward and in good yield (79%).[143] The next stage was to attempt asymmetric addition with a good nucleophile. Initially, 1-bromopentane was chosen, due to the ease of availability and to see the compatibility with (-)-dictyosphaerin. The reaction was carried out with MeLi and DPA with varying temperature from -78 °C to room temperature over 24 h.[143] The addition was successful and gave diastereomeric mixture **118** in a moderate yield of 45%. The final step was to hydrolyse the hydrazone back to the diastereomeric mixture of ketone **119** using MeI and a mixture of 3M HCl/pentane.[143] The desired oxidation did not occur and on purification only the crude *meso*-ketone **69** was isolated. This methodology was then discontinued.



Scheme 3.12 Attempted enantioselective desymmetrisation on *meso*-ketone **69** employing the SAMP methodology with 1-Bromopentane.

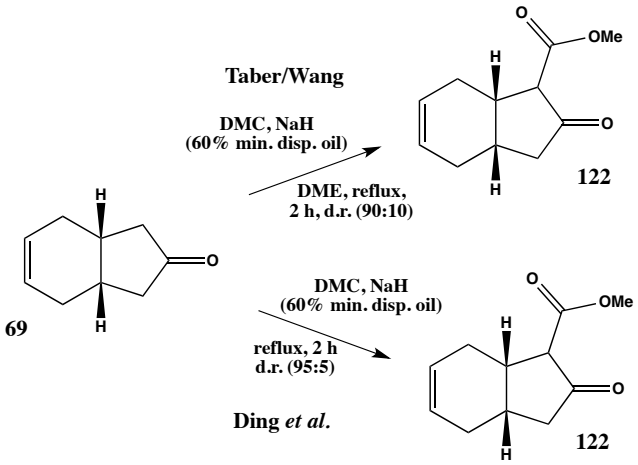
3.2.3 Attempts Towards Racemic Esterification Incorporating 69

Following on from this outcome, a strategy incorporating NaH (4.0 equiv.), dimethyl carbonate (DMC) (3.0 equiv.) and dimethoxyethane (DME) as a solvent was envisioned. The *meso*-ketone **69** was used in a model reaction to insert a methyl ester at the α-position to the ketone to form a racemic β-keto ester. The reaction itself was performed by Taber and Wang[145] and utilised a very similar *trans*-fused bicyclo[4.3.0]nonane ketone **120** to form a racemic diastereomeric mixture of β-keto ester **121** in a moderate yield of 55%, as shown in Scheme 3.13.



Scheme 3.13 Racemic esterification of ketone **120**, to form β-keto ester diastereomeric mixture **121**.

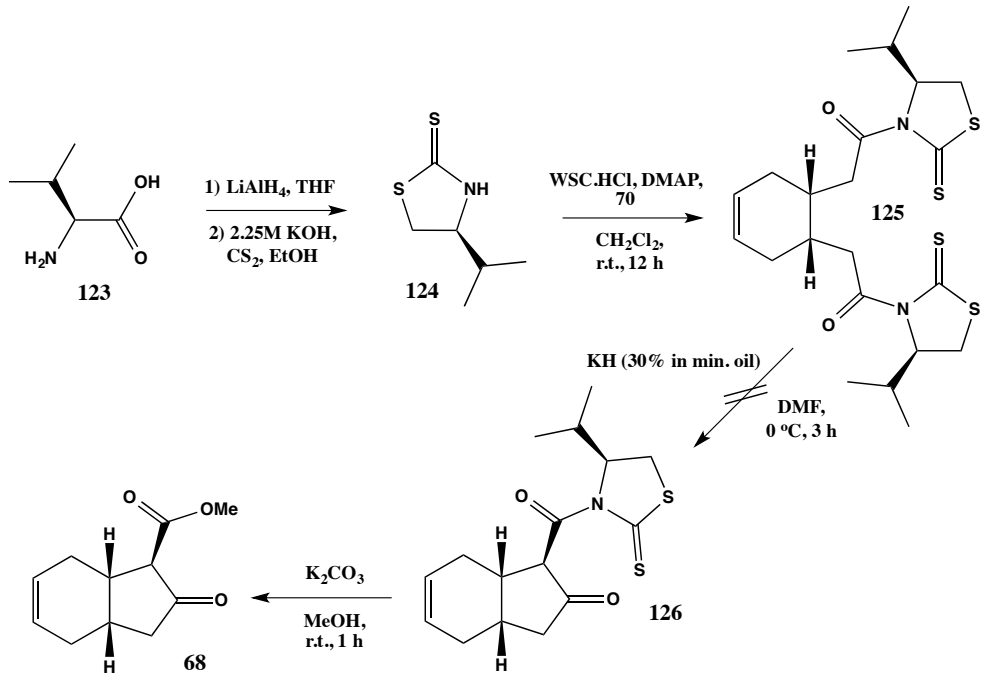
Focusing on this procedure, which would have been beneficial, as the ester could then be manipulated to the necessary C_7 side-chain, the *meso*-ketone **69** was subjected to this acetylation, as shown in Scheme 3.14. Initial attempts proved successful, with yields varying from 50-68% as a mixture of racemic diastereoisomers of the β-keto ester **122** (d.r. 90:10). An improved method of forming the β-keto ester with fewer diastereomeric impurities **122** was also exercised. This new method, by Ding *et al*.,[146] which does not utilize DME as a solvent, and also reduces the equivalents of NaH (1.1 equiv.) and DMC (0.2 M), led to a better d.r. (95:5) but no improvement in yield (45-55%).[146] Even with this amalgamation of esters it was decided that this could be an acceptable method for asymmetric induction of the *meso*-ketone for elaboration to (-)-mucosin. This was on the basis that a future synthon for the racemic β-keto ester **122** would be centered on an enantiomeric strategy to form the optically active β-keto ester **68**.



Scheme 3.14 Attempted racemic esterification on **69** employing the Taber/Wang and Ding *et al.*, protocols.

3.2.4 Attempted Literature Synthesis of the Optically Active β-Keto Ester 68

Subsequent reactions with the racemic diastereomeric β-keto ester mixture **122** led to promising results for the formation of (-)-mucosin. These will be discussed later in detail. The initial problem of forming the enantiomeric β-keto ester **68**, as discussed in the retrosynthesis, still remained. Investigation into the literature revealed that the desired chiral β-keto ester **68** had been synthesised previously by Nagao *et al*.[107,108] This procedure was a five-step synthesis starting from the aforementioned *bis*-acid **70**, made via the Mundy and Theodore procedure[111] and the amino acid L-valine (**123**) depicted in Scheme 3.15. The amino acid was converted into 4(*S*)-iso-propyl-1-3-thiazolidinone-2-thione (**124**), (4(*S*)-IPTT), in a two-step synthesis with high yields (83%) and identical optical rotation ($[\alpha]^{20}_{D}$ -36.81°).[147] A subsequent reaction between *bis*-acid **70**, 4(*S*)-IPTT (**124**) and *N*-Ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride, (WSC hydrochloride), led to the *bis*-amide **125**. The final two steps to form the optically active β-keto ester **68** from the *bis*-amide **125** involved the use of KH (30% disp. min. oil) and DMF to form a β-keto amide **126**, which should then be refluxed in K_2CO_3 and MeOH to form the enantiomeric β-keto ester **68**.[107]



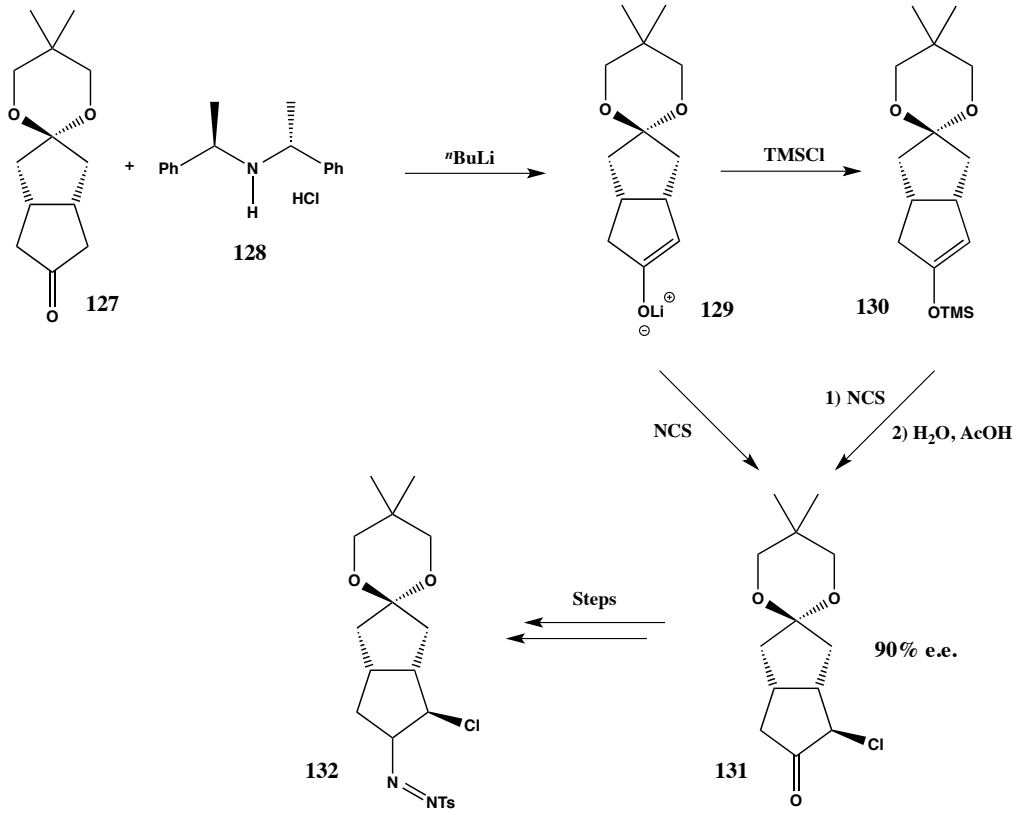
Scheme 3.15 Nagao *et al*. protocol for synthesizing the optically active β-keto ester **68**.

The *bis*-amide **125** was formed in acceptable yields and in high purity. The cyclisation step to form the β-keto amide **126** failed repeatedly with the use of KH at varying concentrations and temperatures. Other bases were tried with **125**, but all failed to form the desired mono amide.

The resulting compound was usually impure *bis*-acid **70**, so this methodology was abandoned.

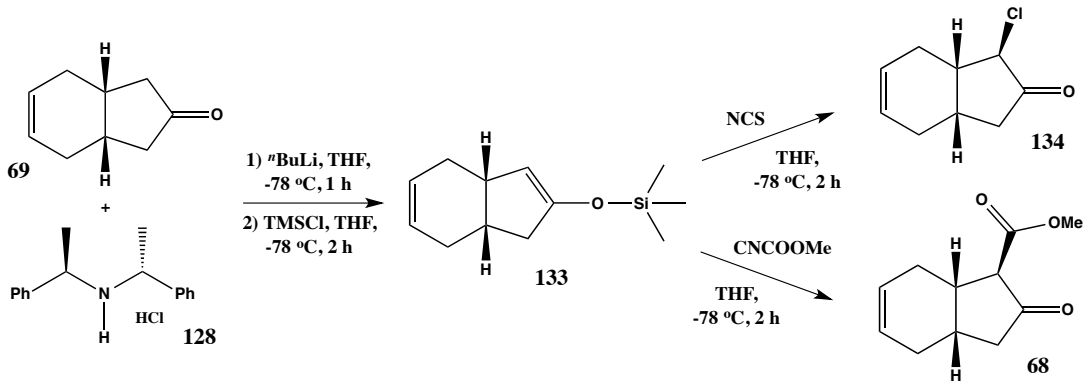
3.2.5 Attempted Synthesis of Optically Active 68 via Employment of a Chiral Base

Due to these results another program of reactions were then focused towards an enantiomeric formation of the racemic enolate **109** as shown above in Scheme 3.10. This time a chiral base would be employed to ensure correct enantioselective formation of the lithium enolate on the *exo*-face of the five-membered ring. Following on from this a nucleophile would then be inserted into the reaction to form a stable intermediate. This intermediate could then be converted into a more versatile structure to produce (-)-mucosin. Gais *et al.*,[110] successfully attempted an analogous reaction pathway previously forming a chiral polycyclic azoene **132** from ketone **127** as seen in Scheme 3.16. The use of the chiral base (+)-*bis*[(*R*)-1-phenylethyl]amine hydrochloride **128** and ^{n}BuLi to form a lithium amide allows for the enantioselective deprotonation of ketone **127** to form the enantiomeric enolate **129**. From here the addition of TMSCl gives the stable intermediate **130**, which can then be transformed into the enantiomeric chloride **131** via NCS with a 90% *e.e.*[110] Direct addition of NCS to the enantiomeric lithium enolate **129** can also be undertaken but the *e.e.* is dramatically reduced.



Scheme 3.16 Gais *et al.* protocol, employing a chiral base **128**, for an enantioselective formation of a lithium enolate **129** via ketone **127**.

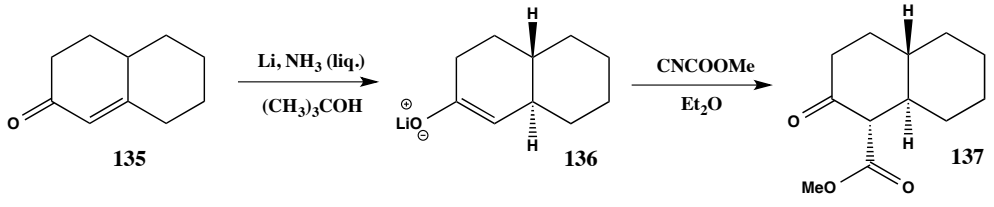
Elaboration to the analogous chloride **134** when employing the *meso*-ketone **69**, as a starting precursor with the Gais *et al*. protocol, would allow for a non-racemic synthesis of (-)-mucosin. This was attempted and the crude chloride **134** was formed in around 70% yield in three-steps via intermediate silyl enol ether **133**, as shown in Scheme 3.17. The decision was then taken to investigate the reaction of other nucleophiles with the enantioselective enolate. Our first choice was Mander's reagent, methyl cyanoformate, as mentioned in the retrosynthesis. The initial preference was to isolate the silyl enol ether **133** first and undertake the subsequent methyl ester addition reaction with MeLi as a base.[148] This was due to the poor reactivity and low yield of methyl cyanoformate in THF compared to Et_2O. However, the silyl enol ether **133** decomposes on silica column purification quite rapidly and removal of the chiral base **128** is necessary not only for recovery but also to reduce the unwanted side-reactions with MeLi in the next step. In spite of this, initial test reactions to form the β-keto ester **68** with a small amount of the recovered silyl enol ether **133** looked encouraging.



Scheme 3.17 Attempted synthesis of optically active β-keto ester **68** via the Gais *et al*. protocol.

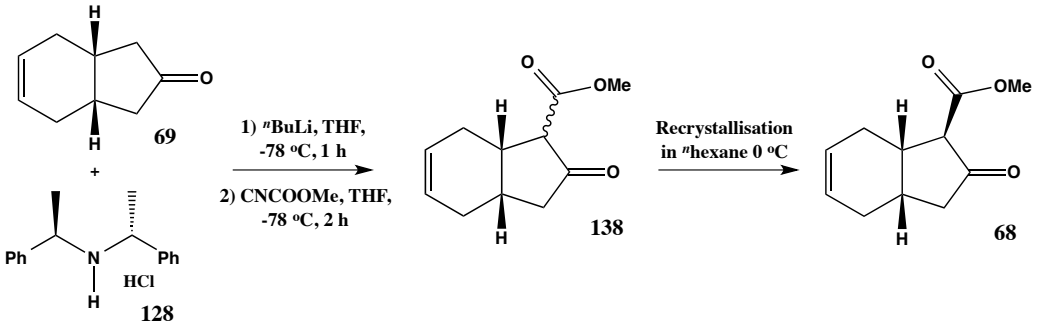
3.2.6 A More Effective Synthesis Towards the Optically Active β-Keto Ester 68

Further studying of the literature proved fruitful, as a model acetylation reaction employing ketone **135** performed by Mander *et al.*,[149] allows for direct stereoselective addition of a methyl ester onto a preformed lithium enolate **136**, as shown in Scheme 3.18, without the need for a stable intermediate such as the silyl enol ether **133**. This stereoselective acetylation gave a yield of 81-84% as pale yellow crystals of the pure β-keto ester **137**.



Scheme 3.18 Racemic synthesis of β-keto ester **136** via a lithium enolate and methyl cyanoformate.

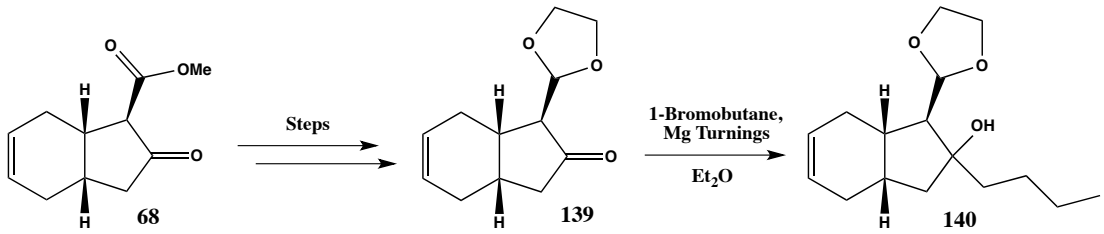
A test reaction was then carried out on the enantiomeric lithium enolate of **69**, formed by the chiral base **128** and ^{n}BuLi, and substituting TMSCl for methyl cyanoformate, as shown in Scheme 3.19. After purification, by silica column and NMR analysis, the optically active β-keto ester **68** was identified as a mixture of optically active diastereoisomers **138** and analysis by ^{1}H and ^{13}C NMR spectra proved that the crude mixture was a duplicate of the racemic diastereomeric mixtures of the racemic β-keto ester **122** formed in Scheme 3.14. Further purification by crystallization in ^{n}hexane afforded pure white crystals of the desired (*R*)-diastereoisomer **68** and analysis by optical rotation gave a value of $[\alpha]^{26}_{D}$ -161.2°, which is close to the literature value of -159.9° for the enantiomeric (*R*)-isomer.[107,108,150] The yield was also acceptable at 83%.



Scheme 3.19 Successful one-step synthesis of the optically active β-keto ester **68** from racemic **69.**

3.3 Efforts Towards the Elaboration of the *trans*-Related Side-Chains

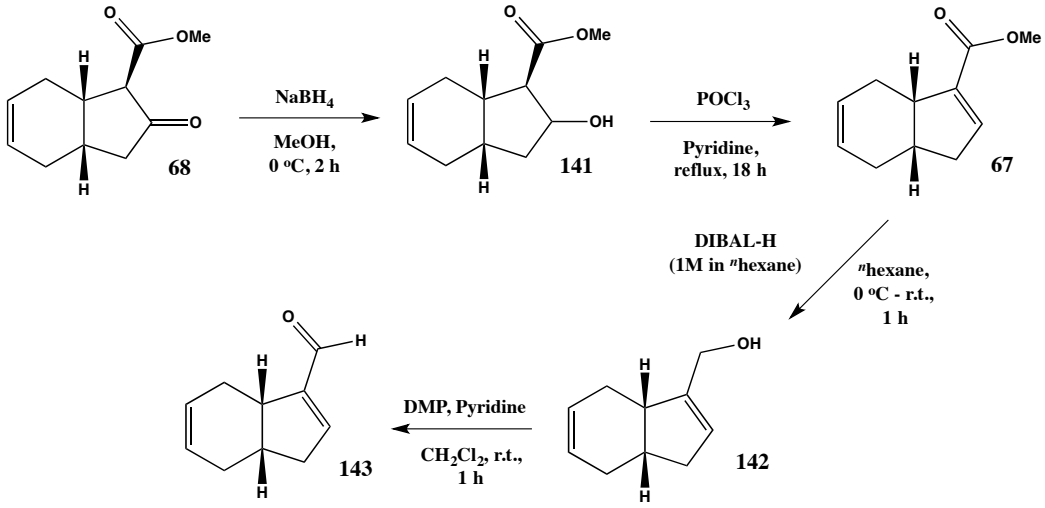
The next stage was to convert this enantiomeric β-keto ester **68** into an intermediate that could be stereoselectively augmented to form the correct chiral configuration at the two side-chains. One option would be to carry out a nucleophilic addition reaction with a butyl Grignard reagent with the ketone to insert the necessary butyl side-chain. However, this leads to problems with the ester group as this would mean the ester would have to be converted into an acetal/ortho-ester or other protecting group, as shown with compound **139**, to shield itself from the Grignard reagent. The other issue is that the Grignard reaction itself is non-stereospecific and would form a mixture of diastereoisomers as shown in Scheme 3.20. The tertiary alcohol in structure **140** would also have to be removed at some stage. Based on this, another methodology would have to be arranged.



Scheme 3.20 Postulated butyl chain insertion via a Grignard reaction from **68**.

3.3.1 Elaboration of the C_4 Side-Chain via a Stereoselective Michael Addition

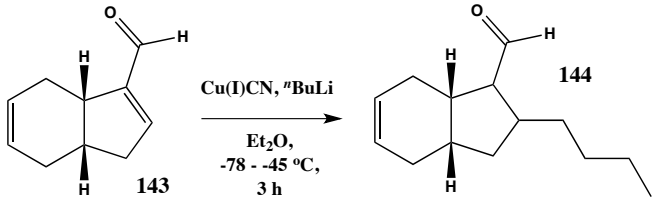
One methodology for a stereoselective nucleophilic addition is a Michael addition, as shown in the retrosynthesis. This uses a carbanion and an α,β-unsaturated carbonyl compound which also has the ability to be highly stereospecific if a chiral imine intermediate is employed.[106] To exploit this idea with the β-keto ester **68** the ketone would have to be reduced to an olefin and the ester converted to a chiral aldimine. Initial attempts of ketone reduction, on **68** via $NaBH_4$ to the diastereomeric alcohol **141** proved successful, as did the reduction to the α,β-unsaturated ester **67** with $POCl_3$ and pyridine.[151,152] The next phase was to convert the ester **67** to an aldehyde **143**, which could then be easily converted to a chiral aldimine. Initial attempts with DIBAL-H at low temperatures to form the aldehyde were successful but over-reduction to the alcohol **142** lowered the yield significantly.[153] It was then decided to reduce the ester **67** completely to the corresponding alcohol **142** and then oxidize **142** to the aldehyde **143** with DMP.[154] This proved a less problematic approach with regards to purification and there was also a significant improvement in the yield.



Scheme 3.21 Synthesis of the α,β-unsaturated aldehyde intermediate **143** for the postulated Michael addition reaction.

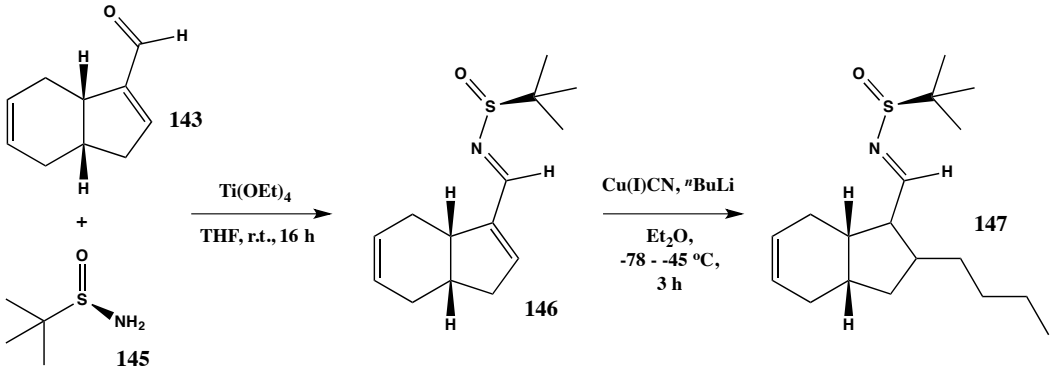
Initially, the Michael addition reaction was tested on the α,β-unsaturated ester **67** without any chiral modification, with Cu(I)CN and ^{n}BuLi, but no reaction occurred.[105] The next attempt

was on the α,β-unsaturated aldehyde **143** again without any chiral modification and this proceeded successfully, but as a mixture of four alkylated diastereoisomers **144** (d.r. = 15:15:35:25), as shown in Scheme 3.22.[105]



Scheme 3.22 Michael addition reaction utilizing **143** without chiral modification.

Subsequently, the α,β-unsaturated aldehyde **143** could then be altered into a chiral aldimine **146** by a simple condensation reaction with $Ti(OEt)_4$ and an optically active *tert*-butanesulfinamide **145** of our choosing.[106] A conjugate addition protocol by McMahon and Ellman,[106] employing the use of a conjugated chiral (*R*)-*tert*-butanesulfinyl aldimine with Cu(I)CN and ^{n}BuLi, as reagents showed the propensity for this reaction to give high diastereoselectivity (d.r. 85:15) once the conjugate addition has occurred. Using this as a template for a diastereoselective Michael addition the (*R*)-aldimine **146** was reacted with Cu(I)CN and ^{n}BuLi to form diastereomeric mixture **147**, as shown in below in Scheme 3.23.

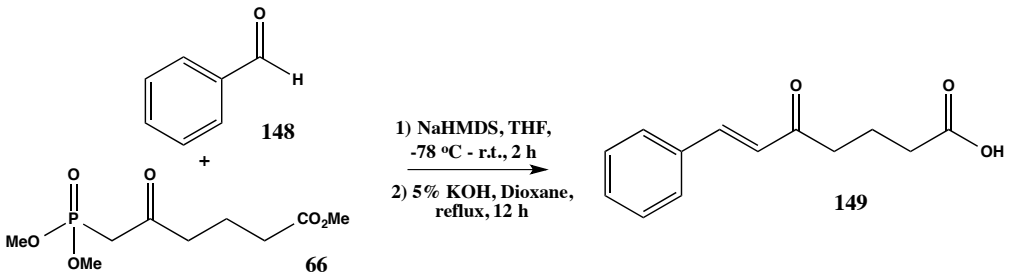


Scheme 3.23 Michael addition, utilizing **143** as a chiral aldimine **146**, to form **147**.

A preliminary result of the conjugate addition showed that mainly one diastereoisomer (d.r. 80:20), in mixture 147, was formed in satisfactory yields (≈60%). This was purified to the main diastereoisomer **147a** but stereochemical analysis by NMR proved difficult so no formal stereochemical assignment could be made with regards to the butyl and (*R*)-aldimine side-chains. The decision was then taken to continue with the extension of the aldimine **147a** to the C_7 side-chain and examine if the stereochemistry of the final compound equates with the literature of the target molecule (-)-mucosin.

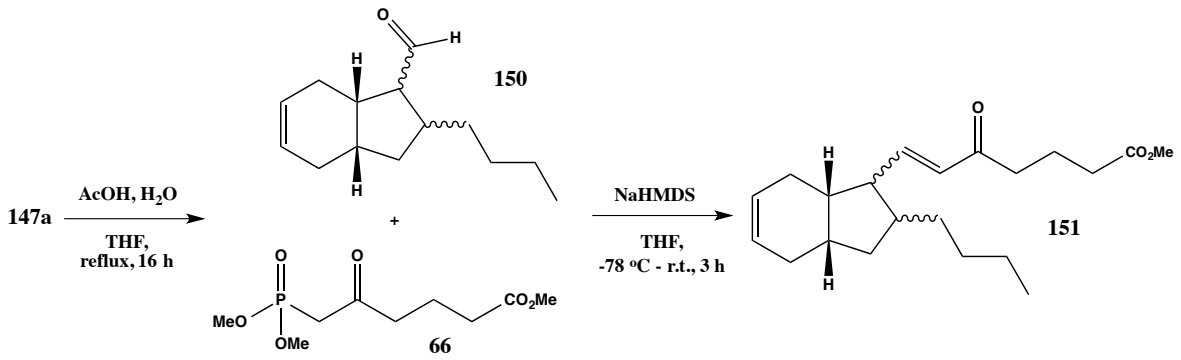
3.3.2 Elaboration of the C_7 Side-Chain via a Horner-Wadsworth-Emmons Reaction

Elongation of the (*R*)-aldimine side-chain should proceed, initially, by removal of the imine with $AcOH/H_2O$ to form an aldehyde.[106] This aldehyde should then have the flexibility to undergo C-C addition reactions such as the Horner-Wadsworth-Emmons, as shown in the retrosynthesis, to yield a C_7 side-chain containing the necessary *E*-olefin at $\Delta^{6,7}$ as shown on intermediate **64**. Precedence for this chain elongation, via the Horner-Wadsworth-Emmons protocol employing a C_6 phosphonate **66**, has been attempted on the synthesis of conformationally restricted analogues, similar in shape to Leukotrienes B_4 and B_3, by Gore *et al.*[104] One of these conformationally restricted analogues **149**, synthesised from the C_6 phosphonate **66** and benzaldehyde (**148**), is shown in Scheme 3.24.[104]



Scheme 3.24 Chain elongation protocol via a Horner-Wadsworth-Emmons reaction by Gore *et al.*

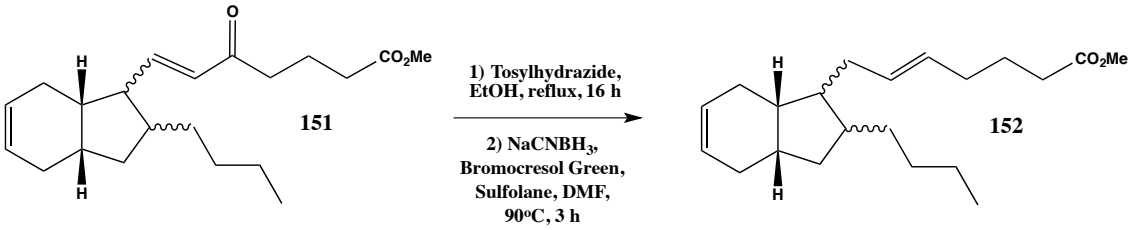
Removal of the imine substituent on **147a** progressed efficiently to an aldehyde in good yields of around 70%. However, an inseparable diastereomeric mixture of two aldehydes **150** (d.r. = 75:25) (two of which were identical to those found in diastereomeric mixture **144**) was seen by analysis with NMR, similar to the ratio found for **147** after the stereoselective Michael addition in Scheme 3.23. This was puzzling as only one diastereoisomer was expected, but due to the acidic nature of the α-proton adjacent to the aldimine moiety an equilibration must occur under the acidic conditions and produce two sets of diastereoisomers with respect to the subsequent aldehyde side-chain. After these initial results a preliminary reaction was carried out, as shown in Scheme 3.25, with the mixture of aldehydes **150** and the suitable phosphonate intermediate **66**. The resulting keto-ester **151** was made as an inseparable mixture of two diastereoisomers, corresponding to the diastereomeric aldehyde precursors **150**, both exclusively with an *E*-olefin in an acceptable yield of 62%. The phosphonate intermediate **66** was made in a three-step synthesis from commercially available methyl 5-chloro-5-oxovalerate in a 91% overall yield comparable to the literature yield of 94%.[104]



Scheme 3.25 Chain elongation via the Horner-Wadsworth-Emmons reaction to form **151**.

3.3.3 Attempted Synthesis of the *E*-Olefin via a Modified Wolff-Kishner Reaction.

The next stage was to perform a reduction protocol, as shown in the retrosynthesis, on the ketone of the C_7 side-chain on **151**. This would allow the *E*-olefin to be selectively transferred across to the ketone carbon ($\Delta^{5,6}$) via 1,3-sigmatropic rearrangement in a modified Wolff-Kishner reaction, as attempted by Hutchins and Majetich.[102,103] This would hopefully provide the correct geometric isomer for the C_7 side-chain of (-)-mucosin. An initial reaction was attempted on the mixture of diastereoisomers **151**, formed by the previous Horner-Wadsworth-Emmons reaction, to form the diastereomeric methyl ester mixture **152** with the *E*-olefin situated at $\Delta^{5,6}$, as shown in Scheme 3.26.



Scheme 3.26 Attempted Wolff-Kishner reduction protocol on diastereomeric mixture **151**.

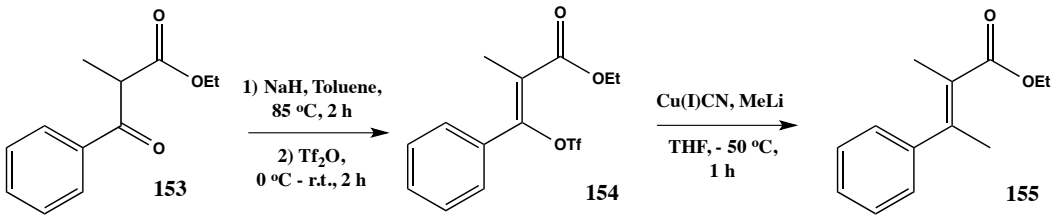
The initial formation of the hydrazone moiety and subsequent purification by column chromatography, although facile, gave repeatedly poor yields (≈25%). The next stage was the reduction of the hydrazone under basic conditions employing $Na(CN)BH_3$. This would allow for the 1,3-sigmatropic rearrangement to be initiated and transfer the hydride across to the $\Delta^{5,6}$ position. The hope was that, in line with the literature precedent, the *E*-olefin would be retained during the rearrangement and the hydrazone would be completely reduced to form (-)-mucosin (**23**) as one of the two diastereoisomers in the final crude diastereomeric mixture of **152**. On analysis of the crude spectra and on comparison with the literature spectra of (-)-mucosin it was discovered that neither of the two diastereoisomers formed gave the matching

stereochemical structure, especially with regard to the ^{13}C NMR. The other major complication with this reaction was the low yield of around 15%.

3.3.4 Review and Improvement of the Michael Addition Strategy

The next alternative was to reinvestigate the Michael addition again, but this time using the opposite enantiomer of (*R*)-*tert*-butanesulfinamide (**145**). The employment of the (*S*)-*tert*-butanesulfinamide with the α,β-unsaturated aldehyde **143** gave the corresponding (*S*)-aldimine in good yields. The ensuing conjugate addition with Cu(I)CN and ^{n}BuLi gave a large excess of the minor diastereoisomer formed when using the (*R*)-aldimine. Successive reactions towards the final product of diastereomeric mixture **152** also gave a mixture of the same diastereoisomers as previously isolated but in the reverse quantities. This adverse development meant that a new pathway had to be sought.

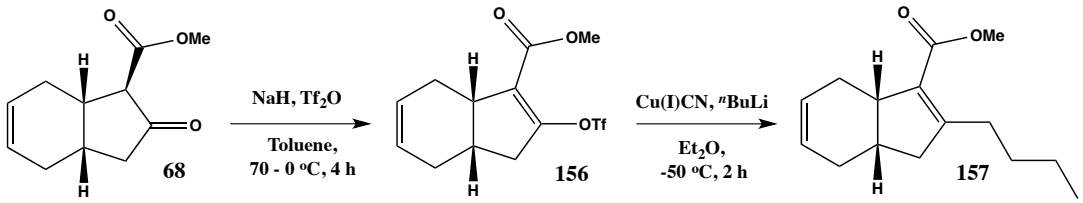
Reviewing the previous strategy it was agreed to continue with attaching the butyl substituent on an α,β-unsaturated precursor but via a contrasting modified intermediate. A paper published in 2014 by Jolit *et al*.,[118] displays an alternative to the conjugate addition approach. Employing vinylic triflate **154** with an ester moiety, formed from β-keto ester **153**, undergoes a copper mediated cross-coupling with an alkylated lithium reagent (here MeLi), to form an α,β-unsaturated ester **155** in an overall yield of 82%, as shown in Scheme 3.27.[118] This attachment of the desired alkyl chain substituent occurs without formation of any unwanted diastereoisomers as seen with the Michael addition in Scheme 3.23. The other benefit of forming this electron poor olefin is that it can now be selectively reduced from the least hindered face using well-known literature protocols. Once reduced this resulting saturated ester could then be equilibrated to one isomer using NaOMe, if necessary, which should yield one thermodynamically stable diastereoisomer in an *anti*-configuration. This will be explained in detail in the next section.



Scheme 3.27 Copper mediated cross-coupling protocol via use of a vinylic triflate **154**.

Based on this concept the β-keto ester **68** was reacted directly with NaH and Tf_2O to form the vinylic triflate **156**. This triflate could then be subjected to the copper mediated cross-

coupling with Cu(I)CN and ^{n}BuLi to form the desired α,β-unsaturated ester **157** with the C_4 side-chain at C-16, as shown below in Scheme 3.28.

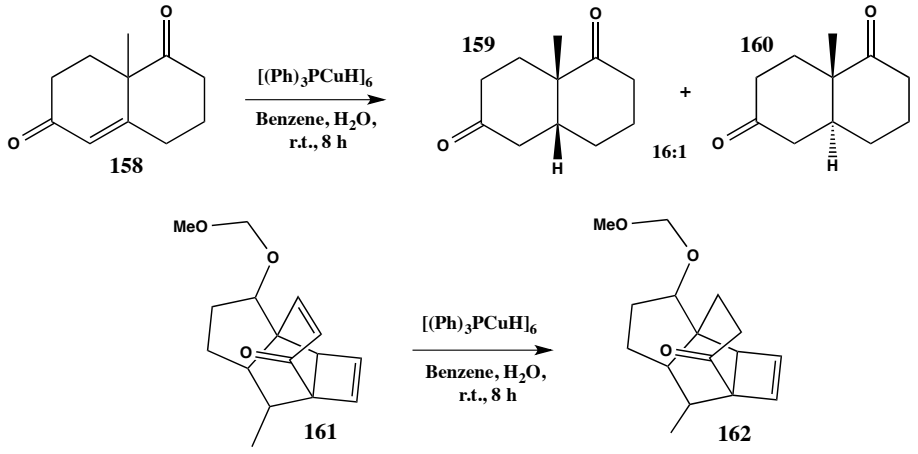


Scheme 3.28 Triflation and Copper mediated cross-coupling of **68** to form the α,β-unsaturated ester **156**.

Conversion to the triflate **156** from **68** proved successful with facile purification and in a good yield of 83%. The following cross-coupling reaction with Cu(I)CN and ^{n}BuLi also proved fruitful and in a high yield 88%. This allowed us to progress rapidly to the next stage, which was the selective reduction of the olefin adjacent to the ester on **157**.

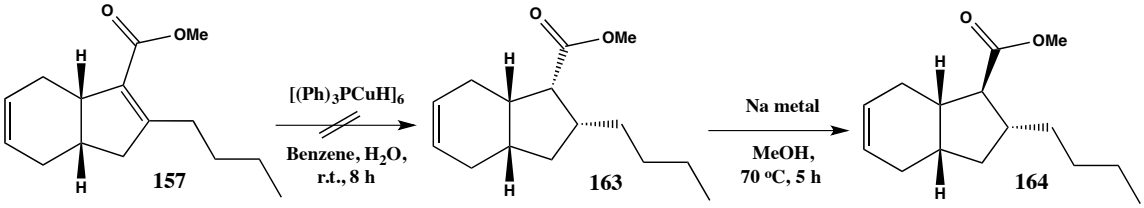
3.3.5 Selective Reduction of the Electron-Poor Olefin

This selective reduction could have been undertaken in many ways due to the olefins electron-poor nature. One way is the use of (triphenylphosphine)copper hydride hexamer or Stryker's reagent, which has the propensity to selectively reduce electron poor olefins from the least sterically hindered face to give the *syn* product as the more favored diastereoisomer.[155] An example of this is shown in Scheme 3.29, where reduction occurs at the least sterically hindered face of **158** to yield the *syn* product **159** as the favored diastereoisomer over the *anti* product **160** (d.r. = 16:1). The other benefit of this reaction is the olefin reduction selectivity with regard to α,β-unsaturated carbonyl compounds with additional olefin moieties.[155] The formation of **162** from **161** leaves the cyclobutene moiety intact and selectively reduces the olefin adjacent to the carbonyl also displayed in Scheme 3.29.



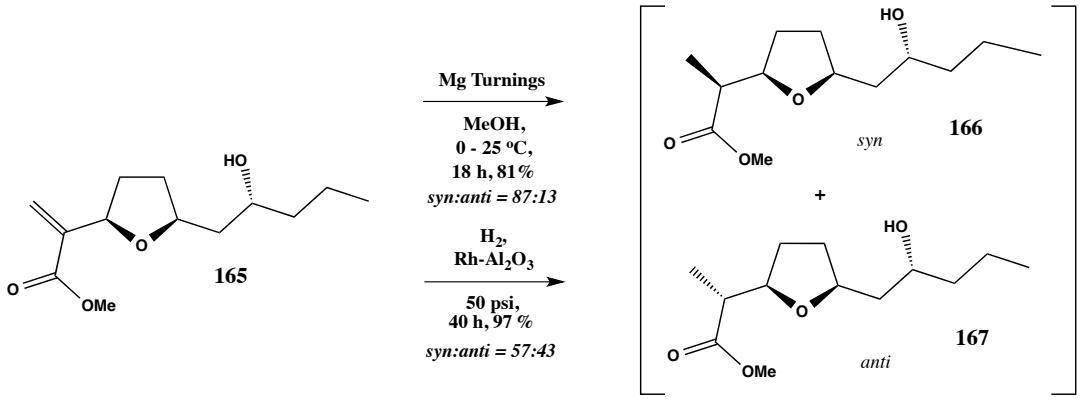
Scheme 3.29 Examples of the reduction of electron-poor olefins by Stryker's reagent.

With respect to compound **157**, the reduction of the electron-poor olefin, adjacent to the ester, by Stryker's reagent would be expected to proceed via the least hindered face and give the *syn* product **163** as the major isomer as well as leaving the cyclohexene moiety intact. The advantage of this reaction also meant that we could equilibrate the *syn* product **163** to the thermodynamically more favored *anti* isomer **164** via NaOMe. This methodology is shown in Scheme 3.30. This would then give the desired correct stereochemistry with regards to the side-chains, as that of (-)-mucosin. The decision was then taken to attempt this reaction using ester **157**. Unfortunately, the Stryker method failed to yield the desired product and only left starting material as the only isolated compound.



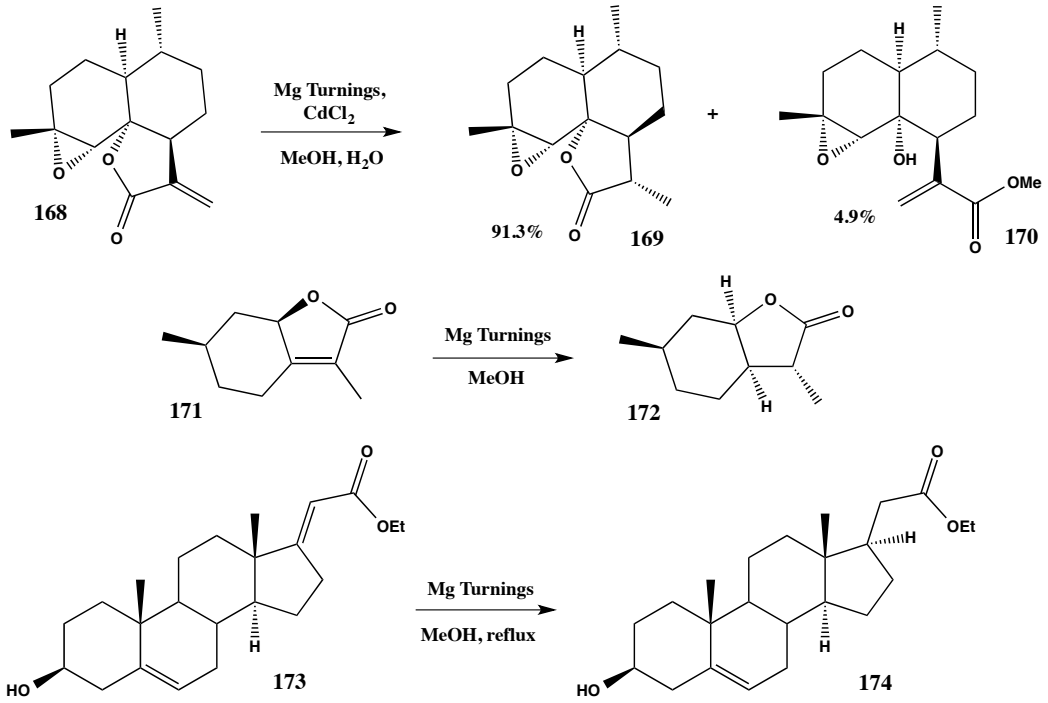
Scheme 3.30 Attempted reduction of **157** by Stryker's reagent to form the *syn* saturated ester **163** and the postulated *anti* structure **164** after NaOMe equilibration.

Another reduction protocol,[156] employing magnesium turnings and MeOH as a solvent, selectively reduces α,β-unsaturated carbonyls keeping other olefin moieties intact. This method has also been used to reduce various other conjugated olefins such as α,β-unsaturated nitriles, amides and lactones. Analyzing the stereoselectivity of this reduction using various unsaturated analogues has led to an interesting development, especially when compared to the equivalent catalytic hydrogenation reduction. Walkup *et al*.,[157] attempted the olefin reduction on polyoxygenated systems using both Mg/MeOH and catalytic hydrogenation. An example of this can be seen in Scheme 3.31 with an α,β-unsaturated lactone **165**. Using the Mg/MeOH protocol gave moderate stereoselectivity towards the *syn* diastereoisomer **166** in a ratio of 87:13 compared to the *anti* diastereoisomer **167**.[157] Conversely, the catalytic hydrogenation protocol gave a mixture of *syn* and *anti* diastereoisomers in a 57:43 ratio.[158] This increased stereoselectivity during the Mg/MeOH reduction is most likely due to the formal delivery of hydrogen to the less hindered face of a cyclic complex between the substrate and a Mg(II) species.[156]



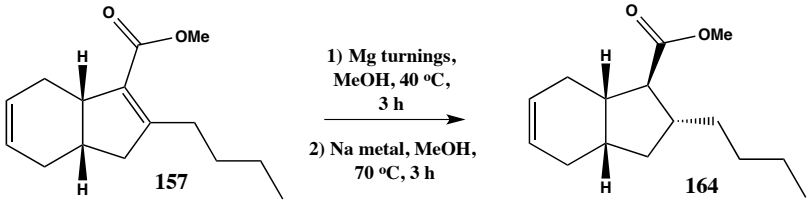
Scheme 3.31 Stereoselective Mg/MeOH reduction of an α,β-unsaturated lactone **165** favoring the least hindered face.

Another Mg/MeOH reduction carried out on highly strained lactone systems (**168** and **171**) by Bhattacharya and co-workers[159] also gave stereoselective reduction from the least hindered face, as shown in Scheme 3.32, with only minor side-products **170** or single isomers **172**. An analogous stereoselective reduction via Mg/MeOH, as demonstrated by Zarecki and Wicha,[160] selectively reduces an α,β-unsaturated ester. Their attempts to reduce the pregnadienoic ester **173** to **174** proved successful with no unwanted side-products and in quantitative yields (99%), as shown in Scheme 3.32.[160] The reduction also leaves the cyclohexene moiety, on the B-ring, intact, which has an analogous cyclohexene moiety to the α,β-unsaturated ester **157**.



Scheme 3.32 Stereoselective Mg/MeOH reduction of various α,β-unsaturated carbonyls favoring the least hindered face.

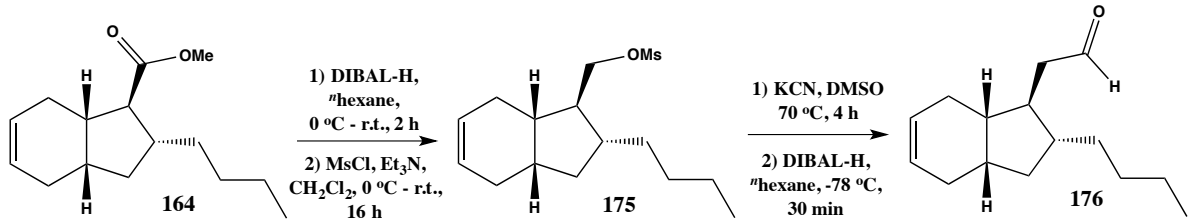
The protocol was attempted on the ester **157** (Scheme 3.33) and gave a mixture of two diastereoisomeric methyl esters, in a 91% yield, with complete consumption of the starting material. This was then equilibrated, as postulated in Scheme 3.30, with NaOMe, under reflux, to yield only one diastereoisomer of saturated methyl ester **164** in an excellent yield (93%). This equilibrated diastereoisomer was presupposed to be the thermodynamically favored *anti*-configuration.



Scheme 3.33 Successful stereoselective Mg/MeOH reduction and subsequent NaOMe equilibration of **157** to form the *anti*-configured saturated ester **164**.

3.3.6 Elaboration of Equilibrated Ester 164 to (-)-mucosin

The next issue to contemplate was the elongation of the equilibrated ester **164** to the C_7 side-chain. Retrospectively, using the Horner-Wadsworth-Emmons protocol with the corresponding aldehyde of **164** and phosphonate intermediate **66** was not ideal due to equilibration issues during reductive conversion as shown previously with the aldimine **147**. Another strategy, employing the corresponding alcohol of **164**, could have been the simple homologation to an aldehyde via a mesylation, cyanation and a modified reduction protocol (replacing Et_2O for ^{n}hexane and reacting at -78 °C rather than 0 °C), as performed by Filippova *et al.*[129] This would allow the removal of the equilibration issues involving the α-proton of the ester **164**. Proceeding from this, a simple Wittig reaction, employing the Schlosser modification with a premade phosphonium halide, could have been adopted to yield the necessary *E*-olefin in the correct position ($\Delta^{5,6}$). The initial conversion to the homologated aldehyde **176**, via mesylate **175**, was attempted as shown in Scheme 3.34. These four steps were all successfully completed and resulted in an overall yield of 91%.

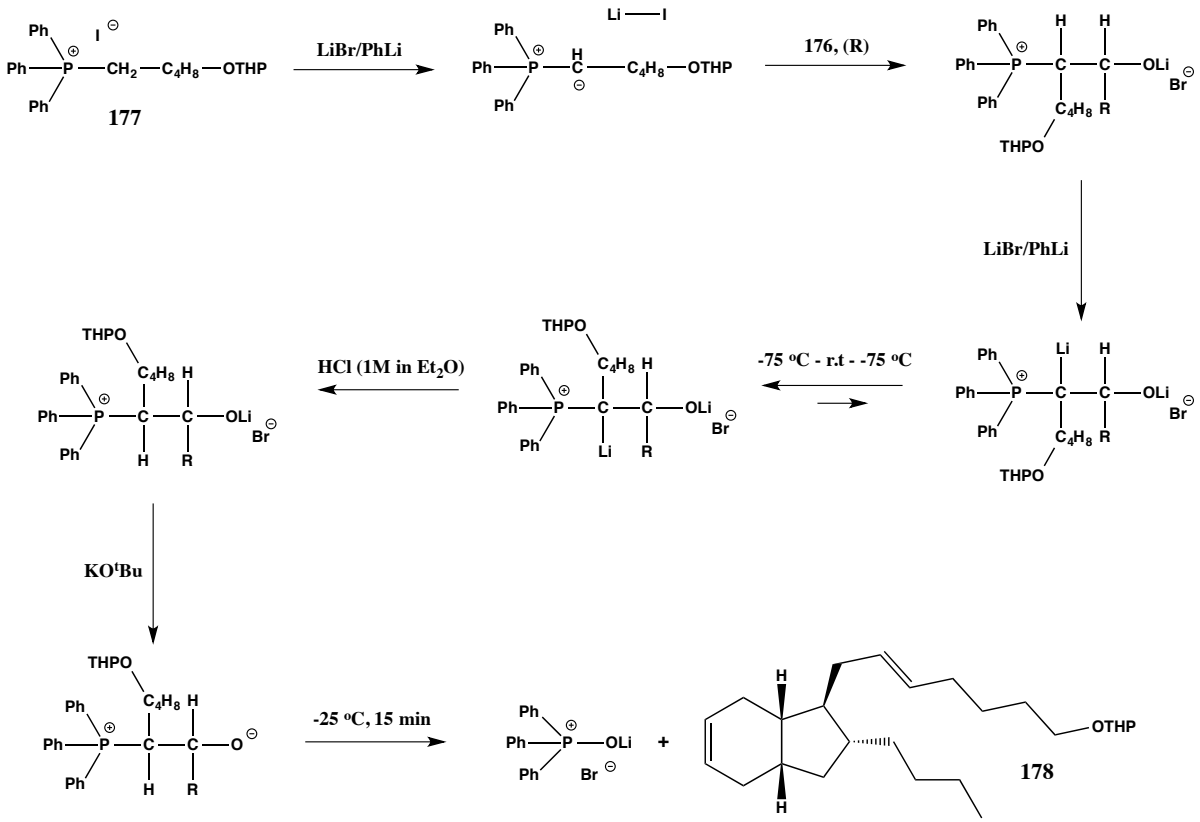


Scheme 3.34 Elaboration of **164** via a reduction and three-step homologation to form aldehyde **176**.

3.3.7 Attempted Synthesis of the *E*-Olefin via a Schlosser-Wittig Reaction

The production of this aldehyde **176** meant that elongation to a C_7 side-chain containing an *E*-olefin could now take place. Many methods were considered, such as Pd(0) catalysed cross-coupling reactions and the Schlosser-Wittig reaction. Primarily, the shorter route was the use of the Schlosser-Wittig reaction. This reaction is shown to be *E*-selective with respect to olefin formation, which is contradictory to the *Z*-selective Wittig reaction. This Schlosser-Wittig reaction could be performed directly on the aldehyde with a preformed C_5 phosphonium ylide and yield the elongated C_7 side-chain in one step. Precedence for this reaction dates back to 1967,[161,162] but employment of the protocol is not as common.

The mechanism of the Schlosser-Wittig reaction differs from the typical Wittig reaction due to the use of halide salts such as LiBr. This suppresses the dissociation of the α-lithiated betaine ylide to an α-metal-free betaine ylide, which occurs readily in the normal Wittig reaction.[163] The mechanism for the Schlosser-Wittig, using aldehyde **176** and a preformed C_5 phosphonium iodide **177**, is shown in Scheme 3.35.



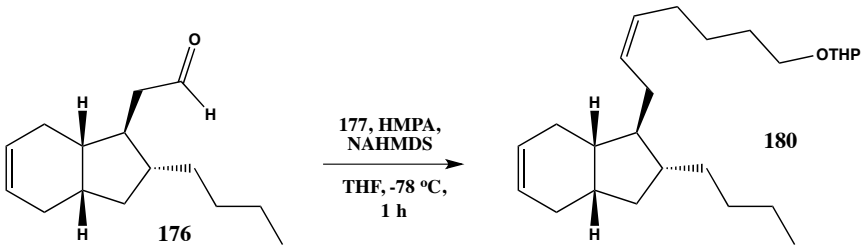
Scheme 3.35 Mechanism of the Schlosser-Wittig reaction, employing aldehyde **176** and the C_5 phosphonium iodide **177**, to form coupling compound **178** containing an *E*-olefin.

Initial attempts at the Schlosser-Wittig reaction using a protocol by Pettit *et al.*,[164] with aldehyde **176** and the preformed C_5 phosphonium iodide **177** gave good yields of around

70% of the assumed *E*-olefinic structure **178** as only one geometric isomer was formed.[164] This intermediate was then subjected to subsequent deprotection,[165] oxidation[166] and esterification[167] reactions to yield the assumed (-)-mucosin methyl ester structure **179**.

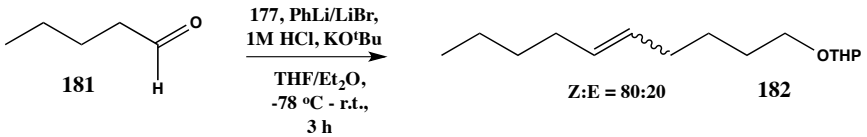
Scheme 3.36 Elaboration of **177** to the postulated (-)-mucosin methyl ester **179**.

On further analysis of this final compound **179**, by ^{1}H and ^{13}C NMR, with the literature natural product, it was observed that certain peaks in the ^{1}H and ^{13}C NMR had subtle differences. This was especially noticeable in the ^{13}C NMR with respect to the bridgehead carbons at C-8, C-9 and C-16. The next stage was to try and decipher which olefin was present on the C_7 side-chain. A Wittig reaction could be carried out on aldehyde **176** and the results could be directly compared with those previously obtained from the Schlosser-Wittig reaction on **176**. This was attempted, as shown in Scheme 3.37, and gave the *Z*-olefinic structure **180** in 63% yield.[168] On comparison with the previous olefin NMR data of **178**, they were found to be identical.



Scheme 3.37 Wittig reaction of aldehyde **176** to form the *Z*-olefinic compound **180**.

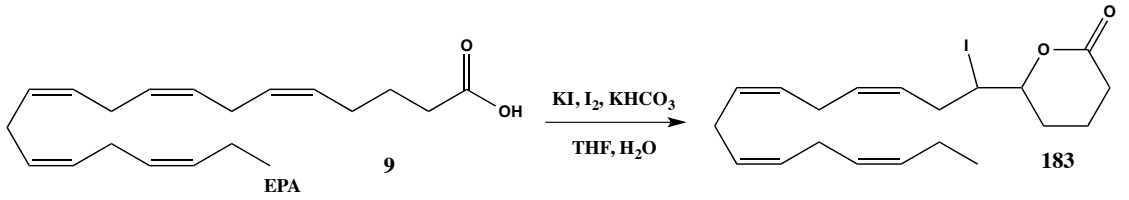
One foreseeable problem with the literature method by Pettit *et al.*,[164] was the lack of LiBr present during the reaction. Another method, by Wang *et al.*,[163] incorporating LiBr, was attempted and yielded differing results. This was only attempted on the commercially available valeraldehyde (**181**) and **177** as shown in Scheme 3.38. The reaction itself was very temperamental and the results gave the isomeric mixture **182**, the majority of which was the unwanted *Z*-olefin in a ratio of 80:20 (*Z*/*E*). This methodology was eventually disregarded after multiple attempts under different anhydrous conditions and temperatures.



Scheme 3.38 Attempted Schlosser-Wittig reaction following the Wang *et al*. protocol.

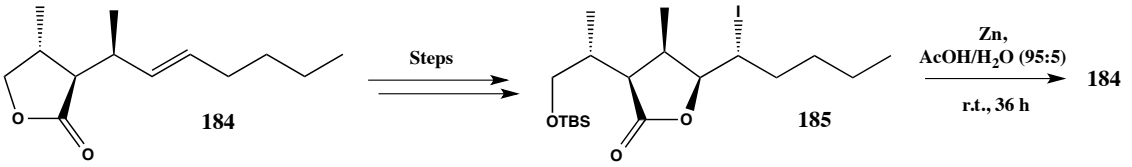
3.3.8 Attempted Synthesis of the *E*-Olefin via an Iodolactonization

The decision was then taken to choose another method to generate the *E*-olefin. Another reaction protocol was formulated by utilizing the iodolactone formation procedure with naturally occurring fatty acids like EPA (**9**) to form iodolactone **183** as shown by Filippova *et al.,*[129] and Flock *et al.,*[126] in Scheme 3.39.



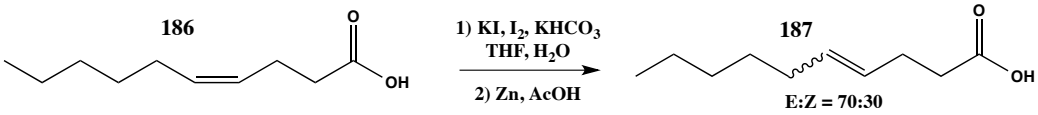
Scheme 3.39 Iodolactonization reaction by the Filippova and Flock protocols employing EPA (**9**).

The concept was to apply this iodolactonization reaction with the *Z*-olefin acid analogue of **179** and promote the ring-opening of the iodolactone via the use of Zn and AcOH. The ambition was to only form the *E*-olefin, as this is always the thermodynamically favored of the two geometric isomers. A number of protocols were found that employ the use of 3-methyl-γ-butyrolactones **184**, which after initial conversion to an iodolactone **185**, proceed via a ring-opening reaction with Zn and AcOH to furnish the *E*-olefin and the original γ-butyrolactone **184**, as shown in Scheme 3.40.[169,170]



Scheme 3.40 Iodolactonization and subsequent ring-opening of the iodolactone **185** to furnish the initial *E*-olefinic lactone present on **184**.

The perception was that the reformation of the olefin from the iodolactonization of the *Z*-olefin acid analogue of **179** via the use of Zn and AcOH would give the more thermodynamically favored *E*-olefin, which could then be developed to the (-)-mucosin methyl ester. The iodolactonization procedure and subsequent iodolactone ring-opening, as shown in Schemes 3.39 and 3.40 respectively, was initially performed on commercially available *cis*-4-decenoic acid (**186**), as shown in Scheme 3.41.

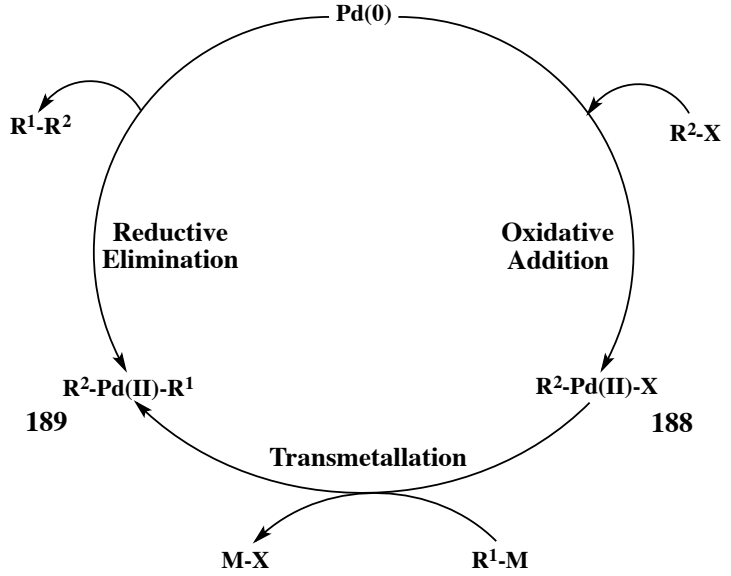


Scheme 3.41 Iodolactonization/iodolactone ring-opening procedure employing **186**.

After the initial iodolactonization/ring-opening a mixture **187** was formed and the ratio of *E*/*Z* isomers was 70:30. This seemed very promising, so the procedure was repeated on the initial product **187** twice more. The concept was to reduce the amount of *Z*-olefin each time to give a final ratio, which is comparable to 99:1 (*E*/*Z*). Unfortunately, this was not the case, as the ratio of *E*/*Z* remained the same after repeating the procedure on **187**. This reaction was attempted at many different temperatures (-78 – 50 °C) and co-solvents (EtOH and THF) but no noticeable improvement was seen in the *E*/*Z* ratio. After these results it was decided to halt with this protocol and look towards cross-coupling approaches.

3.3.9 Synthesis of the *E*-Olefin via a One-Pot Negishi Cross-Coupling Reaction

Cross-coupling reactions to form a carbon-carbon bond employing a transition metal catalyst is a well-established method. Many cross-coupling reactions have been utilised to selectively synthesize *E*-olefins. The majority of cross-coupling protocols employ Pd(0) or Ni(0) catalysts to couple with an organo-halide, which then in turn reacts with an organometallic compound to form a carbon-carbon bond. A general catalytic cycle for a cross-coupling reaction involving Pd(0) as the catalyst can be seen in Scheme 3.42.[171]

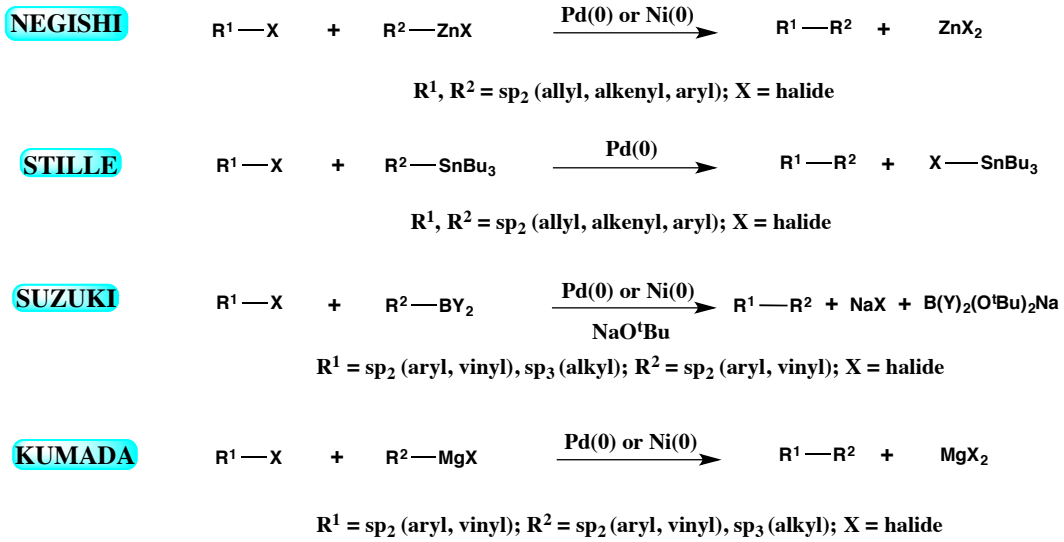


Scheme 3.42 General catalytic cycle for metal mediated cross-coupling reactions.

The reaction mechanism begins with an oxidative addition of an organic halide (R^2X) to the catalyst (Pd(0)), to form an intermediate **188**. Intermediate **188** then undergoes transmetallation with R^1-M to place both coupling partners on the same metal center to form intermediate **189** with elimination of the functional groups M-X.[171] The final step is the

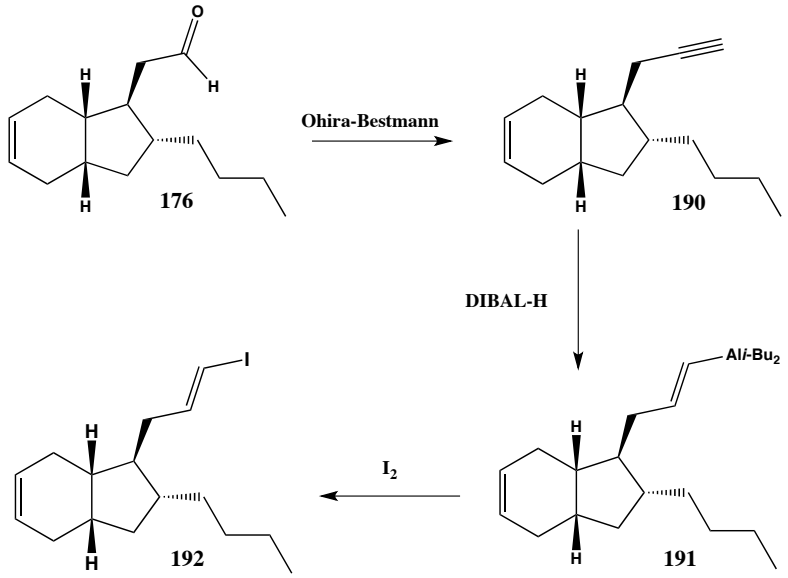
reductive elimination of the two coupling fragments to regenerate the catalyst (Pd(0)) and the new cross-coupled compound R^1-R^2.[171]

Synthesizing *E*-olefins from using various cross-coupling protocols is well known and has been previously carried out on numerous occasions to help synthesize natural products. Cross-coupling protocols such as the Negishi coupling,[172,173] Stille coupling,[174] Suzuki coupling[175] and Kumada coupling[176] reactions all have the propensity to produce *E*-olefins with an organohalide and varying organometallic intermediates. The Negishi coupling employs an organozinc intermediate, the Stille coupling employs an organotin intermediate, and the Suzuki coupling employs an organoboron intermediate with base, whilst the Kumada coupling employs an organomagnesium intermediate. All three have a general outline in Scheme 3.43 below. Choosing which one to incorporate into our strategy would be crucial in selectively furnishing the *E*-olefin.



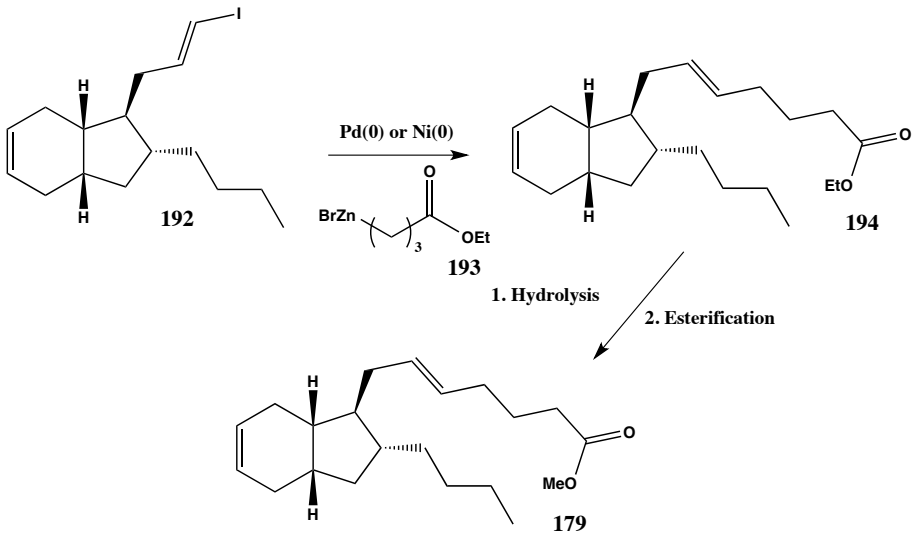
Scheme 3.43 General outline for the Negishi, Stille, Suzuki and Kumada cross-coupling reactions.

To construct the *E*-olefin, the corresponding halide, on the (-)-mucosin intermediate, would have to be synthesised first. The most sensible method would be to make an *E*-vinyl iodide from **176** and then cross-couple it to the necessary C_4 organometallic intermediate via a Pd(0) of Ni(0) catalyst to form a C_7 chain with the *E*-olefin at $\Delta^{5,6}$. Making a vinyl iodide from an aldehyde can occur in three steps. The first step would be a homologation to an alkyne **190** using the Ohira-Bestmann protocol.[177] The second step is the reduction of the alkyne via DIBAL-H to an *E*-specific alkenylaluminum species **191**, which can then be converted to an *E*-vinyl iodide **192** with addition of iodine as the final step, as shown in Scheme 3.44.[178]



Scheme 3.44 Postulated synthesis of the *E*-vinyl iodide **192** from aldehyde **176**.

The simplest method of forming the necessary *E*-olefin from **192** and an organometallic species would be to use the commercially available 4-ethoxy-4-oxobutylzinc bromide (**193**) with a Pd(0) or Ni(0) catalyst in a Negishi-type cross-coupling. This will be able to furnish the necessary *E*-olefin in a stereospecific manner at $\Delta^{5,6}$ and also result in the formation of an ethyl ester **194**, which can easily be converted to the corresponding acid and methyl ester of (-)-mucosin **179**, as shown in Scheme 3.45.

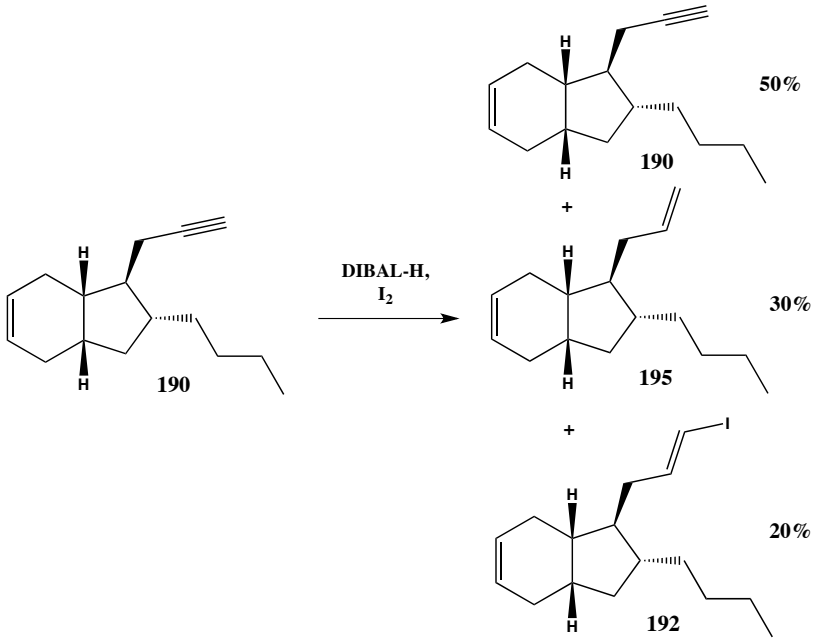


Scheme 3.45 Postulated synthesis towards mucosin methyl ester **179** via a Pd(0) catalysed Negishi cross-coupling with **192**.

Alternatives, such as the employment of the corresponding Stille, Suzuki and Kumada cross-coupling reagents instead of the Negishi-type reagent, could also lead to the correct *E*-olefin. However, the use of the Stille protocol with a suitable organotin reagent can be quite toxic, involves quite a high carbon economy, and the reagent itself would have to be synthesized, as

it is not commercially available. The same could be said with regards to the Suzuki reaction, as the cross-coupling reagent would have to be synthesised beforehand. The use of the Kumada magnesium reagents with an ethyl ester terminus is more likely to lead to unwanted side products as well as the desired ester and are therefore incompatible with these reagents. The ease of availability of the commercially available 4-ethoxy-4-oxobutylzinc bromide (**193**) and the stability of the ethyl ester during the reaction meant that producing the *E*-olefinic ethyl ester **194** via the Negishi reagent was the preferred method.

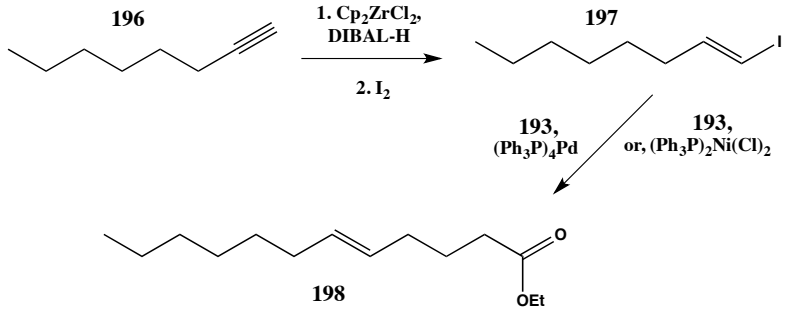
The homologation to the alkyne **190** with the Ohira-Bestmann reagent and aldehyde **176** proceeded successfully and in a high yield of 86%. The next stage was to reduce the resulting alkyne **190** with the use of DIBAL-H and then convert that directly in one-pot to the *E*-vinyl iodide **192**. After initial attempts with a model compound of 1-octyne and **193** the *E*-vinyl iodide of 1-octyne was afforded in good yields (72%). However, by using this method on the alkyne **190** the reaction did not go to completion and afforded a mixture of starting material **190** (50%), terminal olefin **195** (30%) and only a small amount of the necessary iodide **192** (20%) as shown in Scheme 3.46.



Scheme 3.46 Initial attempt at formation of the *E*-vinyl iodide **192** from alkyne **190**.

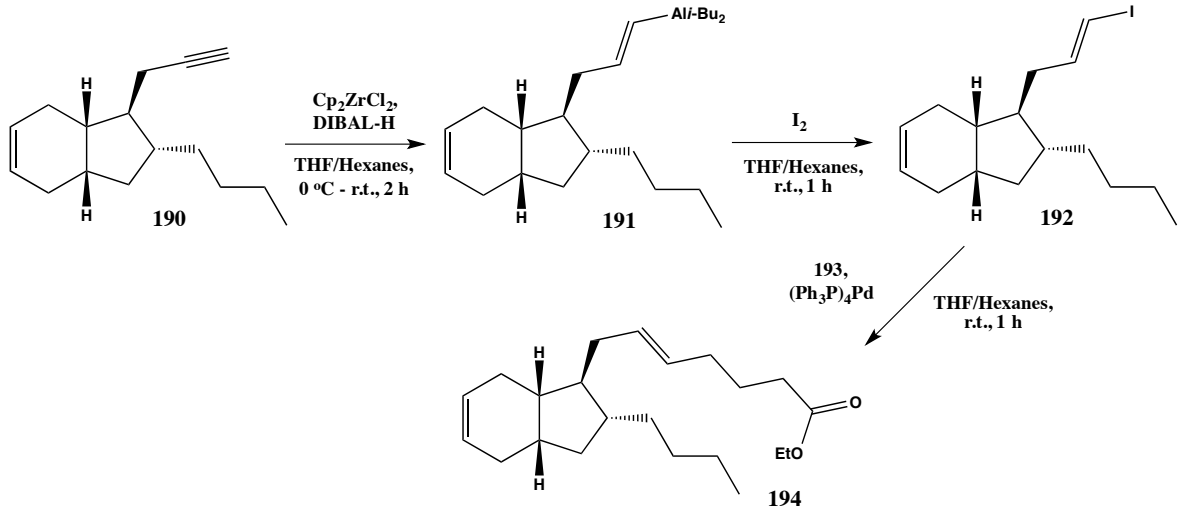
It was then decided to use zirconocene dichloride (Cp_2ZrCl_2) with DIBAL-H to increase the reactivity and help in the formation of the iodide.[179] This was then tested again on 1-octyne (**196**) and the formation again proceeded in high yields and *E*-selectivity. Subsequently, an attempt was made to use two different catalysts; a Pd(0) species and a Ni(0) species to cross-couple the *E*-vinyl iodide **197** of 1-octyne (**196**) and 4-ethoxy-4-oxobutylzinc bromide (**193**).

The hope was to selectively furnish the desired *E*-olefin **198** of the model *E*-vinyl-iodide **197** and the zinc-bromide reagent **193**. Both catalytic species were tested separately and both gave the desired *E*-olefin **198** in quantitative yields with no measurable amount of the *Z*-isomer. This methodology is shown in Scheme 3.47.



Scheme 3.47 Attempted Negishi cross-coupling with 1-octyne (**196**) and **193** via Cp_2ZrCl_2.

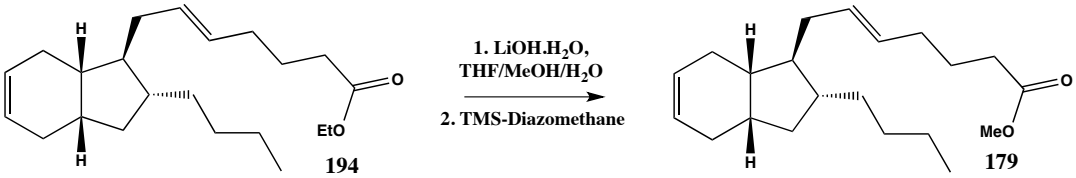
The next aim was to try all three steps (reduction, iodination and catalysed cross-coupling) in a one-pot synthesis, as shown in Scheme 3.48. This was to try and increase the yield and would also mean not having to isolate the unstable *E*-vinyl iodide intermediate **192**. Another added benefit of this is the reduction in steps in the overall synthesis towards (-)-mucosin. This was again attempted on 1-octyne (**196**) and gave the same result as previously stated in Scheme 3.47. Employment of this protocol was then used with the alkyne **190**, Cp_2ZrCl_2, DIBAL-H, iodine, $(Ph_3P)_4Pd$ and **193**. The reaction gave the *E*-ethyl ester of (-)-mucosin **194** in 51% yield with no detection of the *Z*-olefin we had seen previously using the Schlosser-Wittig reaction.



Scheme 3.48 Successful synthesis of **194** via a one-pot Negishi cross-coupling protocol.

3.3.10 Conversion of Ethyl Ester 194 to (-)-mucosin

After this success, the ethyl ester **194** was converted in quantitative yields (97%) to the corresponding acid **23** with a lithium hydroxide monohydrate protocol.[180] The acid **23** was then readily converted to the methyl ester of (-)-mucosin **179**, which was the isolated natural product of (-)-mucosin (**23**), in nearly quantitative yields (92%), via TMS diazomethane.[167] The total yield for the synthesis, over seventeen steps, of the methyl ester of (-)-mucosin **179**, with this synthetic strategy, is 4%.

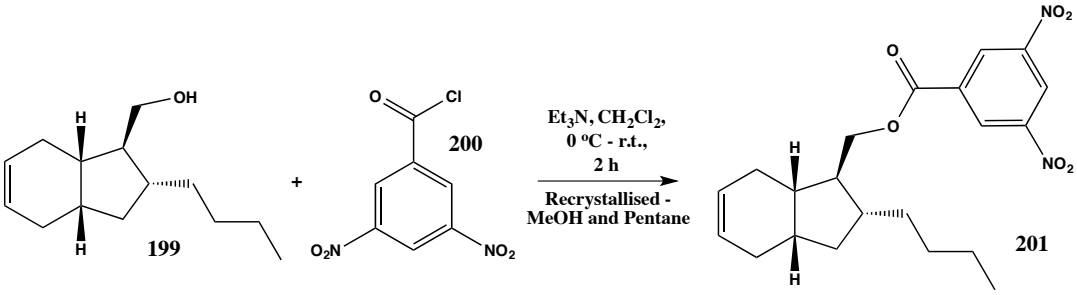


Scheme 3.49 Conversion of ethyl ester **194** to the corresponding acid **23** and methyl ester **179**.

The ^{1}H and ^{13}C NMR spectral data of this methyl ester of (-)-mucosin **179** was then directly compared to the literature spectra of the methyl ester of the natural product (-)-mucosin (**23**) by Casapullo and the methyl ester of enantiomer (+)-mucosin (***ent*-23**) synthesised by Whitby. Again it was discovered, that on comparison of the spectra compound **179** with the literature spectra, there were noticeable peak shift differences, especially in the ^{13}C NMR. A NOESY NMR was also carried out on the methyl ester **179** and directly compared with the tabulated Casapullo NOESY data, but no conclusive stereochemical interactions could be deduced from the data.

3.3.11 Conversion of the Intermediate Alcohol for X-Ray Crystal Analysis

Based on this analysis it was concluded that we have made another diastereoisomer of the methyl ester of (-)-mucosin (**23**), which is different than that found in literature.[58,59] Due to the complexity of the spectra themselves it was decided to synthesize a crystal from a late stage intermediate to determine the relative stereochemistry with X-ray crystallography. The intermediate alcohol **199** was chosen and reacted with 3,5-dinitrobenzoyl chloride (**200**) to form a dinitro-ester **201**, which was re-crystallized with MeOH and pentane, as shown in Scheme 3.50.



Scheme 3.50 Synthesis of **201** from alcohol **199** via 3,5-dinitrobenzoyl chloride (**200**).

These crystals were then taken for X-ray analysis and gave the structure for the relative stereochemistry of **201** in Figure 3.4 below. The alcohol **199** was chosen to be the intermediate for crystal analysis for two reasons. Firstly, with respect to the chiral centers at C-8, C-9, C-14 and C-16 on **199** are all fixed and cannot be changed with any subsequent reaction steps to form the *E*-olefin. Secondly, the structure of **199** is very similar, apart from the butyl moiety at C-16, to the β-keto ester **68**, which is crystalline. This would mean that with the correct dinitro auxiliary attached to the alcohol **199** there would be a high probability to form a crystalline structure. As explained previously, the elucidation of the *E*-olefin produced from the alcohol **199** had been completed so it was not necessary to incorporate this into the crystal intermediate.

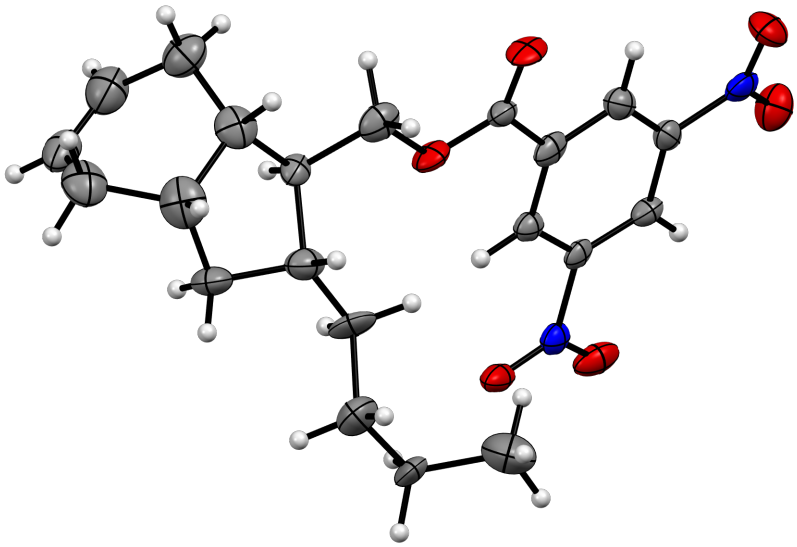


Figure 3.4 Crystal structure of dinitro-ester **201**.

As seen in Figure 3.4, the crystal structure of dinitro-ester **201** has the correct relative stereochemistry that was aimed for from the outset with this synthesis for (-)-mucosin. However, the ^{1}H and ^{13}C NMR spectral data does not match favorably with the published data of Casapullo[58] and Whitby[59]. Therefore, the assumption is that the published data is incorrect with respect to the assigned stereochemistry. This will be discussed in more detail in the next chapter.

4. Structural Analysis

The relative stereochemistry of the current methyl ester stereoisomer **179** of (-)-mucosin has been elucidated to be the identical structure to the one given in the Casapullo[58] natural product paper and the mucosin enantiomer given by Whitby[59]. This structure is shown with the dinitro-ester **201**, made from the late stage intermediate alcohol **199**, shown in Figure 4.1 below.

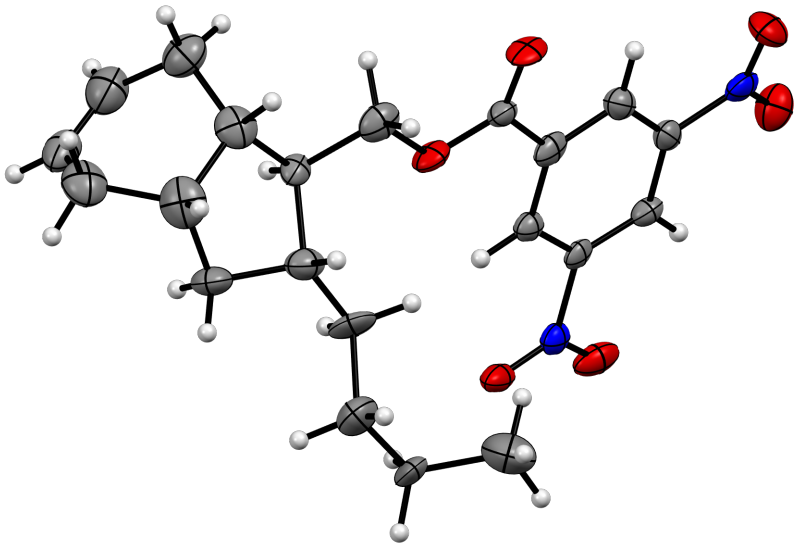


Figure 4.1 Crystal structure of dinitro-ester **201**.

However, on analysis of the ^{1}H and ^{13}C NMR data for the (-)-mucosin methyl ester stereoisomer **179** it was noticed that it was very different to the NMR data produced by Casapullo and Whitby. The comparisons between all three sets of NMR data are given in two tables below. The notable differences between the current stereoisomer and published methyl esters are highlighted.

^{1}H NMR ($CDCl_3$) – mucosin methyl ester

Chem. Commun.[59] **–** **(400 MHz)**	**Natural Product**[58] **–** **(500 MHz)**	**Compound 179 –** **(400 MHz)**
5.73-5.60 (m, 2H)	5.67 (m, 2H)	5.70-5.64 (m, 2H)
5.46 (m, 1H)	5.45 (dt, 1H)	5.46-5.33 (m, 2H)
5.38 (m, 1H)	5.39 (dt, 1H)	3.67 (s, 3H)
3.67 (t, 3H)	3.66 (s, 3H)	2.31 (t, 2H)
2.31 (t, 2H)	2.31 (t, 2H)	***2.22-2.15 (m, 1H)***
2.23 (m, 1H)	2.25 (m, 1H)	***2.12-2.01 (m, 6H)***
2.17 (m, 1H)	2.19 (m, 1H)	***1.89-1.75 (m, 3H)***
2.15-2.08 (m, 2H)	2.12 (m, 2H)	***1.73-1.65 (m, 3H)***
2.03 (q, 2H)	2.02 (q, 2H)	***1.54-1.44 (m, 2H)***
1.80-1.65 (m, 4H)	1.72 (m, 1H)	***1.34-1.16 (m, 6H)***
1.64-1.48 (m, 3H)	1.70 (m, 1H)	***1.12-1.04 (m, 1H)***
1.46-1.33 (m, 2H)	1.69 (m, 2H)	0.88 (t, 3H)
1.33-1.23 (m, 3H)	1.59 (m, 1H)	
1.19-1.13 (m, 2H)	1.55 (m, 1H)	
1.13-1.08 (m, 2H)	1.50 (m, 1H)	
0.89 (t, 3H)	1.40 (m, 2H)	
	1.33 (m, 1H)	
	1.28 (m, 2H)	
	1.15 (m, 2H)	
	1.12 (m, 1H)	
	1.11 (m, 1H)	
	0.88 (t, 3H)	

Table 4.1 ^{1}H NMR environments of the three methyl ester stereoisomers of (-)-mucosin (**23**).

^{13}C NMR ($CDCl_3$) – mucosin methyl ester

Chem. Commun.[59] – (100 MHz)	**Natural Product[58] – (125 MHz)**	**Compound 179 – (100MHz)**
174.2	174.2	174.2
130.3	130.0	130.4
129.8	129.8	129.9
127.3	127.0	***126.3***
127.1	127.0	***126.1***
52.2	52.1	51.4
51.4	51.4	***51.0***
47.2	47.1	***44.0***
42.3	42.1	***40.3***
40.1	39.9	***38.1***
37.0	36.7	***37.7***
36.74	36.5	***37.1***
36.68	36.4	***34.9***
33.4	33.2	33.4
32.4	32.0	31.9
31.9	31.7	***31.0***
31.6	31.5	***27.8***
30.7	30.7	***27.7***
24.7	24.5	24.8
22.9	22.6	22.9
14.1	13.8	14.1

Table 4.2 ^{13}C NMR environments of the three methyl ester stereoisomers of (-)-mucosin (**23**).

Contained in the two tables above are the ^{1}H and ^{13}C NMR data for the elucidated (-)-mucosin methyl esters; firstly the methyl ester enantiomer of (+)-mucosin (***ent*-23**) synthesised by Whitby *et al*.,[59] secondly the methyl ester of the (-)-mucosin natural product (**23**) isolated from *Reniera mucosa* by Casapullo *et al.*,[58] and the methyl ester stereoisomer of (-)-mucosin **179** previously discussed in this thesis.

At first glance, comparing the Whitby and Casapullo ^{1}H, ^{13}C NMR spectra and optical rotation values show that they are most likely enantiomers. This is especially noticeable when comparing the ^{13}C NMR data. On comparison with the current methyl ester stereoisomer of (-)-mucosin **179** and the Whitby and Casapullo methyl ester enantiomers both the ^{1}H and ^{13}C NMR spectra have glaring differences, which have been ***highlighted*** in both tables. In the ^{1}H NMR spectra, the cyclohexene and *E*-olefin proton shifts at 5.70-5.64 ppm and 5.46-5.33 ppm respectively, the methoxy proton shift at 3.67 ppm, the α-methylene shift adjacent to the ester at 2.31 ppm and the methyl proton shift at 0.88 ppm all match very closely. However, the alkyl proton shifts between 2.22-1.04 ppm differ, especially with respect to multiplicity and integration values. This is mainly due to the complex alkyl region of **179**, as shown in Figure 4.2. The Casapullo methyl ester of the natural product **23** gives a much more detailed alkyl region with individual proton environments. The Whitby methyl ester of enantiomer ***ent*-23**, although identical in relative stereochemistry to the Casapullo structure, gives a much more generalized alkyl region, but again this is contrasting with the current methyl ester stereoisomer of (-)-mucosin **179**.

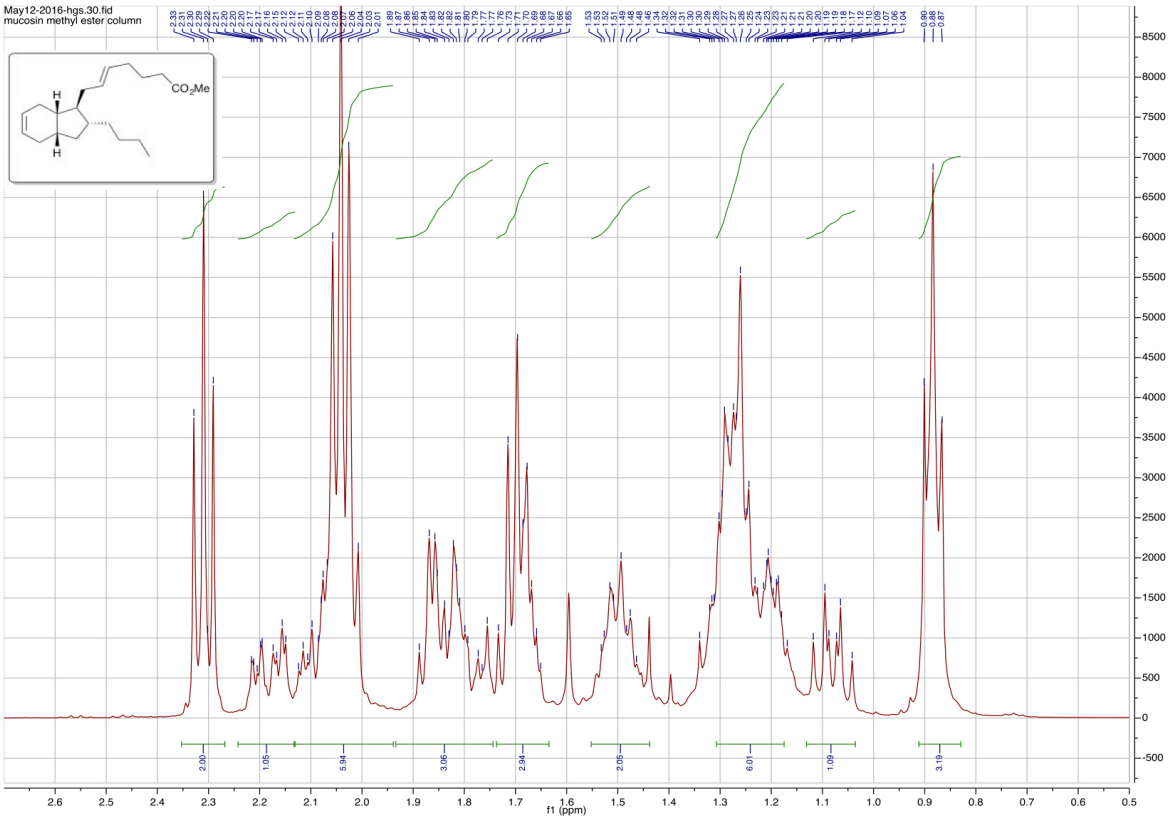


Figure 4.2 Alkyl region of the ^{1}H NMR of diastereoisomer 179.

These differences are also portrayed in the ^{13}C NMR data shown in the table above. The majority of the carbon environments differ dramatically apart from the *E*-olefin, carbonyl, methoxy, methyl and a few sp^2 hybridized moieties. The other dissimilar carbon peaks relate to alkyl sp^3 or sp^2 hybridized regions only. This is almost a parallel of the differences with the ^1H NMR data. One noticeable difference that is not seen in the ^1H NMR data is the shift values for the cyclohexene moiety as they match up on comparison with each other. For the current methyl ester stereoisomer **179** they are given as 126.3 ppm and 126.1 ppm. With the Whitby methyl ester enantiomer of ***ent*-23** they are given as 127.3 ppm and 127.1 ppm and with the Casapullo methyl ester of natural product **23** they are given at exactly 127.0 ppm for both carbons. This implies that the structural environment around these carbons, when comparing **179** with the Whitby and Casapullo methyl esters, is considerably different.

A NOESY NMR, as shown in Figure 4.2, was also performed on the (-)-mucosin methyl ester stereoisomer **179**. The results from the analysis showed extensive overlaps in the aliphatic region (2.31-1.04 ppm), which meant that it was impossible to disseminate every separate correlation clearly. This is at odds with the Casapullo paper for the methyl ester of **23**. He is clearly able to distinguish correlations at all the aliphatic regions on the cyclopentane ring apart from the H-8 and H-9 environments, which seem to overlap on the natural product. Close analysis of the NOESY data and the correlations elucidated by Casapullo leads to some discrepancies with the structure he has given, especially when *cis*-fused bridgehead environments are analysed. The H-9 environment correlates to both the H-14 and H-16 environments, which corresponds to the structure given by Casapullo. However, H-16 gives no correlation to the H-14 environment, which is what one would expect for a *cis*-fused system. The same can be said with respect to the H-14 environment, as there is no correlation with the H-16 environment. Futhermore, the overlap of the NOESY environments with the H-8 and H-9 environments leads to a disparity when assigning the stereochemical configuration of the methyl ester of (-)-mucosin **23**. This could mean that both correlations seen between the H-9 to H-14 environments and H-14 to H-9 environments could both actually be from the H-8 environment instead of the H-9. Based on this analysis of the NOESY spectrum and the inconsistencies around the *cis*-fused bridgehead it seems that a more likely candidate for the structure would be a *trans*-fused bridgehead system. A question remains as to whether the NOESY spectrum, taken by Casapullo of the methyl ester of **23**, gives a clear indication of the environments given in the structure he is portraying.

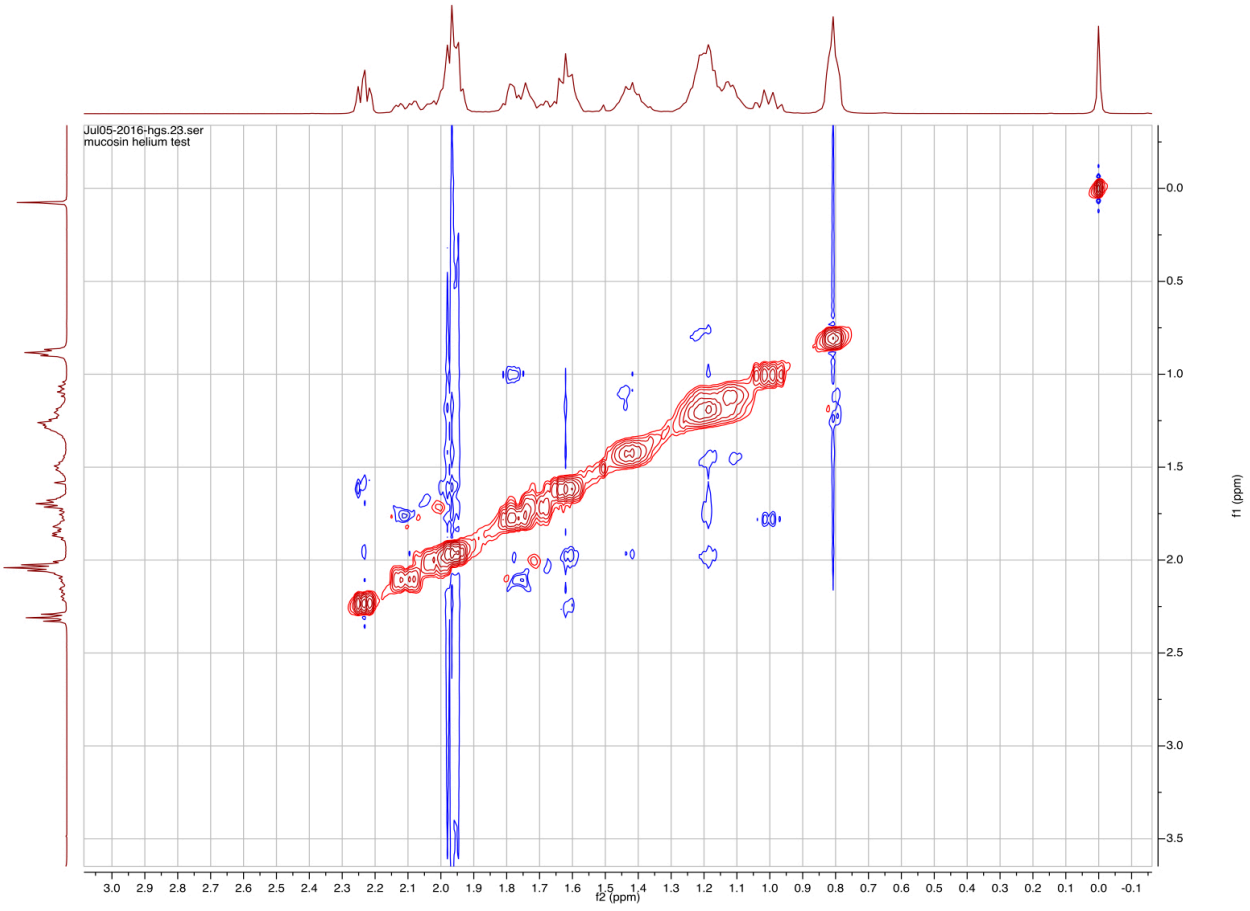


Figure 4.2 NOESY NMR of the aliphatic region (2.3-0.7ppm) of **179**.

This leads to the conclusion that although the general structure of the methyl ester of (-)-mucosin **23** is correct but the relative and absolute configuration given by Casapullo is not and must be investigated. This error with the absolute configuration is not uncommon as seen with other isolated natural products and their final elucidation.[181] This can be seen notably when comparing the current stereoisomers' (**179**) side-chain bridgehead ^{13}C NMR shift values at 51.0 ppm and 44.0 ppm in Table 4.2. A note must also be made with respect to the optical rotation values of **23**, ***ent*-23** and **179**. It must be concluded that the Whitby enantiomer ***ent*-23** and the Casapullo natural product **23** must be enantiomers due to their specific optical rotation values of $[\alpha]^{26}_D$ = +38.2° (c = 0.8, ^{n}hexane) and $[\alpha]^{26}_D$ = -35.5° (c = 0.8, ^{n}hexane), respectively, and their complementary ^1H and ^{13}C NMR shifts. The current stereoisomer **179** has a specific optical rotation value, which is markedly different, at $[\alpha]^{26}_D$ = -9.8° (c = 0.8, ^{n}hexane) as are the ^1H and ^{13}C NMR shifts for the chiral centers.

Using the elucidated crystal structure of the dinitro compound **201** it must be deduced that Casapullo has not assigned the correct stereocenters in his natural product elucidation of the methyl ester of **23**, even with NOESY analysis. As a comparison, the NOESY and Mosher

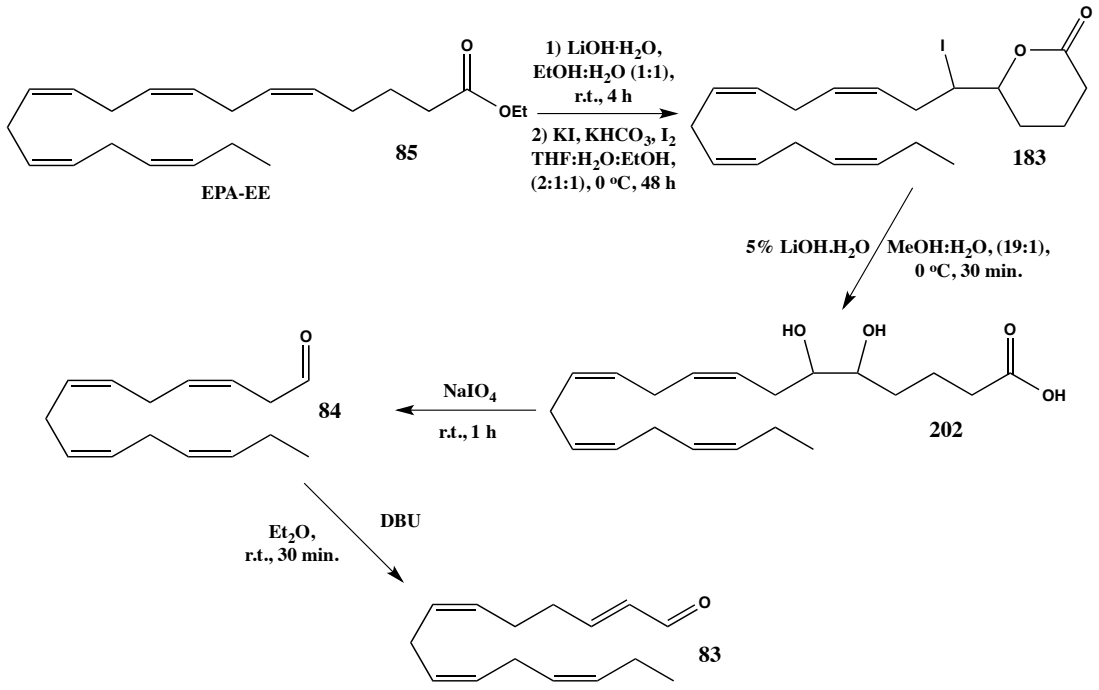
analysis of (-)-dictyosphaerin (**42**), by Rochfort and co-workers,[75] also gave inconclusive absolute or relative stereochemistry at the bicyclic fused-bridgeheads and alcohol moiety for **42**. This means, that due to the structural similarity of the ring system and side-chain lengths of both **23** and **42** it is not inconceivable that Casapullo has made a mistake with assigning the stereocenters of **23**.

Whitby, on closer analysis of his publication, has used an intermediate for crystallographic analysis that is not directly related to the intermediates used in his final synthetic pathway towards his (+)-mucosin (***ent*-23**) enantiomer. On closer inspection of the synthesis carried out by Whitby *et al.*, it seems that he ends up with a mixture of diastereoisomers, which are then separated. It is possible that he might have proceeded with the wrong diastereoisomer, which might also indicate that the appendage points have been erroneously assigned. This is not so difficult to envision as the NOESY data obtained for the current mucosin methyl ester stereoisomer **179** is almost impossible to decipher with respect to the bridgehead stereochemistry, as shown in Figure 4.2. Based on this argument, it appears that the elucidated structure of **179** discussed here and the elucidated structures of the methyl esters of **23** and ***ent*-23** discussed in the literature are not the correct naturally occurring C_{20} fatty acid found in *Reniera mucosa*.

5. Results & Discussion of the Synthesis of (+)-crucigasterin 277

5.1 Literature Synthesis of Aldehyde 83

For the total synthesis of (+)-crucigasterin 277 there were two routes (**A** and **B**) postulated in the retrosynthesis discussion in Chapter 2. Both **Route A** and **Route B** involved a common precursor, a C_{15} ω-3 allylic aldehyde, **83**, which is found in literature.[125,126]



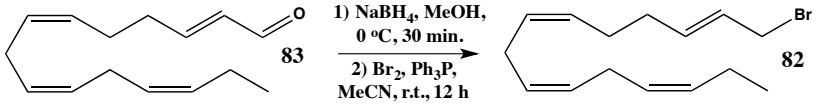
Scheme 5.1 Five-step synthesis of the allylic aldehyde **83** from EPA-EE (**85**).

To synthesize this ω-3 allylic aldehyde **83**, with a *E,Z,Z,Z*-olefinic configuration, it was necessary to use commercially available EPA-EE (**85**) as the starting material. In a five-step synthesis, starting with **85**, **83** was made by a hydrolysis and iodolactonization to form iodolactone **183**, hydrolysis of **183** with $LiOH{\cdot}H_2O$ to form diol acid **202** and periodate oxidation of diol acid **202** to form aldehyde **84**. The final step, which isomerized the *Z*-olefin to an *E*-olefin, involves the use of DBU. The ω-3 allylic aldehyde, **83**, was given in a 73% total yield over five steps, as shown above in Scheme 5.1.

5.2 Synthesis of Bromide 82 and *N*-Boc-D-alaninal for a Grignard Reaction

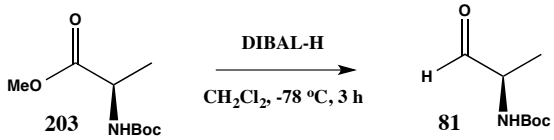
The next stage was to create an intermediate from this ω-3 allylic aldehyde **83** that would yield a carbanion species that could directly attack the aldehyde on the *N*-Boc-protected alaninal (**81**). Using the **Route A** retrosynthetic strategy, a Grignard procedure[119] with a C_{15}

allylic bromide intermediate **82** was postulated with *N*-Boc-protected alaninal (**81**). Employing the C_{15} allylic aldehyde **83**, a reduction with $NaBH_4$ and bromination with Br_2 and Ph_3P under Appel conditions gave the bromide **82** as shown in Scheme 5.2, which could be used directly in a Grignard reaction[119] with the *N*-Boc-protected alaninal (**81**).



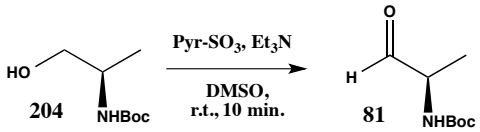
Scheme 5.2 Synthesis of allylic bromide **82** from **83**.

The next step was forming *N*-Boc-D-alaninal (**81**), which could be synthesised in a number of methods from the commercially available *N*-Boc-D-alanine methyl ester (**203**) or *N*-Boc-D-alaninol (**204**). Synthesis of **81** was first attempted by reducing the commercially available *N*-Boc-D-alanine methyl ester (**203**) with DIBAL-H at -78 °C in toluene/^{n}hexane following the literature procedure by Rinehart *et al*.[100] After multiple attempts at replicating the yield and optical purity reported, we obtained crystals of **81** in moderate optical purity and poor yield (29%). The optical purity was then improved (lit.[100] $[\alpha]^{26}_D$ = +40.7°, exp. $[\alpha]^{26}_D$ = +41.0°) by substituting toluene for CH_2Cl_2, as shown in Scheme 5.3, but again the yield remained moderate at 39% due to the lack of full conversion.



Scheme 5.3 Synthesis of *N*-Boc-D-alaninal (**81**) from *N*-Boc-D-alanine methyl ester (**203**).

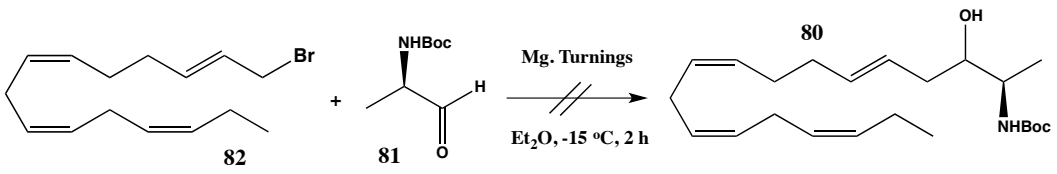
Other reducing protocols were also attempted to improve the yield but without success. It was then decided to attempt oxidation protocols on the commercially available *N*-Boc-D-alaninol (**204**). Several oxidation procedures, including TEMPO[121-123] and DMP, were tried but with no success. Following on from this, another protocol by Hamada and co-workers,[124] treats **204** with a Parikh-Doering oxidation using DMSO, a Pyr-SO_3 complex and Et_3N to yield **81**, as shown in Scheme 5.4.



Scheme 5.4 Synthesis of *N*-Boc-D-alaninal (**81**) from *N*-Boc-D-alaninol (**204**).

This oxidation proceeded without racemization, good yields (63%) and excellent optical purity (lit.[100] $[\alpha]^{26}_D$ = +40.7°, exp. $[\alpha]^{26}_D$ = +39.0°). This method was then chosen to

synthesize *N*-Boc-D-alaninal for use with the allylic bromide **82** and the Grignard reaction postulated in the retrosynthesis, which was attempted as shown in Scheme 5.5 below.

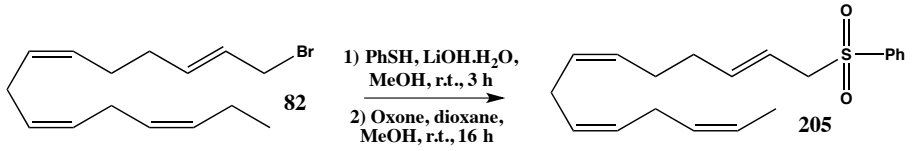


Scheme 5.5 Attempted Grignard reaction between **82** and **81** to form diastereomeric amino alcohols mixture **80**.

Unfortunately, on analysis of results from the Grignard reaction with the *N*-Boc-protected alaninal **81** and the bromide **82** the major products were the Wurtz coupling products in a 70% yield and not diastereomeric mixture **80**. Another organometallic coupling reaction using either Zn or Sn is the Barbier reaction,[182] which is a reaction with an alkyl halide and a carbonyl group in the presence of the aforementioned metals. This was attempted on the bromide **82** and *N*-Boc-protected alaninal **81** but to no avail. The next attempt was to use a Rieke metals procedure[183] with highly activated magnesium. This is reported to reduce the Wurtz coupling effect. However, this did not give any desired results as the Wurtz product still prevailed.

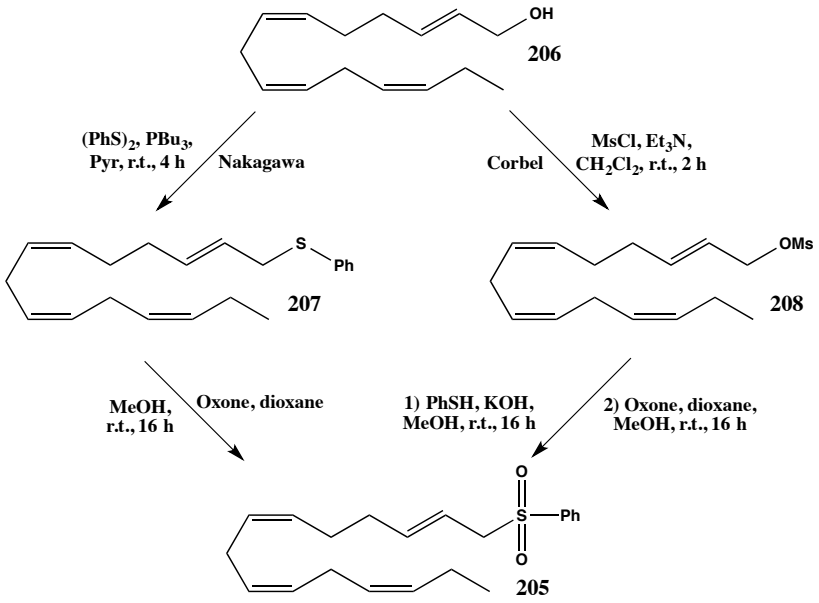
5.3 Conversion of Bromide 82 to Phenyl Sulfone 205

The next concept was to convert the bromide **82** to a phenyl sulfone **205**, which could then undergo a similar nucleophilic attack on the *N*-Boc-protected alaninal (**81**). This would then form four diastereoisomers but a subsequent desulfonation would leave only two diastereoisomers, which could then possibly be separated by silica column chromatography. A literature procedure by Trost and Curran[184] shows the ability to synthesize a phenylsulfone from a bromide in two steps using thiophenol and oxone. This procedure was attempted on the bromide **82** firstly, with thiophenol in the presence of $LiOH{\cdot}H_2O$ and secondly oxidizing the subsequent phenylsulfide with oxone to form the phenylsulfone **205** in 78% yield as shown in Scheme 5.6.



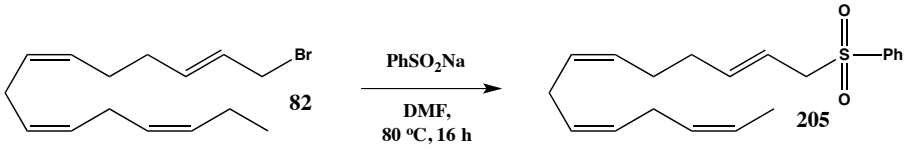
Scheme 5.6 Trost and Curran protocol to form phenyl sulfone **205** from bromide **82**.

Another second shorter literature method, to make the sulfone **205** in two steps rather than three steps compared to the first method, is by Nakagawa and co-workers.[185] Taking the alcohol precursor **206** of the bromide **82** and reacting it with diphenyl disulfide to form the sulfide **207** directly and again use oxone to make the desired sulfone **205**. However, this protocol was discarded due to a low conversion of the alcohol and a mixture of final product and starting material. A third literature method by Corbel *et al*.,[186] employed a mesylate **208**, also synthesized from the alcohol **206**, and reacted it with thiophenol together with KOH in MeOH to give quantitative yields of the corresponding sulfide **207**. Subsequent oxidation with oxone leads to the desired sulfone **205** in a total yield of 83%. These protocols were both attempted on the alcohol **206**, as shown in Scheme 5.7. The Corbel *et al*. protocol gave a much better reproducibility and yield compared to the first two methods.



Scheme 5.7 Nakagawa and Corbel protocols to form phenyl sulfone **205** from alcohol **206**.

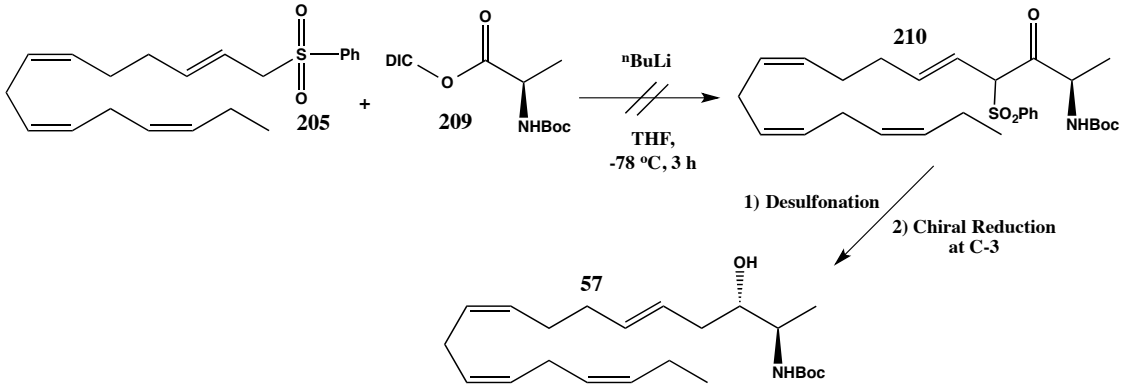
A final method to make the sulfone in one step, by Guerrero *et al*.,[187] directly from the bromide **82** involves the use of sodium phenyl sulfinate in DMF. This was successfully attempted on **82** to yield sulfone **205**, as shown in Scheme 5.8 in an 85% yield.



Scheme 5.8 Guerrero *et al*. one-step protocol to form sulfone **205** from bromide **82**.

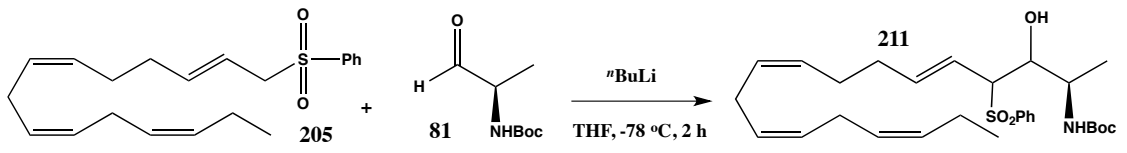
5.4 Nucleophilic Addition Between *N*-Boc-D-alaninal and Phenyl Sulfone 205

An initial nucleophilic addition reaction with a *N*,*N'*-diisopropylcarbodiimide (DIC) activated *N*-Boc-D-alanine precursor **209** and the deprotonated sulfone **205** via ^{n}BuLi was attempted as shown in Scheme 5.9.[188] This was attempted to make the diastereomeric ketone **210**, which could then be desulfonated and stereoselectively reduced at C-3 to make the necessary (*S*)-isomer at C-3, which would yield (+)-crucigasterin 277 (**57**). However, this reaction proved ineffective. It was then envisaged that a similar reaction could occur between a corresponding Weinreb amide, or a thioacetal or protected cyanohydrin analogues of *N*-Boc-D-alanine. However, these three methods seemed problematic. The Weinreb amide could be susceptible to epimerisation of the keto-amine moiety and possible racemization of the *N*-Boc-amine once the reaction had taken place with the deprotonated sulfone. The thioacetal and cyanohydrin analogues would have to be synthesised from the aldehyde directly as they are not commercially available. This leaves the synthesis and employment of the aldehyde as the preferred method, especially as it is made in literature.



Scheme 5.9 Attempted reaction between phenyl sulfone **205** and DIC-activated *N*-Boc-D-alanine **209**.

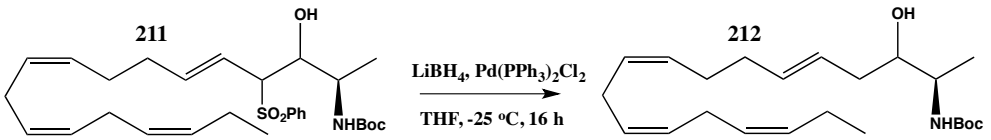
Subsequently, a reaction between the deprotonated sulfone and the optically active *N*-Boc-D-alaninal (**81**) was initiated. This was again attempted by first deprotonating the phenyl sulfone **205** with ^{n}BuLi at -78 °C in THF to form the α-sulfonyl carbanion. The *N*-Boc-D-alaninal (**81**) was then added to the reaction and after workup furnished the *N*-Boc-protected amino alcohol phenyl sulfone diastereomeric mixture **211** in 69% yield as a mixture of four diastereoisomers. This is shown in Scheme 5.10.



Scheme 5.10 Attempted reaction between phenyl sulfone **205** and *N*-Boc-D-alaninal (**81**) via ^{n}BuLi.

5.5 Desulfonation and Purification of Diastereoisomers

The next stage was to remove the sulfone group from all four of the diastereoisomers in the mixture of **211** and separate the remaining two by column chromatography. The desulfonation, as shown in Scheme 5.11, was successfully accomplised by using a protocol, by Kotake and co-workers, consisting of $LiBH_4$ and $Pd(Ph_3P)_2Cl_2$.[189,190] However, separation of the corresponding diastereoisomeric mixture **212** proved extremely difficult as both diastereoisomers always co-eluted by column chromatography. It was then decided to separate the four diastereoisomers in **211** before desulfonation and desulfonate each isomer separately. This also proved problematic as two of the diastereoisomers always co-eluted via column chromatography, which in turn reduced the yield dramatically. It is also noteworthy that the reactions of the sulfone are rather sensitive as elimination of the hydroxyl group to give the conjugated sulfone occurred when trying different temperatures and increasing reaction time.

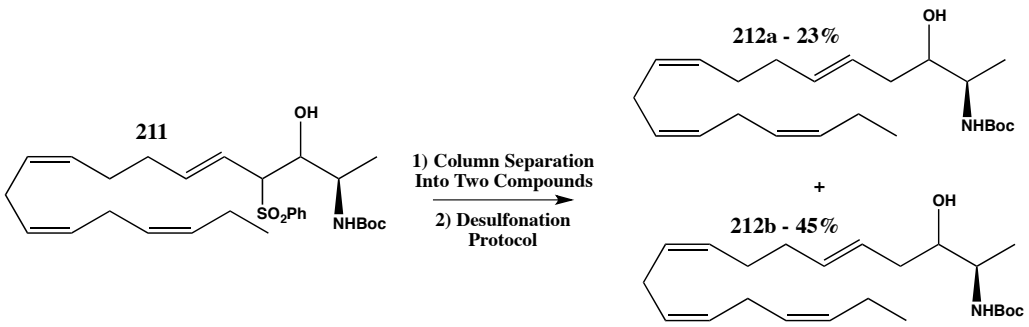


Scheme 5.11 Desulfonation of diastereomeric mixture **211**, via $LiBH_4$ and $Pd(Ph_3P)_2Cl_2$, to form diastereoisomeric mixture **212**.

Another option was to stop the oxidation of the sulfide **207** with oxone before full conversion to the sulfone **205** and obtain the sulfoxide analogue.[184] The hope was that the change in polarity from sulfoxide to sulfone would make separation on the column more manageable. However, separation of the four diastereoisomers of the sulfoxide amino alcohol analogues was even more problematic to separate by column chromatography, so this methodology was abandoned.

Following on from this, the sulfone methodology was then repeated to form **205** and this was subsequently reacted with *N*-Boc-D-alaninal (**81**) to form the same mixture of the four diastereoisomers **212**. Column chromatography was carefully repeated on this diastereomeric mixture and two pure compounds were eluted from the column in a 23% and 45% yield, respectively. When the two pure isomers were separately desulfonated via the Kotake protocol one single amino alcohol was obtained in each case **212a** and **212b**, as shown in Scheme 5.12. Based on the NMR data for these diastereoisomers (**212a** and **212b**) they only differed in configuration at C-3, but the absolute configuration of each one could not be

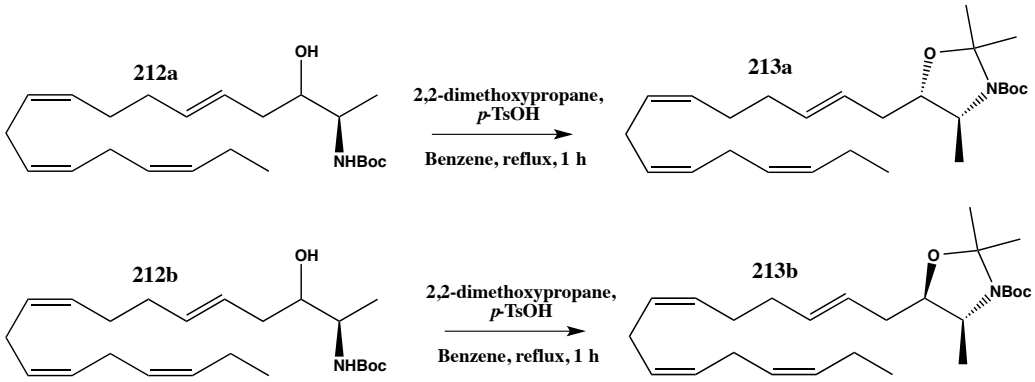
identified. This pointed towards both isomers being the 3*R* and 3*S* *N*-Boc-protected analogues of (+)-crucigasterin 277.



Scheme 5.12 The two diastereoisomers (**212a** and **212b**) resulting from the column separation and desulfonation of diastereomeric mixture **211**.

5.6 Differentiation of Diastereoisomers 212a and 212b

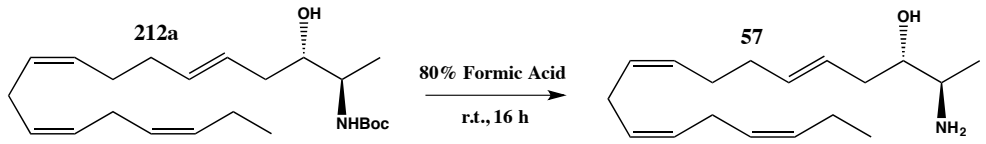
The next stage was to differentiate which diastereoisomer of **212a** and **212b** was the correct analogue to take forward to the final stage. In literature, *cis*- and *trans*-substituted isomers of 1,3-oxazolidones exhibit significantly different values for the vicinal coupling constants of the protons at C-4 and C-5, with the coupling constant being smaller for the *trans* isomer[191] as would also be anticipated from the Karplus rule. Both diastereoisomers were then separately converted to their corresponding 1,3-oxazolidones **213a** and **213b** with 2,2-dimethoxypropane in the presence of *p*-TsOH in a 74% yield respectively, as shown in Scheme 5.13. NMR analysis of both compounds was perturbed at room temperature by the presence of rotamers, which is a common phenomena for 1,3-oxazolidones.[192] To remove this problem the NMR spectra were recorded at 60 °C, which was above the coalescence temperature. The vicinal coupling constants for the protons at C-4 and C-5 of the ring were 5.0 Hz for isomer **213a** and 6.3 Hz for isomer **213b**. This result was certainly not conclusive, and the spectra were further analysed by NOESY experiments. They showed for isomer **213a** a correlation of the methylene protons attached to C-4 and the methyl protons at C-5, while no correlation was observed for **213b**. This result was further confirmed by ROESY experiments. This concluded that the correct diastereoisomer for the final conversion to (+)-crucigasterin 277 would be **213a**.



Scheme 5.13 Structures of the 1,3-oxazolidones formed from the diastereoisomers **213a** and **213b**.

5.7 Removal of the *N*-Boc-Protecting Group to Form (+)-crucigasterin 277

The final stage of the synthesis was then to simply remove the *N*-Boc-protecting group on **212a**. Literature precedents to remove the *N*-Boc-group with TFA and HCl caused partial isomerization of the olefins.[193] However, stirring **212a** in 80% formic acid or 1% HF overnight gave the correct compound of (+)-crucigasterin 277 (**57**) in 82% yield, giving a total yield of 7%.[194] The optical rotation of the diacetyl derivatives of the natural product and the synthesised analogue of (+)-crucigasterin 277 (**57**) were also in accordance with the literature (lit.[100] $[\alpha]^{26}_{D}$ = +45.0°, exp. $[\alpha]^{26}_{D}$ = +41.0°). This confirmed the absolute configuration of the natural product (+)-crucigasterin 277 (**57**).

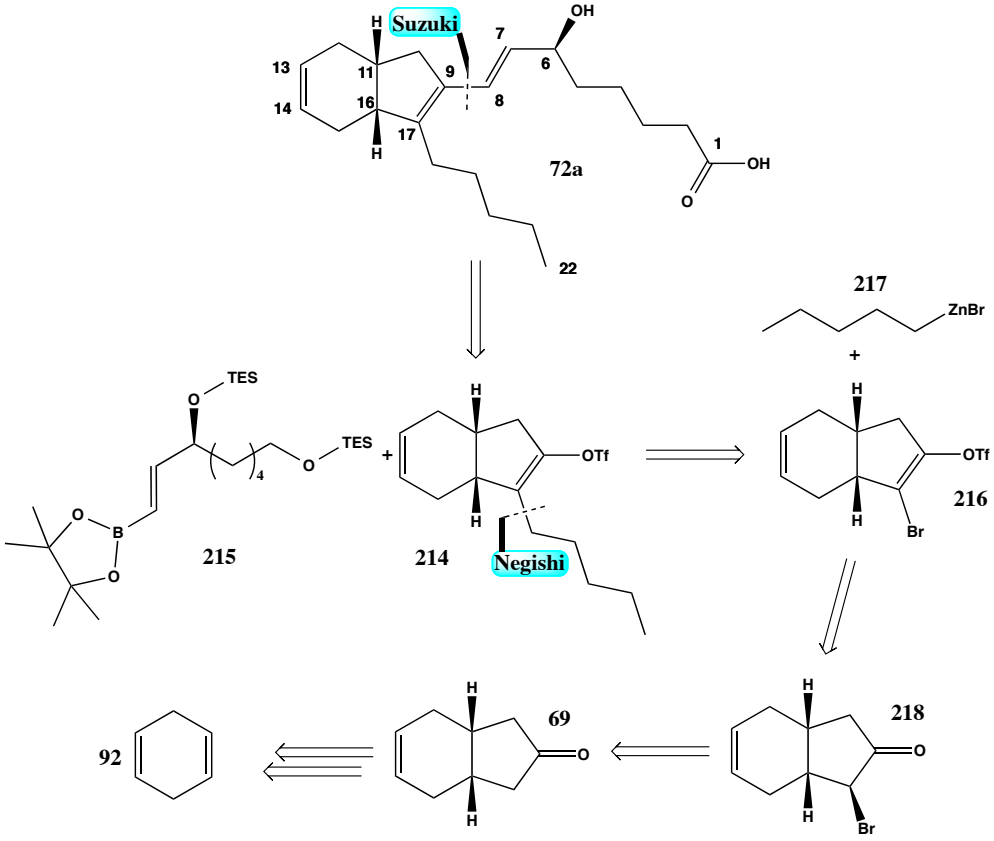


Scheme 5.14 Boc-deprotection procedure on **212a** to yield (+)-crucigasterin 277 (**57**).

6. Future Work and Biosynthetic Speculations

6.1 Remodeled Retrosynthesis of (-)-dictyosphaerin

The retrosynthesis of the second target molecule, (-)-dictyosphaerin (**42**), can be remodeled based on the knowledge gained by the total synthesis of (-)-mucosin (**23**). A much shorter retrosynthesis, than the one explained previously in Chapter 2, can be formulated to give the *cis*-fused diastereoisomers of (-)-dictyosphaerin, with varying alcohol chiral centers. This is shown in Scheme 6.1 below using the (*S*)-configured C-6 diastereoisomer **72a**.



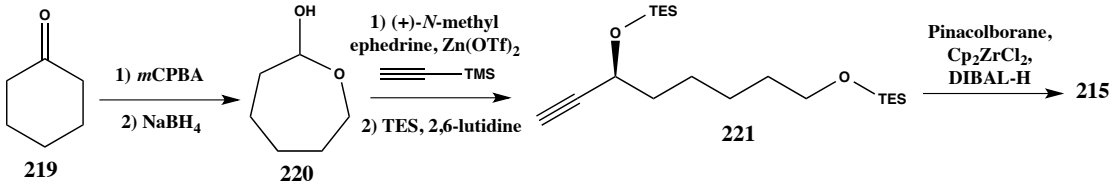
Scheme 6.1 Remodeled retrosynthesis of (-)-dictyosphaerin (**42**).

Starting with the (*S*)-alcohol diastereoisomer of (-)-dictyosphaerin **72a** it is possible to make a scission at $\Delta^{8,9}$ and form a Suzuki cross-coupling reaction, using the Littke and Fu protocol.[195] This can be undertaken with a vinylic triflate **214** and a preformed pinacolborane interemediate **215** with an *E*-olefin and (*S*)-configured chiral center at C-6. This Suzuki cross-coupling between **214** and **215** allows for the full skeleton of (-)-dictyosphaerin diastereoisomer **72a** to be formed. Subsequent selective deprotection of the TES group at C-1 with Swern oxidation[196] to the aldehyde, Pinnick oxidation of the aldehyde to the carboxylic acid and the subsequent deprotection of the TES group at C-6 with TBAF[196] will yield the

(*S*)-alcohol of (-)-dictyosphaerin **72a** with the *cis*-fused hydrogens on the bicyclo[4.3.0]nonene ring.

Following on from the Suzuki cross-coupling, the vinylic triflate **214** will be dissected at $\Delta^{17,18}$ to form an unsaturated bromo-triflate intermediate **216**. This intermediate can undergo a selective Negishi cross-coupling, again using the Littke and Fu protocol[195], with commercially available pentylzinc bromide **217** to form intermediate **214** with retention of the triflate. This gives an orthogonal reaction pathway and there is also scope to do a two-step one-pot synthesis similar to the cross-coupling procedure, as shown in Scheme 3.48 that forms the *E*-olefin. The formation of the unsaturated bromo-triflate **216** can be formed from the enantiomeric keto-bromide **218** using a bulky base and LiHMDS with Tf_2O.[197] The synthesis of the enantiomeric bromo-ketone **218** can be made via the Gais *et al*. protocol.[110] This will involve the use of the already synthesised *meso*-ketone **69**, converting it into the corresponding silyl-enol ether and adding NBS to form **218**. The synthesis of *meso*-ketone **69** can be undertaken by utilizing the aforementioned three-step synthesis starting from 1,4-cyclohexadiene (**92**).

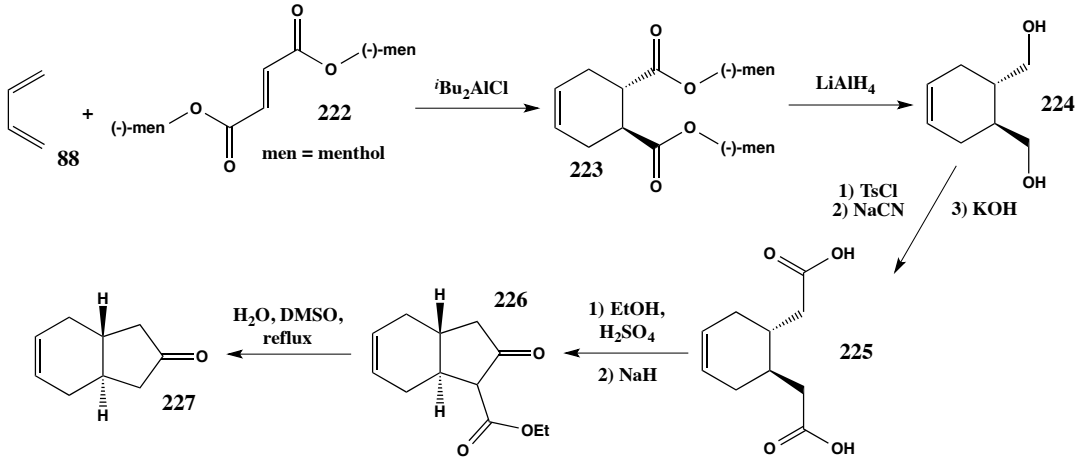
The synthesis of the pinacolborane intermediate **215**, for the Suzuki cross-coupling, can be formed in five steps as shown in Scheme 6.2. The commercially available cyclohexanone (**219**) can be converted directly to the lactol **220** using the Bayer-Villiger reaction with *m*CPBA and $NaBH_4$. The lactol can then undergo an enantioselective addition protocol,[198] with a TMS-acetylene and (+)-*N*-methyl ephedrine, and a subsequent TES protection reaction at the alcohol moieties to form **221**. The use of (-)-*N*-methyl ephedrine would lead to the (*R*)-analogue, which would be useful to form the (*R*)-alcohol diastereoisomer of (-)-dictyosphaerin **72b**. The pinacolborane intermediate **215** can be formed via the alkyne **221** using a catalytic amount of Cp_2ZrCl_2 and pinacolborane.[199] This reaction will selectively give the desired *E*-olefin for the Suzuki cross-coupling with the triflate **214**.



Scheme 6.2 Postulated synthesis for the side-chain **215**.

6.2 Postulated Syntheses of the *trans*-Fused Bicyclo[4.3.0]nonene Skeleton

Another possibility for the structure of (-)-mucosin would of course be a *trans*-fused bicyclo[4.3.0]nonene ring system. In literature, there are two identical methods[109,200] to produce the enantiomeric *trans*-fused version (**227**) of the *meso*-ketone **69**, which is shown in Scheme 6.3. Both start with an asymmetric Diels-Alder reaction using commercially available 1,3-butadiene (**88**), (-)-dimenthyl fumarate (**222**) and diisobutylaluminium chloride. This forms enantiomer **223**, a cyclohexene ring with two menthol esters in an *anti*-relationship to each other in a very high d.e. of ≥ 96%. From here the next reactions involving **223** are analogous to the Mundy and Theodore procedure[111] to produce the *meso*-ketone **69**. However, they seem to elongate the ring-closing procedure by ignoring the Ruzicka-type cyclisation with the *bis*-acid **225**, formed from diol **224**, and continue with an esterification, Dieckmann cyclisation with NaH to give **226** in a mixture of epimers (*R*:*S* = 11:1), sequential hydrolysis and decarboxylation of **226** to form the *trans*-fused bicyclo[4.3.0]nonene analogue **227** with an *e.e* of ≥ 96%.[109]

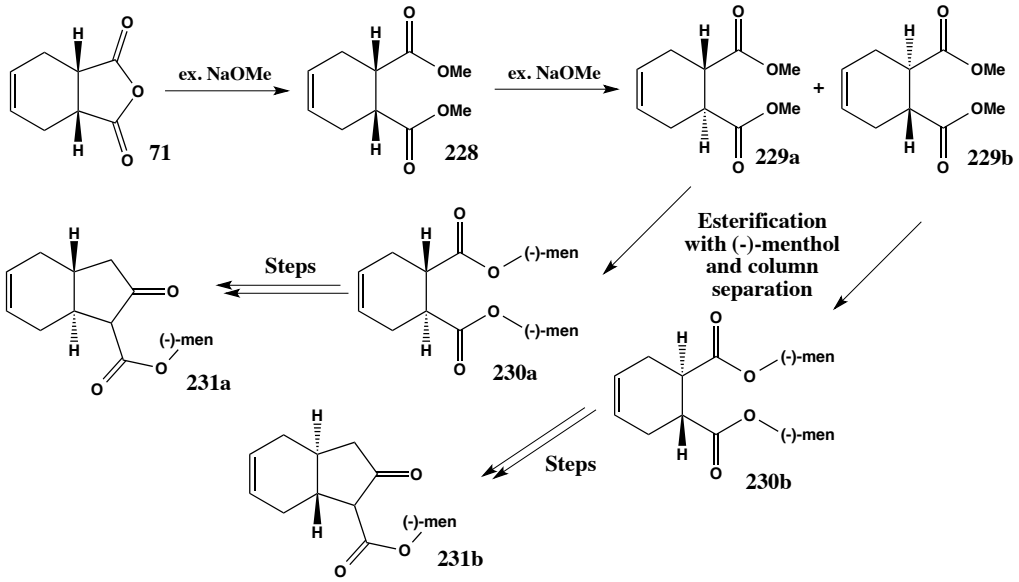


Scheme 6.3 Postulated synthesis of the *trans*-fused ketone **227**.

Using the same procedure to make the methyl ester **179** with the *trans*-fused bicyclo[4.3.0]nonene skeleton can now take place. However, employment of this procedure to synthesize the *trans*-fused **179** does not have to begin with ketone **227**. The possibility of using β-keto ester **226** as the starting point and using this to form the vinyl-triflate species removes any stereochemical issues with epimers from **226** and gives the opportunity for the synthesis of the *trans*-fused bicyclo[4.3.0]nonene diastereoisomer of **179** based on the current pathway. Future conversion of the vinyl-triflate to the saturated butyl ester and the subsequent analysis of the stereochemistry cannot be discussed in detail here and will only

become apparent once attempted. There is also the contingency here to employ the opposite enantiomer of (-)-dimenthyl fumarate and undertake the identical synthesis to give the opposite *trans*-fused bridgehead configuration to the one in Scheme 6.3.

Another method to make both the *trans*-fused bridgehead analogues of the keto-ester, shown in Scheme 6.4, would be to employ the commercially available acid anhydride **71** as discussed in Chapter 2. This could be reacted with an excess of NaOMe to create a ring opened *bis*-methyl ester analogue **228**. This could then be treated again with excess NaOMe to equilibrate itself to a mixture of racemic *trans*-fused *bis*-methyl ester analogues **229a** and **229b**. This mixture of two racemic esters can undergo chiral resolution with an optically active reagent, such as (-)-menthol, via an esterification to form two diastereoisomers, which could then hopefully be separated by column chromatography. There is also an option of converting the racemic mixture to the acid and making chiral amine salts from the acid, which could be separated by crystallization. This would furnish the two desired analogues of the *trans*-fused ring system **230a** and **230b** separately, both of which can then be taken forward to their corresponding keto-ester epimer analogues **231a** and **231b** by the method explained in Scheme 6.3. These keto-ester analogues **231a** and **231b** could then of course be converted to the desired *trans*-fused bridgehead (-)-mucosin structures and analysed accordingly.

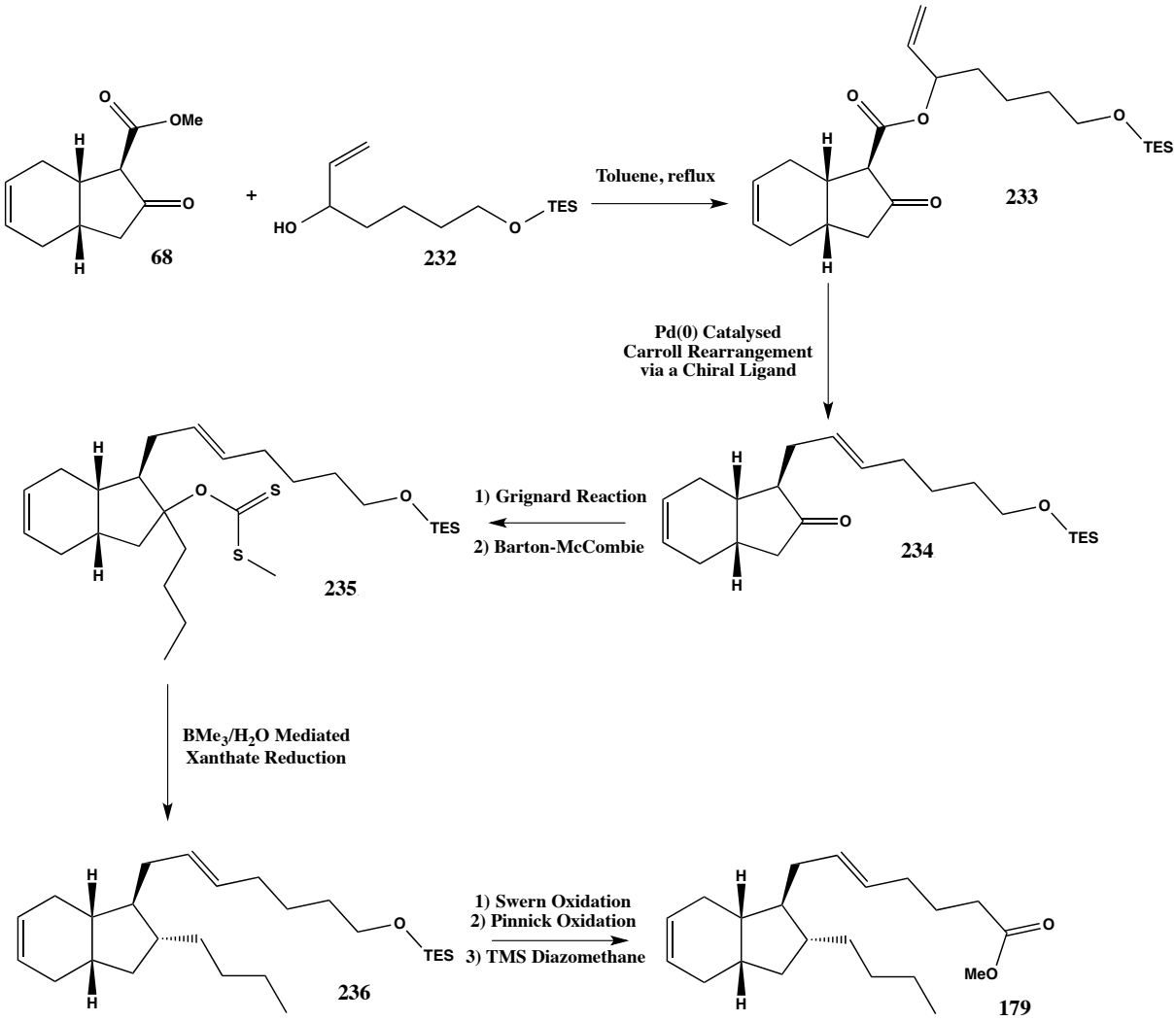


Scheme 6.4 Postulated synthesis towards both sets of *trans*-fused esters **231a** and **231b**.

6.3 A Postulated Shorter Synthon Towards the (-)-mucosin Diastereoisomer 179

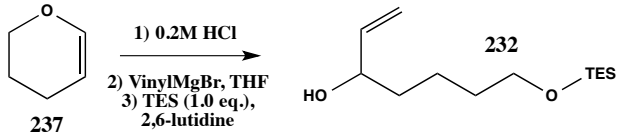
Future synthons on synthesizing the correct structure of (-)-mucosin will be focused towards shortening the number of steps in the total synthesis. One postulated synthon, as shown in Scheme 6.5, would be the direct addition of a C_7 side-chain directly onto the aforementioned optically active β-keto-ester **68**. An interesting and novel method of doing this would be undertaking an enantioselective Carroll-rearrangement reaction[201] with the pre-made trans-esterified intermediate **233**, which is made from **68** and alcohol **232**. Using a complex of a chiral ligand and a Pd(0) catalyst, such as $[Pd_2(dba)_3]$, with **233** should form an intermediate π-allyl species with the terminal olefin. Once formed, this can then undergo the Carroll-rearrangement with the neighboring ester carbonyl and form the intermediate compound **234** with extrusion of CO_2. The hope is, based on the previous (-)-mucosin synthon, is that the reaction will favor the *exo*-face of the bicyclic skeleton and the β-position, with respect to the ketone moiety, will remain in the same (*R*)-configuration as shown on compound **234**. This would be analogous to what we have found when equilibrating with NaOMe to form **164**. The other hope is that the olefin at $\Delta^{5,6}$ will be the *E*-isomer. This is based on the fact that the bulky Pd(0) catalyst and the steric hindrance of the side-chain should favor the *E*-isomer.

A subsequent Grignard reaction[202] with ^{n}butylmagnesium bromide on **234** will give an alcohol as a mixture of diastereoisomers. The alcohol can then be deoxygenated by a Barton-McCombie protocol,[203,204] by first forming the xanthate intermediate **235**, which then can be subsequently reduced by BMe_3 and H_2O rather than Bu_3SnH to give intermediate **236**. This is to stop any unwanted lipophilic side-products from the use of Bu_3SnH being present in the final compound. Once the xanthate has been removed the C_4 butyl side-chain should be *trans*-related to the C_7 side-chain, again due to steric hindrance and the results obtained on the previous synthon with NaOMe. Final removal of the TES-group at C-1 by Swern oxidation[196] to an aldehyde, oxidation of the aldehyde to the corresponding acid by a Pinnick protocol and esterification by TMS diazomethane[167], as previously discussed, should give the methyl ester **179** in fewer steps and hopefully a better yield.



Scheme 6.5 Postulated shorter synthesis towards mucosin methyl ester **179**.

The C_7 side-chain intermediate **232** can be made via a simple three-step reaction using commercially available 3,4-Dihydro-*2H*-pyran (**237**) as a starting material. The first step involves the treatment of **237** with aq. HCl to form a lactol,[205] which subsequently undergoes a ring-opening Grignard reaction with vinylmagnesiumbromide in THF.[205] The final step protects the terminal alcohol moiety with a TES-group via a reaction with 1.0 equiv. of TES and 2,6-lutidine to give the C_7 side-chain intermediate **232** as shown in Scheme 6.6.



Scheme 6.6 Postulated synthesis of the C_7 side-chain intermediate **232**.

6.4 Scope of Biological Testing on (-)-mucosin Diastereoisomer 179

The bioactivity of the (-)-mucosin diastereoisomer **179** will also have to be addressed. Both the methyl ester and acid analogues could be biologically evaluated as well as the *Z*-isomer **238** and alkyne derivative **239** of **179**, as shown in Figure 6.1. There is also the scope to synthesize the 15 stereoisomers of **179** with varying hybridization on the C_7 side-chain at $\Delta^{5,6}$ to see the complete range of bioactivity across all structures. The hope is that they will have some bioactivity, which could be comparable to the natural products with bicyclic ring systems in Figures 1.9 and 1.10 as well as the PGs as discussed in Chapter 1.

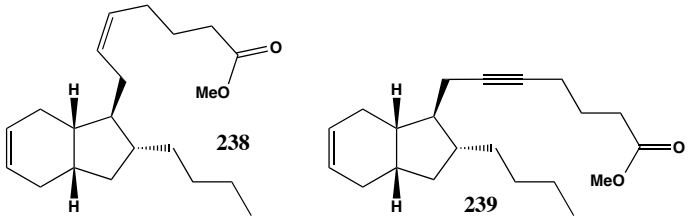


Figure 6.1 Structures of the *Z*-isomer **238** and alkyne analogue **239** of (-)-mucosin diastereoisomer **179**.

Another interesting side-note, with regards to the bioactivity of (-)-mucosin and its analogues, are the computerized docking experiments carried out (-)-dictyosphaerin (**42**). These experiments show the propensity for a chosen molecule to interact with an array of amino acids, which would mimic the active sites of enzymes in the body. When the *cis*-fused bicyclo[4.3.0]nonene structure of (-)-dictyosphaerin, with an (*R*)-configured alcohol moiety at C-6, was tested using this program the results showed a very close match with a bioactive prostanoid called (+/-)-rosaprostol (**240**).[206] The structure of this C_{18} prostanoid with *trans*-related side-chains is shown in Figure 6.2. The sodium salt of (+/-)-rosaprostol is marketed as Rosal®, which is a mixture of racemic 1,2-*trans*-1,5-*cis* and 1,2-*trans*-1,5-*trans* diastereoisomers.[207] Rosal® is used for the treatment of gastric duodenal ulcers. This could give an indication of the type of bioactivity that could be expected for (-)-dictyosphaerin and (-)-mucosin as they both have very similar structures.

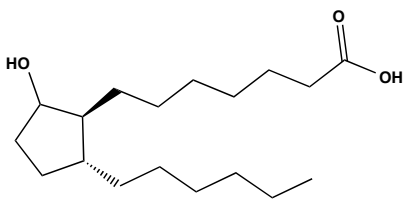
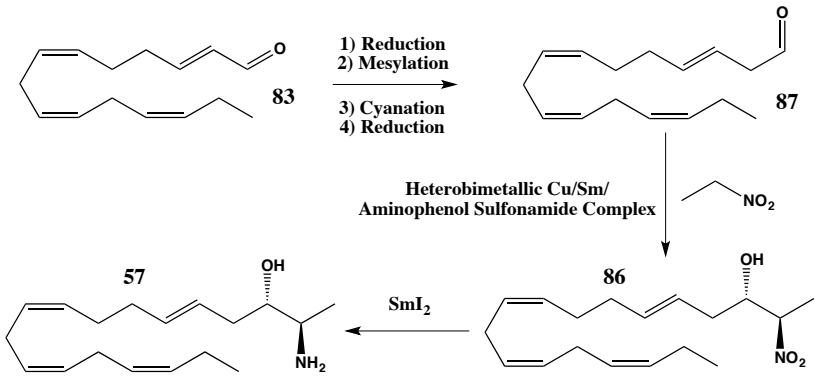


Figure 6.2 Structure of (+/-)-rosaprostol (**240**).

6.5 A More Effective Postulated Synthesis of (+)-crucigasterin 277

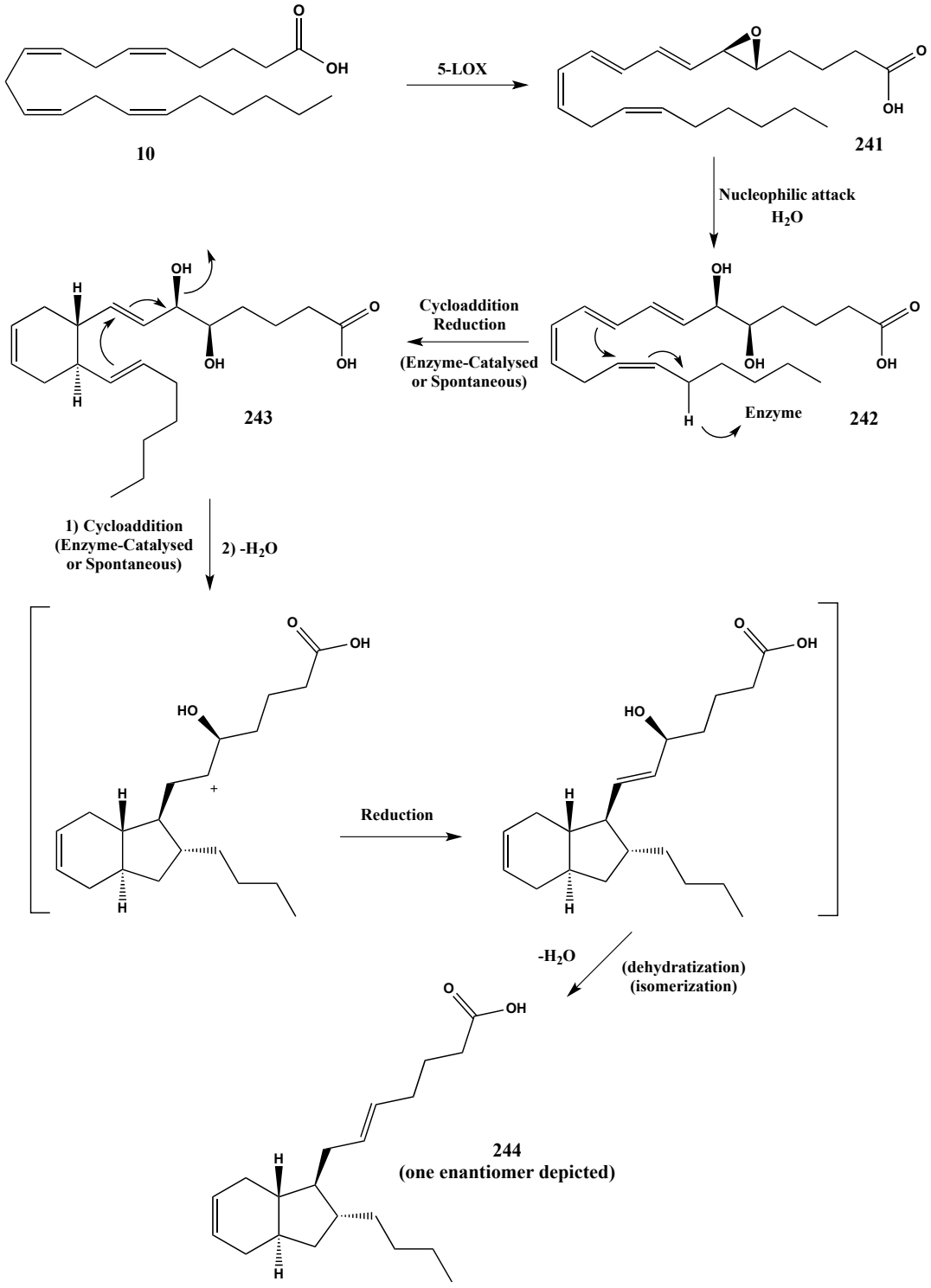
Another aspect of future work will be the exploration of a more efficient and diastereoselective method to synthesize (+)-crucigasterin 277 (**57**). The current method produces four diastereoisomers that are difficult to separate via silica-gel chromatography. Whilst it is notable that one of the two of the diastereoisomers purified by column chromatography, **213a**, can ultimately give the correct compound there are still problems with co-elution, which has an adverse effect on the yield. One method shown in Scheme 6.7, as explained in Chapter 2 with **Route B** in Scheme 2.3, would be the employment of an *anti*-selective Henry reaction protocol[127] between an aldehyde **87** and nitroethane with employment of a heterobimetallic Cu/Sm/aminophenol sulfonamide complex. This reaction has the propensity to give better yields (99%), a high d.r. (30:1) and an excellent *e.e.* (98%).[127] This is shown by the postulated formation of nitro alcohol **86** with the correct 2*R* and 3*S* absolute stereochemistry. The aldehyde **87**, the homologated version of aldehyde **83**, used to synthesize bromide **82** in **Route A**, could be synthesised via a similar homologation using the reduction/mesylation/cyanation/modified reduction protocol[129] employed during the total synthesis of the (-)-mucosin stereoisomer **179**. The subsequent reduction of the nitro group on **86** via a SmI_2 protocol[128] would yield (+)-crucigasterin 277 (**57**) in conceivably improved yields and no racemization.



Scheme 6.7 Postulated, more effective, synthesis towards (+)-crucigasterin 277 (**57**).

There has already been a similar organocatalysed synthesis, employing a stereoselective *anti*-Henry reaction by our group at UiO and NMBU, to synthesize Obscuraminol A.[96]

6.6 Postulated Biosynthesis of a *trans*-Fused Diastereoisomer of (-)-mucosin



Scheme 6.8 Postulated biosynthesis of the *trans*-fused diastereoisomer **244** of (-)-mucosin (**23**).

One postulated biosynthesis of a *trans*-fused diastereoisomer of (-)-mucosin **244** is shown above in Scheme 6.8. Starting from AA (**10**) this will undergo the well-known oxygenation at C-5 by 5-LOX in the 5-lipoxygenase pathway to form leukotriene A_4 (LTA_4) (**241**).[208] LTA_4

(**241**) is then subjected to a nucleophilic attack by water to form 5*S*,6*S*-diHETE (**242**) that has been reported in the marine environment.[209] Intermediate **242** is now ready to undergo an enzyme-catalysed or spontaneous cycloaddition reaction to form **243** with the cyclohexene moiety, which is present on the (-)-mucosin structure. This also leaves a *trans*-fused bridgehead system, which is what we would now expect with the true structure of (-)-mucosin. Following on from this the cyclopentyl moiety is formed from another enzyme-catalysed or spontaneous cycloaddition reaction via a dehydratization and migration, to give the *trans*-diastereoisomer **244** of (-)-mucosin (**23**). This is one of several possible biogenesis for (-)-mucosin, but as mentioned above, **242** is found in the ocean.

Another postulated biogenesis could be with use of Diels-Alderase enzymes (Diels-Alderases) as discussed in Chapter 1. These Diels-Alderases have the propensity to from a *trans*-fused bicyclic ring system with four-contiguous stereocenters. Many examples of this have been found in nature as shown in Schemes 1.2-1.4. However, as discussed by Minami and Oikawa, the Diels-Alderases are not necessarily participating in just a concerted process. Formal Diels-Alderases catalyze formation of a cyclohexene ring from a conjugated diene and olefin in a stereoselective manner.[210] Yet, this may include double Michael reactions, aldol-Michael reactions, bi-radical reactions and other stepwise reactions.[210] Based on the structure of **244**, with the four chiral centers and *trans*-fused bicyclic ring system, it is entirely possible that a Diels-Alderase could be involved in the biosynthesis of **244** and its diastereoisomers as well as (-)-dictyosphaerin.

7. Conclusion

This thesis describes the successful synthesis of a methyl ester (-)-mucosin diastereoisomer **179** in a seventeen-step synthesis, with 4% total yield. The synthesis also has a high optical purity with respect to the β-keto ester intermediate **68** and an excellent enantiomeric excess of ≥99% on comparison of the chiral and racemic alcohol intermediate **199**. The total synthesis consists of a novel three-step one-pot reaction to obtain the desired *E*-olefin in a combined total yield of 51%. The total synthesis also consists of an improved methodology to synthesize the *meso*-ketone **69** in three steps rather than five and almost doubling the total yield when compared to the literature methods.

After analysis of the spectral data from the methyl ester analogues of **23** and ***ent*-23** in the literature and the synthesized methyl ester stereoisomer **179** of **23** in this thesis, it has been deduced that true structure of (-)-mucosin is yet to be elucidated. This is due to Casapullo incorrectly assigning the stereocenters during his analysis of the natural product methyl ester of **23**. Similarly, Whitby *et al.* has most likely incorrectly assigned the stereocenters of his methyl ester enantiomer ***ent*-23** as he has taken forward the incorrect diastereoisomer during his synthesis and he has also undertaken X-ray crystallography on an intermediate, which has no direct relation to the synthesis of ***ent*-23**. This leaves the interesting question as to the true structure of (-)-mucosin. The same question is also true of (-)-dictyosphaerin and will become apparent when the all the stereoisomers of **42** have been synthesized. The same can also be said of the biogenesis for both (-)-mucosin and (-)-dictyosphaerin, whether they utilize an enzyme-catalysed intramolecular cycloaddition or a Diels-Alderase type formation still needs to be resolved. The bioactivity of (-)-mucosin and (-)-dictyosphaerin also needs to be tested and see whether they will have a similar bioactive range as PGs such as (+/-)-rosaprostol in Figure 6.2 and/or the sesquiterpenoids in Figures 1.9 and 1.10.

Another added benefit of this total synthesis is that 23 mg of the pure methyl ester (-)-mucosin diastereoisomer **179** was obtained. When compared to the amount of the (-)-mucosin methyl ester isolated from *Reniera mucosa*, by Casapullo *et al.*,[58] (8.5 mg from 1.35 Kg of sponge) this is nearly three times the amount, even synthesizing on a small scale. The total synthesis of **179** and its postulated analogues already seems to be beneficial towards

protecting the marine environment using this method and also has the scope to undertake in-depth biological testing when synthesised on a larger scale.

This thesis also describes the total synthesis of the cytotoxic amino alcohol (+)-crucigasterin 277 (**57**) via a ten-step synthesis in a 7% total yield. The diacetyl synthetic analogue of (+)-crucigasterin 277 also has a high optical purity on comparison with the diacetyl derivative found from nature. However, the synthon itself is not optimized, especially with respect to the lack of stereoselectivity of the reactions chosen and the total yield. The prospect now is to attempt the synthesis in Scheme 6.7 to make (+)-crucigasterin 277 and improve the diastereoselectivity of the reaction, reduce the number of reactive steps and increase the total yield.

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Appendix

PAPER I

Total synthesis based on the originally claimed structure of mucosin

Harrison C. Gallantree-Smith, Simen G. Antonsen, Carl H. Görbitz, Trond V. Hansen, Jens M. J. Nolsøe and Yngve H. Stenstrøm, *Org. Biomol. Chem.*, **2016**, Accepted; 09/08/2016

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Total synthesis based on the originally claimed structure of mucosin†

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The first total synthesis aimed at the naturally occurring eicosanoid bicycle mucosin is reported. A practical route has been devised allowing the issues relating to the previous assignment of stereochemistry to be examined. X-ray crystallography was performed on a late stage intermediate to pinpoint the topological relationship displayed by the featured bicyclo[4.3.0]non-3-ene scaffold.

Marine organisms continue to be a rich source of novel natural products,[1] constituting a pharmacopeia of biological response and evolutionary adaption that is yet to be explored within the context of human physiology. In particular, compounds originating from polyunsaturated fatty acid metabolites, such as eicosanoids and docosanoids, have a key role in the cascade triggered by injurious stimuli, both in terms of the inflammatory feedback as well as the catabasis leading to the restoration of homeostasis.[2]

Against this setting, our attention has been drawn towards the structure of mucosin (**1**), an unusual bicyclic lipid surmised to be the progeny of arachidonic acid (**3**) (Fig. 1). Isolated as methyl ester **2** from the Mediterranean sponge *Reniera mucosa*, Casapullo *et al.* assigned the structure and relative stereochemistry by applying several analytical techniques (MS, IR and various NMR experiments).[3] Given the assumed biogenetic origin, the most noticeable feature is the *cis*-fused bicyclo[4.3.0]non-3-ene core, indicating a formal disrotatory ring closure.[4] In view of mounting evidence implicating enzymes effectuating the Diels–Alder reaction in biological systems,[5] one might also venture this to be the case for a host of other pericyclic transformations, like the ene reaction.

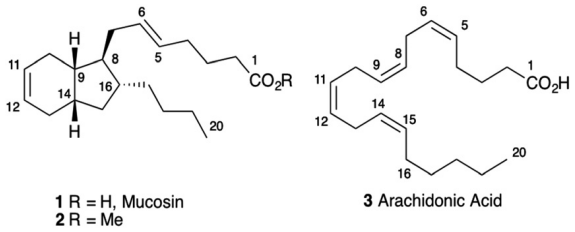

Fig. 1 Relationship between the mucosin scaffold and arachidonic acid.

Thus, allylic carbocations have been invoked as conceptual intermediates in the biogenesis of marine carbocyclic oxylipins, such as prostaglandin A_2 (PGA_2).[6] From a biological perspective, the structural resemblance between mucosin (**1**) and the prostane scaffold calls for attention, considering the vasomodulation associated with the latter compound class.[2a] Prompted by the aspects related above and, in particular, the intriguing findings presented by Casapullo *et al.*,[3] we herein wish to report the first total synthesis of mucosin (**1**) according to the nominal structure.

As a pivotal point in our synthetic strategy (Scheme 1), it was envisioned that desymmetrization of *meso*-ketone **4** could be used as a means to furnish a chiral handle.[7] With this installed, the plan was to further elaborate the functional pattern and prepare the route to the remaining stereocenters contained within the ring system. Taking advantage of the innate conformational aspects, it was anticipated that hydride addition from the less hindered *exo* face of the conjugated ester **7** would establish the chiral configuration attributed to mucosin (**1**).

Synthesis of *meso*-ketone **4** was executed in three steps according to a novel protocol, starting from the commercially available 1,4-cyclohexadiene (**9**) (Scheme 2). Commencing with a [2 + 2] cycloaddition between the preformed dichloroketene and **9**,[8] the *cis*-fused junction was installed. Next, the resulting 8,8-dichlorobicyclo[4.2.0]oct-3-en-7-one (**10**) was subjected to ring expansion by applying the Büchner–Curtius–Schlotterbeck reaction.[9] Finally, zinc mediated hydrodehalogenation of *gem*-dichloroketone **11** completed the sequence

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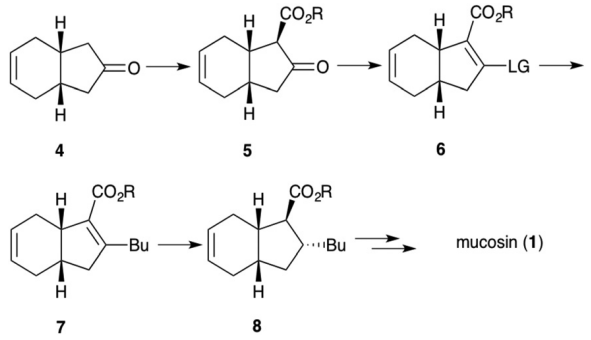
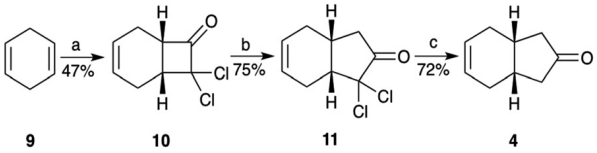
† Electronic supplementary information (ESI) available: Experimental procedures and characterisation data for all new compounds; crystal data and refinement details are included to give evidence of the relative stereochemistry of a late stage intermediate. CCDC 1484546. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c6ob01511e



Scheme 1 Key strategic points towards synthesis of mucosin (**1**).



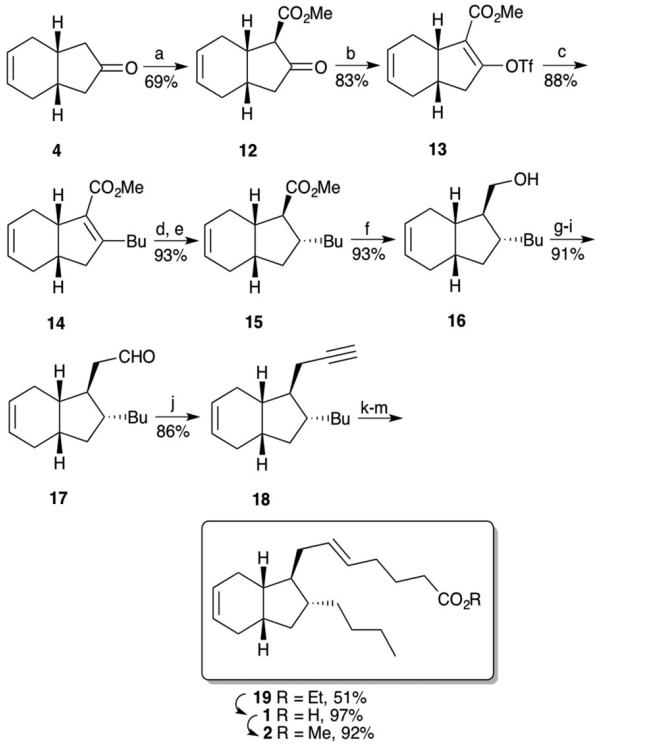
Scheme 2 Synthesis of *meso*-ketone **4**. Reagents and conditions: (a) Zn in Et_2O with sonication, 0 °C, 0.25 h, then slow addition of trichloroacetyl chloride, 0 °C, 2 h, then $NaHCO_3$ (aq., satd.); (b) slow addition of CH_2N_2 in Et_2O, 0 °C, 0.25 h, then 0.5 h, 0 °C, followed by 2 h, rt, then abs. AcOH; (c) Zn in abs. AcOH, 70 °C, 16 h.

and provided an expedient entry to the desired starting material **4**.

The planned stereochemical iteration of *meso*-ketone **4** to obtain mucosin (**1**) (Scheme 3) was undertaken by nominating a chiral base with some suitable electrophiles. Combining the lithium amide derived from (+)-bis[(*R*)-phenylethyl]amine with methyl cyanoformate achieved desymmetrization to yield the known ketoester **12** in excellent agreement with reported values.[10] Considering the more encumbered six step procedure of Nagao *et al.*, which is hinged on asymmetric Dieckmann condensation of a chiral diamide,[10a] the preparation of the optically active ketoester **12** delineated in the present work is less involved.

Converting **12** to the corresponding enol triflate **13**, and subsequent cross coupling to introduce the alkyl group at C16, gave a conjugated ester **14**. Rather than taking advantage of some Pd-catalysed protocols, concomitant addition/elimination *via* a stoichiometric cyanocuprate was opted for.[11]

Given that the two diastereotopic faces of the conjugated system in **14** are distinguishable, due to the different steric requirements of the *exo* and *endo* stance, one would expect this to be borne out during a hydride reduction. Thus, *a priori*, it was assumed that addition from the *exo* face would be highly preferred and give rise to the projected stereoisomer **15** (Fig. 2). The constellation of magnesium in methanol[12] proceeded to afford a 2 : 1 mixture of C8 epimers, which could be equilibrated to the desired diastereomer **15** in the presence of sodium methoxide. Further reduction of ester **15** yielded an advanced intermediate **16**, which was also found amenable for derivatization in order to obtain crystals for X-ray analysis. This established the relative stereochemistry of the four contig-



Scheme 3 The total synthesis of mucosin (**1**) and its methyl ester **2**. Reagents and conditions: (a) (+)-bis[(*R*)-phenylethyl]amine hydrochloride with BuLi in THF, −78 °C, 0.25 h, then briefly at rt, re-cooled to −78 °C, dropwise addition of **4**, −78 °C, 0.75 h, then methyl cyanoformate, −78 °C, 2 h, water quenching; (b) NaH in toluene, rt, 10 min, then 85 °C, 1.5 h, Tf_2O added under cold conditions, 0 °C, 1 h, water quenching; (c) CuCN in Et_2O, −50 °C, then BuLi, 1 h, added to **13**, −50 °C, 1 h, then NH_4Cl (aq., satd.); (d) Mg in MeOH, 40 °C, 3h, then abs. AcOH, rt; (e) Na in MeOH, Δ, 3h (f); DIBAL-H in hexane, 0 °C to rt, 0.5 h, then Rochelle's salt (aq., satd.), 0 °C; (g) Et_3N in CH_2Cl_2, followed by MsCl, briefly 0 °C then rt, 2 h; (h) KCN in DMSO, 70 °C, 2 h; (i) DIBAL-H in hexane, −78 °C, 0.33 h, then Rochelle's salt (aq., satd.) under cold conditions; (j) the Ohira–Bestmann reagent and K_2CO_3 in MeOH, rt, overnight; (k) (1) DIBAL-H added to Cp_2ZrCl_2 in THF, 0 °C, 1 h, then **18**, 0 °C, 1 h. (2) I_2 added under cold conditions, then rt, 1 h. (3) 4-Ethoxy-4-oxobutylzinc bromide and $(Ph_3P)_4Pd$ (10 mol%), rt, 1 h, then HCl (1 M, aq.); (l) LiOH in THF/MeOH/H_2O, rt, 3 h; (m) $TMSCHN_2$ in toluene/MeOH, rt, 3 h.

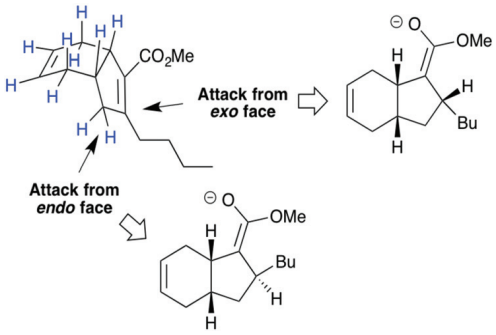


Fig. 2 Hydride attack on the distinguishable diastereotopic faces of conjugated ester **14**.

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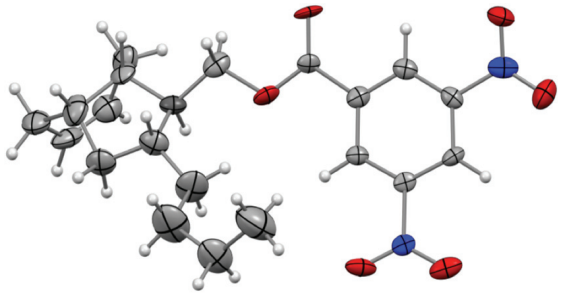

Fig. 3 Single crystal X-ray structure obtained from the 3,5-dinitrobenzoate of alcohol **16** at 110 K. The *n*-butyl conformation is shown with a *trans, trans, gauche*+ orientation relative to the dark C atom. For clarity, a minor *trans*, *gauche*−, *gauche*− orientation with occupancy 0.26(3) has been omitted.

uous centres (Fig. 3). Moreover, analysis of **16** by GLC on a chiral column showed an enantiomeric excess better than 99%.

Having installed the assigned topology, **16** was sequentially elaborated into alkyne **18** *via* a four step procedure, featuring Ohira–Bestmann homologation as the key.[13] The final step was executed in an untested three step one-pot approach, taking advantage of the facile alkyne hydrometallation,[14] followed by halodemetallation[15] to form the corresponding *E*-vinyl halide as a substrate for Pd-catalysed cross-coupling with a commercial zinc reagent.[16] Finally, the portrayed natural compound mucosin (**1**) was obtained after hydrolysis of ethyl ester **19**. In order to compare with data cited in the literature, the free acid was re-esterified to methyl ester **2**.

The recordings made on the material furnished by our synthetic route are at odds with the values reported in the literature,[3,17] both with regard to optical rotation and resonances quoted in ^{13}C NMR. Thus Casapullo *et al.* have provided $[\alpha]_D^{26}$ = −35.5° (*c* = 0.8, hexane),[3] while Whitby and co-workers have found $[\alpha]_D^{26}$ = +38.2° (*c* = 0.8, hexane) for the enantiomer.[17] We, on the other hand, have observed a reading of $[\alpha]_D^{26}$ = −9.8° (*c* = 0.8, hexane). However, the physical difference between our compound and the methyl ester of naturally occurring mucosin is more tangible when the ^{13}C NMR spectra are placed side by side: of the 20 atoms that constitute the carbon framework, excluding the methoxy group, there are 12 resonances that deviate (Table 1).

To summarise, based on the reported structure of mucosin (**1**), we have devised a synthetic strategy to access the four contiguous stereocentres. A novel route was conceived for the known *meso*-ketone **4**, which was manipulated during the course of 11 discrete steps to achieve the target molecule **1** in an overall yield of 16.9%, delivering multi-milligram quantities. With the cumulative information obtained in this process, and in particular underpinned by X-ray crystallography of the late stage intermediate **16**, we raise the question about the true identity of mucosin. In the absence of a thorough discussion of stereochemistry[3,18] and the lack of structural evidence directly linked to the main synthetic events,[17] the risk of misassignment is a looming peril.[19] Thus,

Table 1 Observed ^{13}C NMR resonances for methyl ester **2** (*δ*-values)

Casapullo *et al.*[3]	Whitby *et al.*[17]	This work
174.2	174.2	174.2
130.0	130.3	130.4
129.8	129.8	129.9
127.0	127.3	***126.3***
127.0	127.1	***126.1***
52.1	52.2	***51.4***
51.4	51.4	51.0
47.1	47.2	***44.0***
42.1	42.3	***40.3***
39.9	40.1	***38.1***
36.7	37.0	***37.7***
36.5	36.74	***37.1***
36.4	36.68	***34.9***
33.2	33.4	33.4
32.0	32.4	31.9
31.7	31.9	***31.0***
31.5	31.6	***27.8***
30.7	30.7	***27.7***
24.5	24.7	24.8
22.6	22.9	22.9
13.8	14.1	14.1

by executing our sequence, it has been demonstrated that structure **1** does not represent naturally occurring mucosin.

The *cis*-fused bicyclo[4.3.0]non-3-ene system inferred to be present in mucosin by Casapullo *et al.*[3] is not a widespread motif in nature. In fact, to our knowledge, the only other example surmised to incorporate the pertinent structural element is dictyosphaerin, a marine natural product isolated from the green algae *Dictyosphaeria sericea*.[20] However, in this case, neither the absolute nor the relative stereochemistry has been established.

Considering mucosin, depending on the extent of concert between enzyme promoted and spontaneous processes, the biogenetic machinery leading to the formation of a *cis*-configured bicyclo[4.3.0]non-3-ene system may involve one or several of the following basic steps: (i) a disrotatory ring closure,[4] (ii) a Diels–Alder reaction,[5] or (iii) an ene reaction.[21] In all events, arachidonic acid (**3**) is the most plausible substrate, based on its presence in the marine environment[22] and the structural elements identified in mucosin.[3] Regardless of the cycloaddition invoked, it is necessary that **3** is biosynthetically transformed into a suitable conjugated precursor, in terms of the downstream process leading to the claimed structure **1** of mucosin. Interconversion of **3** into *E*,*E*- and *E*,*Z*-conjugated olefins is known to occur in several marine species.[22,23] The resulting conjugated C20 fatty acids are obtained either by an enzymatic isomerization of the skipped all-*Z* polyenic system,[24] or by dehydration of the corresponding *E*- or *Z*-allylic carbinol derivative.[6,22,23] The action of lipoxygenase enzymes on the polyene backbone of **3** leads to the stereoselective formation of secondary alcohols,[6,24] providing natural products that incorporate mono- and dihydroxylated *E*,*E*-diene motifs. Such conjugated motifs participate in enzyme catalysed Diels–Alder reactions to form bicyclo[4.4.0]deca-3-ene systems. The action of a Diels–Alderase in the biosynthesis of natural products is exemplified by statins,[25] anthramycins,[26] solano-

pyrones,[27] spinosyns[28] and macrophomic acids.[29] Most conspicuously, all of the listed compound classes display a *trans*-disubstituted cyclohexene. In contrast, the involvement of a Diels–Alderase in the formation of *cis*-bicyclo[4.4.0]deca-3-ene systems has not yet been reported.

In the instance of non-enzymatic cycloaddition, formation of the marine carbocyclic oxylipins takes place *via* an allylic carbocation.[6,22] For the overwhelming majority of the provided examples, the outcome of the annealing process is a *trans*-1,2-disubstituted cyclopentane ring.[30]

In light of the discussion above and the flawed assignment of mucosin, it is clear that thorough biosynthetic studies and/or preparation of stereochemical permutations based on structure **1** are needed.

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18 To provide a convincing rational for the proposed structure of mucosin (**1**), similar NOESY/ROESY correlations between protons along the bisecting plane of the conjectured pseudo-symmetric configuration must be expected. However, this is not the case, since only correlations have been reported for H9/H16 and not for H14/H16. Moreover, no correlations have been reported for H14/H15ab. Thus, one can neither infer topology at the points of fusion nor at the points of appendage. It is also disconcerting that there is no reciprocal correlation reported for H8/H17 nor for H7/H10b, which is ought to be the case for a *cis*-fused/*trans*-substituted system.
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Supporting Information for:

Total Synthesis Based on the Originally Claimed Structure of Mucosin

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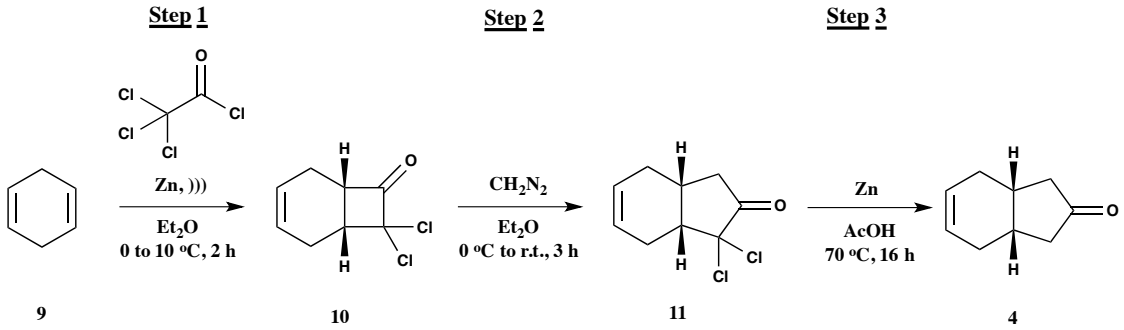
Table of Contents:

General Information:

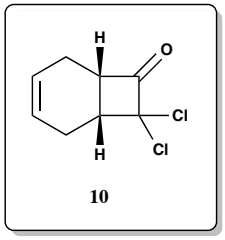
All commercially available reagents and solvents were used in the form they were supplied without any further purification. (+)-*bis*[(*R*)-1-phenylethyl]amine hydrochloride (optical purity ≥ 99% *e.e.* by GLC) was purchased from Sigma-Aldrich. The stated yields are based on isolated material. The melting points are uncorrected. Thin layer chromatography was performed on silica gel 60 F_{254} aluminium-backed plates fabricated by Merck. Flash column chromatography was performed on silica gel 60 (40-63 μm) fabricated by Merck. NMR spectra were recorded on a Bruker Ascend^{TM} 400 at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR. Coupling constants (*J*) are reported in hertz and chemical shifts are reported in parts per million (δ) relative to the central residual protium solvent resonance in ^{1}H NMR ($CDCl_3$ = δ 7.27) and the central carbon solvent resonance in ^{13}C NMR ($CDCl_3$ = δ 77.00 ppm). Mass spectra were recorded at 70 eV on Waters Prospec Q spectrometer using EI as the method of ionization. IR spectra (4000–600 cm−1) were recorded on a Perkin-Elmer Spectrum BX series FT-IR spectrophotometer using a reflectance cell (HATR). Optical rotations were measured using a 1 mL cell with a 1.0 dm path length on a Perkin Elmer 341 polarimeter using the stated solvents. Determination of enantiomeric excess was performed by GLC on an Agilent Technologies 7820A GC instrument with split (1:30) injection, FID detector and equipped with a chiral stationary phase (Agilent J&W GC columns, CP-Chirasil-DEX CB, 25 m, 0.25 mm, 0.25 μm) applying the conditions stated. X-ray crystallography was performed on a Bruker D8 Venture diffractometer with InCoatec ImuS Microfocus radiation source and Photon 100 CMOS detector. Data collection with Apex2,[1] data integration and cell refinement with SAINT,[1] absorption correction by SADABS,[1] structure solution with SHELXT,[2] structure refinement with SHELXL.[3] Molecular graphics from Mercury.[4]

Preparation of *meso*-ketone (4):



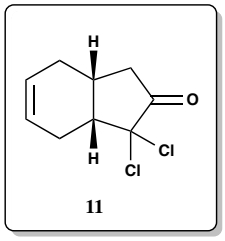
Scheme S-1 Synthetic route to *meso*-ketone **4**.

***rac*-(1*R*,6*S*)-8,8-Dichlorobicyclo[4.2.0]oct-3-en-7-one (10).[5]**



1,4-Cyclohexadiene **9** (5 g, 62.5 mmol, 1.0 equiv.) was added to a suspension of zinc powder (8.2 g, 125 mmol, 2.0 equiv.) in dry Et_2O (100 mL) and sonicated at 0 °C for 15 min. Then trichloroacetylchloride (22.75 g, 125 mmol, 2.0 equiv.) in dry Et_2O (100 mL) was added dropwise over 2 h while the reaction mixture was still sonicating. After complete addition the reaction mixture was sonicated for a further 2 h at 0-10 °C. The colour changed from colourless to dark yellow. The sonication was then stopped and the reaction mixture filtered and concentrated *in vacuo*. The resulting orange slurry was diluted in Et_2O (400 mL) and washed with H_2O (2 x 400 mL) and sat. aq. $NaHCO_3$ (1 x 400 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The resulting crude dark yellow oil was purified by column chromatography on silica (^{n}hexane/EtOAc 99:1) to afford the title compound as a colourless oil. All spectroscopic and physical data were in full agreement with those reported in the literature.[5] Yield: 2.25 g (47%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.90-5.81 (m, 2H), 4.07-4.01 (m, 1H), 3.32 (ddt, J = 2.0, 7.9, 10.4 Hz, 1H), 2.63-2.50 (m, 2H), 2.39-2.32 (m, 1H), 2.17-2.10 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.3, 127.3, 126.3, 88.5, 53.7, 45.2, 23.1, 21.3; IR (neat, cm^{-1}) 3041 (w), 2939 (w), 2895 (w), 2841 (w) 1799 (s), 1644 (w), 1433 (m); HRMS (EI+): Exact mass calculated for $C_8H_8OCl_2$ $[M]^+$: 189.9952, found 189.9953; TLC (^{n}hexane/EtOAc 4:1, $KMnO_4$ stain): R_f = 0.65.

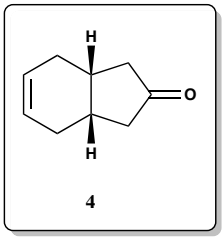
***rac*-(1*R*,6*R*)-7,7-Dichlorobicyclo[4.3.0]oct-3-en-7-one (11).**



11

To a stirring solution of *rac*-(1*R*,6*S*)-8,8-dichlorobicyclo[4.2.0]oct-3-en-7-one **10** (2 g, 10.47 mmol, 1.0 equiv.) in dry Et_2O (50 mL), at 0 °C, was added diazomethane (2.47 g, 104.7 mmol, 10.0 equiv.) in dry Et_2O (50 mL) dropwise over 15 min. The reaction mixture bubbled and turned a deep golden yellow colour. After 30 min the reaction was warmed to room temperature and left to stir for 2 h. The reaction was then quenched with glacial AcOH (5 mL) dropwise until there was no more gas evolution and the colour changed from golden yellow to almost colourless. The resulting mixture was then washed with H_2O (2 x 300 mL), sat. aq. $NaHCO_3$ (1 x 300 mL), brine (1 x 300 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The resulting dark yellow oil was purified by column chromatography on silica (^{n}hexane/EtOAc 9:1) to afford the title compound as a colourless oil. Yield: 1.6 g (75%); ^{1}H NMR (400 MHz, $CDCl_3$) δ; 5.64-5.57 (m, 2H), 2.86-2.80 (m, 1H), 2.74-2.69 (m, 1H), 2.54 (dd, *J* = 7.5, 19.2 Hz, 1H), 2.38-2.29 (m, 2H), 2.07-2.00 (m, 2H), 1.72-1.64 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.7, 123.9, 123.1, 89.5, 46.7, 36.6, 28.1, 25.8, 23.5; IR (neat, cm^{-1}) 3033 (m), 2916 (m), 2842 (m), 1764 (s) 1662 (w) 1434 (m), 1402 (m); HRMS (EI+): Exact mass calculated for $C_9H_{10}OCl_2$ $[M]^+$: 204.0109, found 204.0103; TLC (^{n}hexane /EtOAc 4:1, $KMnO_4$ stain): R_f = 0.60.

***meso*-(1*S*,6*R*)-Bicyclo[4.3.0]non-3-ene-8-one (4).[6]**



4

To a stirring suspension of zinc powder (1.71 g, 26.34 mmol, 2.0 equiv.) in glacial AcOH (50 mL) was added *rac*-(1*R*,6*R*)-7,7-dichlorobicyclo[4.2.0]oct-3-en-7-one (**11**) (2.7 g, 13.17 mmol, 1.0 equiv.) in glacial AcOH (30 mL) dropwise. The resulting reaction mixture was stirred for 16 h at 70 °C. The reaction mixture was then cooled to room temperature and filtered to remove the resulting solid. The filtrate was diluted with CH_2Cl_2 (200 mL) and

washed with H_2O (2 x 300 mL), sat. aq. $NaHCO_3$ (1 x 300 mL), brine (1 x 300 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The resulting crude pale yellow oil was purified by column chromatography on silica (^{n}hexane/EtOAc 95:5) to give the *meso* compound as a colourless oil. All spectroscopic and physical data were in full agreement with those reported in the literature.[6] Yield: 2.45 g (72%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.70-5.69 (m, 2H), 2.46-2.41 (m, 2H), 2.34-2.25 (m, 4H), 2.10 (dd, J = 6.4, 18.6 Hz, 2H) 1.89-1.83 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 219.6 124.6 (2C), 44.6 (2C), 32.3 (2C), 26.2 (2C); IR (neat, cm^{-1}) 3024 (m), 2834 (m), 2901 (s), 1744 (s), 1655 (w), 1439 (m), 1407 (s); HRMS (EI+): Exact mass calculated for $C_9H_{12}O$ $[M]^+$: 136.0888, found 136.0983; TLC (^{n}hexane /EtOAc 4:1, $KMnO_4$ stain): R_f = 0.51.

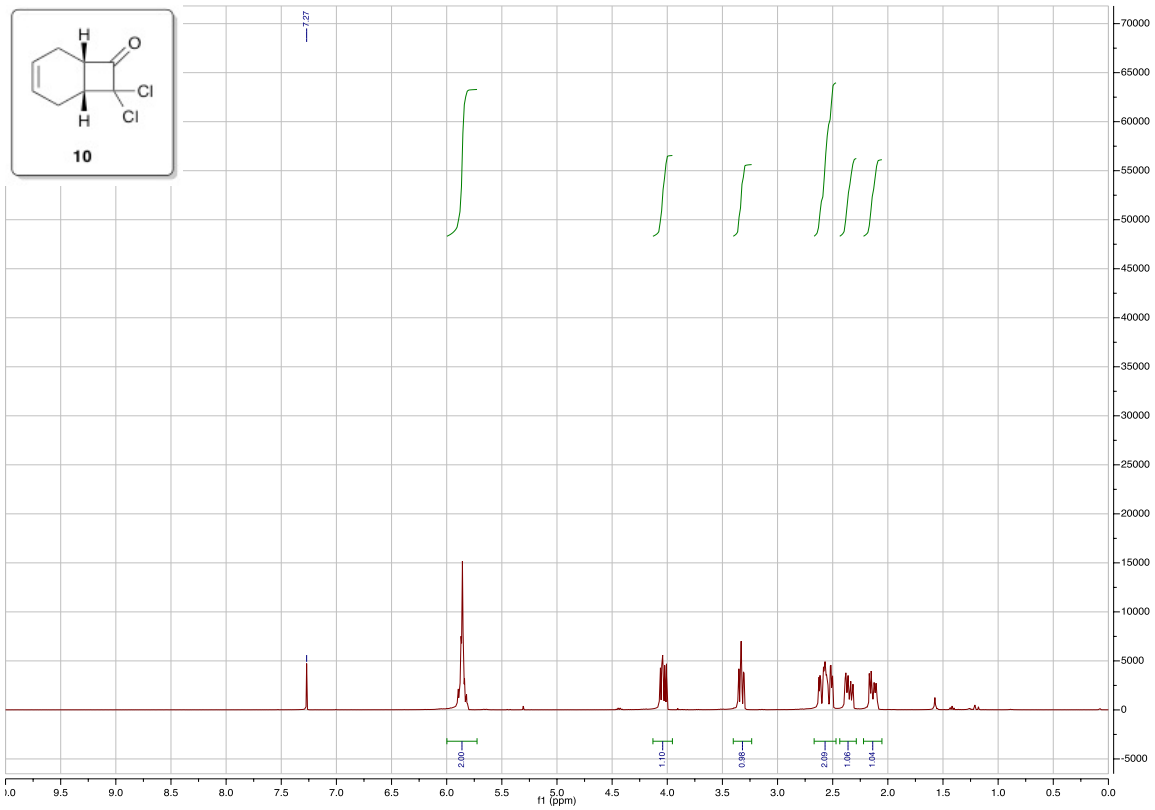


Figure S-1 ^{1}H-NMR spectrum of compound **10**.

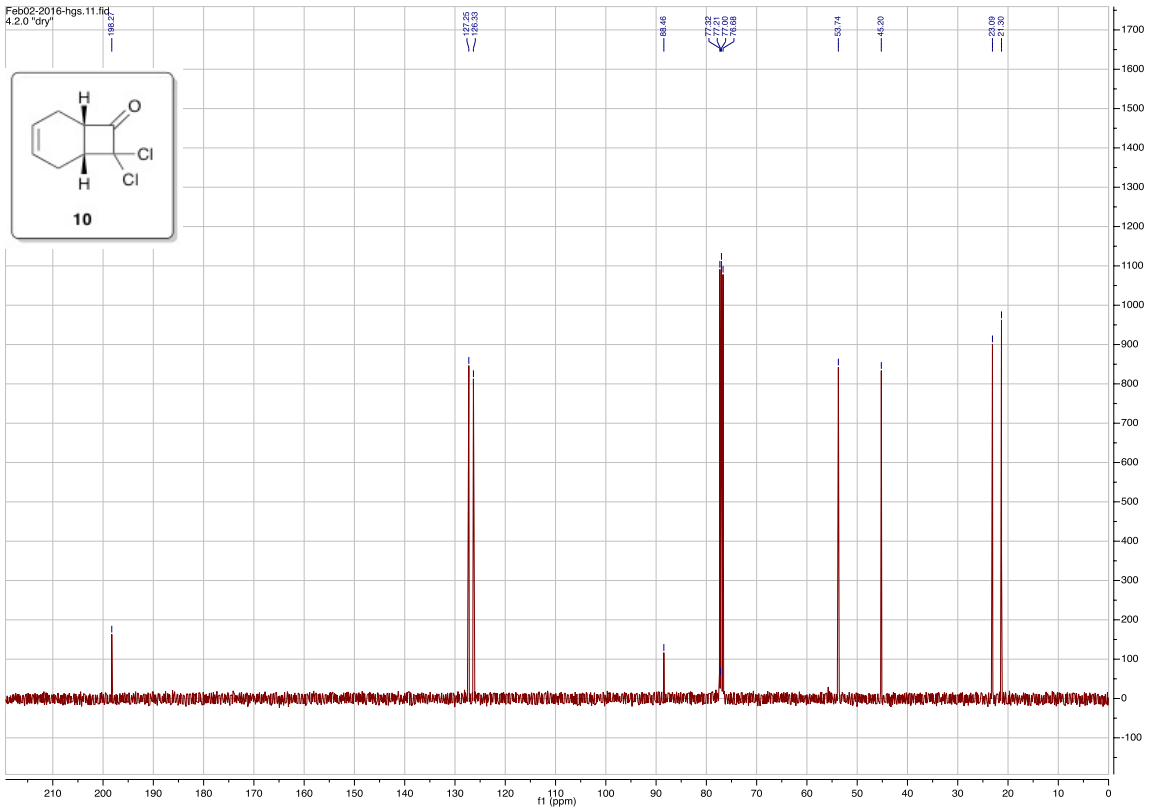


Figure S-2 ^{13}C-NMR spectrum of compound **10**.

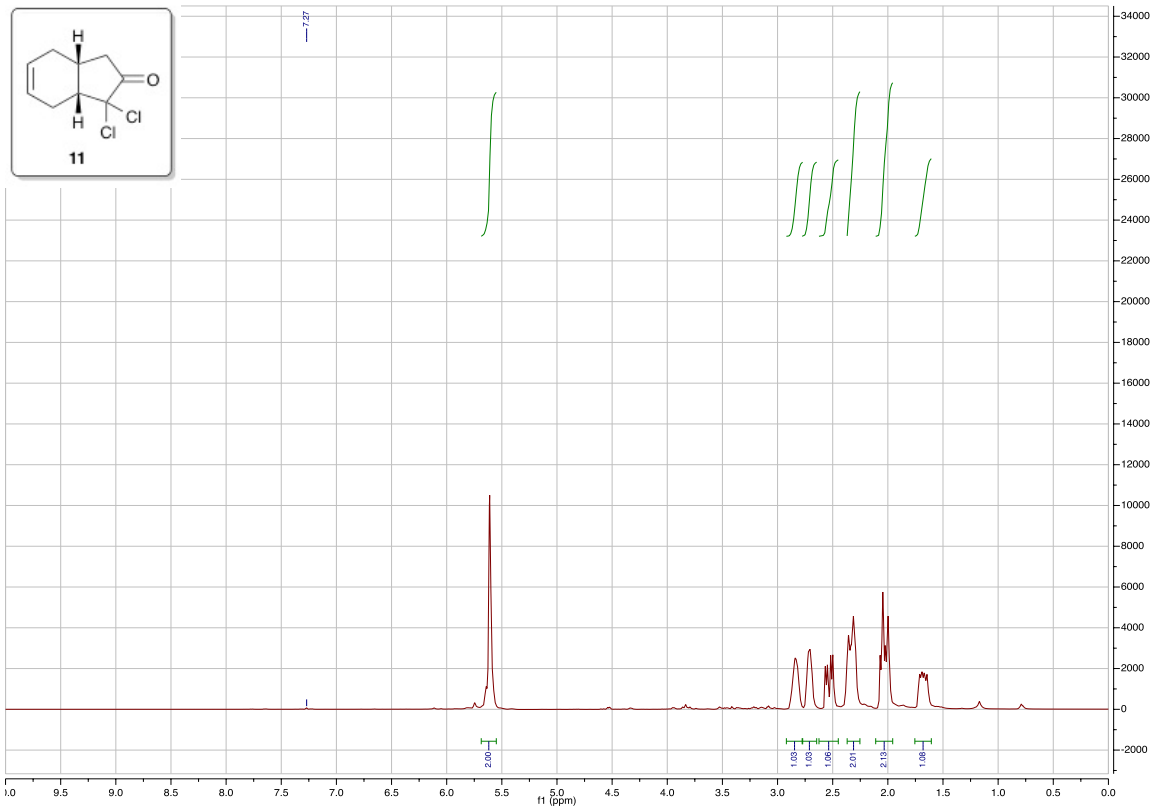


Figure S-3 ^{1}H-NMR spectrum of compound **11**.

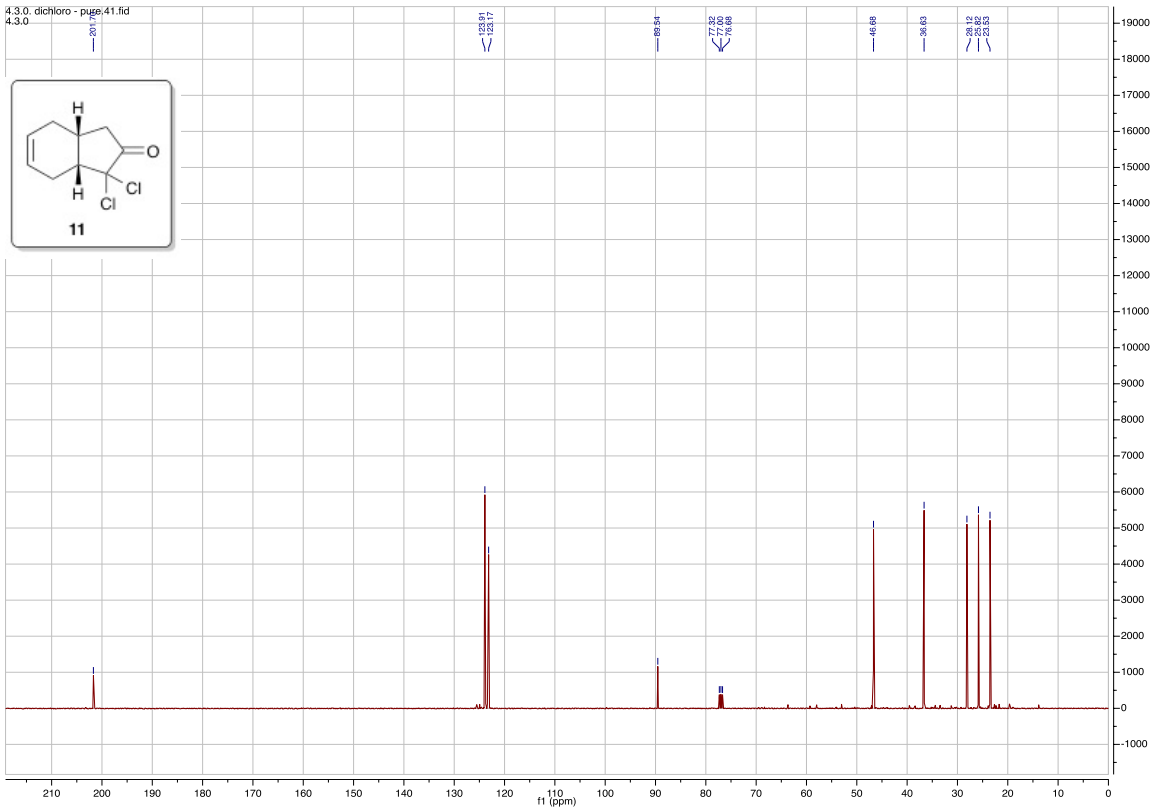


Figure S-4 ^{13}C-NMR spectrum of compound **11**.

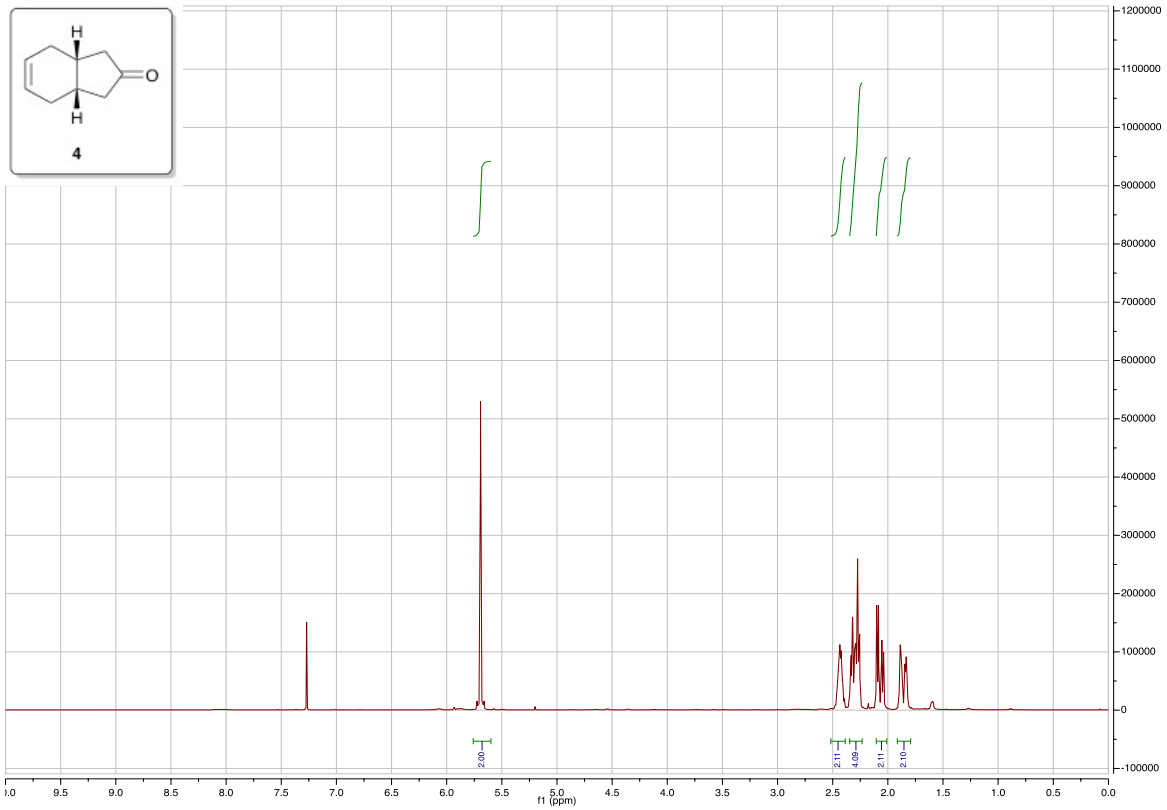


Figure S-5 ^{1}H-NMR spectrum of compound **4**.

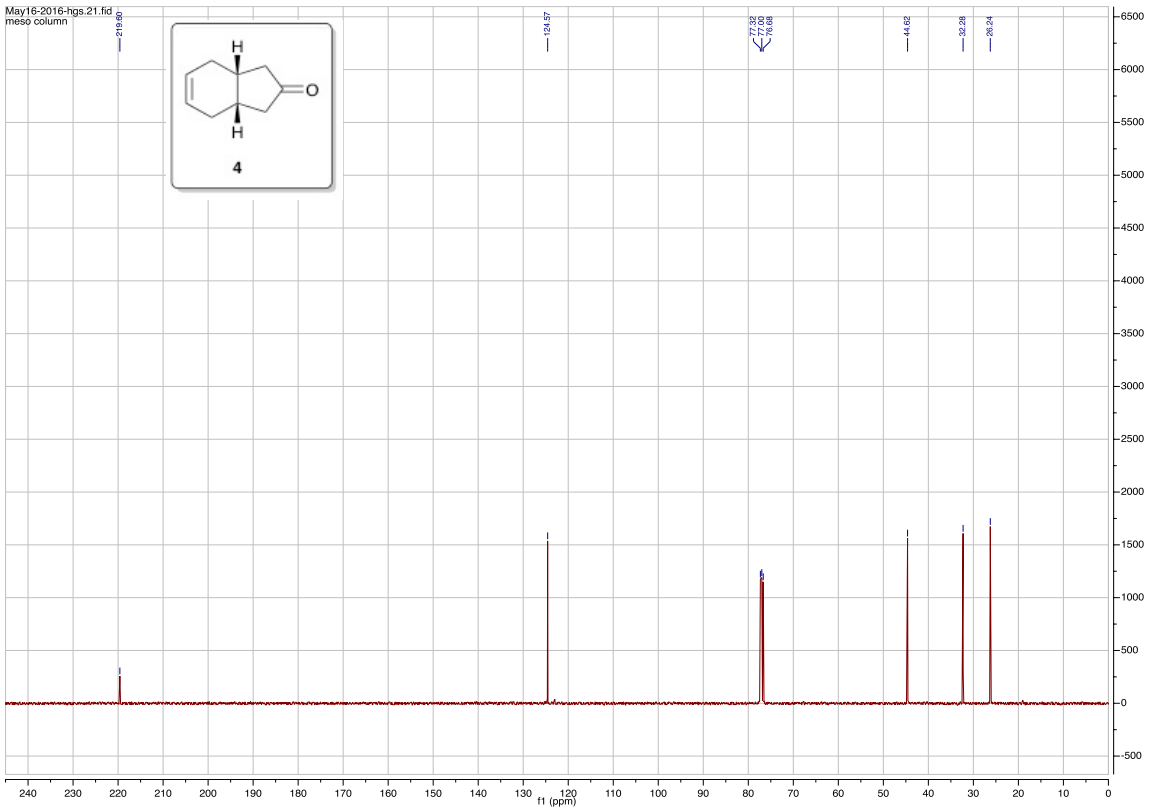


Figure S-6 ^{13}C-NMR spectrum of compound **4**.

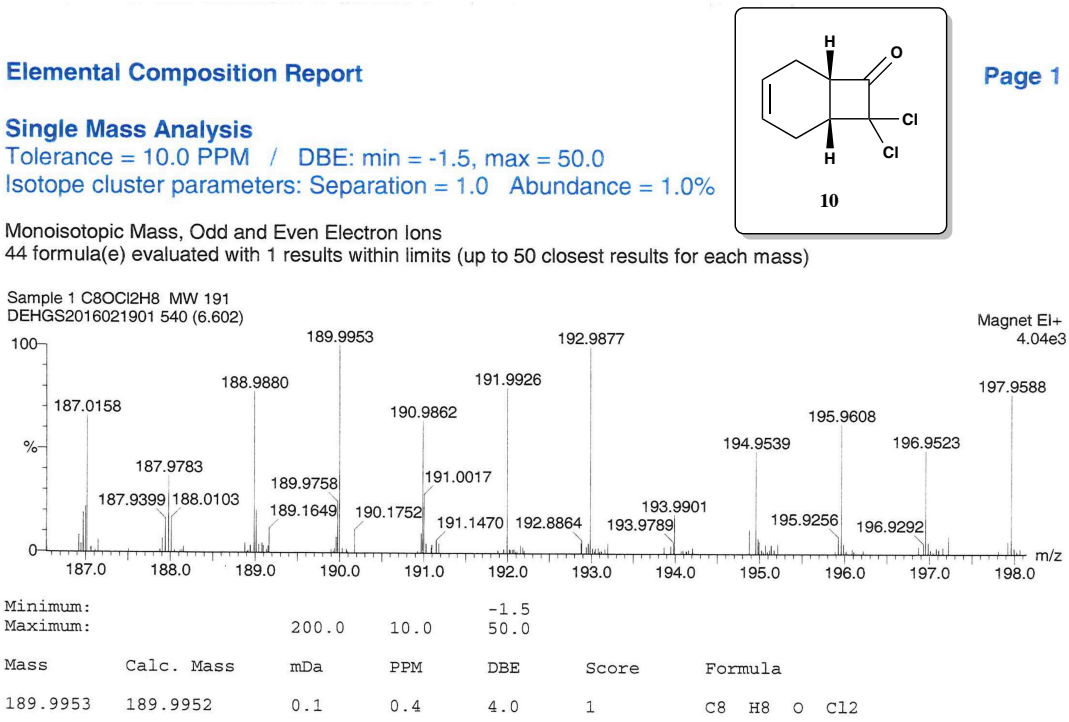


Figure S-7 HRMS of compound **10**.

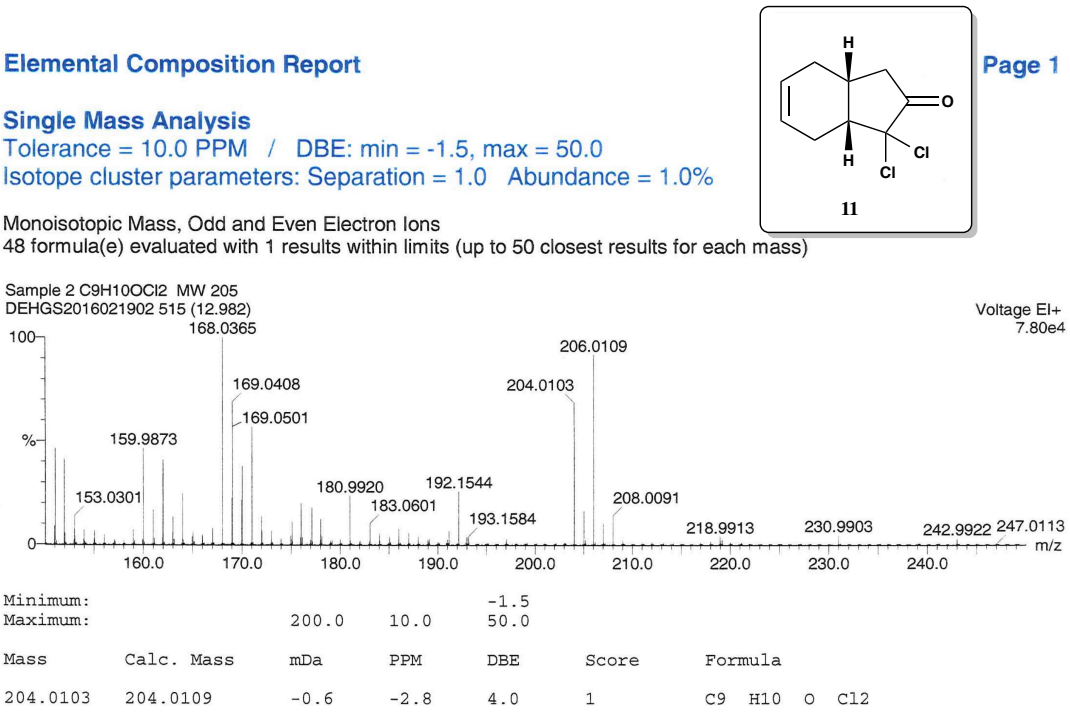


Figure S-8 HRMS of compound **11**.

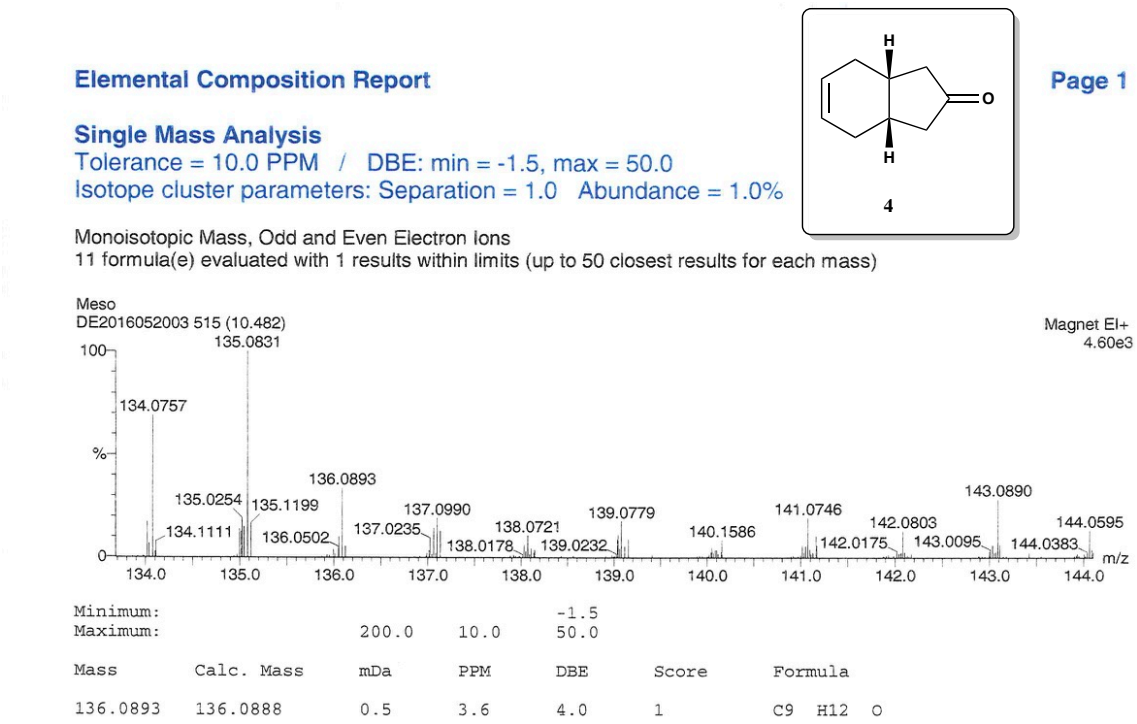


Figure S-9 HRMS of compound **4**.

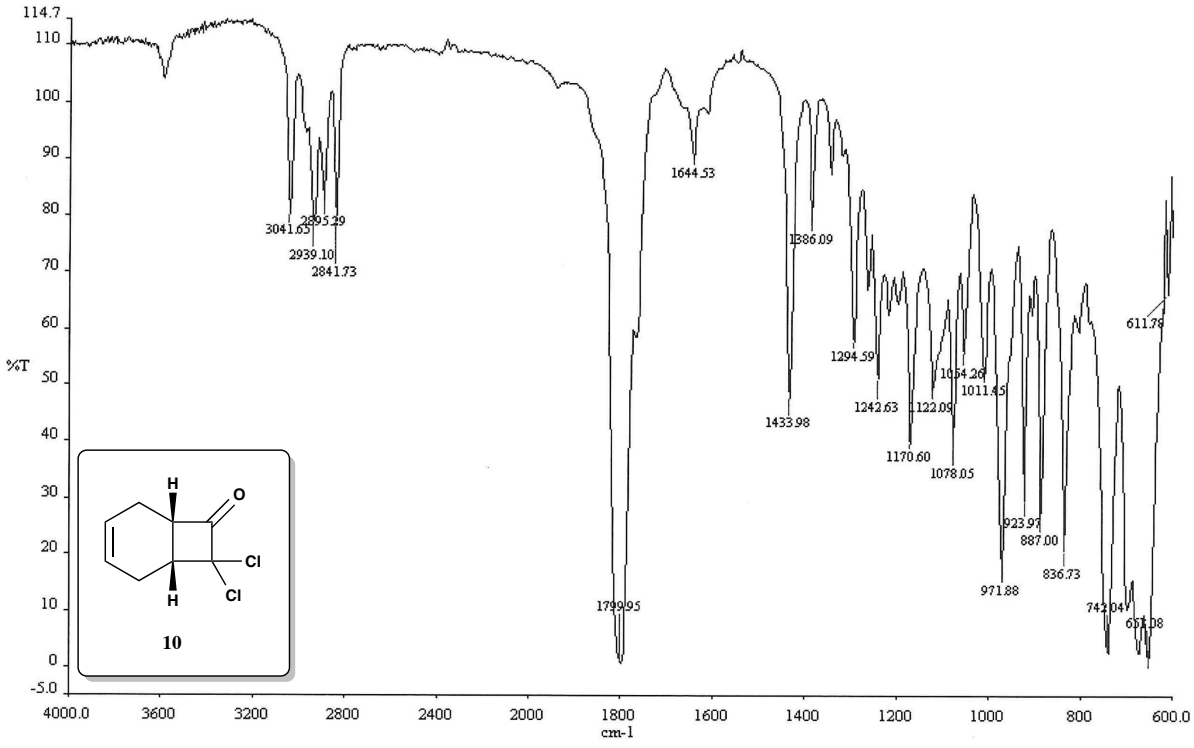


Figure S-10 IR of compound **10**.

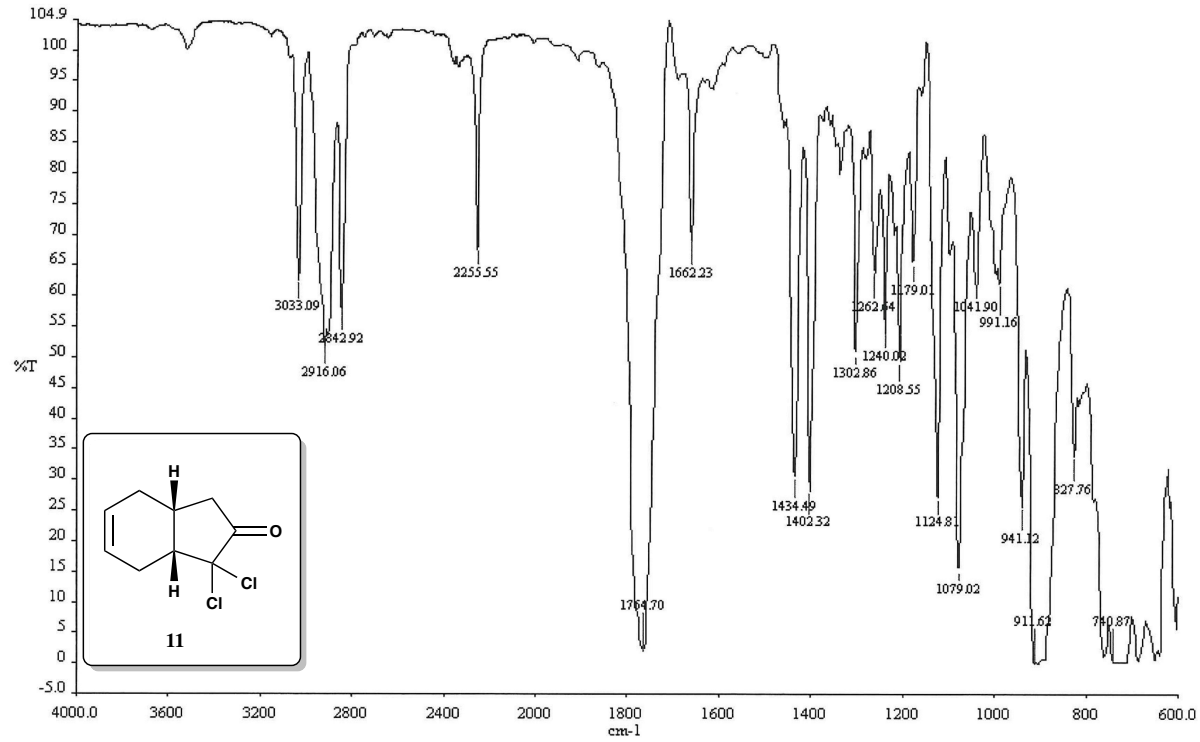


Figure S-11 IR of compound **11**.

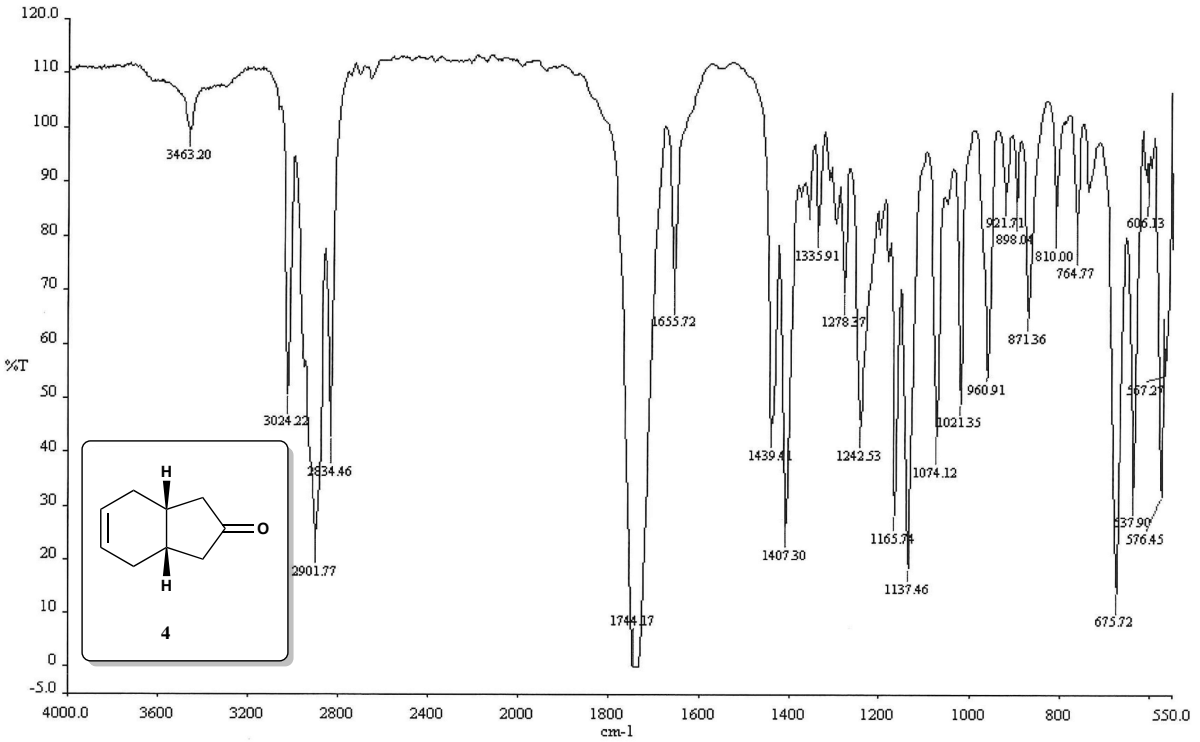
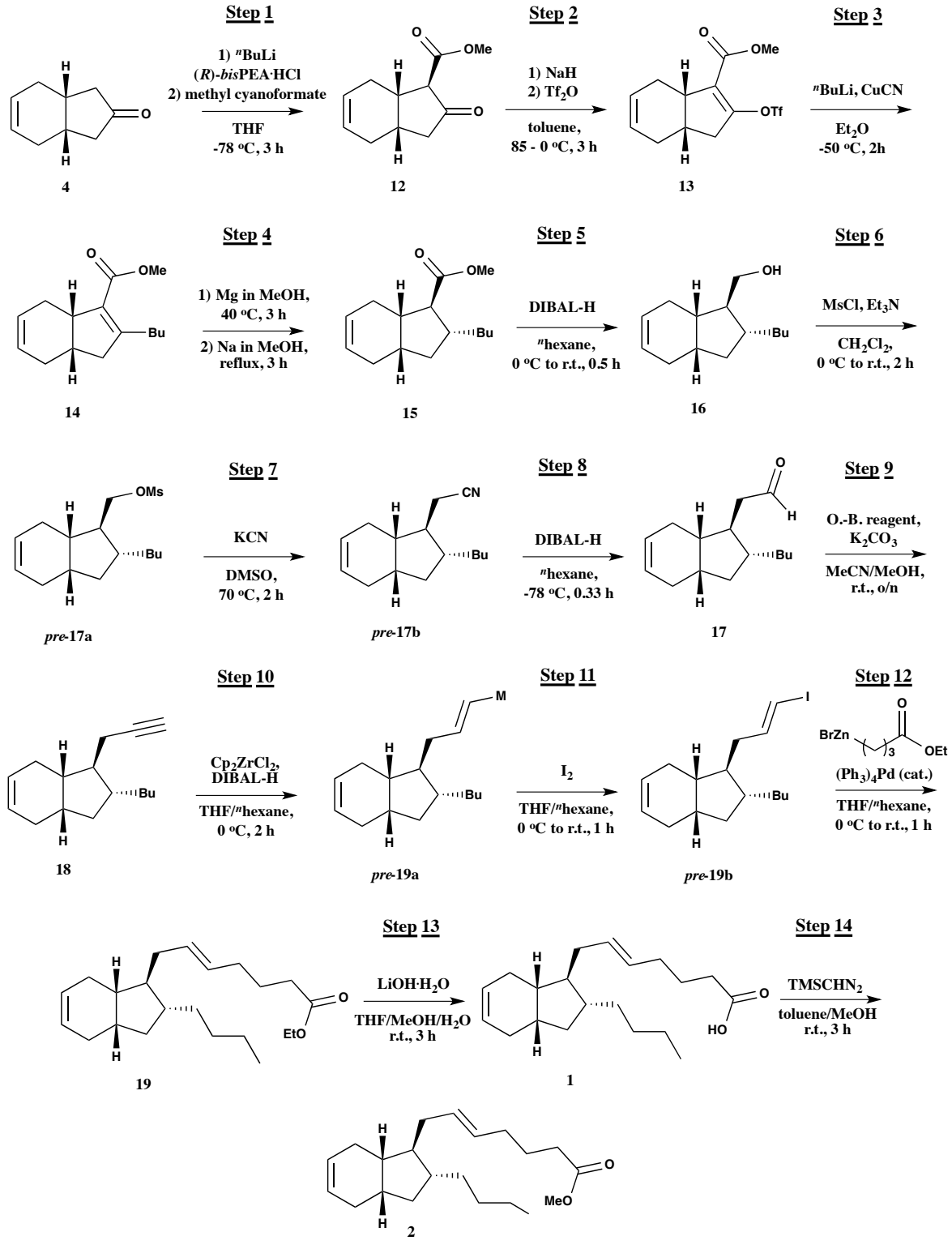


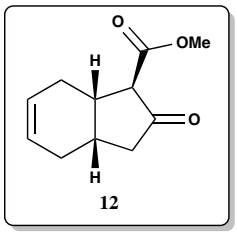
Figure S-12 IR of compound **4**.

Preparation of mucosin (1) and the methyl ester (2):



Scheme S-2 Synthetic route to mucosin (**1**) and its methyl ester **2**.

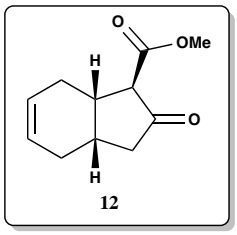
Methyl (1*S*,6*S*,7*R*)-8-oxobicyclo[4.3.0]non-3-ene-7-carboxylate (12).[7]



12

(+)-*Bis*[(*R*)-1-phenylethyl]amine hydrochloride (2.5 g, 9.60 mmol, 1.58 equiv.) was added in one portion to dry THF (10 mL) at room temperature and stirred for 5 min. The stirring suspension was then cooled to -78 °C and ^{n}BuLi (2.5M in ^{n}hexane, 7.67 mL, 19.18 mmol, 3.16 equiv.) was added dropwise. The suspension changed colour from cloudy white to pale orange. After stirring at -78 °C for 15 min the suspension was warmed to room temperature whereby a transparent yellow solution was formed. This was then cooled to -78 °C again and *meso*-(1*S*,6*R*)-bicyclo[4.3.0]non-3-ene-8-one **4** (826 mg, 6.07 mmol, 1.0 equiv.) was added dropwise over 10 min in dry THF (10 mL). This mixture was then stirred for 45 min whereby a purple colour evolved. Methyl cyanoformate (0.96 mL, 12.14 mmol, 2.0 equiv.) was then added dropwise over 5 min. and the mixture immediately turned bright yellow in colour. This mixture was left stirring for 2.5 h and then quenched by addition of H_2O (2 mL) at -78 °C. The mixture was then warmed to r.t and extracted with EtOAc (2 x 50 mL). The resulting organic layer was then washed with H_2O (2 x 100 mL), 0.5 M HCl (1 x 100 mL) and brine (1 x 100 mL). The organic layer was then dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting crude keto-ester was purified by column chromatography (^{n}hexane/EtOAc 5:1) to form a colourless oil. This oil was then recrystallised from ^{n}hexane at 0°C, filtered and air dried to obtain the title compound as white crystals. All spectroscopic and physical data were in full agreement with those reported in the literature.[7] Yield: 812 mg (69%); $[\alpha]_D^{26}$ -161.2° (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73-5.66 (m, 2H), 3.76 (s, 3H), 3.04 (d, *J* = 11.1 Hz, 1H), 2.88-2.83 (m, 1H), 2.52-2.38 (m, 3H), 2.33-2.21 (m, 2H), 2.04 (dd, *J* = 1.9, 18.2 Hz, 1H), 1.67-1.61 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.6, 169.7, 124.9, 123.9, 57.7, 52.4, 46.6, 37.3, 29.7, 26.8, 25.3; IR (neat, cm^{-1}) 3034 (w), 2945 (m), 2908 (m), 2837 (w), 1751 (s), 1718 (s) 1656 (w) 1433 (s) 1404 (m); HRMS (EI+): Exact mass calculated for $C_{11}H_{14}O_3$ $[M]^+$: 194.9033, found 194.0943; m.p.: 59-61 °C; TLC (^{n}hexane/EtOAc 4:1, $KMnO_4$ stain): R_f = 0.42.

***rac*-Methyl (1*S*,6*S*,7*R*)-8-oxobicyclo[4.3.0]non-3-ene-7-carboxylate (12).**

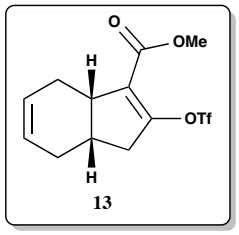


12

LDA (1M in THF/^{n}hexane) (1.65 mL, 1.65 mmol, 1.5 equiv.) was added dropwise to dry THF (5 mL) at -78 °C and stirred for 30 min. Then *meso*-(1*S*,6*R*)-bicyclo[4.3.0]non-3-ene-8-one **4** (150 mg, 1.10 mmol, 1.0 equiv.) was added dropwise in dry THF (5 mL) over 5 min and left to stir for 45 min. To the resulting yellow solution was added methyl cyanoformate (0.174 mL, 2.2 mmol, 2.0 equiv.) dropwise over 5 min and the reaction changed from yellow to colourless. After 30 min and monitoring the reaction via TLC the reaction was quenched at -78 °C by sat. aq. NH_4Cl (2 mL) and the reaction mixture was left to slowly warm to room temperature. The reaction mixture was then poured over H_2O (1 x 20 mL) and the organic phase separated. The aqueous phase was then extracted with EtOAc (2 x 20 mL). The organic phases were then combined, washed with H_2O (2 x 50 mL), brine (1 x 50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to from a crude yellow oil. This yellow oil was purified by column chromatography on silica (^{n}hexane/EtOAc, 5:1) to afford the racemic keto-ester. This was recrystallised in the same fashion as the optically active keto ester to afford pure white crystals. Yield: 166 mg, (78%).

The material was used in the preparation of racemic reference material for chiral GLC analysis.

Methyl (1*S*,6*S*)-8-(((trifluoromethyl)sulfonyl)oxy)bicyclo[4.3.0]non-3,7-diene-7-carboxylate (13).



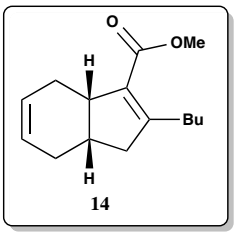
13

NaH (60% disp. in min. oil, 148 mg, 3.71 mmol, 1.8 equiv.) was added to dry toluene (10 mL). The suspension was stirred for 5 min and then methyl (1*S*,6*S*,7*R*)-8-oxobicyclo[4.3.0]non-3-ene-7-carboxylate **12** (400 mg, 2.06 mmol, 1.0 equiv.), dissolved in

dry toluene (7 mL), was added dropwise over 10 min during which bubbling occurred. After the full addition of **12** the reaction mixture was heated to 85 °C for 1.5 h during which time the mixture turned to a brown colour. The reaction mixture was then cooled to 0 °C and the triflic anhydride (0.52 mL, 3.09 mmol, 1.5 equiv.), was added dropwise. The reaction mixture changed colour from brown to a pale yellow/white slurry. After stirring at 0 °C for 1 h and monitoring by TLC the reaction mixture was quenched carefully with H_2O (10 mL). The resulting mixture was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with H_2O (1 x 150 mL), brine (1 x 150 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. This afforded a brown oil, which was purified by column chromatography on silica (^{n}hexane/EtOAc 95:5) to afford the unsaturated triflate as a colourless oil. Yield: 527 mg (83%); $[\alpha]_D^{26}$ +100.8° (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.95-5.89 (m, 1H), 5.86-5.81 (m, 1H), 3.81 (s, 3H), 3.10 (q, J = 6.7 Hz, 1H), 2.84-2.77 (m, 1H), 2.72-2.63 (m, 1H), 2.57-2.42 (m, 2H), 2.34-2.26 (m, 1H), 2.05-1.96 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.6, 153.9, 127.9, 127.3, 126.6, 118.3 (q, J_{CF} = 320 Hz), 51.8, 39.5, 38.9, 32.0, 27.3, 26.2; IR (neat, cm^{-1}) 3036 (w), 2953 (m), 2845 (w), 1723 (s), 1662 (m), 1425 (s); HRMS (EI+): Exact mass calculated for $C_{12}H_{13}O_5SF_3$ $[M]^+$: 326.0436, found 326.0438; TLC (^{n}hexane /EtOAc 4:1, $KMnO_4$ stain): R_f = 0.75.

Following the same procedure as above, racemic synthesis was performed to obtain reference material for chiral GLC analysis.

Methyl (1*S*,6*S*)-8-butylbicyclo[4.3.0]non-3,7-diene-7-carboxylate (14).



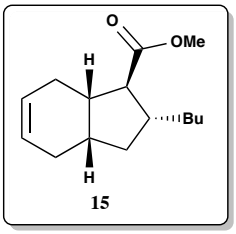
14

Solid Cu(I)CN (1.45 g, 16.18 mmol, 2.5 equiv.) was added to dry Et_2O (5mL) at room temperature. This was stirred for 5 min, cooled to -50 °C and then ^{n}BuLi (2.5 M in hexane, 6.47 mL, 16.18 mmol, 2.5 equiv.) was added dropwise over 5 min. This mixture was stirred for 1 h at -50 °C and a dark brown suspension occurred. Methyl (1*S*,6*S*)-8-(((trifluoromethyl)sulfonyl)oxy)bicyclo[4.3.0]non-3,7-diene-7-carboxylate **13** (1.98 g, 6.47 mmol, 1.0 equiv.) was then added via cannula at -50 °C in dry Et_2O (5 mL). The reaction changed from a dark brown suspension to black slurry and was left to stir for 1 h whilst

monitoring by TLC. Once the reaction was finished sat. aq. NH_4Cl (5 mL) was added dropwise. The reaction turned from black to dark purple and was left to warm to room temperature. The subsequent ethereal slurry was filtered through celite and the celite filter washed with EtOAc (3 x 15 mL). The organic layer was then separated and the aqueous layer extracted with EtOAc (2 x 15 mL). The organic layers were then combined, washed with H_2O (1 x 100 mL), brine (1 x 100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. This afforded a crude yellow oil, which was purified by column chromatography in silica (^{n}hexane/EtOAc 98:2) to afford the unsaturated butyl diene as a colourless oil. Yield: 1.33 g (88%); $[\alpha]_D^{26}$ +124.5° (c = 3.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.88-5.83 (m, 1H), 5.79-5.73 (m, 1H), 3.72 (s, 3H), 2.94 (q, *J* = 7.6 Hz, 1H), 2.64-2.54 (m, 1H), 2.52-2.38 (m, 4H) 2.32-2.23 (m, 2H), 1.97-1.90 (m, 1H), 1.84-1.77 (m, 1H), 1.46-1.28 (m, 4H), 0.91 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6, 159.9, 132.5, 127.9, 126.4, 50.8, 43.7, 42.3, 34.2, 30.1, 29.8, 27.5, 27.2, 22.7, 13.9; IR (neat, cm^{-1}) 3025 (w), 2926 (s), 1709 (s), 1630 (w), 1433 (s); HRMS (EI+): Exact mass calculated for $C_{15}H_{22}O_2$ $[M]^+$: 234.1620, found 234.1628; TLC (^{n}hexane/EtOAc 9:1, $KMnO_4$ stain): R_f = 0.85.

Following the same procedure as above, racemic synthesis was performed to obtain reference material for chiral GLC analysis.

Methyl (1*S*,6*S*,7*S*,8*R*)-8-butylbicyclo[4.3.0]non-3-ene-7-carboxylate (15).



15

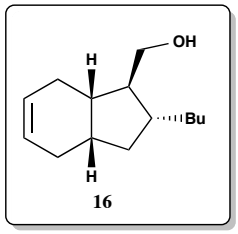
1) Methyl (1*S*,6*S*)-8-butylbicyclo[4.3.0]non-3,7-diene-7-carboxylate **14** (1.33 g, 5.68 mmol, 1.0 equiv.) was dissolved in MeOH (5 mL) at room temperature. This was stirred for 5 min then magnesium turnings (3.75 g, 156.31 mmol, 27.5 equiv.) were added in one portion. The turnings were stirred at room temperature for 10 min and then heated to 40 °C. A violent reaction occurs with lots of bubbling. After all the magnesium turnings had been consumed the addition of 27.5 equiv. of magnesium turnings in one portion was repeated at 40 °C. After 3 h the reaction was then cooled to r.t to give a white cloudy mixture. Glacial AcOH (5mL) was added dropwise until the cloudy suspension had dissolved to leave a colourless solution. The reaction mixture was then concentrated *in vacuo* to leave a white slurry, which was

poured over EtOAc/H_2O 1:1 (100 mL). The organic phase was separated and the aqueous layer was extracted again with EtOAc (2 x 50 mL). The organic phases were combined and washed with sat. aq. $NaHCO_3$ (1 x 100 mL), brine (1 x 100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*, to give a crude product. This crude product was analysed by ^1H and ^{13}C NMR to show the reaction had gone to completion by the formation of two unconjugated diastereomeric esters in a 2:1 ratio, no further purification was carried out. The crude diastereomeric esters were then equilibrated with NaOMe as shown below.

2) To MeOH (10 mL) at room temperature was added sodium metal (760 mg, 33.05 mmol, 6.0 equiv.). This was left to stir until all the sodium metal had dissolved. The crude diastereomeric esters were then added dropwise in MeOH (5 mL) and the reaction mixture was heated to 70 °C and monitored by TLC. After 3 h the reaction had gone to completion, was cooled to r.t and concentrated *in vacuo* but not to dryness. The crude mixture was then poured over Et_2O (50 mL) and H_2O (50 mL). The organic layer was separated and the aqueous layer was extracted with Et_2O (2 x 50 mL). The organic layers were then combined, washed with H_2O (1 x 50 mL), brine (1 x 50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to form a crude yellow oil. This crude yellow oil was purified by column chromatography on silica (^{n}hexane/EtOAc 98:2) to give the title compound as a colourless oil. Yield: 1.24 g (93%); $[\alpha]_D^{26}$ -4.32° (c = 2.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.73-5.66 (m, 2H), 3.69 (s, 3H), 2.32-2.22 (m, 1H) 2.22-2.14 (m, 5H), 2.09-2.02 (m, 1H), 1.91-1.77 (m, 2H), 1.54-1.49 (m, 1H), 1.36-1.21 (m, 5H), 1.15-1.09 (m, 1H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.2, 126.3, 125.5, 55.5, 51.5, 42.7, 41.2, 38.3, 36.9, 35.4, 30.6, 28.0, 26.5, 22.8, 14.0; IR (neat, cm^{-1}) 3024 (m), 2927 (s), 2856 (s), 1726 (s), 1657 (w), 1628 (w), 1541 (w), 1520 (w), 1458 (m), 1434 (s); HRMS (EI+): Exact mass calculated for $C_{15}H_{24}O_2$ $[M]^+$: 236.1776, found 236.1783; TLC (^{n}hexane/EtOAc 9:1, $KMnO_4$ stain): R_f = 0.85.

Following the same procedure as above, racemic synthesis was performed to obtain reference material for chiral GLC analysis.

(1*S*,6*S*,7*S*,8*R*)-8-Butyl-7-(hydroxymethyl)bicyclo[4.3.0]non-3-ene (16).

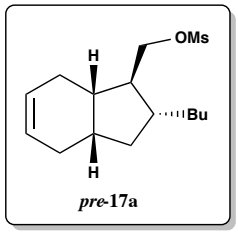


16

Methyl (1*S*,6*S*,7*S*,8*R*)-8-butylbicyclo[4.3.0]non-3-ene-7-carboxylate **15** (1.24 g, 5.25 mmol, 1.0 equiv.) was dissolved in ^{n}hexane (20 mL) at room temperature and stirred for 5 min. The solution was then cooled to 0 °C and DIBAL-H (1M in ^{n}hexane) (10.51 mL, 10.51 mmol, 2.0 equiv.) was added dropwise over 5 min. The reaction was then left to warm to r.t. After 30 min the reaction was cooled back to 0 °C and quenched with sat. aq. NH_4Cl (6 mL). The reaction mixture was allowed to warm to room temperature whereby a cloudy suspension occurred. This suspension was poured over sat. aq. NH_4Cl (30 mL) and the organic layer separated. The aqueous layer was extracted with EtOAc (2 x 25 mL) and the organic layers combined, washed with H_2O (1 x 100 mL), brine (1 x 100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give a crude cloudy oil. This was then purified by column chromatography on silica (^{n}hexane/EtOAc 95:5) to afford the title compound as a colourless oil. Yield: 1.01 g, (93%); $[\alpha]_D^{26}$ -10.33° (c = 8.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 5.70-5.64 (m, 2H), 3.61-3.52 (m, 2H), 2.24-2.03 (m, 3H), 1.92-1.81 (m, 4H), 1.57-1.42 (m, 4H), 1.33-1.22 (m, 5H), 1.18-1.11 (m, 1H), 0.89 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 126.0, 125.8, 65.7, 54.0, 41.0, 37.8, 37.7, 37.4, 35.0, 31.0, 27.7, 27.3, 22.9, 14.1; IR (neat, cm^{-1}) 3316 (br.), 3020 (m), 2918 (s), 1657 (m), 1464 (m), 1433 (m); HRMS (EI+): Exact mass calculated for $C_{14}H_{24}O$ $[M\text{-}H_2O]^+$: 190.1722, found 190.1723; TLC (^{n}hexane/EtOAc 4:1, $KMnO_4$ stain): R_f = 0.35. The enantiomeric excess was determined by chiral GLC analysis (CP-Chirasil-DEX CB, using the following program: 80 ^{o}C (30 min) - 3 degrees/min to 150 ^{o}C - 150 ^{o}C (5 min)): t_r(e_1, major) = 38.97 min and t_r(e_2, minor) = 39.95 min; *e.e.*: > 99%.

Following the same procedure as above, racemic synthesis was made to obtain reference material for chiral GLC analysis.

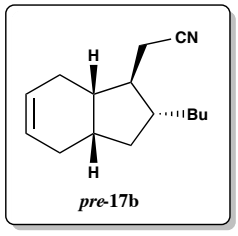
(1*S*,6*S*,7*S*,8*R*)-8-Butyl-7-((methylsulfonyl)oxymethyl)bicyclo[4.3.0]non-3-ene (*pre*-17a).



pre-17a

To a stirring solution of (1*S*,6*S*,7*S*,8*R*)-8-butyl-7-(hydroxymethyl)bicyclo[4.3.0]non-3-ene **16** (1.01 g, 4.81 mmol, 1.0 equiv.) in dry CH_2Cl_2 (10 mL) at room temperature, was added Et_3N (1.34 mL, 9.62 mmol, 2.0 equiv.) dropwise. This solution was left stirring for 5 min then cooled to 0 °C. Then methanesulfonyl chloride (1.12 mL, 14.43 mmol, 3.0 equiv.) was added dropwise and the reaction was left at 0 °C for 10 min then warmed to room temperature and left for 2 h. The reaction mixture turned colourless to yellow. After 2 h brine (5 mL) was added dropwise and the volatiles concentrated *in vacuo* to afford a yellow liquid. This was poured over EtOAc (50 mL) and sat. aq. $NaHCO_3$ (50 mL) was added. The organic layer was separated and the aqueous layer extracted with EtOAc (2 x 50 mL). The organic layers were combined and washed with brine (1 x 50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a crude yellow oil. This was then purified by column chromatography on silica (^{n}hexane/EtOAc 95:5) to afford the title compound as a colourless oil. Yield: 1.30 g, (94%); $[\alpha]_D^{26}$ -11.65° (c = 8.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.69-5.62 (m, 2H), 4.18-4.10 (m, 2H), 2.99 (s, 3H), 2.24-2.07 (m, 3H), 1.94-1.79 (m, 4H), 1.68-1.50 (m, 3H), 1.33-1.22 (m, 5H), 1.19-1.12 (m, 1H), 0.88 (t, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 126.0, 125.2, 72.0, 50.3, 40.9, 38.0, 37.5, 37.2, 36.8, 34.9, 30.7, 27.2, 27.1, 22.7, 14.0; IR (neat, cm^{-1}) 3024 (w), 2926 (s), 1657 (w), 1464 (m) 1435 (w); HRMS (EI+): Exact mass calculated for $C_{15}H_{26}O_3S_2$ $[M]^+$: 286.1603, found 286.1606; TLC (^{n}hexane/EtOAc 4:1, $KMnO_4$ stain): R_f = 0.45.

(1*S*,6*S*,7*S*,8*R*)-8-Butyl-7-(cyanomethyl)bicyclo[4.3.0]non-3-ene (pre-17b).

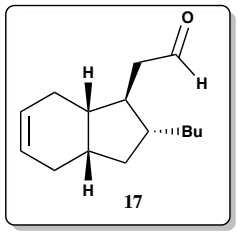


pre-17b

To a stirring solution of (1*S*,6*S*,7*S*,8*R*)-8-butyl-7-((methylsulfonyl)oxymethyl)bicyclo[4.3.0]non-3-ene ***pre*-17a** (1.30 g, 4.55 mmol, 1.0 equiv.)

in dry DMSO (10 mL) was added solid KCN (1.77 g, 27.27 mmol, 6.0 equiv.) in one portion. The reaction mixture was then heated to 70 °C for 2 h. The reaction mixture changed from colourless to yellow. After 2 h the reaction was cooled to r.t and H_2O (5 mL) was added dropwise. The reaction mixture turned from yellow to colourless. This was then poured over EtOAc (20 mL) and the organic layer separated. The aqueous layer was then extracted with EtOAc (2 x 20 mL) and the organic layers combined. They were then washed with brine (1 x 50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a crude brown oil. This was then purified by column chromatography on silica (^{n}hexane/EtOAc 98:2) to give the title compound as a colourless oil. Yield: 906 mg, (92 %); $[\alpha]_D^{26}$ -19.15° (c = 8.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73-5.65 (m, 2H), 2.41 (d, *J* = 6.7 Hz, 2H), 2.27-2.14 (m, 3H), 2.04-1.96 (m, 1H), 1.93-1.76 (m, 3H), 1.67-1.49 (m, 3H), 1.36-1.19 (m, 5H), 1.15-1.08 (m, 1H), 0.90 (t, *J* = 6.8 Hz, 3H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 126.5, 125.4, 119.0, 46.4, 43.9, 41.3, 37.9, 36.0, 34.6, 30.6, 27.9, 26.3, 22.8, 21.0, 14.0; IR (neat, cm^{-1}) 3024 (m), 2921 (s), 1658 (w), 1465 (m) 1436 (m); HRMS (EI+): Exact mass calculated for $C_{15}H_{23}N$ $[M]^+$: 217.1830, found 217.1827; TLC (^{n}hexane/EtOAc 4:1, $KMnO_4$ stain): R_f = 0.82.

(1*S*,6*S*,7*S*,8*R*)-8-Butyl-7-(formylmethyl)bicyclo[4.3.0]non-3-ene (17).

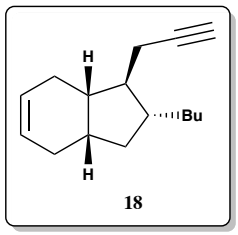


17

A stirring solution of (1*S*,6*S*,7*S*,8*R*)-8-butyl-7-(cyanomethyl)bicyclo[4.3.0]non-3-ene ***pre*-17b** (906 mg, 4.18 mmol, 1.0 equiv.) in ^{n}hexane (10 mL) was cooled to -78 °C. Then DIBAL-H (1M in ^{n}hexane) (6.26 mL, 6.26 mmol, 1.5 equiv.) was added dropwise over 5 min and the reaction left to stir for 20 min. Then sat. aq. Rochelle salt (5 mL) was added dropwise to the reaction mixture and then left to warm to room temperature. The resulting cloudy suspension was poured over EtOAc (20 mL) and sat. aq. Rochelle salt (20 mL). The organic layer was separated and the aqueous phase extracted with EtOAc (2 x 20 mL). The organic phases were combined and washed with brine (1 x 50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a crude cloudy oil. This was then purified by column chromatography on silica (^{n}hexane/EtOAc, 95:5) to afford the aldehyde as a colourless oil. Yield: 813 mg, (88%); $[\alpha]_D^{26}$ -14.40° (c = 8.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (t, *J* = 2.5 Hz, 1H), 5.71-5.64 (m, 2H), 2.44 (dd, *J* = 2.5, 6.5 Hz, 2H), 2.22-2.08 (m, 3H), 1.96-1.91 (m, 1H), 1.89-1.67

(m, 4H), 1.55-1.49 (m, 2H), 1.33-1.15 (m, 5H), 1.13-1.08 (m, 1H), 0.88 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.0, 126.3, 125.6, 49.5, 45.3, 45.0, 41.8, 37.9, 36.4, 34.9, 30.8, 27.7, 27.1, 22.8, 14.0; IR (neat, cm^{-1}) 3023 (m), 2923 (s), 2718 (m), 1720 (s), 1657 (w), 1465 (m), 1434 (m); HRMS (EI+): Exact mass calculated for $C_{15}H_{24}O$ $[M]^+$: 220.1827, found 220.1828; TLC (^{n}hexane/EtOAc 4:1, $KMnO_4$ stain): R_f = 0.82.

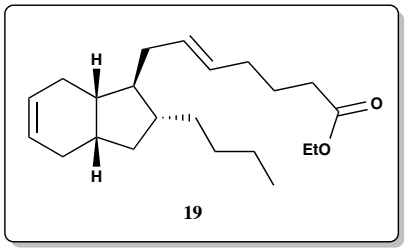
(1*S*,6*S*,7*S*,8*R*)-8-Butyl-7-(prop-2'-yn-1'-yl)bicyclo[4.3.0]non-3-ene (18).



18

To a stirring solution of (1*S*,6*S*,7*S*,8*R*)-8-Butyl-7-(formylmethyl)bicyclo[4.3.0]non-3-ene **17** (300 mg, 1.36 mmol, 1.0 equiv.) in dry MeOH (15 mL) at 0 °C was added solid K_2CO_3 (451 mg, 3.27 mmol, 2.4 equiv.) in one portion and Ohira-Bestmann reagent (10% w/w in MeCN, 4.9 mL, 3.9 g, 2.05 mmol, 1.5 equiv.). The suspension was then warmed to room temperature and left stirring overnight. After analysis by TLC the mixture was treated with sat. aq. $NaHCO_3$ (20 mL), and the resulting mixture poured over CH_2Cl_2 (20 mL). The organic phase was separated and the aqueous phase was washed with CH_2Cl_2 (3 x 10 mL). The organic phases were then combined, dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford a crude oil. This was purified by column chromatography on silica (^{n}hexane/EtOAc, 95:5) to afford title compound as a colourless oil. Yield: 253 mg, (86%); $[\alpha]_D^{26}$ -16.95° (c = 8.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73-5.66 (m, 2H), 2.28 (dd, J = 2.6, 5.9 Hz, 2H), 2.23-2.08 (m, 3H), 1.97-1.79 (m, 5H), 1.70-1.53 (m, 2H), 1.44-1.38 (m, 1H), 1.36-1.16 (m, 5H), 1.13-1.06 (m, 1H), 0.90 (t, J = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ; 126.4, 126.0, 83.6, 68.6, 49.0, 43.5, 40.6, 38.2, 36.6, 34.8, 30.9, 28.1, 27.0, 22.9, 22.3, 14.1; IR (neat, cm^{-1}) 3310 (m), 3021 (m), 2954 (s), 2915 (s), 1657 (w), 1465 (m), 1435 (m); HRMS (EI+): Exact mass calculated for $C_{16}H_{24}$ $[M]^+$: 216.1878, found 216.1870; TLC (^{n}hexane, $KMnO_4$ stain and anisaldehyde dip): R_f = 0.24.

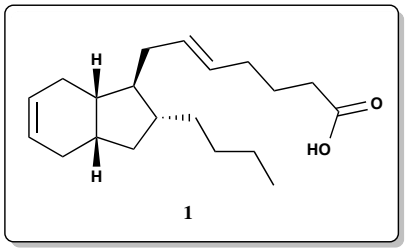
(1*S*,6*S*,7*S*,8*R*)-8-Butyl-7-((*E*)-7'-ethoxy-7'-oxohept-2'-enyl)bicyclo[4.3.0]non-3-ene (19).



19

To a stirring solution of Cp_2ZrCl_2 (95 mg, 0.324 mmol, 2.0 equiv.) in dry THF (2 ml) at 0 °C was added DIBAL-H (1M in ^{n}hexane) (0.32 mL, 0.324 mmol, 2.0 equiv.) via dropwise addition. The resulting homogenous mixture was then protected from light and stirred at 0 °C for 1 h after which time a colourless heterogeneous mixture formed. Then (1*S*,6*S*,7*S*,8*R*)-8-butyl-7-(prop-2'-yn-1'-yl)bicyclo[4.3.0]non-3-ene **18** (35 mg, 0.162 mmol, 1.0 equiv.) dissolved in dry THF (2 mL) was added dropwise to the reaction mixture at 0 °C. After 1 h at 0 °C iodine (63 mg, 0.248 mmol, 1.5 equiv.) was added in one portion to the homogeneous yellow reaction mixture. The reaction mixture was then warmed to room temperature and stirred for 1 h. To the preformed vinyl iodide was successively added 4-ethoxy-4-oxobutylzinc bromide solution (0.5M in THF) (0.648 mL, 0.324 mmol, 2.0 equiv.) dropwise and $(Ph_3P)_4Pd$ (19 mg, 0.016 mmol, 0.01 equiv.) in one portion. The resulting tea brown mixture was stirred at room temperature for 1 h and monitored by TLC. Once the reaction had gone to completion 1M HCl (10 mL) was added dropwise and the reaction poured over Et_2O (15 mL). The aqueous phase was extracted with Et_2O (3 x 15 mL) and the organic phases combined, dried over $MgSO_4$, filtered and concentrated *in vacuo* to form a crude brown oily mixture. This oily mixture was purified by column chromatography on silica (^{n}hexane/EtOAc, 95:5) to afford the title compound as a colourless oil. Yield: 27 mg, (51%); $[\alpha]_D^{26}$ -11.15° (c = 2.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.70-5.64 (m, 2H), 5.47-5.33 (m, 2H), 4.13 (q, *J* = 7.1 Hz, 2 H), 2.29 (t, *J* = 7.5 Hz, 2H), 2.22-2.15 (m, 1H), 2.12- 2.01 (m, 6H), 1.89-1.76 (m, 3H), 1.73-1.66 (m, 3H), 1.53-1.47 (m, 2H), 1.34-1.16 (m, 9H), 1.12-1.04 (m, 1H), 0.88 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8, 130.3, 129.9, 126.2, 126.1, 60.2, 51.0, 44.0, 40.3, 38.1, 37.7, 37.1, 34.9, 33.7, 31.9 31.0, 27.8, 27.7, 24.8, 22.9, 14.2, 14.1; IR (neat, cm^{-1}) 3021 (m), 2920 (s), 1734 (s), 1657 (w), 1438 (m); HRMS (EI+): Exact mass calculated for $C_{22}H_{36}O_2$ $[M]^+$: 332.2715, found 332.2722; TLC (^{n}hexane/EtOAc 95:5, $KMnO_4$ stain): R_f = 0.65.

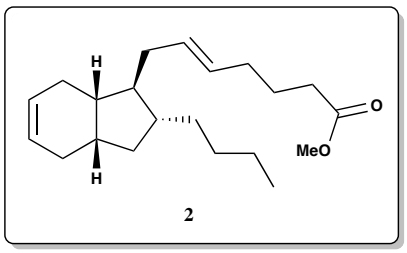
(1*S*,6*S*,7*S*,8*R*)-8-Butyl-7-((*E*)-7'-hydroxy-7'-oxohept-2'-enyl)bicyclo[4.3.0]non-3-ene (1).



1

To a stirring solution of the (1*S*,6*S*,7*S*,8*R*)-8-butyl-7-((*E*)-7'-ethoxy-7'-oxohept-2'-enyl)bicyclo[4.3.0]non-3-ene **18** (27 mg, 0.081 mmol, 1.0 equiv.) in THF/MeOH/H_2O (2:2:1) (5 mL) at room temperature was added lithium hydroxide monohydrate (119 mg, 2.84 mmol, 35.0 equiv.) in one portion. The reaction mixture was left stirring and monitored by TLC. After 3 h the reaction had gone to completion and was acidified to pH 2 by 1M HCl (5 mL). The reaction mixture was then poured over EtOAc (5 mL) and the aqueous phase extracted with EtOAc (3 x 5 mL). The organic phases were then combined and washed with brine (1 x 20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to form a colourless oil. This was then purified by column chromatography on silica (^{n}hexane/EtOAc, 3:2) to afford the title compound as a colourless oil. Yield: 24 mg, (97%); $[\alpha]_D^{26}$ -10.19° (c = 2.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.24 (br, 1H), 5.71-5.64 (m, 2H), 5.48-5.33 (m, 2H), 2.35 (t, J = 7.5 Hz, 2H), 2.23-2.14 (m, 1H), 2.13-2.02 (m, 6H), 1.89-1.75 (m, 3H), 1.73-1.65 (m, 3H), 1.55-1.44 (m, 2H), 1.36-1.15 (m, 6H), 1.12-1.05 (m, 1H), 0.89 (t, J = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.3, 130.6, 129.7, 126.2, 126.0, 51.0, 44.0, 40.3, 38.1, 37.7, 37.1, 34.9, 33.3, 31.8, 31.0, 27.8, 27.7, 24.4, 22.9, 14.1; IR (neat, cm^{-1}) 3021 (m), 2920 (s), 1708 (s), 1457 (m), 1436 (m), 1412 (m); HRMS (EI+): Exact mass calculated for $C_{20}H_{32}O_2$ $[M]^+$: 304.2402, found 304.2391; TLC (^{n}hexane/EtOAc 3:2, $KMnO_4$ stain): R_f = 0.40.

(1*S*,6*S*,7*S*,8*R*)-8-Butyl-7-((*E*)-7'-methoxy-7'-oxohept-2'-enyl)bicyclo[4.3.0]non-3-ene (2).



2

To a stirring solution of (1*S*,6*S*,7*S*,8*R*)-8-butyl-7-((*E*)-7'-hydroxy-7'-oxohept-2'-enyl)bicyclo[4.3.0]non-3-ene **1** (24 mg, 0.079 mmol, 1.0 equiv.) in toluene/MeOH (3:2) (5 mL) at room temperature was added TMS diazomethane solution (2M in ^{n}hexane) (0.06 mL,

0.119 mmol, 1.5 equiv.) dropwise over 2 min. The reaction mixture bubbled and turned transparent yellow. The reaction was monitored by TLC and after 1 h had gone to completion. The reaction mixture was then concentrated *in vacuo* and directly purified by column chromatography on silica (^{n}hexane/EtOAc, 95:5) to afford the title compound as a colourless oil. Yield: 23 mg, (92%); $[\alpha]_D^{26}$ -9.8° (c = 0.8, ^{n}hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.70-5.64 (m, 2H), 5.46-5.33 (m, 2H), 3.67 (s, 3H), 2.31 (t, *J* = 7.6 Hz, 2H), 2.22-2.15 (m, 1H), 2.12-2.01 (m, 6H), 1.89-1.75 (m, 3H), 1.73-1.65 (m, 3H), 1.54-1.44 (m, 2H), 1.34-1.16 (m, 6H), 1.12-1.04 (m, 1H), 0.88 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ; 174.2, 130.4, 129.9, 126.3, 126.1, 51.4, 51.0, 44.0, 40.3, 38.1, 37.7, 37.1, 34.9, 33.4, 31.9, 31.0, 27.8, 27.7, 24.8, 22.9, 14.1; IR (neat, cm^{-1}) 3020 (w), 2952 (m), 2923 (s), 1741 (s), 1657 (w), 1603 (w), 1541 (w), 1508 (w), 1458 (m), 1436 (m); HRMS (EI+): Exact mass calculated for $C_{21}H_{34}O_2$ $[M]^+$: 318.2559, found 318.2544; TLC (^{n}hexane/EtOAc 95:5, $KMnO_4$ stain): R_f =0.65.

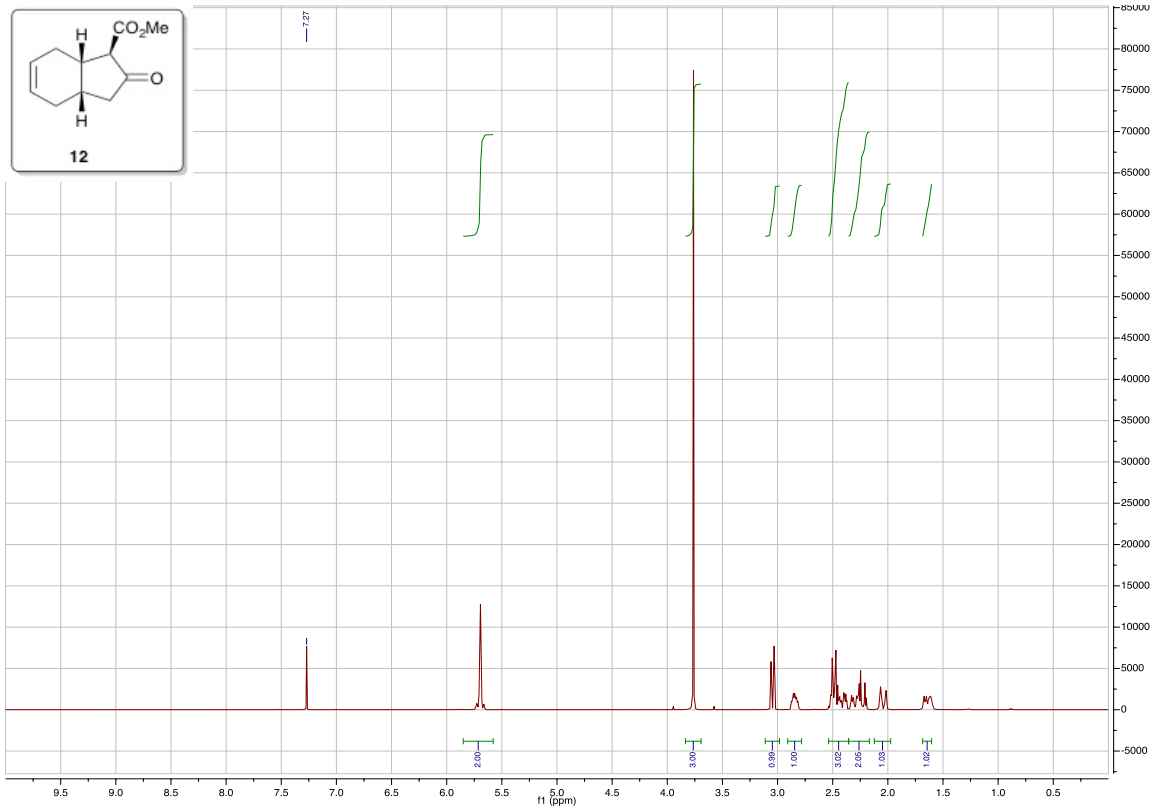


Figure S-13 ^{1}H-NMR spectrum of compound **12**.

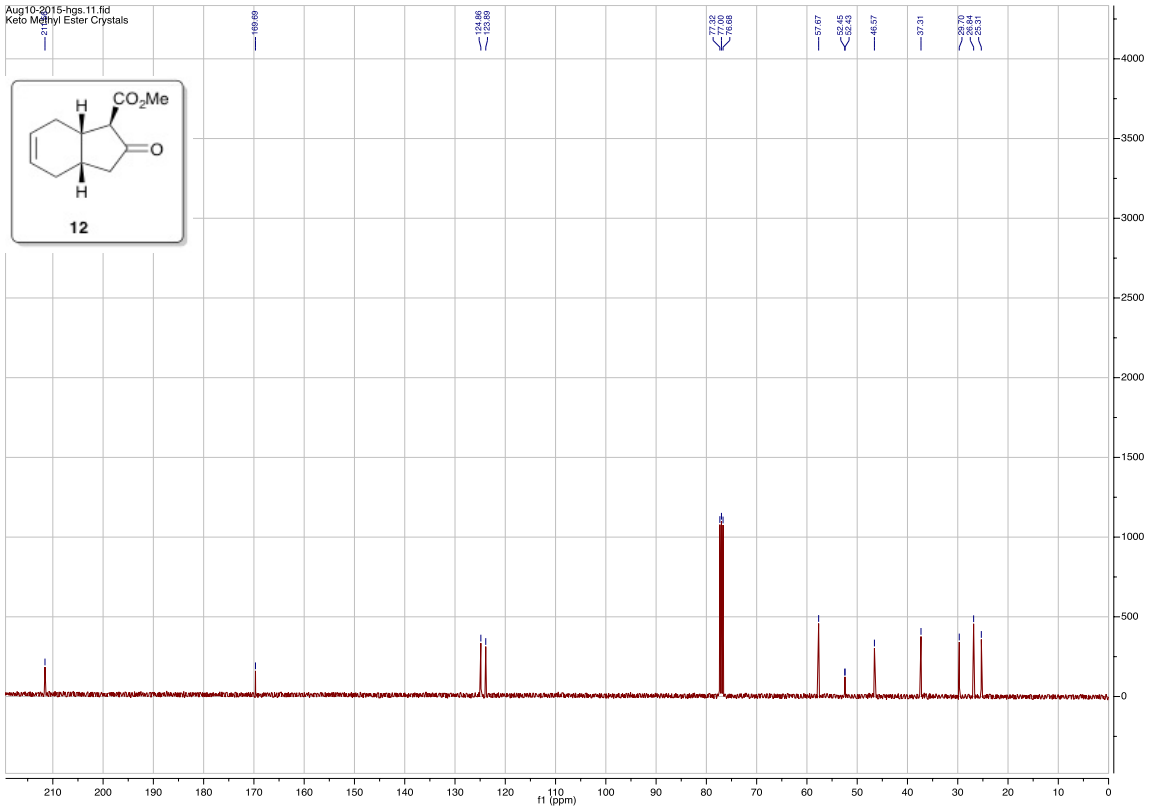


Figure S-14 ^{13}C-NMR spectrum of compound **12**.

Figure S-15 ^{1}H-NMR spectrum of compound **13**.

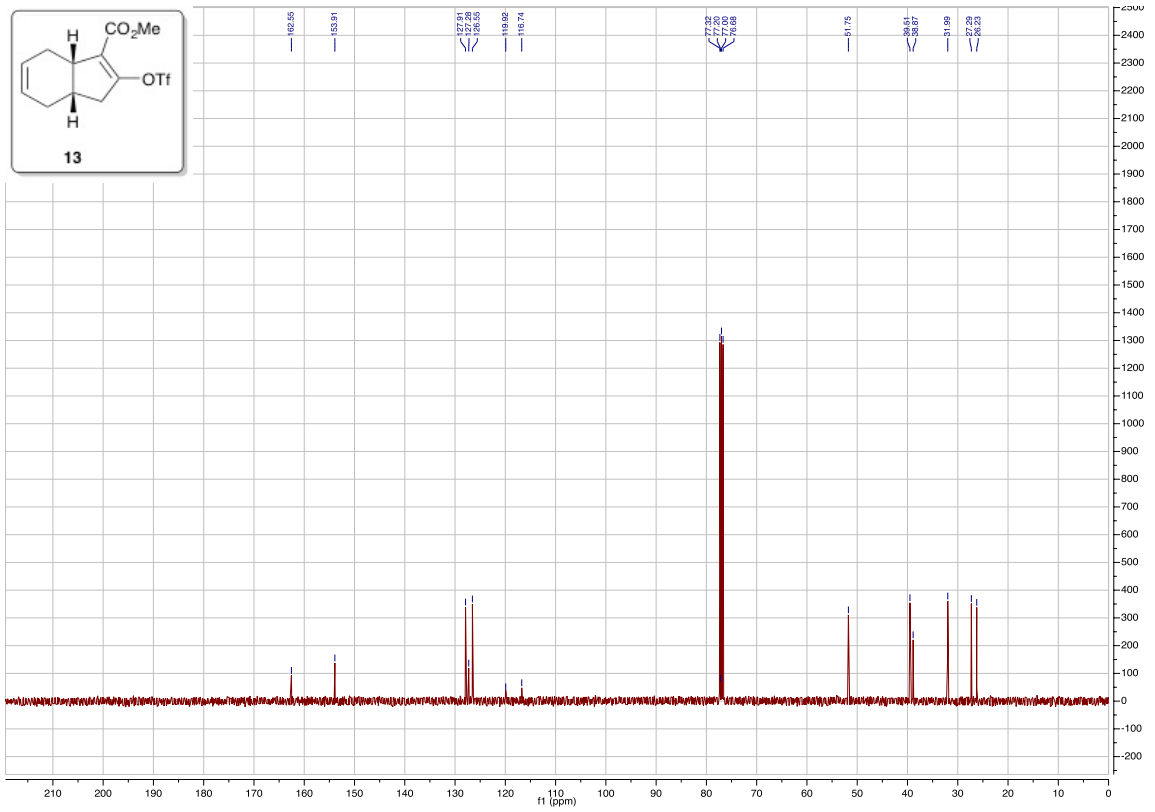


Figure S-16 ^{13}C-NMR spectrum of compound **13**.

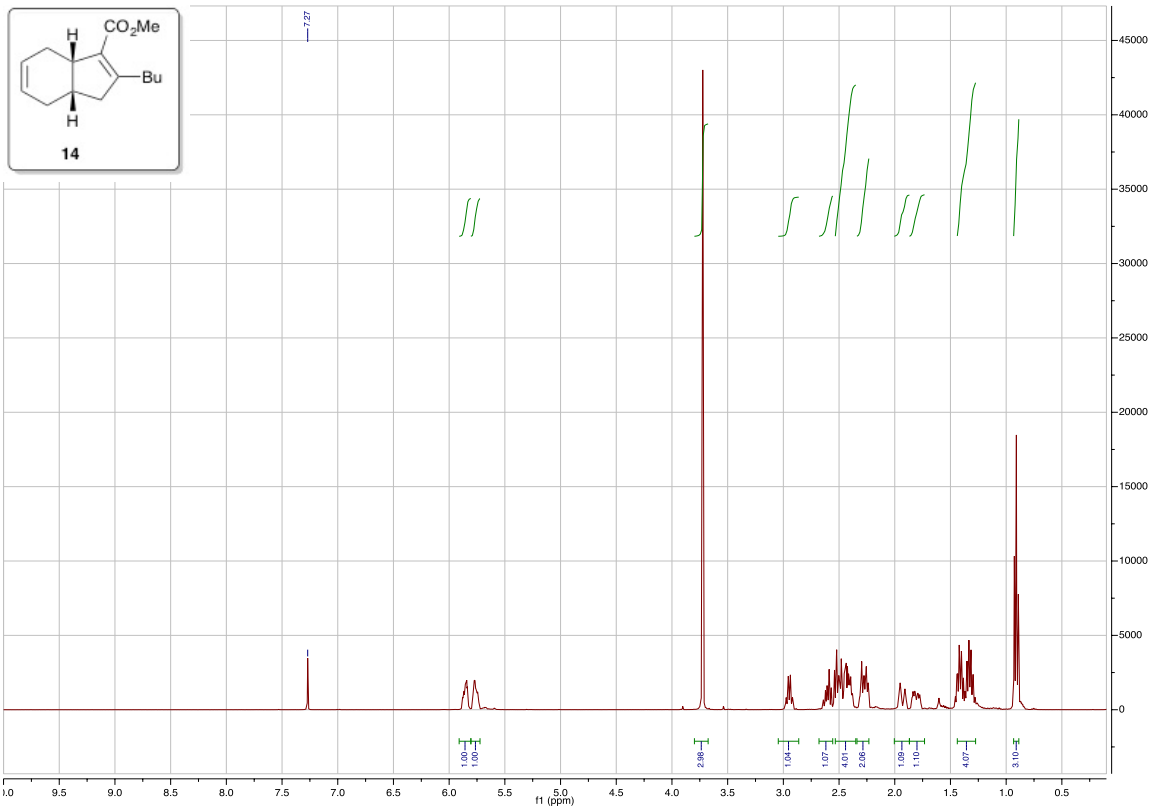


Figure S-17 ^{1}H-NMR spectrum of compound **14**.

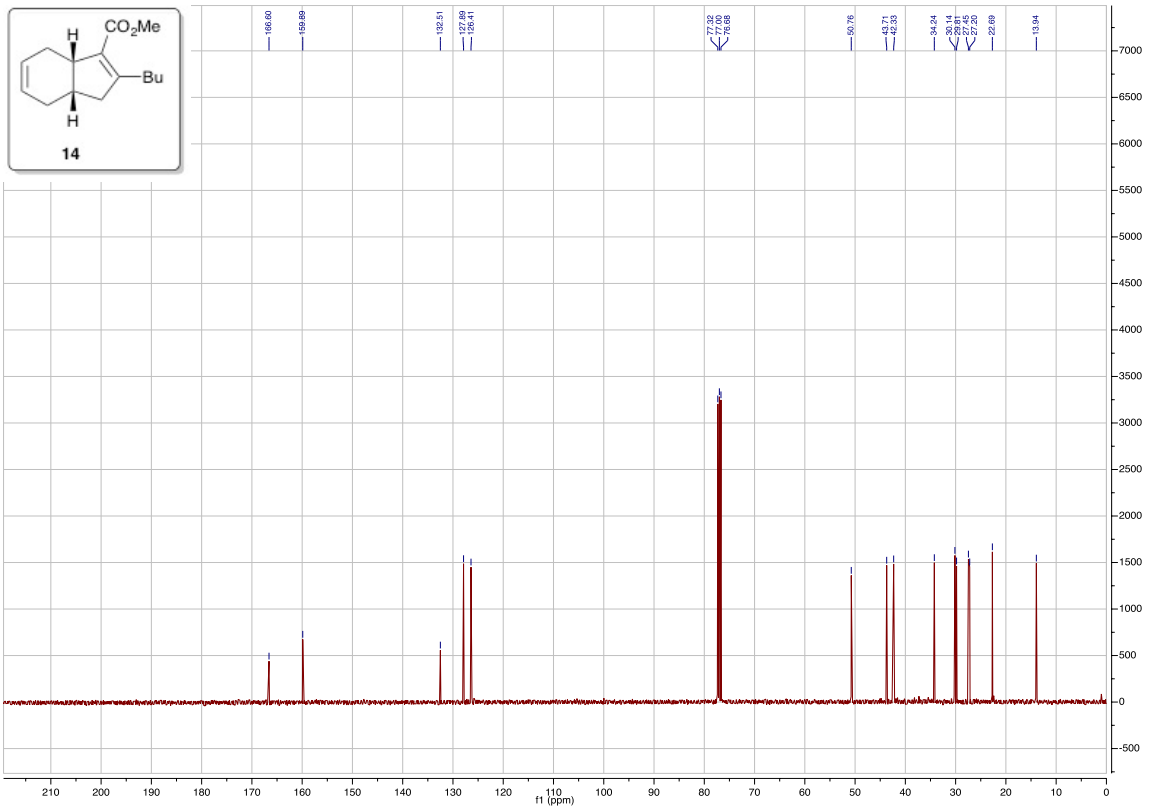


Figure S-18 ^{13}C-NMR spectrum of compound **14**.

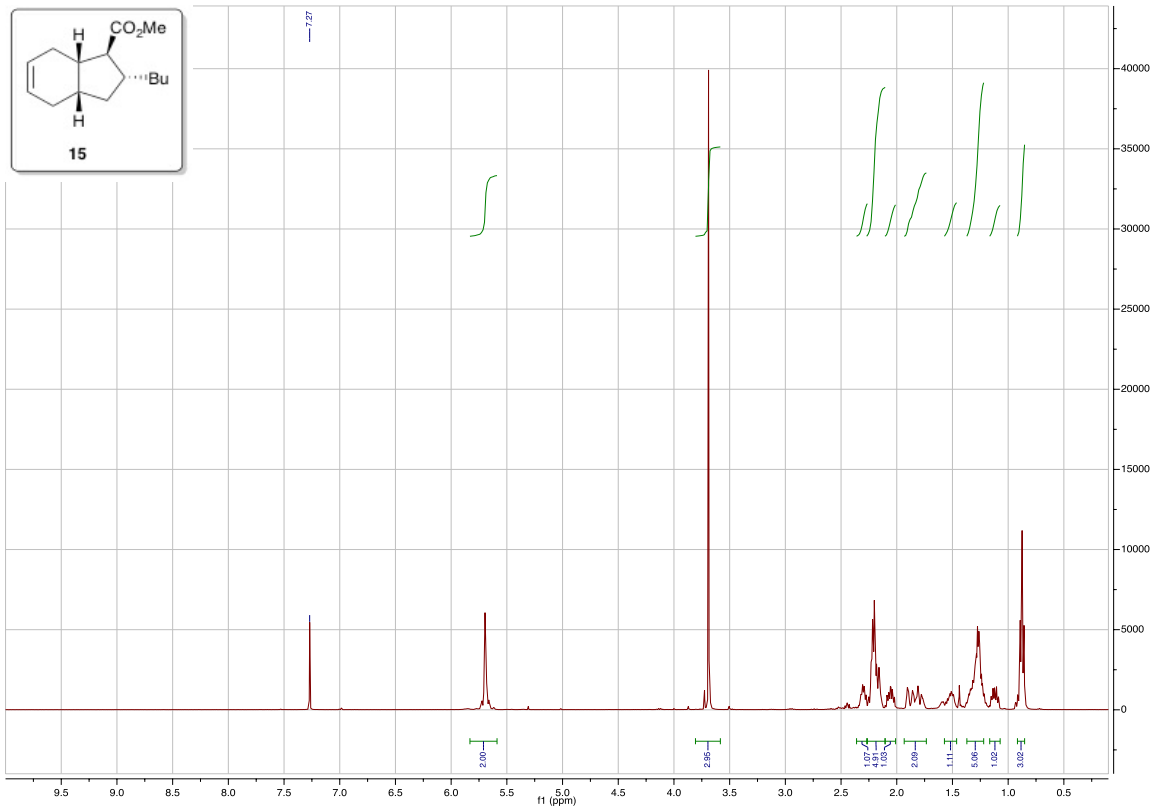


Figure S-19 ^{1}H-NMR spectrum of compound **15**.

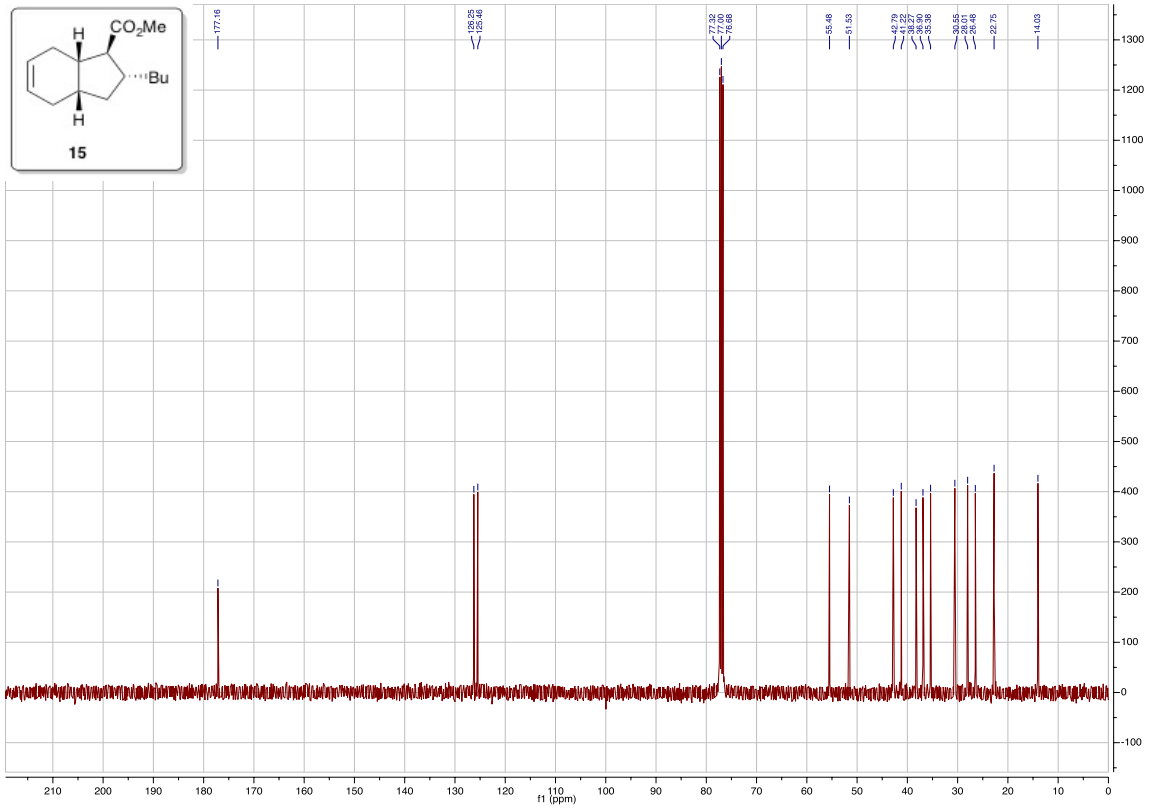


Figure S-20 ^{13}C-NMR spectrum of compound **15**.

Figure S-21 ^{1}H-NMR spectrum of compound **16**.

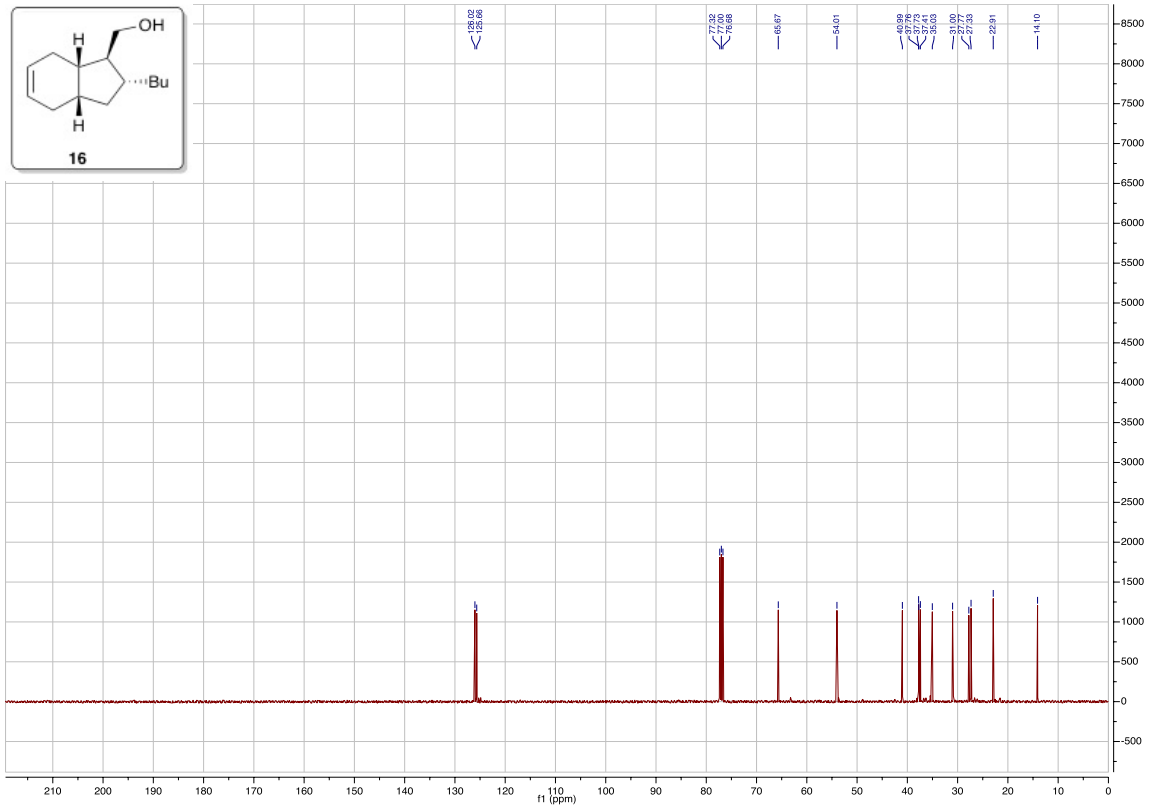


Figure S-22 ^{13}C-NMR spectrum of compound **22**.

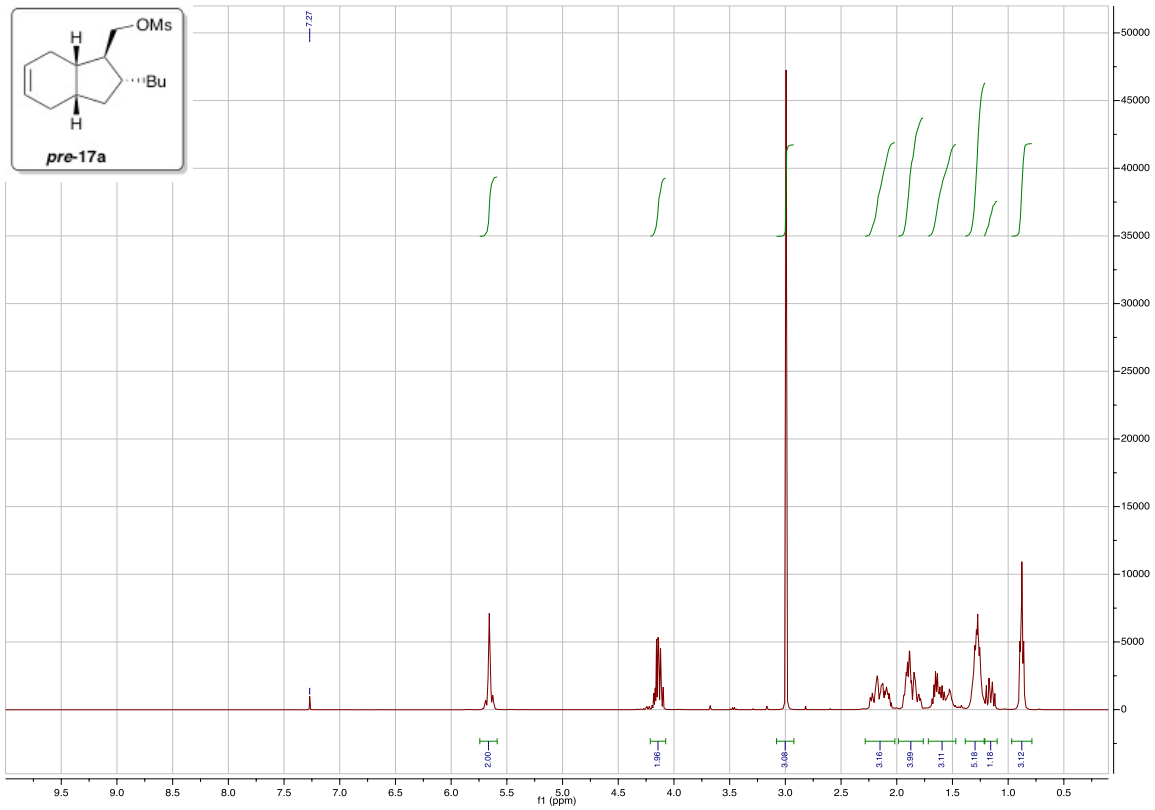


Figure S-23 ^{1}H-NMR spectrum of compound ***pre*-17a**.

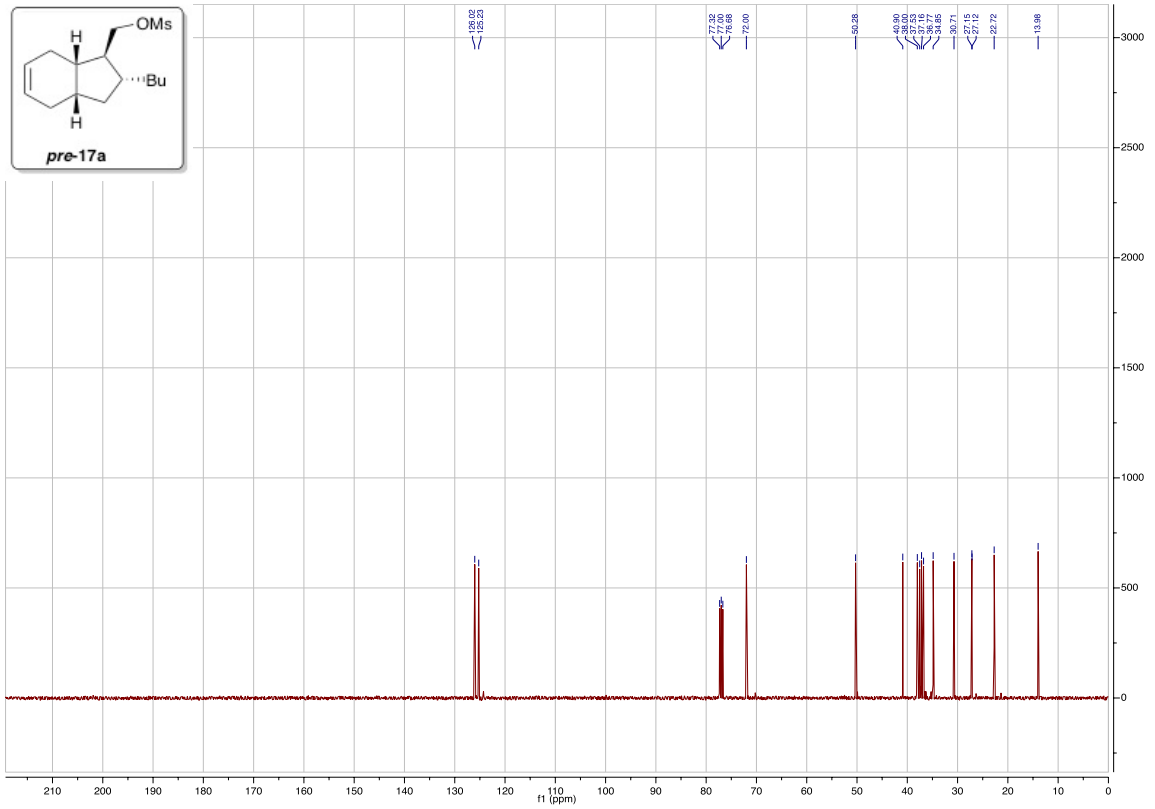


Figure S-24 ^{13}C-NMR spectrum of compound ***pre*-17a**.

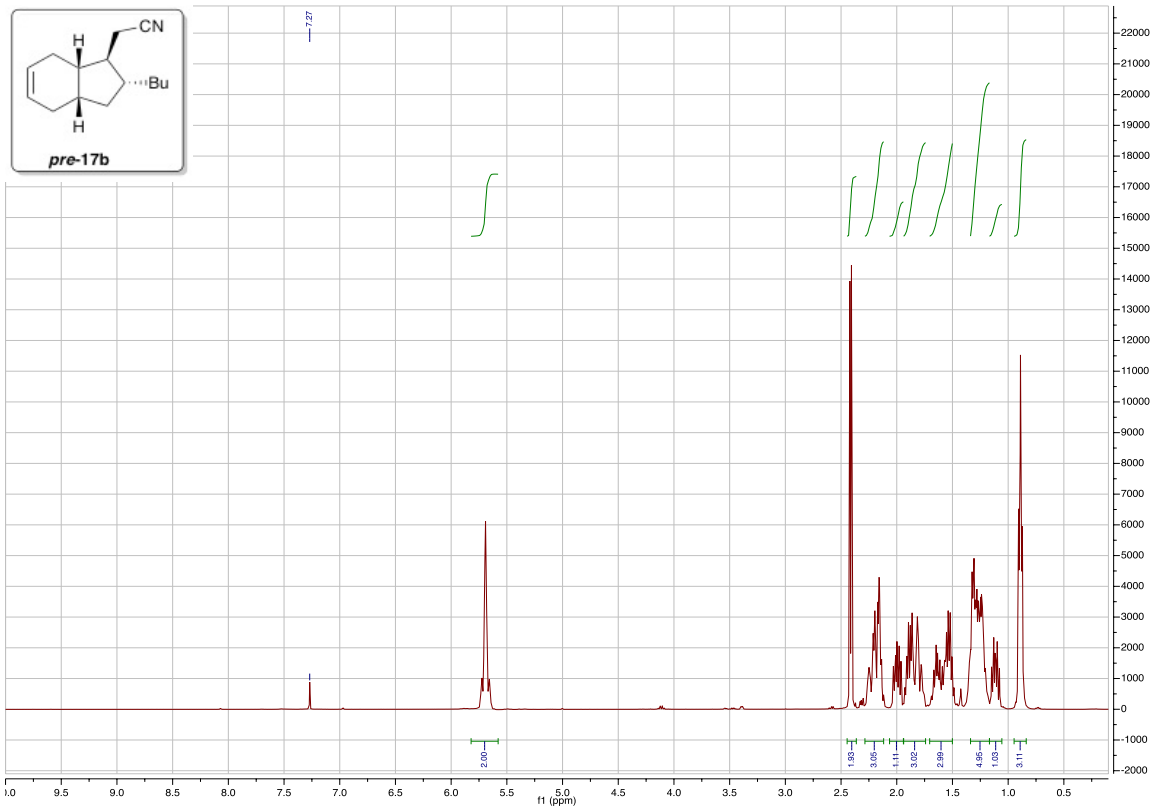


Figure S-25 ^{1}H-NMR spectrum of compound ***pre*-17b**.

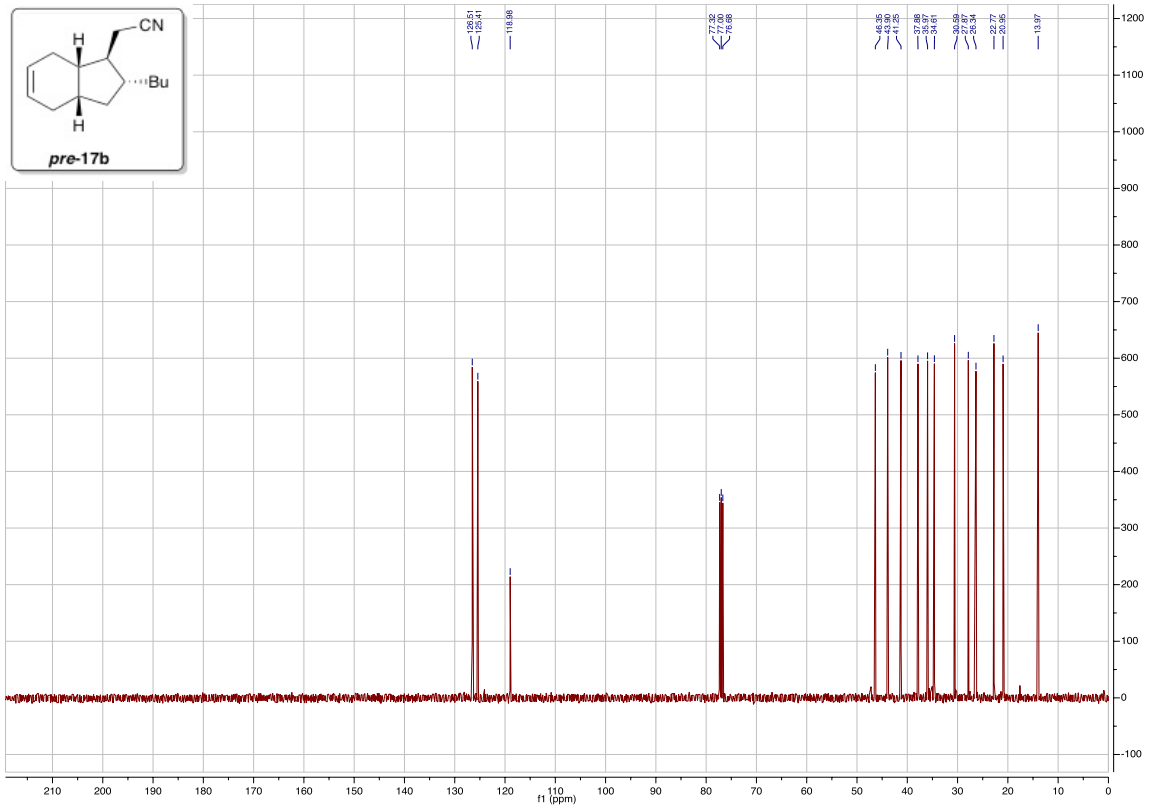


Figure S-26 ^{13}C-NMR spectrum of compound ***pre*-17b**.

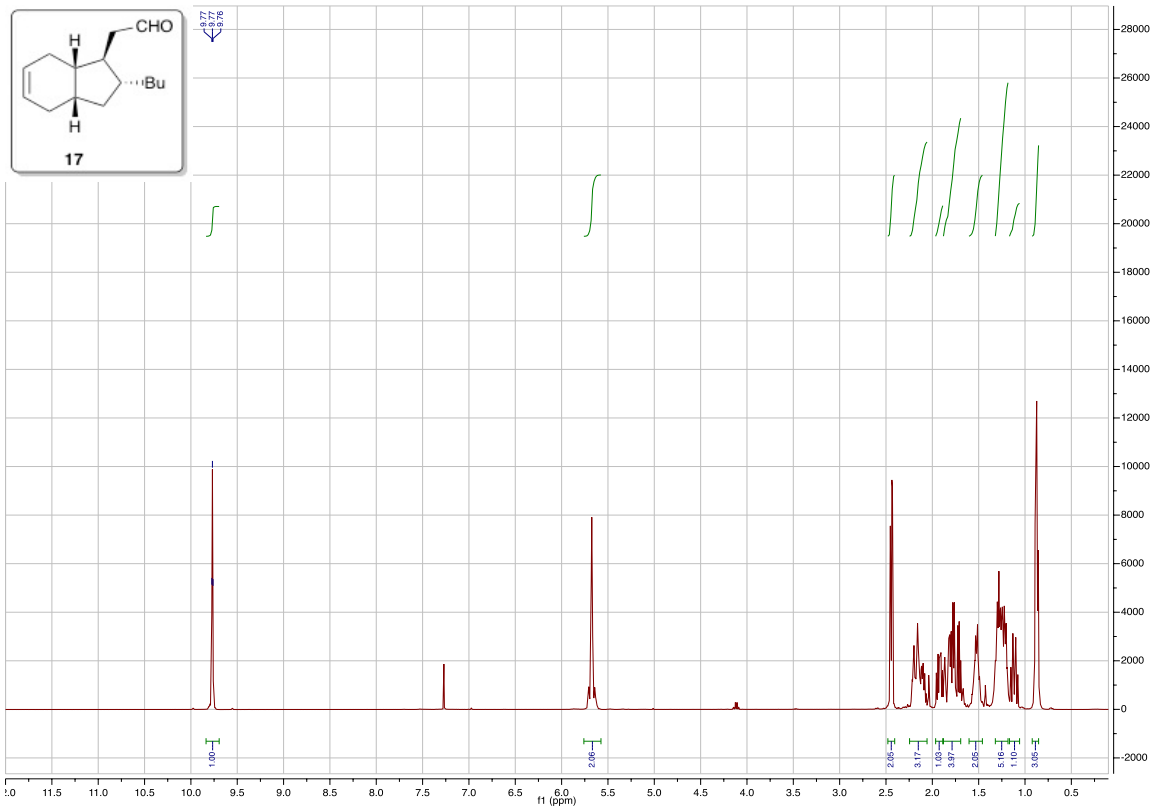


Figure S-27 ^{1}H-NMR spectrum of compound **17**.

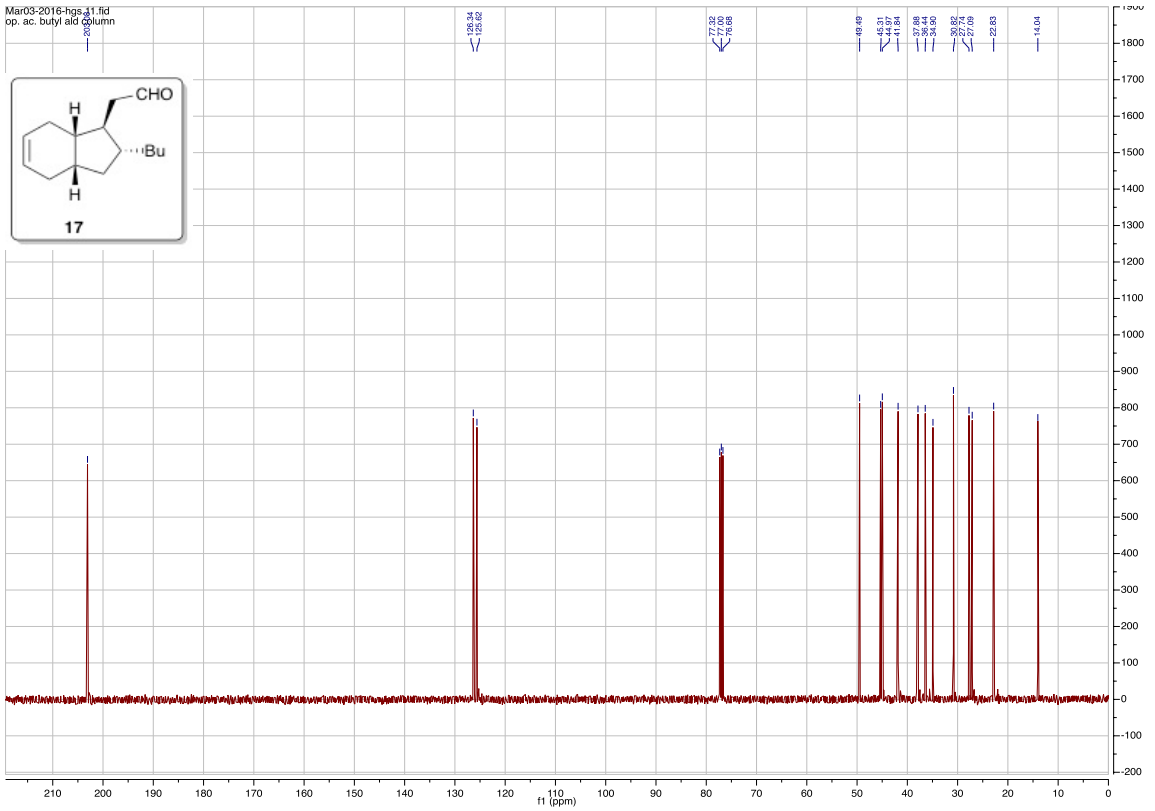


Figure S-28 ^{13}C-NMR spectrum of compound **17**.

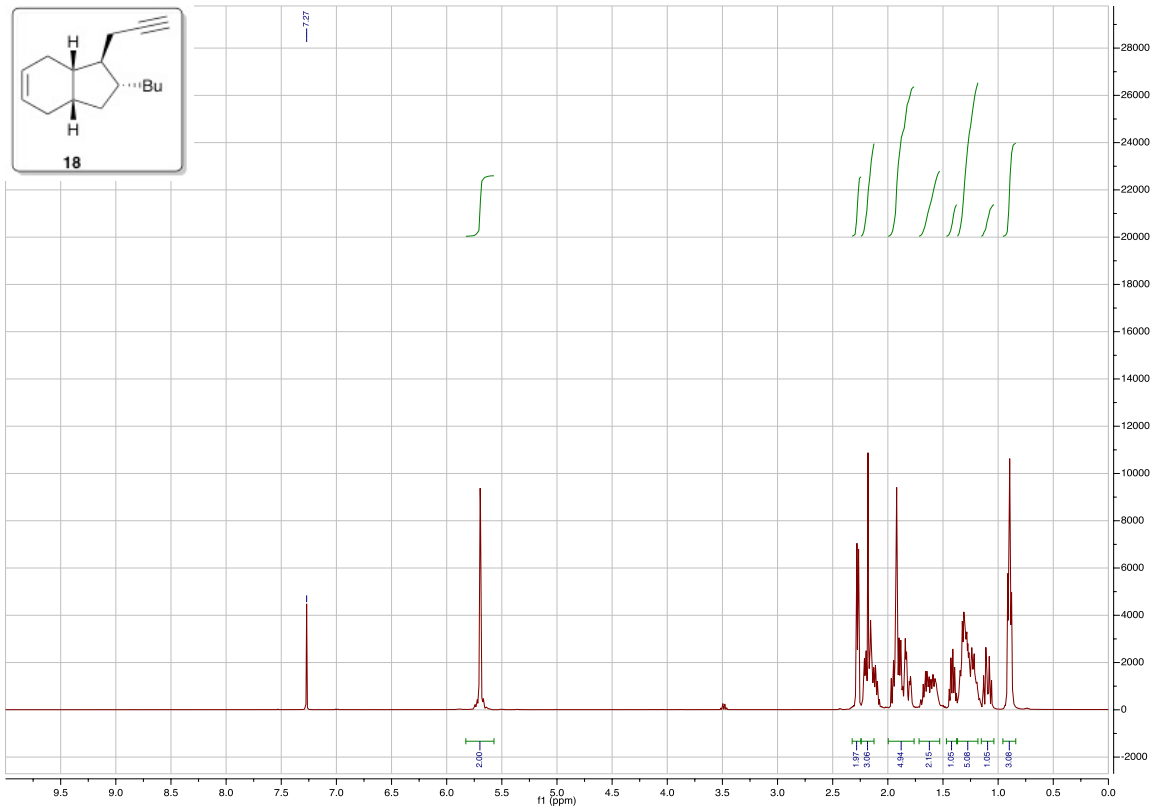


Figure S-29 ^{1}H-NMR spectrum of compound **18**.

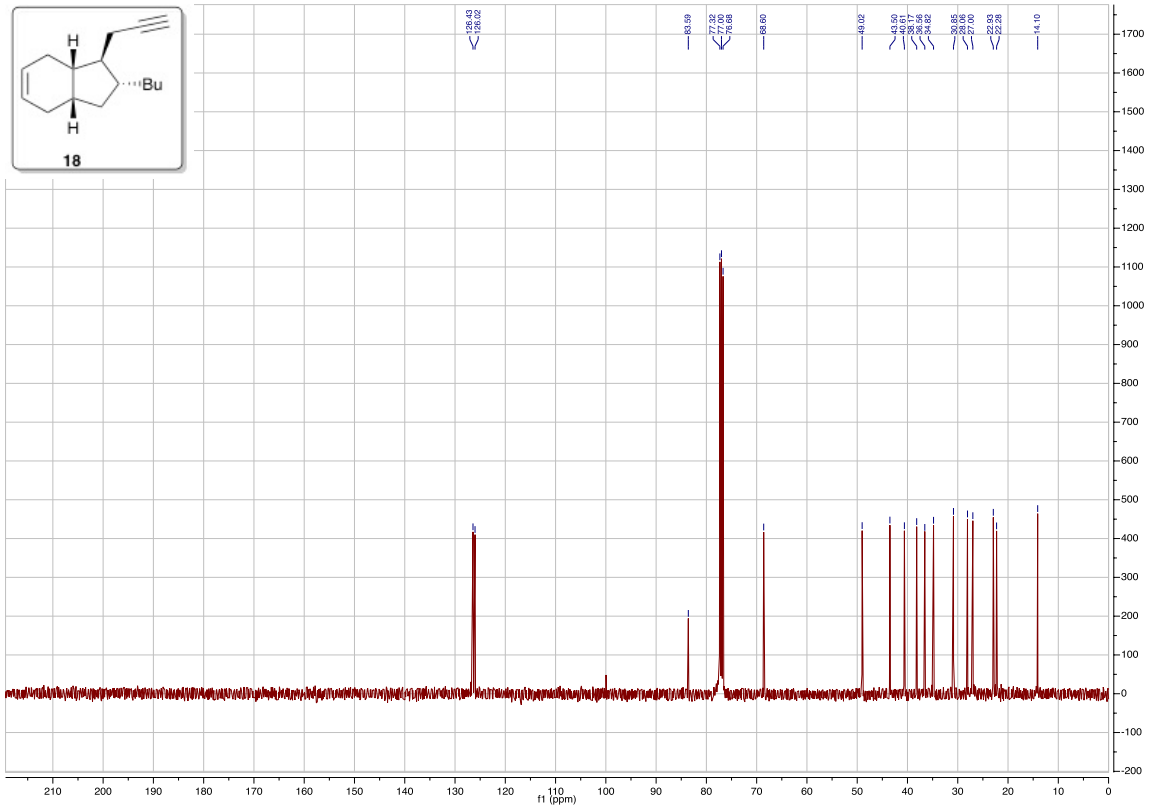


Figure S-30 ^{13}C-NMR spectrum of compound **18**.

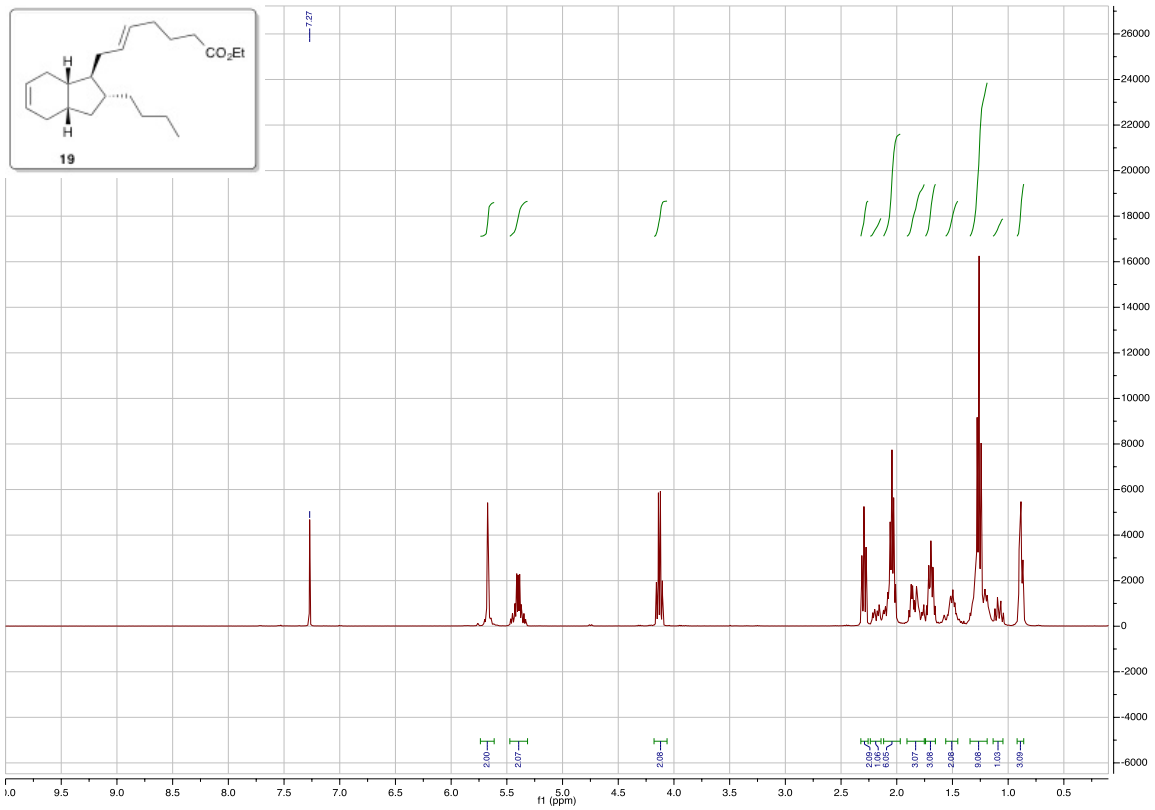


Figure S-31 ^{1}H-NMR spectrum of compound **19**.

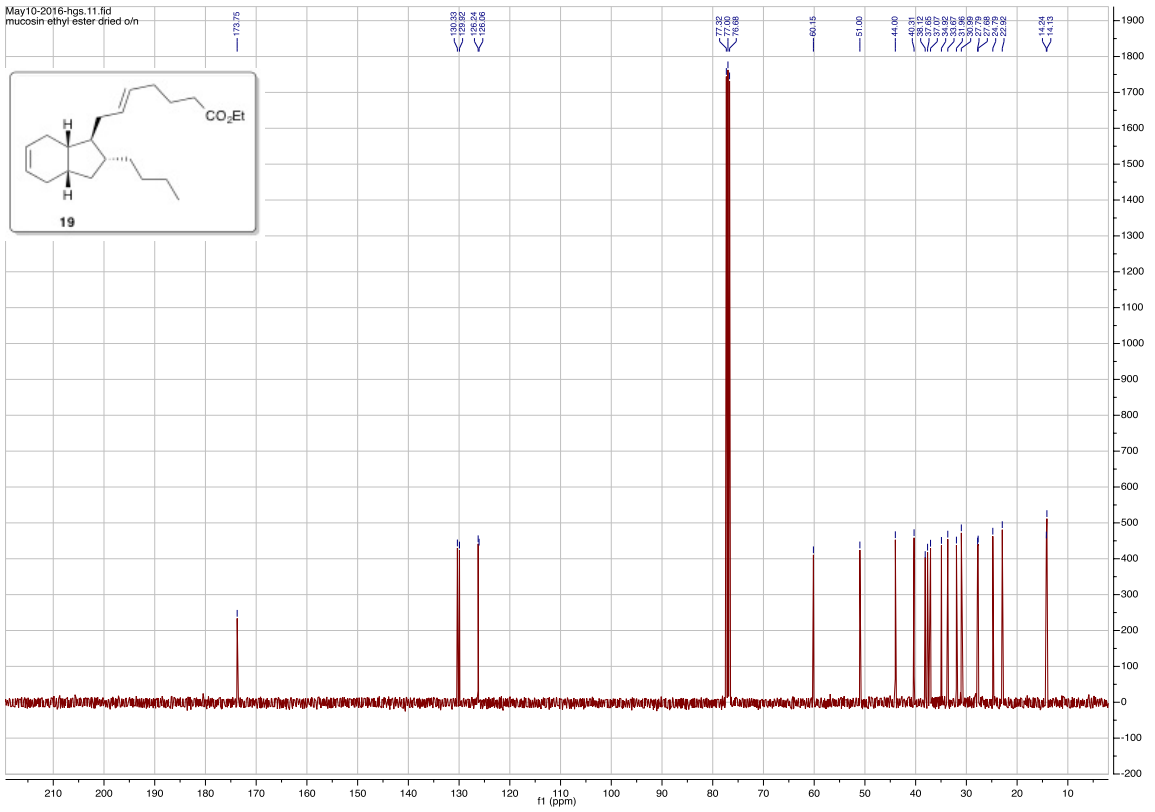


Figure S-32 ^{13}C-NMR spectrum of compound **19**.

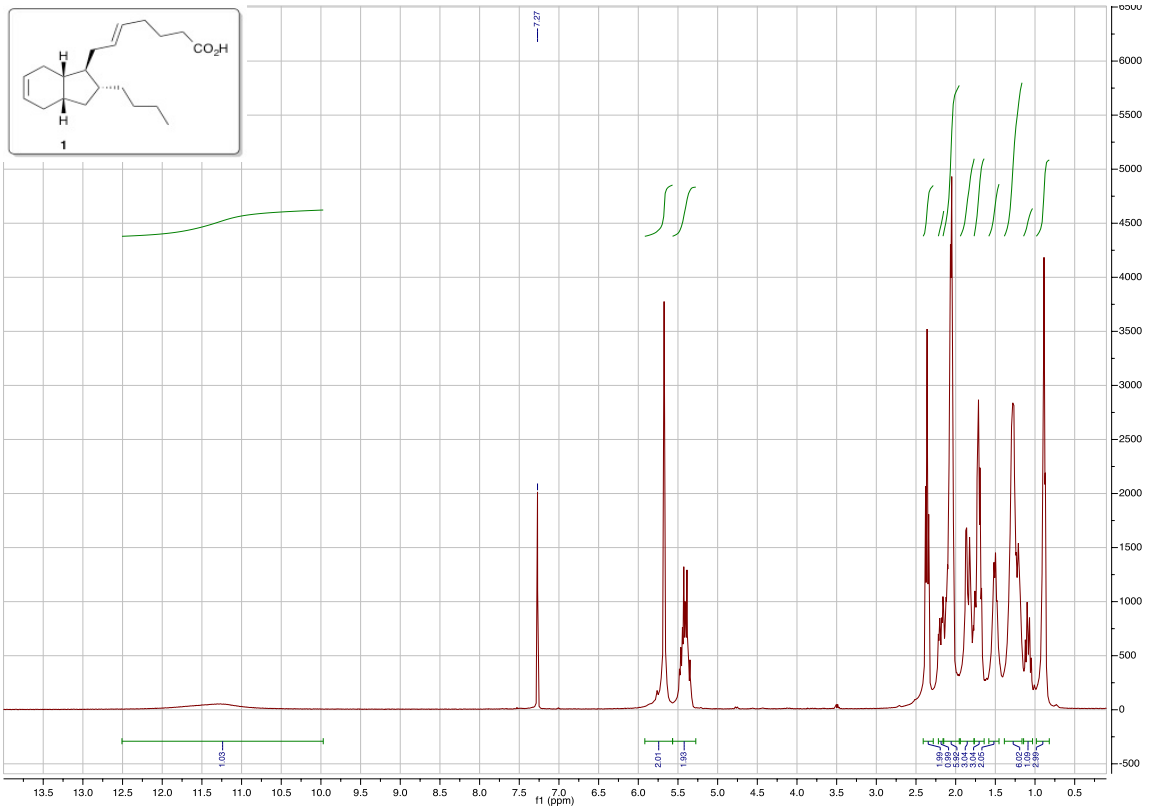


Figure S-33 ^{1}H-NMR spectrum of compound **1**.

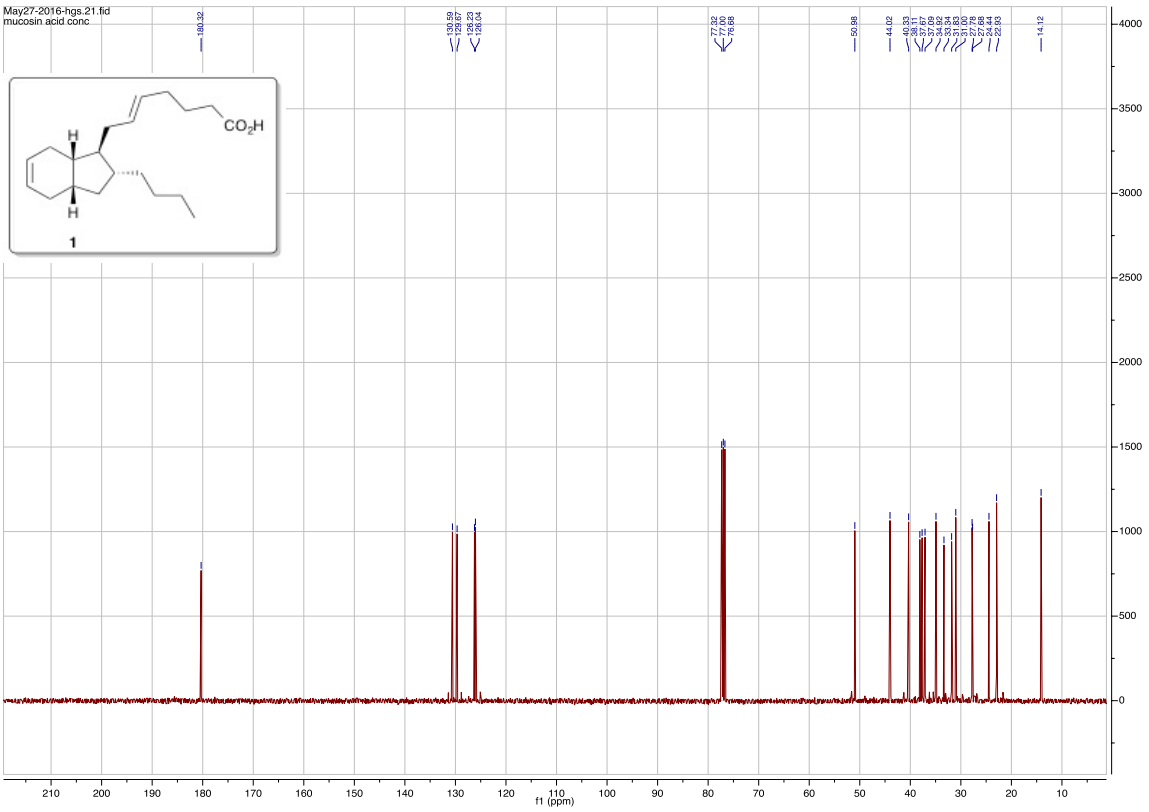


Figure S-34 ^{13}C-NMR spectrum of compound **1**.

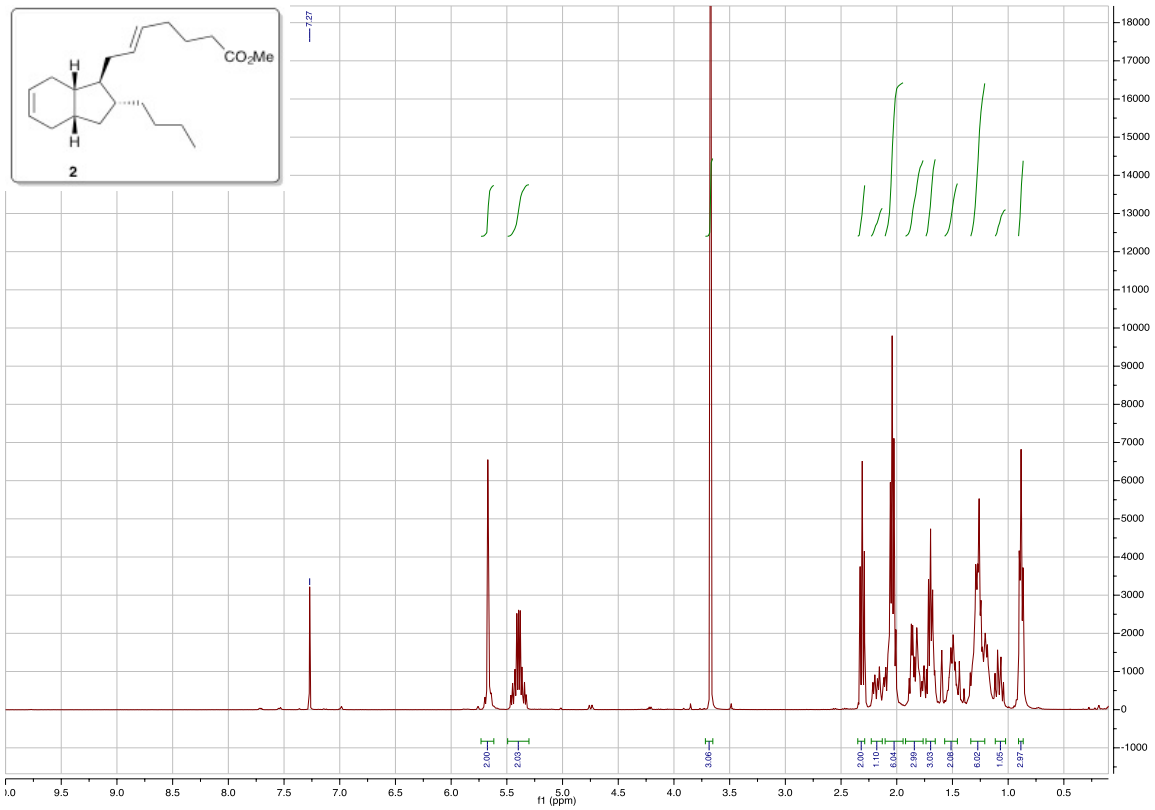


Figure S-35 ^{1}H-NMR spectrum of compound **2**.

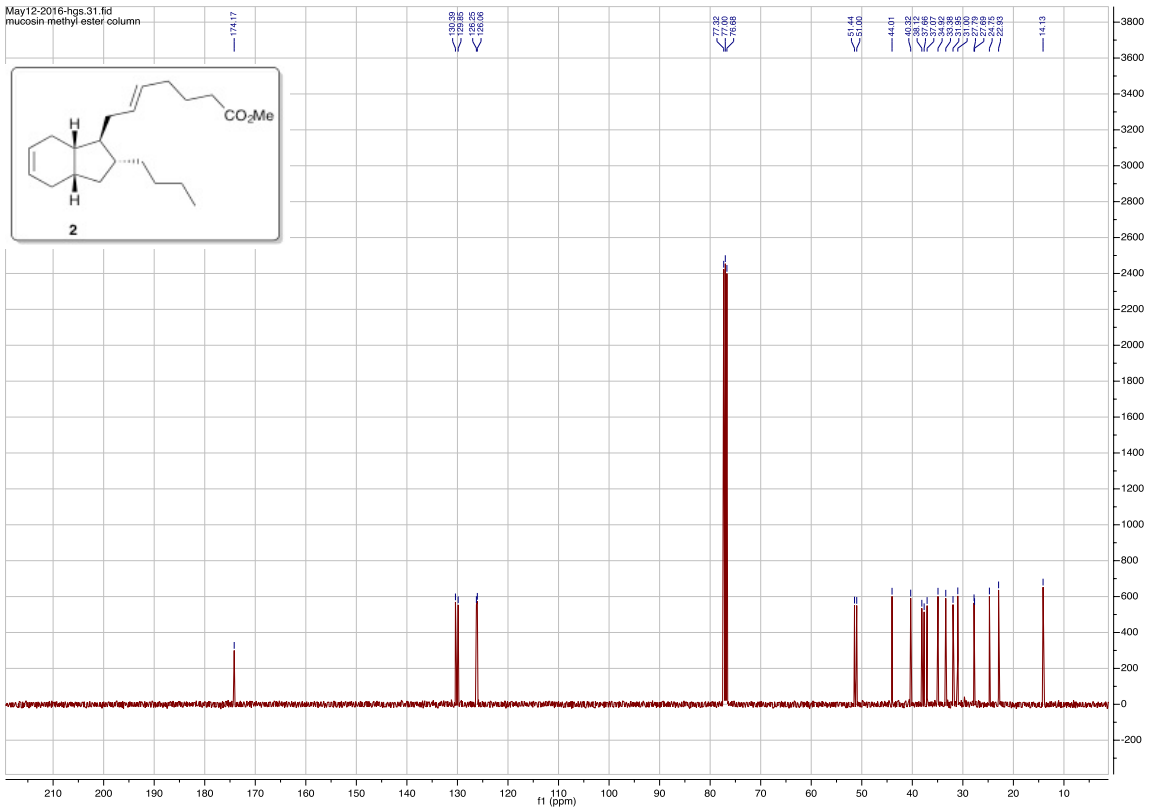


Figure S-36 ^{13}C-NMR spectrum of compound **2**.

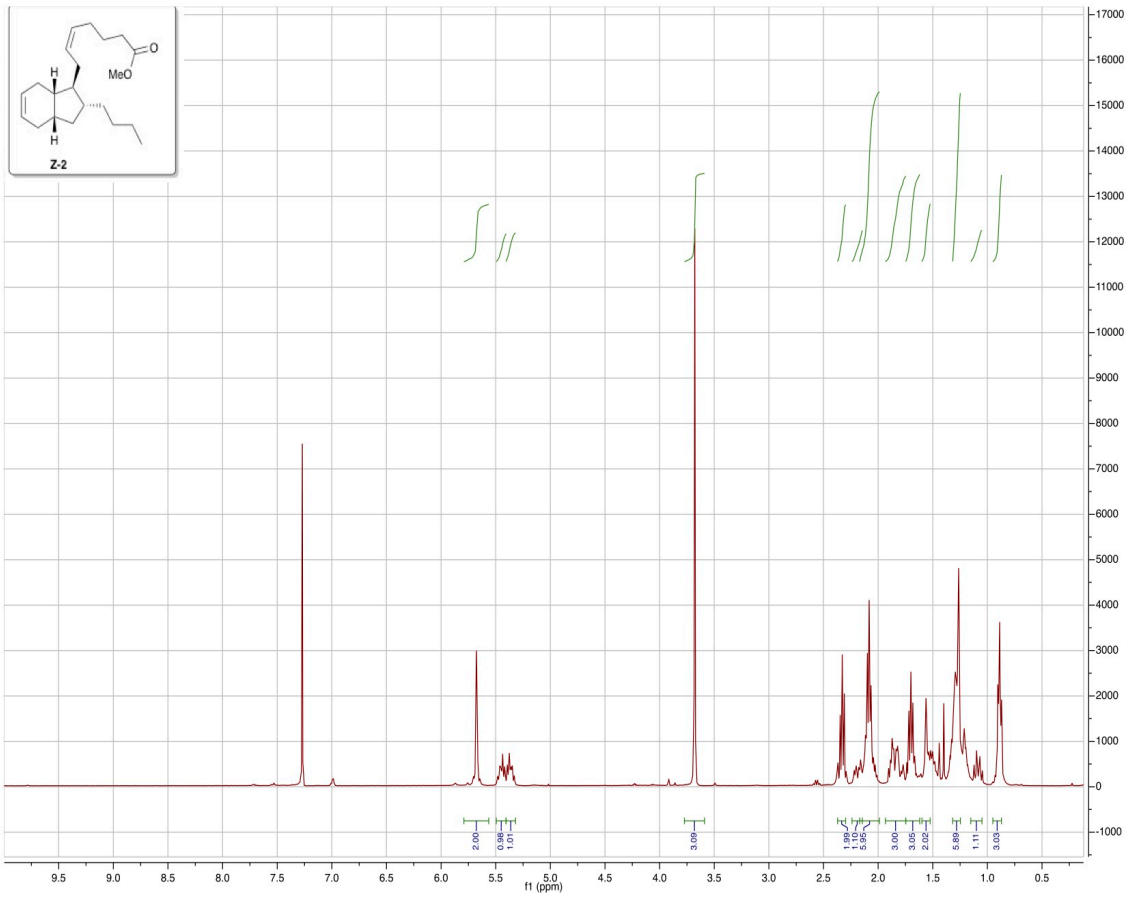


Figure S-37 ^{1}H-NMR spectrum of compound **2*Z*** for comparison with **2.**

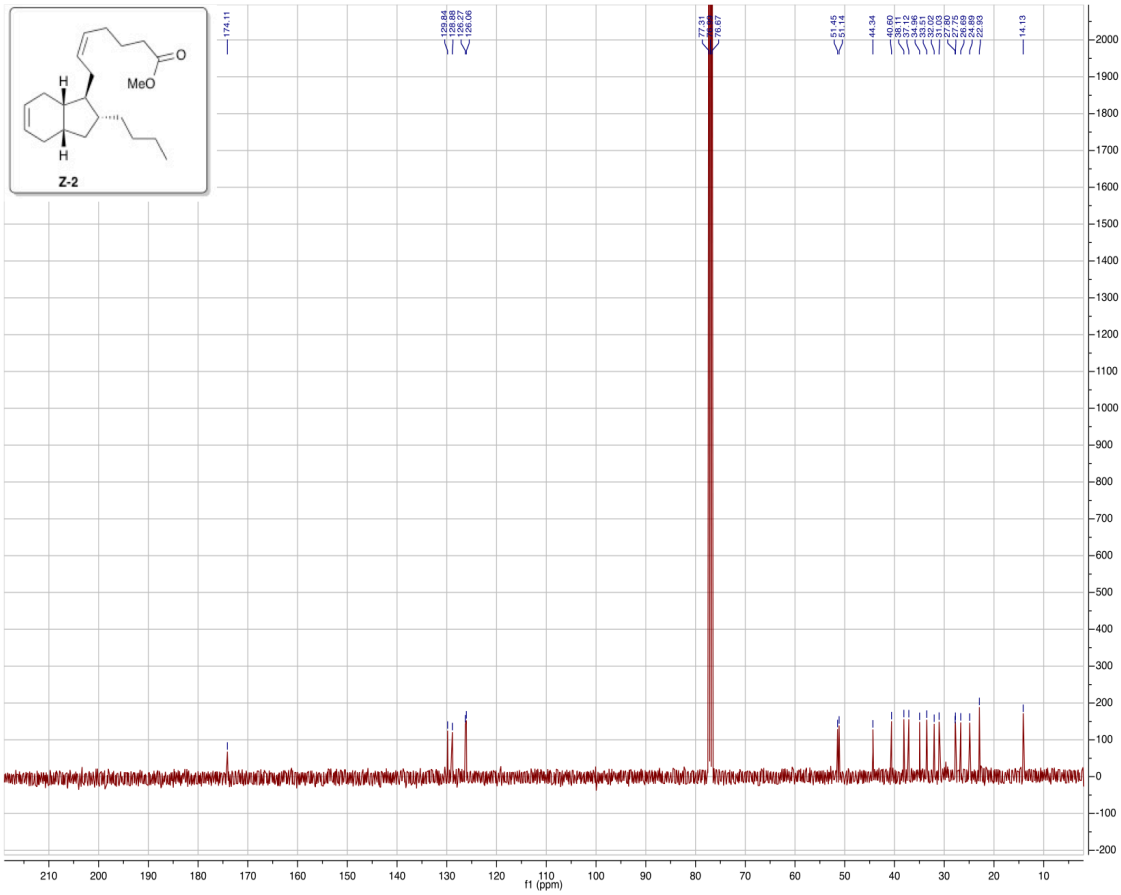


Figure S-38 ^{13}C-NMR spectrum of compound **2*Z*** for comparison with **2**.

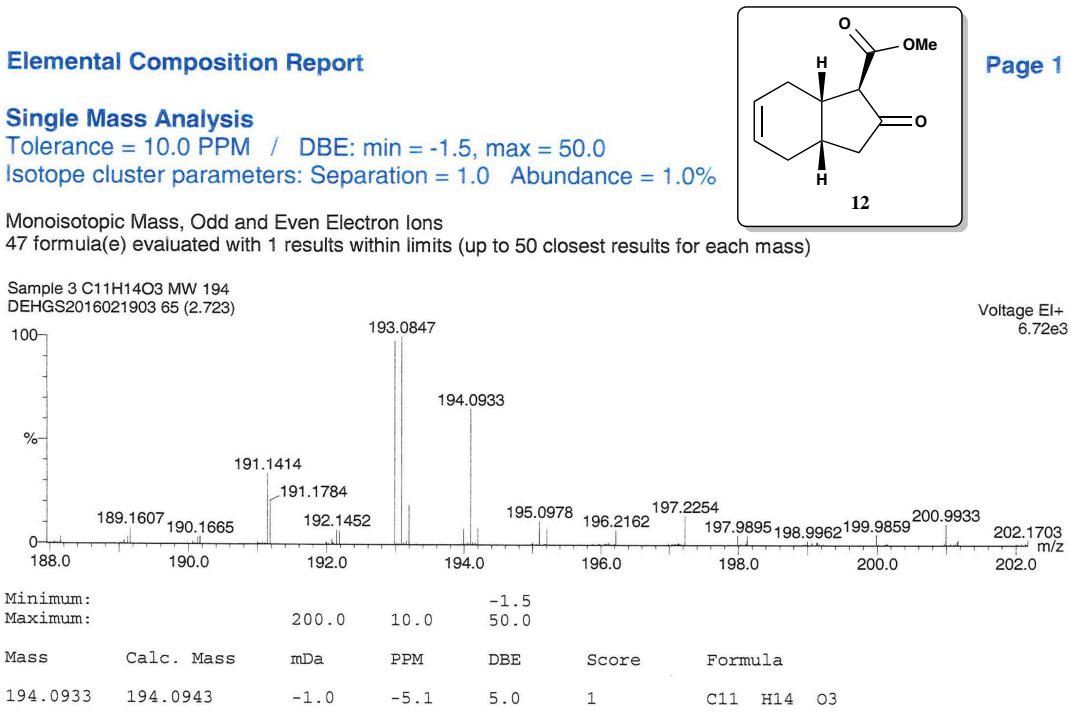


Figure S-39 HRMS of compound **12**.

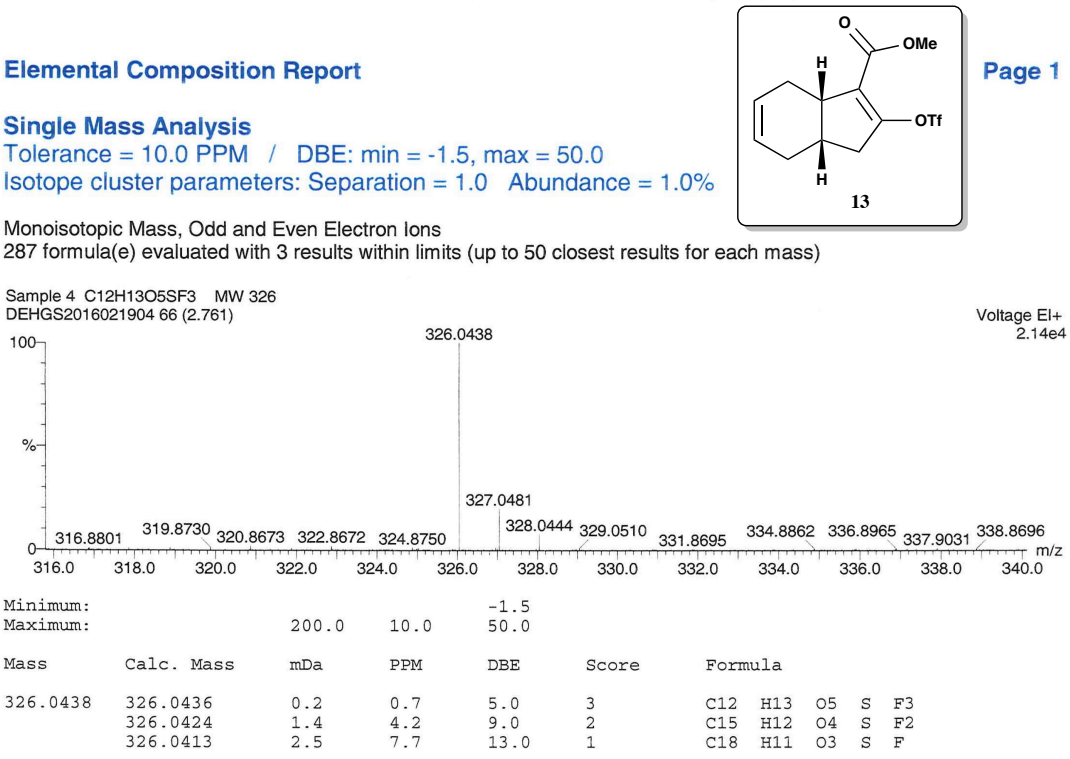


Figure S-40 HRMS of compound **13**.

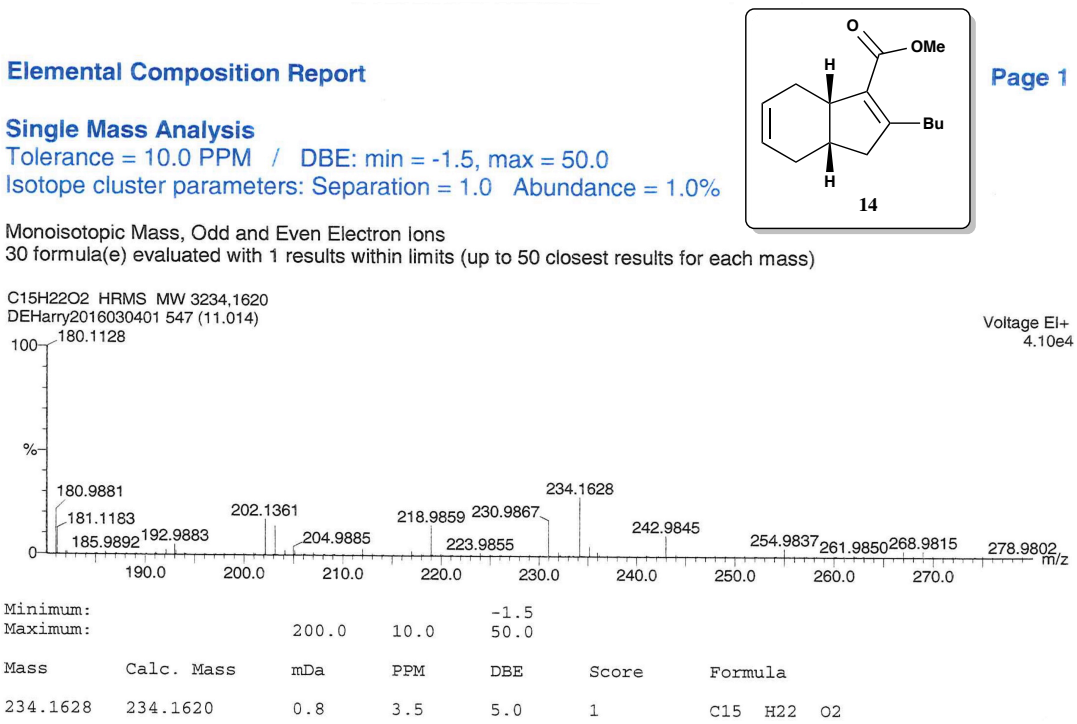


Figure S-41 HRMS of compound **14**.

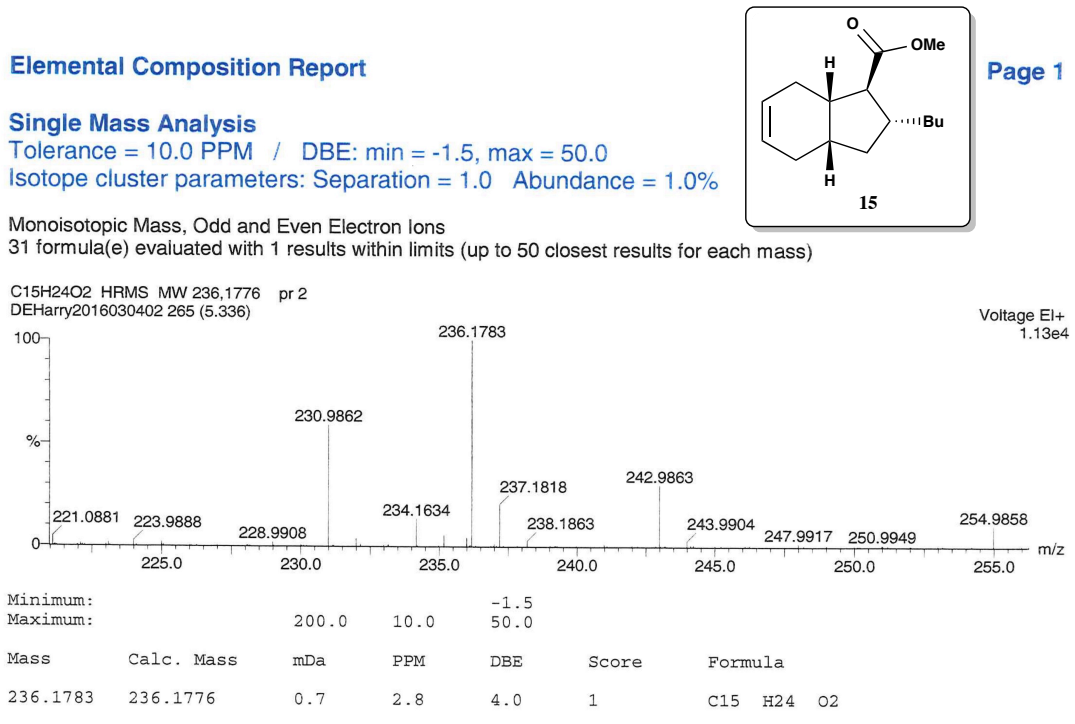


Figure S-42 HRMS of compound **15**.

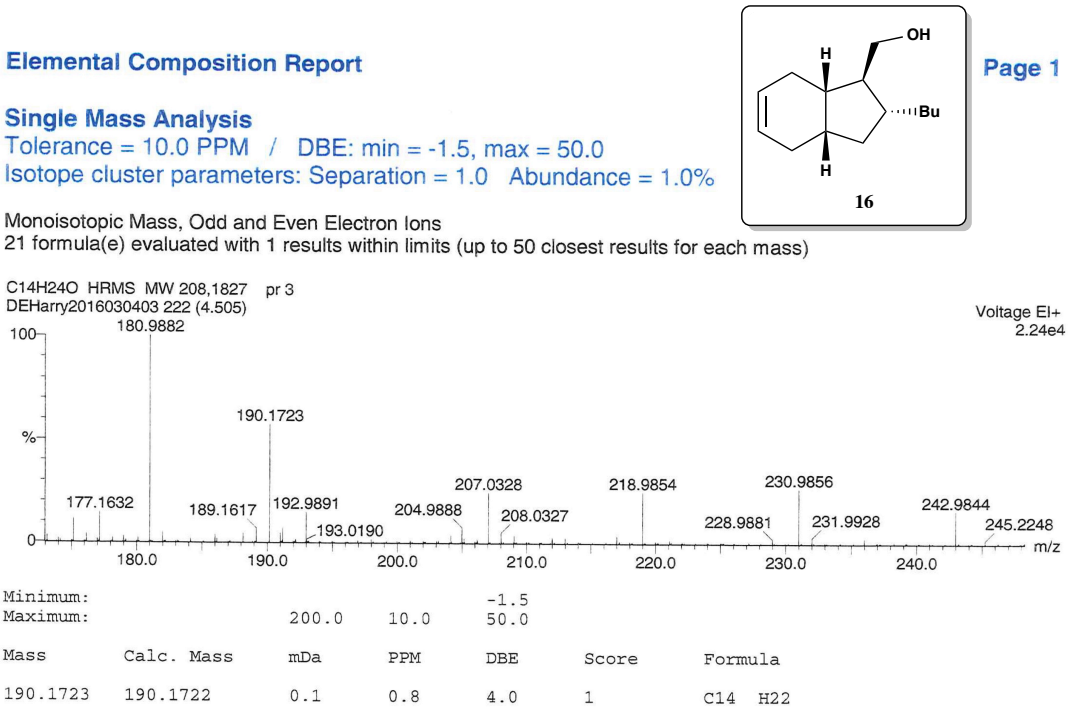


Figure S-43 HRMS of compound **16**.

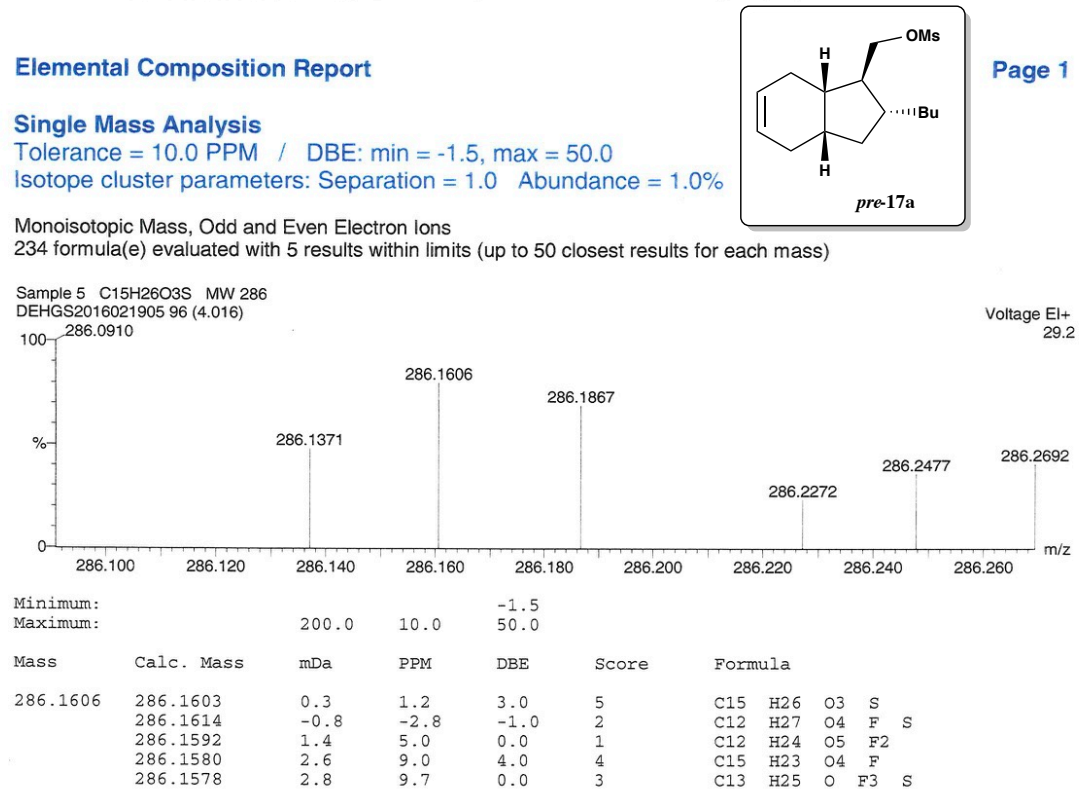


Figure S-44 HRMS of compound ***pre*-17a**.

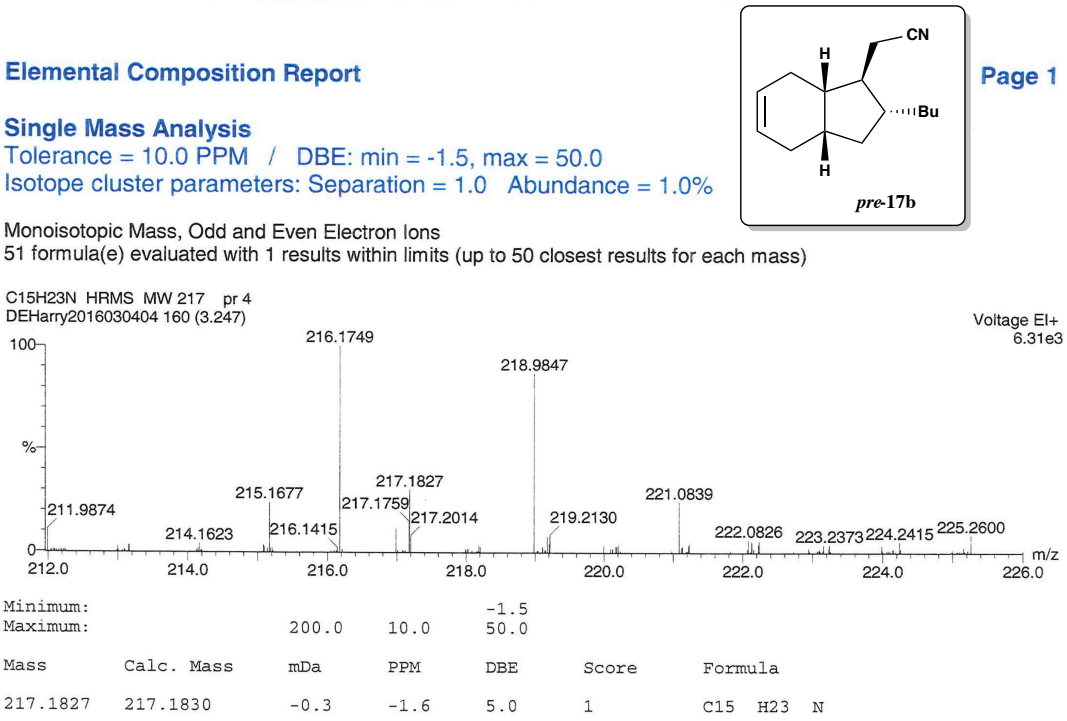


Figure S-45 HRMS of compound ***pre*-17b**.

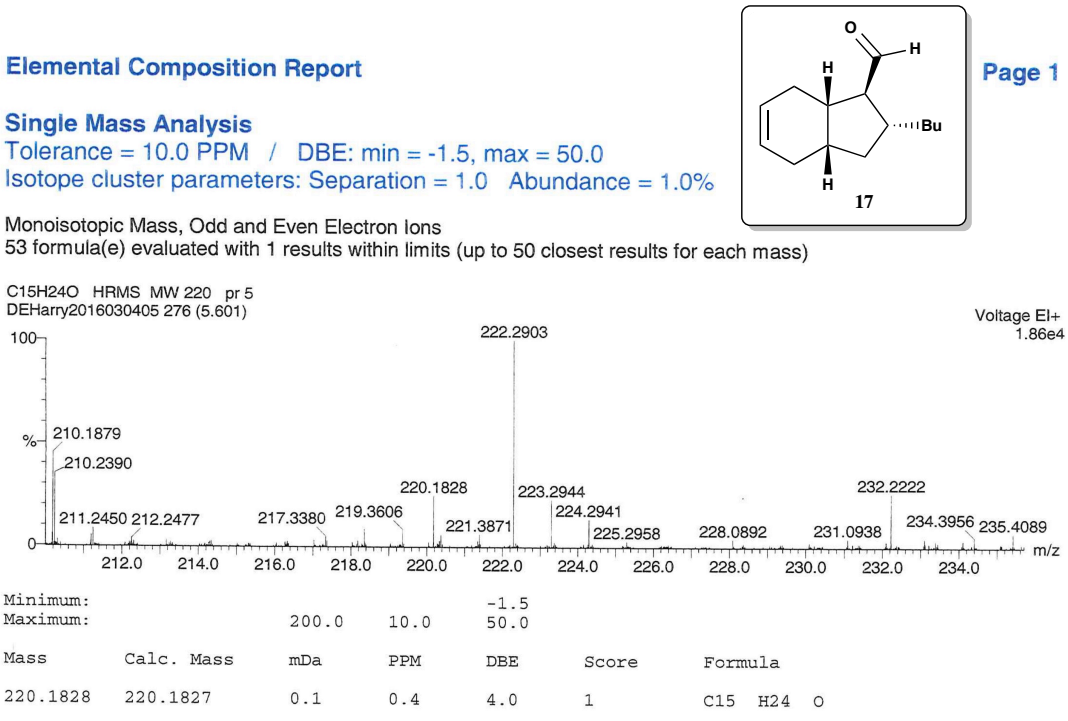


Figure S-46 HRMS of compound **17**.

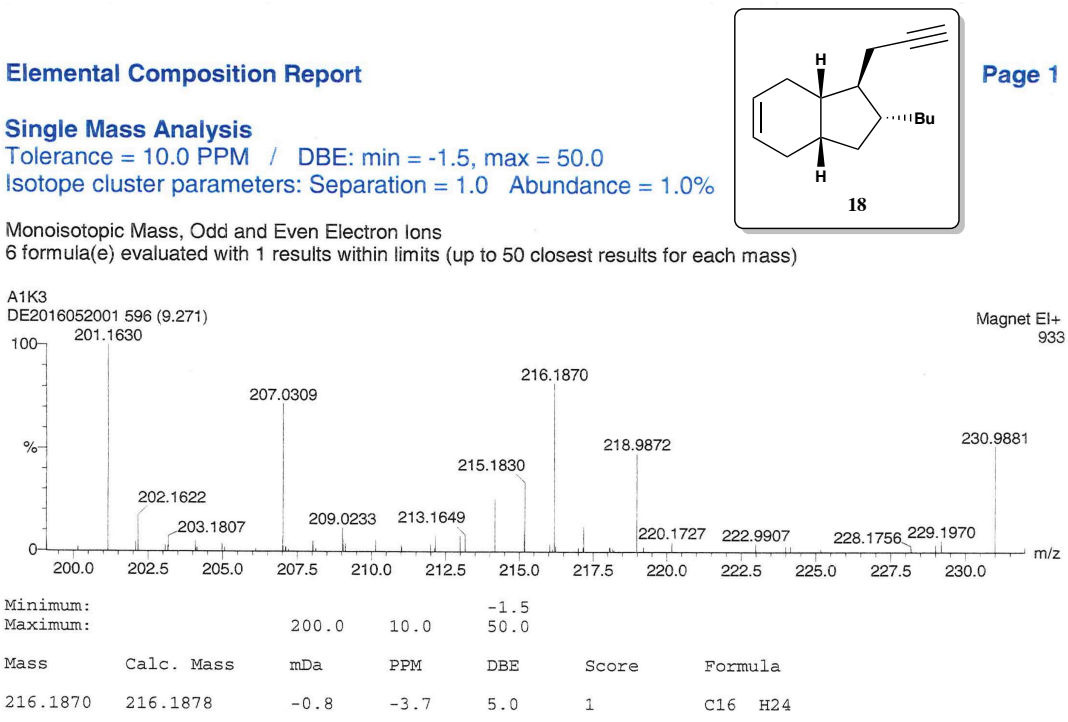


Figure S-47 HRMS of compound **18**.

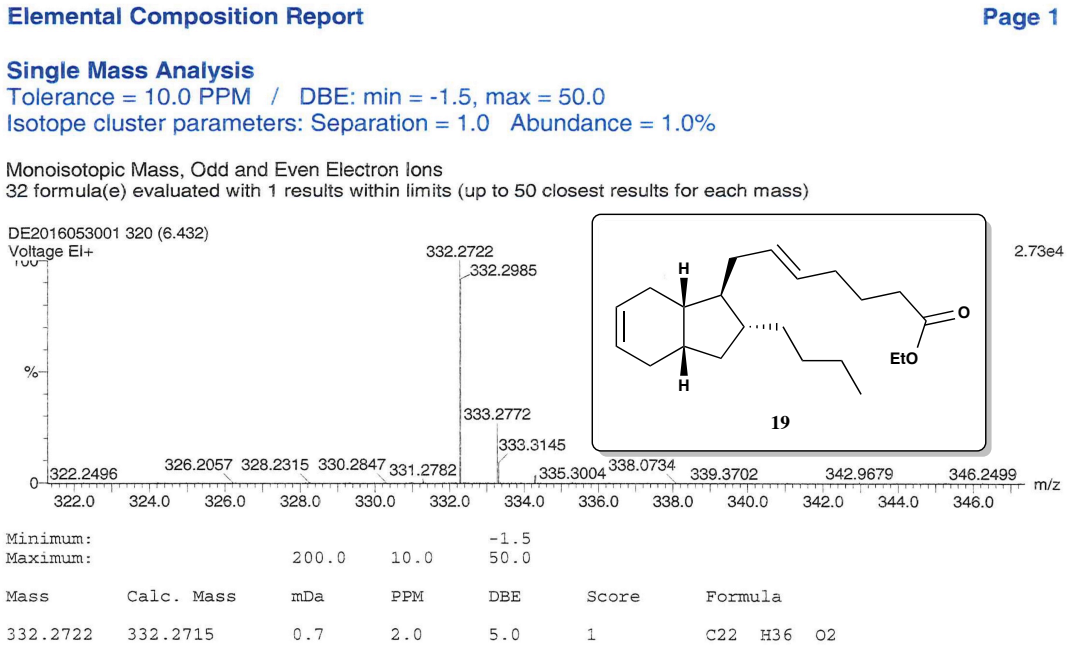


Figure S-48 HRMS of compound **19**.

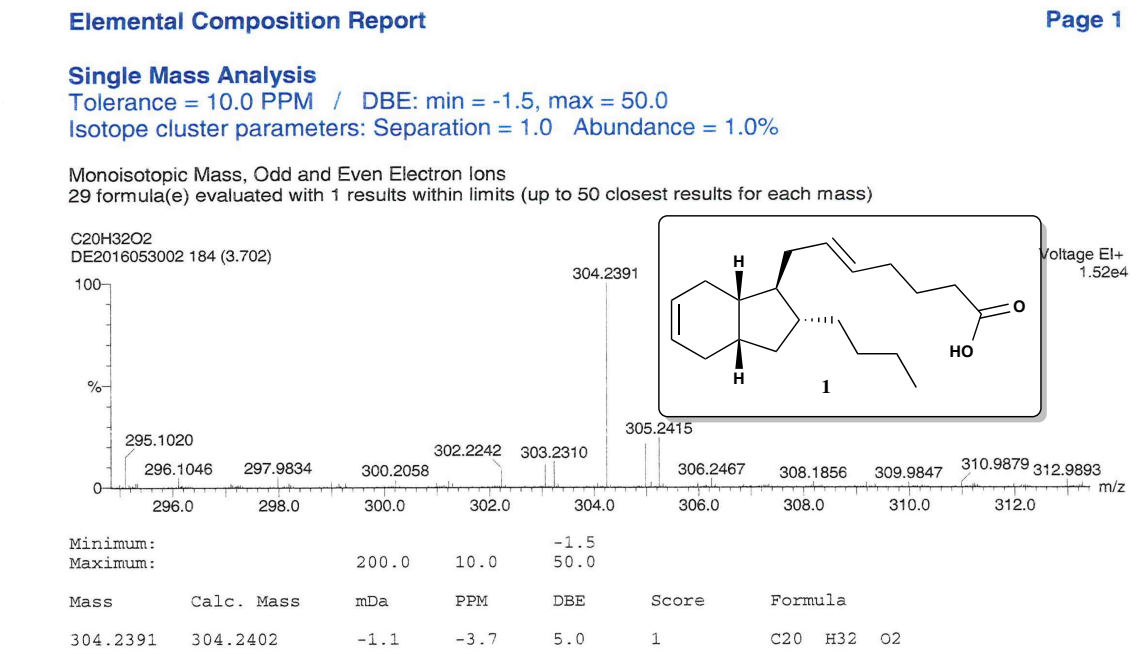


Figure S-49 HRMS of compound **1**.

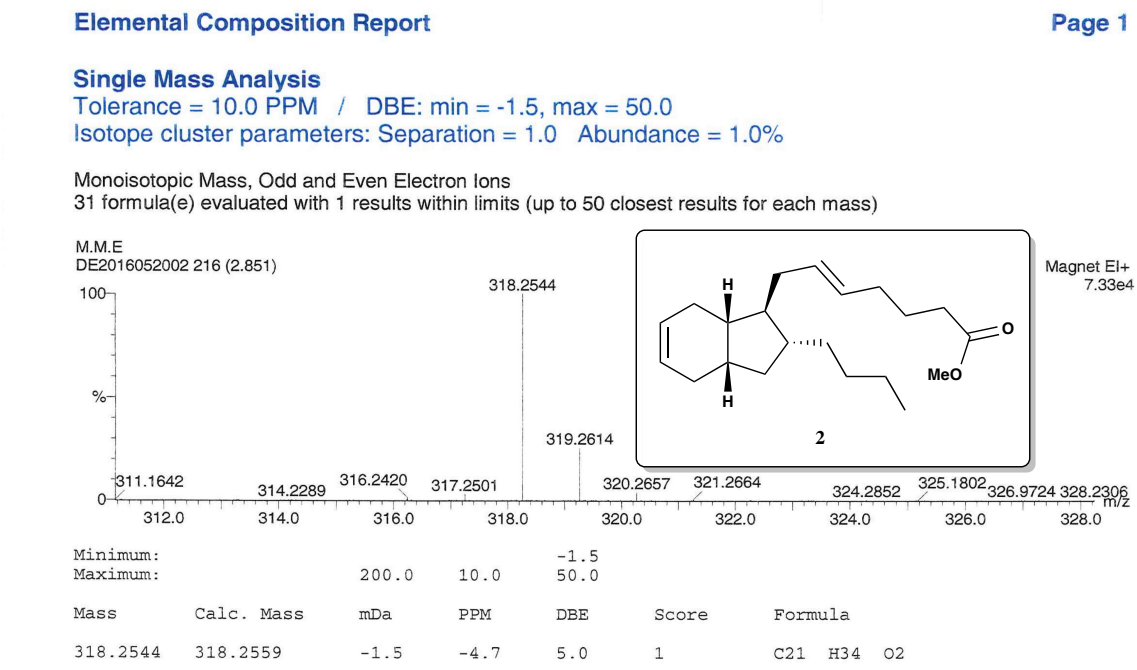


Figure S-50 HRMS of compound **2**.

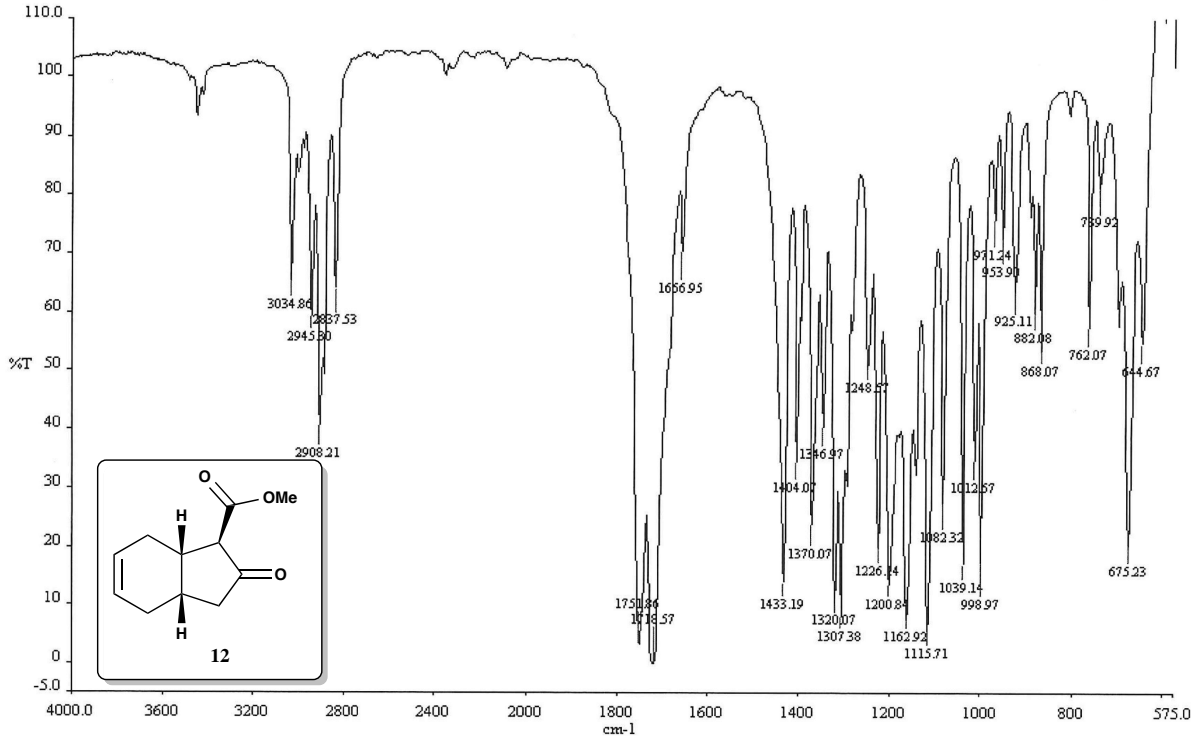


Figure S-51 IR of compound **12**.

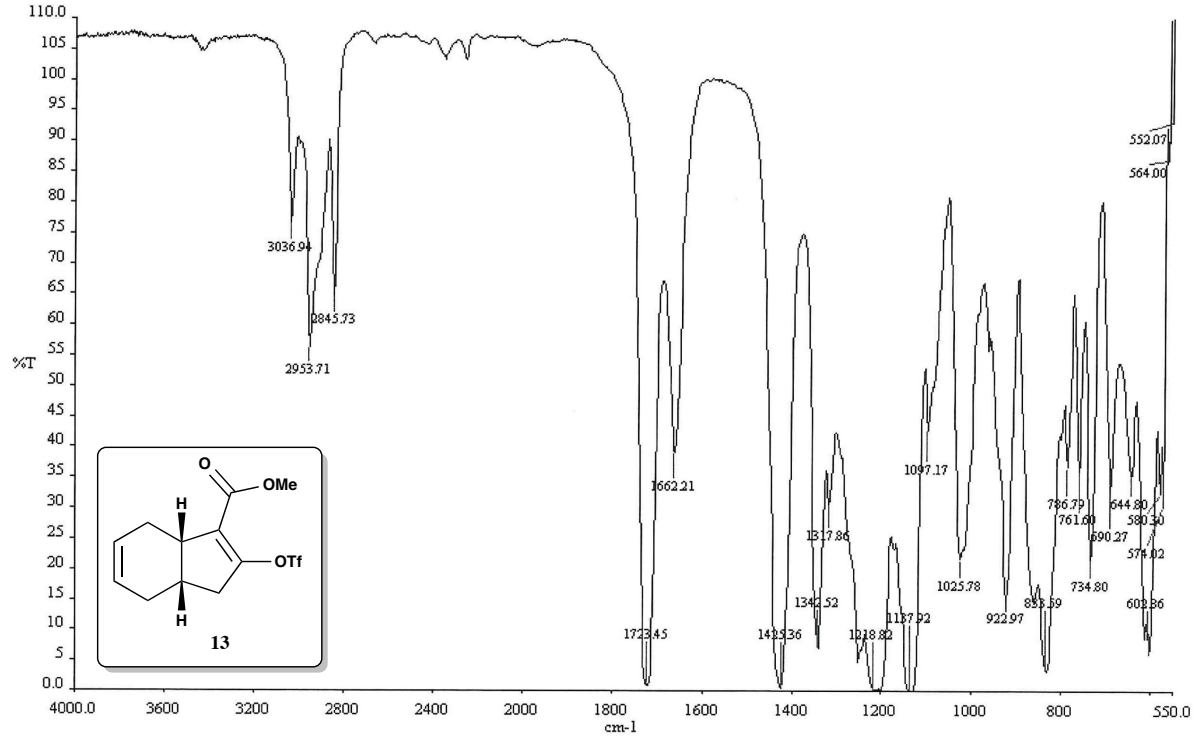


Figure S-52 IR of compound **13**.

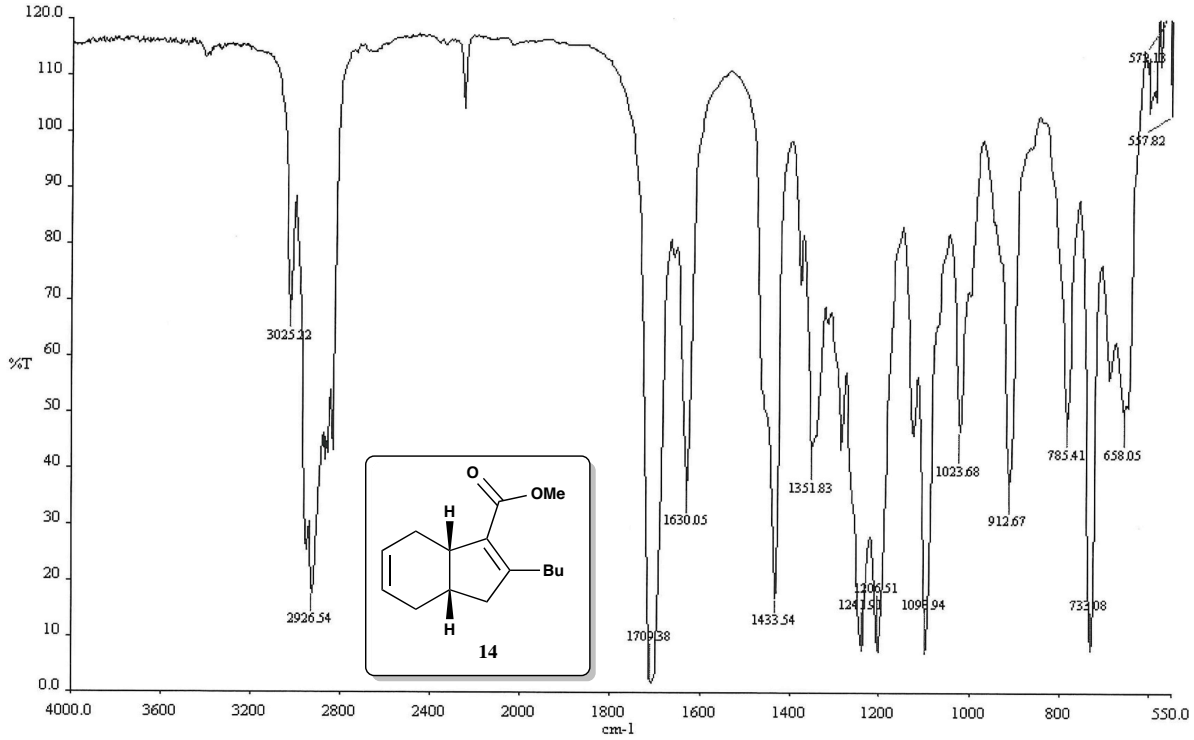


Figure S-53 IR of compound **14**.

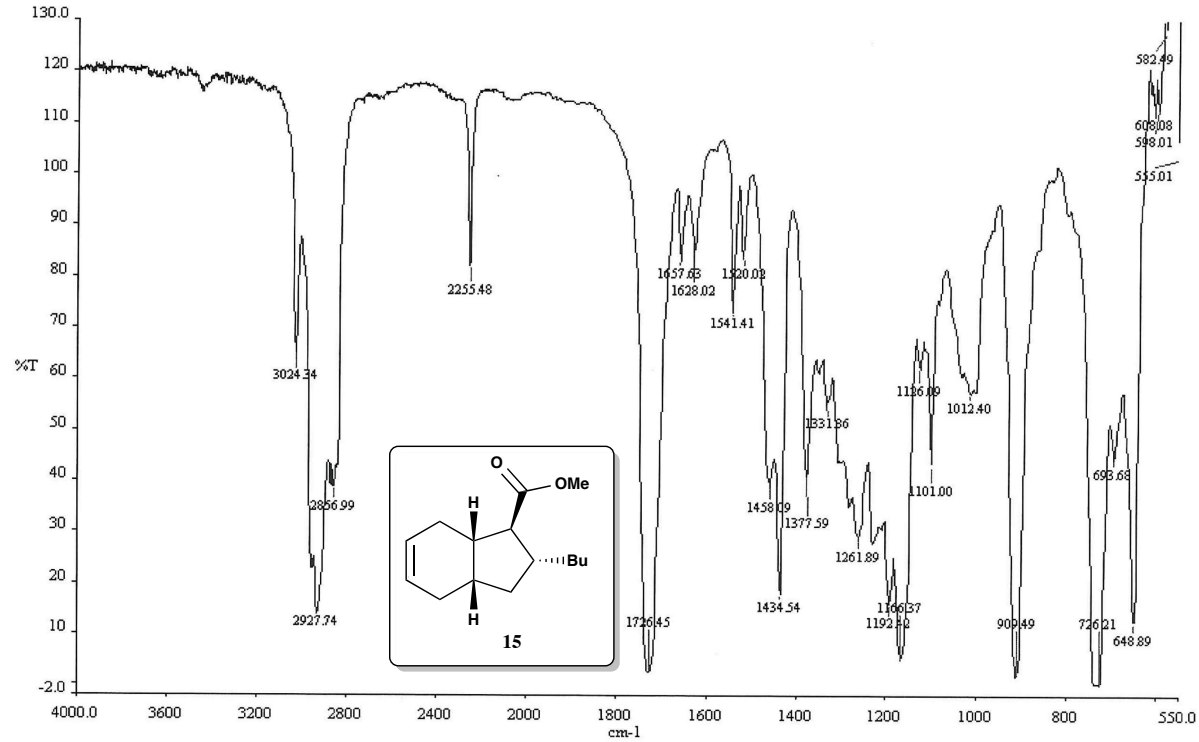


Figure S-54 IR of compound **15**.

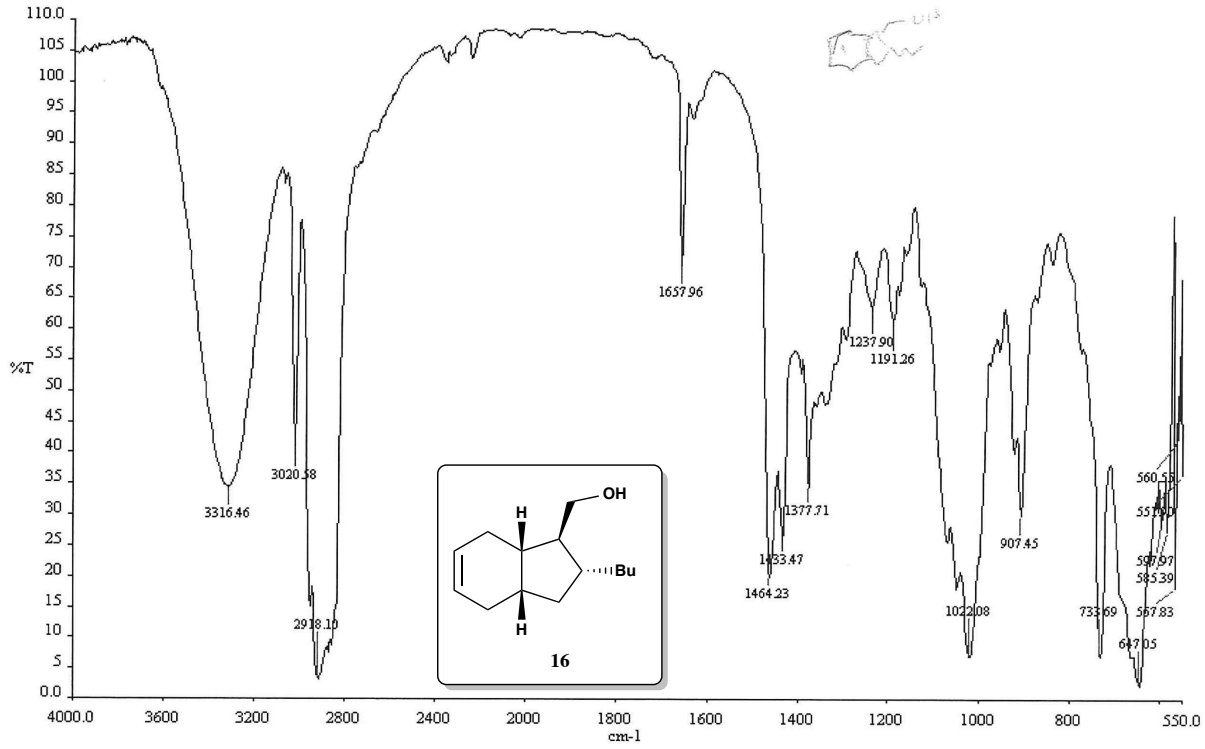


Figure S-55 IR of compound **16**.

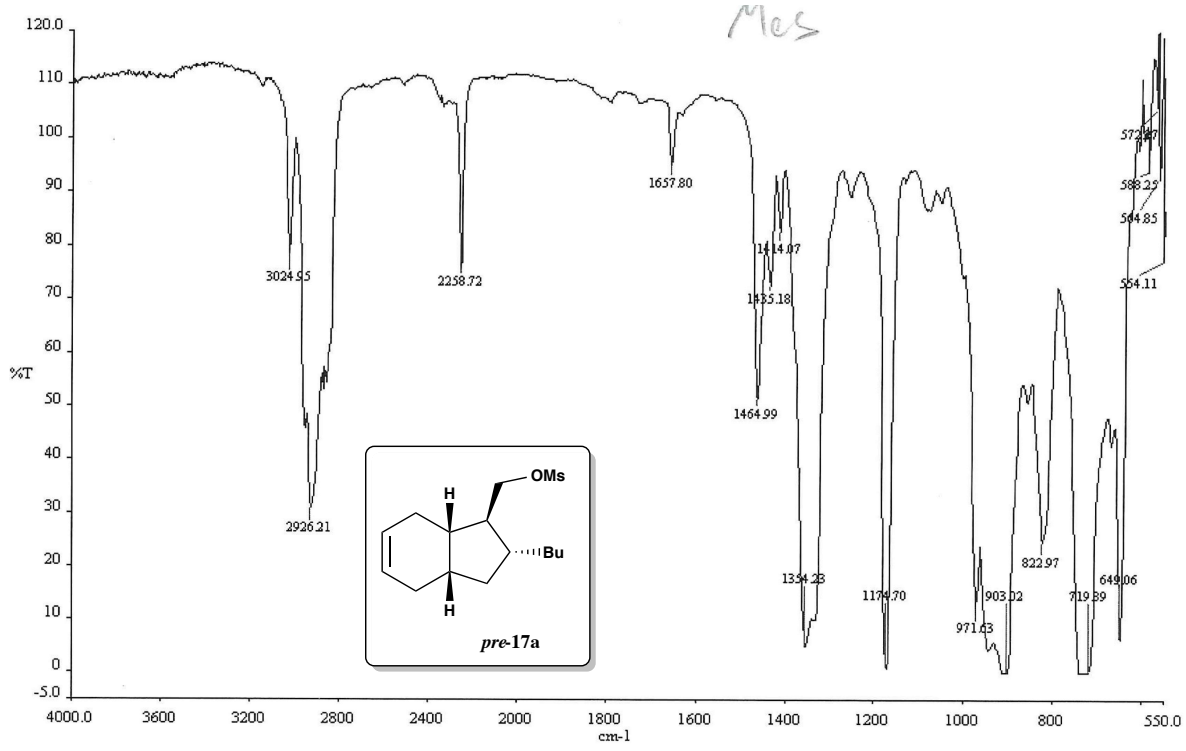


Figure S-56 IR of compound ***pre*-17a**.

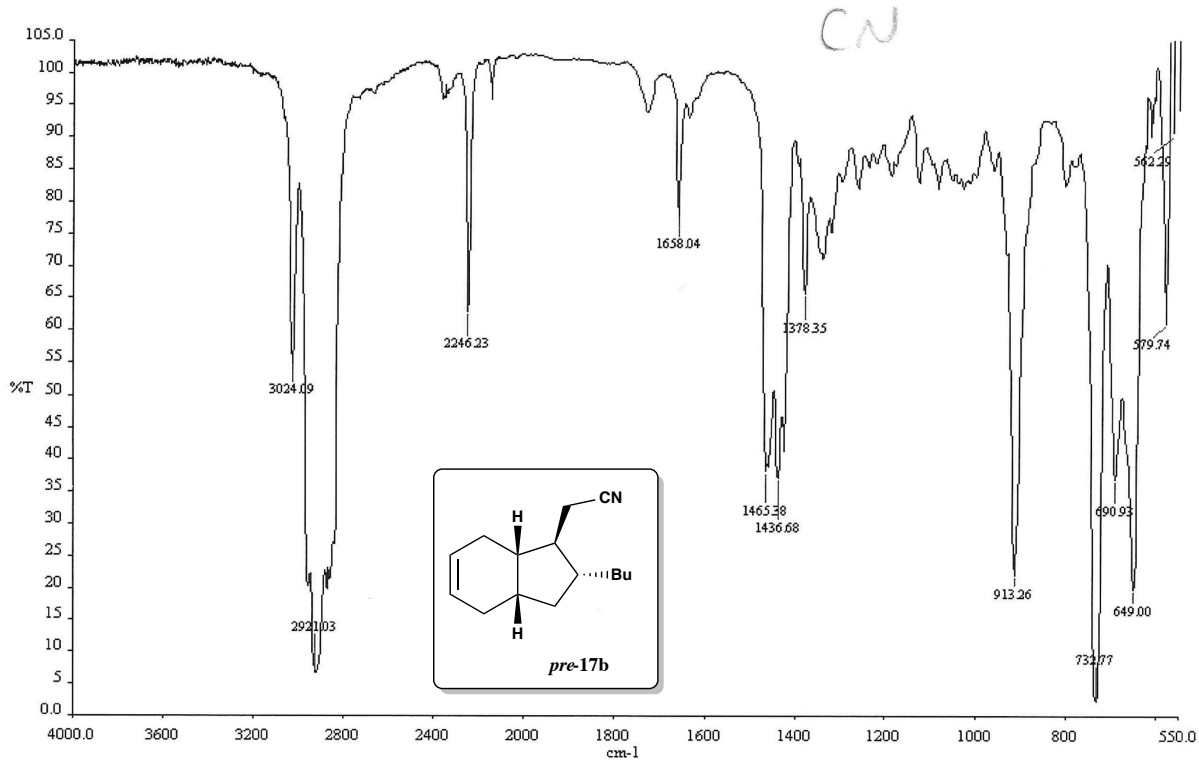


Figure S-57 IR of compound ***pre*-17b**.

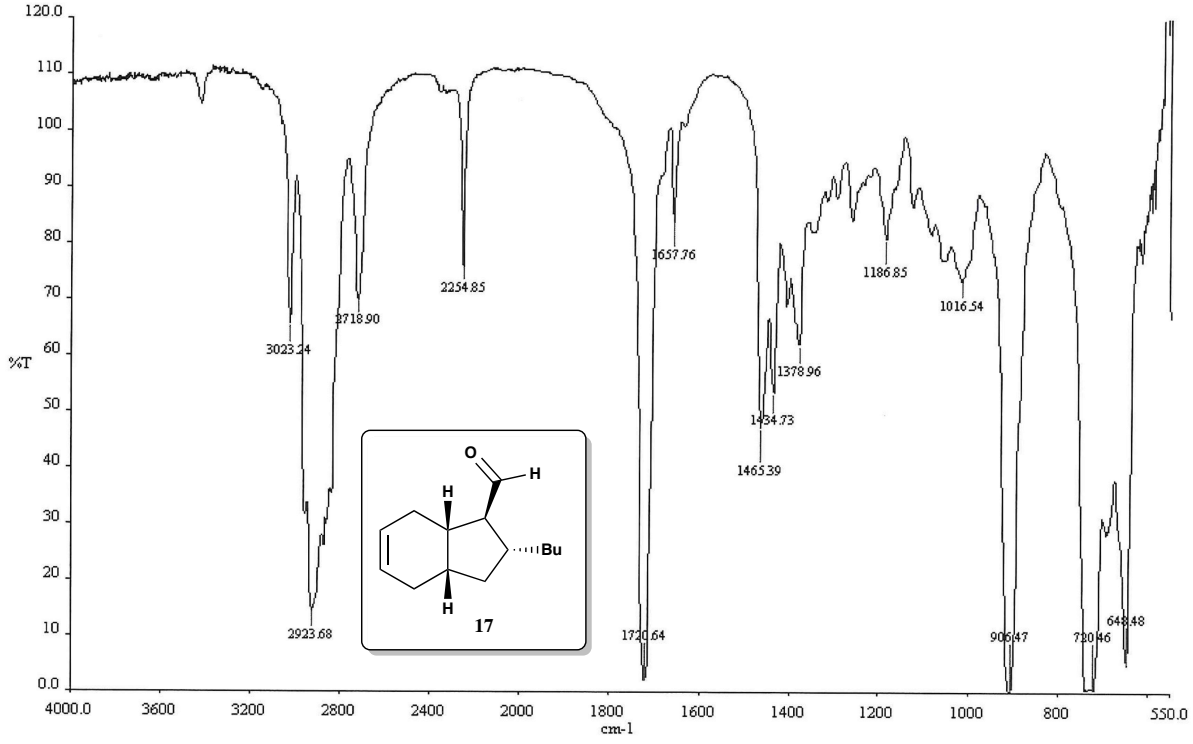


Figure S-58 IR of compound **17**.

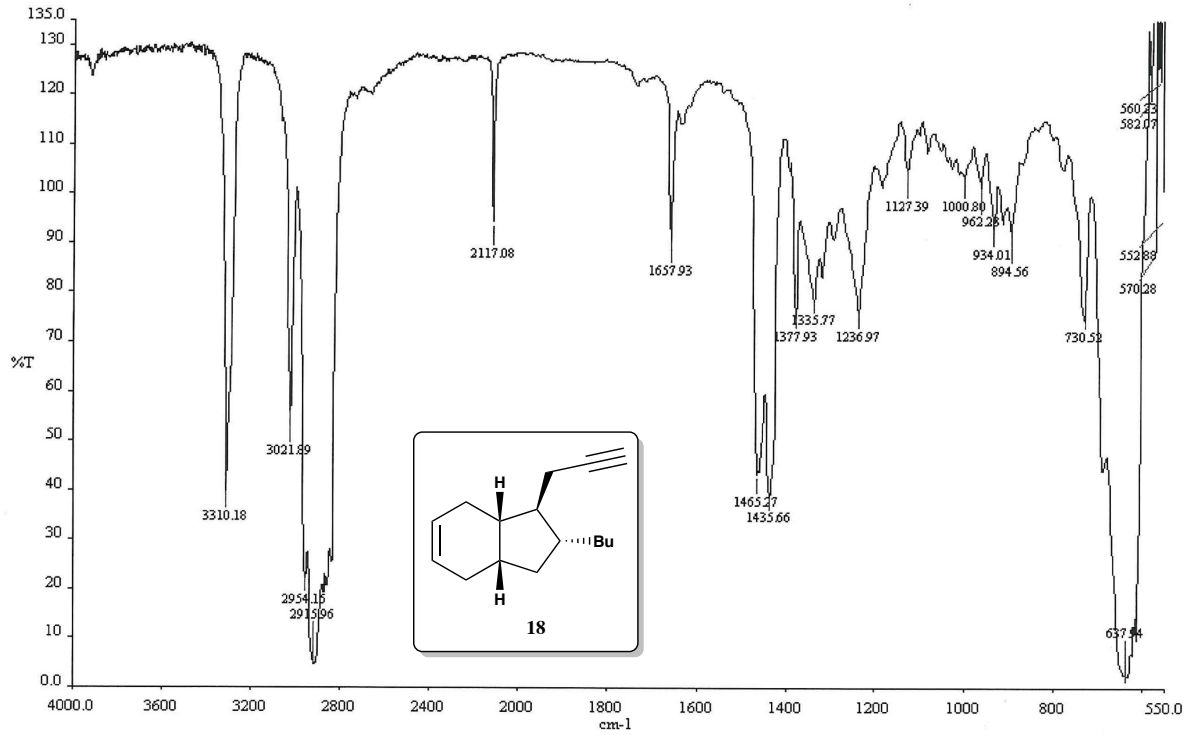


Figure S-59 IR of compound **18**.

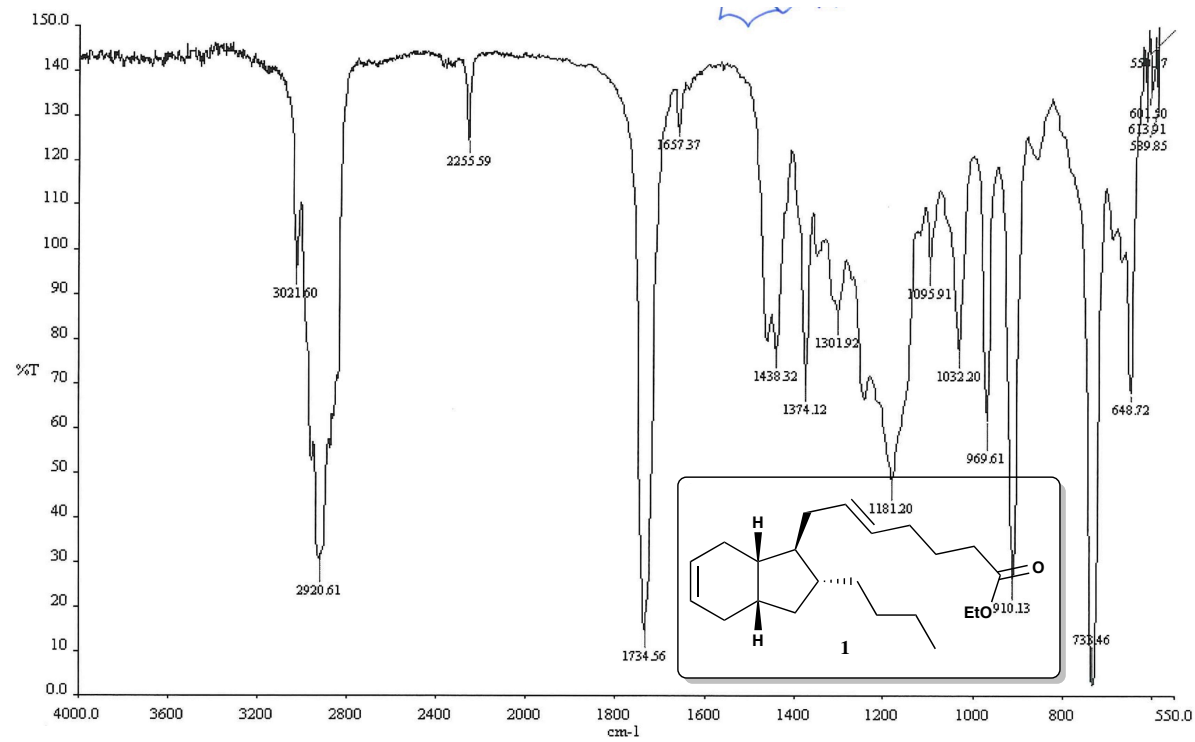


Figure S-60 IR of compound **19**.

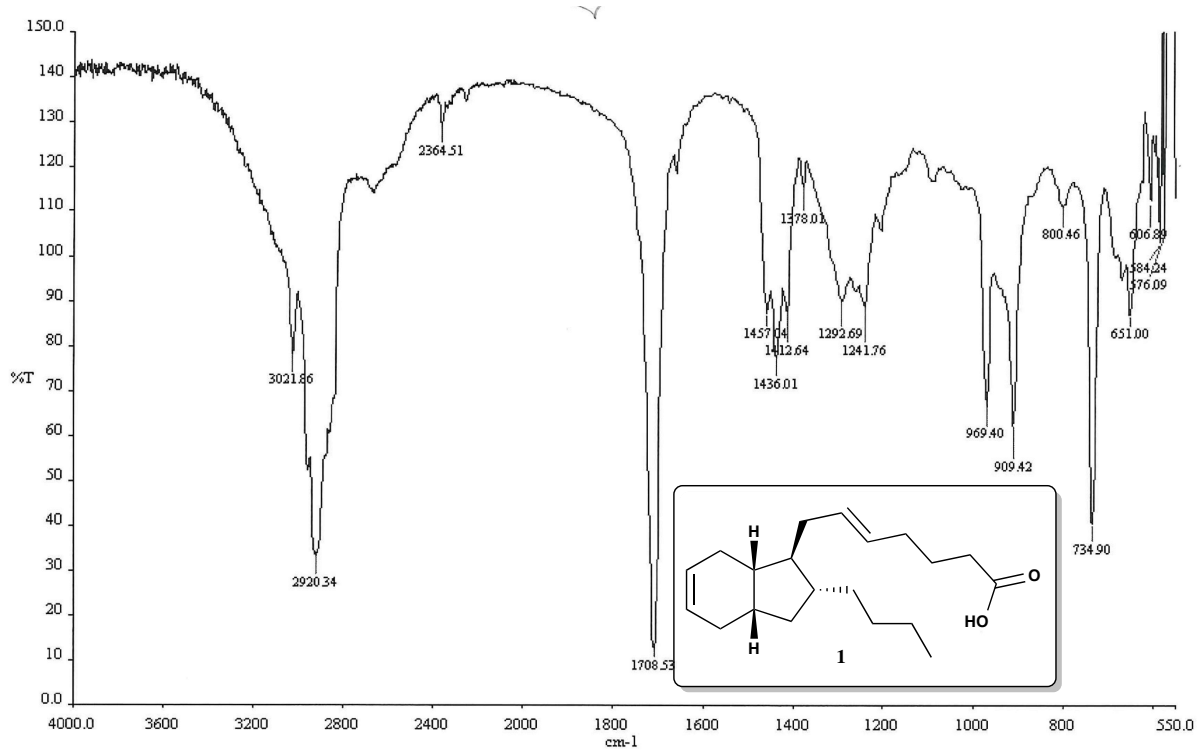


Figure S-61 IR of compound **1**.

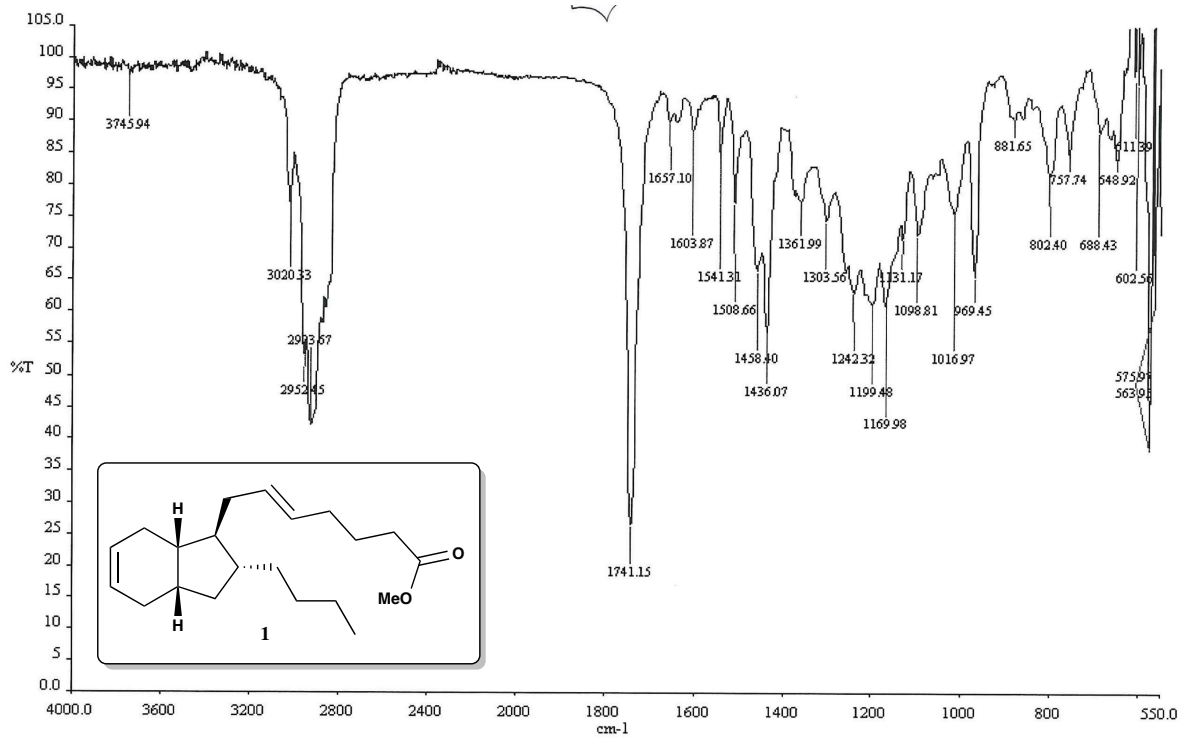
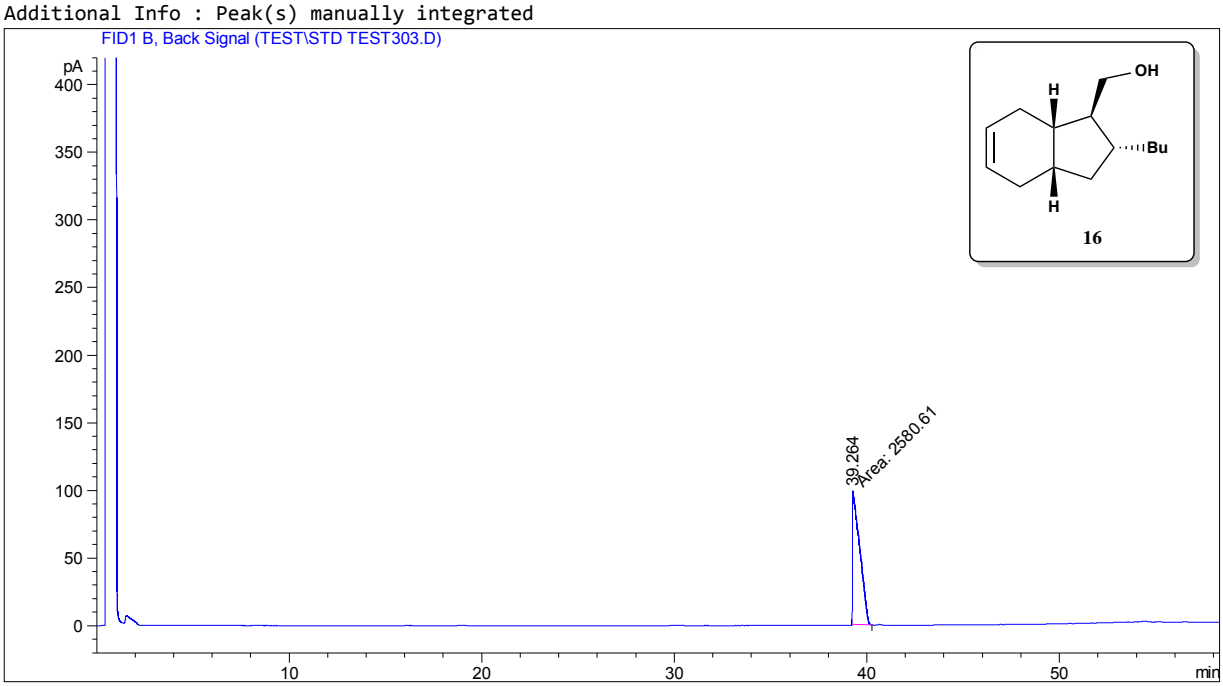


Figure S-62 IR of compound **2**.

Data File C:\CHEM32\1\DATA\TEST\STD TEST303.D
Sample Name: RAC

```
======================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : 7820 GC                       Location : Vial 1
Injection Date  : 5/2/2016 15:28:06
                                                Inj Volume : Manually
Method          : C:\CHEM32\1\METHODS\CP7502\CP7502.M
Last changed    : 5/2/2016 14:05:55 by SYSTEM
Sample Info     : 80 grader 30 min, 3 grader/min til 150 grader, 5 min hold time

Additional Info : Peak(s) manually integrated
```




```
======================================================================
                    External Standard Report
======================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Tuesday, August 20, 201311:04:49
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 B, Back Signal

RetTime  Type     Area     Amt/Area    Amount   Grp   Name
 [min]          [pA*s]                [ng/ul]
-------|------|----------|----------|----------|--|------------------
  3.710            -          -          -         tridekan
  4.351            -          -          -         tetradekan
  4.970            -          -          -         pentadekan
  5.557            -          -          -         hexadekan

Totals :                                0.00000
```


Figure S-63 Chiral GLC of compound **16**.

Data File C:\CHEM32\1\DATA\TEST\STD TEST303.D
Sample Name: RAC

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========
==========
Area Percent Report
==========

Sorted By : Signal
Calib. Data Modified : Tuesday, August 20, 201311:04:49
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 B, Back Signal

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	3.710		0.0000	0.00000	0.00000	tridekan
2	4.351		0.0000	0.00000	0.00000	tetradekan
3	4.970		0.0000	0.00000	0.00000	pentadekan
4	5.557		0.0000	0.00000	0.00000	hexadekan
5	39.264	MM	0.4356	2580.60645	1.000e2	?

Totals : 2580.60645

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

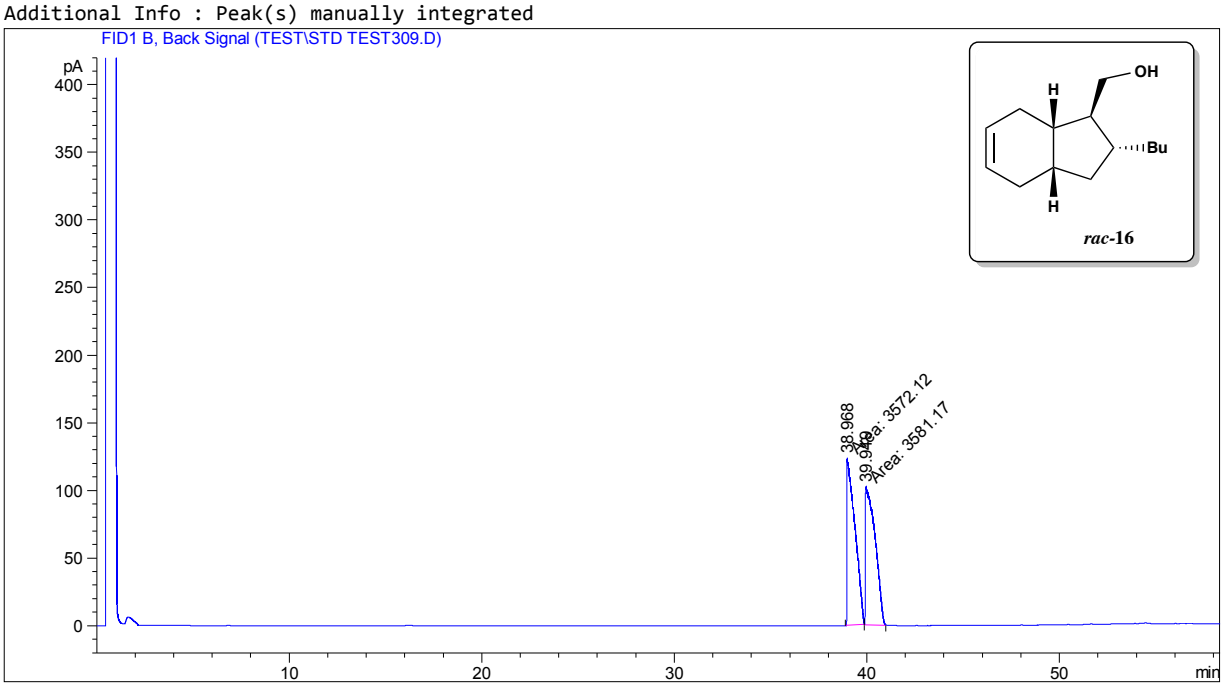
==========
*** End of Report ***

Figure S-64 Report from chiral GLC of compound **16**.

Data File C:\CHEM32\1\DATA\TEST\STD TEST309.D
Sample Name: Rac fort 1:5

```
==================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : 7820 GC                       Location : Vial 1
Injection Date  : 5/10/2016 14:46:51
                                                Inj Volume : Manually
Method          : C:\CHEM32\1\METHODS\CP7502\CP7502.M
Last changed    : 5/2/2016 14:05:55 by SYSTEM
Sample Info     : 80 grader 30 min, 3 grader/min til 150 grader, 5 min hold time

Additional Info : Peak(s) manually integrated
```




```
==================================================================
                    External Standard Report
==================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Tuesday, August 20, 201311:04:49
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 B, Back Signal

RetTime  Type     Area     Amt/Area    Amount   Grp   Name
 [min]          [pA*s]                [ng/ul]
-------|------|----------|----------|----------|--|------------------
  3.710            -          -          -         tridekan
  4.351            -          -          -         tetradekan
  4.970            -          -          -         pentadekan
  5.557            -          -          -         hexadekan

Totals :                               0.00000
```


Figure S-65 Chiral GLC of compound *rac*-**16**.

Data File C:\CHEM32\1\DATA\TEST\STD TEST309.D
Sample Name: Rac fort 1:5

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========
==========
Area Percent Report
==========

Sorted By : Signal
Calib. Data Modified : Tuesday, August 20, 201311:04:49
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 B, Back Signal

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	3.710		0.0000	0.00000	0.00000	tridekan
2	4.351		0.0000	0.00000	0.00000	tetradekan
3	4.970		0.0000	0.00000	0.00000	pentadekan
4	5.557		0.0000	0.00000	0.00000	hexadekan
5	38.968	MM	0.4812	3572.11621	49.93671	?
6	39.949	MM	0.5835	3581.17065	50.06329	?

Totals : 7153.28687

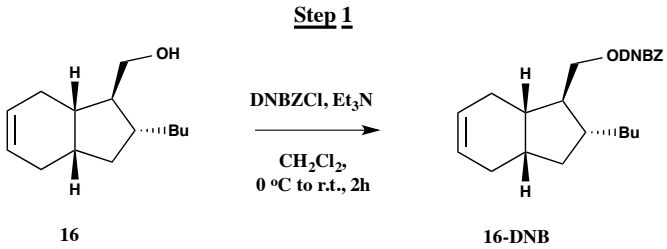
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========
*** End of Report ***

Figure S-66 Report from chiral GLC of compound *rac*-**16**.

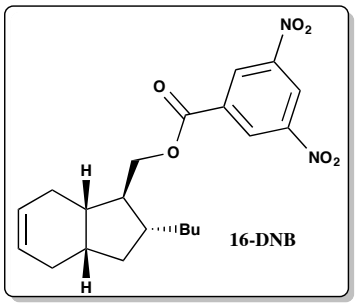
Preparation 3,5-dinitrobenzoate derivative of (16).



16 16-DNB

Scheme S-3 Derivatization of advanced intermediate **16** to 3,5-dinitrobenzoate **16-DNB**.

(1*S*,6*S*,7*S*,8*R*)-8-Butyl-7-((3,5-dinitrobenzoyl)oxymethyl)bicyclo[4.3.0]non-3-ene (16-DNB).



A stirring solution of (1*S*,6*S*,7*S*,8*R*)-8-butyl-7-(hydroxymethyl)bicyclo[4.3.0]non-3-ene **16** (120 mg, 0.577 mmol, 1.0 equiv.) in dry DCM (10 mL) was added Et_3N (0.241 mL, 1.73 mmol, 3.0 equiv.) dropwise. The solution was then cooled to 0 °C and 3,5-dinitrobenzoyl chloride (173 mg, 0.75 mmol, 1.3 equiv.) was added in one portion. The reaction was slowly warmed to room temperature and monitored by TLC until completion. After 2h, the reaction mixture was poured over H_2O (10 mL) and the organic layer separated. The aqueous layer was then extracted with DCM (2 x 10 mL) and the organic layers combined. The organic layers were then washed with H_2O (1 x 30 mL), brine (1 x 30 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo* to form a crude orange oil. This was purified by column chromatography on silica (^{n}hexane/EtOAc, 95:5) to afford the title compound as a slightly off-white powder. Yield: 185 mg, (82%), $[\alpha]_D^{26}$ -3.67 (c = 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25-9.24 (m, 1H), 9.16-9.15 (m, 2H), 5.74-5.67 (m, 2H), 4.45-4.37 (m, 2H), 2.27-2.14 (m, 3H), 2.01-1.90 (m, 4H), 1.85-1.79 (m, 1H), 1.74-1.68 (m, 1H), 1.66-1.60 (m, 1H), 1.42-1.19 (m, 6H), 0.89-0.88 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.6, 148.6, 134.1, 129.3 (2C), 126.2, 125.2, 122.3, 69.7, 50.0, 41.8, 38.7, 37.7, 37.2, 35.1, 30.9, 27.4, 27.3, 22.8, 14.0; IR (neat, cm^{-1}) 3012 (m), 3022 (m), 2922 (s), 1728 (s), 1628 (m), 1597 (w), 1540 (s), 1460 (m); HRMS (EI+): Exact mass calculated for $C_{21}H_{26}N_2O_6$ $[M]^+$: 402.1791, found 402.1788; m.p.: 45-47 °C; TLC (^{n}hexane/EtOAc 9:1, $KMnO_4$ stain): R_f =0.55.

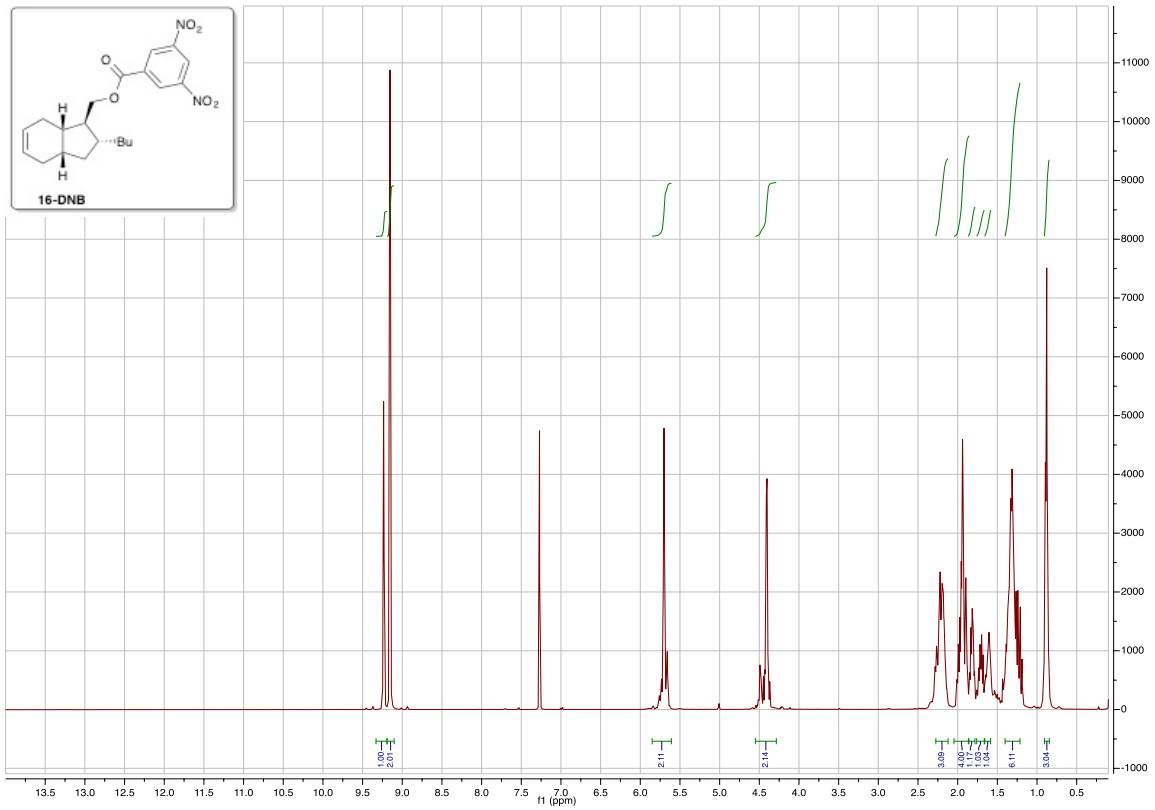


Figure S-67 ^{1}H-NMR spectrum of compound **16-DNB**.

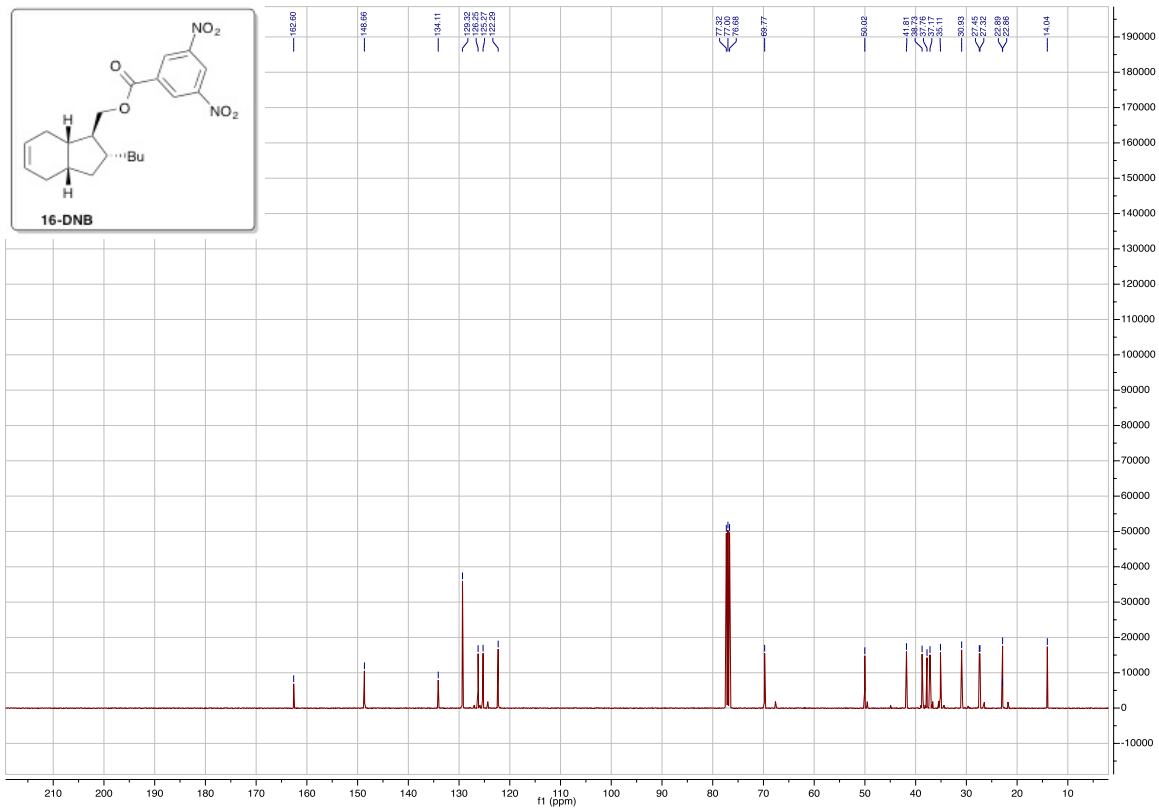


Figure S-68 ^{13}C-NMR spectrum of compound **16-DNB**.

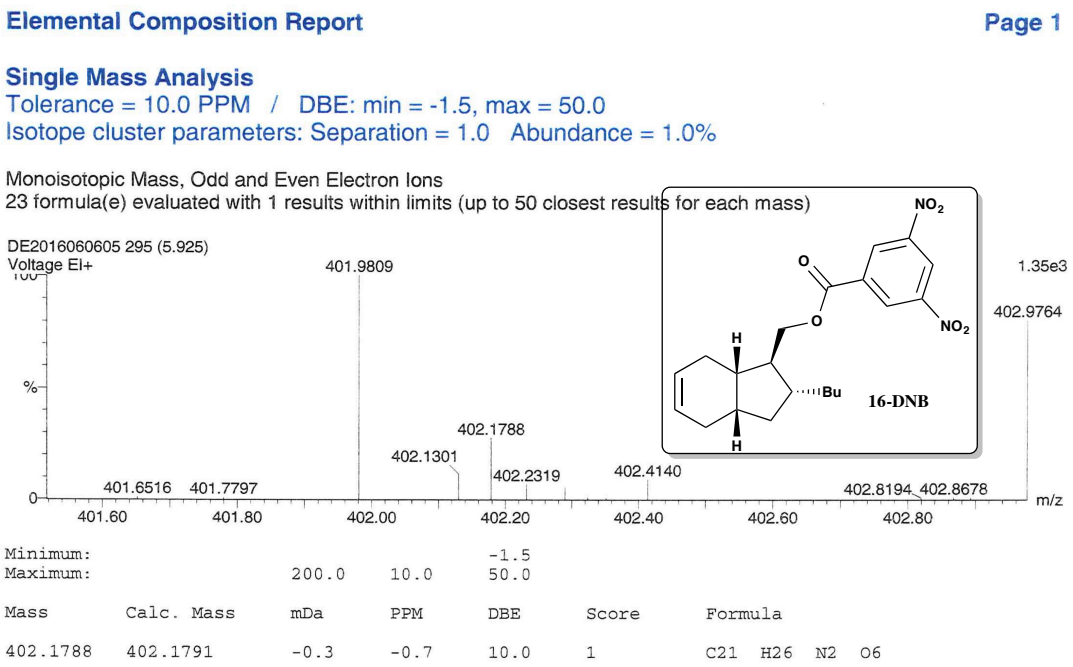


Figure S-69 HRMS spectrum of compound **16-DNB**.

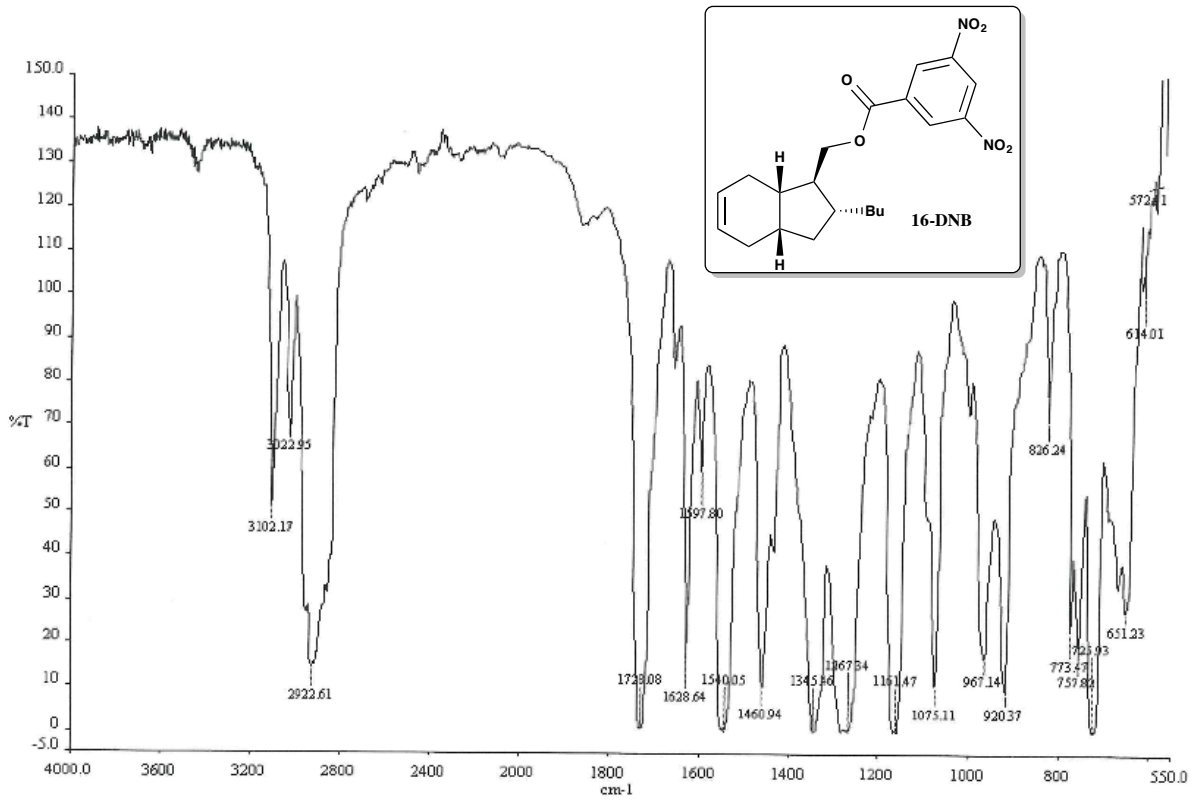


Figure S-70 IR spectrum of compound **16-DNB**.

X-ray crystallography on compound (16-DNB):

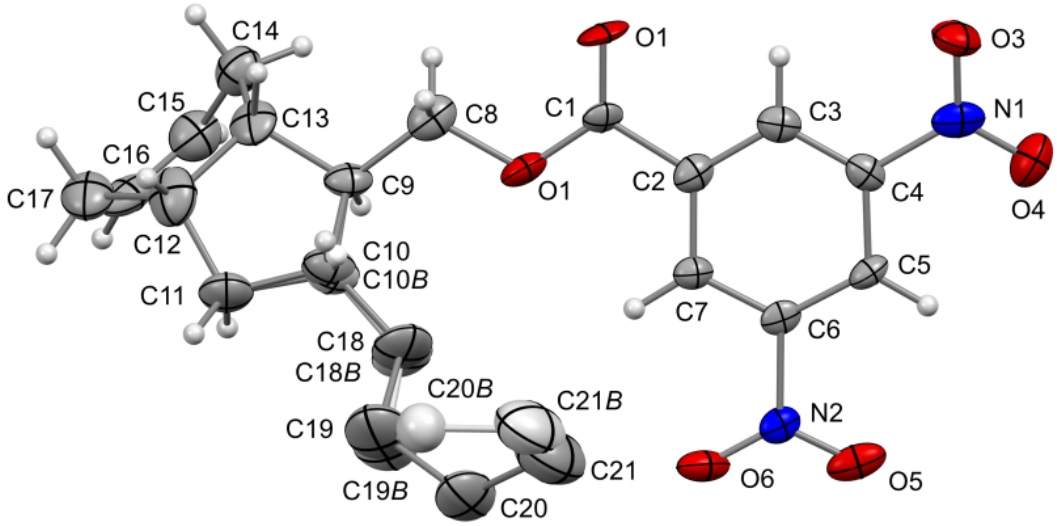


Figure S-71 Single crystal X-ray structure of the 3,5-dinitrobenzoate of alcohol **16** at 110 K. The disordered ^{n}butyl group (H-atoms omitted) has a major orientation [occupancy 0.74(3)] with *trans,trans,gauche+* torsion angles along C9-C10-C18-C19-C20-C21, while the minor orientation (atoms in lighter tone) is *trans,gauche–,gauche–*.

Table S-1 Crystal Data for **16-DNB**.*

	(I)
Crystal data	
Chemical Formula	$C_{21}H_{26}N_2O_6$
M_r	402.44
Crystal system, Space Group	Monoclinic, $P2_1$
a (Å)	10.866(5)
b (Å)	5.196(2)
c (Å)	18.617(10)
β (°)	106.703(11)
V (Å^3)	1006.8(9)
Z	2
Radiation	Mo Kα
Wavelength (Å)	0.71073
μ (mm^{-1})	0.098
Temperature (K)	110(2)

Crystal Size (mm)	0.21 × 0.19 × 0.01
T_{min}, T_{max}	0.692, 1.000
No. of Measured, Independent and Observed [$I > 2\sigma(I)$] Reflections	5899, 2089, 1718
R_{int}	0.140
θ_{max} (°)	20.86
Refinement $R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.085, 0.225, 1.03
No. of Reflections	2089
No. of Parameters	278
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.30, −0.28
CCDC	1484546

*Bruker D8 Venture diffractometer with InCoatec ImuS Microfocus radiation source and Photon 100 CMOS detector. Data collection with Apex2,[1] data integration and cell refinement with SAINT,[1] absorption correction by SADABS,[1] structure solution with SHELXT,[2] structure refinement with SHELXL.[3] Molecular graphics from Mercury.[4]

supporting information

First total synthesis of mucosin based on its structural assignment

Harrison C. Gallantree-Smith, Simen. G Antonsen, Carl Henrik Görbitz,* Trond V. Hansen, Jens M. J. Nolsøe and Yngve H. Stenstrøm

Experimental

Very fragile, small platelets were obtained by slow evaporation from a methanol/pentane mixture.

Refinement

Two disorder positions, with occupancies 0.74 (3) and 0.26 (3) were refined for the C18—C21 n-butyl group attached to C10. Their covalent geometries were restrained by a SHELXL SAME 0.004 0.008 command, additional restraints were imposed on the C—C bond lengthes in this substituent. Corresponmding atoms in the two conformations shared the same displacement parameteres, except that a fixed value was used for C20B, which is separted from C20 by 1.38 Å.

name

Crystal data

$C_{21}H_{26}N_2O_6$
M_r = 402.44
Monoclinic, $P2_1$
a = 10.866 (5) Å
b = 5.196 (2) Å
c = 18.617 (10) Å
β = 106.703 (11)°
V = 1006.8 (9) Å^3
Z = 2
$F(000)$ = 428
D_x = 1.327 Mg m^{-3}
Mo $K\alpha$ radiation, λ = 0.71073 Å
Cell parameters from 2295 reflections
θ = 2.3–20.9°
μ = 0.10 mm^{-1}
T = 110 K
Plate, colourless
0.21 × 0.19 × 0.01 mm

Data collection

Bruker D8 Venture with Photon 100 CMOS detector diffractometer
Radiation source: InCoatec ImuS Microfocus
Graphite monochromator
Detector resolution: 8.3 pixels mm^{-1}
Sets of exposures each taken over 0.5° ω rotation scans
Absorption correction: multi-scan
SADABS (Bruker, 2014)
T_{min} = 0.692, T_{max} = 1.000
5899 measured reflections
2089 independent reflections
1718 reflections with $I > 2\sigma(I)$
R_{int} = 0.214
θ_{max} = 20.9°, θ_{min} = 2.3°
h = −10→10
k = −5→5
l = −18→18

Refinement

Refinement on F^2
Least-squares matrix: full
$R[F^2 > 2\sigma(F^2)]$ = 0.085
$wR(F^2)$ = 0.225
S = 1.03
2089 reflections
278 parameters
77 restraints
Primary atom site location: structure-invariant direct methods
Secondary atom site location: difference Fourier map
Hydrogen site location: inferred from neighbouring sites
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.0936P)^2 + 2.6032P]$ where $P = (F_o^2 + 2F_c^2)/3$

$(\Delta/\sigma)_{max} < 0.001$
$\Delta\rho_{max} = 0.30$ e Å^{-3}
$\Delta\rho_{min} = -0.28$ e Å^{-3}

Special details

Geometry. All esds (except the esd in the dihedral angle between two l.s. planes) are estimated using the full covariance matrix. The cell esds are taken into account individually in the estimation of esds in distances, angles and torsion angles; correlations between esds in cell parameters are only used when they are defined by crystal symmetry. An approximate (isotropic) treatment of cell esds is used for estimating esds involving l.s. planes.
Refinement. Two disorder positions refined for n-butyl group attached to C10. Occupancies 0.74 (3) and 0.26 (3).

Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (Å^2)

	x	*y*	*z*	U_{iso}*/U_{eq}	Occ. (<1)
O1	0.4965 (7)	0.0115 (19)	0.3931 (4)	0.034 (2)	
N1	0.7700 (10)	0.288 (2)	0.6525 (7)	0.034 (3)	
C1	0.5467 (10)	0.212 (3)	0.3832 (7)	0.023 (3)	
O2	0.5244 (7)	0.3323 (19)	0.3180 (5)	0.032 (2)	
N2	0.8755 (9)	0.896 (2)	0.4771 (7)	0.025 (2)	
C2	0.6411 (11)	0.353 (3)	0.4451 (7)	0.028 (3)	
O3	0.7262 (8)	0.077 (2)	0.6594 (5)	0.040 (2)	
C3	0.6602 (11)	0.265 (2)	0.5163 (7)	0.028 (3)	
H31	0.6121	0.1220	0.5254	0.033*	
C4	0.7491 (10)	0.382 (3)	0.5752 (6)	0.025 (3)	
O4	0.8317 (8)	0.420 (2)	0.7046 (5)	0.044 (2)	
C5	0.8206 (10)	0.596 (2)	0.5648 (7)	0.024 (3)	
H51	0.8798	0.6815	0.6053	0.029*	
O5	0.9445 (7)	1.0018 (19)	0.5311 (5)	0.039 (2)	
C6	0.7990 (10)	0.672 (2)	0.4921 (7)	0.021 (3)	
O6	0.8619 (7)	0.9540 (18)	0.4128 (5)	0.036 (2)	
C7	0.7120 (10)	0.562 (2)	0.4316 (6)	0.023 (3)	
H71	0.7003	0.6265	0.3823	0.028*	
C8	0.4292 (12)	0.208 (3)	0.2553 (7)	0.036 (3)	
H81	0.3491	0.1772	0.2692	0.044*	
H82	0.4626	0.0397	0.2445	0.044*	
C12	0.3164 (15)	0.342 (3)	0.0499 (8)	0.055 (4)	
H121	0.3329	0.1800	0.0253	0.066*	
C13	0.2879 (13)	0.270 (3)	0.1245 (7)	0.041 (4)	
H131	0.2890	0.0776	0.1285	0.049*	
C14	0.1617 (12)	0.364 (3)	0.1326 (8)	0.046 (4)	
H141	0.0929	0.2417	0.1076	0.055*	
H142	0.1663	0.3703	0.1865	0.055*	
C15	0.1292 (13)	0.625 (3)	0.0990 (9)	0.050 (4)	
H151	0.0928	0.7505	0.1241	0.059*	
C16	0.1501 (12)	0.682 (3)	0.0365 (10)	0.049 (4)	
H161	0.1334	0.8508	0.0167	0.059*	
C17	0.2006 (14)	0.483 (3)	−0.0052 (8)	0.053 (4)	
H171	0.2286	0.5653	−0.0458	0.063*	
H172	0.1321	0.3573	−0.0281	0.063*	
C9	0.4010 (11)	0.375 (3)	0.1869 (7)	0.032 (3)	0.74 (3)
H91	0.3797	0.5522	0.2003	0.038*	0.74 (3)
C11	0.4409 (12)	0.494 (3)	0.0739 (8)	0.048 (4)	0.74 (3)
H111	0.4230	0.6802	0.0769	0.058*	0.74 (3)

H112	0.4911	0.4696	0.0377	0.058*	0.74 (3)
C10	0.5144 (12)	0.391 (4)	0.1509 (8)	0.044 (4)	0.74 (3)
H10	0.5419	0.2108	0.1440	0.053*	0.74 (3)
C18	0.6307 (14)	0.537 (6)	0.1938 (9)	0.060 (6)	0.74 (3)
H181	0.6668	0.4529	0.2431	0.072*	0.74 (3)
H182	0.6039	0.7130	0.2033	0.072*	0.74 (3)
C19	0.7354 (15)	0.558 (7)	0.1554 (11)	0.084 (7)	0.74 (3)
H191	0.7465	0.3889	0.1335	0.101*	0.74 (3)
H192	0.7092	0.6839	0.1139	0.101*	0.74 (3)
C20	0.8620 (16)	0.641 (4)	0.2089 (12)	0.067 (8)	0.74 (3)
H201	0.8470	0.8021	0.2333	0.081*	0.74 (3)
H202	0.9211	0.6827	0.1787	0.081*	0.74 (3)
C21	0.932 (4)	0.453 (7)	0.271 (2)	0.096 (13)	0.74 (3)
H211	1.0170	0.5212	0.2964	0.145*	0.74 (3)
H212	0.9403	0.2862	0.2482	0.145*	0.74 (3)
H213	0.8819	0.4328	0.3066	0.145*	0.74 (3)
C9B	0.4010 (11)	0.375 (3)	0.1869 (7)	0.032 (3)	0.26 (3)
H91B	0.3750	0.5474	0.2013	0.038*	0.26 (3)
C11B	0.4409 (12)	0.494 (3)	0.0739 (8)	0.048 (4)	0.26 (3)
H11B	0.4217	0.6809	0.0720	0.058*	0.26 (3)
H12B	0.4929	0.4588	0.0392	0.058*	0.26 (3)
C10B	0.5162 (13)	0.418 (5)	0.1535 (9)	0.044 (4)	0.26 (3)
H10B	0.5570	0.2473	0.1510	0.053*	0.26 (3)
C18B	0.619 (2)	0.598 (8)	0.1954 (11)	0.060 (6)	0.26 (3)
H18B	0.6533	0.5355	0.2476	0.072*	0.26 (3)
H19B	0.5803	0.7695	0.1975	0.072*	0.26 (3)
C19B	0.730 (3)	0.630 (9)	0.162 (3)	0.084 (7)	0.26 (3)
H20B	0.6960	0.6514	0.1073	0.101*	0.26 (3)
H21B	0.7786	0.7876	0.1829	0.101*	0.26 (3)
C20B	0.820 (3)	0.402 (9)	0.179 (3)	0.050*	0.26 (3)
H22B	0.8733	0.4081	0.1438	0.060*	0.26 (3)
H23B	0.7673	0.2438	0.1673	0.060*	0.26 (3)
C21B	0.911 (13)	0.37 (3)	0.258 (5)	0.096 (13)	0.26 (3)
H24B	0.9659	0.2227	0.2605	0.145*	0.26 (3)
H25B	0.8605	0.3521	0.2939	0.145*	0.26 (3)
H26B	0.9645	0.5281	0.2714	0.145*	0.26 (3)

Atomic displacement parameters (Å²)

	U^{11}	U^{22}	U^{33}	U^{12}	U^{13}	U^{23}
O1	0.026 (4)	0.016 (5)	0.058 (6)	−0.013 (4)	0.011 (4)	−0.002 (4)
N1	0.017 (6)	0.025 (7)	0.061 (9)	0.008 (5)	0.014 (6)	0.009 (6)
C1	0.011 (5)	0.026 (5)	0.034 (6)	0.001 (5)	0.008 (4)	0.002 (5)
O2	0.020 (4)	0.027 (5)	0.047 (6)	−0.008 (4)	0.005 (4)	−0.005 (4)
N2	0.017 (4)	0.019 (5)	0.037 (5)	0.006 (4)	0.005 (4)	0.001 (5)
C2	0.021 (5)	0.023 (5)	0.037 (6)	0.004 (5)	0.005 (5)	0.000 (5)
O3	0.045 (5)	0.031 (6)	0.047 (6)	−0.004 (5)	0.017 (5)	0.012 (4)
C3	0.024 (5)	0.023 (5)	0.038 (6)	0.006 (4)	0.013 (5)	0.004 (5)
C4	0.022 (5)	0.025 (5)	0.030 (6)	0.009 (5)	0.011 (5)	0.007 (5)
O4	0.039 (5)	0.043 (6)	0.043 (6)	0.002 (5)	0.000 (5)	−0.012 (5)
C5	0.017 (5)	0.017 (5)	0.035 (6)	−0.001 (4)	0.003 (4)	−0.004 (5)

O5	0.023 (5)	0.024 (5)	0.073 (7)	0.001 (4)	0.019 (5)	−0.007 (5)
C6	0.011 (5)	0.019 (5)	0.034 (6)	0.007 (4)	0.007 (4)	0.000 (4)
O6	0.021 (4)	0.026 (5)	0.062 (7)	0.001 (4)	0.013 (4)	0.015 (4)
C7	0.018 (5)	0.019 (5)	0.033 (6)	0.003 (4)	0.008 (5)	0.003 (5)
C8	0.029 (7)	0.028 (7)	0.051 (9)	0.006 (6)	0.009 (7)	−0.006 (7)
C12	0.072 (12)	0.044 (9)	0.045 (9)	0.003 (9)	0.011 (9)	−0.014 (7)
C13	0.057 (10)	0.018 (7)	0.044 (8)	−0.005 (6)	0.009 (7)	−0.010 (6)
C14	0.040 (8)	0.052 (9)	0.046 (9)	−0.001 (7)	0.012 (7)	−0.007 (8)
C15	0.045 (9)	0.042 (10)	0.059 (11)	0.004 (7)	0.011 (9)	−0.001 (8)
C16	0.038 (9)	0.022 (8)	0.075 (12)	−0.005 (7)	−0.004 (8)	0.006 (8)
C17	0.067 (10)	0.029 (8)	0.057 (9)	−0.006 (7)	0.010 (8)	0.000 (8)
C9	0.029 (7)	0.021 (7)	0.047 (8)	0.001 (6)	0.014 (7)	0.005 (7)
C11	0.035 (8)	0.060 (9)	0.057 (10)	−0.008 (8)	0.023 (7)	0.002 (8)
C10	0.034 (8)	0.048 (9)	0.056 (10)	0.001 (7)	0.021 (8)	0.006 (8)
C18	0.029 (8)	0.089 (15)	0.071 (10)	−0.013 (9)	0.028 (8)	−0.007 (10)
C19	0.041 (10)	0.132 (19)	0.087 (13)	−0.005 (12)	0.031 (9)	0.019 (13)
C20	0.026 (12)	0.099 (19)	0.078 (17)	0.002 (12)	0.016 (11)	0.021 (14)
C21	0.09 (2)	0.10 (3)	0.104 (19)	0.00 (2)	0.029 (14)	0.045 (19)
C9B	0.029 (7)	0.021 (7)	0.047 (8)	0.001 (6)	0.014 (7)	0.005 (7)
C11B	0.035 (8)	0.060 (9)	0.057 (10)	−0.008 (8)	0.023 (7)	0.002 (8)
C10B	0.034 (8)	0.048 (9)	0.056 (10)	0.001 (7)	0.021 (8)	0.006 (8)
C18B	0.029 (8)	0.089 (15)	0.071 (10)	−0.013 (9)	0.028 (8)	−0.007 (10)
C19B	0.041 (10)	0.132 (19)	0.087 (13)	−0.005 (12)	0.031 (9)	0.019 (13)
C21B	0.09 (2)	0.10 (3)	0.104 (19)	0.00 (2)	0.029 (14)	0.045 (19)

Geometric parameters (Å, º) for (I)

O1—C1	1.213 (14)	C17—H171	0.9900
N1—O3	1.218 (13)	C17—H172	0.9900
N1—O4	1.219 (12)	C9—C10	1.565 (17)
N1—C4	1.474 (15)	C9—H91	1.0000
C1—O2	1.326 (14)	C11—C10	1.524 (18)
C1—C2	1.498 (16)	C11—H111	0.9900
O2—C8	1.467 (14)	C11—H112	0.9900
N2—O5	1.202 (11)	C10—C18	1.495 (19)
N2—O6	1.202 (11)	C10—H10	1.0000
N2—C6	1.502 (15)	C18—C19	1.512 (17)
C2—C3	1.361 (16)	C18—H181	0.9900
C2—C7	1.397 (16)	C18—H182	0.9900
C3—C4	1.377 (16)	C19—C20	1.51 (2)
C3—H31	0.9500	C19—H191	0.9900
C4—C5	1.403 (15)	C19—H192	0.9900
C5—C6	1.364 (16)	C20—C21	1.53 (2)
C5—H51	0.9500	C20—H201	0.9900
C6—C7	1.368 (15)	C20—H202	0.9900
C7—H71	0.9500	C21—H211	0.9800
C8—C9	1.497 (17)	C21—H212	0.9800
C8—H81	0.9900	C21—H213	0.9800
C8—H82	0.9900	C10B—C18B	1.50 (2)
C12—C11	1.52 (2)	C10B—H10B	1.0000
C12—C13	1.55 (2)	C18B—C19B	1.512 (18)

C12—C17	1.558 (19)	C18B—H18B	0.9900
C12—H121	1.0000	C18B—H19B	0.9900
C13—C14	1.504 (19)	C19B—C20B	1.51 (2)
C13—C9	1.530 (17)	C19B—H20B	0.9900
C13—H131	1.0000	C19B—H21B	0.9900
C14—C15	1.49 (2)	C20B—C21B	1.53 (2)
C14—H141	0.9900	C20B—H22B	0.9900
C14—H142	0.9900	C20B—H23B	0.9900
C15—C16	1.28 (2)	C21B—H24B	0.9800
C15—H151	0.9500	C21B—H25B	0.9800
C16—C17	1.49 (2)	C21B—H26B	0.9800
C16—H161	0.9500		

O3—N1—O4	124.5 (11)	C13—C9—C10	104.6 (10)
O3—N1—C4	116.4 (11)	C8—C9—H91	109.0
O4—N1—C4	119.0 (10)	C13—C9—H91	109.0
O1—C1—O2	125.0 (11)	C10—C9—H91	109.0
O1—C1—C2	122.8 (11)	C12—C11—C10	105.9 (11)
O2—C1—C2	112.2 (10)	C12—C11—H111	110.5
C1—O2—C8	114.9 (9)	C10—C11—H111	110.5
O5—N2—O6	125.9 (10)	C12—C11—H112	110.5
O5—N2—C6	116.5 (10)	C10—C11—H112	110.5
O6—N2—C6	117.7 (10)	H111—C11—H112	108.7
C3—C2—C7	120.0 (11)	C18—C10—C11	117.2 (12)
C3—C2—C1	117.6 (11)	C18—C10—C9	116.5 (11)
C7—C2—C1	122.3 (11)	C11—C10—C9	99.0 (10)
C2—C3—C4	120.2 (11)	C18—C10—H10	107.8
C2—C3—H31	119.9	C11—C10—H10	107.8
C4—C3—H31	119.9	C9—C10—H10	107.8
C3—C4—C5	122.0 (11)	C10—C18—C19	114.7 (13)
C3—C4—N1	120.6 (11)	C10—C18—H181	108.6
C5—C4—N1	117.5 (10)	C19—C18—H181	108.6
C6—C5—C4	115.0 (10)	C10—C18—H182	108.6
C6—C5—H51	122.5	C19—C18—H182	108.6
C4—C5—H51	122.5	H181—C18—H182	107.6
C5—C6—C7	125.3 (10)	C20—C19—C18	112.0 (14)
C5—C6—N2	117.5 (10)	C20—C19—H191	109.2
C7—C6—N2	117.2 (10)	C18—C19—H191	109.2
C6—C7—C2	117.5 (11)	C20—C19—H192	109.2
C6—C7—H71	121.2	C18—C19—H192	109.2
C2—C7—H71	121.2	H191—C19—H192	107.9
O2—C8—C9	110.3 (10)	C19—C20—C21	118 (2)
O2—C8—H81	109.6	C19—C20—H201	107.8
C9—C8—H81	109.6	C21—C20—H201	107.8
O2—C8—H82	109.6	C19—C20—H202	107.8
C9—C8—H82	109.6	C21—C20—H202	107.8
H81—C8—H82	108.1	H201—C20—H202	107.1
C11—C12—C13	104.7 (10)	C20—C21—H211	109.5
C11—C12—C17	115.6 (12)	C20—C21—H212	109.5
C13—C12—C17	111.8 (12)	H211—C21—H212	109.5
C11—C12—H121	108.2	C20—C21—H213	109.5

C13—C12—H121	108.2	H211—C21—H213	109.5
C17—C12—H121	108.2	H212—C21—H213	109.5
C14—C13—C9	111.3 (10)	C18B—C10B—H10B	107.8
C14—C13—C12	115.6 (11)	C10B—C18B—C19B	114.8 (15)
C9—C13—C12	105.6 (11)	C10B—C18B—H18B	108.6
C14—C13—H131	108.0	C19B—C18B—H18B	108.6
C9—C13—H131	108.0	C10B—C18B—H19B	108.6
C12—C13—H131	108.0	C19B—C18B—H19B	108.6
C15—C14—C13	111.5 (12)	H18B—C18B—H19B	107.5
C15—C14—H141	109.3	C20B—C19B—C18B	112.1 (16)
C13—C14—H141	109.3	C20B—C19B—H20B	109.2
C15—C14—H142	109.3	C18B—C19B—H20B	109.2
C13—C14—H142	109.3	C20B—C19B—H21B	109.2
H141—C14—H142	108.0	C18B—C19B—H21B	109.2
C16—C15—C14	120.3 (14)	H20B—C19B—H21B	107.9
C16—C15—H151	119.9	C19B—C20B—C21B	118 (2)
C14—C15—H151	119.9	C19B—C20B—H22B	107.8
C15—C16—C17	120.2 (13)	C21B—C20B—H22B	107.8
C15—C16—H161	119.9	C19B—C20B—H23B	107.8
C17—C16—H161	119.9	C21B—C20B—H23B	107.8
C16—C17—C12	109.4 (11)	H22B—C20B—H23B	107.1
C16—C17—H171	109.8	C20B—C21B—H24B	109.5
C12—C17—H171	109.8	C20B—C21B—H25B	109.5
C16—C17—H172	109.8	H24B—C21B—H25B	109.5
C12—C17—H172	109.8	C20B—C21B—H26B	109.5
H171—C17—H172	108.3	H24B—C21B—H26B	109.5
C8—C9—C13	111.3 (10)	H25B—C21B—H26B	109.5
C8—C9—C10	113.6 (10)		

O1—C1—O2—C8	1.1 (15)	C17—C12—C13—C9	−128.2 (11)
C2—C1—O2—C8	−178.0 (8)	C9—C13—C14—C15	83.5 (14)
O1—C1—C2—C3	−6.8 (16)	C12—C13—C14—C15	−37.0 (16)
O2—C1—C2—C3	172.3 (10)	C13—C14—C15—C16	40.9 (18)
O1—C1—C2—C7	170.7 (10)	C14—C15—C16—C17	3 (2)
O2—C1—C2—C7	−10.2 (14)	C15—C16—C17—C12	−47.5 (18)
C7—C2—C3—C4	0.2 (16)	C11—C12—C17—C16	−74.5 (15)
C1—C2—C3—C4	177.7 (10)	C13—C12—C17—C16	45.1 (15)
C2—C3—C4—C5	1.2 (16)	O2—C8—C9—C13	−171.4 (9)
C2—C3—C4—N1	179.8 (10)	O2—C8—C9—C10	70.8 (13)
O3—N1—C4—C3	14.6 (14)	C14—C13—C9—C8	86.1 (13)
O4—N1—C4—C3	−167.3 (10)	C12—C13—C9—C8	−147.7 (10)
O3—N1—C4—C5	−166.7 (9)	C14—C13—C9—C10	−150.8 (11)
O4—N1—C4—C5	11.4 (14)	C12—C13—C9—C10	−24.6 (13)
C3—C4—C5—C6	−2.4 (14)	C13—C12—C11—C10	29.4 (14)
N1—C4—C5—C6	179.0 (9)	C17—C12—C11—C10	152.9 (12)
C4—C5—C6—C7	2.5 (15)	C12—C11—C10—C18	−169.6 (13)
C4—C5—C6—N2	−178.4 (8)	C12—C11—C10—C9	−43.5 (14)
O5—N2—C6—C5	−4.6 (13)	C8—C9—C10—C18	−70.5 (16)
O6—N2—C6—C5	175.9 (9)	C13—C9—C10—C18	167.9 (14)
O5—N2—C6—C7	174.6 (9)	C8—C9—C10—C11	163.0 (11)
O6—N2—C6—C7	−4.8 (13)	C13—C9—C10—C11	41.4 (13)

C5—C6—C7—C2	−1.2 (15)	C11—C10—C18—C19	−60 (2)
N2—C6—C7—C2	179.6 (8)	C9—C10—C18—C19	−177.4 (19)
C3—C2—C7—C6	−0.2 (15)	C10—C18—C19—C20	−164 (2)
C1—C2—C7—C6	−177.7 (10)	C18—C19—C20—C21	68 (4)
C1—O2—C8—C9	173.8 (9)	C9B—C10B—C18B—C19B	178 (2)
C11—C12—C13—C14	121.2 (13)	C10B—C18B—C19B—C20B	−76 (4)
C17—C12—C13—C14	−4.7 (16)	C18B—C19B—C20B—C21B	−75 (10)
C11—C12—C13—C9	−2.3 (14)		

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PAPER II

The first synthesis of Crucigasterin 277 - a polyunsaturated C-18 amino alcohol from the Mediterranean tunicate Pseudodistoma crucigaster

Solveig Flock, Simen Antonsen, Harrison Gallantree-Smith, Anne Marie Langseter, Lars Skattebøl, Yngve Stenstrøm, *Tetrahedron* **2016**, *72*, 4518-4522

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The first synthesis of Crucigasterin 277—a polyunsaturated C-18 amino alcohol from the Mediterranean tunicate *Pseudomonas crucigaster*

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ABSTRACT

Starting from eicosapentaenoic acid (EPA) and D-alanine, the first synthesis of (2*R*, 3*S*)-crucigasterin 277, a polyunsaturated C-18 amino alcohol from the Mediterranean tunicate, *Pseudodistoma crucigaster*, is described.

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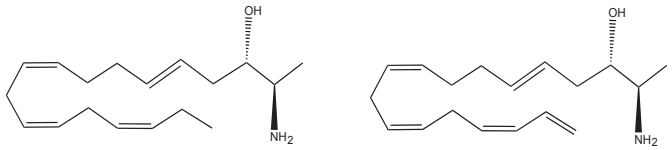
1. Introduction

The α-amino alcohol structural moiety is present in a large number of naturally occurring compounds.[1] Amino alcohols related to sphingosines have been isolated from marine organisms,[2] and several of these compounds have in common a polyunsaturated carbon chain with methylene interrupted double bonds, terminated by either one or two α-amino alcohol residues.

Some years ago Rinehart and co-workers[3] isolated two polyunsaturated C-18 amino alcohols from the Mediterranean tunicate, *Pseudodistoma crucigaster*. The compounds named crucigasterin 277 (**1**) and 275 (**2**), shown in Fig. 1, exhibited moderate cytotoxic and antimicrobial activity. All but one of the double bonds of **1** have the *Z*-configuration, and at first glance it seems that the compound is derived biosynthetically from D-alanine and an ω-3 polyunsaturated fatty acid. The absolute configuration was assigned based on comparison of a degradation product with that of an authentic compound.[4] The compounds **1** and **2** have not yet been synthesized. The present paper describes the synthesis of crucigasterin 277 (**1**), starting from commercially available *N*-Boc-protected D-alanine (**3**) and (all-*Z*)-eicosa-5,8,11,14,17-pentaenoic acid (EPA, **4**) (see Scheme 2).



Crucigasterin 277 (1) Crucigasterin 275 (2)

Fig. 1. Structures of crucigasterins.[3]

2. Results and discussion

Retrosynthetic analysis suggested cleavage of the C3–C4 bond in the target molecule 1 introducing chirality utilizing an α-amino acid derivative as outlined in Scheme 1. In the synthesis it was contemplated that this bond would be formed by reaction of the *N*-Boc-protected aldehyde 5 derived from 3, with a carbanion equivalent corresponding to the polyunsaturated C-15 residue. We

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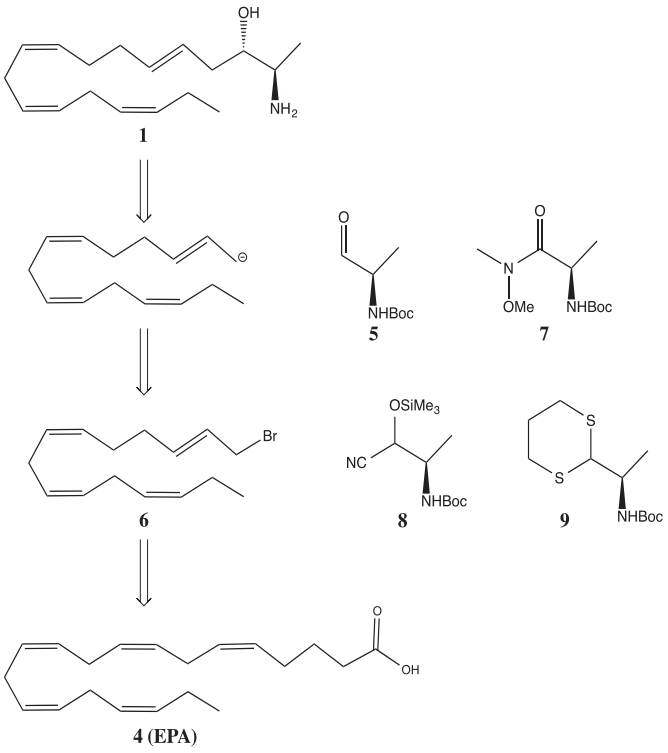
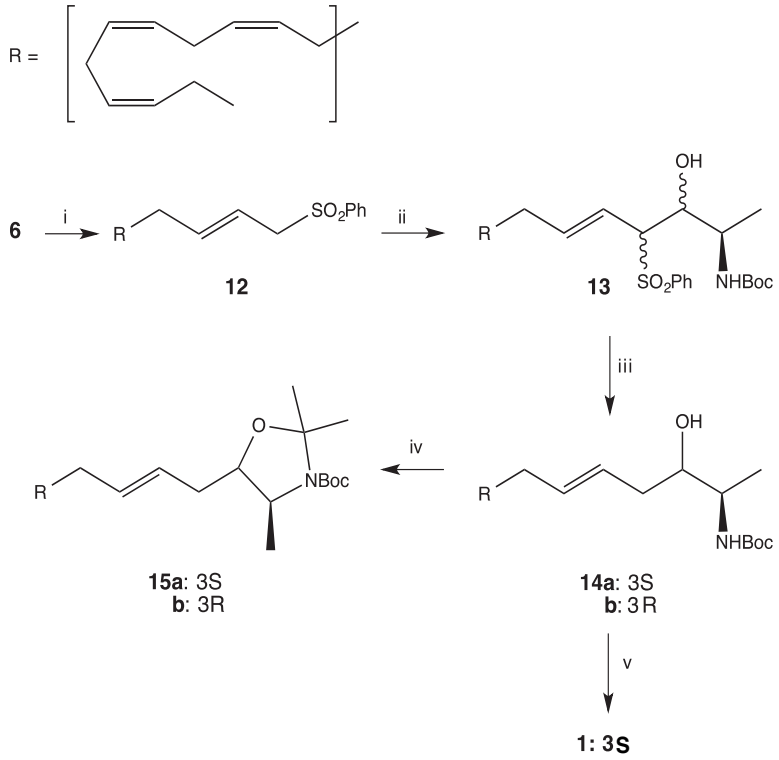


Scheme 1. Retrosynthetic analysis for the synthesis of **1**.

carbanion derived either from the protected cyanohydrin **8** or the thioacetal **9**. Subsequent reduction should yield the target amino alcohol. It was also observed that preparation of the compounds with the stereogenic center α to a carbonyl group was problematic due to fast racemization. The first alternative seemed the most promising, particularly since it would lead directly to the *N*-Boc-protected amino alcohol. It was anticipated that the protecting group could be removed without problems.

The *N*-Boc-protected D-alaninal (**5**) is known in the literature,[3,4] and several methods for the preparation **5** are reported. Accordingly commercially available *N*-Boc-protected D-alanine was converted to the methyl ester by standard conditions.[3,4] Reduction of the ester with DIBAL-H in THF at −78 °C furnished the aldehyde **5** in moderate yield.[4] The main problem with this reaction was the lack of reproducibility with regard to optical purity because of racemization. A second approach involved the oxidation of commercially available *N*-Boc-protected D-alaninol. Use of the Dess-Martin reagent furnished the aldehyde **5** in 80% yield, but with considerable racemization, but switching to the Parikh-Doering oxidation method gave *N*-Boc D-alaninal in excellent optical purity and moderate to good yield.[7] The optical purity was determined by optical rotation and comparison with the literature value. The aldehyde was used immediately to avoid any racemization.

Having the precursors for the vicinal amino alcohol moiety in place, we turned to the problem of transforming the bromide **6** into a carbanion equivalent, corresponding to the C-15 polyunsaturated residue.



Scheme 2. Synthesis of crucigasterin 277.

envisioned that the latter could be obtained from 1-bromo-2*E*,6*Z*,9*Z*,12*Z*-pentadecatetraene (6), a compound that is readily available from EPA in better than 50% overall yield.[5,6]

A second approach to the target molecule would involve reaction of the carbanion with the commercially available *N*-Boc-protected reactive amide **7**. This should afford the amino ketone and subsequently the amino alcohol by reduction. A third way of forming the C3–C4 bond involves reversal of polarities; the amino ketone might be formed by reaction of the bromide **6** with the

We anticipated that the bromide **6** could be converted into the corresponding Grignard, zinc or lithium derivatives, which are all allylic carbanion equivalents. Reactions at both the α- and γ-carbon atoms were expected, the latter being actually the preferred reaction mode with aldehydes,[8] but examples of α preference have been reported.[9] Reaction of the bromide **6** with magnesium metal in THF proceeded smoothly with partial consumption of the metal, and was followed by addition of the aldehyde **5**. However, the product obtained consisted according to GLC analysis of essentially

two compounds which we have tentatively assigned the dimeric structures **10** and **11** (see Fig. 2). The same dimers were also the sole products from reactions of the bromide with either zinc metal or methyllithium. We were unable to separate the dimers by chromatography and the assignments are based on spectral data obtained on the mixture. It has been reported[1,11,12] that the use of Rieke magnesium in this kind of reactions caused less Wurtz coupling, but insignificant change was observed in the present case.

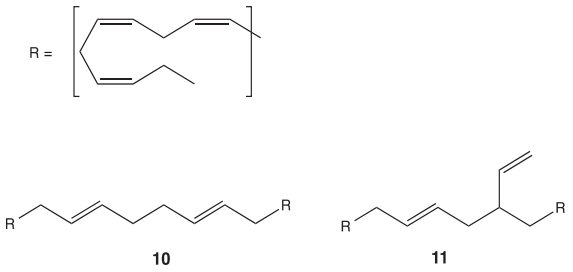


Fig. 2. Dimeric structures from Grignard reaction.

The Grignard type approach was abandoned and different ways of generating the carbanion analog were explored.

It is well known that allylic α-sulfonyl carbanions react with aldehydes at the α-carbon furnishing the straight chain compound,[13] usually as an *erythro-threo* product mixture. A reductive elimination of the sulfonyl group should then provide the desired alcohol **13** as outlined in Scheme 2.

The phenyl sulfone **12** was originally obtained in 58% overall yield from reaction of the bromide **6** with thiophenol, followed by oxidation of the intermediate sulfide with oxone, but subsequently we found that the sulfone was formed in one step and higher yield by reacting the same bromide with $NaSO_2Ph$ in DMF.[14]

The α-sulfonyl carbanion was generated in THF with *n*-butyllithium at −78 °C, and the reaction with aldehyde **5** furnished the *N*-Boc-protected amino alcohol **13** in 69% yield, as a mixture of four stereoisomers. Only the two major isomers were obtained pure by column chromatography, in 45% and 23% yield, respectively. Several methods are known to reductively remove the phenylsulfonyl group, but in the case of compound **13** the possibilities of elimination and double bond migration had to considered when choosing a reagent. The use of sodium amalgam was excluded (Julia elimination) and magnesium metal elimination as well. On the other hand, the palladium(II) catalyzed reduction with lithium borohydride as reported by Kotake and co-workers appeared successful.[14–16]

When the two pure isomers were reacted with this constellation of reagents, one single product was obtained in each case. According to spectral data the compounds were the *N*-Boc amino alcohols **14a** and **14b**, differing only in the configuration at C-3. Based on the NMR data, we were unable to establish their absolute configurations. However, according to the literature *cis*- and *trans*-substituted isomers of 1,3-oxazolidines exhibit significantly different values for the vicinal coupling constants of the protons at C-4 and C-5, the constant being smaller for the *trans* isomer.[17] Hence, the two diastereoisomers were converted with 2,2-dimethoxypropane in the presence of PTSA in 74% yield to the 1,3-oxazolidines **15a** and **15b**, respectively. The NMR measurements were hampered by the presence of rotamers at room temperature, which was particularly apparent for the isomer **15a**. This is apparently a common phenomenon for 1,3-oxazolidines,[18] and the spectra were therefore recorded at about 60 °C, above the coalescence temperature. The vicinal coupling constants for the protons at C-4 and C-5 of the ring were 5.0 Hz for isomer **15a** and 6.3 Hz for isomer **15b**. This result was certainly not conclusive, and the spectra were further analyzed by NOESY experiments. They showed for isomer **15a** a correlation of the methylene protons attached to C-4 and the methyl protons at C-5, while no correlation was observed for **15b**. This result was further confirmed by ROESY experiments.

Reagents and conditions: i) $PhSO_2Na$, DMF, 80 °C.; ii), n-BuLi, THF, 0 °C, **5**; iii) $LiBH_4$, $Pd(PPh_3)_2Cl_2$, −25 °C; iv) 2,2-dimethoxypropane, PTSA, PhH, Δ; v) 80% aq formic acid, 82% (only on **14a**).

It remained only to remove the protecting group of the (2*R*, 3*S*) isomer **14a** in order to complete the synthesis of crucigasterin 277 (**1**). This was expected to be a simple operation, but standard conditions for deprotection, including reactions with either trifluoroacetic acid or hydrochloric acid, caused partial isomerization of the double bonds. However, the removal of the *N*-Boc group of the isomer **14a** was successfully achieved using 80% formic acid giving crucigasterin 277 (**1**) in 82% yield. The diacetate was prepared according to the procedure reported previously.[3] The spectroscopic and physical data of both **1** and the corresponding diacetate was in agreement with data reported by Rinehart and co-workers[3] and thus confirmed the absolute configuration of the natural product.

3. Experimental section

The NMR spectra were recorded in $CDCl_3$, with a Bruker Avance DPX 200 or DPX 300 instruments. The IR spectra were obtained with a Perkin–Elmer 1310 infrared spectrophotometer or a Nicolet Magna-IR 550 spectrometer. Mass spectra were recorded at 70 eV with a Fisons VG Pro spectrometer. Optical rotations were measured with a Perkin Elmer 241 Polarimeter. Optical purity was determined using a Chrompack GC column: 3% OV 17 ON CHROM WHP. All reactions were performed under a nitrogen or argon atmosphere. The synthesis of **6** has previously been described by our group.[5,19]

3.1. *N*-Boc-D-Alaninal (5)

3.1.1. Method A. The compound was prepared from *N*-Boc-D-alanine methyl ester, according to literature[3] to give the D-alaninal (39% total yield) as crystals. Mp 74–76 °C; $[\alpha]_D^{25}$ =+41 (*c*=0.10, MeOH) (lit.[4] mp 76–78 °C and $[\alpha]_D^{25}$ =+40.8 (*c*=1.10, MeOH)). ^1H NMR (300 MHz, $CDCl_3$) δ 1.31 (d, *J*=7.3 Hz, 3 H), 1.43 (s, 9 H), 4.21 (m, 1 H), 5.17 (br s, 1 H, NH), 9.54 (s,1 H).

3.1.2. Method B. The compound was prepared from D-alaniol, according to literature to give the D-alaninal in 63% yield as crystals.[7] Mp 75–77, $[\alpha]_D^{25}$ =+39 (*c*=0.10, MeOH).

3.2. C-30 Hydrocarbons 10 and 11

A solution of the bromide **6** (0.64 g, 2.3 mmol) in dry ether (4 mL) was added dropwise to a stirred mixture of magnesium turnings (0.082 g, 3.4 mmol) and dry ether (1 mL). Once the reaction had started, the remaining bromide solution was added dropwise over a period of 20 min. After 2 h of gentle reflux, the mixture was cooled to −50 °C. A solution of *N*-Boc D-alaninal (**5**) (0.078 g, 0.45 mmol) in anhydrous THF (5 mL) was added. The mixture was left stirring at ambient temperature for 1 h. The reaction mixture was poured into a solution of 1 M aq NaH_2PO_4 and extracted with ether. The extract was washed with 1 M aq NaH_2PO_4, water, brine and dried ($MgSO_4$). Evaporation of solvents followed by flash chromatography (silica gel, 95:5 hexane/EtOAc) gave a mixture of dimers **10** and **11** (0.60 g, 65%). *m*/*z* (EI): 406 (1.0), 108 (48), 93 (63), 79 (100), 67 (83), 41 (54); (HRMS: found: M^+406.3607, $C_{30}H_{46}$ requires 406.3600).

3.3. (2*E*,6*Z*,9*Z*,12*Z*)-1-Phenylsulfonyl-2,5,9,12-pentadecatetraene (12)

3.3.1. Method A. To a stirred solution of LiOH×H_2O(1.1 g, 26.2 mmol) and thiophenol (1.7 g, 15.5 mmol) in MeOH (130 mL) was added, at room temperature, a solution of the bromide **6** (4.0 g, 14.1 mmol) in MeOH (20 mL). After stirring for 3 h at rt, water (100 mL) was added and the mixture extracted with hexane. The extract was washed with brine and dried ($MgSO_4$). Evaporation of solvents under reduced pressure followed by filtration through a plug of silica (hexane as eluent) gave (2*E*, 6*Z*, 9*Z*, 12*Z*)-1-Phenylthio-2,5,9,12-pentadecatetraene (4.1 g, 93%) as an oil. R_f (20% EtOAc/hexane): 0.80; IR: 3011, 2962, 2931, 1652, 1585, 1480, 1452 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, *J*=7.5 Hz, 3 H), 2.03–2.21 (m, 6 H), 2.75–2.93 (m, 4 H), 3.43–3.52 (m, 2 H), 5.20–5.44 (m, 7 H), 5.45–5.55 (1 H), 7.17–7.25 (m, 1 H), 7.27–7.40 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ14.2 (CH_3), 20.5, 25.5, 25.6, 26.8, 32.2, 36.4 (6×CH_2), 125.4, 126.0, 127.0, 128.0, 128.2, 128.3 (6×CH═), 128.6 (2×CH═), 129.1 (CH═), 129.8 (2×CH═), 131.9, 133.5 (2×CH═), 136.2 (C═); *m*/*z* (EI) 312 (M^+, 8%), 203 (22), 79 (100) (HRMS: found: M^+ 312,1919. $C_{21}H_{28}S$ requires 312, 1912).

To an ice-cooled solution of the sulfide (4.0 g, 12.8 mmol) in MeOH (30 mL) and dioxane (20 mL) was added dropwise an aqueous solution of oxone (23.7 g, 38.4 mmol). The mixture was left stirring at rt overnight. Water (50 mL) was added and the mixture extracted with $CHCl_3$. The extract was washed with brine and dried ($MgSO_4$). Evaporation of solvents under reduced pressure followed by flash chromatography (silica gel, 8:2 hexane/EtOAc) gave the sulfone **12** (2.7 g, 62%) as an oil. R_f (30% EtOAc/hexane): 0.45; IR: 3000, 2965, 2923, 1445, 1318, 1306, 1152 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.92 (t, *J*=7.5 Hz, 3 H), 1.91–2.08 (m, 6 H), 2.62–2.81 (m, 4 H), 3.71 (d, *J*=7.2 Hz, 2 H), 5.12–5.51 (m, 8 H), 7.49–7.57 (m, 2 H), 7.58–7.62 (m, 1 H), 7.78–7.86 (m, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ14.2 (CH_3), 20.4 (CH_2), 25.4 (CH_2), 25.5 (CH_2), 26.2 (CH_2), 32.4 (CH_2), 59.9 (CH_2), 116.3, 126.8, 127.7 (3×CH═), 128.4 (2×CH═), 128.5 (2×CH═), 128.5 (CH═), 128.9 (2×CH═), 131.9, 133.5 (2×CH═), 138.2 (C═), 140.8 (CH═); *m*/*z* (EI) 344 (M^+, 3%), 203 (82), 79 (100) (HRMS: found: M^+344,1802. $C_{21}H_{28}O_2S$ requires 344, 1810).

3.3.2. Method B. A solution of bromide **6** (4.0 g, 17.0 mmol) and $PhSO_2Na$ (5.55 g, 34.0 mmol) in DMF (100 mL) was heated at 80 °C for 15 h. Mixture was cooled to rt followed by addition of water (100 mL) and DCM (100 mL). Phases were separated, and the organic phase was washed with water (3×100 mL). Purification by chromatography (silica gel, 8:2 hexane/EtOAc) gave the sulfone **12** (4.1 g, 85%) as an oil.

3.4. (2*R*,5*E*,9*Z*,12*Z*,15*Z*)-*N*-Boc-2-Amino-3-hydroxy-4-phenysulfonyl-5,9,12,15-octadecatetraene (13)

n-BuLi (1.3 M in hexane, 5.50 mL, 7.15 mmol) was added to a solution of the sulfone **12** (2.67 g, 7.76 mmol) in THF (25 mL) at −78 °C. The mixture was stirred for 30 min before a solution of *N*-Boc-D-alaninal (**5**, 0.80 g, 4.62 mmol) in THF (5 mL) was added dropwise (0.5 mL/min using a syringe pump). The reaction mixture was stirred at −78 °C for 1.5 h before the reaction was quenched by addition of water and the mixture extracted with ether. The extract was washed with water (3×), brine and dried ($MgSO_4$). Evaporation of solvents under reduced pressure gave a residue that contained a mixture of unreacted **12** and four diastereomers. Purification by flash chromatography (silica gel, 8:2 hexane/EtOAc) gave the sulfone **12** (1.05 g), diastereomer **13a** (0.33 g), diastereomer **13b** (0.62 g), and an inseparable mixture of diastereomers **13c** and **13d** (0.61 g, 54:46 according to NMR). The combined yield of **13** was 69% based on alaninal.

3.5. Diastereomer 13a

R_f (20% EtOAc/hexane): 0.32; $[\alpha]_D^{25}$ =+4.6 (*c*=0.32, CH_3OH); IR: 3506 (broad), 3443, 3388, 3008, 2973, 2933, 1710, 1501, 1447, 1299, 1167, 1144 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.93 (t, *J*=7.5 Hz, 3 H), 1.17 (d, *J*=6.0 Hz, 3 H), 1.39 (s, 9 H), 1.86–2.10 (m, 6 H), 2.63–2.81 (m, 4 H), 3.46 (t, *J*=9.8 Hz, 1 H, H-4), 3.58–3.74 (m, 1 H, H-2), 4.17 (s, 1 H, OH), (4.26 m, 1 H, H-3), 4.26 (d, *J*=9.8 Hz, 1 H, H-3), 5,01–5.46 (m, 9 H), 7.44–7.54 (m, 2 H), 7.57–7.65 (m, 1 H), 7.73–7.86 (m, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ12.3, 14.2 (2×CH_3), 20.6, 25.4, 25.5, 26.1 (4×CH_2), 28.3 (3×CH_3), 32.5 (CH_2), 47.6 (CHNH), 70.1 (CHOH), 72.8 ($CHSO_2Ph$), 79.3 (CO), 118.4, 126.7, 127.7, 128.3, 128.5 (5×CH═), 128.8 (3×CH═), 129.3 (2×CH═), 132.0 (CH═), 134.1 (CH═), 136.5 (C), 141.2 (CH═), 155.0 (C═O). *m*/*z* (CI): 518 (M^++1, 0.9), 57 (100); (HRMS (Electrospray): found: M^+ +1, 518.2938. $C_{29}H_{44}NO_5S$ requires 518.2935).

3.6. Diastereomer 13b

R_f (20% EtOAc/hexane): 0.20; $[\alpha]_D^{25}$ =−2.45 (*c*=0.41, CH_3OH); IR: 3501(broad), 3447, 2970, 2932, 1716, 1499, 1441, 1297, 1158, 1136 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ0.92 (t, *J*=7.5 Hz, 3 H), 1.17 (d, *J*=6.7 Hz, 3 H), 1.34 (s, 9 H), 1.83–2.08 (m, 6 H), 2.60–2.78 (m, 4 H), 3.56–3.77 (m, 2 H, H-2 and H-4), 4.09 (d, *J*=9.5 Hz, 1 H, H-3), 4.33 (s, 1 H, OH), 4.72–4.93 (m, 1 H, NH), 4,95–5.43 (m, 8 H), 7.41–7.52 (m, 2 H), 7.53–7.64 (m, 1 H), 7.77–7.84 (m, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ14.2, 18.7 (2×CH_3), 20.5, 25.4, 25.5, 25.9 (4×CH_2), 28.2 (3×CH_3), 32.5 (CH_2), 47.7 (CHNH), 71.4 (CHOH), 72.9 ($CHSO_2Ph$), 78.9 (CO), 117.9, 126.9, 127.8, 128.4, 128.7 (5×CH═), 128.8 (3×CH═), 129.1 (2×CH═), 132.0, 133.9 (2×CH═), 137,0 (C), 141.6 (CH═), 154.8 (C═O). *m*/*z* (CI): 518 (M^++1, 0.6), 57 (100). *m*/*z* (EI): 461 (1.4), 44 (100); (HRMS (Electrospray): found: M^+ +1, 518.2934. $C_{29}H_{44}NO_5S$ requires 518.2935).

3.7. Mixture of diastereomers 13c and 13d

R_f (20% EtOAc/hexane): 0.12; ^1H NMR (300 MHz, $CDCl_3$) δ0.92 (t, *J*=7.5 Hz, 2×3 H), 1.12 (d, *J*=6.7 Hz, 3 H), 1.34 (d, *J*=6.5 Hz, 3 H), 1.37 (s, 9 H), 1.39 (s, 9 H), 1.29–2.15 (m, 2×6 H), 2.58–2.28 (m, 2×4 H), 3.38–3.77 (m, 2×2 H), 4.26–4.45 (m, 2×1 H), 4.58 (d, *J*=8.6 Hz, 1 H), 4.82 (d, *J*=9.4 Hz, 1 H), 5.05–5.48 (m, 2×8 H), 5.49–5.64 (m, 2×1 H), 7.38–7.62 (m, 2×3 H), 7.72–7.81 (m, 2×2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ14.1, 18.3, 20.4, 25.4 (2×CH_2), 26.1, 32.5, 32.6, 49.1, 50.3, 70.4, 70.5, 71,2, 72.0, 77.2, 79.4, 117.4, 117.6, 126.8, 127.7, 128.4, 128.5, 128.6 (2×CH), 128.7, 128.8, 129.0, 129.2, 131.8, 131.9, 133.5, 133.6, 137.2, 137.3, 140.8, 142.1, 155.2, 155.7.

3.8. (2*R*,5*E*,9*Z*,12*Z*,15*Z*)-*N*-Boc-2-Amino-3-hydroxy-5,9,12,15-octadecatetraene (14a)

To a solution of compound **13a** (0.150 g, 0.29 mmol) and $LiBH_4$ (0.032 g, 1.45 mmol) in dry THF (10 mL), cooled to −25 °C, was added a suspension of $Pd(PPh_3)_2Cl_2$ in THF (5 mL).[10] The mixture was left stirring at −25 °C overnight. 0.1 M aq NaOH (3 mL) was added and the mixture extracted with ether/hexane 1:1. The extract was washed with water until neutral, then with brine and dried ($MgSO_4$). Evaporation of solvents followed by flash chromatography (silica gel, 8:2 hexane/EtOAc) gave the compound **14a** (0.09 g, 82%) as an oil; *Rf* (40% EtOAc/hexane): 0.60; $[\alpha]_D^{25}$ =+11.6 (*c*=0.17, $CHCl_3$) IR:3439 (broad), 3010, 2974, 2932, 1690 (broad), 1505, 1366, 1170 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ0.94 (t, *J*=7.5 Hz, 3 H), 1.06 (d, *J*=6.7 Hz, 3 H), 1,42 (s, 9 H), 1.90–2.28 (m, 9 H), 2.62–2.81 (m, 4 H), 3.51–3.72 (m, 2 H, H-2 and H-3), 4.68–4.82 (m, 1 H, NH), 5.21–5.60 (m, 8 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 14.5 (2×CH_3), 20.5, 25.5, 25.6, 27.0 (4×CH_2), 28.4 (3×CH_3), 32.5, 37.2 (2×CH_2), 50.1 (CHNH), 73.4 (CHOH), 79.3 (CO), 126.3, 127.0, 128.0,

128.3, 128.4, 129.3, 132.0, 133.7 (8×CH=), 155.7 (C=O); *m/z* (CI): 378 (M^++1, 2.6), 278 (100); (HRMS (Electrospray): found: M^+ +1, 378.3000. $C_{23}H_{40}NO_3$ requires 378.3003).

3.9. (*2R,5E,9Z,12Z,15Z*)-*N*-Boc-2-Amino-3-hydroxy-5,9,12,15-octadecatetraene (14b)

Reduction of compound **13b** (0.35 g, 0.67 mmol), as described above for **13a,** gave compound **14b** (0.19 g, 75%) as an oil; *Rf* (40% EtOAc/hexane): 0.60; $[\alpha]_D^{25}$ =+9.3 (*c*=0.37, CH_3OH); *IR:* 3390 (broad), 2988, 2935, 2900, 1670 (broad), 1485, 1350, 1155 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ0.94 (t, *J*=7.5 Hz, 3 H), 1.14 (d, *J*=6.8 Hz, 3 H), 1,41 (s, 9 H), 1.90–2.32 (m, 9 H), 2.66–2.83 (m, 4 H), 3.38–3.52 (m, 1 H, H-3), 3.53–3.69 (m, 1 H, H-2), 4.66–4.82 (m, 1 H, NH), 5.20–5.58 (m, 8 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2 (CH_3), 18.5 (CH_3), 20.5, 25.5, 25.6, 27.0 (4×CH_2), 28.3 (3×CH_3), 32.5, 37.7 (2×CH_2), 49.7 (CHNH), 73.8 (CHOH), 79.2 (CO), 126.1, 127.0, 128.0, 128.1, 128.2, 128.3, 128.4, 129.2, 129.3, 131.9, 133.9 (11×CH=), 156.0 (C=O); *m/z* (EI): 321 (3.4), 44(100); *m/z* (CI): 378 (M^++1, 11.6), 278 (100). HRMS (Electrospray): found: M^++1, 378.3005, $C_{23}H_{40}NO_3$ requires 378.3003.

3.10. 2,2-Dimethyl-1,3-oxazolidine (15a)

A solution of the amino alcohol **14a** (52 mg, 0.14 mmol), 2,2-dimethoxypropane (0.035 mL, 0.028 mmol) and *p*-toluenesulfonic acid (cat. amount) in benzene (5 mL) was heated under reflux for 1 h. Ether (10 mL) was added and the mixture neutralized with satd aq $NaHCO_3$, washed with brine and dried ($MgSO_4$). Evaporation of solvents followed by flash chromatography (silica gel, 95:5 hexane/EtOAc) gave the oxazolidine **15a** (45 mg, 78%) as an oil; R_f (40% EtOAc/hexane): 0.60; $[\alpha]_D^{25}$ =+0.50 (*c*=0.77, $CHCl_3$); ^1H NMR (500 MHz, C_6D_6, 79 °C) δ 0.91 (t, *J*=7.5 Hz, 3 H), 1.13 (bd, *J*=5.3 Hz, 3 H), 1.43 (s, 9 H), 1.56 (br s, 3 H), 1.68 (br s, 3 H), 1.93–2.14 (m, 7 H), 2.07–2.15 (m, 1 H), 2.66–2.86 (m, 4 H), 3.78–4.01 (m, 1 H), 3.92 (m, 1 H), 5.31–5.52 (m, 8 H); ^{13}C NMR (75 MHz, C_6D_6, 79 °C) δ 13.9, 14.2 (2×CH_3), 20.9, 26.0, 26.1, 27.5 (4×CH_2), 28.6 (5×CH_3), 32.9 (CH_2), 55.7 (CH–N), 76.7 (CH–O), 79.0, (N–C–O), 92.8 (CO), 126.4, 127.6, 128.5, 128.6, 128.8, 129.7, 132.2, 132.6 (8×CH=), 151.8 (C=O).

3.11. 2,2-Dimethyl-1,3-oxazolidine (15b)

The amino alcohol **14b** (0.13 g, 0.34 mmol) was treated with 2,2-dimethoxypropane (0.085 mL, 0.069 mmol) and *p*-toluenesulfonic acid (cat. amount) as described for **15a** to give the oxazolidine **15b** (0.11 g, 76%) as an oil. $[\alpha]_D^{25}$ =–10.5 (*c*=0.57, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, 35 °C) δ 0.94 (t, *J*=7.5 Hz, 3 H), 1.25 (d, *J*=5.9 Hz, 3 H), 1,45 (s, 12 H), 1,55 (s, 3 H), 1.88–2.13 (m, 6 H), 2.21–2.36 (m, 2 H), 2.68–2.82 (m, 4 H), 3.52 (br s, 1 H), 3.67 (q, *J*=6.2 Hz, 1 H), 5.23–5.54 (m, 8 H); ^{13}C NMR (75 MHz, $CDCl_3$, 35 °C) δ 14.2,18.5 (2×CH_3), 20.5, 24.5, 25.6, 26.1 (4×CH_2), 28.4 (5×CH_3), 32.6, 36.8 (2×CH_2), 57.2, (CH–N), 79.3 (N–C–O), 81.2 (CH–O), 93.8 (CO), 125.5, 127.0, 128.1, 128.2, 128.3, 129.3, 131.8, 132.9 (8×CH=), 152.1 (CH=O); HRMS (Electrospray): found: M^+ +1, 418.3315, $C_{26}H_{44}NO_3$ requires 418.3316.

(2*R*,3*R*,5*E*,9*Z*,12*Z*,15*Z*)-2-aminooctadeca-5,9,12,15-tetraen-3-ol (Crucigasterin 277, **1**). The amino alcohol **14a** (0.18 g, 0.48 mmol) was dissolved in 80% formic acid (20 mL). The reaction mixture was left stirring at room temperature overnight. Water (20 mL) was added and most of the formic acid was removed by evaporation. Saturated $NaHCO_3$ was added to neutral pH and then the reaction mixture was extracted with EtOAc (3×20 mL). The extract was washed with water (20 mL) and brine (20 mL) and dried ($MgSO_4$). Evaporation of solvents under reduced pressure gave the amino alcohol **1** (0.11 g, 82%) as an oil. $[\alpha]_D^{25}$ =+4.7 (*c*=0.60, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, *J*=6, 3 H), 1.13–1.42 (m, 6H), 1.90–2.24 (m, 9H), 2.39–2.24 (m, 2 H), 2.69–2.84 (m, 4 H), 3.14 (br s, 1H), 3.55 (s, 2H), 4.74 (br s, 3H), 5.21–5.57 (m, 8 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.47, 18.74, 20.75, 25.73, 25.86, 27.29, 32.89, 38.21, 52.11, 73.27, 125.35, 127.25, 128.24, 128.48, 128.64, 129.51, 132.19, 134.07.

The diacetyl derivative was made according to literature[3] giving $[\alpha]_D^{25}$ =+41 (*c*=0.62, CH_3OH); that is in accord with the published one. All spectral data were consistent with the published ones.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2016.06.009.

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Supporting Information for:

The First Synthesis of Crucigasterin 277 – a Polyunsaturated C_{18} Amino Alcohol from the Mediterranean Tunicate *Pseudomonas crucigaster*.

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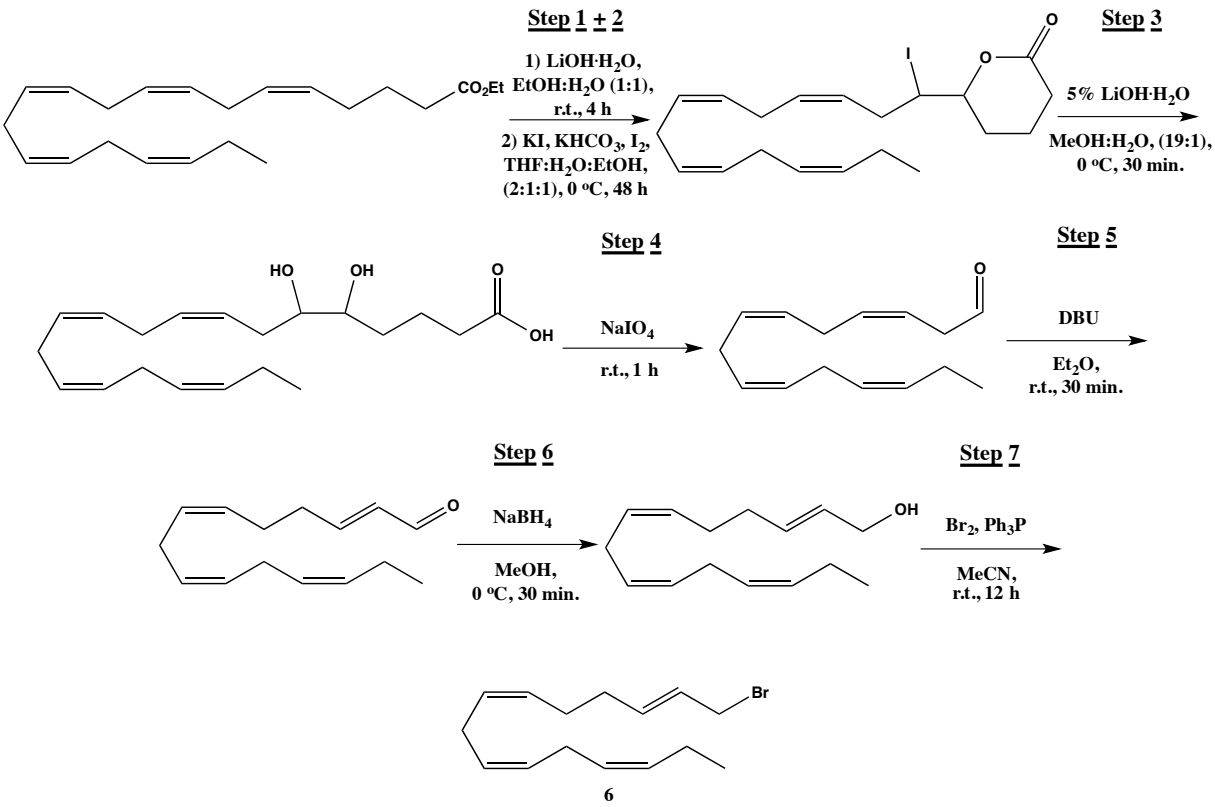
General Information:

The NMR spectra were recorded in $CDCl_3$, with a Bruker Avance DPX 200 or DPX 300 instruments. The IR spectra were obtained with a Perkin-Elmer 1310 infrared spectrophotometer or a Nicolet Magna-IR 550 spectrometer. Mass spectra were recorded at 70 eV with a Fisons VG Pro spectrometer. Optical rotations were measured with a Perkin Elmer 241 Polarimeter. Optical purity was determined using a Chrompack GC column: 3% OV 17 ON CHROM WHP. All reactions were performed under a nitrogen or argon atmosphere. Our group has previously reported the synthesis of **6**.[1,2]



Scheme S-1: Preparation of bromide **6**.

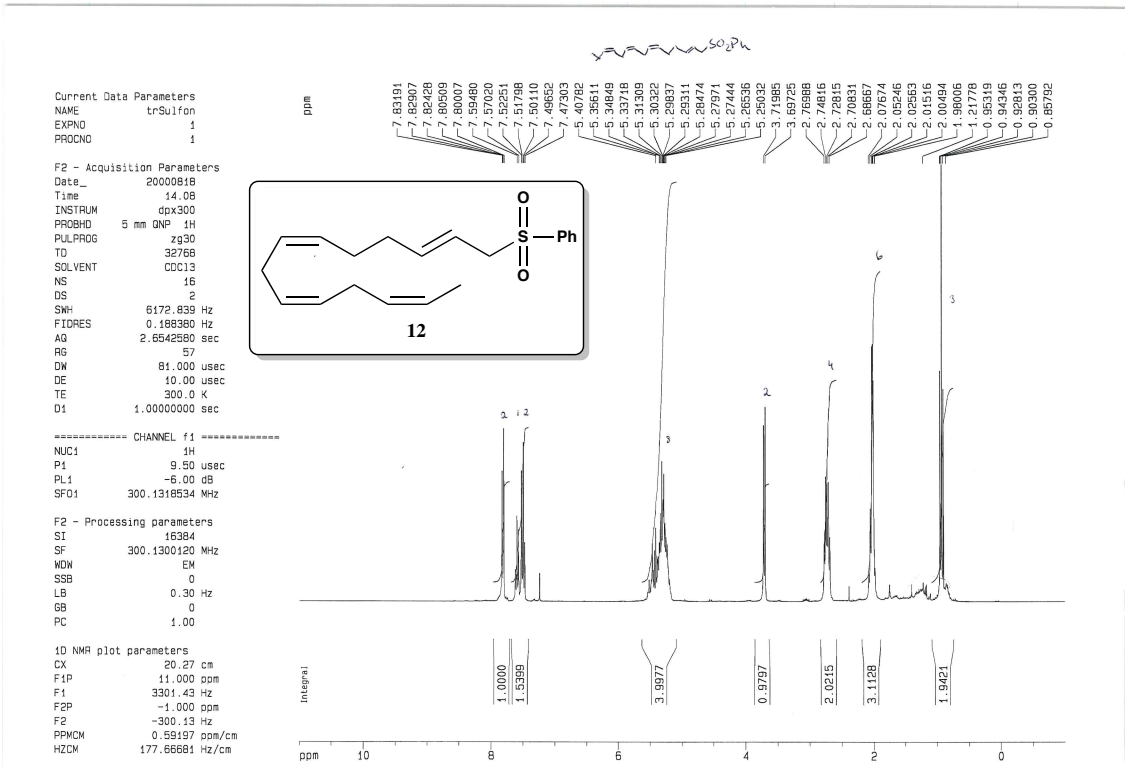


Figure S-1 ^{1}H-NMR spectrum of compound **12**.

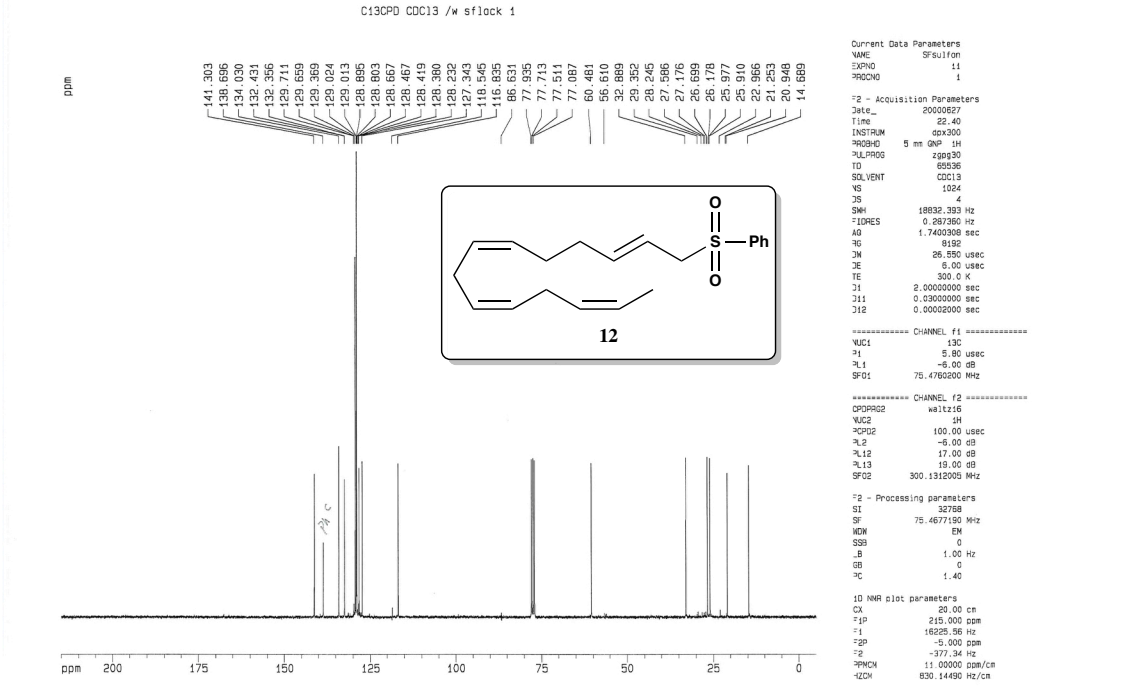


Figure S-2 ^{13}C-NMR spectrum of compound **12.**

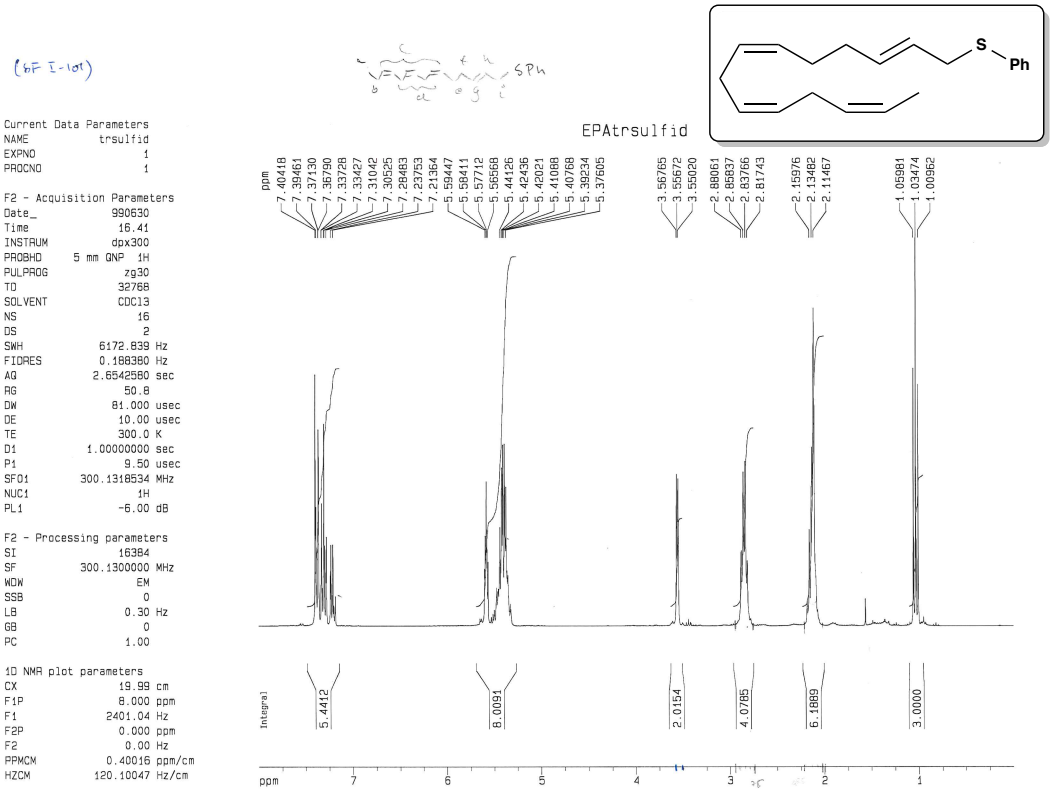


Figure S-3 ^{1}H-NMR spectrum of intermediate sulfide.

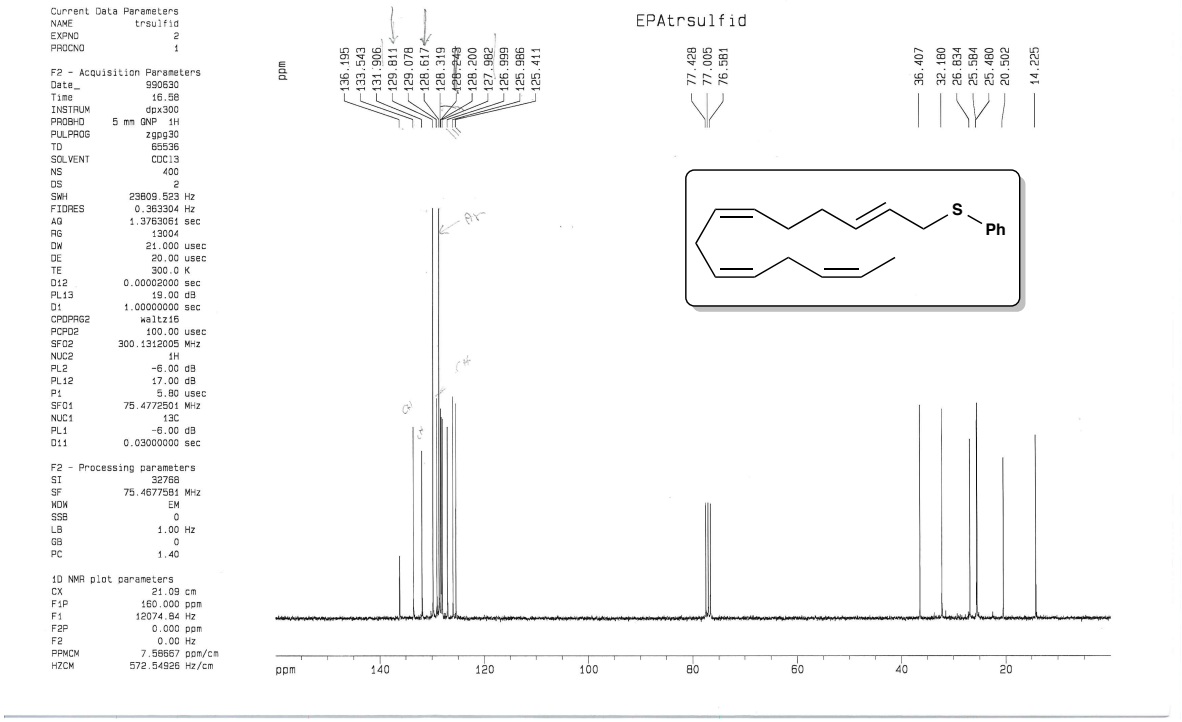


Figure S-4 ^{13}C-NMR spectrum of intermediate sulfide.

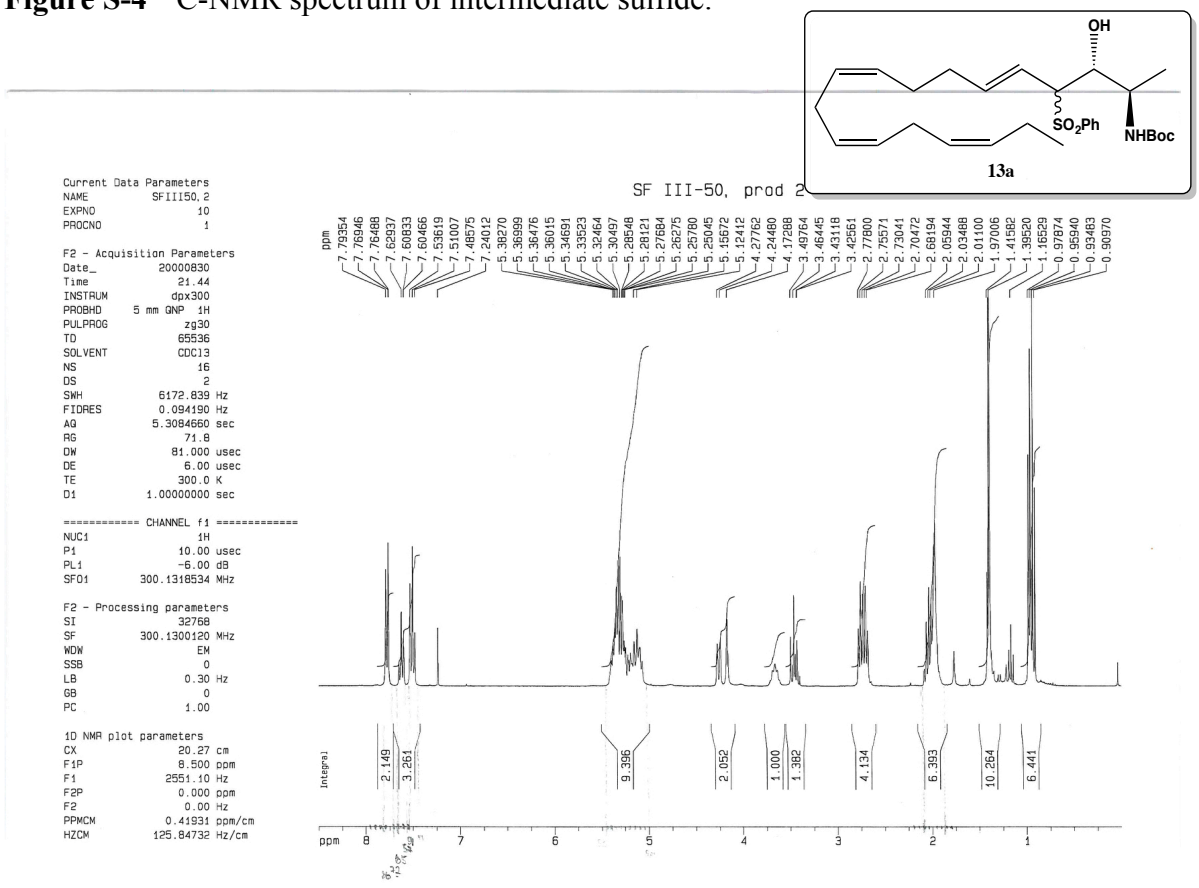


Figure S-5 ^{1}H-NMR spectrum of compound **13a**.

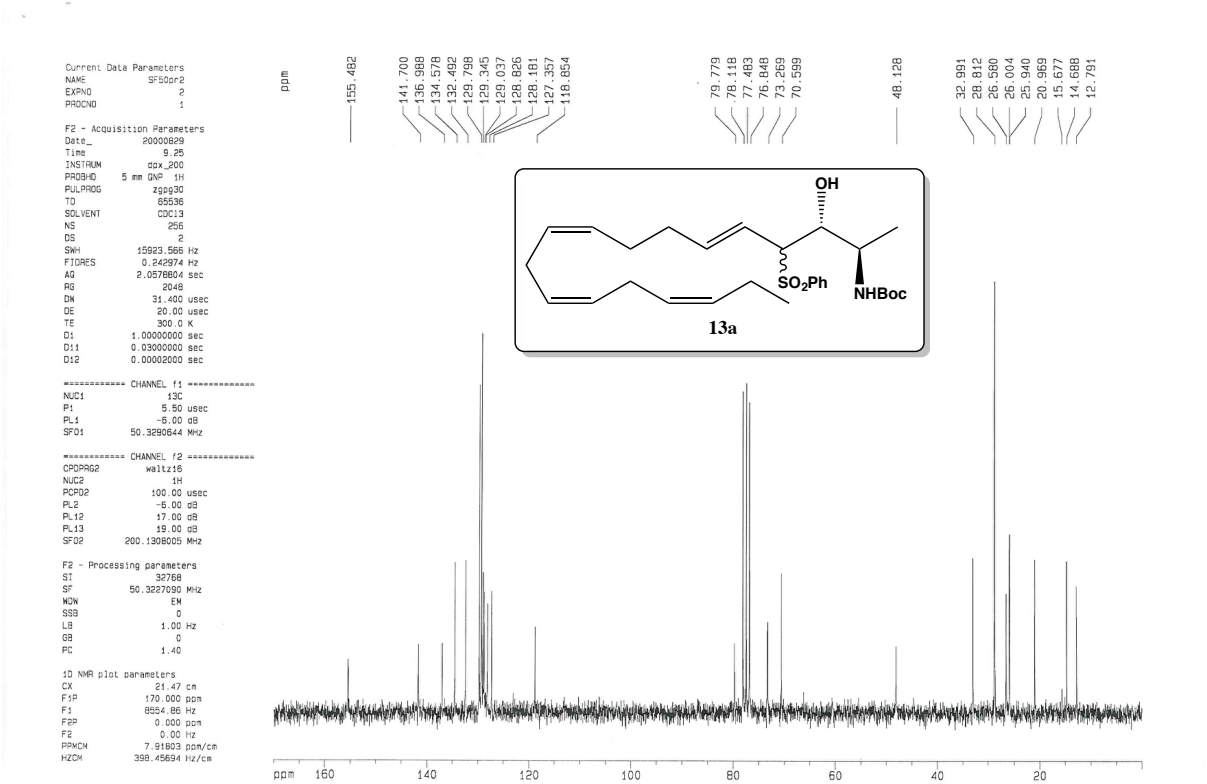


Figure S-6 ^{13}C-NMR spectrum of compound **13a**.

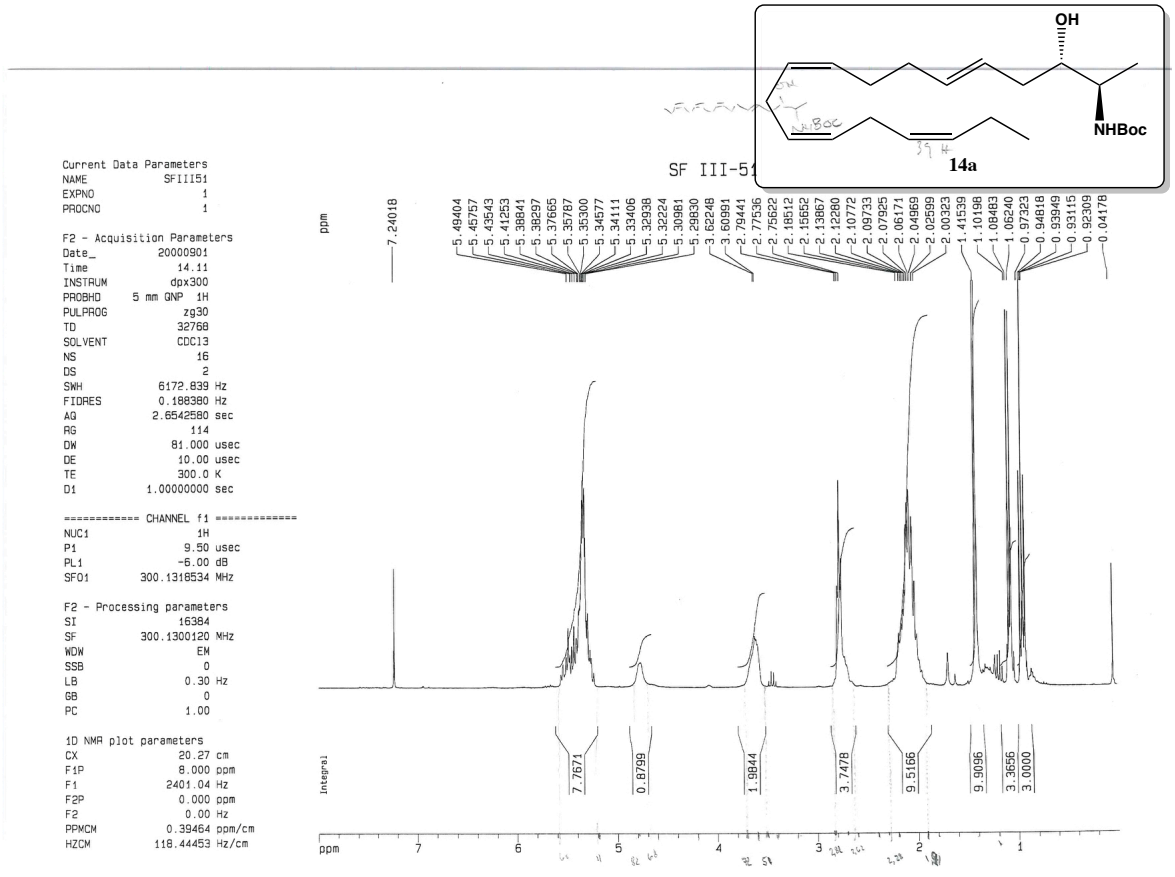


Figure S-7 ^{1}H-NMR spectrum of compound **14a**.

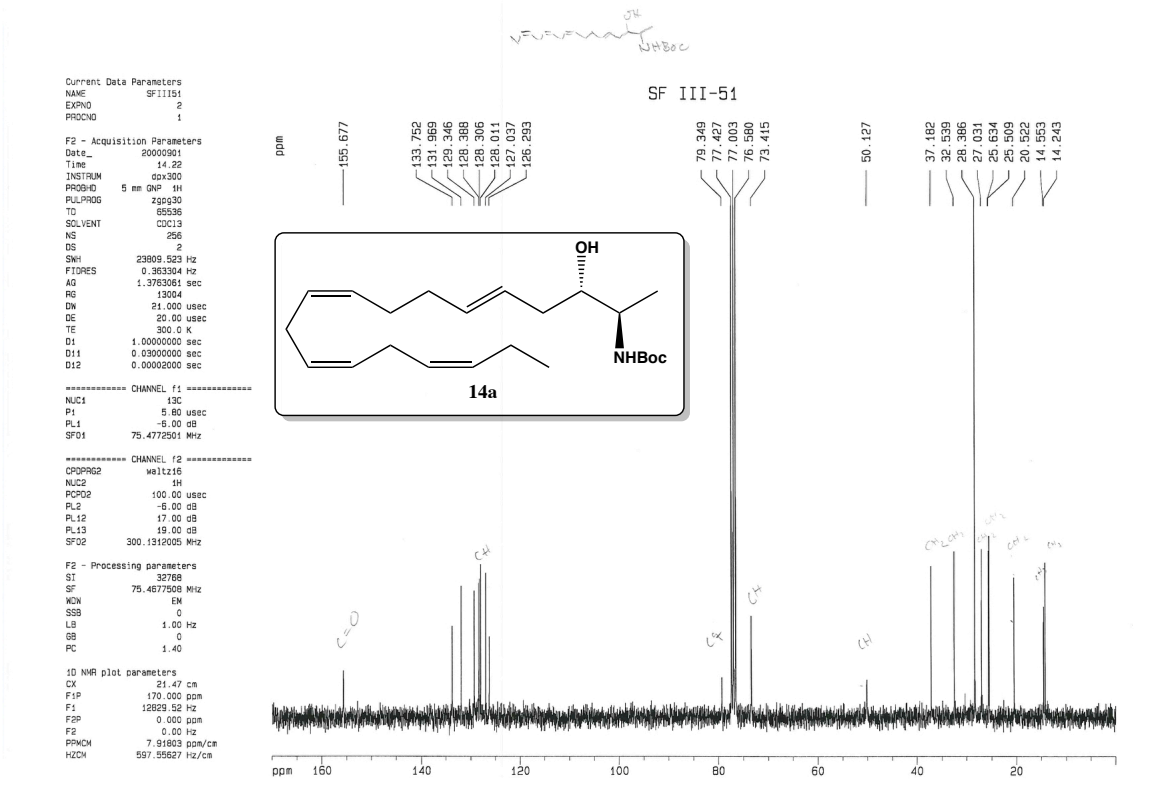


Figure S-8 ^{13}C-NMR spectrum of compound **14a**.

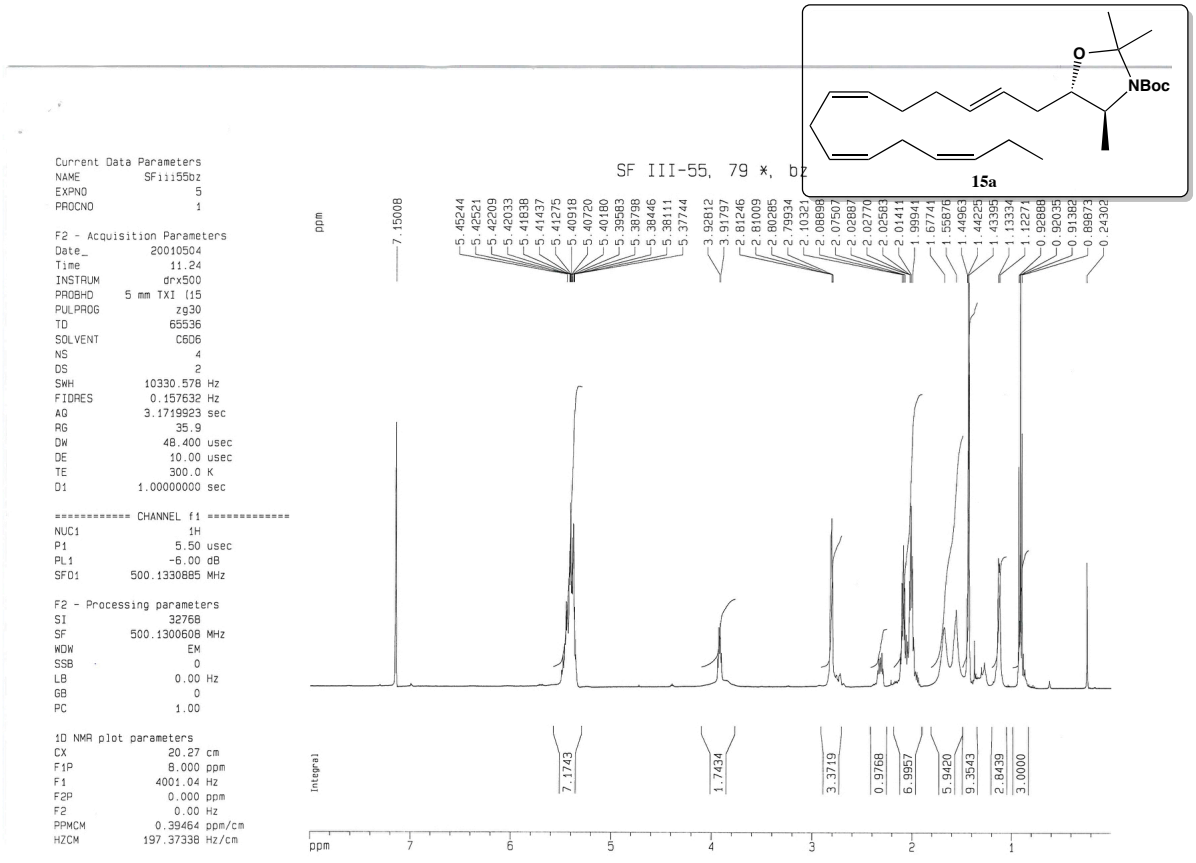


Figure S-9 ^{1}H-NMR spectrum of compound **15a**.

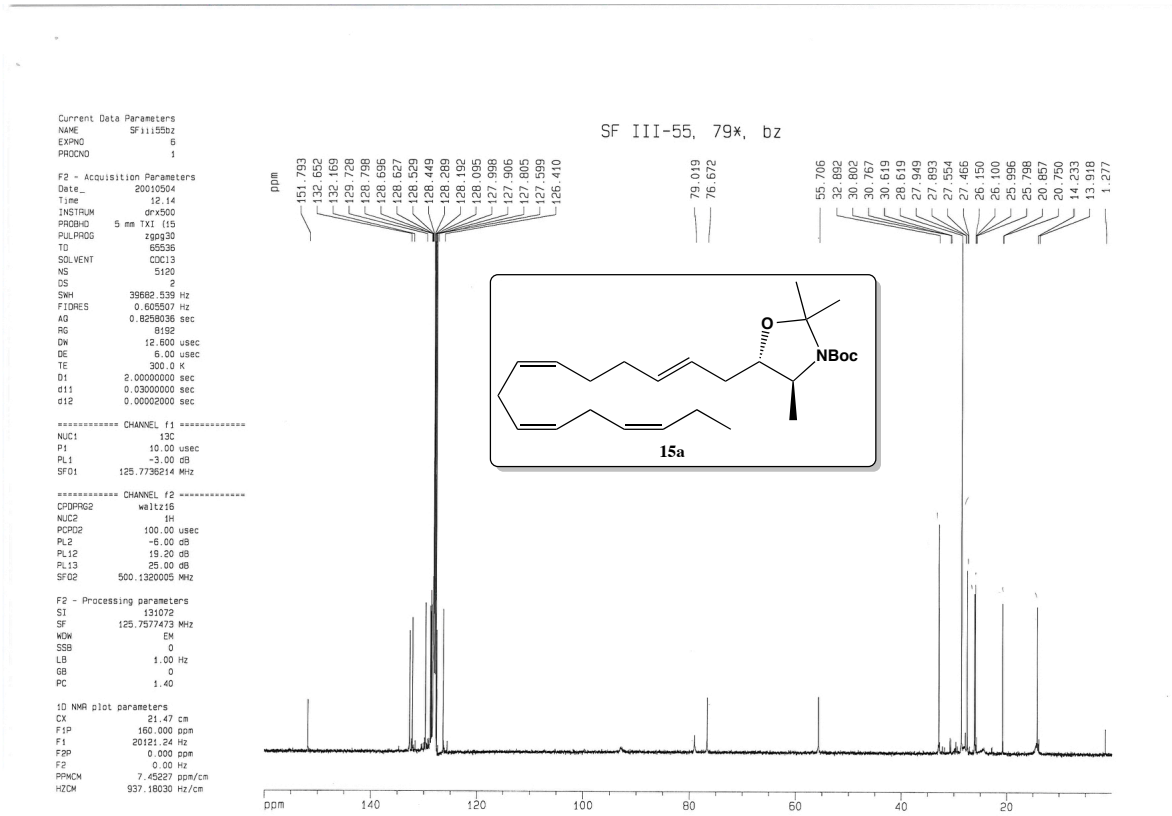


Figure S-10 ^{13}C-NMR spectrum of compound **15a**.

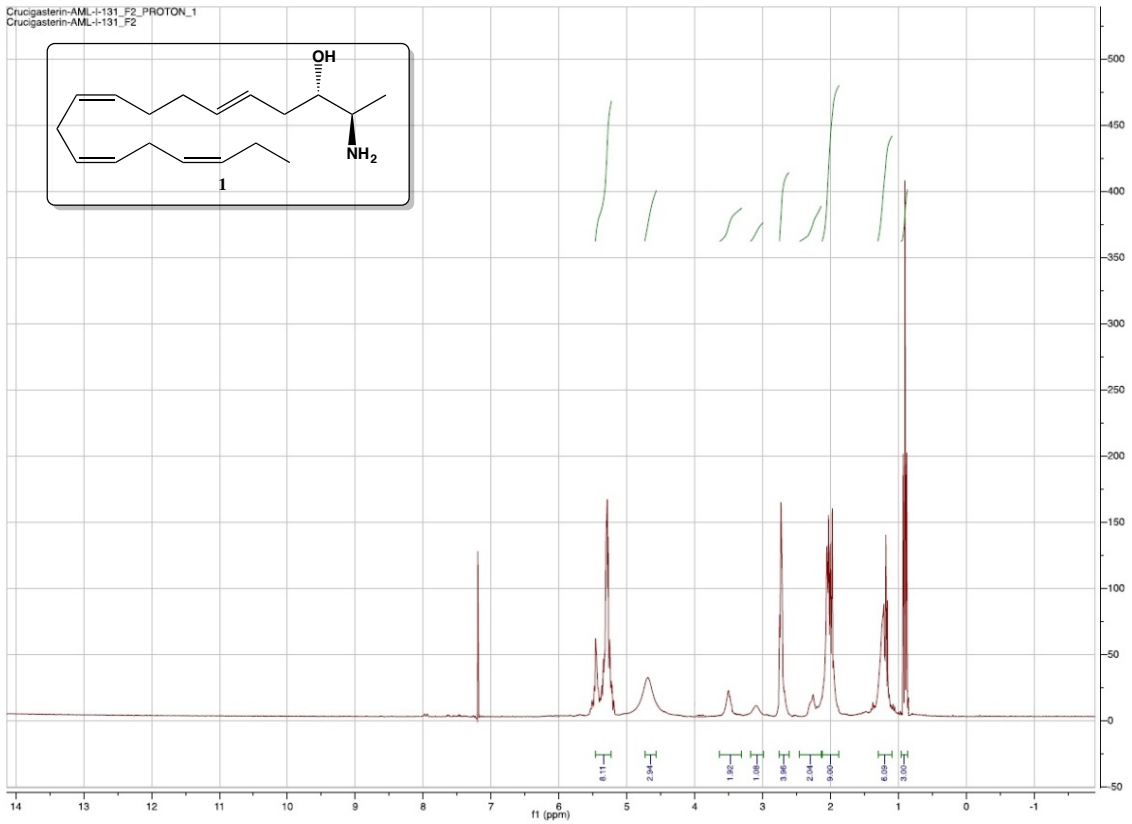


Figure S-11 ^{1}H-NMR spectrum of compound **1**.

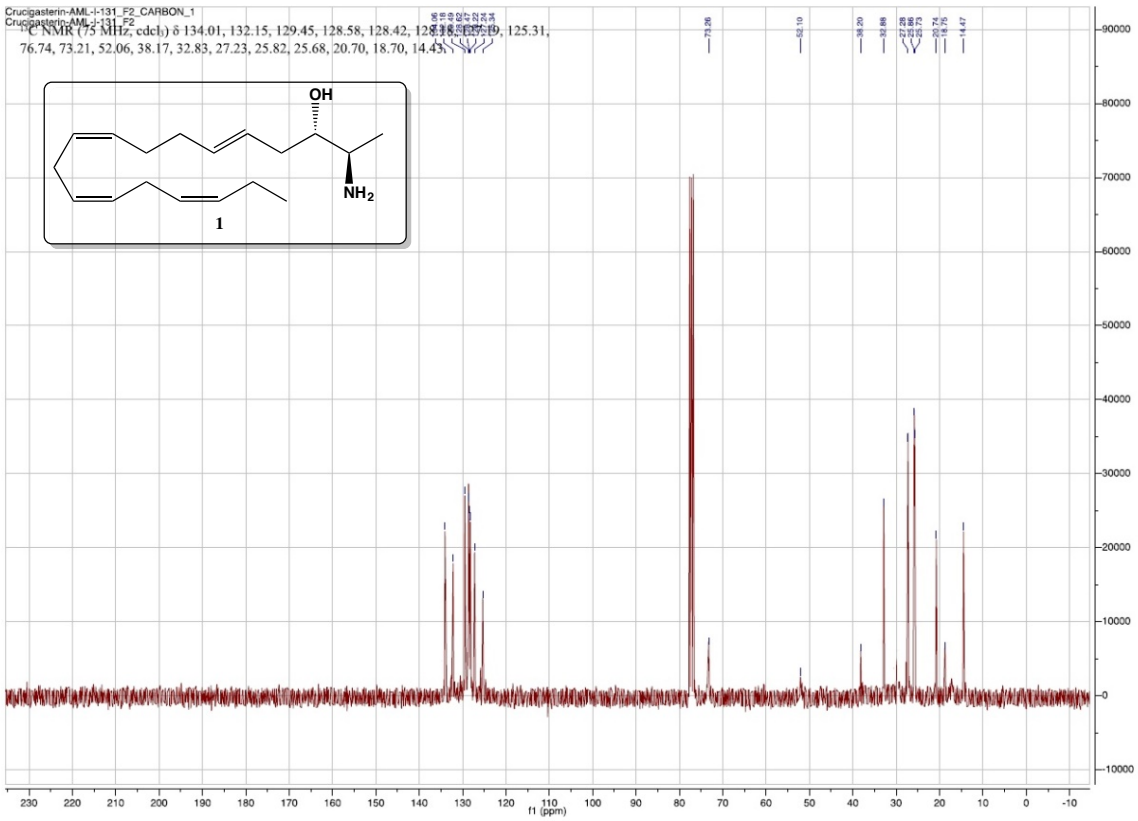


Figure S-12 ^{13}C-NMR spectrum of compound **1**.

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