Total synthesis as a tool for structural elucidation of some marine lipid natural products

Totalsyntese som et verktøy for strukturoppklaring av noen marine naturprodukter med lipid struktur

Philosophiae Doctor (PhD) Thesis

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Day after day, day after day,
We stuck, nor breath nor motion;
As idle as a painted ship
Upon a painted ocean.

Water, water, every where,
And all the boards did shrink;
Water, water, every where,
Nor any drop to drink.

The Rime of the Ancient Mariner, Samuel Taylor Coleridge, 1798

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Finally, thanks to my friends and family, who have all supported me!

Simen Antonsen
Ås, February 2017

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List of papers

I. *Total synthesis based on the originally claimed structure of mucosin.* Harrison Gallantree-Smith, Simen Gjelseth Antonsen, Carl Henrik Görbitz, Trond Vidar Hansen, Jens Mortansson Jelstrup Nolsøe and Yngve Stenstrøm. *Organic and Biomolecular Chemistry*, **2016**,14, 8433.

II. *Pursuing the true structure of marine natural product mucosin: Part 2.* Simen Gjelseth Antonsen, Harrison Gallantree-Smith, Carl Henrik Görbitz, Trond Vidar Hansen, Yngve Stenstrøm and Jens Mortansson Jelstrup Nolsøe. Manuscript.

III. *The first synthesis of Crucigasterin 277—a polyunsaturated C-18 amino alcohol from the Mediterranean tunicate Pseudomonas crucigaster.* Solveig Flock, Simen Antonsen, Harrison Gallantree-Smith, Anne Marie Langseter, Lars Skattebøl and Yngve Stenstrøm. *Tetrahedron*, **2016**, 72, 4518.

IV. *Synthesis of Obscuraminol A using an organocatalyzed enantioselective Henry reaction.* Liudmila Filippova, Simen Gjelseth Antonsen, Yngve; Hansen Stenstrøm and Trond Vidar Hansen. *Tetrahedron*, **2016**, 72, 6572.

List of papers not included in the thesis:

I. *Synthesis of racemic β-chamigrene, a spiro[5.5]undecane sesquiterpene.* Simen Gjelseth Antonsen, Lars Skattebøl and Yngve Stenstrøm. *Molecules*, **2014**, 19, 20664.

II. *The binding of pentapeptides to biological and synthetic high affinity heparin.* Ragnar Flengsrud and Simen Gjelseth Antonsen. *Bioorganic & Medicinal Chemistry Letters*, **2015**, 25, 4774.

III. *Safe Synthesis of Alkylhydroxy and Alkylamino Nitramines*. Simen Antonsen, Marius Aursnes, Harrison Gallantree-Smith, Christian Dye and

Yngve Stenstrøm. *Molecules*, **2016**, 21, 1738.

IV. Naturally occurring cyclobutanes, their biological significance and synthesis. Simen Antonsen, Runa Berg Østby and Yngve Stenstrøm. *Studies in Natural Products Chemistry*, Accepted.

V. *Synthetic Studies towards Cyclobutanes by Microwave Assisted Intramolecular [2+2] Cycloaddition of Allene-Ene Esters*. Runa B. Østby, Rita Sørensen, Yngve H. Stenstrøm, Stephen Westerås, Simen Antonsen. Manuscript.

Aim of study

The aim was to synthesize the following lipids:

- Mucosin and dictyosphaerin, prostaglandin-like structures of marine origin.
- Crucigasterin 277 and obscuraminol A, unsaturated sphingoid bases.

These efforts should provide sufficient amounts of material for biological evaluation.

Abstract

Paper I describes the total synthesis of the proposed structure of mucosin, a C_{20} fatty acid, isolated from a Mediterranean sponge, *Reniera mucosa*. The synthetic pathway started with the transformation of 1,4-cyclohexadiene to a known *meso*-ketone in three steps.

Employing a chiral base, an optically active β-keto ester, also previously described in literature, was obtained in excellent stereochemical purity. In a thirteen-step linear sequence, including a copper mediated cross-coupling, one-pot alumination/halodemetallation/Negishi cross-coupling protocol, gave the proposed structure of (-)-mucosin. Comparison of spectral data with the literature reported values revealed discrepancies. By employing X-ray crystallography on a late stage intermediate, it was demonstrated that the relative stereochemistry assigned to mucosin had been achieved. This also proved that the structure claimed in literature is most likely incorrect.

Paper II describes the total synthesis of the diastereomer of the proposed structure of mucosin. Taking advantage of the same keto ester from paper I, we prepared an α,β-unsaturated ester enabling an inverted motive for the conjugate addition. By this strategy we made the *anti*-diastereomer of the proposed structure for mucosin. This demonstrated that the natural product most likely does not contain a *cis*-fused bicyclic core, since the prepared material did not match the reported data.

Paper III describes the synthesis of crucigasterin 277, a sphingolipid isolated from the Mediterranean tunicate, *Pseudomonas crucigaster*. The compound was prepared via a chiral pool approach. An allylic C_{15} bromide, prepared from eicosapentaenoic acid (EPA) following a published procedure, was used as the ω-end fragment. It was attempted to transform the resulting bromide into the corresponding magnesium bromide for the planned reaction with *D*-**125**. With the Wurtz coupling of the C_{15} bromide being the major product, it was concluded that the addition to *D*-**125** failed. In previous work in our group a α-sulfonyl carbanion has been added to an aldehyde. The sulfone was prepared and

reacted with *D*-**125**. The addition resulted in a mixture of four isomers of the 4-sulfonyl-3-hydroxyl-2-amine. These were separated, desulfonated and deprotected to give crucigasterin 277, and thereby confirming the suggested structure.

Paper IV describes the synthesis of obscuraminol A, a sphingolipid. Instead of using the chiral pool approach, we planned a strategy utilizing an asymmetric nitroaldol reaction (Henry reaction). The corresponding C_{15} alcohol was made according to published procedures starting with commercially available EPA. Homologation gave a C_{16} aldehyde. This aldehyde was subjected to nitroethane, in the presence of base, and a chiral proline-derived catalyst. This resulted in a nitro alcohol, which was reduced using SmI_2 to give the sphingolipid obscuraminol A.

Sammendrag

Artikkel I beskriver totalsyntesen av mucosin, en C_{20} fettsyre, isolert fra middelhavssvampen, *Reniera mucosa*. Syntesen startet med en tre-trinns syntese av et kjent *meso*-keton. Dette *meso*-ketonet ble omdannet til en optisk aktiv β-ketoester, også kjent fra litteraturen. I en tretten trinns sekvens, som blant annet inkluderer en kobber-katalysert krysskobling og kombinert aluminering/halodemetallering/Negishi krysskobling, ble den foreslåtte strukturen av (-)-mucosin syntetisert. Sammenligning av spektrale data fra litteraturen avdekket uoverensstemmelser. Røntgenkrystallografi av et mellomprodukt viste at den foreslåtte strukturen mest sannsynlig ikke kan være korrekt.

Artikkel II beskriver den totale syntese av diastereomeren av den foreslåtte strukturen til mucosin. Vi dro nytte av den samme ketoesteren fra artikkel I, for å lage en α, β-umettede ester. Denne ble benyttet i konjugert addisjon for å skape et invertert motiv. Ved denne strategien klargjorde vi anti-diastereomeren av den foreslåtte strukturen for mucosin. Dette viser at naturproduktet mest

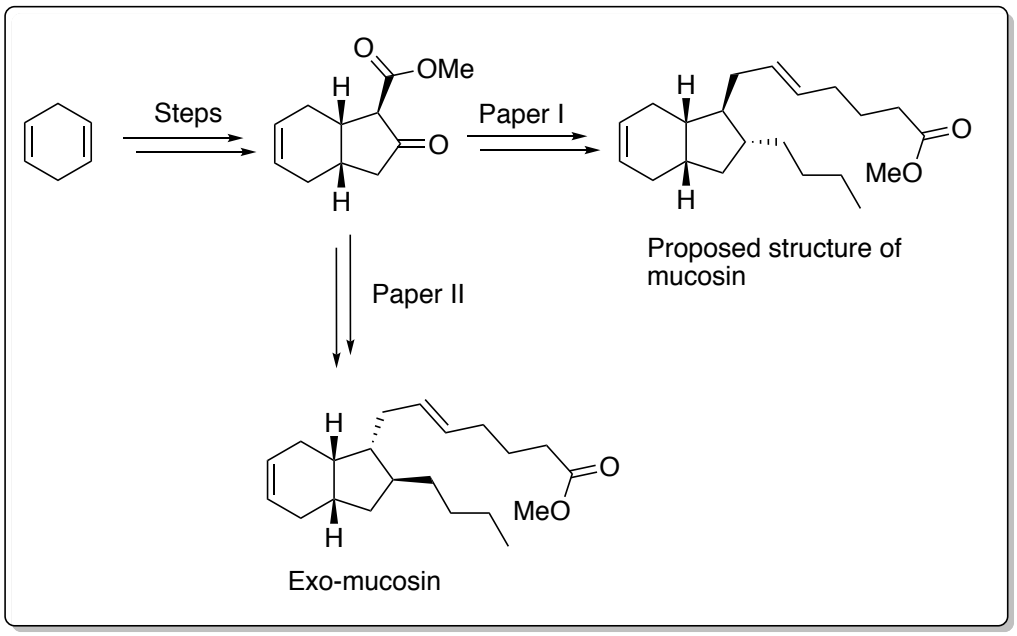
sannsynlig ikke inneholder en *cis*-fusjonert, bisyklisk kjerne. Dette fordi det fremstilte materialet ikke samsvarte med de rapporterte data.

Artikkel III beskriver syntesen av crucigasterin 277, et sfingolipid isolert fra et kappedyr fra middelhavsområdet, *Pseudomonas crucigaster*. Forbindelsen ble fremstilt via en "chiral pool" tilnærming, der aminoalkohol-delen ble hentet fra *D*-alaninal. Et umettet, allylisk C_{15}-bromid, fremstilt fra eikosapentaensyre (EPA) ved å følge litteraturprosedyrer ble anvendt som ω-fragment. Denne ble forsøkt omdannet til det tilsvarende allyliske Grignard-reagenset, etterfulgt av reaksjon med *D*-alaninal. Dette ga Wurtz-produkt av C_{15}-bromid som hovedprodukt. Gruppen vår har tidligere erfaringer med å benytte sulfoner i slike koblinger, og C_{15}-sulfonet ble laget fra C_{15}-bromidet. Dette sulfonet ble reagert med alaninal, og resulterte i en blanding av fire isomerer av sulfoyl hydroksylamin. Disse ble separert, desulfonert og avbeskyttet for å gi sfingolipidet crucigasterin 277. Dermed ble strukturen av crucigasterin 277 bekreftet.

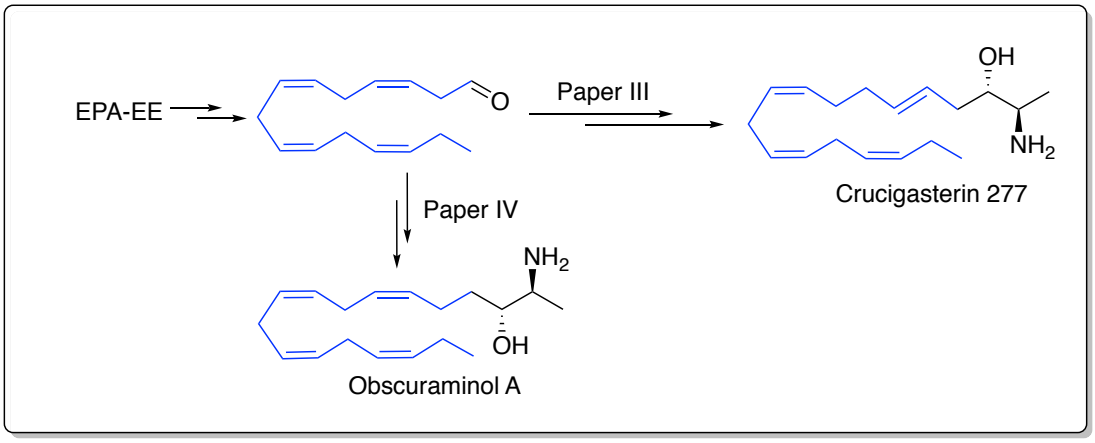
Artikkel IV beskriver syntesen av obscuraminol A, en sfingolipid med likheter til crucigasterin 277. I stedet for å bruke «chiral pool» tilnærmingen benyttet i Paper II, ble en asymmetrisk nitroaldol reaksjon benyttet. Mye av de samme reaksjonene som ble benyttet i foregående publikasjoner ("Paper II"), ble anvendt for å fremstille en C_{15} alkohol. Denne alkoholen ble homologert til et C_{16} aldehyd. Dette aldehydet ble reagert med nitroetan i nærvær av base og en kiral katalysator basert på prolin. Dette resulterte i en nitroalkohol, som ble redusert ved bruk av SmI_2 for å gi sfingolipidet obscuraminol A.

Graphical Abstracts

Paper I and II:



Paper III and IV:



Abbreviations

9-BBN	9-Borabicyclo[3.3.1]nonan
AA	Arachidonic acid
Ac	Acetyl
Aq	Aqueous
BINOL	1,1'-Bi-2-naphthol
BOC	tert-Butyloxycarbonyl protecting group
CBS	Corey-Bakshi-Shibata catalyst
CDI	Carbonyldiimidazole
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CM	Cross metathesis
CoA	Coenzyme A
COX	Cyclooxygenase
CSA	Camphorsulfonic acid
Cp	Cyclopentadienyl complex
Cy	Cyclohexyl
Cyp	Cytochrome P450 isoforms
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DET	Diethyl tartrate
DHA	Docosahexaenoic acid
$(DHQ)_2PHAL$	Hydroquinine 1,4-phthalazinediyl diether
DIBAL-H	Diisobutylaluminium hydride
DMAP	4-Dimethylaminopyridine
DME	Dimethoxyethane
DMF	Dimethylformamide
DMP	Dess–Martin periodinane
DMSO	Dimethyl sulfoxide
DPA	Docosapentaenoic acid
dr	Diastereomeric ratio
ee	Enantiomeric excess

EPA	Eicosapentaenoic acid
HMPA	Hexamethylphosphoramide
HWE	Horner–Wadsworth–Emmons reaction
Im	Imidazole
KHMDS	Potassium bis(trimethylsilyl)amide
LDA	Lithium diisopropylamide
LOX	Lipoxygenase
LT	Leukotriene
LX	Lipoxine
Ms	Methanesulfonyl
NCS	N-Chlorosuccinimide
p-Ts	Para-toluensulfonyl
PG	Prostaglandins
PGI	Prostacyclins
PUFA	Polyunsaturated fatty acid
RCM	Ring-closing metathesis
TBDMS/TBS	tert-Butyldimethylsilyl ether
TBDPS	tert-Butyldiphenylsilyl ether
TEA	Triethylamine
Tf	Trifluoromethanesulfonyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TMS	Trimethylsilyl ether
TX	Thromboxanes
Δ	Heat

1. Introduction

1.1 Natural products

Natural product chemistry is the study of chemical compounds produced by living organisms. Natural products are often divided into primary and secondary metabolites. The former are the compounds directly involved with normal growth, development and reproduction, thus being key components in maintaining normal physiological processes. In contrast, the secondary metabolites are not directly involved in these processes. While primary metabolites are found in a wide set of species, secondary are often restricted to only one or a small group of individual species or cells.

Currently, one third of clinically used drugs originate from natural products, either as native compounds or derivatives thereof.[1] These may span the space of simple compounds onto the complex protein structures. Typical examples include morphine (**1**), penicillin G (**2**), paclitaxel (**3**) and botulinum toxin A (**4**).

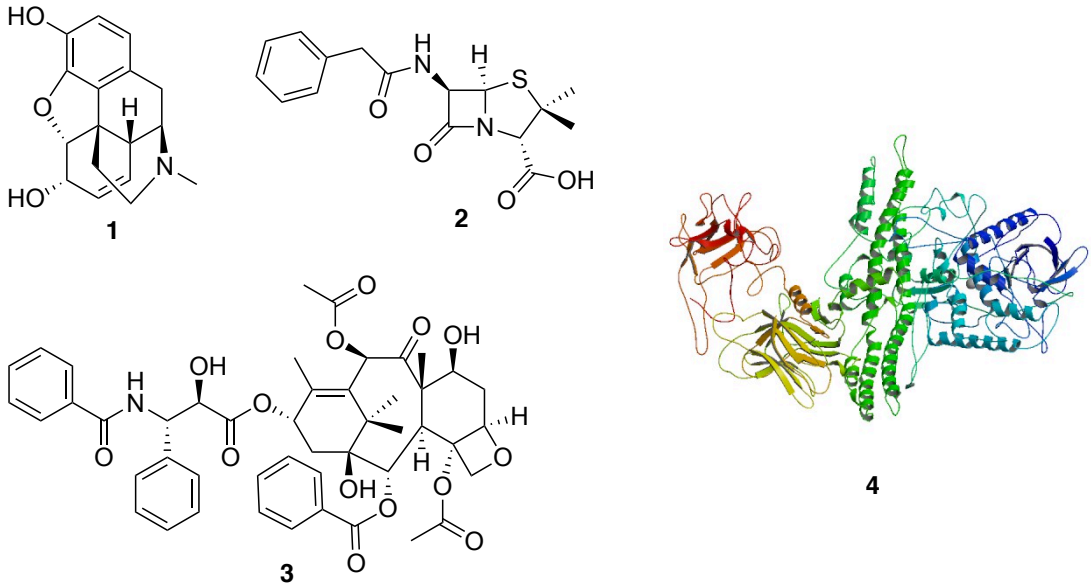


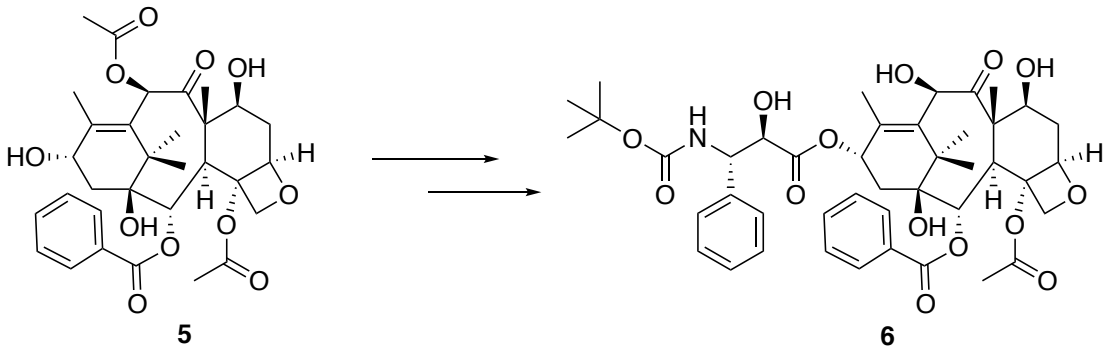
Figure 1 Structures of compounds 1-4. Botulinum toxin A structure from ref 2.

Some natural products are produced for commercial purposes by extraction from their natural source. For other natural products, mass production via extraction can be unsustainable (*e.g.* species listed on the multilateral treaty to protect endangered plants and animals, CITES[3]) or the originating source does not yield sufficient amounts. In these instances, laboratory synthesis of the

natural products will often be the only possibility to obtain sufficient amounts for testing and marketing of the compounds. In most cases, a total synthesis of complex molecules is too expensive on any practical or industrial scale. Possible solutions are semisyntheses or syntheses of structurally simpler analogues. As natural products may only be viewed as prototypical drug candidates, synthetic analogues can be designed to yield higher efficacy than the natural product itself.[4]

Morphine (**1**) is produced commercially by extraction from opium poppy,[5] penicillin G (**2**) from *Penicillium* fungi[6] and botulinum toxins from *Clostridium botulinum,*[7] the latter two by fermentation.

The industrial production of taxol (**3**) is a semisynthesis from the natural product baccatin III (**5**). An analogue, taxotere (**6**), is also approved for clinical use. Taxotere is also prepared via a semisynthetic route.[8]



Scheme 1 Semisynthetic route to 6 from the natural product 5.

On the other hand, latanoprost (**7**), a prostaglandin $PGF_{2\alpha}$-analogue, is mass-produced synthetically using a protocol developed by Corey *et al.*[9,10]

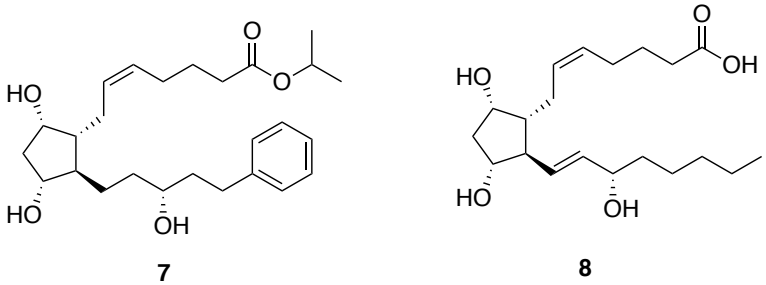


Figure 2 Structure of compounds 7 and 8.

One of the main groups of natural products is the lipids, including steroids and other terpenes, sphingolipids, phospholipids, fatty acids and related structures such as eicosanoids. Several lipids are found as component of cell membranes and used as energy storage, but from a chemist's point of view, the bioactive lipids are of greater interest.

1.1.1 Eicosanoids:

The eicosanoids are signaling molecules derived from polyunsaturated fatty acids (PUFAs) by enzymatic or non-enzymatic oxidations. As the name indicates, they have 20 carbon units in length, like arachidonic acid (AA, **9**) and eicosapentaenoic acid (EPA, **10**). The eicosanoids are important in diverse physiological and pathological systems, such as inflammation, regulation of cell growth, controlling blood pressure *etc*.

There are three main enzymatic pathways for generation of the oxygenated PUFA metabolites governed by 1) cyclooxygenase (COX 1 and COX 2), 2) lipoxygenase (LOX) and 3) cytochrome P450 (CYP) isoforms.[11] Important metabolites biosynthesized via this route compromise leukotrienes (LT), prostaglandins (PG), including the prostacyclins (PGI), and thromboxanes (TX).[12] Some of the pathways are illustrated in Figure 3.[13]

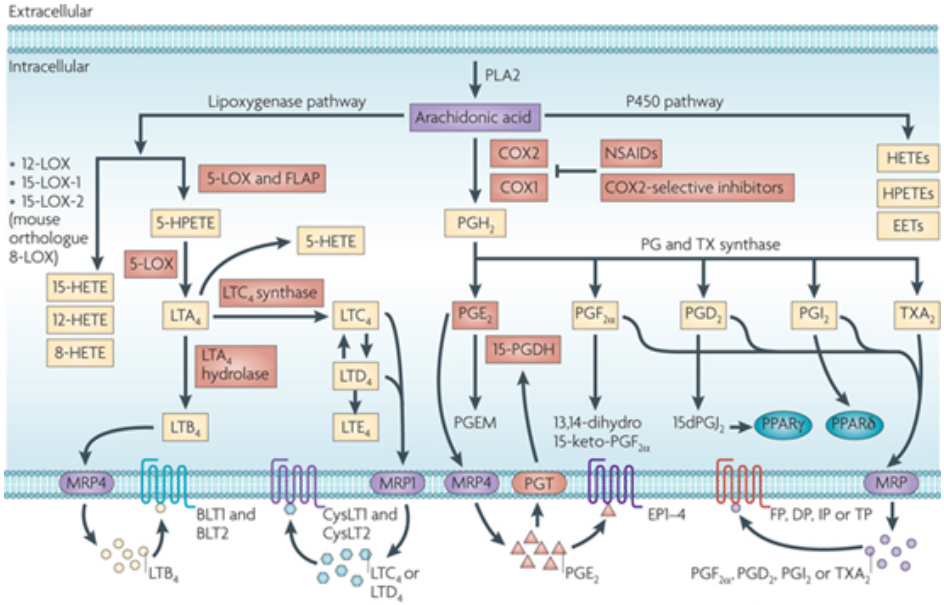


Figure 3 Biosynthesis of prostanoids and leukotrienes (the illustration is from ref. [14])

The prostaglandins were originally found from the prostate glands and seminal plasma of humans.[15-17] Thromboxanes are isolated from platelets (thrombocytes) and leukotrienes from leukocytes, explaining their names. Despite their names, these eicosanoids are now known to be found in almost every tissue in animals.

Structurally, the prostaglandins contain 20 carbon atoms, as with arachidonic acid (**9**). In contrast to the non-cyclic structure of **9**, the prostaglandins contain a cyclopentane moiety and the carbon framework have undergone varying degrees of oxidation. Thromboxanes are similar, but have a 1,2 disubstituted tetrahydropyran motif. Leukotrienes, however, are non-cyclic.

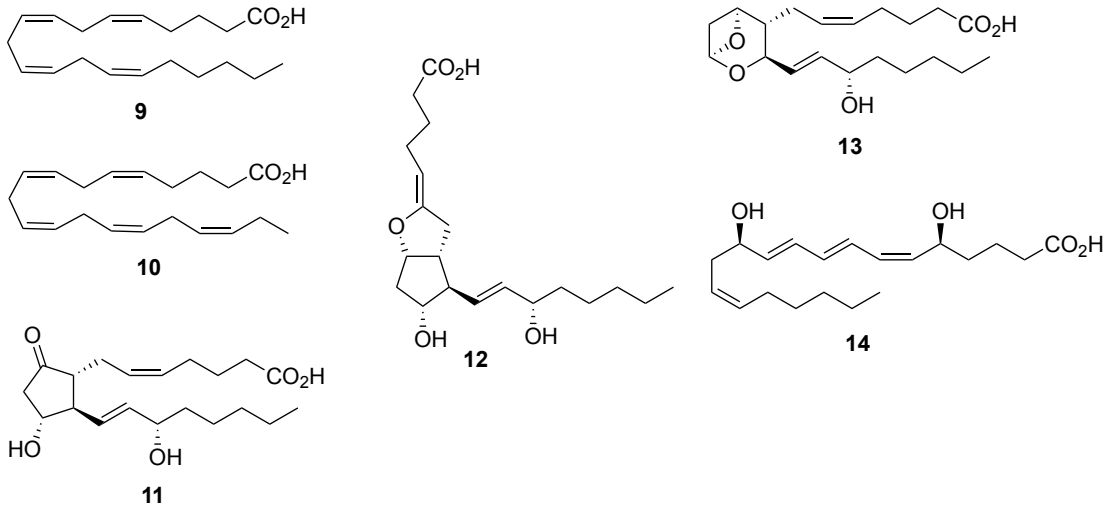


Figure 4 The structure of some eicosanoids.

The different eicosanoids are denoted with an abbreviated name, suffixed by a letter and a number to signify the number of double bonds. Examples include $PGF_{2\alpha}$ (prostaglandin $F_{2\alpha}$, **8**), PGE_2 (prostaglandin E_2, **11**), PGI_2 (prostaglandin I_2, **12**), TXA_2 (thromboxane A_2, **13**) and LTB_4 (leukotriene B_4, **14**). The mentioned structures are shown in Figure 4.

The oxygenated PUFA metabolites, are important in regulation of a wide range of physiological and pathological processes including immune responses, inflammation, cell proliferation and angiogenesis.[12]

It is known that a given prostaglandin may have different effects in different tissues depending on the prostaglandin binding receptors expressed in that particular tissue. They can act both as paracrine (locally active) and autocrine (acting on the same cell from which it is synthesized) factors. The thromboxanes are mediators of vasoconstriction, and the prostacyclins are active in the resolution phase of inflammation.

The prostaglandins are synthesized in the early stage of inflammation, and are essential for controlling blood flow and stimulating neutrophil influx to the tissue. Other oxygenated lipid mediators take over during the later stages of inflammation.[18]

It was observed by von Euler that the prostaglandins could affect the contraction and relaxation of muscles.[19] In 1971 it was also discovered that aspirin-like drugs could inhibit the biosynthesis of these structures.[20]

Several prostanoids are pharmaceutical products. PGE_2 (**11**) is sold under different brand names such as Cervidil, Prepidil and Prostin E_2 and is used to induce labor. $PGF_{2\alpha}$ (**8**), pharmaceutically termed dinoprost, is used for the same purpose, and also as abortifacient.[21] Latanoprost (**7**) is used to control the progression of glaucoma.[22] PGI_2 (**12**), marketed as epoprostenol, reduces blood pressure and inhibits platelet aggregation.

1.1.2 Sphingolipids

The vicinal amino alcohol motif is found in fatty acid derived entities known as sphingoid bases. The first compound of this class to be discovered was sphingosine, originally isolated from brain extract in the 1870s, and structurally elucidated in 1947.[23,24] The related structures are referred to as sphingolipids.

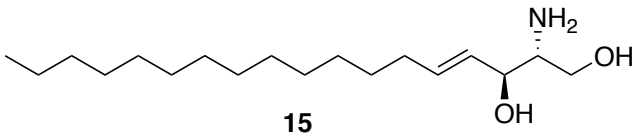


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Figure 5 Structure of sphingosine

Biosynthetically, sphingosine and related sphingolipids, are synthesized *de novo* from palmitoyl-CoA and *L*-serine (*L*-**16**) by the enzyme serine palmitoyltransferase.[25] It has been shown that other fatty acyl-CoA and alternative amino acids can bind to the active sites.[26,27]

The sphingoid bases are often found bonded to other functionalities, such as phosphocholine, sugars moieties, fatty acid residues *etc*. This gives rise to many subclasses of sphingolipids include ceramides, sphingomyelins and cerebrosides (see Figure 6).

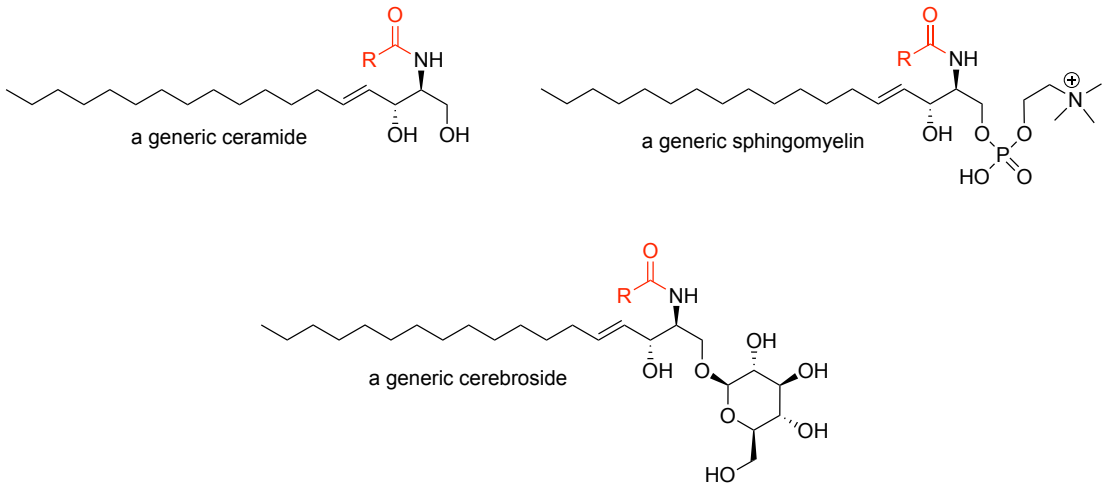


Figure 6 Examples of sphingolipids

The sphingolipids have important roles in signal transmission and cell recognition. Several known disorders of sphingolipid metabolism are known, and many of these are fatal by early age, as they primarily affect the central nervous system,[28] *e.g.* Niemann–Pick disease.[29]

Marine organisms have been found to be a rich source of sphingolipids. In contrast to the common sphingolipids, these are lacking the hydroxy group at C-1, hence possibly being derived from *D*- or *L*-alanine (**17**). Examples includes spisulosine (**18**),[30] the obscuraminols,[31] the crucigasterins,[32,33] the xestoaminols,[34] the clavaminols[35,36] and the halaminols.[37]

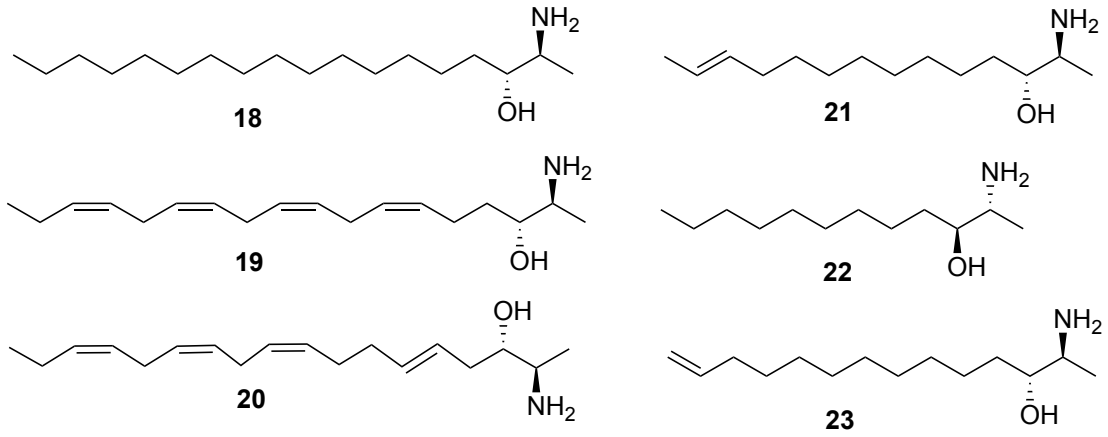


Figure 7 Vicinal amino alcohols isolated from marine organisms

Spisulosine (**18**), isolated from the clam *Spisula polynyma*,[30] is known to induce prostate tumor PC-3 and LNCaP cell death. The mechanism is believed to be linked to *de novo* synthesis of ceramide.[38] The compound was included in clinical trials as a lead candidate, but discontinued after the phase I studies.[39]

Obscuraminol A (**19**), isolated from *Pseudodistoma obscurum*, is mildly cytotoxic.[31] A similar natural product, crucigasterin 277 (**20**), isolated from *Pseudomonas crucigaster,* exhibit antimicrobial activity against *Bacillus subtilis* and cytotoxic activity against mouse lymphocytic leukemia L1210 cells.[33]

The xestoaminols were isolated from Fiji sponges of *Xestospongia* sp. Xestoaminol A (**21**) showed potent antimicrobial activity against several Gram-positive bacteria.

Clavaminols show cytotoxic activity on tumor cell lines, with clavaminol A (**22**) being the most potent, leading to cell death through activation of the apoptotic machinery.[35,36]

The halaminols were isolated from the antifungal extract of a tropical marine sponge of the genus *Haliclona*.[37] Halaminol A (**23**) is shown to have a role in preventing colonization of larvae.[40]

1.2 Organic synthesis as a tool for structural elucidation

In the 19th and early 20th century, structural elucidation depended almost exclusively on chemical synthesis, predominantly in the form of chemical degradation and derivatization reactions. For the testing of a structural hypothesis, grams of material were often needed. The development of better analytical instrumentation has revolutionized the field of structural elucidation with NMR, MS, various chromatographic systems *etc*, often in a combination of two or more of these methods. Often one can today get all the information needed for a structural elucidation from only milligrams of material.

It took more than one hundred years from the time morphine (**1**) was isolated by Sertürner,[41] until Sir Robert Robinson's proposed structure[42] was confirmed by Gates.[43] With present day analytical instrumentation, it would be possible to elucidate the structure in matter of hours or days.

Structural misassignments, however, do still occur. Nicolaou and Snyder reported that more than 300 compound were assigned incorrect structures between 1990 and 2004.[44] These misassignments include, but are not limited to, incorrect absolute stereochemistry. In such cases, total synthesis as a tool for verified or disproving a proposed structure, has proved to be as important as ever.

Examples of structures that have been revised, and later confirmed by total synthesis include the complex structure bryostatin 3. The compound was first found in 1983.[45] The structure was proved to be wrong, and a new structure was proposed.[46] Nine years later, the corrected proposal was verified by synthesis.[47]

However, misassignments are not limited to complex, large structures. The relatively simple natural product ascidiatrienolide A was isolated in 1989.[48] The proposed structure was revised and verified by total synthesis four years later.[49]

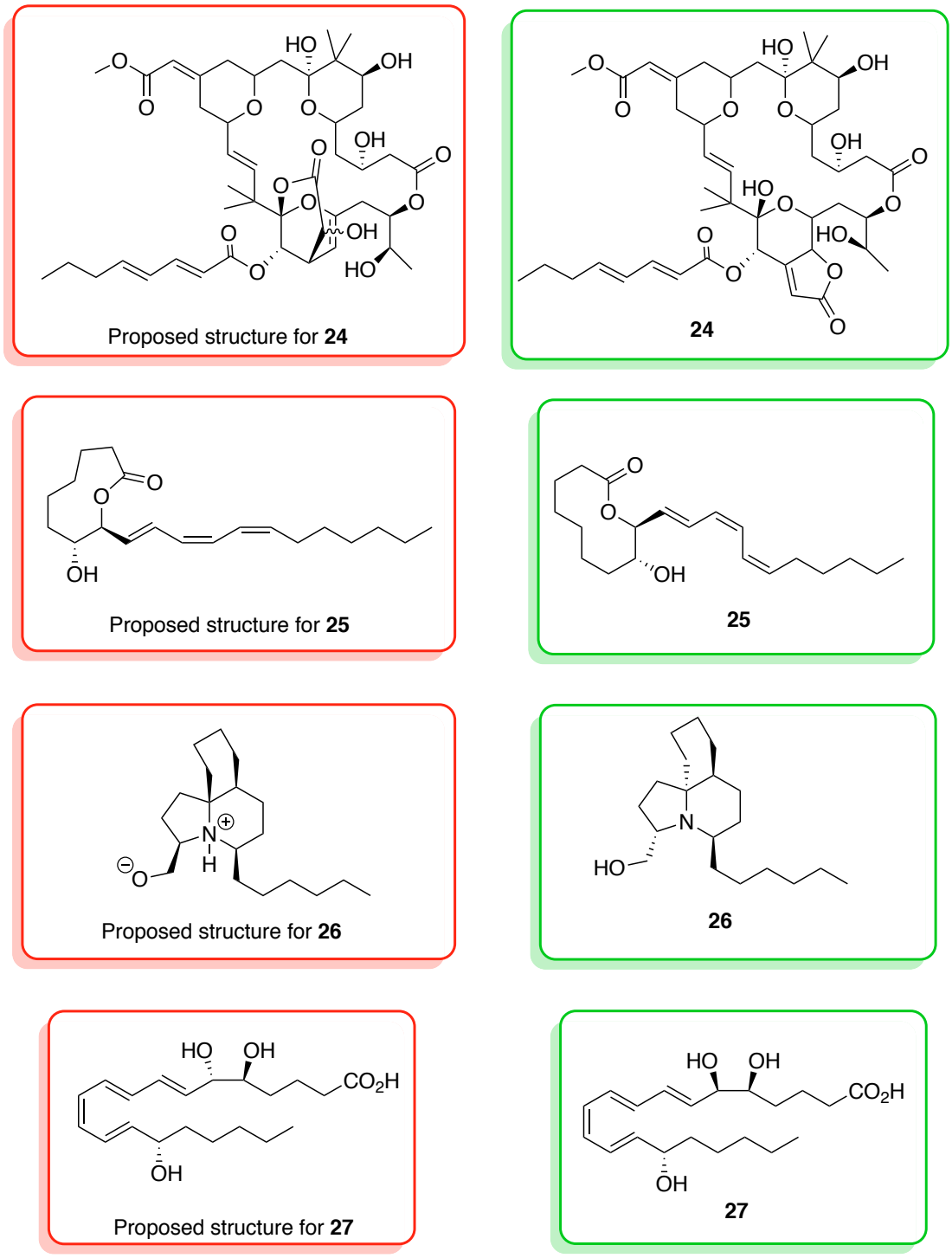


Figure 8 Proposed structures in red frame and revised, verified structures in green.

Another example is the proposed structure of lepadiformine.[50] The proposed structure was later synthesized, and proved wrong. The same authors proposed a revised structure, which they also verified by total synthesis and comparison of data.[51]

The structure of lipoxin A_4,[52,53] was assigned as (5*S*,6*S*,15*S*) by the groups of Corey[54] and Adams,[55] but later proved to be (5*S*,6*R*,15*S*) by Serhan *et al*.[56] This was also confirmed by the fact that 6*S*-epimer does not share the biological activity of natural LXA_4.

Conclusive evidence for the structural assignment of a complex natural product is to preform a matching experiment between the authentic and the synthetic material. The Hansen group has done this on several synthesized lipid mediators.[57-59]

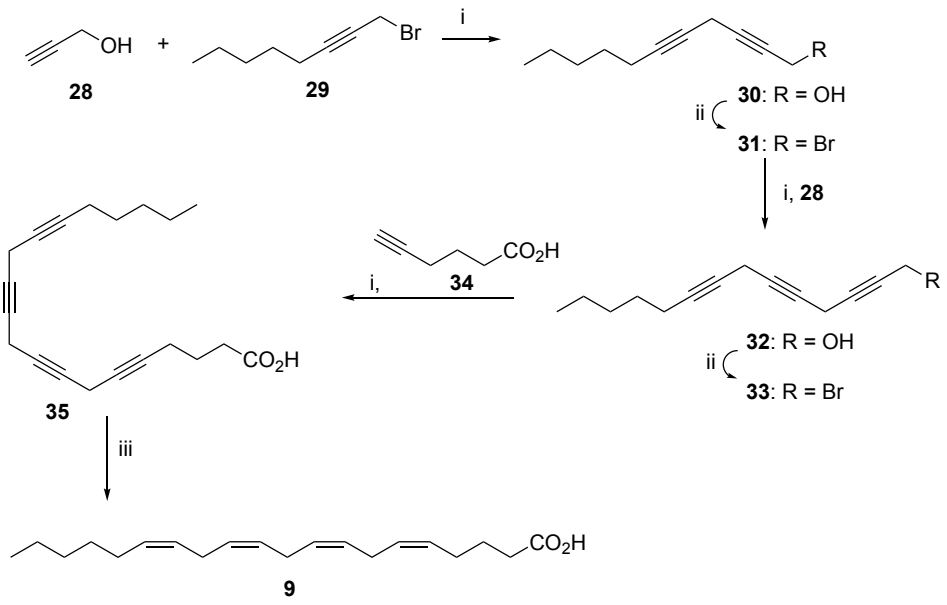
2. General approaches towards synthesis of fatty acids and derived structures

In the PUFAs and their derived structures a common feature found is the presence of multiple double bonds, often skipped *Z*-olefins. In the published strategies for the synthesis of these structures, several different approaches have been utilized. In the following section a selection of some the most frequently applied methods will be discussed.

2.1 Acetylenic approach

One of the most utilized strategies for syntheses of polyenes is through partial reduction of acetylenes. Due to the acidity of the acetylene protons, metal acetylides can easily be formed, and subsequently reacted with a suitable electrophile.

One early example of this is the synthesis of arachidonic acid based on alkylation of terminal alkynes with propagylic electrophiles in the presence of copper (I) salts via Grignard acetylide intermediates.[60] See Scheme 2.

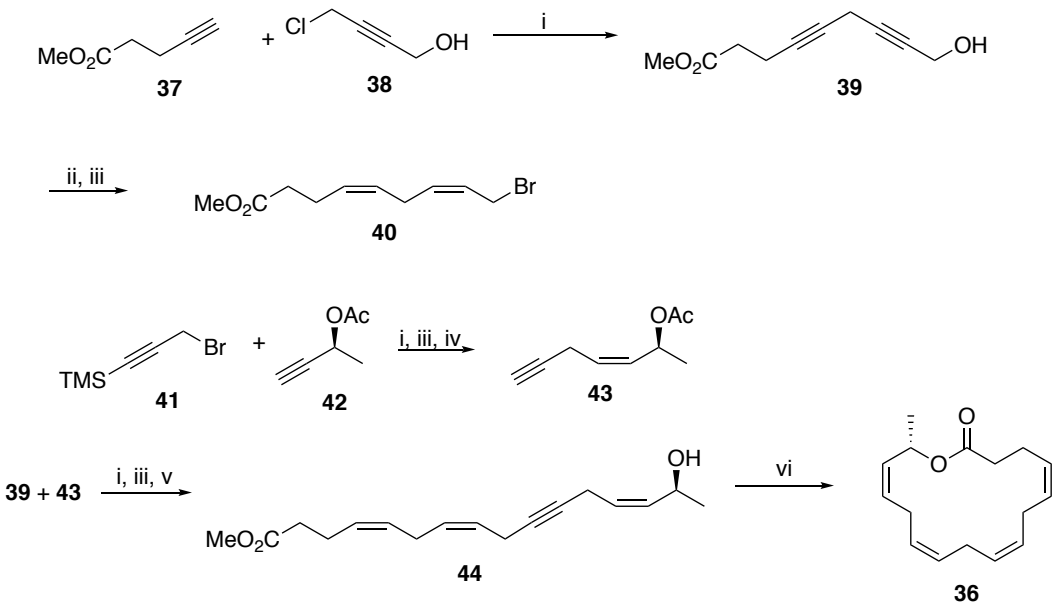


Scheme 2 Synthesis of arachidonic acid.[60] Reagents and conditions: (i) EtMgBr, THF, CuCl; (ii) PBr_3, Et_2O; (iii) H_2, Lindlar catalyst.

More recently, modified protocols employ copper (I) catalyzed cross-coupling of primary propagyl halides (or tosylates) with terminal alkynes in presence of weak base (Na_2CO_3 or Cs_2CO_3) and the constellation of NaI/*n*-Bu_4NCl. In contrast to the protocols of Osbond *et al*., involving high temperature, the more recent modifications make it possible to perform the reaction at room temperature.[61,62]

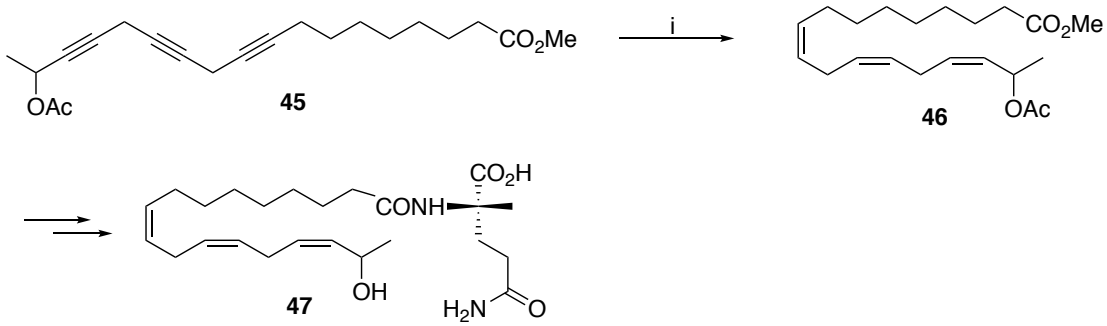
Hansen and Stenstrøm used the latter methodology in their synthesis of (-)-aplyolide A (**36**) , as depicted in Scheme 3.[63]

The remaining step is the partial reduction of the polyyne backbone. If the thermodynamically favored *E*-geometry is preferred, metallic sodium dissolved in cold ammonia may be an option. For the preparation of *Z*-alkenes, palladium, and other transition metals belonging to group 10 are frequently used for the purpose of catalytic hydrogenation.[64,65] One example of such is the Lindlar catalyst, which consists of palladium on a $CaCO_3$ support where the reactivity of the metal center has been modified by addition of *e.g.* $Pb(OAc)_2$, PbO and quinoline.[66] An example of the use of the Lindlar catalyst for partial reduction can be seen in Scheme 2.



Scheme 3 Synthesis of (-)-aplyolide A (36).[63] Reagents and conditions: (i) NaI, CuI, K_2CO_3, DMF; (ii) CBr_4, Ph_3P, CH_2Cl_2; (iii) P-2 Ni, H_2, EtOH; (iv) Bu_4NF, DMF; (v) LiOH, MeOH, H_2O; (vi) 2,2'-dipyridyl disulfide, PPh_3, toluene, Δ.

In addition to palladium, nickel catalysis is also being used extensively, *e.g.* Brown's highly stereospecific catalyst, P-2 nickel.[63,67,68] The use of P-2 nickel is depicted in Scheme 4.



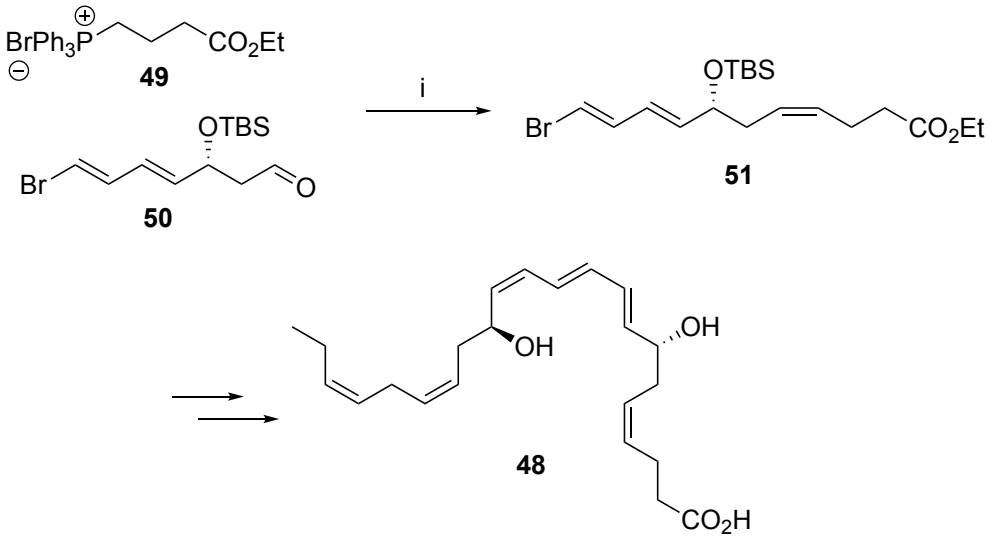
Scheme 4 The use of P-2 Ni catalyst in a formal synthesis of volicitin.[68] Reagents and conditions: (i) $Ni(OAc)_2$, $NaBH_4$, EtOH, H_2.

One of the major drawbacks of this strategy is the possibility of over-reduction and isomerization encountered with the reduction of polyynes.

2.2 Olefination strategy

Wittig reactions are among the most used transformations to construct the double bonds. To construct *Z*-alkenes, as usually found in naturally occurring fatty acid derivatives, unstabilized ylides are reacted with an aldehyde or a

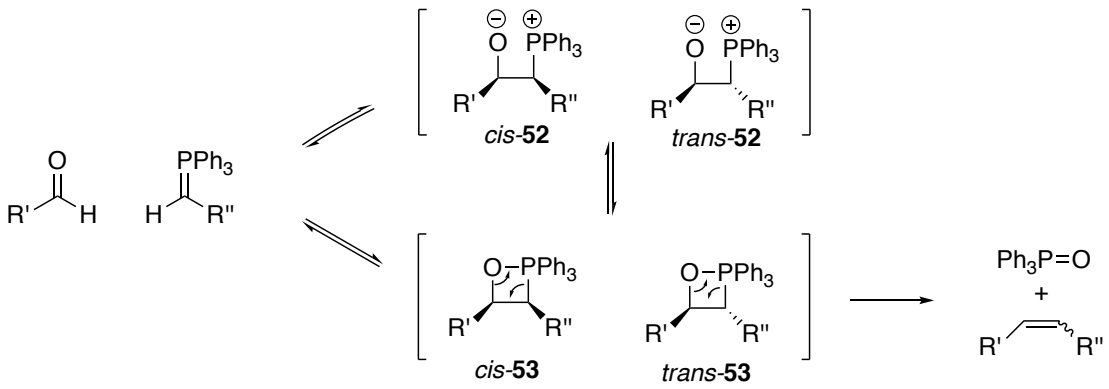
ketone. A *Z*-selective Wittig was employed by Tungen *et al.* in their stereoselective synthesis of maresin 1 (**48**), as depicted in Scheme 5.[59]



Scheme 5 The *Z*-selective Wittig employed by Tungen *et al.* in their stereoselective synthesis of maresin 1 (48).[59] Reagents and conditions: (i) NaHMDS, HMPA, THF, -78^{0}C.

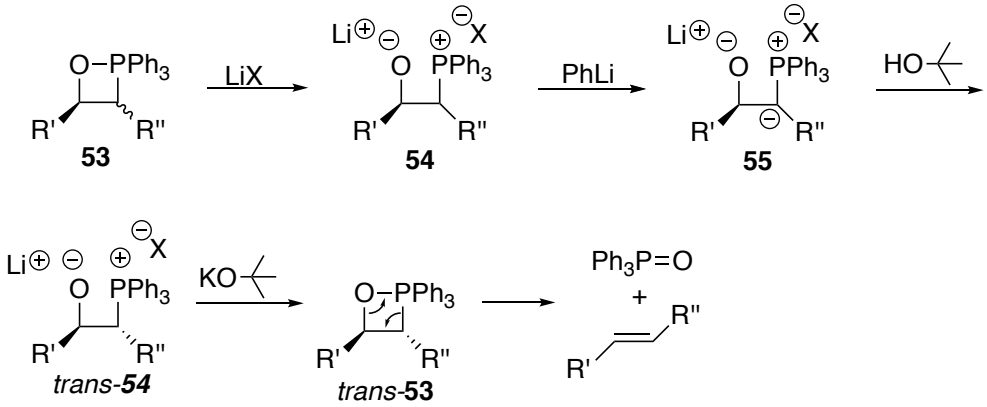
Wittig reactions can provide high stereoselectivity under the right conditions: low temperature, high dilution and absence of lithium can enhance the formation of the *Z*-isomer.[69] In contrast, it has been reported that excess of lithium salt during the addition step give the *E*-isomer as the major product often formed in a ratio larger than 9:1. This is referred to as the Schlosser-Wittig modification.[70] An alternative of this was published by Schlosser and co-workers in 2003.[71]

Looking into the detailed reaction mechanism, as depicted in Scheme 7, can make a rationale for this. Under Wittig reaction conditions, both the *cis*- and *trans*-betaine (**52**) will be formed. *Cis*-betaine is the kinetic product, and hence formed faster while *trans*-betaine is the thermodynamic product. The ring closes to form the oxaphosphetane (**53**), which then collapses into the alkene and triphenylphosphine oxide. The geometry of the oxaphosphetane decides the geometry of the resulting alkene.



Scheme 6 Wittig reaction mechanism

Lithium salts affect the betaine, so that it will not close to the oxaphosphetane (**53**). These lithiobetaines (**54**) are quite stable, and can be deprotonated using PhLi to give **55**. By using a sterically hindered proton donor, *e.g. tert*-butanol, the *trans*-lithiobetaine is formed. Finally, a potassium source is added, resulting in *trans*-oxaphosphetane that eventually will give the *E*-alkene.

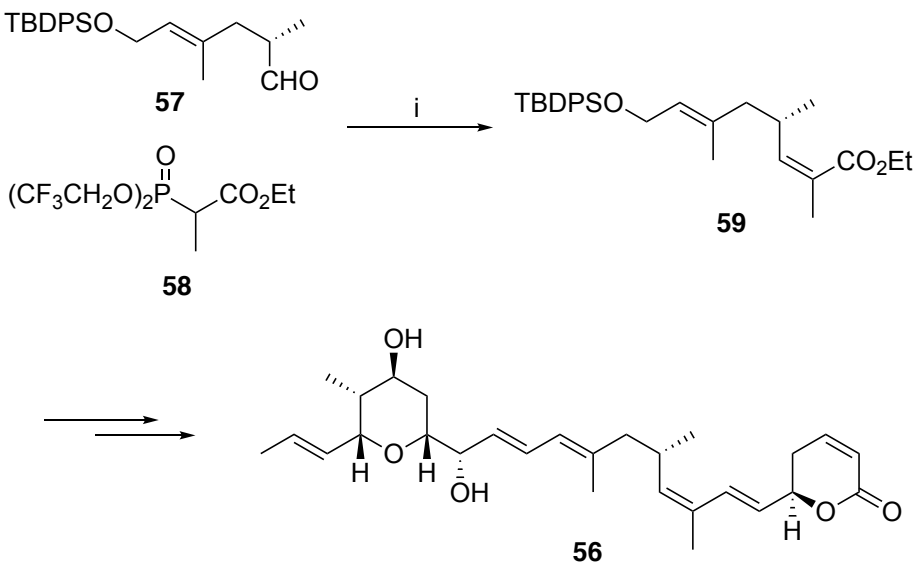


Scheme 7 Schlosser-Wittig mechanism

The Horner-Wadsworth-Emmons (HWE) reaction is another variety of the Wittig reaction. In this protocol, phosphonate-stabilized carbanions are employed. These, compared to triphenyl phosphonium ylide, are more nucleophilic and less basic, and can easily be removed by aqueous extraction. HWE is often employed to prepare *E*-alkenes.[72]

The Still-Gennari[73] and the Ando[74] modifications of HWE lead to *Z*-olefins. In strongly dissociating conditions and by employing phosphonates with electron-withdrawing groups (trifluorethyl is often used) the reaction can yield nearly

exclusively the *Z*-olefin. This in combination with KHMDS and 18-crown-6 has proven itself to be a very effective system. Kalesse *et al*. employed the Still-Genari modification in the synthesis of (+)-ratjadone (**56**), as depicted in Scheme 8.[75,76]



Scheme 8 Still-Genari modification employed in the synthesis of (+)-ratjadone.[76] Reagents and conditions: (i) THF, KHMDS, 18-crown-6

2.3 Olefin metathesis

Olefin metathesis can briefly be described as the interchange of an alkylidene moiety through the intermediacy of a transition metal catalyst.

The modern olefin metathesis reaction has found widespread applications in organic chemistry, including pharmaceutical industry in the synthesis of complex molecules.[77] Modern catalysts are often molybdenum(IV)- and tungsten(IV)-complexes or ruthenium(II) carbenoid complexes. The groups of Schrock,[78] Grubbs'[79,80] and Hoveyda[81,82] should be particularly mentioned regarding the development of several useful catalysts (some examples are shown in Figure 9).

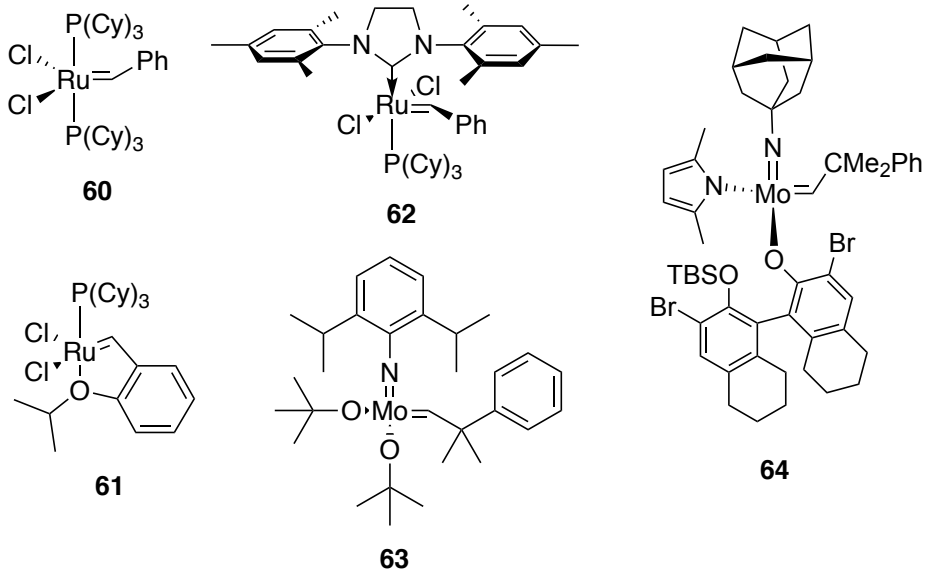
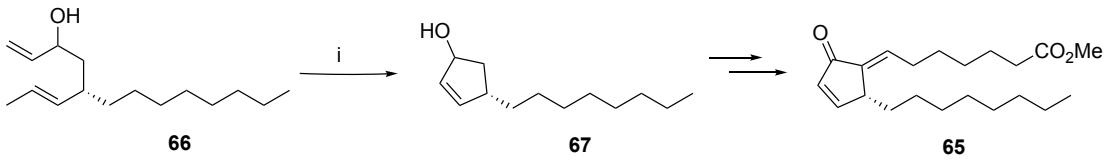


Figure 9 Catalysts used in olefin metathesis

Intramolecular ring-closing metathesis (RCM), which will give rise to unsaturated rings from alkylidenes, is perhaps the most frequently used type of olefin metathesis. For small to intermediate sized rings, only the *Z*-cycloalkene will be formed due to ring strain. Using Grubbs' first generation catalyst (**60**), RCM was employed in the synthesis of prostaglandin TEI 9826 (**65**), an anti-tumor agent, as depicted in Scheme 9.[83]

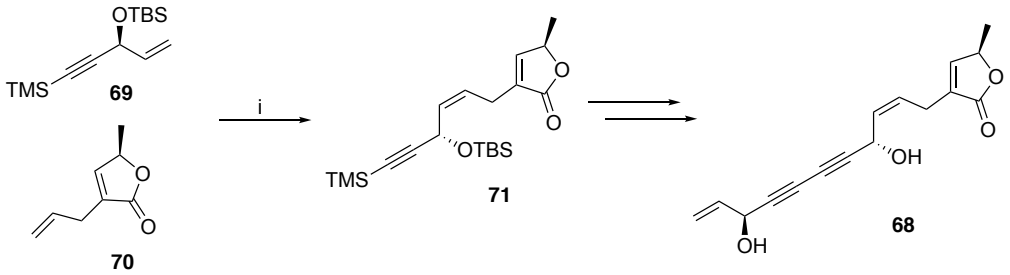


Scheme 9 RCM in the synthesis of prostaglandin TEI 9826 (65).[83] Reagents and conditions: (i) Grubb's catalyst (60).

For larger rings, cross metathesis (CM) thermodynamics may pose a problem. As metathesis reactions are controlled by thermodynamics, most catalysts will give *E*-alkenes as the major product.[84] This has been a limitation, especially for the use in fatty acid derived structures with methylene skipped *Z*-olefins.

Recently, several Ru-, Mo- and W-based catalysts that are able to produce *Z*-alkenes have been developed.[85-89] These catalysts must overcome the formation of the thermodynamically favored *E*-alkenes. It must also inhibit secondary

metathesis that can give isomerization to the more stable *E*-alkene. The catalytic systems reported so far, rely on steric differentiation between axial disposed ligands.[84] In the total synthesis of trocheliophorolide C (**68**), a Mo-catalyzed cross metathesis reaction was employed, see Scheme 10.[87]



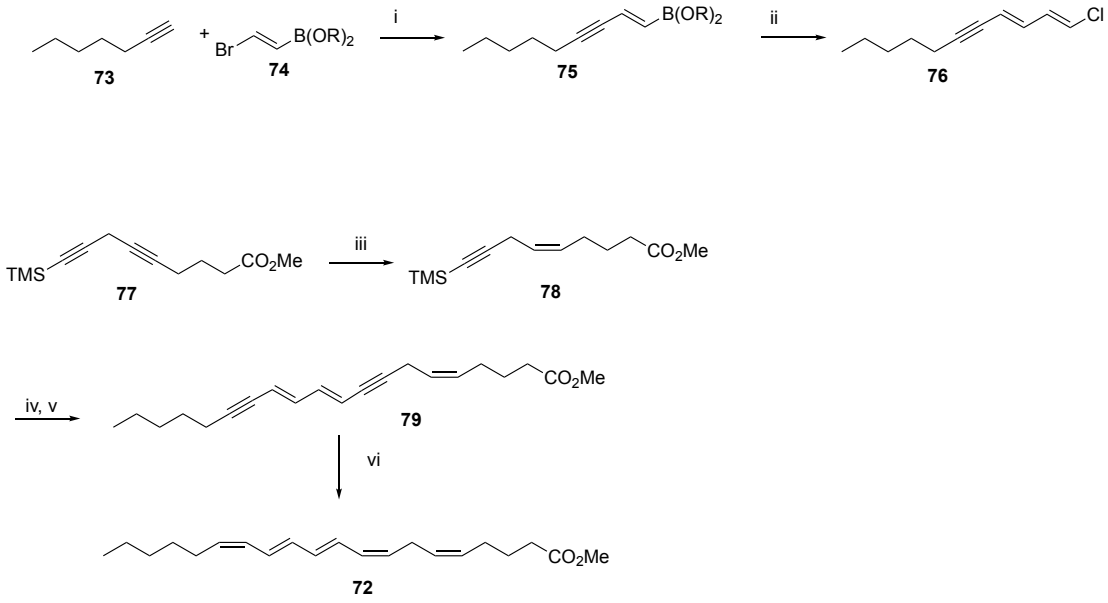
Scheme 10 Olefin metathesis in the synthesis of trocheliophorolide C.[87] Reagents and conditions: (i) 4.5 mol% catalyst 64, PhCl, 7.0 torr, 22^{0}C, 4h.

2.4 Metal-catalyzed cross-couplings

As described above, copper (I) catalyzed cross-couplings of primary propagyl halides can be used in the formation of poly-ynes. More recently, cross-coupling reactions catalyzed by transition metals from group 10, such as palladium and nickel, have nearly become routine in the laboratory. Examples of these include Heck,[90] Sonogashira,[91] Stille,[92,93] Suzuki[94] and Negishi cross-coupling reaction.[95]

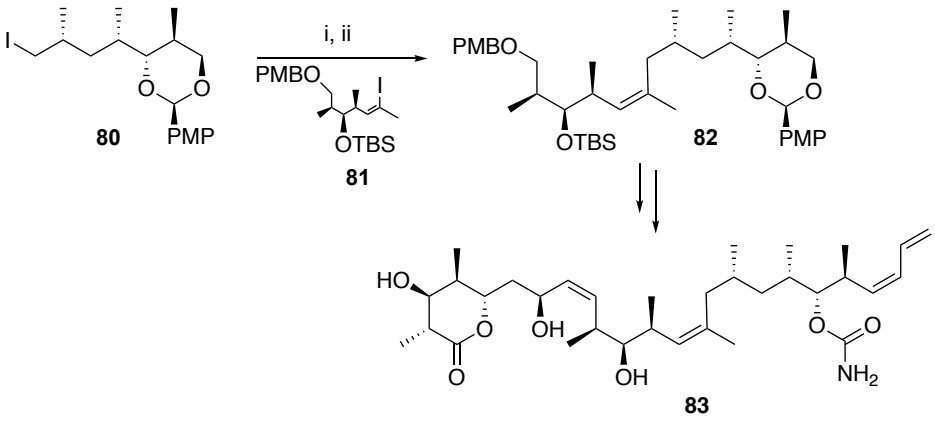
Mohamed and Hansen utilized both Sonogashira and Suzuki coupling reactions in their synthesis of the methyl ester of bosseopentaenoic acid (**72**).[96]

The cross-coupling reactions shown in Scheme 11 are between sp^2-sp^2 and sp-sp^2 hybridized carbon atoms, but it is also possible to use carbons of sp^3-hybridization. [58] An example of a Negish cross-coupling of sp^3-sp^2 hybridized carbons is depicted in Scheme 12.[97,98]



Scheme 11 Synthesis of methyl (5*Z*,8*Z*,10*E*,12*E*,14*Z*)-eicosapentaenoate.[96] Reagents and conditions: (i) $Pd(Ph_3P)_2Cl_2$ (5 mol %), CuI (10 mol %), piperidine, THF (82% yield); (ii) (a) 1 M aq. NaOH (b) *trans*-1,2-dichloroethene (4), $Pd(Ph_3P)_4$ (10 mol %), THF, Δ (70% yield); (iii) H_2, $Pd/CaCO_3$, quinoline, MeOH (71% yield); (iv) KF, MeOH, Δ; (v) 76, $Pd(Ph_3P)_2Cl_2$ (5 mol %), CuI (10 mol %), piperidine, THF (58% yield); (vi) Zn(Cu/Ag), TMSCl, MeOH, H_2O (88% yield).

Generally the geometry of the starting vinyl halide is retained during the course of cross-coupling, irrespective of whether it is *cis* or *trans* configured.



Scheme 12 Synthesis of (+)-Discodermolide (83).[98] Reagents and conditions: (i) *tert*-BuLi, $ZnCl_2$, Et_2O -78 to 25°C; (ii) $Pd(Ph_3)_4$ (5mol%) Et_2O, 25°C (66%).

2.5 Aldol type reactions

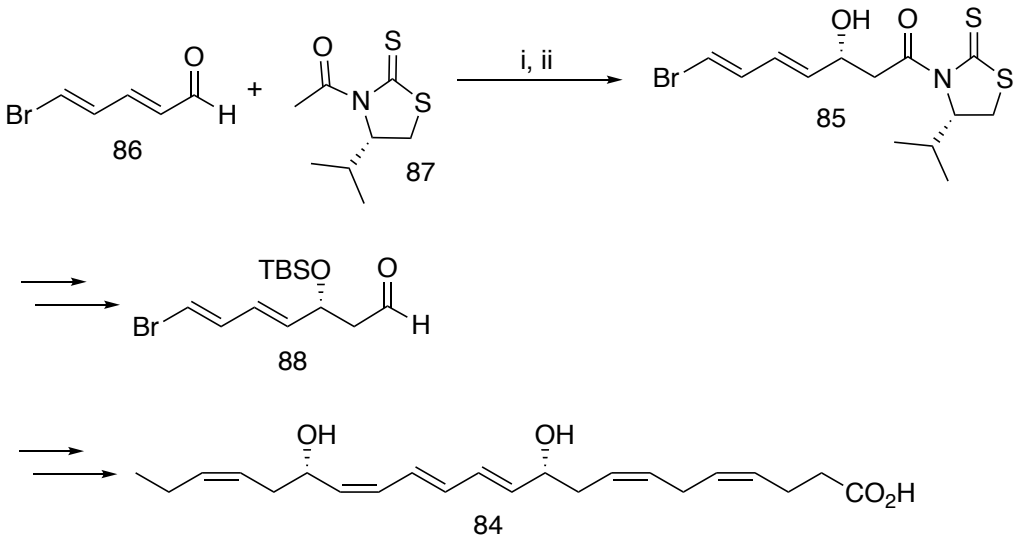
Since the aldol reaction was discovered in the last half of the 19th century,[99,100] as the reaction combining two aldehydes to form a β-hydroxy aldehyde, it has become one of the most utilized carbon-carbon bond forming reactions in organic synthesis. It is now textbook knowledge that the reaction can be

performed on aldehydes and ketones, and several related reactions, such as the Claisen condensation, the Henry reaction and the Dieckmann condensation, are also well known.

Aldol type reactions followed by dehydration, gives a structure consisting of an alkene conjugated to a carbonyl. Both this moiety and the β-hydroxy carbonyl group are commonly found in natural products. Biosynthetically, a type of Claisen condensation is used to prepare polyketides and the fatty acids.

During the last half of the 20th century, groups started to investigate the stereochemical outcome of the aldol reaction.[101,102] It was found that *E*-and *Z*-enolates give the *anti*-and the *syn*-diastereomer, respectively. More recently, several chiral auxiliaries have been developed, *e.g.* Evans' oxazolidinones[103] or Crimmins' thiazolidinethione, to give products with high steresoselectivity".[104]

Aursnes *et al.* used a thiazolidinethione auxiliary in their total synthesis of protectin D1 (**84**), as sketched in Scheme 13, to give a diastereomeric ratio of 15.3:1 of aldol product **85**.[57]



Scheme 13 The use of a thiazolidinethione auxiliary in the total synthesis of protectin D1 (84).[57] Reagents and conditions: (i) $TiCl_4$, CH_2Cl_2, -78°C; (ii) *i*-Pr_2NEt, 86%

2.5.1 Nitro aldol reactions

The nitro aldol condensation, also known as the Henry reaction, is the base catalyzed reaction of a nitro alkane and an aldehyde or ketone, resulting in β-

nitro alcohols. This functionality is quite handy as it can easily be converted to several other useful functionalities, *e.g.* reduction of the nitro group to the hydroxylamine or dehydration to nitroalkenes.

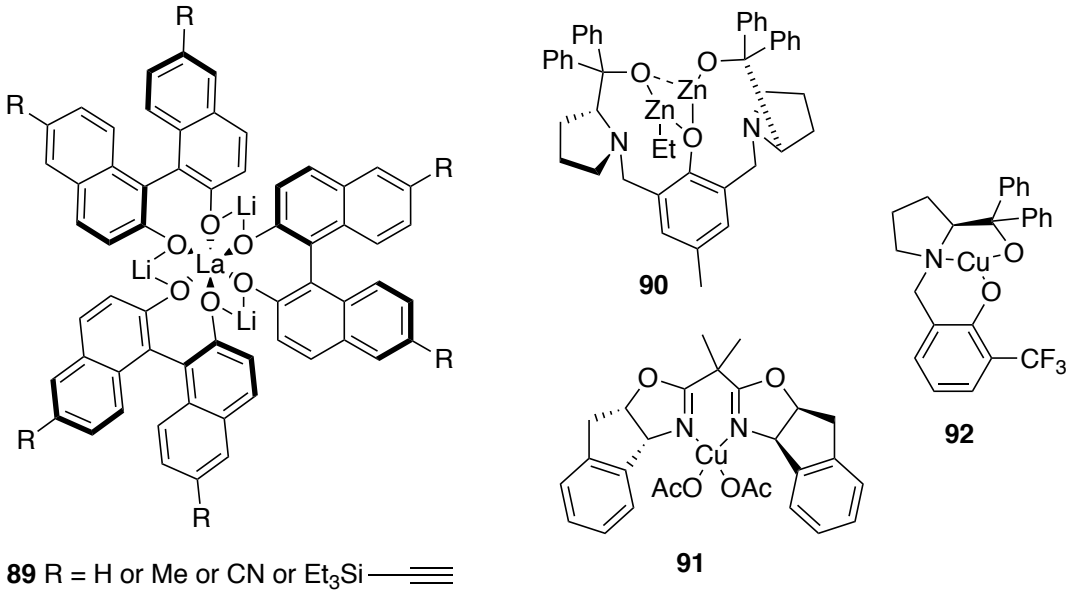


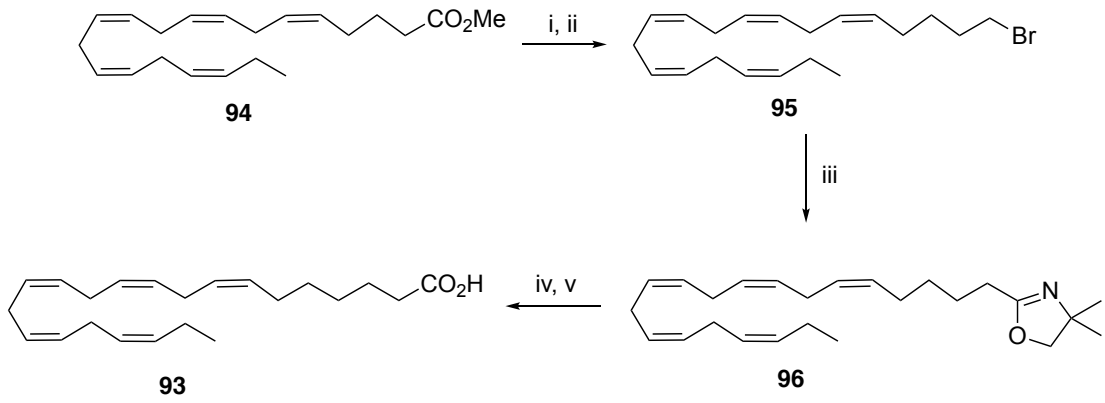
Figure 10 Structure of catalysts 89-92

Stereoselectivity of the Henry reaction is poor without modifications. This can be explained by the fact that the reaction is reversible and epimerization can easily occur on the carbon bearing the nitro group.[105] However, there are several literature procedures on enantioselective nitro aldol reactions. One way to achieve control is via silyl nitroates, which will give *anti*-diastereomers, or lithium nitroates, which will give *syn*-diastereomers.[105]

Since the first catalyst for enantioselective nitroaldol reactions, the Shibasaki catalyst, La-Li BINOL complex **89**, was reported in 1992,[106,107] several catalytic systems have been reported,[107-113] include dinuclear zinc complex **90**[114] and Cu-complexes **91** and **92**.[115,116] The majority of the published catalysts focus on *cis*-induction. Another limitation is that few protocols focus on long-chained aliphatic aldehydes.

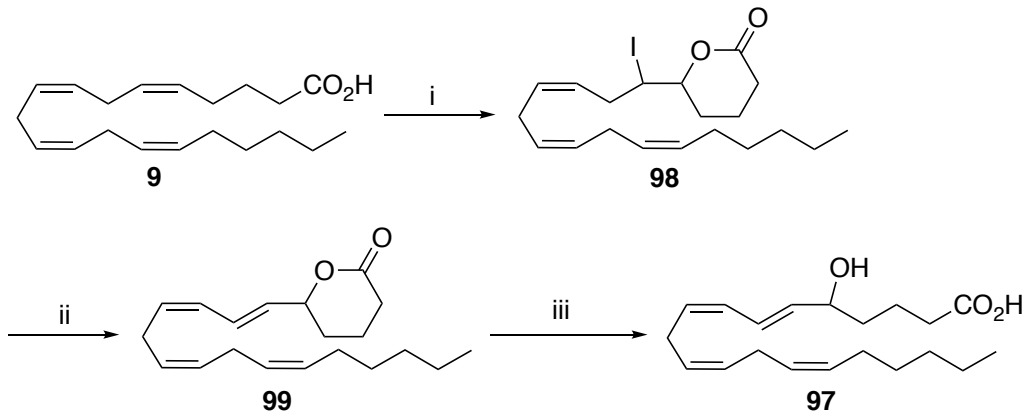
2.6 Hemi-synthesis of PUFAs and natural polyenes

An alternative approach to prepare fatty acid derived structures is to use available PUFAs (*i.e.* AA, EPA, DHA *etc.*) as starting material in the synthesis of polyenes. These can be elongated or degraded, but leaving some, or all of the double bonds unaltered. One example is the C_2 elongation of EPA in the synthesis of DPA (**93**) as depicted in Scheme 14.[117]



Scheme 14 DPA C_2 elongation of the methyl ester of EPA (94) in the synthesis of DPA (93).[117] Reagents and conditions: (i) $LiAlH_4$, 3 eq., ether, rt, 2 h; (ii) Ph_3PBr_2, 1.5 eq., CH_2Cl_2, rt, 30 min; (iii) 2,4,4-trimethyl-2-oxazoline, 1.5 eq., *n*-BuLi, 1.65 eq., −78°C, 2 h; (iv) HCl/MeOH, reflux, 12 h; (v) KOH/ethanol/water, 1.5 eq., rt, 16 h, then 1M HCl

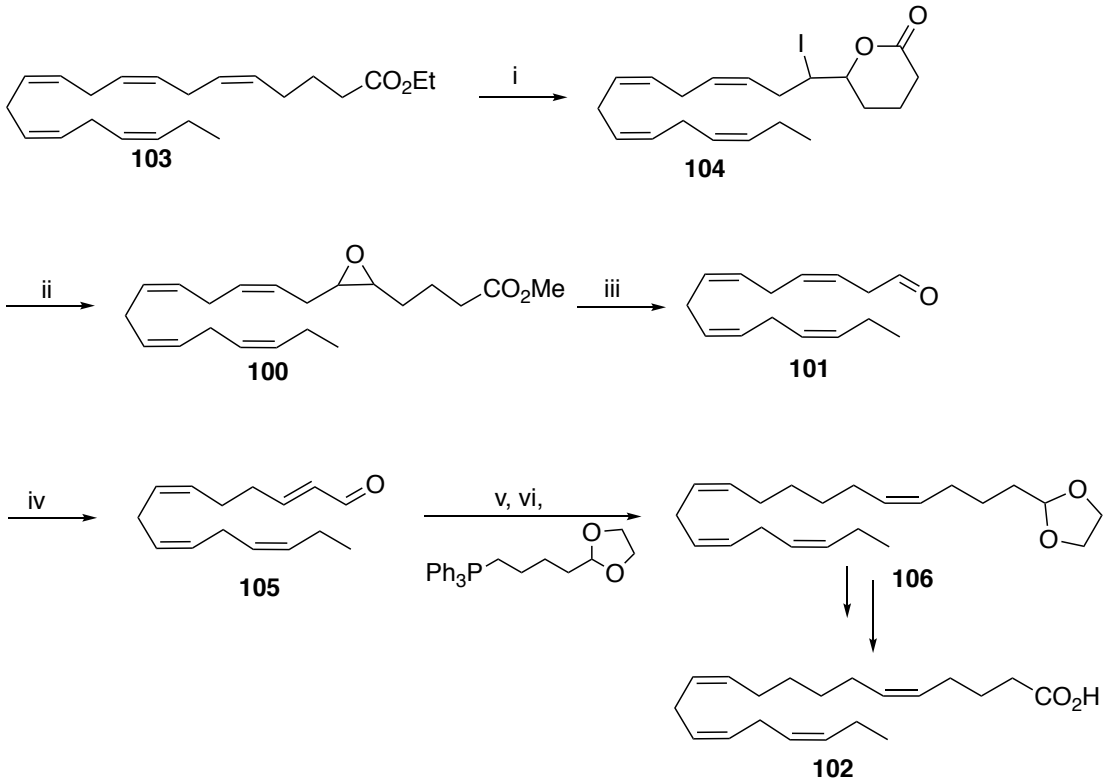
Corey *et al.* employed the iodolactonization protocol in their hemi-synthesis of (±)-5-HETE (**97**), as depicted in Scheme 15.[118] In this synthesis the iodolactone **98** was treated with DBU in benzene to eliminate iodine and provide the tetraene lactone (**99**). Transformation into the methyl ester of 5-HETE was achieved with Et_3N in MeOH. Basic hydrolysis yielded **97**.



Scheme 15 The synthesis of (±)-5-HETE (97).[118] Reagents and conditions: (i) KI, I_2, KHCO3, H_2O, THF, 0 C; (ii) DBU, PhH; (iii) Et3N, MeOH; (iv) LiOH, DME, H_2O

Treatment of iodolactones with potassium carbonate in methanol gives the corresponding epoxy methyl ester (*e.g*. compound ***100***). Oxidative cleavage, employing periodate, of the epoxide gives aldehydes (*e.g*. compound ***101***), as seen in Scheme 16.[119,120]

This methodology has been employed by Skattebøl and co-workers in several hemi-syntheses of natural products,[121-124] including the synthesis of juniperonic acid (**102**) by Vik *et al*. as represented in Scheme 16.[123] This synthesis also features a *Z*-selective Wittig reaction.

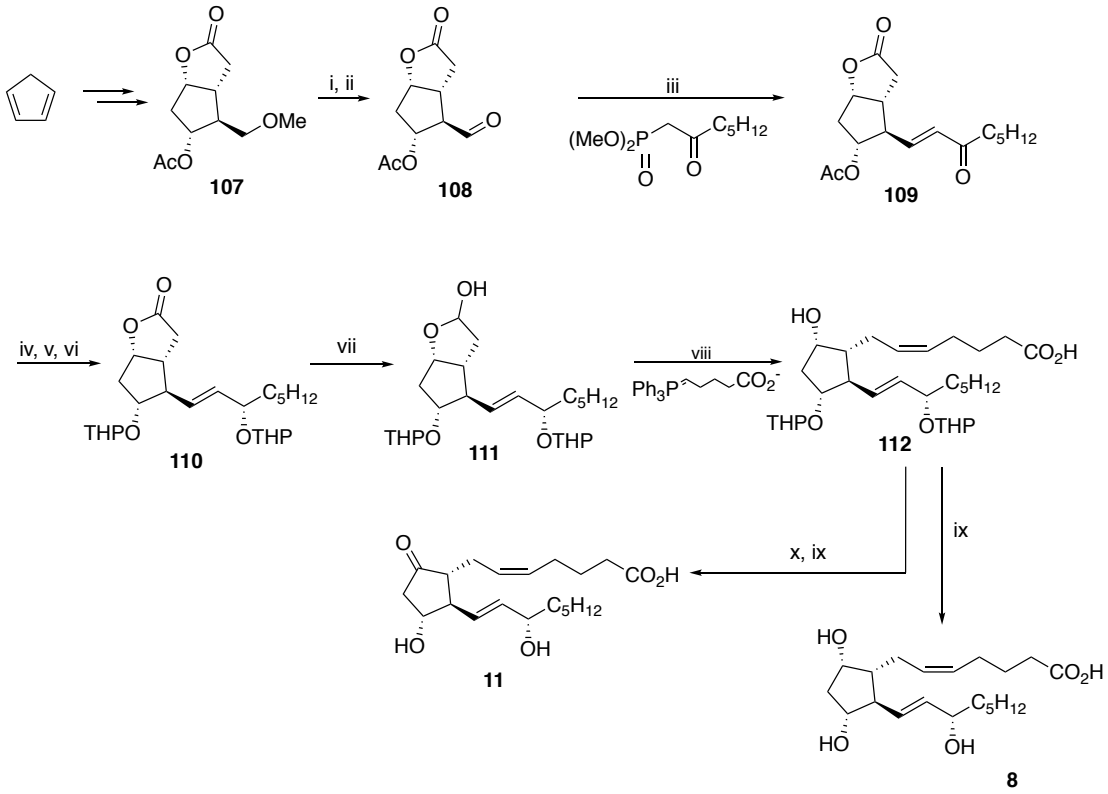


Scheme 16 Synthesis of juniperonic acid (102) by Vik *et al*.[123] Reagents and conditions: (i) I_2, $KHCO_3$, KI, THF/H_2O; (ii) K_2CO_3, MeOH; (iii) H_5IO_6, Et_2O; (iv) DBU, Et_2O; (v) DIBAL-H, CuI, THF/HMPA, -20°C; (vi) NaHMDS, THF, -100°C.

3. Synthesis of prostaglandins

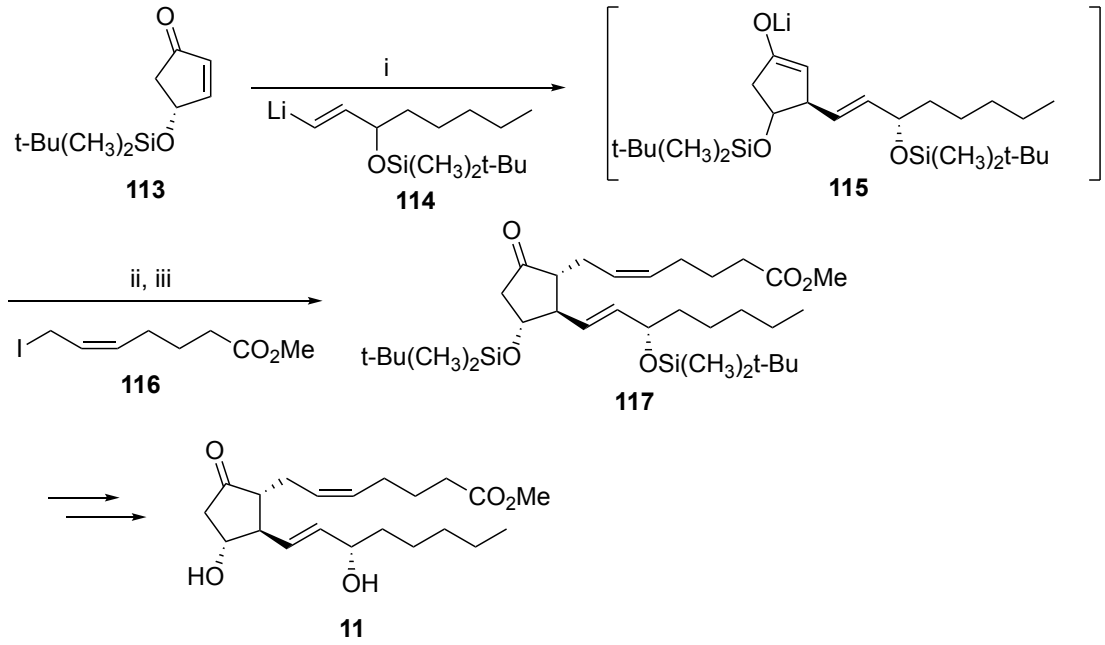
There are a large number of published syntheses of prostanoids, and only a few will be described herein. One of the classics in the field is the synthesis of prostaglandin $PGF_{2\alpha}$ (**8**) by Corey *et al.*[9] The key structure **107**, known as the Corey lactone, was prepared in several steps from cyclopentadiene. The *E*-alkene is formed utilizing HWE methodology, while the *Z*-double bond is added via a

Wittig reaction. Corey's group has published several modifications of this synthesis that include the possibility to prepare the 1- and 2-series of prostaglandins.[125-128] The original synthesis of $PGF_{2\alpha}$ (**8**) is shown in Scheme 17.



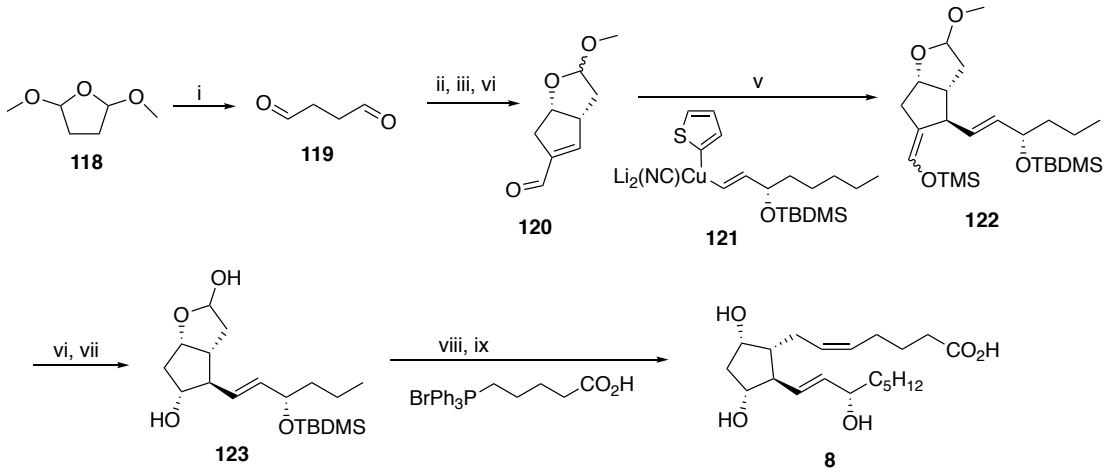
Scheme 17 Corey's synthesis of PGE_2 (11) and $PGE_{2\alpha}$ (8).[9] Reagents and conditions: (i) BBr_3, CH_2Cl_2, 0^0C (>90%); (ii) CrO_3-2Pyr, CH_2Cl_2, 0^0C; (iii) NaH, DME, dimethyl 2-oxoheptylphosphonatel, 25^0C (70% over 2 steps); (iv) $Zn(BH_4)_2$, DME, (>97%); (v) K_2CO_3, MeOH; (vi) THP, CH_2Cl_2, p-TsOH; (vii) DIBAL-H, toluene, -60°C, 0.5h; (viii), CH_3SOCH_2Na, 5-triphenylphosphoniopentanoic acid, DMSO; (ix) 2:1 AcOH:H_2O, 37°C; (x) chromic (two-phase) oxidation.

Noyori and Suzuki published the so-called "Three-component coupling synthesis of prostaglandins". The concept is a tandem conjugate addition/alkylation sequence, as shown in Scheme 18.[129-131]



Scheme 18 The tree-component coupling synthesis of prostaglandins. Condition: (i) CuI, THF, Bu_3P, -78^{0}C XX; (ii) HMPA, Ph_3SnCl, THF, -78^{0}C; (iii) methyl (*Z*)-7-iodo-5-heptenoate, -20^{0}C, 17h.

Recently, Aggarwal *et al.* published a seven-step synthesis of $PGF_{2\alpha}$ (**8**).[132] The synthesis is outlined in Scheme 19. The same strategy was also employed in the synthesis of analogues including latanoprost (**7**).[22]

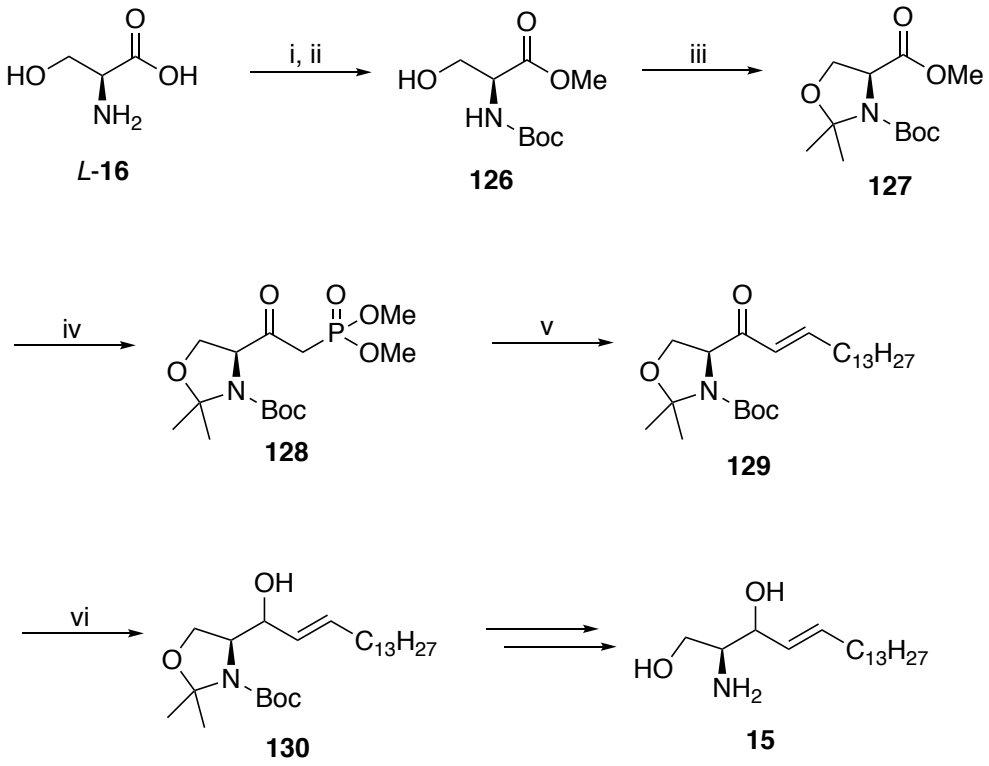


Scheme 19 $PGF_{2\alpha}$.synthesis by Aggarwal *et al.*[132] Reagents and conditions: (i) a: H_2O, 75^{0}C, 4 h, b: 115^{0}C to distil MeOH and H_2O; (ii) a: (*S*)-proline (2mol%),THF (2M) rt, 20 h, b: $[Bn_2NH_2][OCOCF_3]$ (2mol.%), THF (1 M), rt, 14 h; (iii) a) MeOH (2.0 eq), amberlyst 15, $MgSO_4$, CH_2Cl_2, rt, 14 h; (iv) (1.1 eq), THF, b) TMSCl, Et_3N. (v), O_3, CH_2Cl_2/MeOH (3:1), 278^{0}C, c) $NaBH_4$ (3 eq), at 278^{0}C to rt; (vi) 1.5% aq. HCl/THF (3:2), rt, 16 h; (vii) (4-carboxybutyl)(triphenyl)phosphonium bromide (6 eq), KOt-Bu (12 eq), THF, 0^{0}C to rt.

4. Synthesis of sphingolipids

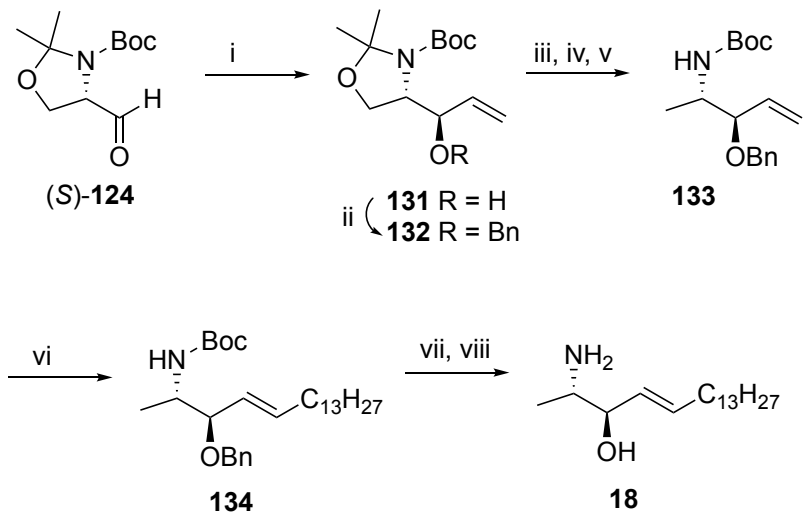
A large number of syntheses of sphingolipids have been reported in the literature. Only the most typical approaches will be listed herein.

Numerous published strategies rely on the chiral pool. These mainly use structures derived from amino acids, *e.g.* Garner's aldehyde (**124**), *N*-BOC *D*-alaninal (*D*-**125**) *etc*. Koskinen *et al.*[133] and Boutin and Rapoport[134] prepared **15** from amino acid *L*-**16**.



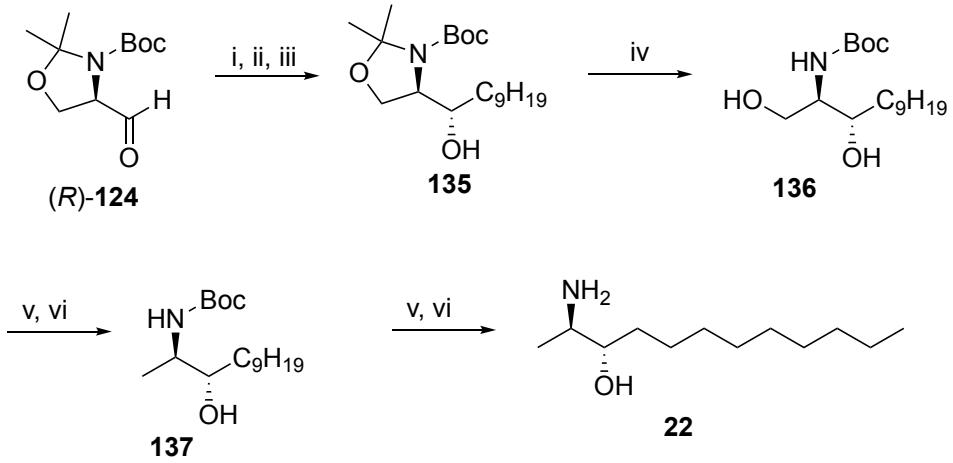
Scheme 20 Synthesis of sphingosine from *L*-16 by Koskinen *et al.*[133] Reagents and conditions: (i) MeOH, HCl; (ii) $(BOC)_2O$, TEA; (iii) 2,2-$(MeO)_2$propane, BF_3-Et_2O, DMF (55% from *L*-16); (iv) $(MeO)_2P(=O)$-CH_2-Li, THF (83%); (v) *n*-$C_{13}H_{27}CHO$, K_2CO_3, CH_3CN (80%); (vi) L-selectride, THF, -78°C.

Oxazolidine *S*-**124**, derived from *L*-**16**, was used in the synthesis of sphingolipid **18**. Aldehyde **124** was transformed into allylic alcohol **131** in a 6:1 ratio of *anti* and *syn* isomers, respectively. The alcohol was protected as the benzyl ether **132**. Olefin metathesis reaction was utilized to elongate the side chain. The synthetic pathway is shown in Scheme 21.[135]



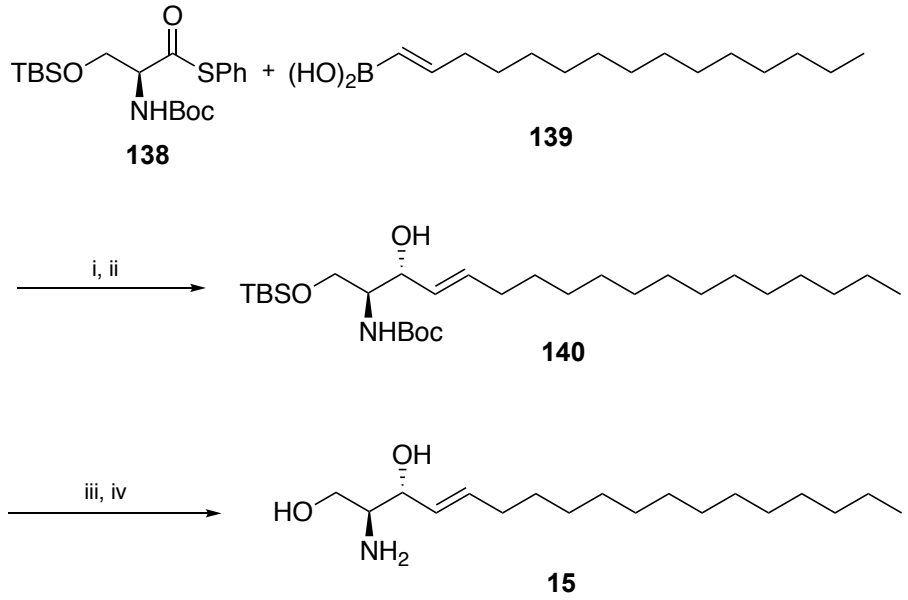
Scheme 21 Synthesis of (+)-18.[135] Reagents and conditions: (i) Vinyl magnesium bromide, THF, 78 °C, 74%; (ii) BnBr, NaH, DMF, 91%; (iii) *p*-TsOH, MeOH, 0 °C, 84%; (iv) *p*-TsCl, Et_3N, CH_2Cl_2, 25 °C, 73%; (v) $LiAlH_4$, THF, 20 °C, 80%; (vi) Grubbs' second generation catalyst (62) (5 mol%), 1-pentadecene, CH_2Cl_2, reflux, 87%; (vii) H_2, Pd/C, MeOH-$CHCl_3$, 91%; (viii) (a) HCl-dioxane, rt; (b) Aq. NaOH, CH_2Cl_2, rt, 90%.

Clavaminol A (**22**) was synthesized from (*R*)-**124**. However, a regular Grignard reaction gave rise to an inseparable mixture of *syn*/*anti* products. Oxidation followed by asymmetric reduction gave **135**, which was subsequently transformed to the target molecule in a few steps, as shown in Scheme 22.[136]



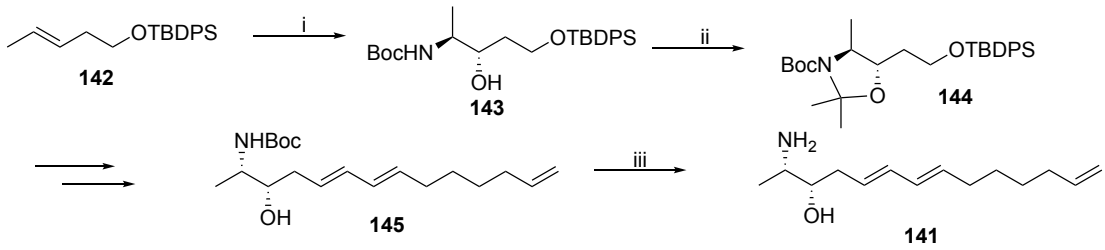
Scheme 22 Synthesis of clavaminol A.[136] Reagents and conditions: (i) Nonylmagnesium bromide, THF, −78-0°C, 30 min, 91%, (*syn*/*anti* = 1:3); (ii) DMP, CH_2Cl_2, 0°C to rt, 30 min, 96%; (iii) (*R*)-CBS, THF, −40°C, 8 h, 92%; (iv) CSA, MeOH, 0°C, 4 h, 90%; (v) *p*-TsCl, Et_3N, CH_2Cl_2, 0°C to rt, 5 h; (vi) $LiAlH_4$, THF, −20°C, 4 h, 79% over two steps; (vii) Et_2O, HCl, 0°C to rt 10 h, 88%.

In another synthesis of **15**, a thioester (**138**), derived from *L*-**16**, was reacted with a boronic acid **139**, prepared from pentadec-1-yne. The synthesis gave **15** in good yield over 7 steps. The coupling is shown in Scheme 23.[137]



Scheme 23 A short synthesis of sphingosine (15) employing palladium coupling.[137] Reagents and conditions: (i) $Pd_2(dba)_3$, CuTC, $P(OEt)_3$, THF, rt, 10h, 94%; (ii) $Li(Ot\text{-}Bu)_3AlH$, EtOH, -78°C, 96%; (iii) HCl, MeOH/H_2O, 0°C to rt, 30min 99%; (iv) CF_3CO_2H, CH_2Cl_2, 0°C to rt, 30min, 90%

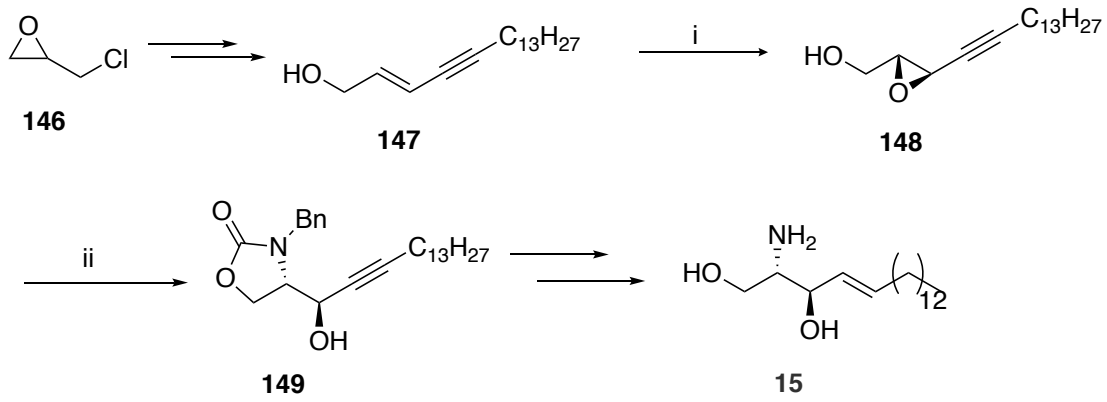
Numerous non-chiral pool approaches have also been reported. One of the direct strategies is the aminohydroxylation. Sharpless asymmetric aminohydroxylation was utilized to prepare crucigasterin A (**141**). Parts of the synthesis of is shown in Scheme 24.[138]



Scheme 24 Synthesis of crucigasterin A (141).[138] Reagents and conditions: (i) *t*-$BuOCONH_2$, NaOH, 1,3-dichloro-5,5-dimethylhydantoin, $(DHQ)_2PHAL$, K_2OsO_4 x $2H_2O$, 21 h, 65%, 94% ee; (ii) 2,2-DMP, CH_2Cl_2, *p*-TsOH (cat), 0°C to rt, 30 min, 82%; (iii) TFA, CH_2Cl_2, 92%.

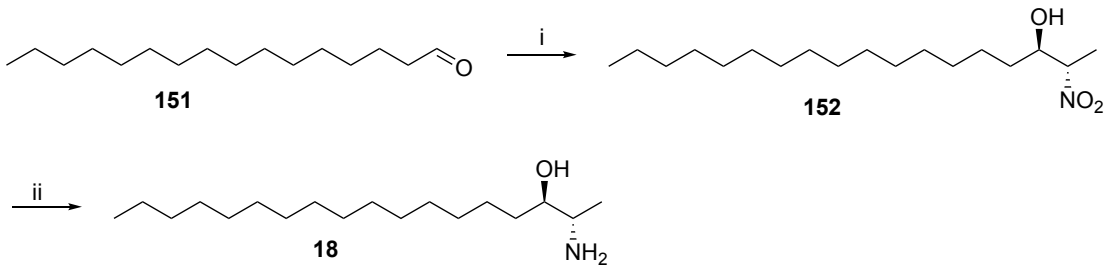
More frequently used is the asymmetric epoxidation, with subsequent ring opening employing some nitrogen source. One example is the synthesis of **15** whereby benzyl isocyanate was employed to open the epoxide.[139,140] Parts of the

synthesis are depicted in Scheme 25. Other research groups have employed similar strategies. [141-143]

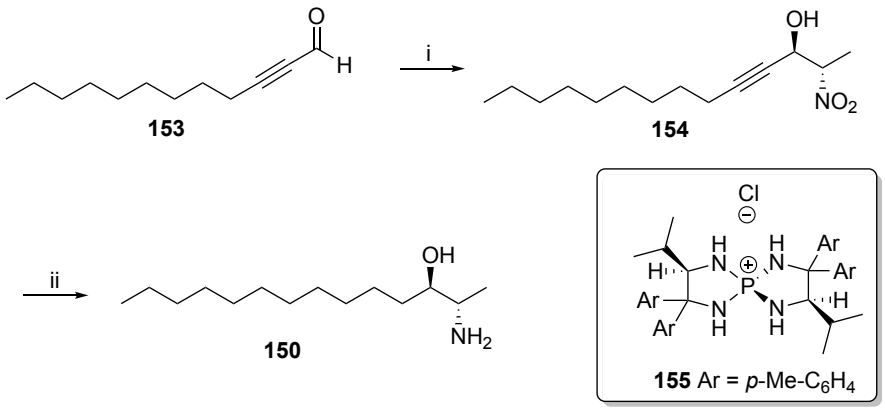


Scheme 25 Asymmetric epoxidation used by Bernet and Vasella in the synthesis of sphingosine.[140,139] Reagents and conditions: (i) (-)-DET, t-BuO$_2$H, Ti(t-BuO)$_4$; (ii) BnNCO, NaH.

Sphingolipids **18**[115] and xestoaminol C (**150**)[108] are reported prepared employing asymmetric nitro aldol reactions. These are shown in Scheme 26 and Scheme 27, respectively.



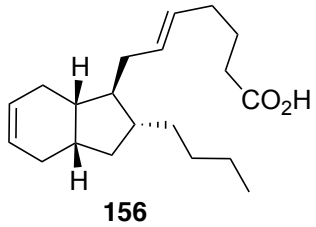
Scheme 26 Synthesis of (+)-spisulosine.[115] Reagents and conditions: (i) EtNO$_2$, catalyst 92 (5 mol%), Cs$_2$CO$_3$ (5 mol%), THF, 0°C, 72h, 82% yield, 95% ee; (ii) 10% Pd/C, H$_2$, MeOH, rt, 12h, 73% yield.



Scheme 27 Synthesis of (+)-xestoaminol C (150).[108] Reagents and conditions: (i) EtNO$_2$, 155/KO*t*Bu (5 mol%), THF/DMF (5%), -78°C, 30min, 94% yield, (*anti*/*syn* >20:1, 98% ee for *anti*-diastereomer); (ii) 10% Pd/C, H$_2$, MeOH, rt, 6h, >99% yield.

5. Mucosin

5.1 Introduction



156

Figure 11 Proposed structure of mucosin

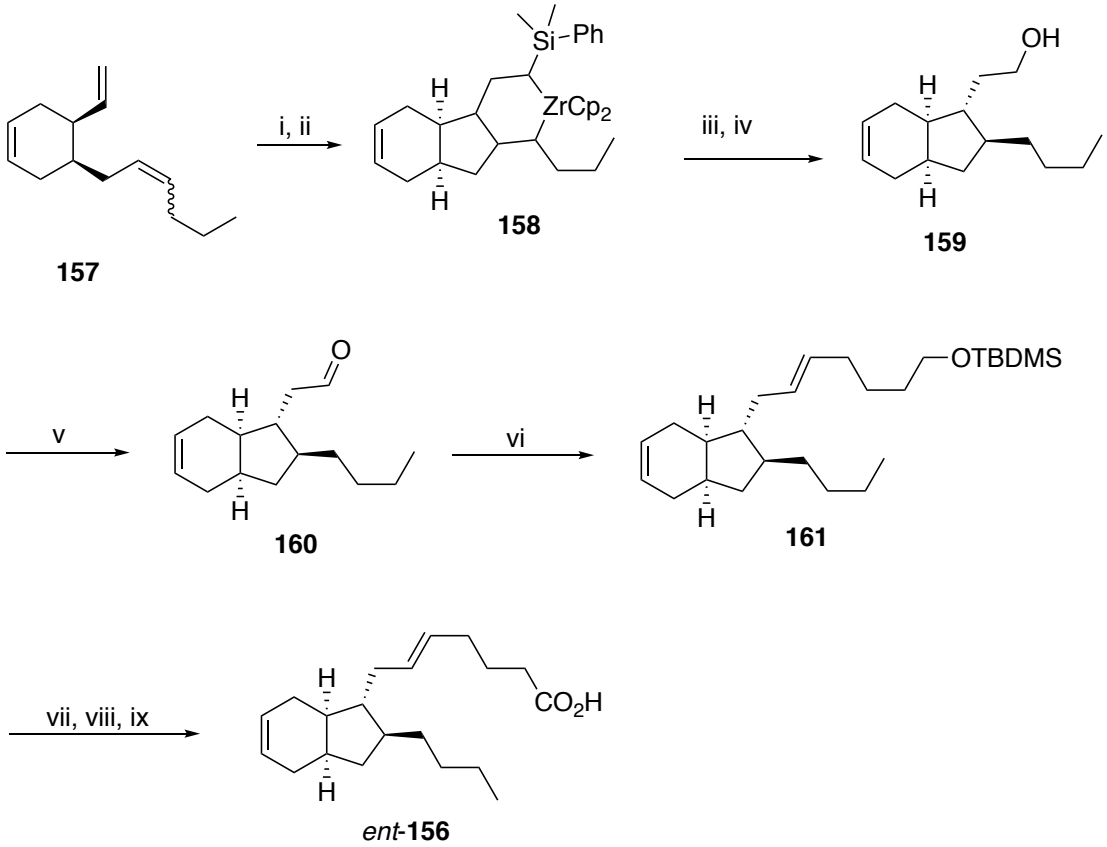
The marine natural product mucosin, was isolated by Casapullo *et al*. from the sponge *Reniera mucosa* harvested in the Mediterranean Sea.[144] The claimed structure (**156**) was elucidated as the optically active methyl ester by ^{1}H and ^{13}C-NMR. However, the absolute configuration was not determined.

Inspection of the structure reveals several interesting features. The structure contains a bicyclo[4.3.0]nonene skeleton. Attached to the cyclopentane are two side-chains with lengths of seven and four carbons, respectively. The former containing a carboxylic acid moiety and an *E*-double bond between C-5 and C-6. The side chains were suggested to be *trans*-related, while the hydrogens on the bridgeheads are suggested to be *cis*-fused. However, Casapullo *et al.* present no conclusive proof for the bridgeheads being *cis*-fused, as correlations for these couplings are not reported.

The compound has a structure with striking similarities to the prostaglandins as seen by the five-membered ring with the side-chains. Casapullo *et al*. postulates that mucosin might be biosynthetically derived from arachidonic acid through an intramolecular cycloaddition, though no proof of this is presented.

In 2012, Whitby *et al.* published a synthesis of the enantiomer of mucosin (*ent*-**156**).[145] Their strategy employed the zirconocene-induced co-cyclization of triene **157**. Zirconacycle **158** was treated with base and TBAF to give **159** with its diastereoisomer in a 2.7:1 ratio. Only **159** was used in the reactions to give aldehyde **160**, which was reacted with $CHI_2(CH_2)_3CH_2OTBDMS$ under the Takai

olefination conditions. Deprotection and oxidation of the resulting alcohol, gave *ent*-**156**. The strategy is outlined in Scheme 28.

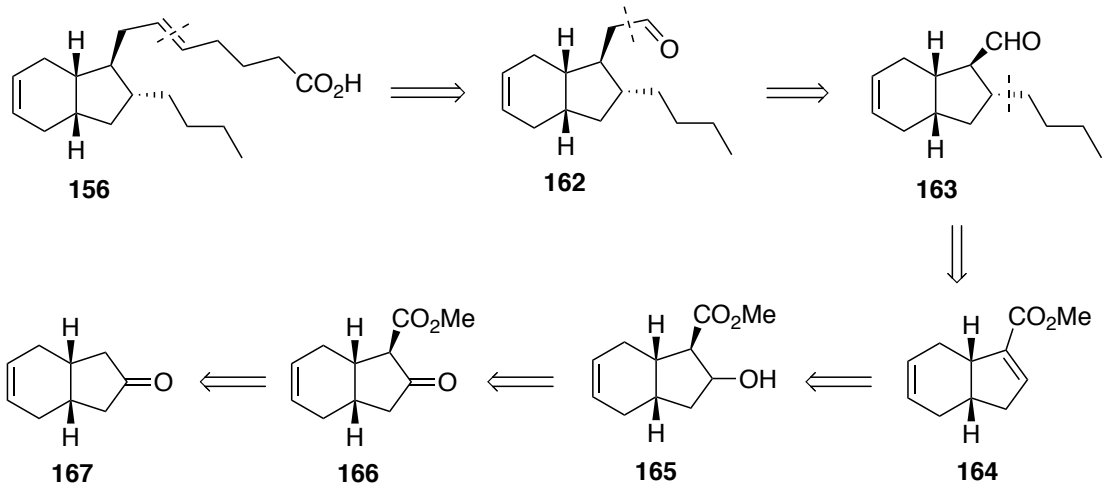


Scheme 28 Whitby's synthesis of the enantiomer of mucosin.[145] Reagents and conditions: (i) Cp_2ZrBu_2; (ii) (chloro(dimethyl(phenyl)silyl)methyl)lithium; (iii) $NaHCO_3$, MeOH, 78 °C to rt, 15 h; (iv). KH, NMP, *t*-BuO_2H, 0 °C to rt, 10 mins then TBAF, 70 °C, 14 h. (v) Me_2SO, CH_2Cl_2, $(COCl)_2$, 60°C, 15 min then Et_3N, 60 °C to rt, 1 h; (vi) $I_2CH(CH_2)_3CH_2OTBDMS$, $CrCl_2$, DMF, THF, rt, 2.5 h; (vii) TBAF, THF, rt, 3h;(ix) PDC,DMF,0°C to rt,15h.

No biological testing has been reported of this stereoisomer, nor on the natural product.

5.2 Retrosynthesis

As a target molecule for total synthesis, there are some challenges that need to be addressed. First of all: there is one carbon-carbon double bond. It has been shown to have *E*-geometry. There are also four stereogenic centers. As mentioned, the absolute configuration is not determined. The two side chains are said to be *trans* related. Casapullo does not present any other conclusive proof of stereochemistry. As Whitby reported the enantiomer of mucosin, we aimed for the opposite absolute stereochemistry of what he reported.



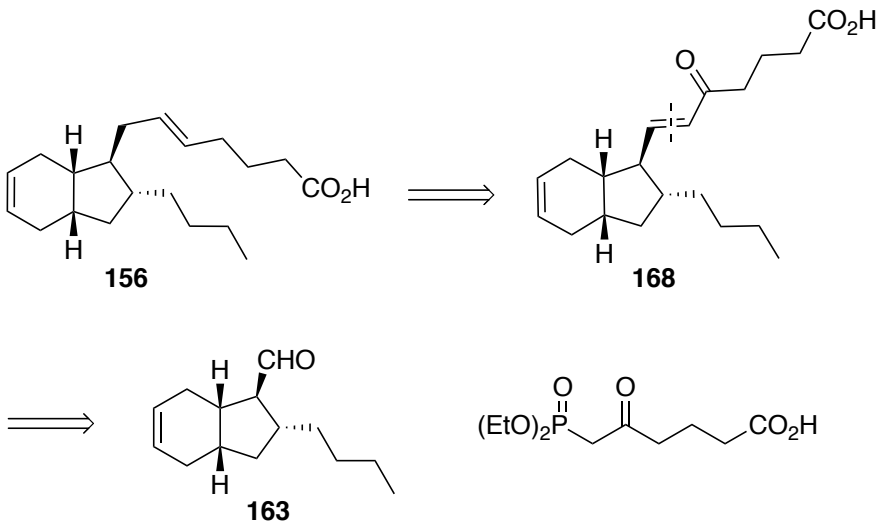
Scheme 29 *E*-selective Wittig retrosynthesis

One possible strategy is presented in Scheme 29. The $\Delta^{5,6}$ double bond found in **156** provides an opportunity for an *E*-selective Wittig type reaction. This would furnish aldehyde **162**. The aldehyde **162** can in turn be prepared via homologation of aldehyde **163**.

The 1,3-relation between the carbomethoxy group and the side-chain found in aldehyde **163** reveals an opening for conjugate addition. This disconnection leads back to the α,β-unsaturated ester **164** and a C_4-carbon nucleophile, *e.g.* butyl lithium or butylmagnesium halide.

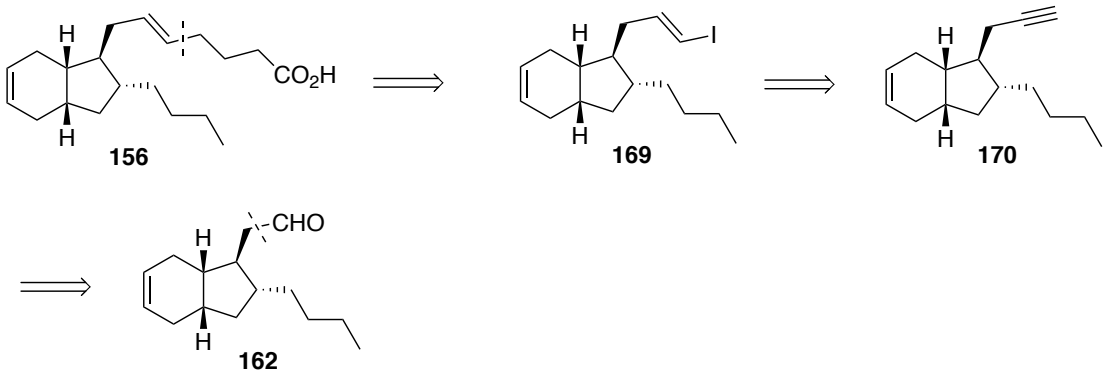
The α,β-unsaturated ester **164** can be prepared from alcohol **165** by dehydration. Alcohol **165** can easily be prepared by reduction of β-keto ester **166**, according to the literature.[146,147] An alternative route to **166** is via *meso*-ketone **167**, also described in the literature.[148,149]

Alternatively, Wolff-Kishner reduction on α,β-unsaturated carbonyl compounds, is known to give double bond migration.[150] Hence, the $\Delta^{5,6}$ double bond can be prepared from $\Delta^{6,7}$ unsaturated ketone **168**. Ketone **168** can be prepared from aldehyde **163** by HWE olefination.



Scheme 30 Retrosynthesis by HWE/Wolff-Kishner strategy

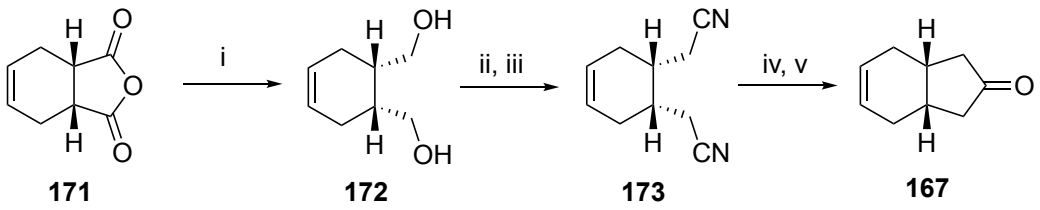
A third option is transition metal catalyzed cross-coupling (*e.g*. Negishi coupling). This would demand preparation of vinylic halide **169**. The vinylic halide can be prepared from the corresponding alkyne **170**, prepared from aldehyde **162**. The retrosynthesis is outlined in Scheme 31.



Scheme 31 Cross-coupling retrosynthesis

5.3 Synthesis of *meso*-ketone and β-keto ester

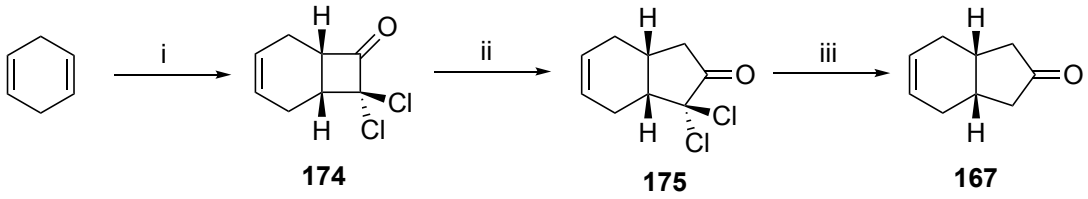
Syntheses of both β-keto ester **166** and *meso*-ketone **167** have been published, but these protocols were found to be laborious.[151,152] After repeating the work of Mundy and Theodore, shown in Scheme 32, we wanted to find a shorter and more efficient synthesis of these.



Scheme 32 Mundy and Theodore's synthesis of *meso*-ketone 166.[151] Reagents and conditions: (i) $LiAlH_4$, THF, reflux, 16h (82%); (ii) *p*-TsCl, pyridine, 0^0C, 2h (76%); (iii) NaCN, EtOH, reflux, 72h (76%); (iv) 40% KOH, reflux, 24h (38%); (v) Ac_2O, pyridine, reflux, 24h (75%).

We decided to attempt a strategy consisting of ketene [2+2] cycloaddition reactions with 1,4-cyclohexadiene and trichloroacetyl chloride with subsequent ring expansion and dehalogenation.

Ketene addition to 1,4-cyclohexadiene, enhanced by sonication, resulted in dichlorobicycloketone **174** along with some *bis*-adduct.[153] The purified mono-adduct was treated with diazomethane to give the dichlorobicyclo[4.3.0]nonone (**175**) by a Buchner–Curtius–Schlotterbeck ring expansion reaction.[154,155] Dehalogenation was achieved by zinc mediated hydro dehalogenation to give the *meso*-ketone **167**.[153]



Scheme 33 Synthesis of *meso*-ketone 167. Reagents and conditions: (i) trichloroacetyl chloride, Et_2O, 0 to 10^0C, 2h (73%); (ii) CH_2N_2, Et_2O, 0^0C to rt, 3h (75%); (iii) Zn, AcOH, 70^0C, 16h (72%).

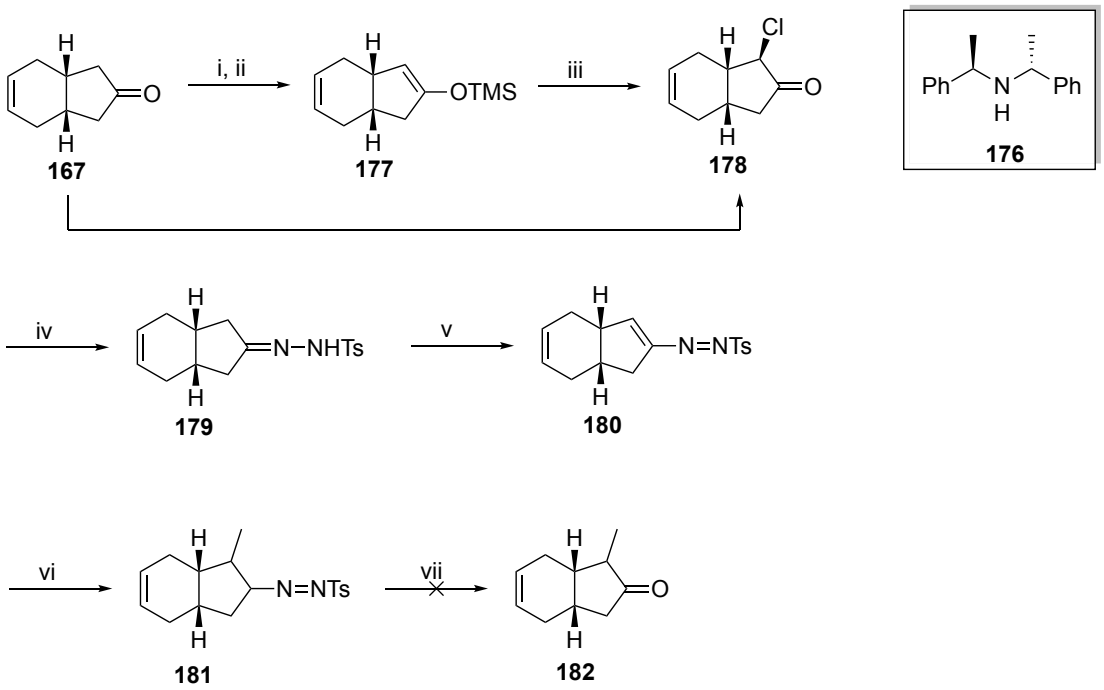
Total yield for the three-step protocol was 39%,* which was a significant improvement compared to Mundy and Theodore's synthesis of the same structure (14% yield).[151]

Next we turned to the preparation of β-keto ester **166** from *meso*-ketone **167**. It was anticipated that forming the lithium enolate, using LDA, followed by addition of dimethyl carbonate would give β-keto ester **166**. The reaction gave

* Ketene addition was reported to proceed in 47% yield, but was later improved to 73%. This resulted in improvement from 28% to 39% total yield.

the desired product, but in moderate to poor yields. The yield was significantly improved when LDA was exchanged for NaH.[156,157]

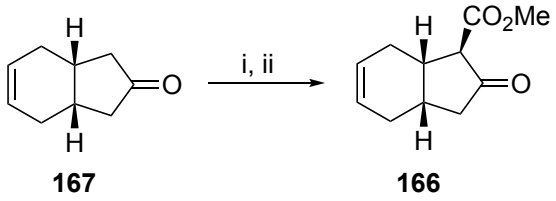
A chiral base, (*R,R*)-*bis*(phenylethyl)lithiumamide, prepared from the corresponding amine **176** and *n*-BuLi, was employed on *meso*-ketone **167** followed by addition of TMSCl and NCS, gave α-chloro ketone **178** in good yields and enantiomeric excess.[158] We also isolated the trimethylsilyl enolate **177**. The corresponding *para*-toluenesulfonyl hydrazone **179** was prepared from α-chloro ketone **178** as crystals. Treatment with aqueous $NaHCO_3$ furnished azoene **180**. Preliminary experiments on the conjugate addition proved to work poorly. By adding MeLi to CuI/Bu_3P with subsequent addition of precooled azoene, we only manage to obtain small amounts (less than 10%) of hydrazone **181**. However, we decided to attempt the hydrolysis of hydrazone **181** employing $(PhSeO)_2O$, but obtained only unreacted starting material. The strategy, depicted in Scheme 34, was eventually abandoned.[158-161]



Scheme 34 Attempt on the Gais procedure. Reagents and conditions: (i) *n*-BuLi, THF, base 176 -78°C, 1 h; (ii) TMSCl, THF, -78°C, 2 h; (iii) NCS, THF, -78°C, 2 h (70% from compound 167); (iv) NH_2NHTs (77%); (v) $NaHCO_3$; (vi) MeI, n-Bu_3P/CuI (54% over two steps); (vii) $(PhSeO)_2O$.

Meso-ketone **167** was treated with the constellation of **176**/*n*-BuLi, and reacted with dimethylcarbonate, which only gave the β-keto ester **166** in low yield.

However, employing Mander's reagent (methyl cyanoformate) worked much better giving the β-keto ester (**166**) in 82% yield.[162] The spectral data and optical rotation was in accordance with the literature value. [99,100,142]

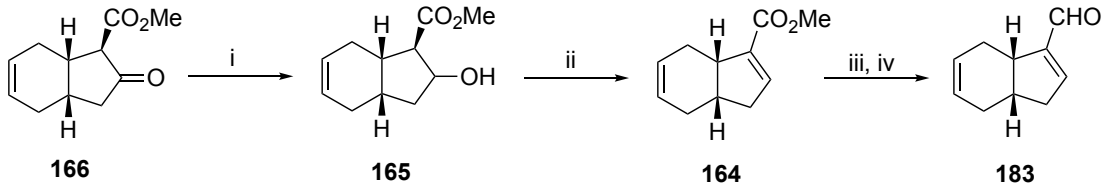


167 **166**

Scheme 35 Reaction with *meso*-ketone and Mander's reagent. (i) *n*-BuLi, THF, 176, -78°C, 1 h, compound 176; (ii) $CNCO_2Me$, THF -78°C, (82%).

5.4 Synthesis of mucosin

Subsequent to this, we wanted to prepare an α,β-unsaturated carbonyl system suitable for a conjugate addition. The ketone was reduced to the alcohol **165** using $NaBH_4$.[155] Dehydrated employing $POCl_3$ in pyridine gave the α,β-unsaturated ester **164**.[163,164]



166 **165** **164** **183**

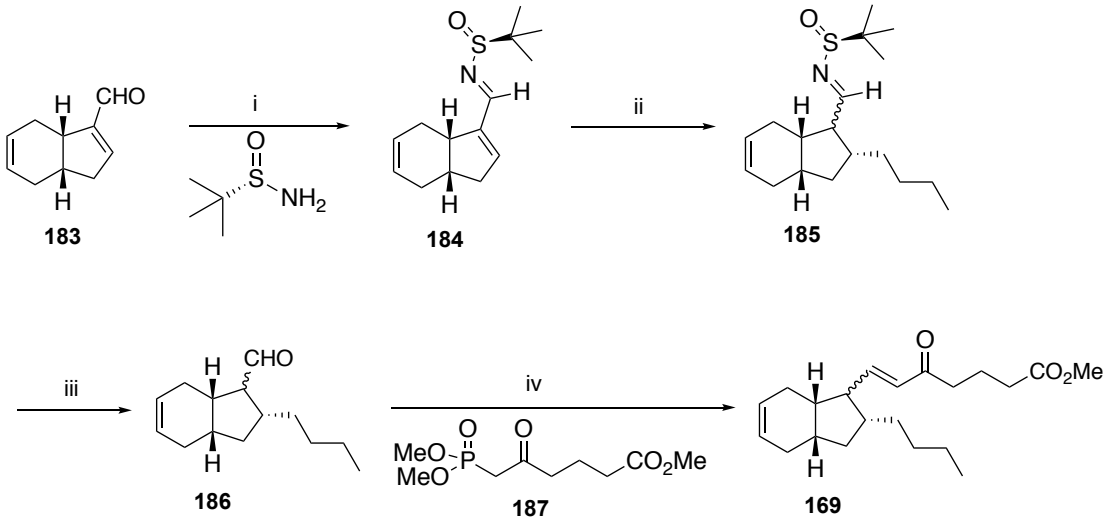
Scheme 36 Preparation of α,β-unsaturated methyl ester 164 and corresponding aldehyde 183. Reagents and conditions: (i) $NaBH_4$, MeOH, 0°C, 2h (84%); (ii) $POCl_3$, pyridine, reflux, 18h (90%); (iii) DIBAL-H, hexane, 0°C to rt, 1h (86%); (iv) DMP, pyridine, CH_2Cl_2, rt, 1h (62%).

One way to render the Michael reaction asymmetric is by employing a chiral auxiliary such as *tert*-butanesulfinamides. According to this the α,β–unsaturated aldehyde **183** was treated with (*S*)-*tert*-butanesulfinamide and $Ti(OEt)_4$, to afford chiral aldimine **184**.[165] Michael conditions were successfully employed to give compound **185**. Hydrolysis of the (*S*)-aldimine, by aqueous solution of AcOH, gave the aldehyde **186**.[165] However, epimerization occurred during hydrolysis.

Employing (*R*)-*tert*-butanesulfinamide in the conjugate addition, the final product obtained was a mixture of the same diastereomers as previously isolated, but in opposite ratio.† It was decided to complete the synthetic

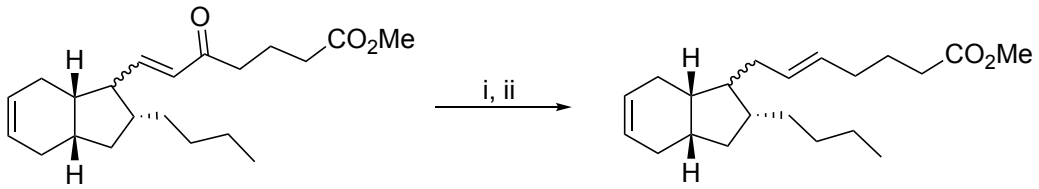
† In hindsight, the information obtained in subsequent experiments, indicates that the addition to the Michael acceptor proceded from the least hindered face irespective of the identity of the chiral sulfonamide.

sequence to investigate if any of the isomers matched the data of the natural product. The mixture of aldehydes **186** from the (*S*)-aldimine **184** was treated with phosphonate **187** to give two diastereomers of *E*-olefin **169**.[166]



Scheme 37 Michael addition, utilizing a chiral aldimine followed by HWE olefination. Reagents and conditions: Ti(OEt)$_4$, THF, r.t., 16 h (79%); (ii) Cu(I)CN, *n*-BuLi, Et$_2$O, -78 to -45°C, 3 h (60%); (iii) AcOH, H$_2$O, THF, reflux, 16 h (70%); (iv) NaHMDS, THF, -78 °C to rt, 3 h

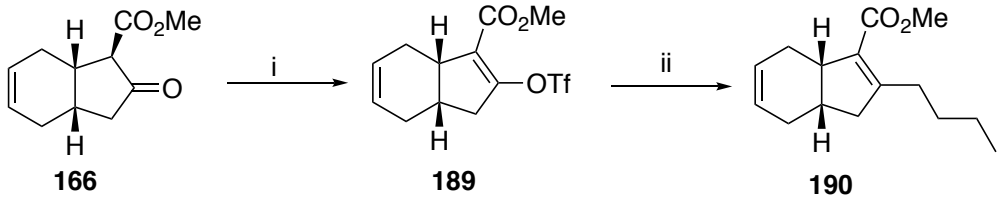
Finally, it was anticipated that Wolff-Kishner conditions on the α,β–unsaturated ketone **169**, would provide us with the methyl ester of mucosin **188**. The formation of the hydrazone of **169** and subsequent reduction, employing $NaBH_3CN$ only worked poorly. Using a stronger and chelating reduction agent, *e.g.* $LiBH_4$ or Luche conditions ($CeCl_3$ x $6H_2O/NaBH_4$),[167] or increasing the reaction time, could probably increase the yield of the reaction, but as none of the two resulting isomers matched the spectral data of the methyl ester of natural product, the strategy was abandoned.



Scheme 38 The Wolff-Kishner approach. Reagents and conditions: (i) TsNHNH$_2$, EtOH, reflux, 16h; (25%) (ii) NaBH$_3$CN, bromocresol green, sulfolane, DMP, 90^{0}C, 3h (15%).

An attractive alternative to the strategy mentioned above is a cross-coupling reaction followed by conjugate reduction of the olefin. The β-keto ester was

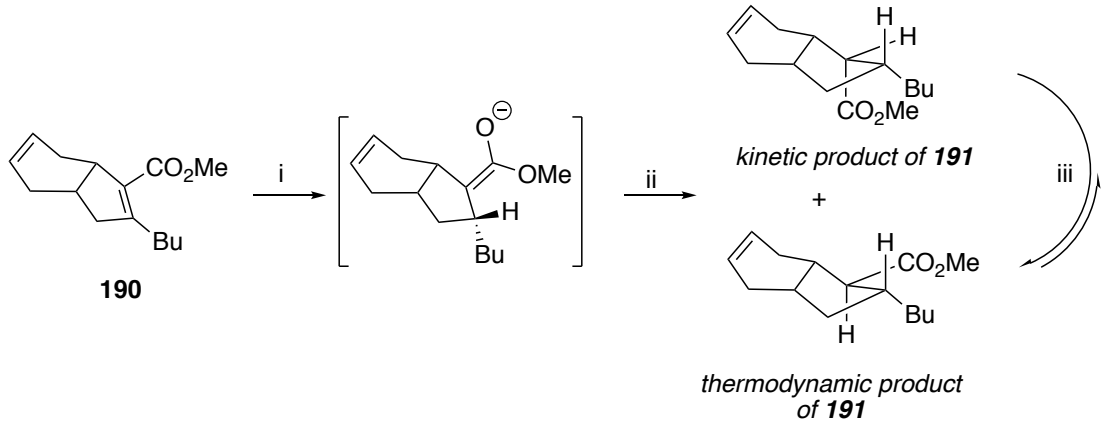
reacted with NaH and Tf_2O to give vinylic triflate **189**. This compound was subjected to copper mediated cross-coupling and *n*-BuLi to yield the desired α,β–unsaturated ester **190**.[168]



Scheme 39 Copper mediated cross-coupling. Reagents and conditions: (i) NaH, Tf_2O, toluene, 70 to 0 °C, 4 h (83%); (ii) Cu(I)CN, *n*-BuLi, Et_2O, -50 °C, 2 h (88%)

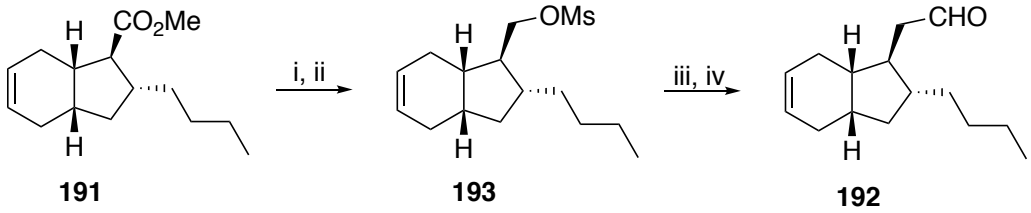
The next step was conjugate reduction of the olefin. Stryker´s reagent, (triphenylphosphine)copper hydride hexamer, is known to effectively give conjugate reductions with high regioselectivity.[169] In our hands the Stryker protocol did not work, as we only isolated the starting material.

A simpler protocol for conjugate reduction employs Mg turnings in MeOH.[170] After 3 hours of reaction time, all the starting material was converted and two diastereomers (in a 1:2 ratio) were isolated in 91% crude yield. The crude mixture of kinetic and thermodynamic product **191** was equilibrated using MeOH/NaOMe at reflux, to give only one isomer of the saturated methyl ester **191** in 93% yield. This isomer was presupposed to be the thermodynamically favored *trans*-isomer.



Scheme 40 Conjugate reduction of α,β-unsaturated ester into ester 191. Reagents and conditions: (i) Mg turnings, MeOH, 40^{0}C, 3h (95%); (ii) abs. AcOH; (iii) MeONa, 70^{0}C, 3h (91%).

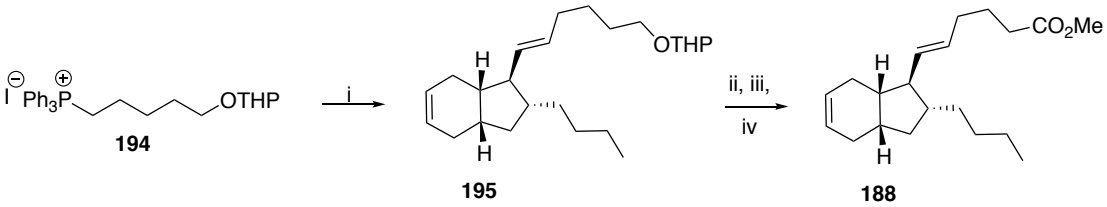
It was decided to attempt the Schlosser modification of the Wittig reaction. A homologation to aldehyde **192** was achieved by reduction of the ester to alcohol, followed by mesylation, cyanation and reduction of nitrile to aldehyde.



191 **193** **192**

Scheme 41 Homologation of ester 191 to aldehyde 192. Reagents and conditions: (i) DIBAL-H, hexane, 0^0C to r.t., 2h; (ii) MsCl, Et_3N, CH_2Cl_2, 0^0C to rt, 16h; (iii) KCN, DMSO, 70^0C, 4h; (iv) DIBAL-H, hexane, -78^0C, 0.5h (91% over four steps).

The olefination reaction was carried out with aldehyde **192** and the C_5 phosphonium iodide **194** in good yields.[171] The tetrahydropyranyl ether **195** was hydrolyzed,[172] and the resulting alcohol was oxidized[173] and esterified to give mucosin.[174] Comparison of spectral data with the isolated material, revealed structural differences.



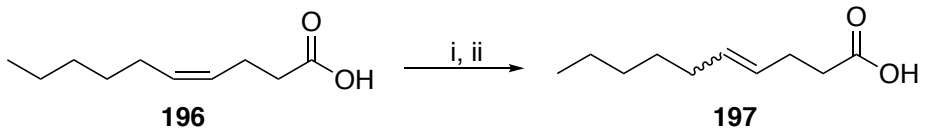
194 **195** **188**

Scheme 42 Schlosser-Wittig on aldehyde 192 towards the mucosin. Reagents and conditions: (i) LiBr,/PhLi, compound 192, -78 to rt to -78^0C, KO^{t}Bu (70%); (ii) AcOH/H_2O/THF; (iii) PDC, DMF; (iv) TMS-CHN_2.

A regular Wittig reaction was carried out on aldehyde **192**, to directly compare to the product of the Schlosser-Wittig reaction. This gave the *Z*-olefin in 63% yield. Comparison of data revealed that the Schlosser-Wittig conditions had mainly given the *Z*-olefin. Several experiments were done, but we were not able to obtain satisfactory proportions of the *E*-geometry. At best, mixtures of *E* and *Z* alkenes were formed with the *Z*-isomer always dominating.[175,71]

Preliminary experiments on several alternative methods to convert the *Z*-olefin to *E*-geometry were tested, including formation of iodolactone with subsequent

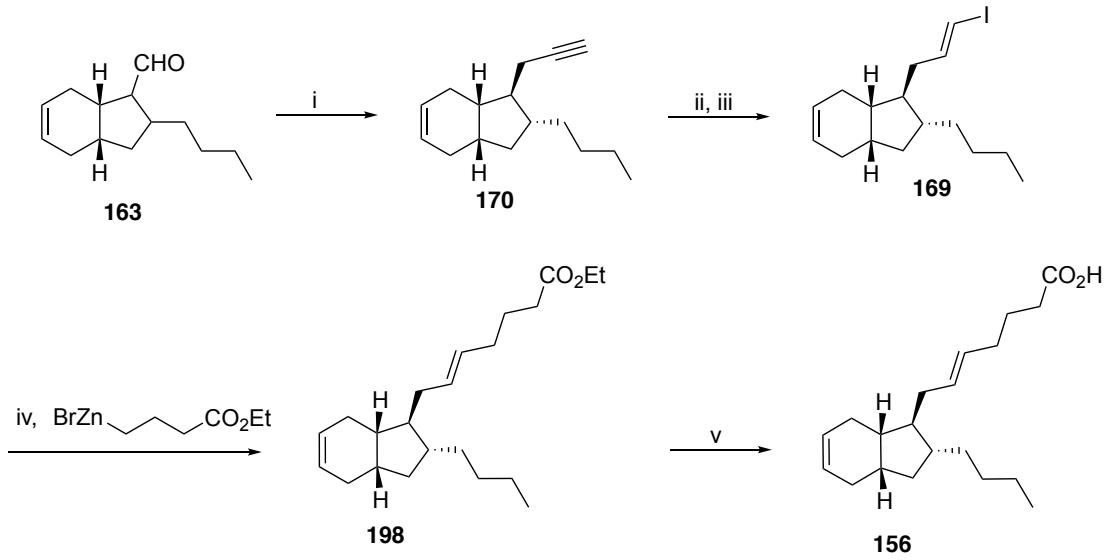
elimination, but we never achieved any better than a 3:1 *Z*/*E*-ratio even after repeating the iodolactone/elimination protocol. [176]



196 **197**

Scheme 43 Preliminary experiments on iodolactone/elimination protocol. Reagents and conditions: (i) KI, I_2, $KHCO_3$, THF, H_2O; (ii) Zn, AcOH.

Eventually, we decided to use a transition metal catalyzed cross-coupling strategy, as transition metal catalyzed cross-couplings have been used many times to yield structures with *E*-olefins exclusively. We decided to use a Negishi cross-coupling reaction as this allows the coupling of a vinyl halide directly with an ester, instead of using protection groups or obnoxious reagents. Aldehyde **163** was successfully transformed into the alkyne employing Ohira-Bestmann methodology, in 85% yield.[177] This alkyne was treated with DIBAL-H, followed by treatment of the reaction with I_2, yielding the *E*-vinyl iodide **196**. The reactions were improved by employing zirconocene dichloride in combination with DIBAL-H.[178]



163 **170** **169**

198 **156**

Scheme 44 Route to mucosin via cross-coupling of vinyl iodide 169 and 4-ethoxy-4-oxo-butylzinc bromide. Reagents and conditions: (i) Ohira-Bestmann reagent, K_2CO_3, MeCN/MeOH, rt; (ii) Cp_2ZrCl_2, DIBAL-H, THF/hexane, 0^{0}C, 2 h; (iii) I_2, THF/hexane, 0^{0}C to rt, 1 h; (iv) $(Ph_3)_4Pd$ (cat.), THF/hexane, 0^{0}C to rt, 1 h (51% from 170); (v) LiOH, THF/MeOH/H_2O, rt, 3 h (100%).

Negishi coupling of (4-ethoxy-4-oxobutyl)zinc(II) bromide with vinyl iodide **196** was catalyzed by palladium tetrakis to give ethyl ester **198**. The synthesis of (4-methoxy-4-oxobutyl)zinc(II) bromide has recently been described by Yamada.[179]

We knew from experience with simpler alkynes that it is possible to prepare vinyl iodide and then do the palladium catalyzed cross-coupling reaction in one-pot. This was indeed achieved starting with alkyne **170** to give the ester **198** in a total yield of 51% of the ethyl ester of mucosin.

The ester was hydrolyzed by LiOH x H_2O to give the acid in quantitative yield. The acid was then converted into the methyl ester, as the data provided for naturally occurring mucosin pertained to this derivative. Total yield from cyclohexadiene is 7%.

5.5 Comparison of spectral data

Spectral data and optical rotation were compared to that of the isolated compound, and disappointingly, mismatches were found. Comparison with the synthetic isomer, prepared by Whitby *et al*., also revealed differences.

The comparison of ^{1}H and ^{13}C NMR of mucosin methyl ester are shown in Table 1 and Table 2, respectively. The notable differences between the isomer we prepared and published methyl esters are highlighted.

A NOESY experiment was performed and the results showed extensive overlap in the aliphatic region, which made it impossible to draw any clear conclusions, see Figure 12. This is in contrast to Whitby and Casapullo, as both groups are able to distinguish correlations in the same region. As mentioned on page 29, there is no real convincing proof for the proposed *cis*-fusion.
The only correlations reported are the ones for H-7/H-9 and H-7/H-16, indicating a *trans*-configuration on the side chains on C-16 and C-8. The expected correlations for H-14/H-16, H-14/H-15ab, H-8/H-17 and H-7/H-10b are not listed.

Table 1 Comparison of ^{1}H NMR

Whitby (400 MHz)	Casapullo (500 MHz)	This work (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)	

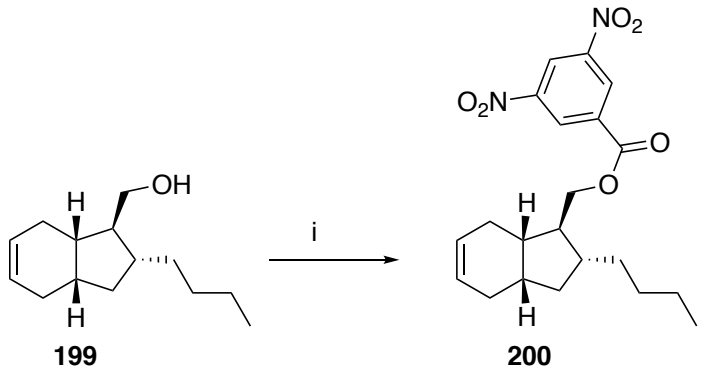
Table 2 Comparison of ^{13}C

Whitby (100 MHz)	Casapullo (125 MHz)	This work (100 MHz)
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

For this reason we needed a reliable method to elucidate the structure and in particular the relative stereochemistry. The one method for this is X-ray crystallography. Therefore we looked for a crystalline intermediate where all the stereochemistry was fixed. Since there is no doubt regarding the *E*-configuration of the alkene, we figured that the alcohol **200** was a good candidate for this. One way to make an alcohol crystalline is to make the corresponding 3,5-dinitrobenzoate.

Figure 12 NOESY NMR spectrum of the methyl ester of 156.

With some effort, we managed to obtain crystals that were suitable for such X-Ray analysis. Results can be seen in Figure 13. The relative stereochemistry was shown to be the one depicted, and clearly revealing that the structure reported for mucosin in the literature to be wrong.



Scheme 45 Formation of dinitrobenzoate 200. Reagents and conditions: (i) 3,5-dinitrobenzoyl chloride.

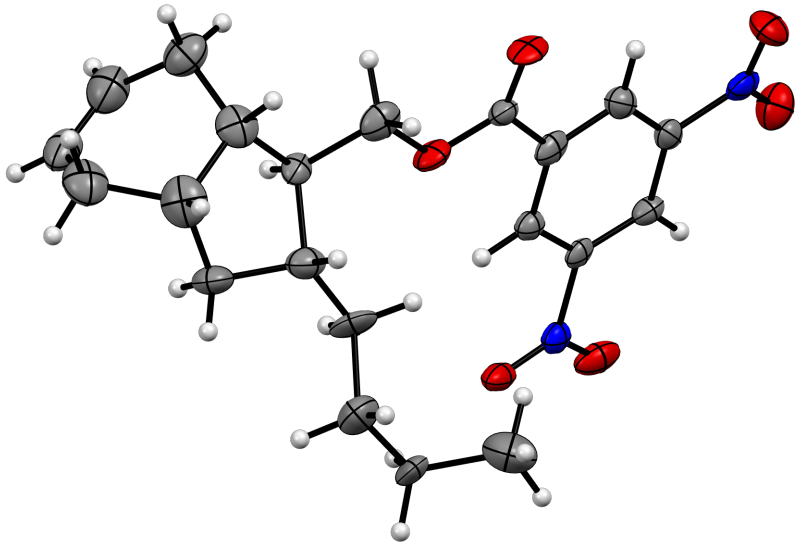
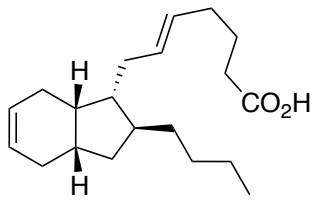


Figure 13 Single-crystal X-ray diffraction structure of dinitro-ester 200

As the structure suggested by Casapullo is proved to be wrong, we wanted to find the correct structure.

As mentioned, the reported data is deficient. In the article, Casapullo is proving that the double bond at $\Delta^{5,6}$ is of *E*-configuration, and that the two alkyl chains are of *trans* configuration. As the absolute configuration is not defined, the natural product might be the 8*R*,16*S* instead of the 8*S*,16*R* *trans* isomer that we prepared. The 8*R*,16*S* can be prepared by small modifications to the strategy applied. Another option is that the H-9 and H-14 are *trans* related.

5.6 Stereopermutation on the bicyclo[4.3.0]non-3-ene scaffold

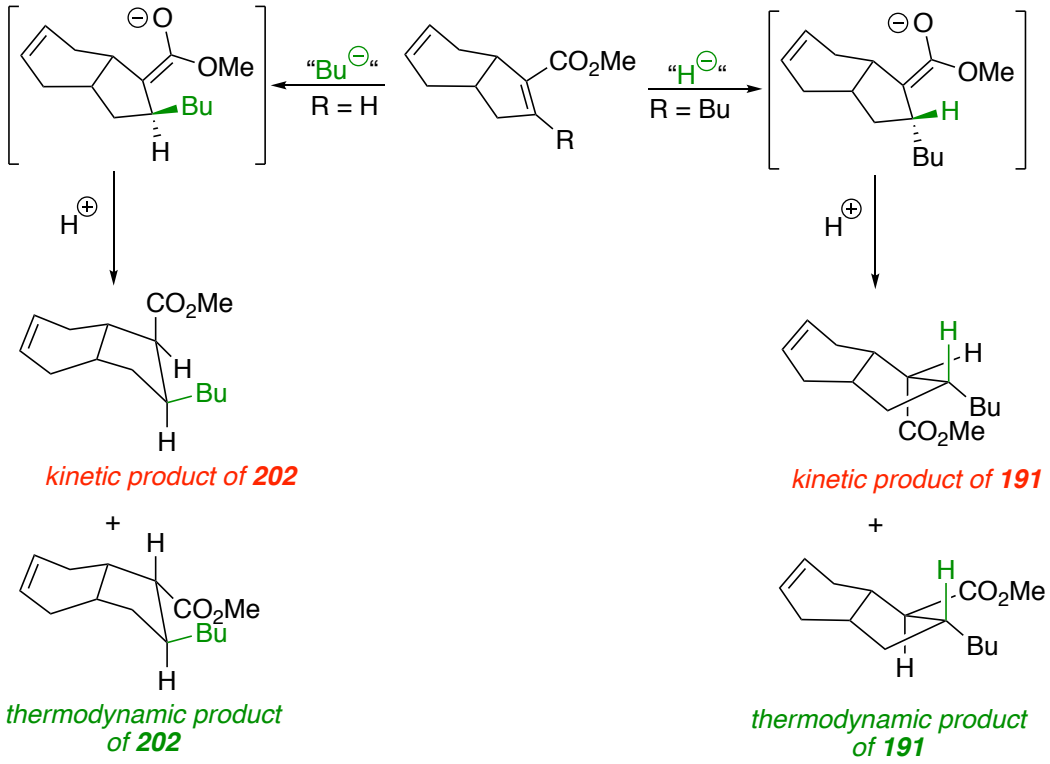


201

Figure 14 Exo-mucosin

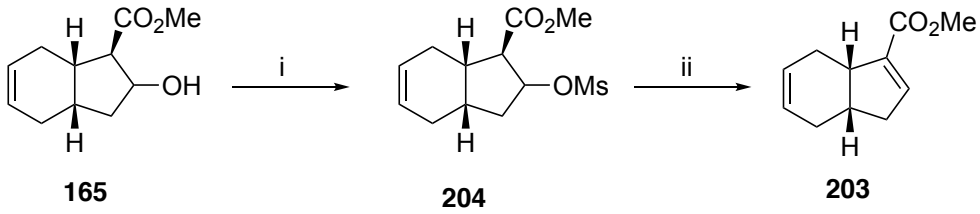
It was observed during the conjugate reduction that addition of hydrogen took place from the least hindered side of the system. As the hydride equivalent is the smallest possible nucleophile, our hypothesis was that a larger nucleophile, such as butylmagnesium halide, would act similarly and specifically merely add to the

least hindered side. That would give us the opposite configuration at C-16 and C-8 in a Michael type reaction.



Scheme 46 Addition of butyl vs. addition of hydrogen

First, the unsaturated system **203** was prepared. We found it easier to convert the alcohol **165** into the corresponding mesylate followed by DBU induced elimination, rather than the constellation of $POCl_3$/pyridine providing the Michael acceptor in one step.

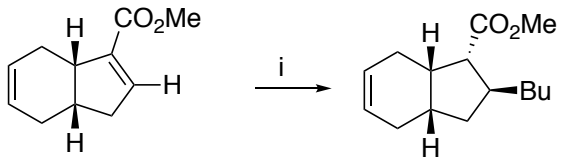


Scheme 47 Preparation of α,β-unsaturated ester 203. Reagents and conditions: (i) MsCl, Et_3N, CH_2Cl_2, 0°C, (77%); (ii) DBU, toluene, rt, (95%).

The Michael reaction was carried out by dropwise addition of butylmagnesium chloride to a solution of α,β-unsaturated ester **203**, TMSCl and catalytic amounts of CuI. TMSCl act both as a Lewis acid to lower the LUMO of the Michael acceptor

and as a stabilizer of the ester enolate. The reaction proceeded with complete diastereoselectivity, providing only one compound. The spectral data and optical rotation were different from **191**, thus confirming our hypothesis.

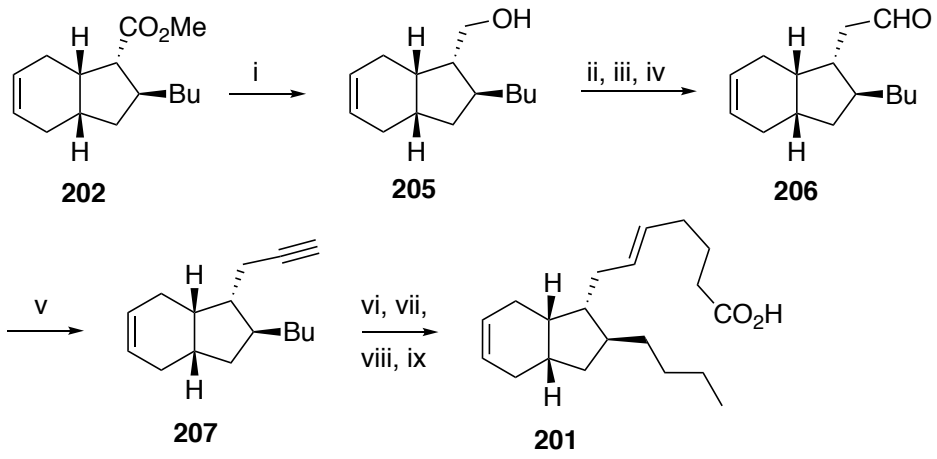
It was expected that the compound was the thermodynamic product. This was verified by subjecting the compound to equilibrating conditions, only to return the material unchanged. Thus, supporting our postulation.



203 **202**

Scheme 48 Conjugate addition. Reagents and conditions: (i) CuI, TMSCl,, THF, -35°C, BuMgCl, 2h (81%).

Taking advantage of this divergent strategy, we prepared the diastereomer of the suggested structure of mucosin, inverting the stereochemistry on C-8 and C-16. The data for this compound was distinctly different from those reported for the natural product. The synthesized compound is therefore not the correct structure for mucosin.



202 **205** **206**

207 **201**

Scheme 49 Final steps of synthesis of diastereomer of *exo*-mucosin (201). Reagents and conditions: (i) DIBAL-H in hexane, 0 °C to rt, 0.5 h, then Rochelle´s salt (aq., satd.), 0 °C; (ii) Et_3N in CH_2Cl_2, followed by MsCl, briefly 0 °C then rt, 2 h; (iii) KCN in DMSO, 70 °C, 2 h; (iv) DIBAL-H in hexane, - 78 °C, 0.33 h, then Rochelle´s salt (aq., satd.) in the cold; (v) Ohira-Bestmann reagent and K_2CO_3 in MeOH, rt, overnight; (vi) DIBAL-H added to Cp_2ZrCl_2 in THF, 0°C, 1h, then14,0°C,1h; (vii) I_2 ,1h; (viii) 4-Ethoxy-4-oxobutylzinc bromide and $(Ph_3P)_4Pd$ (10 mol%), rt, 1 h, then HCl (1 M, aq.); (ix) LiOH in THF/MeOH/H_2O, rt, 3 h.

We prepared single crystals, as described for the proposed structure of mucosin (*vide supra*), for structural analysis by X-ray diffraction, see Figure 15. This confirmed our hypothesis.

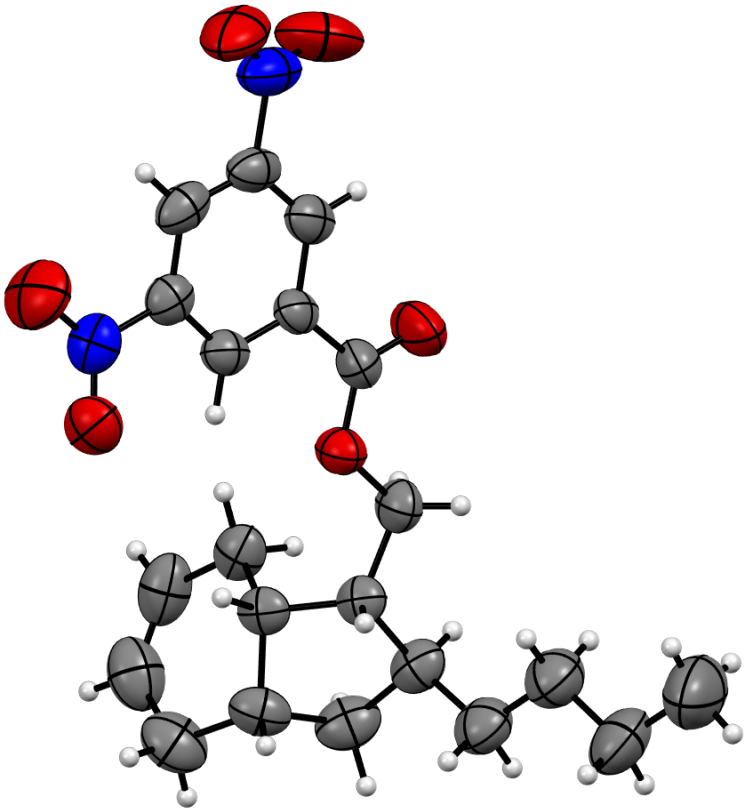


Figure 15 Single-crystal X-ray diffraction to show the relative stereochemistry of *exo*-mucosin.

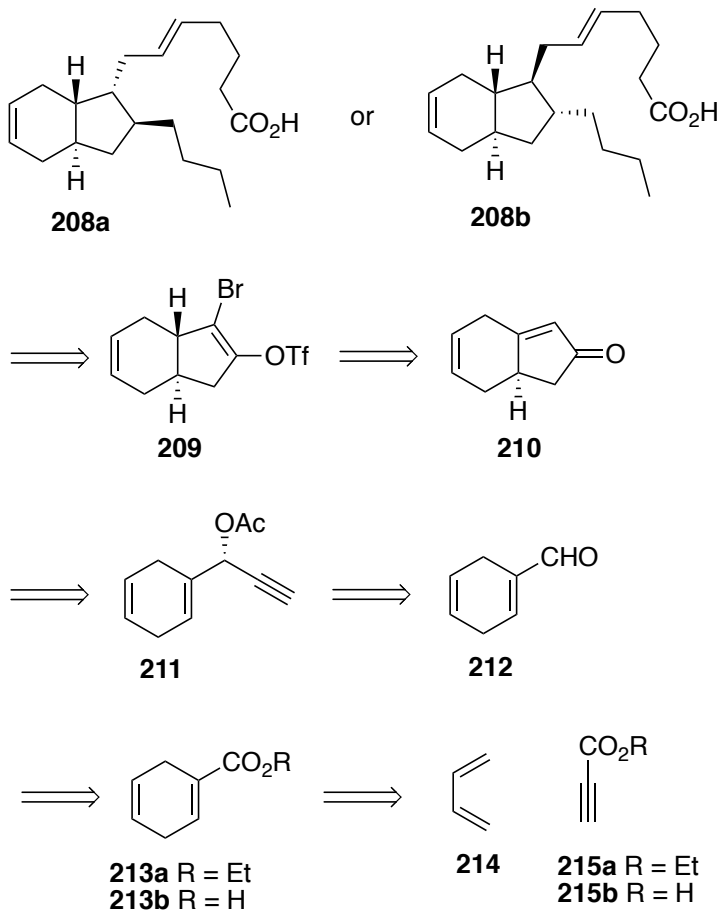
Yet, in terms of the *cis*-fused bicycle, there are permutants still unaccounted for. However, given the obvious sterical encumbrance of the two remaining *syn*-diastereomers, they seemed unlikely candidates considering the biogenesis of marine carbocyclic oxylipins.[180]

5.7 Preparation of *trans*-bridgehead stereoisomers:

The remaining four stereoisomers of mucosin require the preparation of *trans*-fusion over the bridgehead. That demands a different strategy.

One possible route for, two of the four diastereomers, is depicted in Scheme 50. Both isomers can be formed from **209** via cross-coupling reactions. Compound

209 can be prepared from enone **210** via α-bromination[181] with subsequent treatment with *L*-selectride and *N*-Phenyl-*bis*(trifluoromethanesulfonimide).[182]



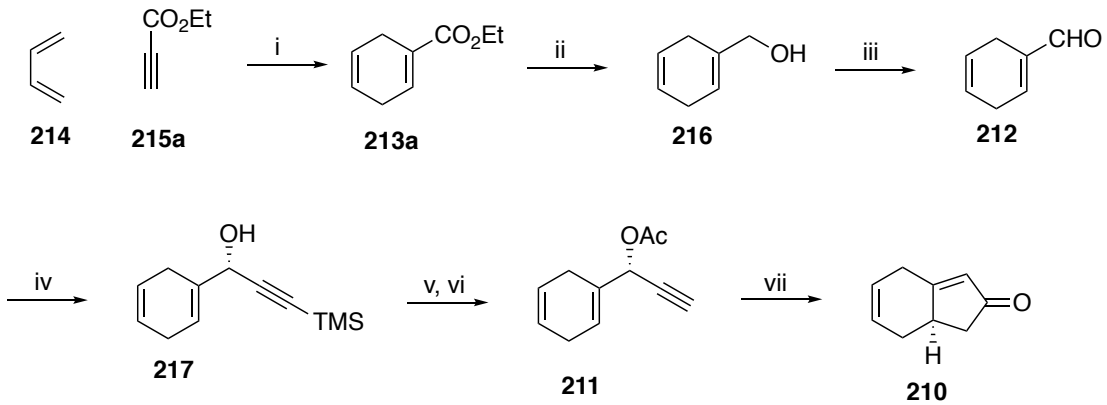
Scheme 50 Retrosynthesis of new strategy

Enone **210** can be prepared via a Rautenstrauch rearrangement from propagyl acetate **211**.[183] Alkynylation of aldehyde **212**, would give **211**. Aldehyde **212** can be prepared from 1,3-butadiene (**214**) and *e.g.* ethyl propiolate (**215a**) or propiolic acid (**215b**) via Diels-Alder reaction.

The Diels-Alder reaction of **214** and **215** resulted in ester **213a**. Ester **213a** was reduced employing $LiAlH_4$, with a subsequent Dess-Martin oxidation. We observed a large amount of benzaldehyde in addition to the desired aldehyde **212** in our first and only trial.

As the mild conditions of Dess-Martin oxidation of alcohol **216** were enough to aromatize, we decided that we needed another route avoiding oxidation.

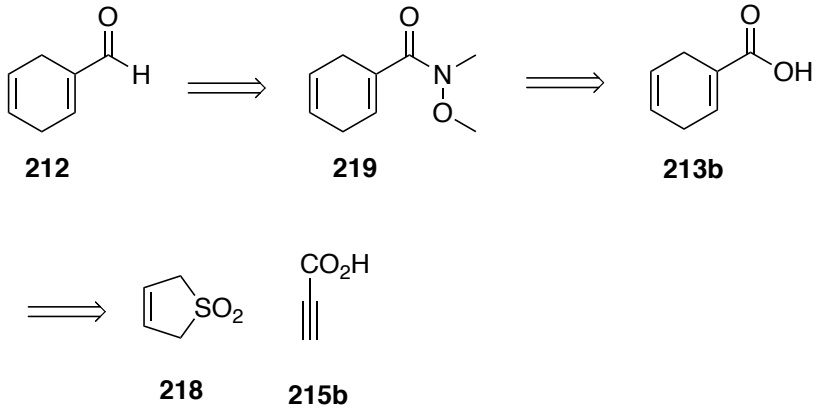
An alternative to the present strategy would be capitalizing on Birch reduction of the appropriate aromatic precursor, but this was not tested because of the problems we encountered.



Scheme 51 Synthesis towards 210. Reagents and conditions: (i) $SnCl_4$, neat, 0°C, 60h; (ii) $LiAlH_4$, Et_2O, rt, 1h; (iii) DMP, CH_2Cl_2, rt, 12h; (iv) TMS-acetylene, BuLi, THF, -78°C to rt, 3h; (v) K_2CO_3, MeOH, rt, 12h; (vi) Ac_2O Et_3N, DMAP, CH_2Cl_2, rt, 3h.

Aldehyde **212** was reacted with trimethylsilylacetylene to give **217**. Compound **217** was desilylated and protected as the acetyl-derivative. We tested a palladium dichloride catalyzed Rautenstrauch rearrangement, but in our first and only attempt only trace amounts of the target molecule **210** was found.

As an alternative to the depicted Diels-Alder reaction, sulfolene (**218**) can be used as an *in situ* precursor for 1,3-butadiene. At approximately 100^{0}C sulfolene will be transformed into SO_2 and 1,3-butadiene via a cheletropic reaction.

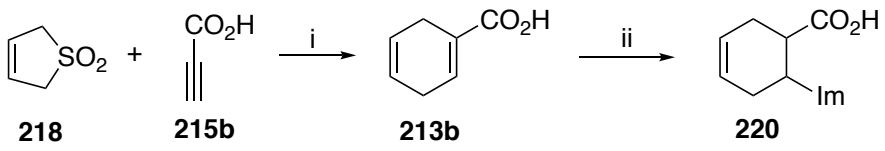


Scheme 52 Reduction of N,O-dimethylhydroxyamide 219.

In order to avoid the oxidation, an optioned is to transform the acid **213b** into the corresponding Weinreb amide **219** followed by reduction. It is known that these amides can be reduced directly to the aldehydes.

Diels Alder reaction with propiolic acid (**215b)** and **218** as the diene precursor gave the acid **213b** in 50% yield unoptimized. The starting acid (**215b**) was also recovered, and we assume that the reaction can be improved substantially. In the literature, xylene is used for this type of reactions, as boiling point is a slightly higher.

We decided to employ a constellation of CDI/*N,O*-dimethylhydroxylamine hydrochloride for the preparation of the amide **219**. We have successfully employed this protocol on several other systems, but in the present case it was unsuccessful. It seemed that the dominating reaction was a conjugate addition of imidazole to the acid. The current approach has been put on a hold in order to pursue a more divergent strategy based on our previous approach (*vide infra*) .

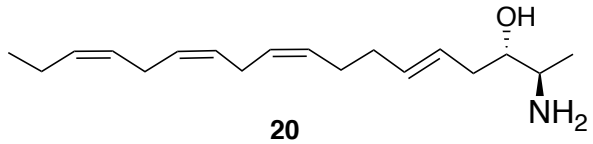


218 **215b** **213b** **220**

Scheme 53 Attempt on transforming acid 213b into corresponding Weinreb amide 220. Reagents and conditions: (i), toluene, slow warming to reflux, 48h (50%); (ii) CDI, DCM, N,O-dimethylhydroxylamine hydrochloride.

6. Crucigasterin 277

6.1 Introduction:



20

Figure 16 Crucigasterin 277

Crucigasterin 277 (**20**) was isolated from the Mediterranean tunicate *Pseudomonas crucigaster*, along with related structures crucigasterin 275 and 225.[33] The numbers refer to the molecular mass of the compounds. Crucigasterin 277 was isolated as the diacetyl derivative.

The structure itself is a C_{18} polyunsaturated sphingolipid. The α-end contains a 2-amino-3-ol moiety, indicating that it is derived from *D*-**17**, rather than *D*-**16**. The absolute stereochemistry of the 2-amino-3-ol moiety was determined based on chiral GC analysis with a chiral column in addition to NMR spectral data of the ozonolysis product of a 2-amino-3-hydroxypentanoic derivative prepared from the natural product itself. This derivative was compared to the four possible isomers prepared from *L*-**17**. This suggested that the compound is derived from *D*-**17**. Absolute stereochemistry was found to be 2*R*,3*S*.

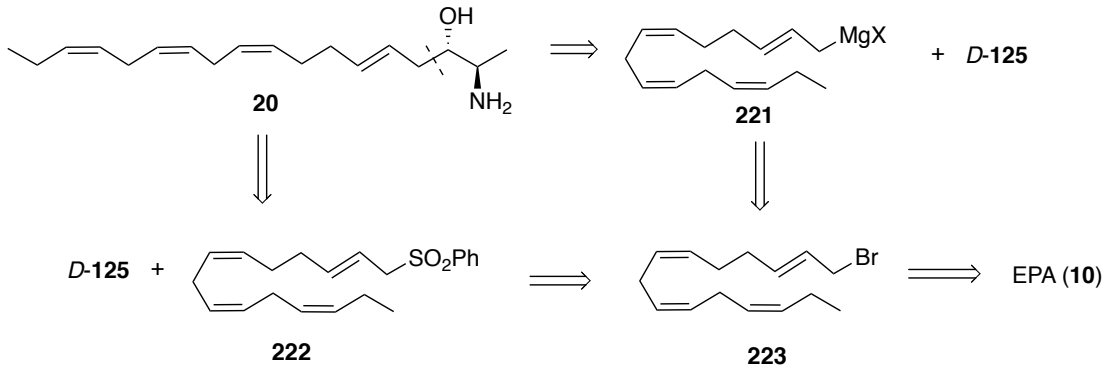
The structural makeup of the C_{18}-framework consists of four double bonds. Starting from the ω-3 terminus, three of these are methylene-interrupted double bonds with *Z*-configuration, while the final double bond is spaced by an ethylene unit and is *E*-configured. The *E*-configured double bond is homoallylic to the hydroxyl group.

6.2 Retrosynthesis

There are already several syntheses of sphingolipids and other amino alcohols found in literature, but no published synthesis of **20** at the time this project was initiated. As many sphingolipids have been synthesized, a closer inspection of the chosen strategies seems prudent.

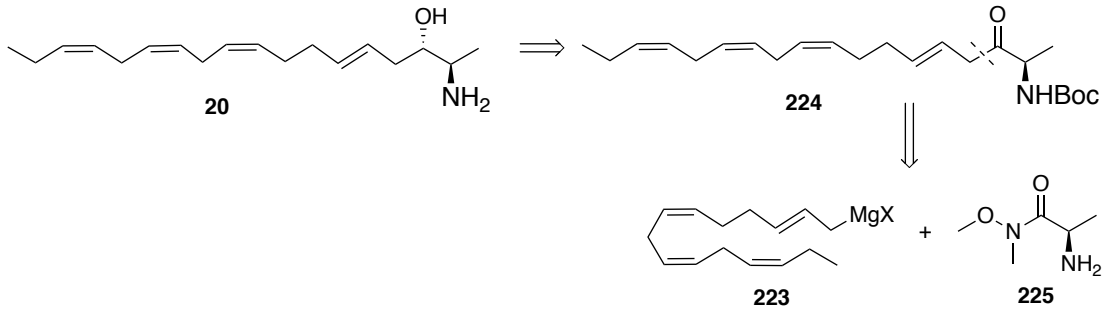
Many published syntheses found in the literature rely on a chiral pool strategy; a disconnection between C-3 and C-4 gives a C_{15}-fragment, and a fragment derived from amino acid. The most straightforward approach would be an alkyl magnesium halide with either *R*-**124** or *D*-**125**, as employed in several protocols discussed on page 25.

One possible reagent for the C_{15}-fragment is the corresponding allylic magnesium halide. These are known to be difficult to prepare,[184] but there are alternative ways to activate the metal *e.g.* Rieke metals[185,186] and sonication[187]. There are also alternatives avoiding organometallic chemistry such as the use of sulfones, as α-sulfonyl carbanions are used as carbon nucleophiles. Both the organometallic compounds and the sulfones can be prepared from C_{15}-bromide **223**, known in literature.[176]



Scheme 54 Retrosynthesis of crucigasterin 277.

Employing a chiral pool approach will obviously control the stereochemistry at C-2, but we will also need a way to control the outcome on C-3. One way to do this would be stereoselectivity reducing amino ketone **224**. Amino ketone **224** can be prepared by either oxidizing the mixture of amino alcohols formed in the nucleophilic attack on *D*-**125** depicted in Scheme 54, or via a similar reaction on the Weinreb amide **225**, as shown in Scheme 55.



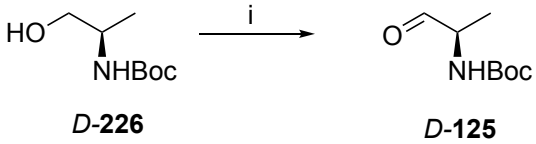
Scheme 55 Retrosynthesis employing Weinreb amide 225.

6.2 Synthesis

The first step was preparing the amino acid derived moiety. Beginning with the corresponding aldehyde seemed to be a logical starting point, but the Weinreb amide would also be a good option, as mentioned in the retrosynthesis.

There are several published procedures on how to prepare *D*-**125**. Most of these are reductions of alaninate or oxidation of *N*-BOC *D*-alaninol (*D*-**226**). Both *D*-**226** and methyl alaninate are commercially available.

Racemization of the amino group is a challenge with α-amino aldehydes. Fortunately, *D*-**125** is a solid, and short reaction times and work up give a structure stable towards racemization. Several methods were tested, [33,188-192] and eventually the Parikh-Doering oxidation, shown in Scheme 56 was found to be the most suitable. Little or no racemization was observed.[193]



D-**226** *D*-**125**

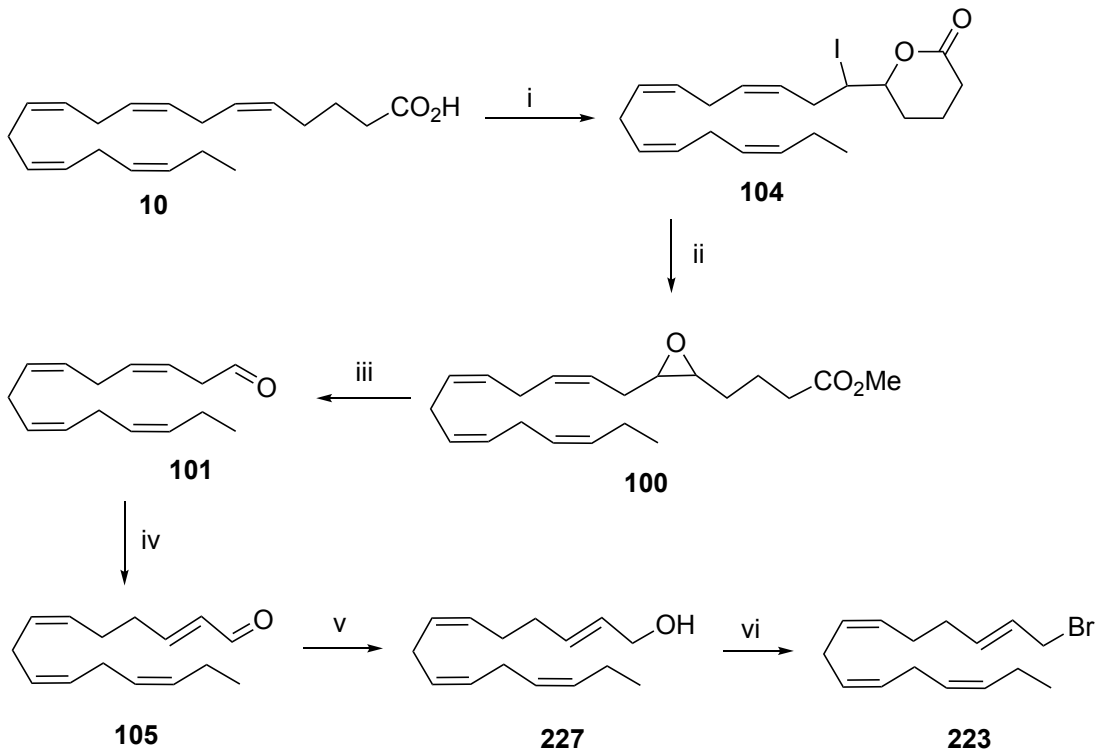
Scheme 56 Parikh-Doering oxidation of *D*-226 to *D*-125. Reagents and conditions: (i) Pyridine-SO_3, DMSO, Et_3N (63%).

6.3 Organometallic couplings

With the *D*-**125** in hand we turned towards the ω-fragment. From the **103**, we employed procedures previously published by our group. Hydrolysis of the ester followed by iodolactonization was done in one-pot with excellent yield. The resulting iodolactone **104** can be opened to yield the epoxide methyl ester **100**, using K_2CO_3 in MeOH. This epoxide was treated with periodic acid in ether followed by isomerization to the α,β-unsaturated aldehyde **105**. Aldehyde **105** was reduced to the corresponding alcohol **227** and a subsequent Appel type reaction gave bromide **223**. This synthetic pathway is presented in Scheme 57.[124,176]

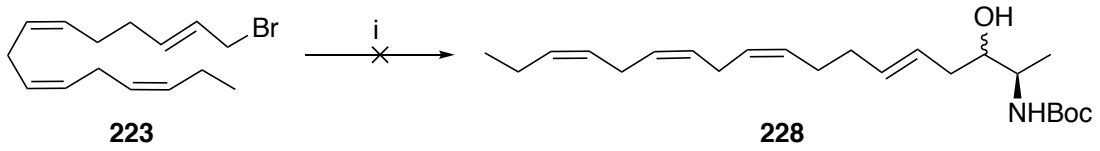
With the C_{15} allylic bromide **223** in hand, our first attempt was to prepare the magnesium bromide, and react this with *D*-**125** using standard Grignard conditions. However, the mixture mainly provided the Wurtz dimerization product, rather than the expected mixture of stereoisomers of C_{18} amino alcohol **228**. The same result was observed for the Weinreb amide.

We also attempted to prepare the lithium equivalent and use Barbier conditions with tin and zinc. Sonication was employed for activation of the metals, but this did not lead to the desired compound either.[194]



Scheme 57 Preparation of bromide 223. Reagents and conditions: (i) HI, $KHCO_3$, I_2, THF/H_2O (95%); (ii) K_2CO_3, MeOH (91%); (iii) H_5IO_6, Et_2O; (iv) DBU/Et_2O (40% over two steps); (v) $NaBH_4$, MeOH (82%); (vi) Ph_3P/Br_2, CH_3CN (85%).

Rieke's method of activating metals was also tested since this has been reported to give reaction and hence diminish the formation of the Wurtz product.[184] None of these attempts led to the desired product.[195,185,186]



Scheme 58 Failed attempts on organometallic chemical coupling reactions. Reagents and conditions: (i) Metal (Li, Mg, Sn, Zn), *D*-125.

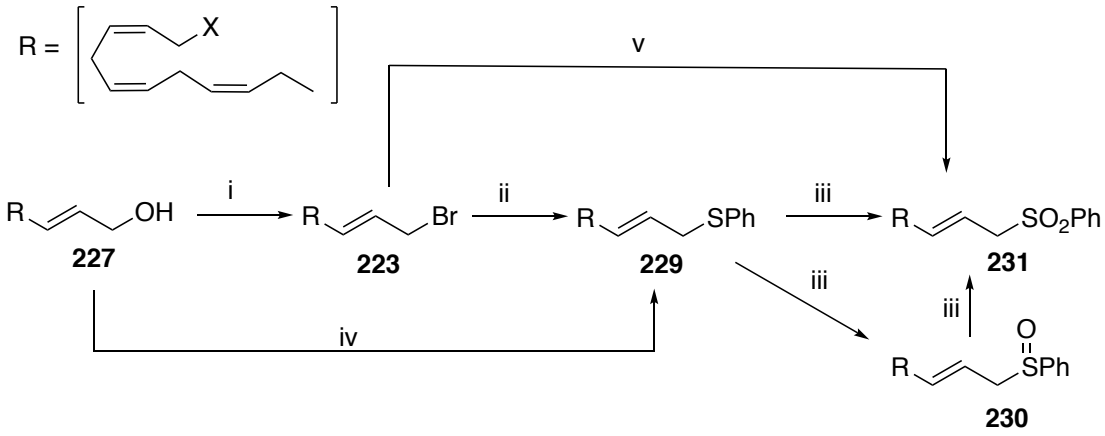
6.4 Sulfone approach

Given these negative results, we decided to prepare the sulfone from the bromide. Similar reactions, with sulfone carbanions acting as nucleophiles on aldehydes, have been reported.[196-199]

Bromide **223** was reacted with thiophenol to yield the corresponding sulfide **229** in good yield.[200] Alternatively, the sulfide can be made in one step from the

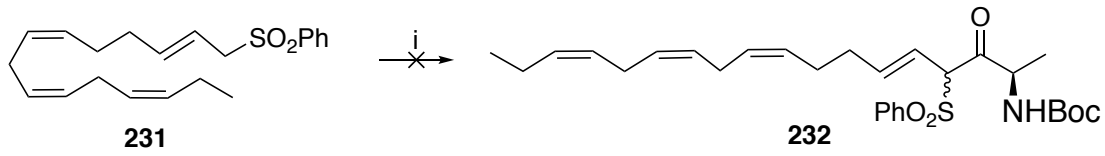
alcohol by reaction with diphenyl disulfide.[201] Both reaction time and yield are approximately the same for the two alternatives. This sulfide was easily oxidized to the sulfoxide **230** by a stoichiometric amount of oxone™, or all the way to the sulfone **231** by adding two equivalences of oxone™.[200]

We discovered that we could improve the yield by reacting the bromide **223** with $NaSO_2Ph$ to yield **231** directly, as presented in Scheme 59.[202]



Scheme 59 Different approaches towards sulfone XX. Reagents and conditions: (i) Ph_3P/Br_2, MeCN (85%); (ii) PhSH, LiOH, MeOH (93%); (iii) Oxone™, MeOH (62%); (iv) $(PhS)_2$, pyridine, Bu_3P (75%); (v) $PhSO_2Na$, DMF, 80°C, 15h (85%).

Sulfone **231** was treated with *n*-BuLi to give the α-sulfonyl carbanion, with subsequent addition of *N*,O-dimethylhydroxyamide **225**, as this would result in a ketone that can be selectively reduced. The sulfonyl keto amine **232** was not observed, but the unreacted sulfone was isolated.



Scheme 60 Attempts on formation of compound 232. Reagents and conditions: (i) *n*-BuLi, -78^{0}C, THF, compound *D*-225.

The reaction with the α-sulfonyl carbanion and *D*-**125**, however, resulted in four diastereomers of the sulfonyl hydroxylamine as shown in Scheme 61. It would be preferable to oxidize the hydroxyl group into the corresponding ketone, followed by stereoselective reduction to the desired isomer, as done by *e.g.* Thirupathi and

co-workers on the synthesis of clavaminol A.[136] Preliminary results proved this difficult, as isomerization to the conjugated system occurred under both the oxidation and subsequent reduction. For this reason, the strategy was abandoned.

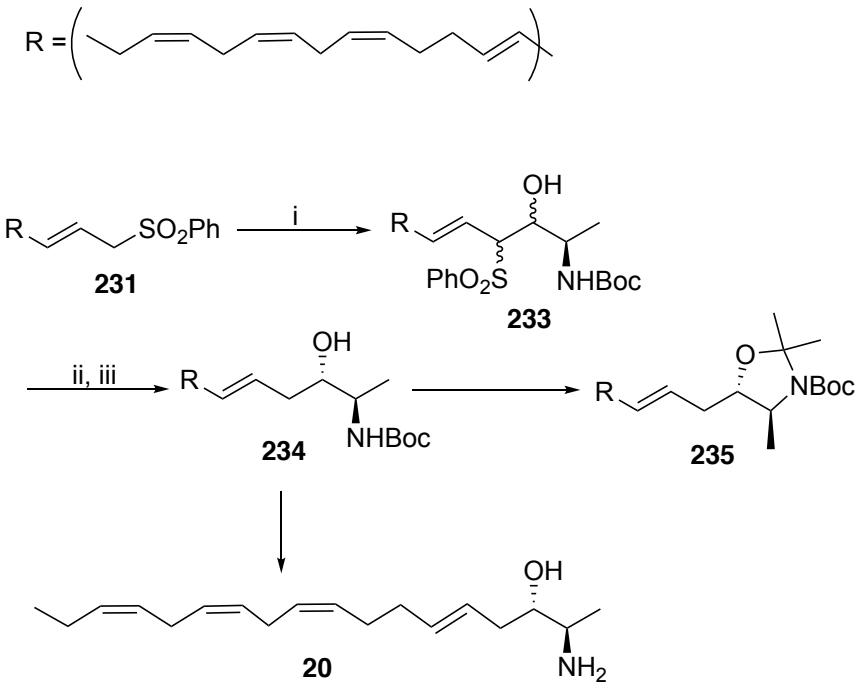
Eventually, we reacted the mixture of diastereomers with $LiBH_3CN$ and $Pd(Ph_3P)_2Cl_2$, an effective protocol for the reductive elimination of allylic sulfones.[203,204]

Separation of the two amino alcohol stereoisomers of **234** did not work either, as we experienced complete co-elution. Eventually, we decided to separate the four diastereomers of sulfone amino alcohol **233**, formed in the previous step. During the purification on column chromatography, 2:1 ratios of two of the four were separated, while the remaining two co-eluted.

In order to improve the overall yield, we investigated whether the sulfoxide **230** could be used instead of sulfones, as the polarity is a bit different and possibly the separation would improve. Unfortunately, this led to all four isomers co-eluting.

To establish the configuration of the two epimers from desulfonation, both epimers of **234** were converted to their respective 1,3-oxazolidines (**235**). NOESY and ROESY experiments showed a clear correlation for protons at C-1 and C-4 with the *cis* configuration between amino and hydroxyl group, and no correlation for the *trans* configuration. These experiments proved the minor isomer to be the desired *trans* isomer.[205]

Removal of the BOC protection group using standard conditions (TFA or HCl) led to isomerization.[206] It was discovered that stirring in 80% formic acid at room temperature gave the desired final product without affecting the double bonds. The amino alcohol **20** was isolated and transformed into the diacetyl derivative and found comparable to the literature value.[33] Hence, the first synthesis of **20** was completed. Total yield is 7% over 10 steps.



Scheme 61 Remaining synthesis of crucigasterin 277. Reagents and conditions: (i) *n*-BuLi, -78^{0}C, THF, compound *D*-125, (69%); (ii) $LiBH_4$, $Pd(Ph_3P)_2Cl_2$, 25^{0}C; (iii) separation on flash column; (iv) 2,2-dimethoxypropane, *p*-TsOH, PhH, Δ; (v) 80% formic acid.

7. Asymmetric synthesis of obscuraminol A and towards crucigasterin 277

7.1 Introduction:

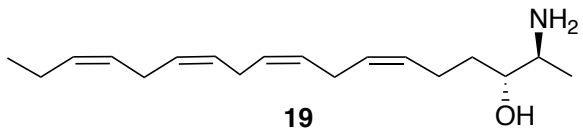


Figure 17 Obscuraminol A

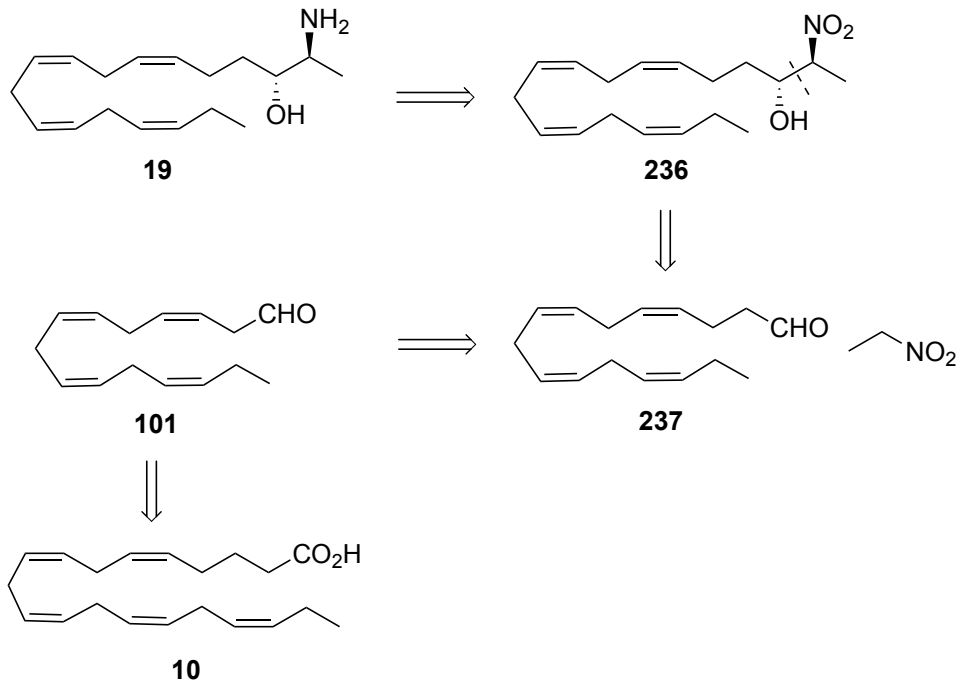
Structural investigation of **19** reveals some differences from **20**. First of all, the configuration of the amino alcohol moiety is *anti*, but with the opposite absolute stereochemistry, 2*S*,3*R*. Second, all the double bonds in **19** have *Z*-geometry, and are skipped, in contrast to **20** with its one *E*-alkene moiety. A consequence of this is that the hydroxyl group in obscuraminol is *bis*-homoallylic, compared to the homoallylic hydroxyl group in **20**.

The homoallylic C_{15} alkyl magnesium halide needed for the synthesis of **19** is already found in literature.[207] It would probably be possible to do the selective reduction of the α-amino ketone as well. Despite this, we wanted to attempt asymmetric synthesis using a nitro aldol reaction.

7.2 Asymmetric strategy retrosynthesis

Vicinal amino alcohols can be prepared employing nitro aldol reaction with subsequent reduction of the nitro group. The disconnection between C-2 and C-3, that corresponds to the nitro aldol reaction, gives nitroethane and a C_{16}-aldehyde **236** found in literature, prepared from C_{15}-aldehyde **101**.[122]

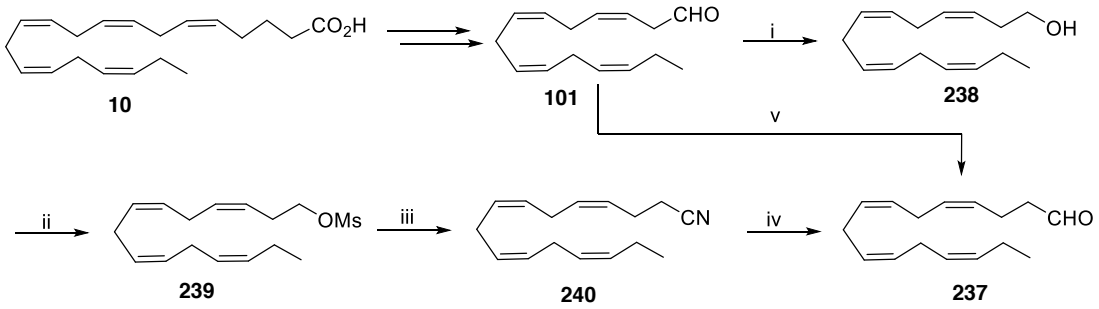
The main challenges in this strategy was to find a suitable catalyst for the asymmetric Henry reaction and reduction of the nitro alcohol to the corresponding amino alcohol. The retrosynthesis is depicted in Scheme 62.



Scheme 62 Retrosynthesis of obscuraminol A

7.3 Synthesis

Aldehyde **101** was prepared using literature procedures. PUFA **10** was transformed into alcohol **238**, with subsequent homologation by mesylation, cyanation and DIBAL-H reduction to C_{16} aldehyde **237**.[122]

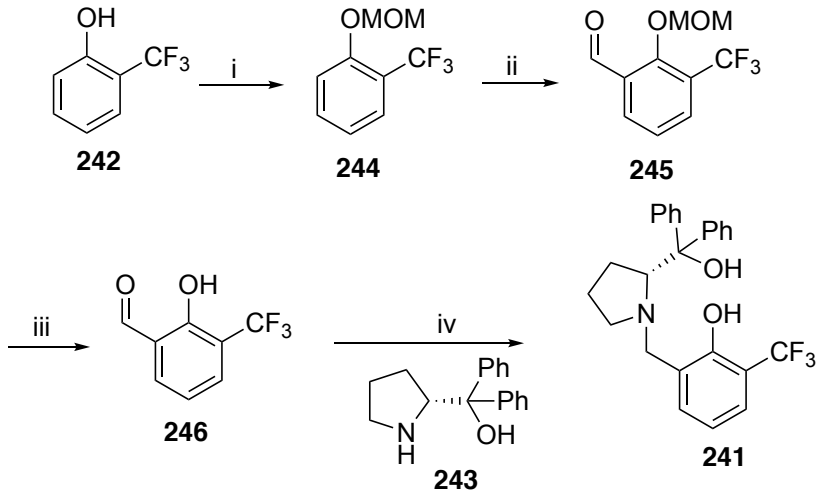


Scheme 63 Synthesis of C16 aldehyde. Reagents and conditions: (i) $NaBH_4$, MeOH, 0°C; (ii) MsCl, CH_2Cl_2, TEA; (iii) NaCN, DMSO, 80°C (60% from 238); (iv) DIBAL-H, hexane, 0°C (66%); (v) $MeOCH_2PPh_3Cl$, *t*-BuOK, overnight; (vi) 80% HCO_2H, overnight (40% from 101).

Aldehyde **237** can also be prepared via a Wittig reaction with aldehyde **101** and methoxymethylenetriphenylphosphine with a subsequent hydrolysis of the enol ether moiety. However, the total yield is approximately the same as for the longer route described above (approximately 40%). The total yield from **10** was 29%.

For our system, we need an *anti*-inducing catalyst for the Henry reaction. The most promising catalysts had been developed by Wang *et al.*[115] and Ooi *et al.*[108] We chose the former, a system catalytic system (**92**) consisting of proline-phenol ligand **241** and copper (II). This system is reported to yield high diastereoselective catalytic activity (above 15:1 in most cases). Another advantage is that the catalyst is available in both enantiomeric forms.

The ligand was prepared in four steps from commercially available 2-trifluoromethylphenol (**242**) and *R*-α,α-diphenyl-2-pyrrolidinementhol (**243**), as seen in Scheme 64.[115,208,209]



Scheme 64 Preparation of the Wang catalyst. Reagents and conditions: (i) NaH (86%); (ii) MOMCl; (iii) *n*-BuLi; (iv) DMF; (v) AcOH, MeOH (43% from 242); (vi) $NaBH_4$, EtOH (64%).

The *R*- and *S*-epimers of **241** should provide the (2*S*, 3*R*) and (2*R*, 3*S*) configuration of the nitroalcohol as the major diastereomers, respectivly.[177]

7.4 Nitroaldol reaction

The nitro aldol reaction was initially performed with 5 mol% *R*-**241**, 5 mol% $CuBr_2$, 7.5 mol% Cs_2CO_3, 10 equivalents of nitroethane in THF. This gave the desired nitro alcohol **236** in 23% yield and 15.6:1 *anti/syn* ratio after 24 hours reaction time.

Doubling the reaction time gave a drop in diastereomeric ratio to 9.6:1, but interestingly, also led to an improvement in optical purity of the major diastereomer (62% *ee* vs. 56% *ee*).

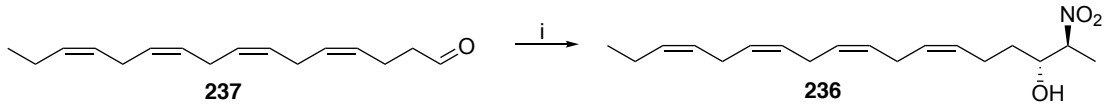
Increase of temperature led to better yield, but a decrease in both diastereomeric ratio (7.3:1 at -10 ^{0}C and 3.4:1 at -5^{0}C) and enantiomeric excess (approximately 40%).

Based on these observations, we assumed that this was due to epimerization of the nitro group of the kinetic (2*R*,3*S*)-**236**.

Based on Wang's proposed mechanism, this isomer was formed though the least favored transition state, in the catalytic reaction pathway.[115] That means that it

was formed via a non-catalytic pathway in the initial stage of the reaction. To decrease the non-catalyzed side reaction, the catalyst-load was doubled, to 10 mol%. It was also discovered that it was of importance that THF/*R*-**92** and nitroethane was pre-cooled to -15^{0}C prior to aldehyde addition.

Reaction time of 120 hours at -15^{0}C gave the thermodynamic nitro-alcohol (2*S*, 3*R*) **236** in 94% yield, 11.5:1 diastereomeric ratio and 82% *ee* of the major diastereomer. The reaction is shown in Scheme 65.

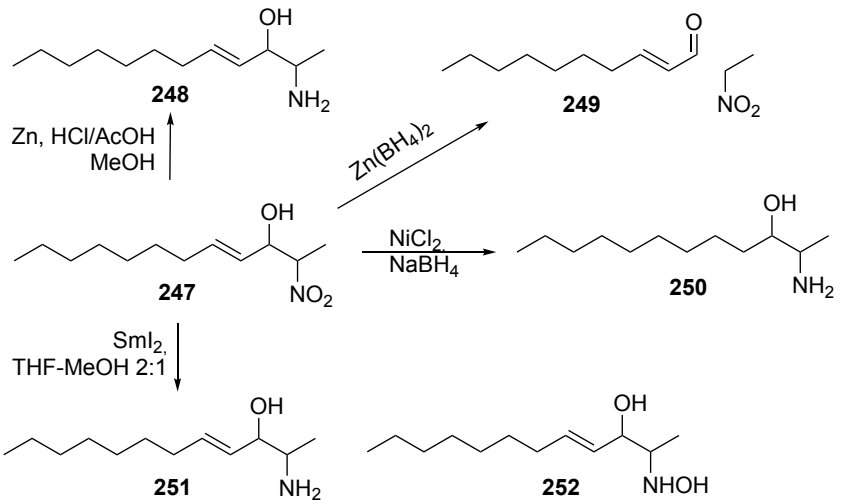


Scheme 65 Henry reaction on C_{16} aldehyde 237. Reagents and conditions: (i) $CH_3CH_2NO_2$ (10 eq), *R*-246 (10 mol%), $CuBr_2$ (10 mol%), Cs_2CO_3 (15 mol%), THF, -15°C, 5 days (94%, 11.5:1, 82% ee).

7.5 Reduction of the nitro group

It was soon discovered that the reduction of the nitro group, without affecting the stereochemistry, was a major challenge. In the syntheses of spisulosine (**18**)[115] and xestoaminol C (**150**),[108] Pd/C in H_2-atmosphere was used, but this would be useless for our unsaturated system.

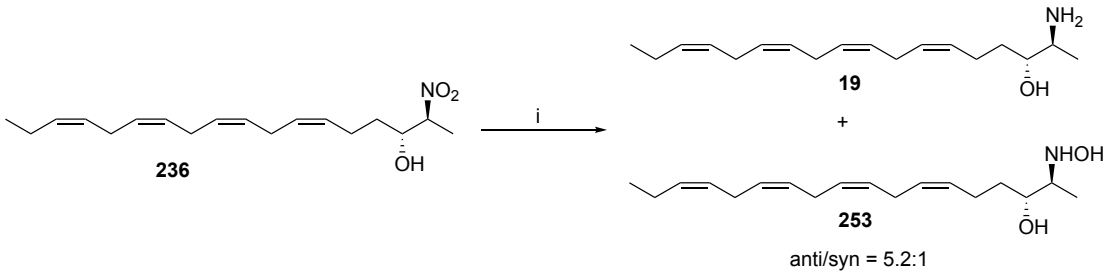
Different reducing agents and conditions were screened on inexpensive, racemic (*E*)-2-nitrododec-4-en-3-ol as depicted in Scheme 66.



Scheme 66 Test reactions on nitro reduction

$NaBH_4/NiCl_2$ resulted in reduction of both the nitro group and the double bond.[210] $NaBH_4/ZnCl_2$ resulted in an inseparable complex mixture including starting material, retro-nitro aldol product **249** and target molecule **248**.[211] Zinc powder in acidic media, HCl/AcOH, in methanol gave **248**, albeit in low yield.[212] A procedure with $SiCl_3H$ was also tested, but neither this gave the desired target molecule.[213]

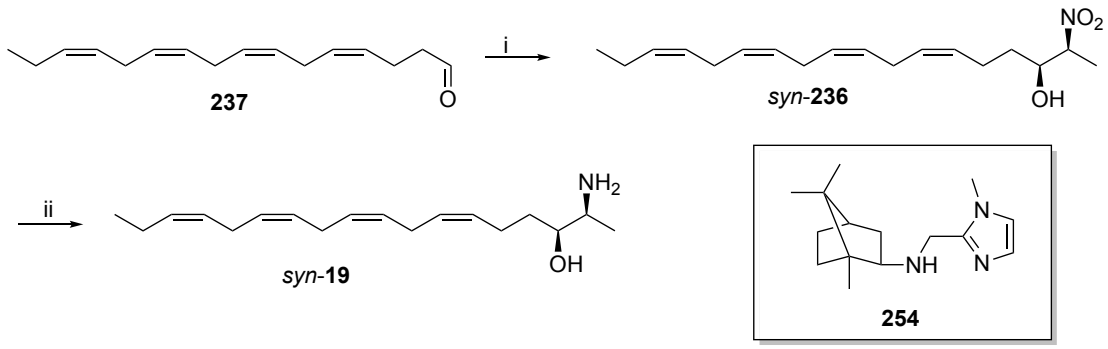
Finally, we found a reduction protocol employing SmI_2 in THF. The nitroaldol **236** was converted to a mixture of amino alcohol **19** and the corresponding hydroxylamine (**253**) as the major byproduct, as shown in Scheme 67. Purification was achieved by column chromatography to give the desired amino alcohol in 59% yield.[214,215]



Scheme 67 Reduction of nitro alcohol 19 with SmI_2. Reagents and conditions: (i) SmI_2, THF-MeOH 2:1, rt (60%).

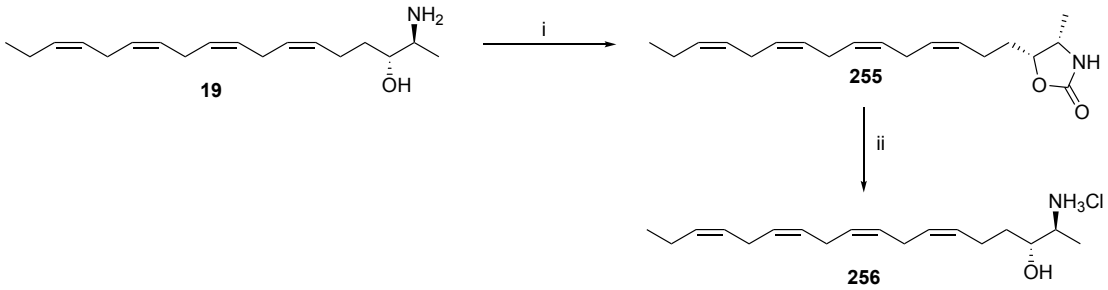
However, it was also discovered that the diastereomeric ratio had dropped to *anti*/*syn* 5.2:1. This can partly be explained by a *syn*-favorable transition state controlled by chelation of Sm(III) with Lewis basic functionalities, rather than by epimerization of the nitro group on an intermediate stage of redution.[216]

In order to evaluate this hypothesis, syn-enriched nitro-alcohol was prepared employing *syn*-selective catalyst **254**,[116] giving the *syn* nitro aldol product *syn*-**236** in 1:2.8 *anti/syn* ratio. SmI_2 reduction gave the resulting amino-alcohol *syn*-**19** with slightly improved *anti/syn* ratio of 1:4, thus strengthening our hypothesis.



Scheme 68 Reagents and conditions: (i) $CH_3CH_2NO_2$ (10 eq), cat. 254 (*anti:syn* 1:2.8). (ii) SmI_2, THF-MeOH 2:1, rt (*anti:syn* 1:4).

Separation of the *syn* and *anti* isomers of **19** from the *anti*-selective Henry reaction was impossible at this stage. The mixture was treated with carbonyl diimidazole (CDI) to yield the corresponding oxazolidinones (**255**), as shown in Scheme 69.



Scheme 69 Preparation of hydrochloride 256 and oxazolidinone 255 for comparison to spectral data. Reagents and conditions: (i) Im_2CO, THF, heat; (ii) 1M KOH, EtOH-H_2O 1:1 (63%); (iii) HCl gas.

NOESY and ROESY experiments were performed. A clear correlation for C-1-C-4 protons indicated a *cis*-configuration on the oxazolidinone, a confirmation that the original amino-alcohol contains the *anti*-configuration.

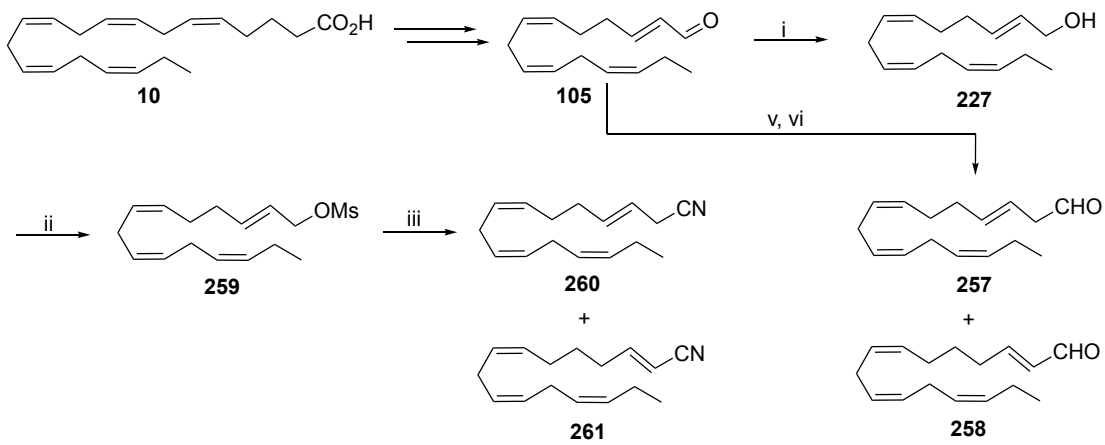
Hydrolysis of oxazolidinone **255** afforded the target molecule. Comparison of NMR data with that of the authentic natural product,[31] were not in accordance. Even though not shown in the reported structure, the data for the natural product is actually for the hydrochloride **256**.[31]

Pure oxazolidinone **255** was thus transformed into hydrochloride **256** by treatment with HCl gas. The resulting data was in accordance with literature value, thus proving the structure of obscuraminol A.

7.5 Asymmetric synthesis towards crucigasterin 277

After the success of synthesizing obscuraminol A (**19**), it was decided to employ the same strategy on crucigasterin 277 (**20**). First we used the same chemistry as described above for the synthesis of the C_{15} conjugated aldehyde **105**. The homologation to C_{16} was more challenging than for the non-conjugated system. The Wittig reaction with methoxymethylenetriphenylphosphine led to a mixture of aldehyde **257** and **258**. Separation of **257** and **258** was impossible. Some time was spent to find the right conditions, but the strategy was eventually abandoned.

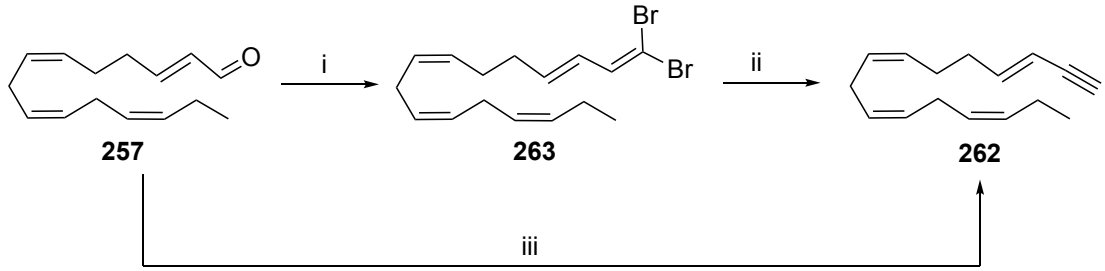
We decided to go via the nitrile as one of the alternatives. The mesylate **259** was formed in good yield. However, cyanation gave isomerization, and a mixture of nitriles **260** and **261** was obtained.[217]



Scheme 70 Synthesis of C_{16} aldehyde. Reagents and conditions: (i) $NaBH_4$, MeOH, 0^0C (82%); (ii) MsCl, CH_2Cl_2, TEA (81%); (iii) NaCN, DMSO, 80^0C; (iv) DIBAL-H, hexane, 0^0C; (v) $MeOCH_2PPh_3Cl$, *t*-BuOK, overnight; (vi) 80% HCO_2H, overnight.

Eventually we decided to transform aldehyde **105** to the terminal alkyne **262** and then hydroborate to give aldehyde **257**. The Corey-Fuchs protocol was employed. The formation of the dibromoolefin **263** exceeded. The second

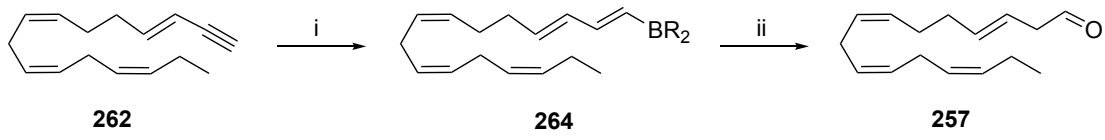
reaction, the Fritsch–Buttenberg–Wiechell rearrangement, resulted in alkyne **262** in 65% yield over two steps.[122,218]



Scheme 71 Synthesis of C_{16} alkyne via the Corey-Fuchs protocol and the Colvin protocol. Reagents and conditions: (i) Zn, PPh_3, CBr_4, CH_2Cl_2, rt; (ii) *n*-BuLi, Et_2O, -78^{0}C (65%); (iii) $TMSCHN_2$, *n*-BuLi, Et_2O/THF/hexane, -78^{0}C to rt (60%).

We did some experiments with the Colvin protocol, which gives the alkyne **257** in one pot.[219-221] The reaction worked well, and the yield is approximately 60%, but the purification was problematic. Eventually, we concluded that the two-step protocol by Corey and Fuchs was less laborious, and the Colvin protocol was abandoned.

The plan was to hydroborate-oxidize the alkyne to give aldehyde **257**. Some different boranes were tested to find a suitable system that will exclusively give the *anti*-Markovnikov product. Attempts with both dicyclohexylborane and 9-BBN were successfully carried out. A subsequent mild oxidation, employing sodium perborate of the organoborane, was found give aldehyde **257**.[222,223]



Scheme 72 Hydroboration of alkyne 259a. Reagents and conditions: (i) 9-BBN, ether, 0^{0}C; (ii) $NaBO_3$ x H_2O (3eq), 1:1 H_2O/THF (52% from 260)

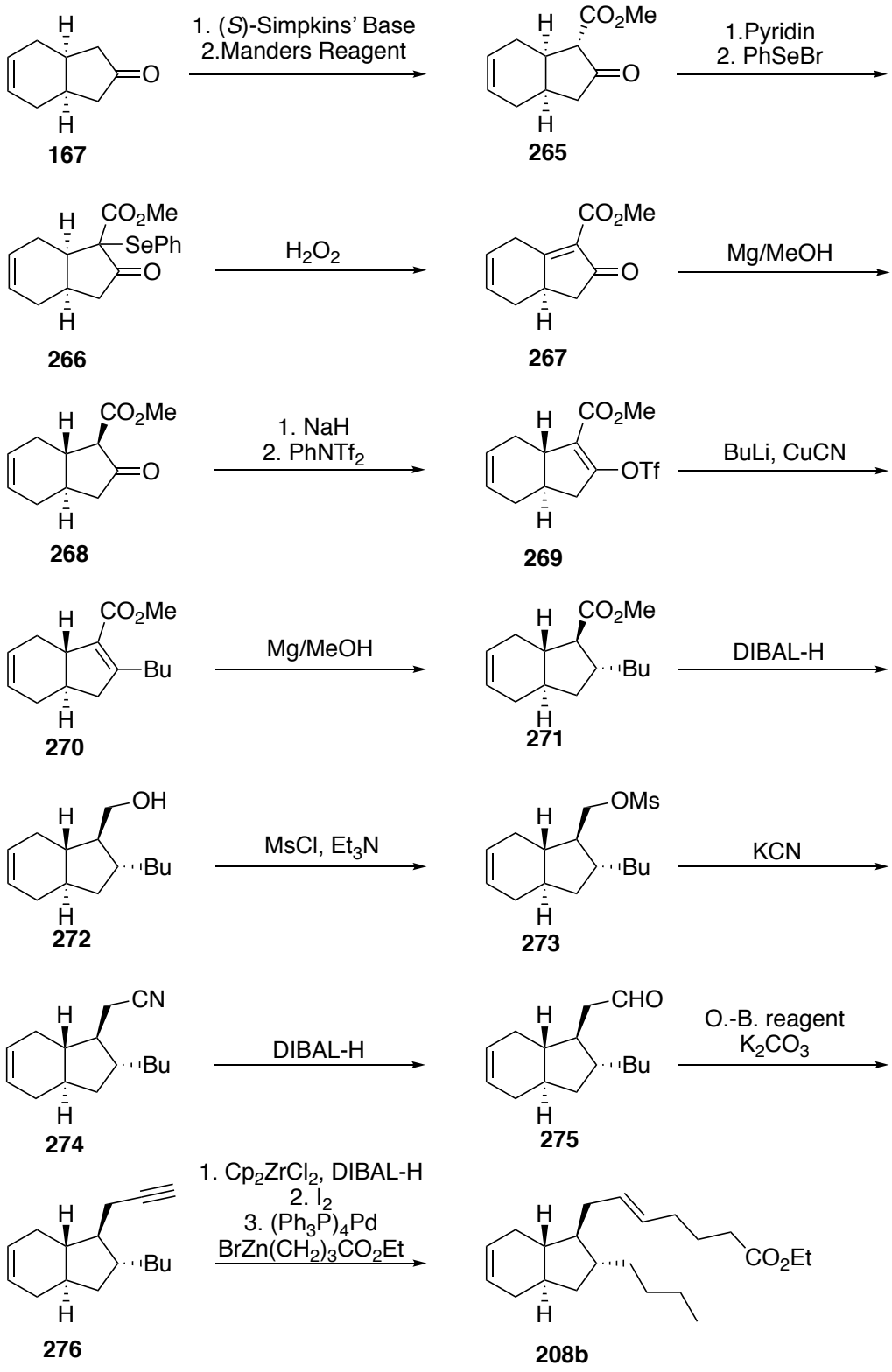
Due to time limitation, we were not able to finish the project.

8. Conclusion and future work

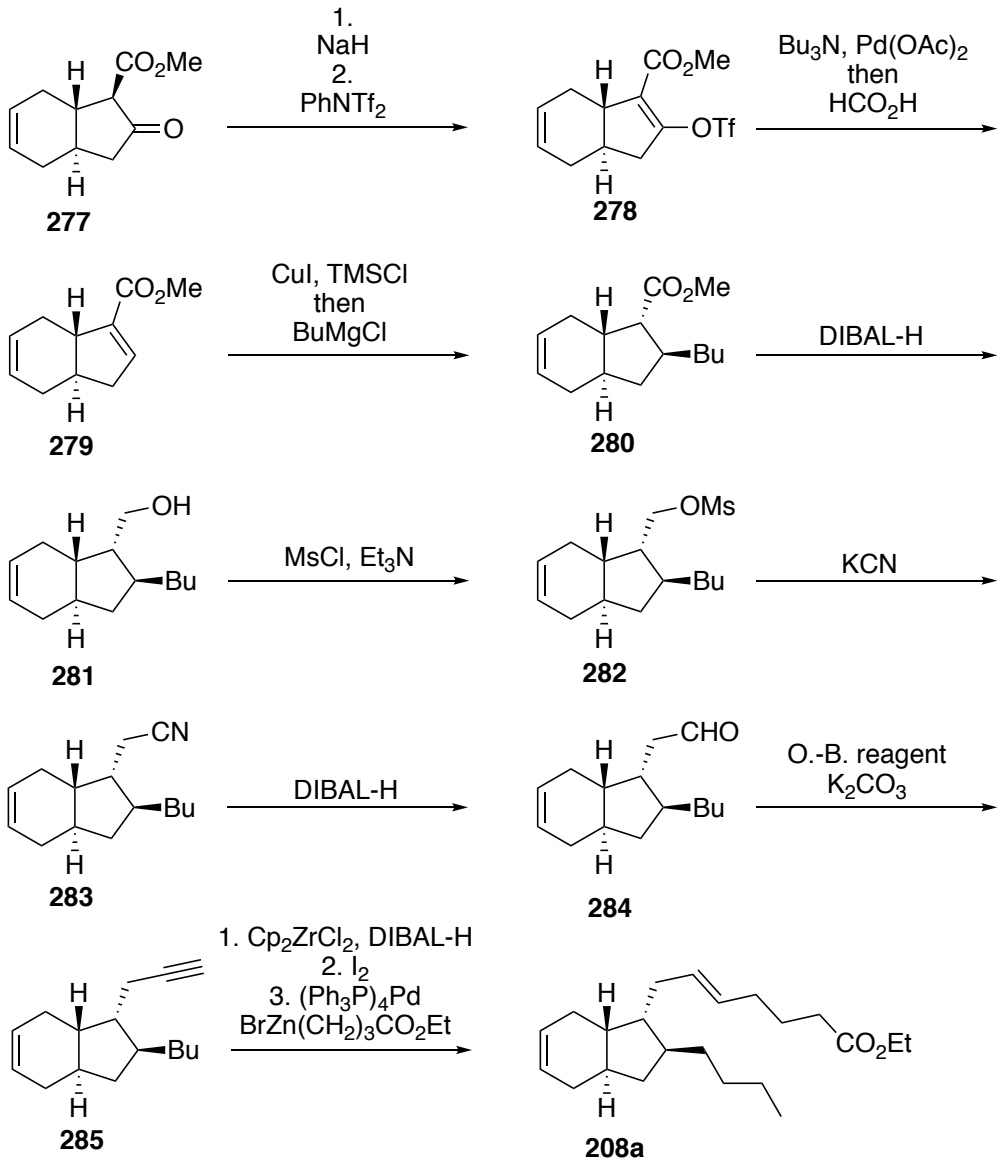
Our efforts towards mucosin gave us the molecule we aimed for, but it was found that the structure reported for mucosin is incorrect.[224] We also prepared the *anti*-diastereomer, called *exo*-mucosin, which was also found to be the incorrect structure. Given the sterical requirements of the remaining two *syn*-diastereomers, it was deemed unlikely that any of these represent the true structure of the natural product.

We are currently working on a synthesis of mucosin with *trans*-fused bridgeheads. Several strategies have been planned, and several preliminary experiments have been conducted.

With the experiences harvested from the present work, we have envisioned a general approach to access the two remaining pairs of *trans*-fused *anti*-diastereomers. From the keto ester **265**, phenylselenid **266** can be prepared. By oxidative elimination of the selenid group, double bond formation will occur towards the bridgehead.[225] *L*-selectride or Mg/MeOH of **267**, will result in *trans*-fusion on the bridgeheads of keto ester **268**. The remaining steps will be executed in accordance with our previously described work to give stereoisomers **208a** and **208b**. The strategy is depicted in Scheme 73 and Scheme 74. If successful, the present approach represents a unified strategy to prepare mucosin and its respective diastereomers, both in terms of control over bridgehead configuration as well as the appended substituents.



Scheme 73 Strategy for new approach towards 208b.



Scheme 74 Strategy for new approach towards 208a

We have also envisioned a strategy for the preparation of dictyosphaerin **(286)**, taking advantage of chiral keto esters.

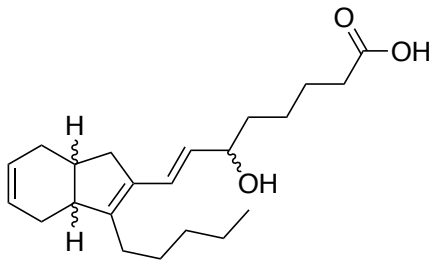
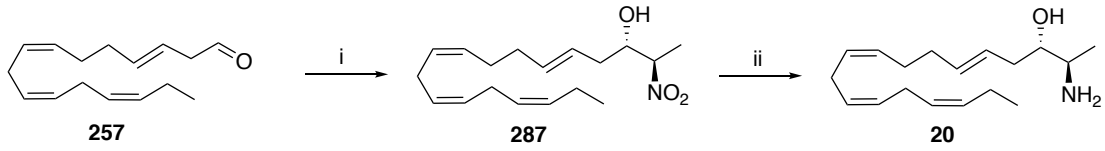


Figure 18 Dictyosphaerin

The first total syntheses of sphingolipids crucigasterin 277 and obscuraminol A are also reported. These projects led to the establishment of the proposed structure for both compounds.[226,227]

We are currently working on the asymmetric synthesis of crucigasterin 277, but due to time limitations we were not able to finish the project. The remaining steps are the nitro aldol reaction and the reduction of the resulting nitro alcohol.



Scheme 75 Final reaction for an asymmetric synthesis of crucigasterin 277.

It is also planned to do biological evaluation of the synthesized compounds. However, these have not been initiated presently.

9. References

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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**,14, 8433.

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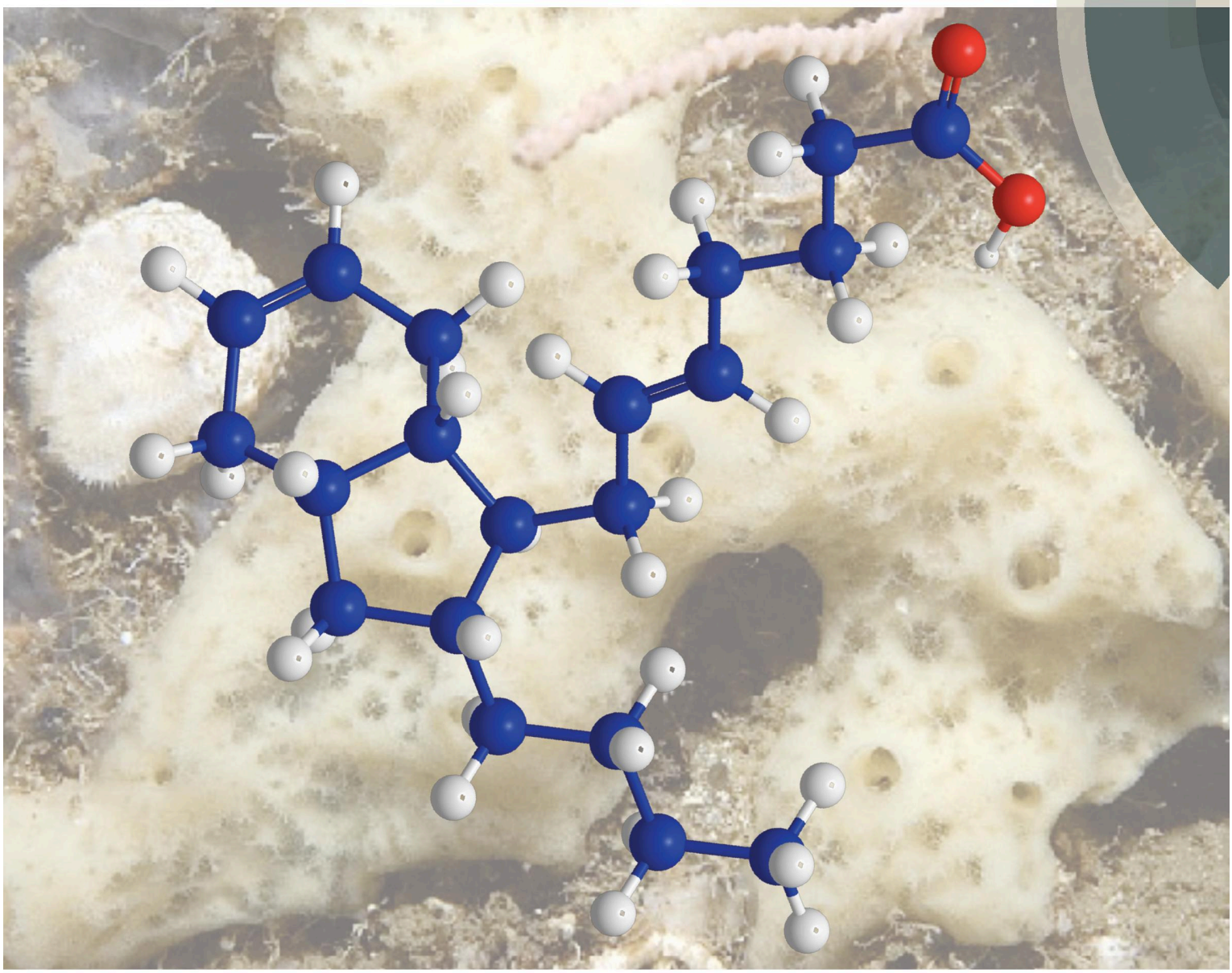

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Jens M. J. Nolsøe, Yngve H. Stenstrøm *et al.*

Total synthesis based on the originally claimed structure of mucosin

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Total synthesis based on the originally claimed structure of mucosin†

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

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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.

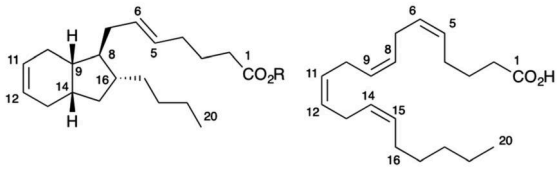

Fig. 1 Relationship between the mucosin scaffold and arachidonic acid.

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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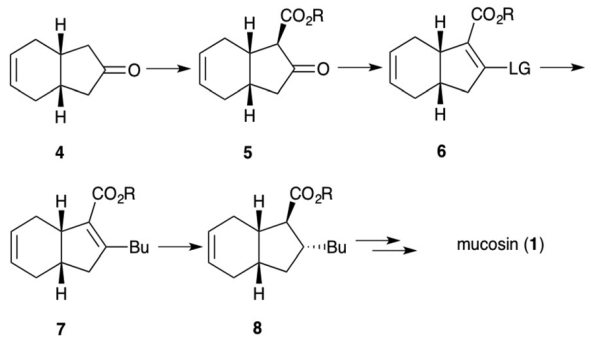
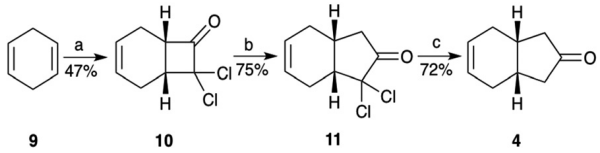
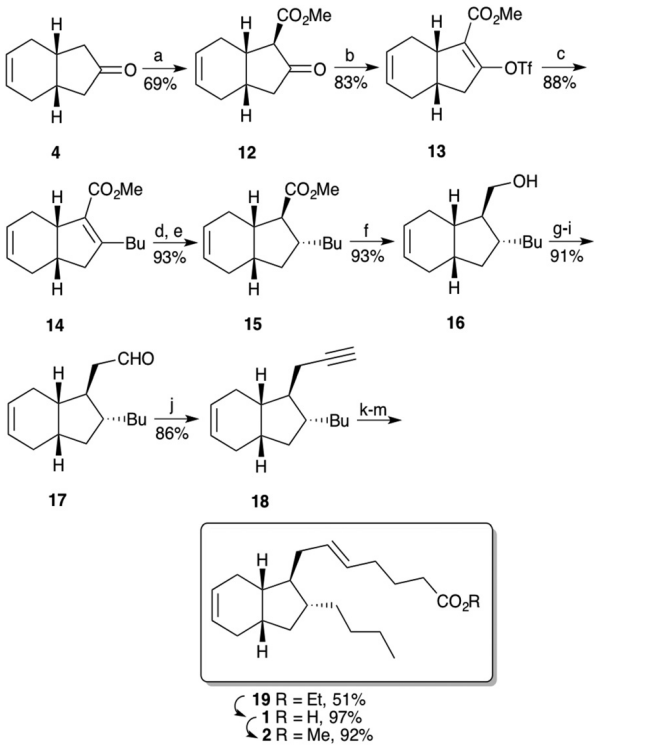
†Electronic supplementary information (ESI) available: Experimental procedures and characterisation data for all new compounds; crystal data and refinement details are included to give evidence of the relative stereochemistry of a late stage intermediate. CCDC 1484546. For ESI and crystallographic data 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.



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.

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-

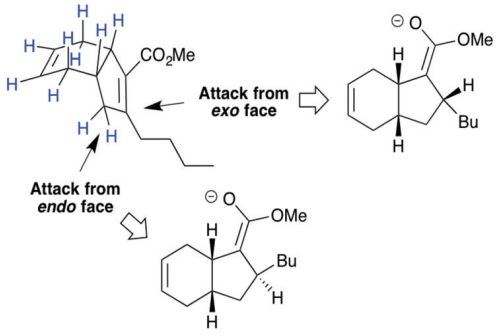


Fig. 2 Hydride attack on the distinguishable diastereotopic faces of conjugated ester **14**.

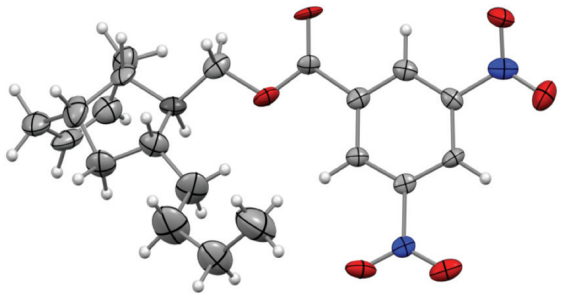


Fig. 3 Single crystal X-ray structure obtained from the 3,5-dinitrobenzoate of alcohol **16** at 110 K. The *n*-butyl conformation is shown with a *trans, trans, gauche*+ orientation relative to the dark C atom. For clarity, a minor *trans, gauche*−, *gauche*− orientation with occupancy 0.26(3) has been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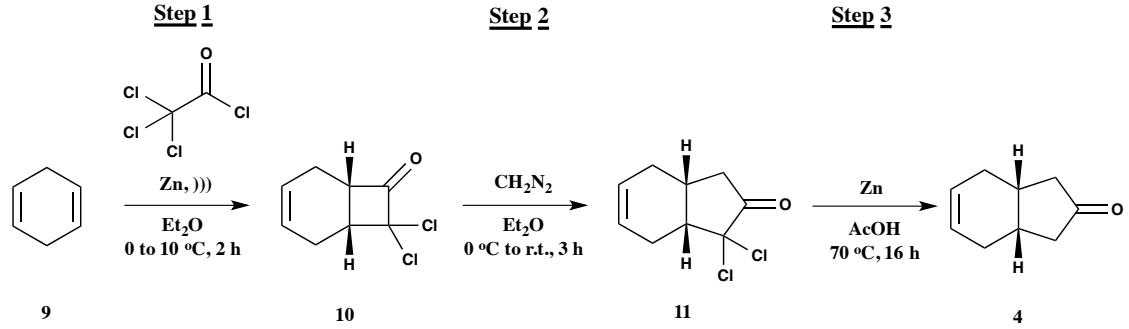
Table of Contents:

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

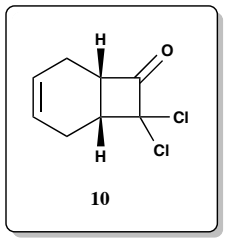
columns, CP-Chirasil-DEX CB, 25 m, 0.25 mm, 0.25 μm) applying the conditions stated. X-ray crystallography was performed on a Bruker D8 Venture diffractometer with InCoatec ImuS Microfocus radiation source and Photon 100 CMOS detector. Data collection with Apex2,[1] data integration and cell refinement 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):



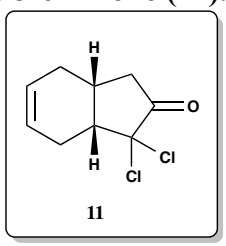
Scheme S-1 Synthetic route to *meso*-ketone **4**.

***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.

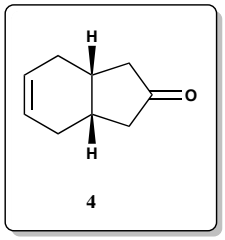
***rac*-(1*R*,6*R*)-7,7-Dichlorobicyclo[4.3.0]oct-3-en-7-one (11).**



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]**



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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.

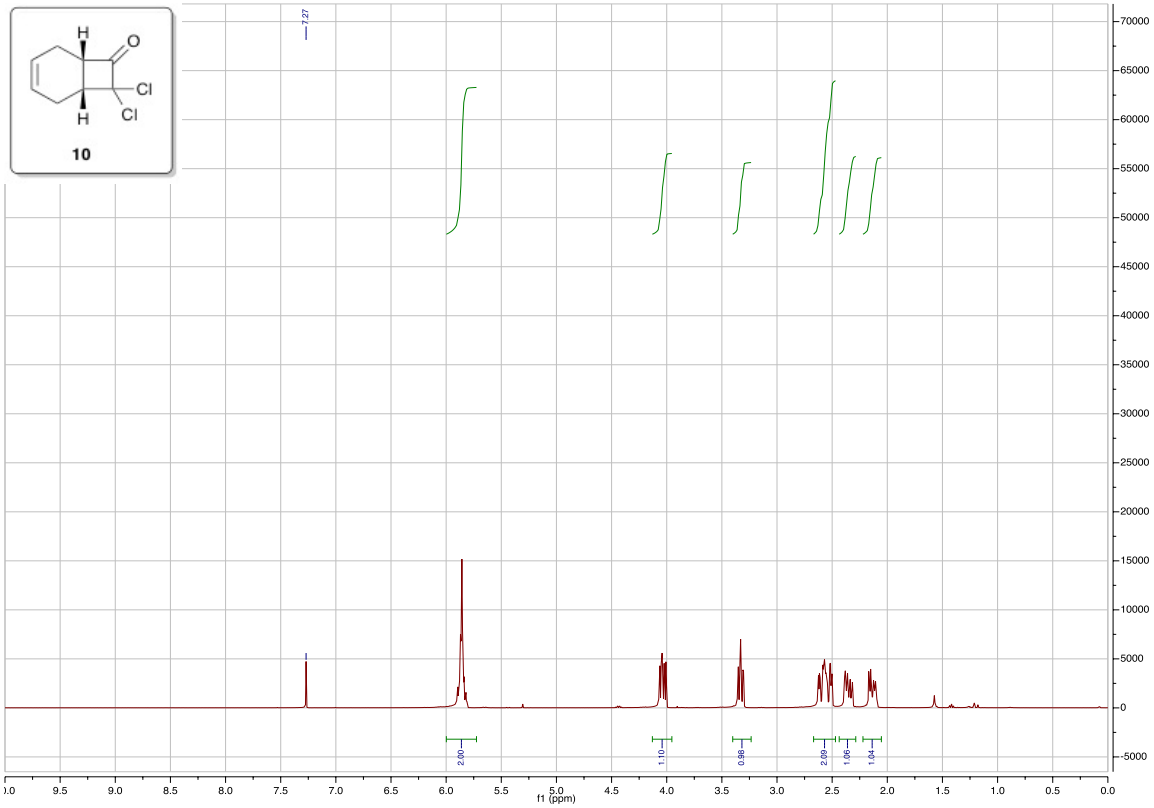


Figure S-1 ^{1}H-NMR spectrum of compound **10**.

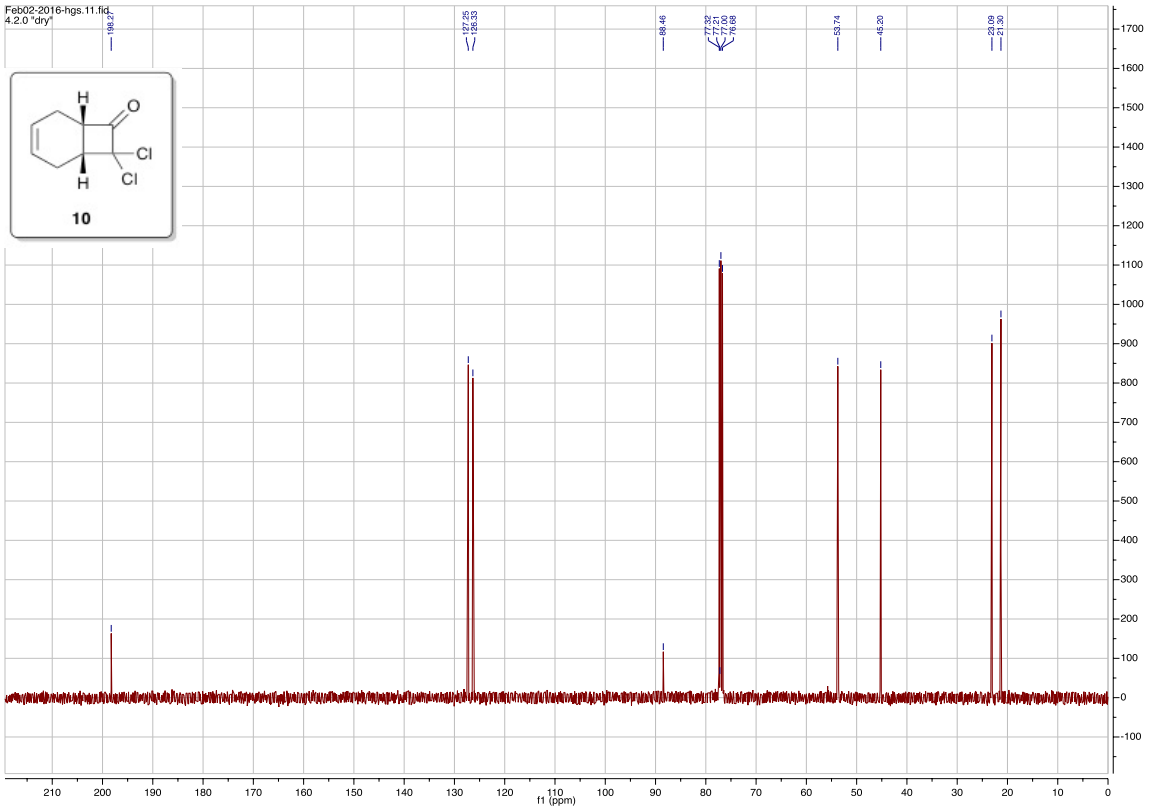


Figure S-2 ^{13}C-NMR spectrum of compound **10**.

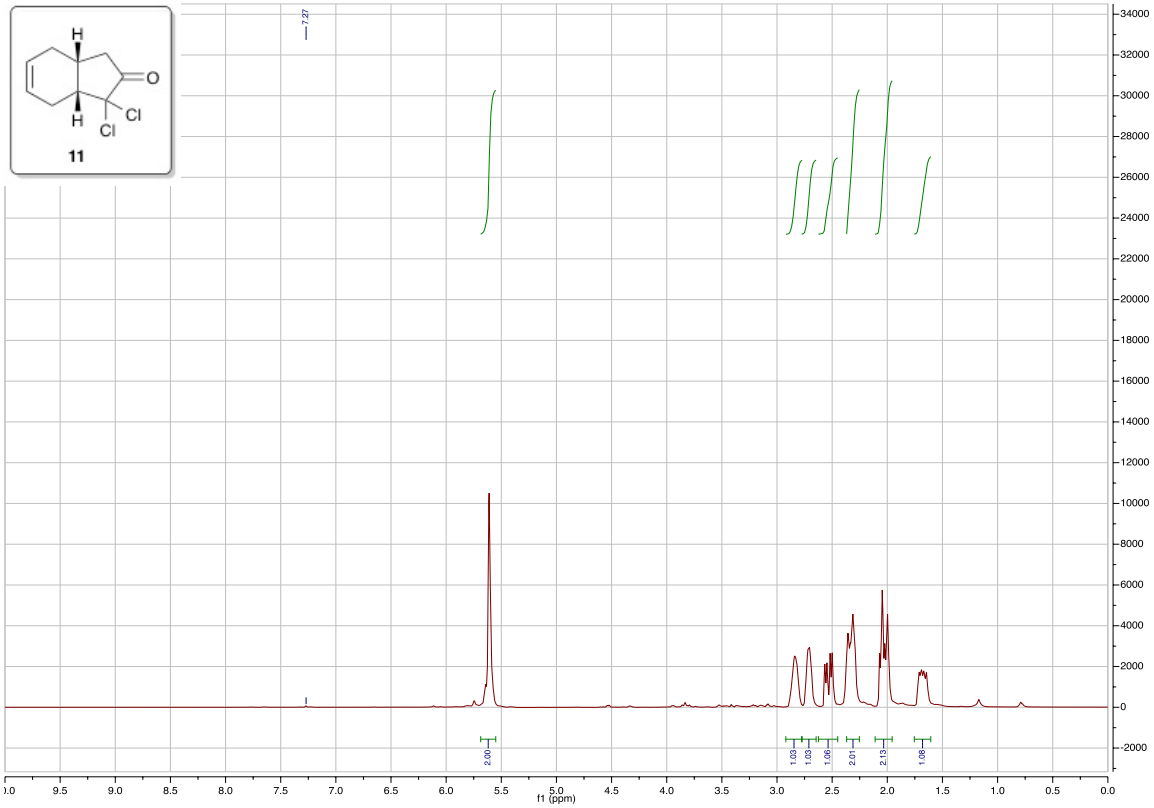


Figure S-3 ^{1}H-NMR spectrum of compound **11**.

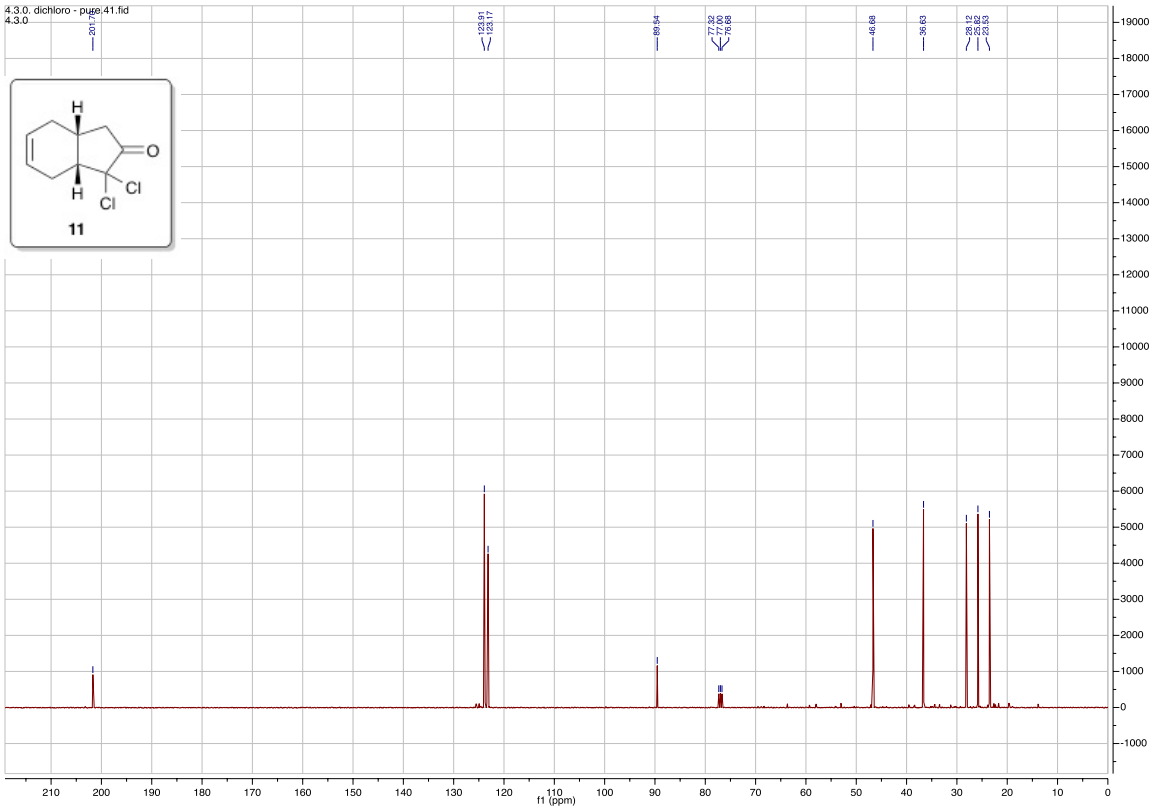


Figure S-4 ^{13}C-NMR spectrum of compound **11**.

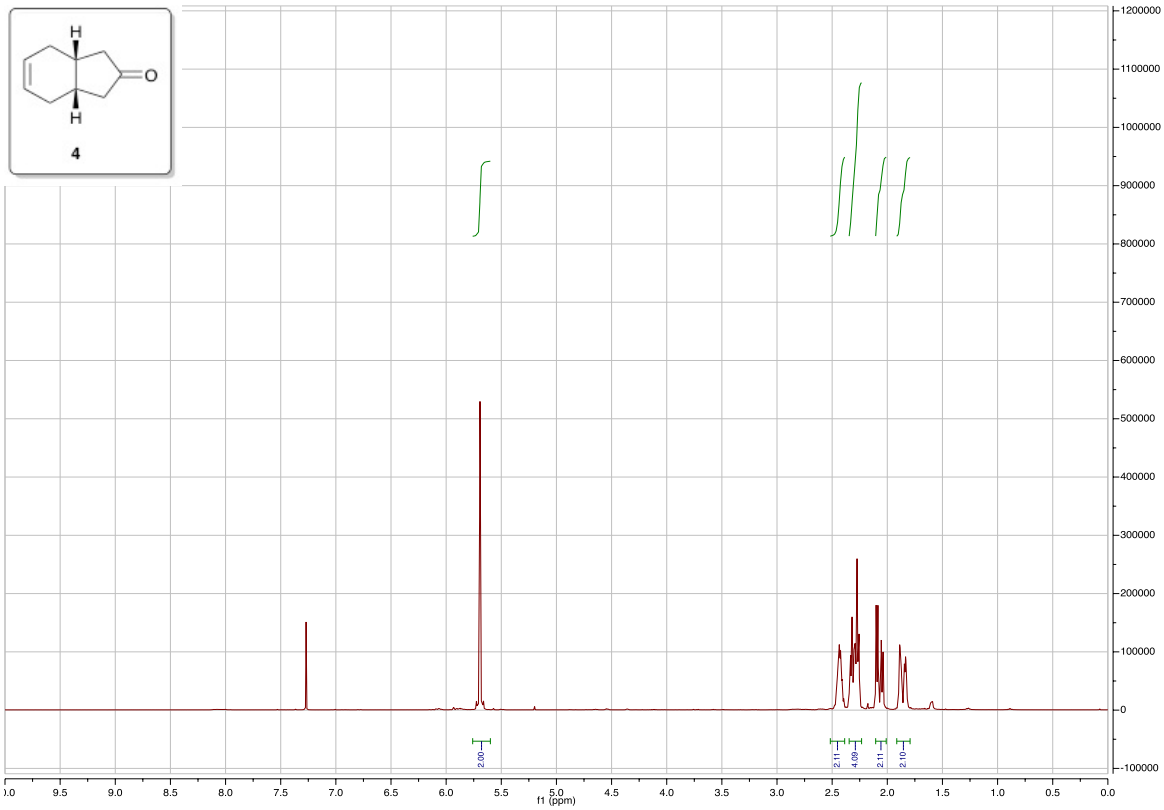


Figure S-5 ^{1}H-NMR spectrum of compound **4**.

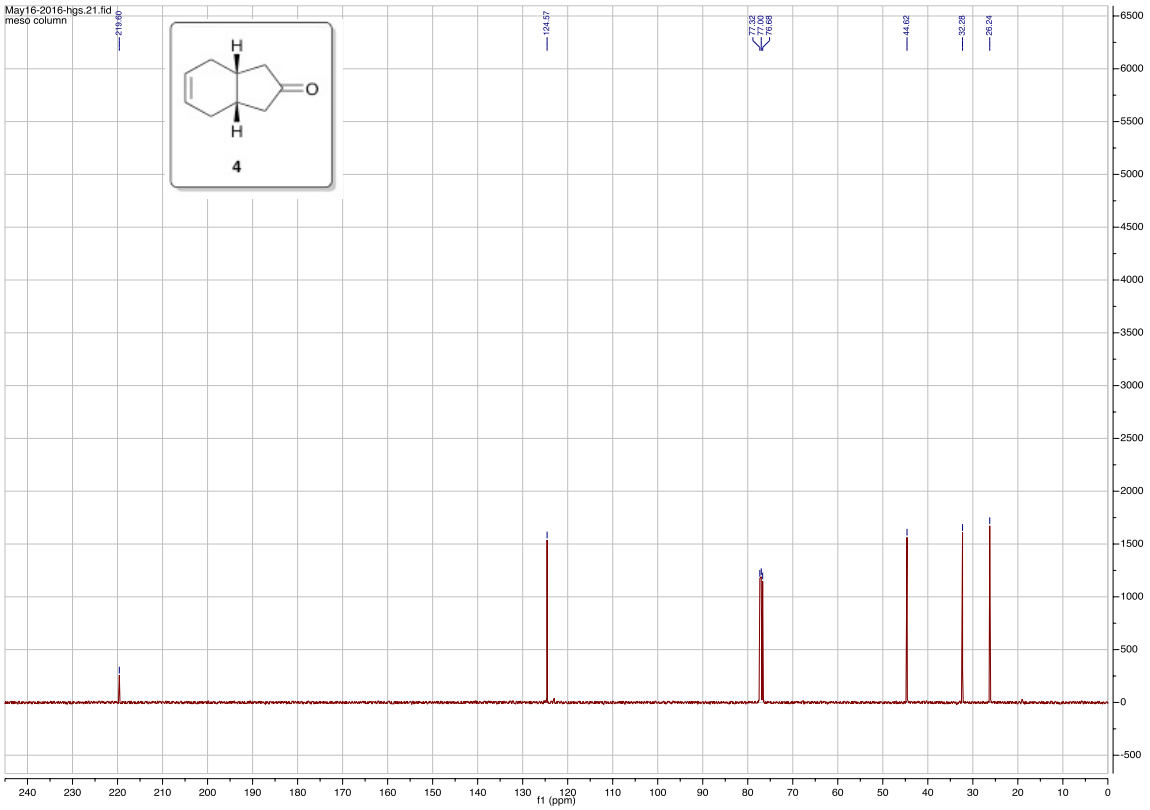


Figure S-6 ^{13}C-NMR spectrum of compound **4**.

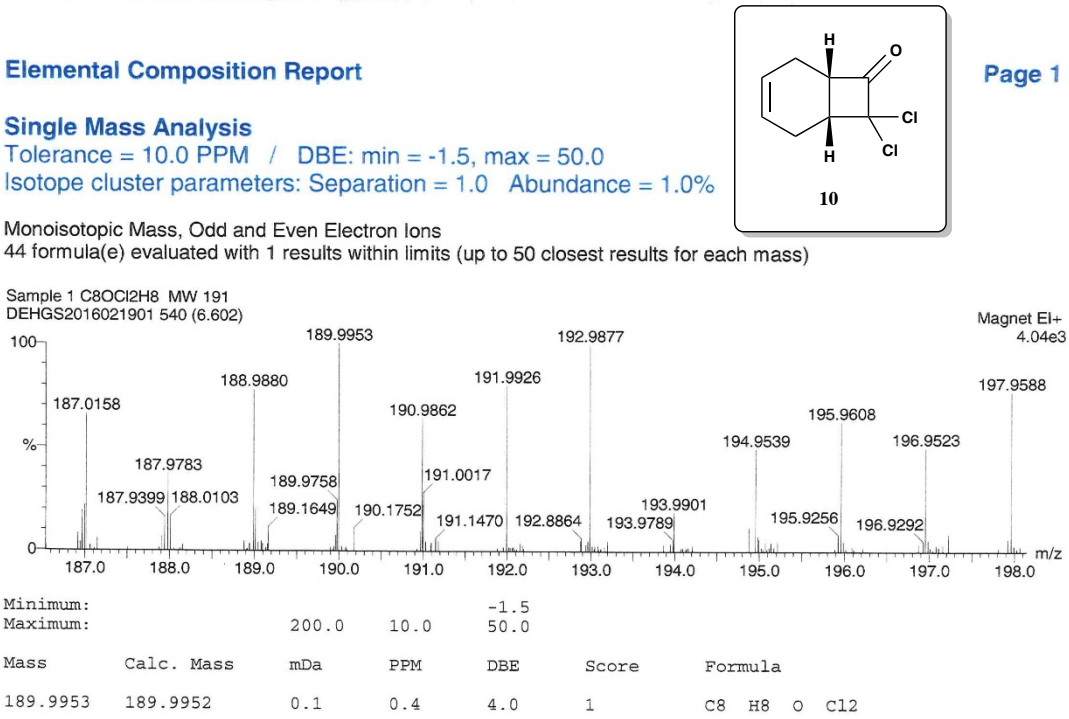


Figure S-7 HRMS of compound **10**.

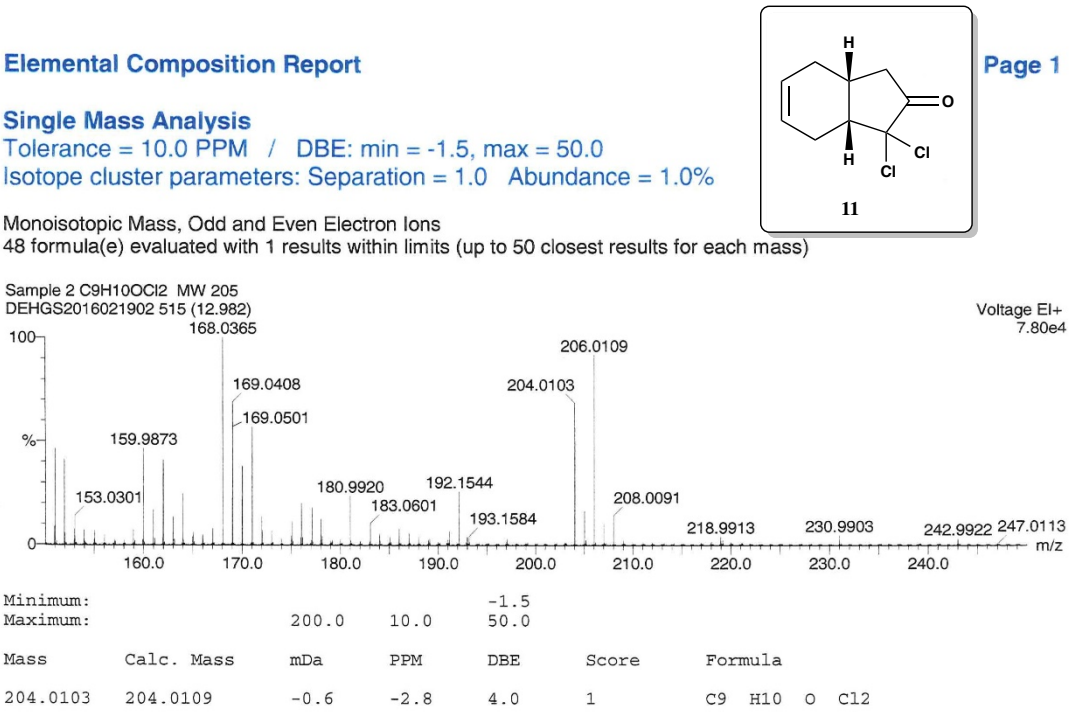


Figure S-8 HRMS of compound **11**.

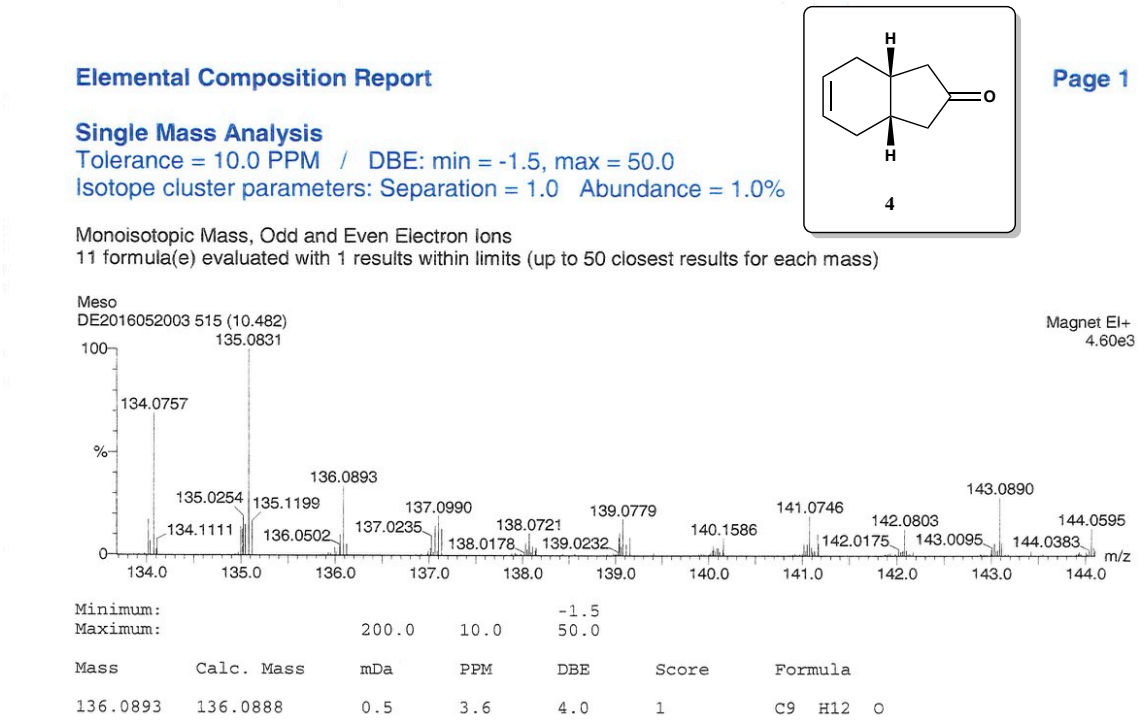


Figure S-9 HRMS of compound **4**.

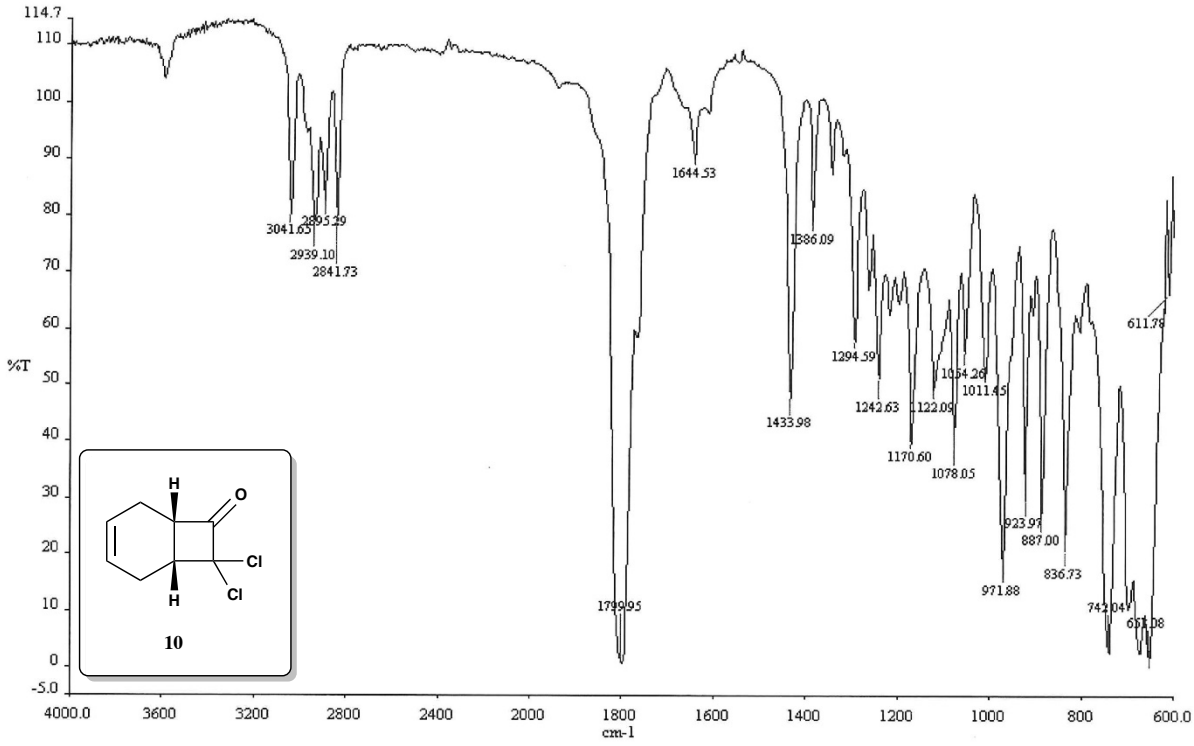


Figure S-10 IR of compound **10**.

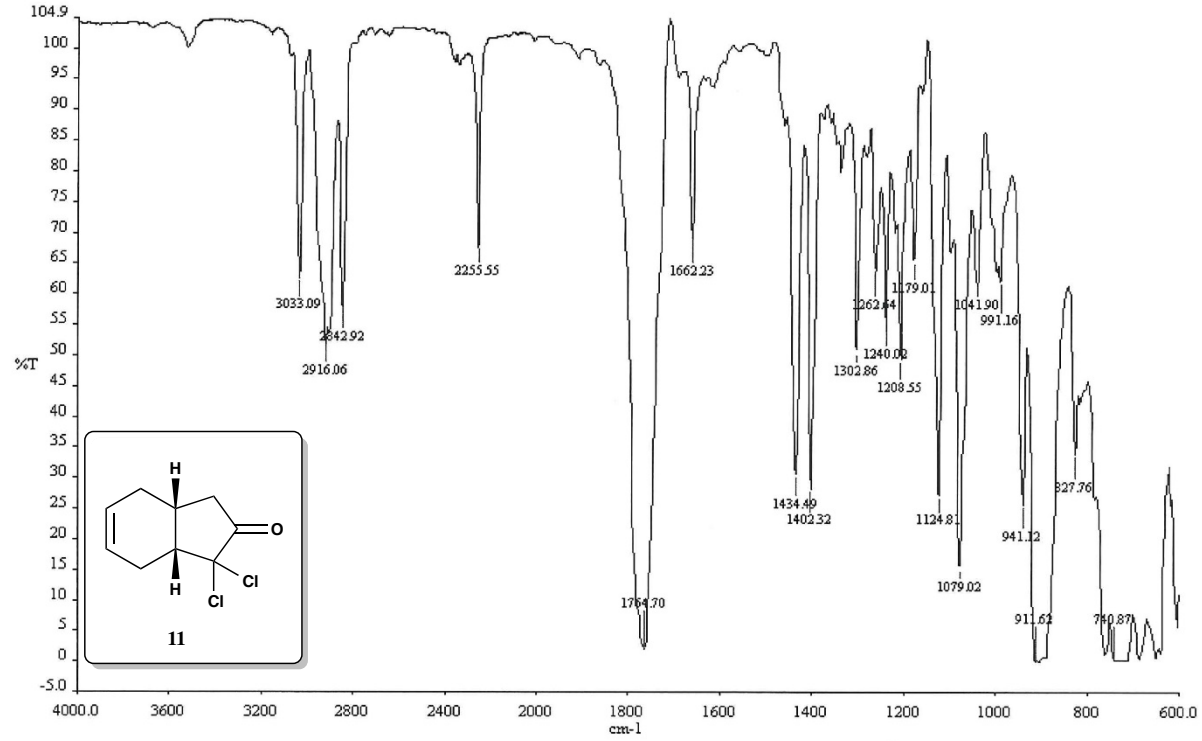


Figure S-11 IR of compound **11**.

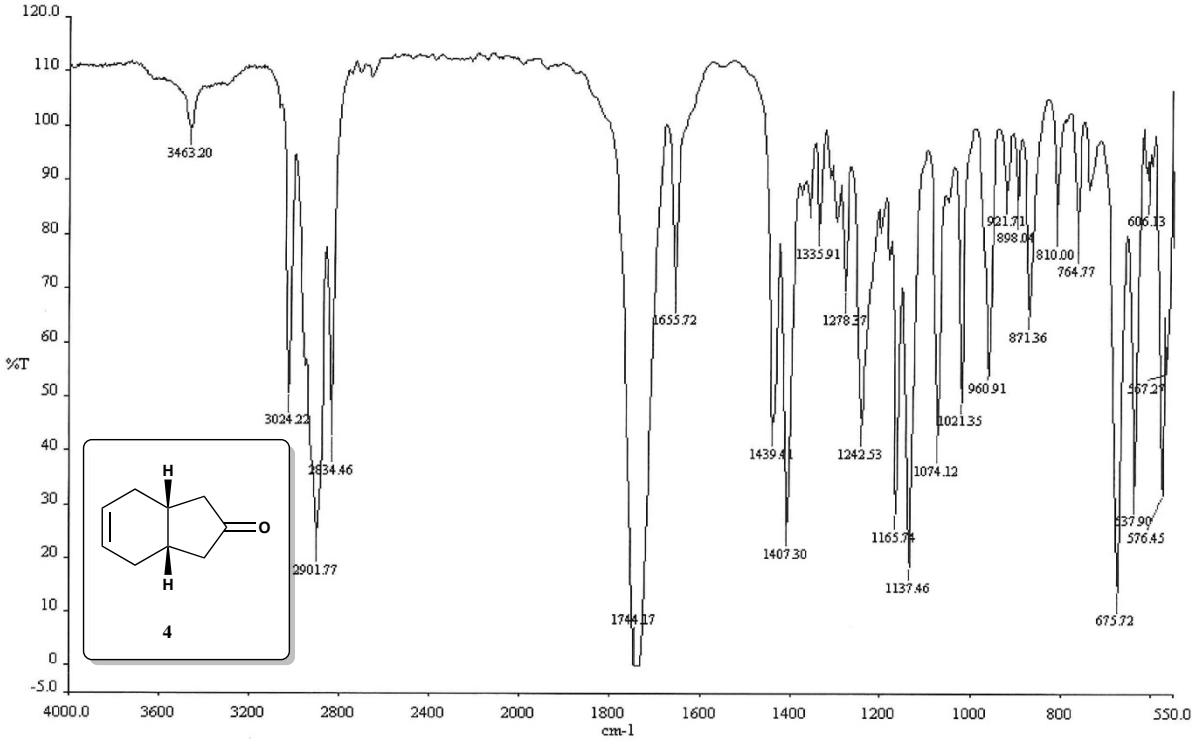
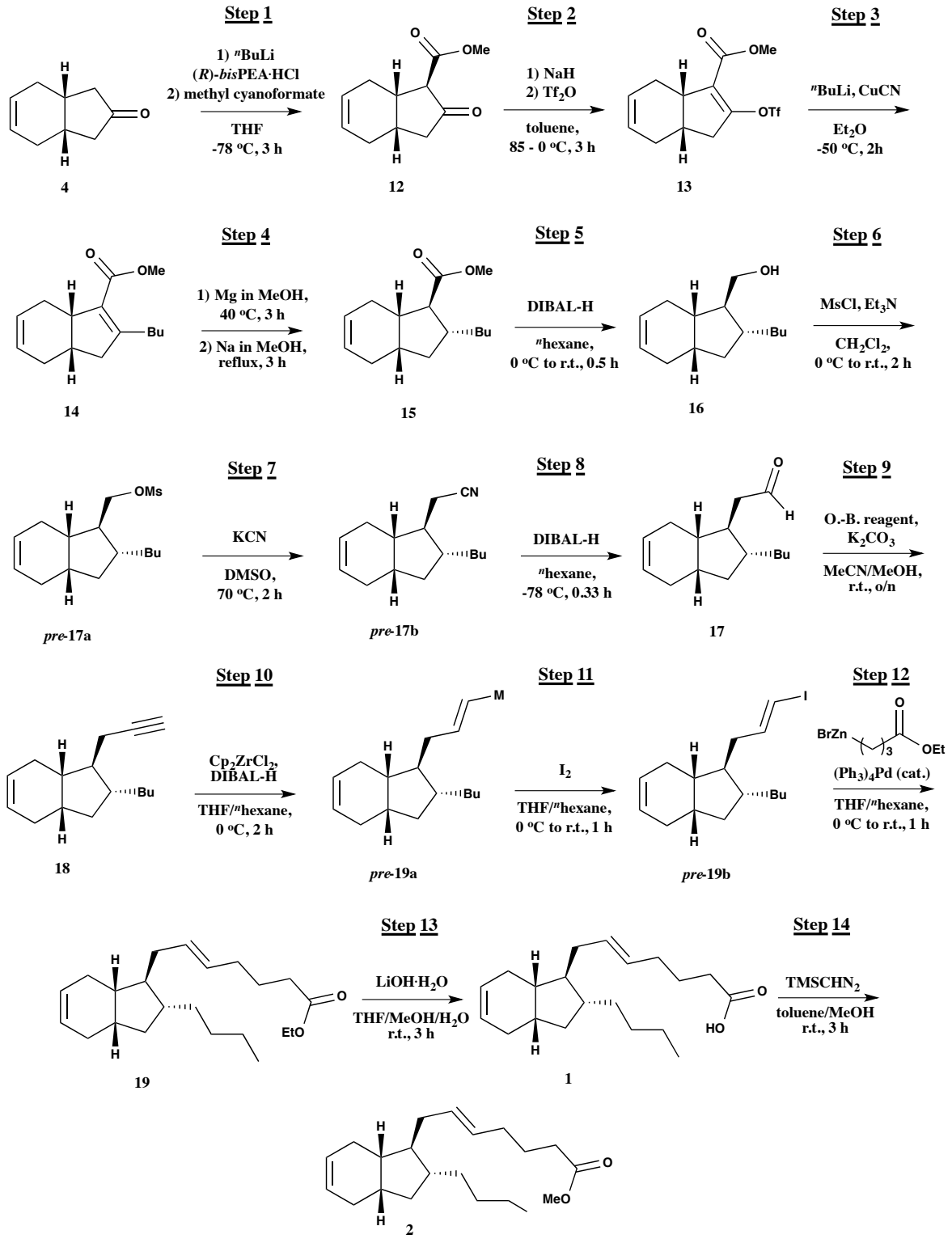


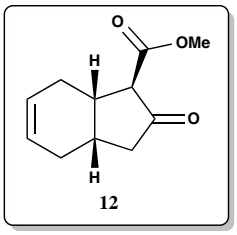
Figure S-12 IR of compound **4**.

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



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

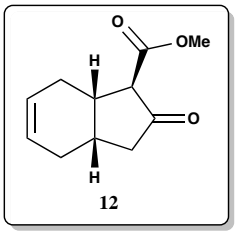
Methyl (1*S*,6*S*,7*R*)-8-oxobicyclo[4.3.0]non-3-ene-7-carboxylate (12).[7]



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).**



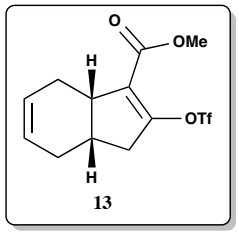
12

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).

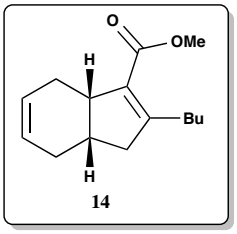


13

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).

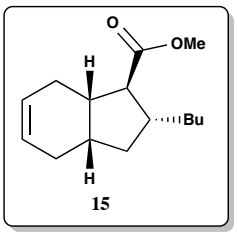


14

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

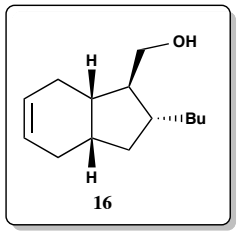
1) Methyl (1*S*,6*S*)-8-butylbicyclo[4.3.0]non-3,7-diene-7-carboxylate **14** (1.33 g, 5.68 mmol, 1.0 equiv.) was dissolved in MeOH (5 mL) at room temperature. This was stirred for 5 min then magnesium turnings (3.75 g, 156.31 mmol, 27.5 equiv.) were added in one portion. The turnings were stirred at 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).



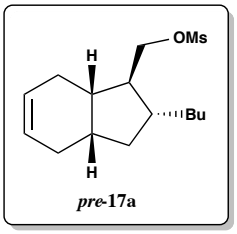
16

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, $CHCl_3$); ^1H 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 °C (30 min) - 3 degrees/min to 150 °C - 150 °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.

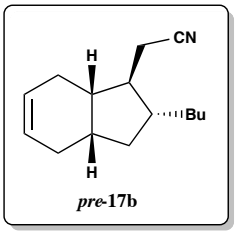
(1*S*,6*S*,7*S*,8*R*)-8-Butyl-7-((methylsulfonyl)oxymethyl)bicyclo[4.3.0]non-3-ene (*pre*-17a).



***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).

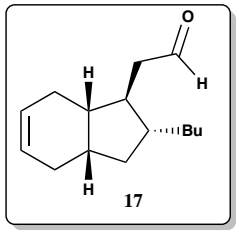


***pre*-17b**

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.

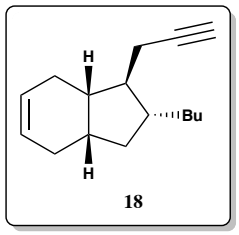
(1*S*,6*S*,7*S*,8*R*)-8-Butyl-7-(formylmethyl)bicyclo[4.3.0]non-3-ene (17).



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).

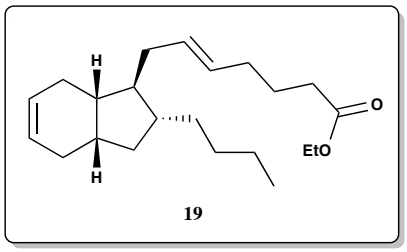


18

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.

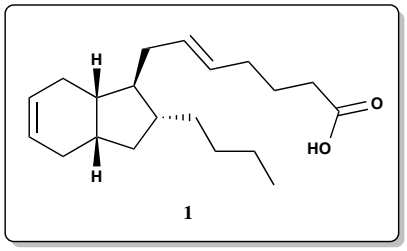
(1*S*,6*S*,7*S*,8*R*)-8-Butyl-7-((*E*)-7'-ethoxy-7'-oxohept-2'-enyl)bicyclo[4.3.0]non-3-ene (19).



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.

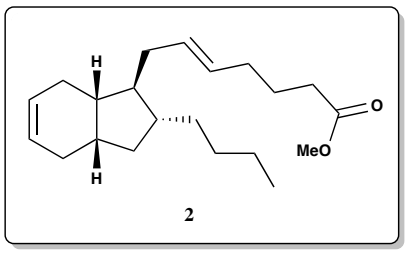
(1*S*,6*S*,7*S*,8*R*)-8-Butyl-7-((*E*)-7'-hydroxy-7'-oxohept-2'-enyl)bicyclo[4.3.0]non-3-ene (1).



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.

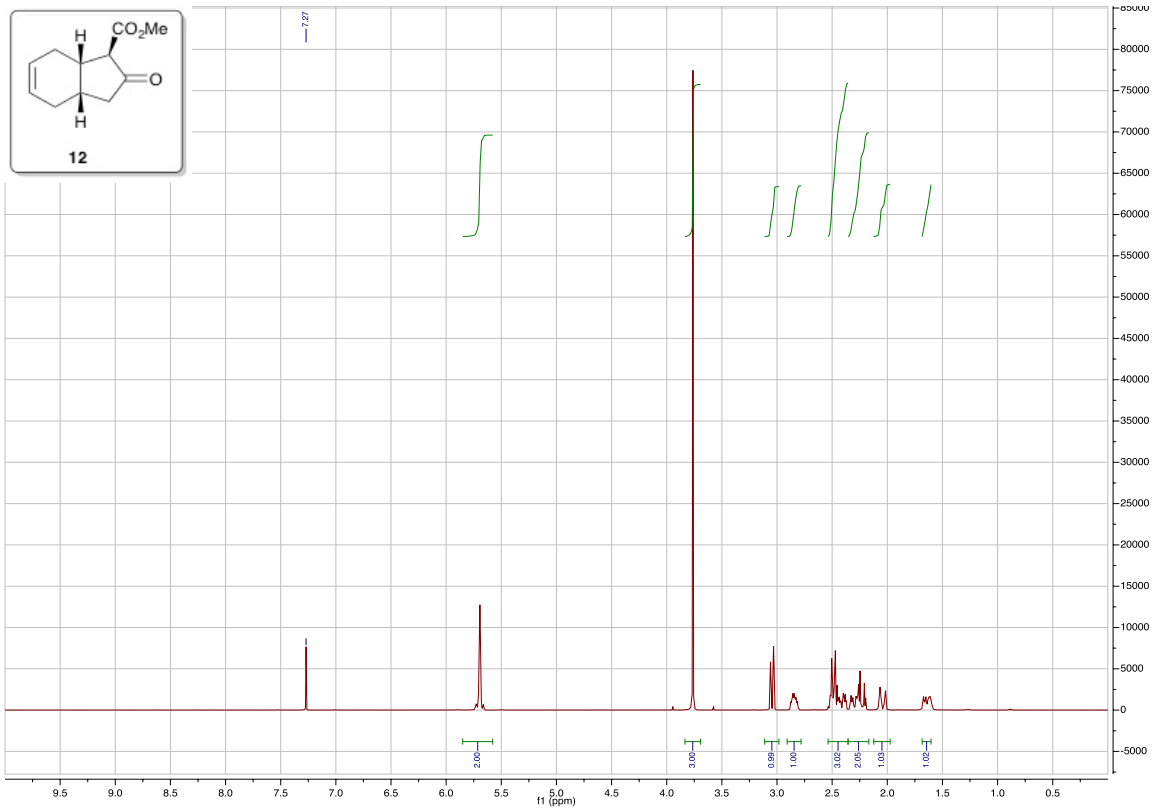


Figure S-13 ^{1}H-NMR spectrum of compound **12**.

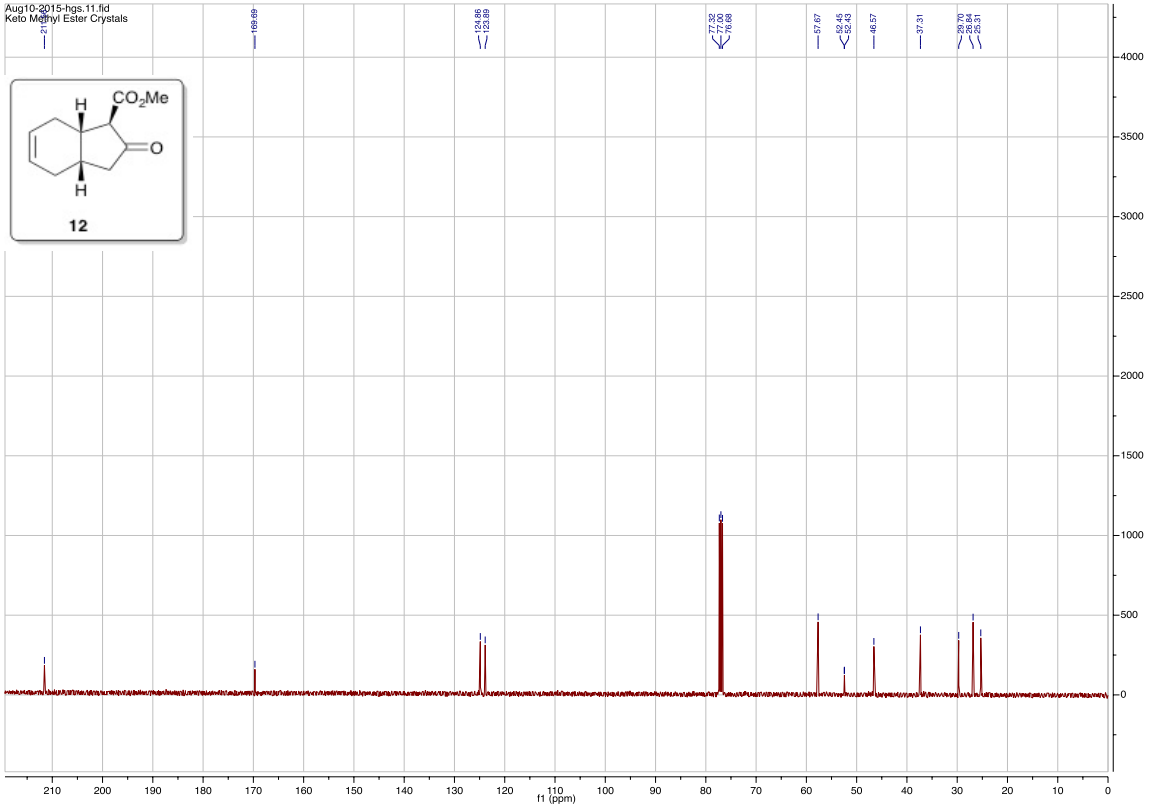


Figure S-14 ^{13}C-NMR spectrum of compound **12**.

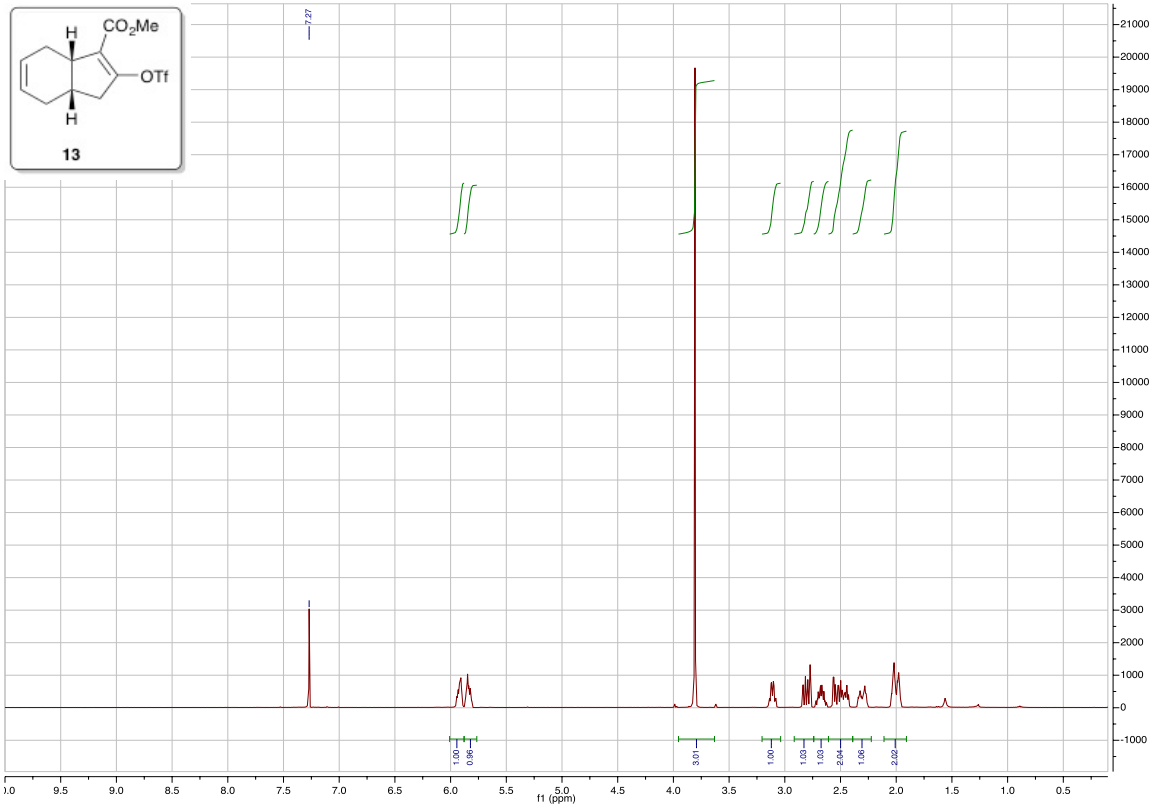


Figure S-15 ^{1}H-NMR spectrum of compound **13**.

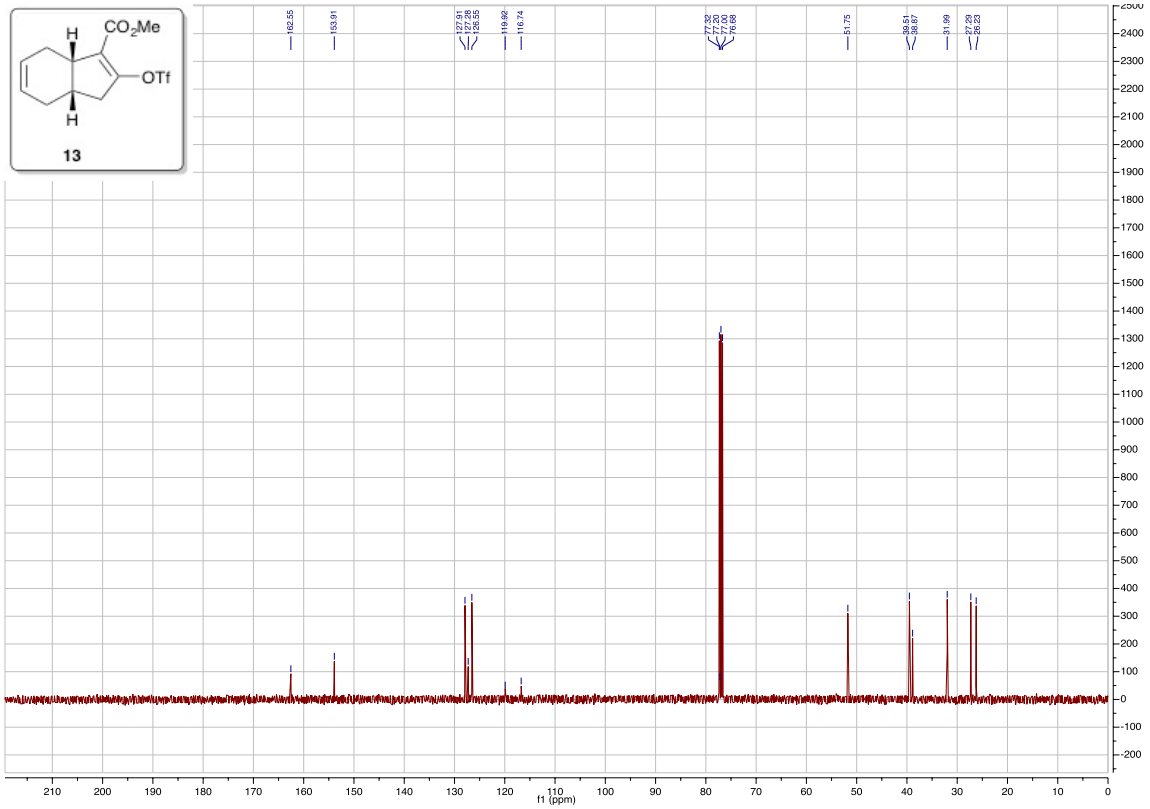


Figure S-16 ^{13}C-NMR spectrum of compound **13**.

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

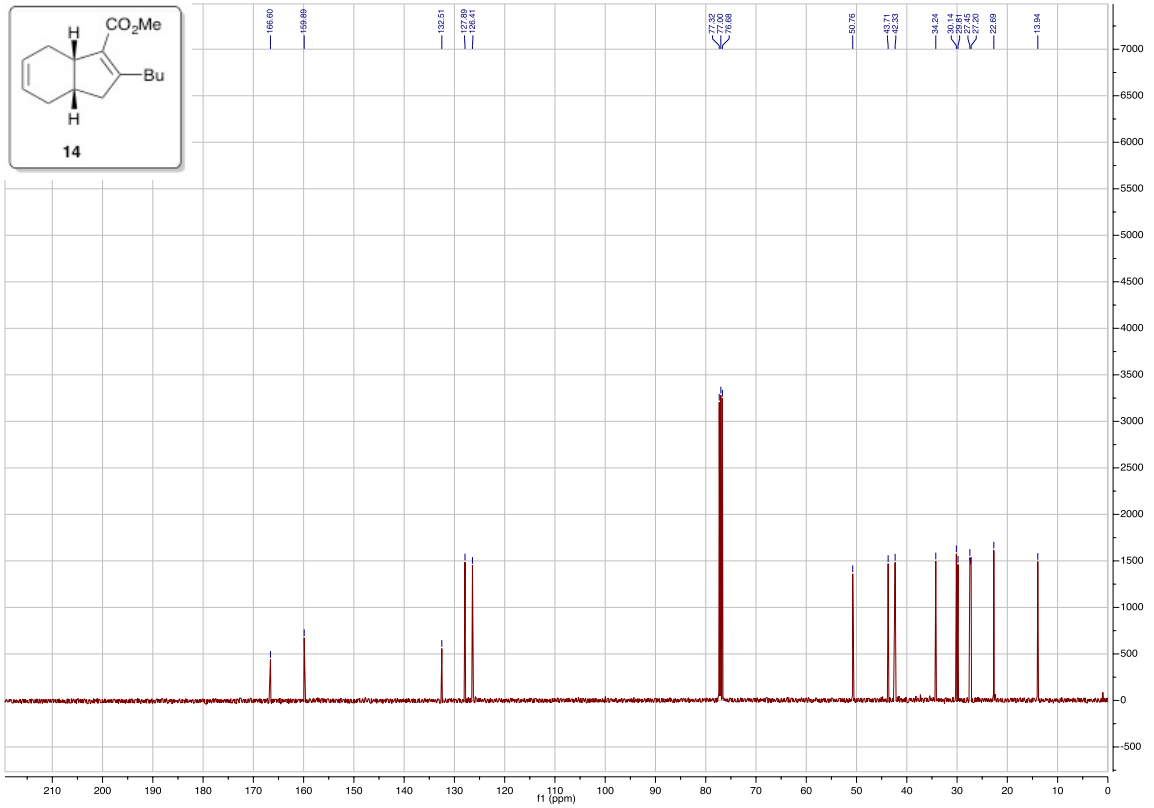


Figure S-18 ^{13}C-NMR spectrum of compound **14**.

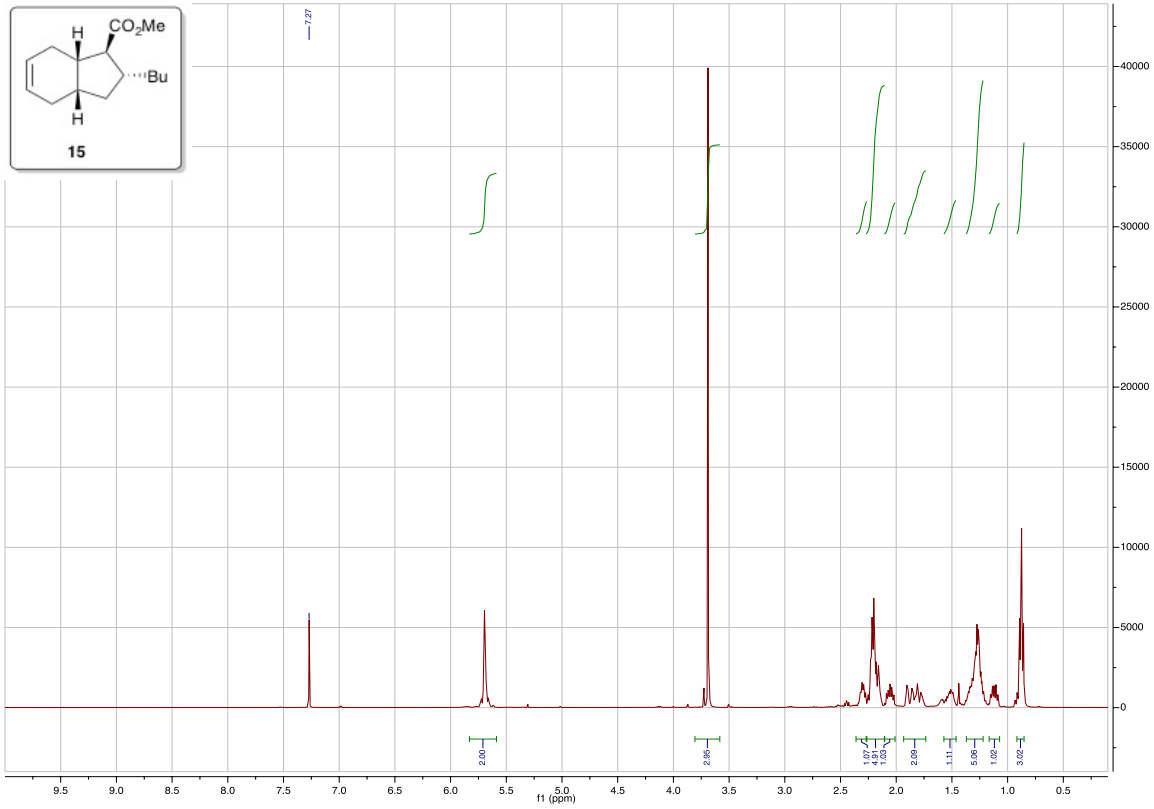


Figure S-19 ^{1}H-NMR spectrum of compound **15**.

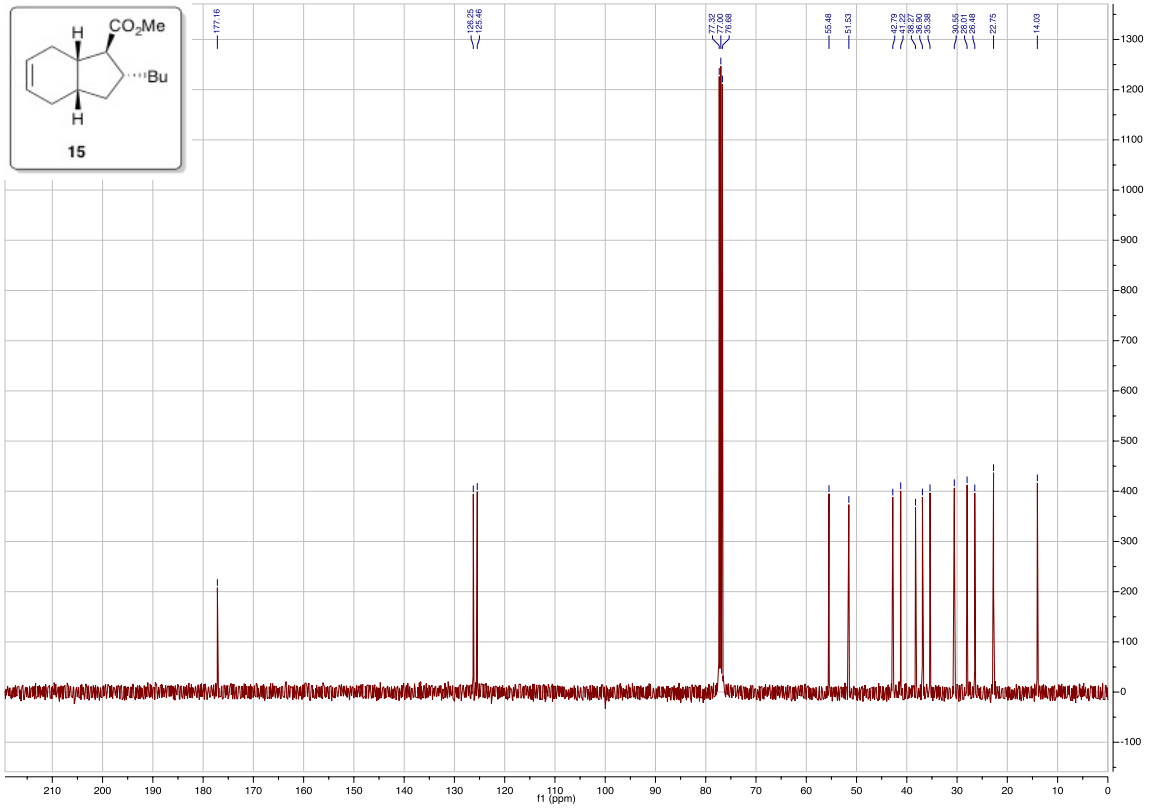


Figure S-20 ^{13}C-NMR spectrum of compound **15**.

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

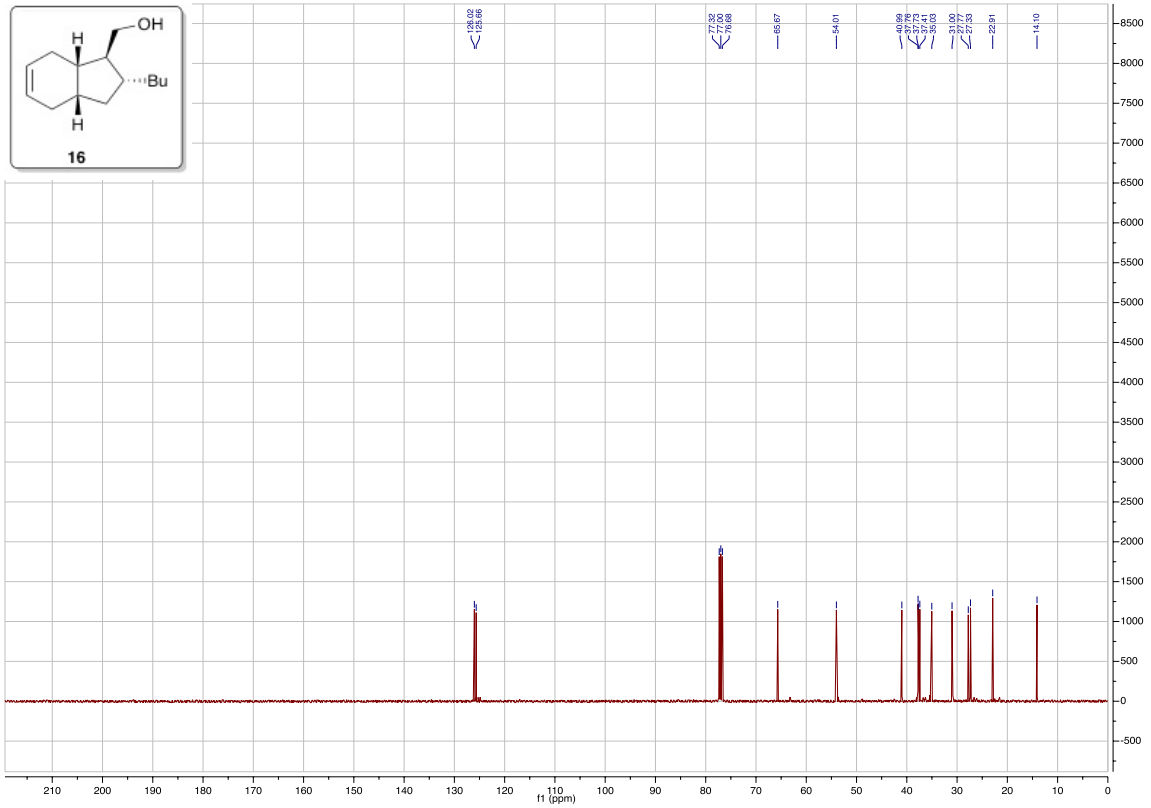


Figure S-22 ^{13}C-NMR spectrum of compound **22**.

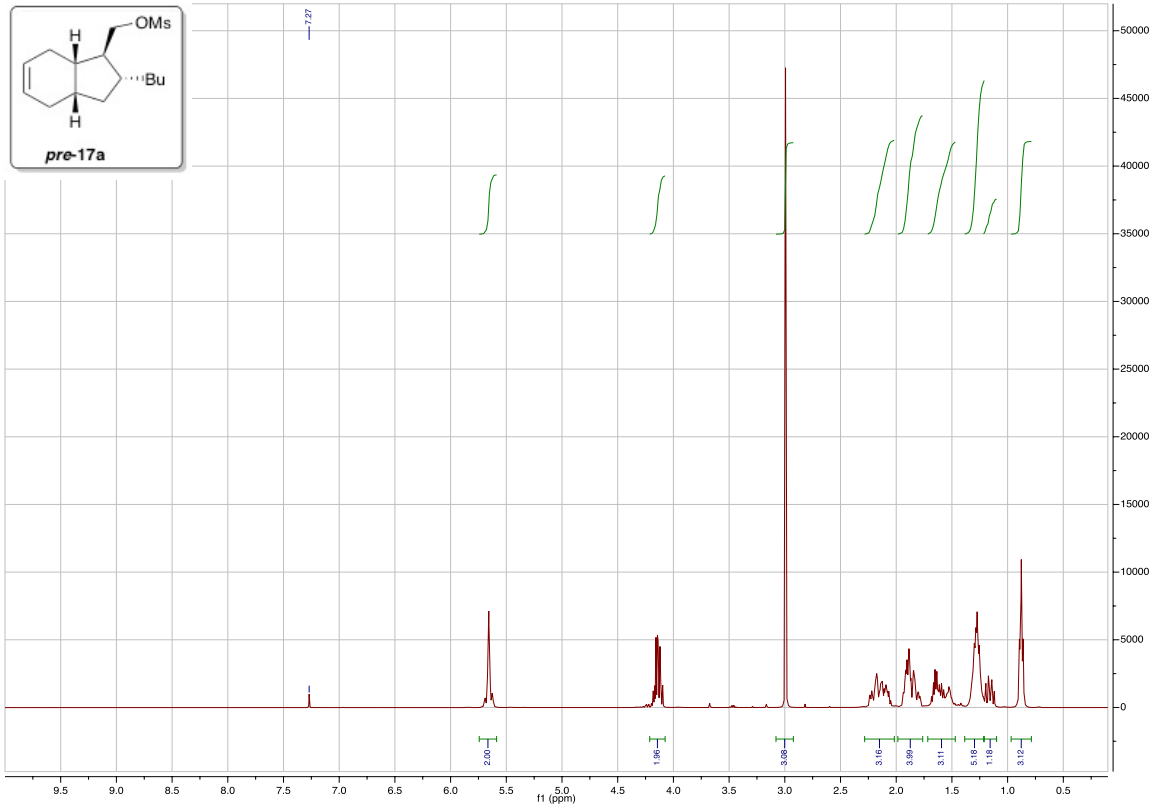


Figure S-23 ^{1}H-NMR spectrum of compound ***pre*-17a**.

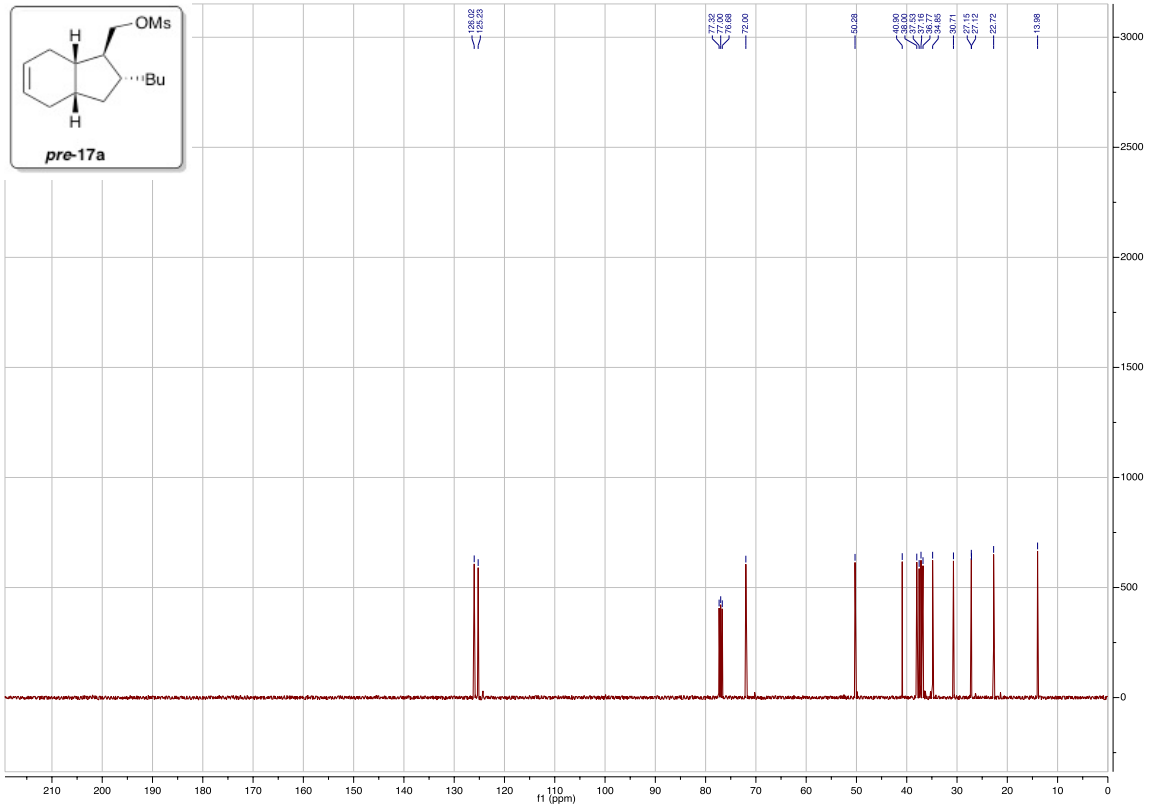


Figure S-24 ^{13}C-NMR spectrum of compound ***pre*-17a**.

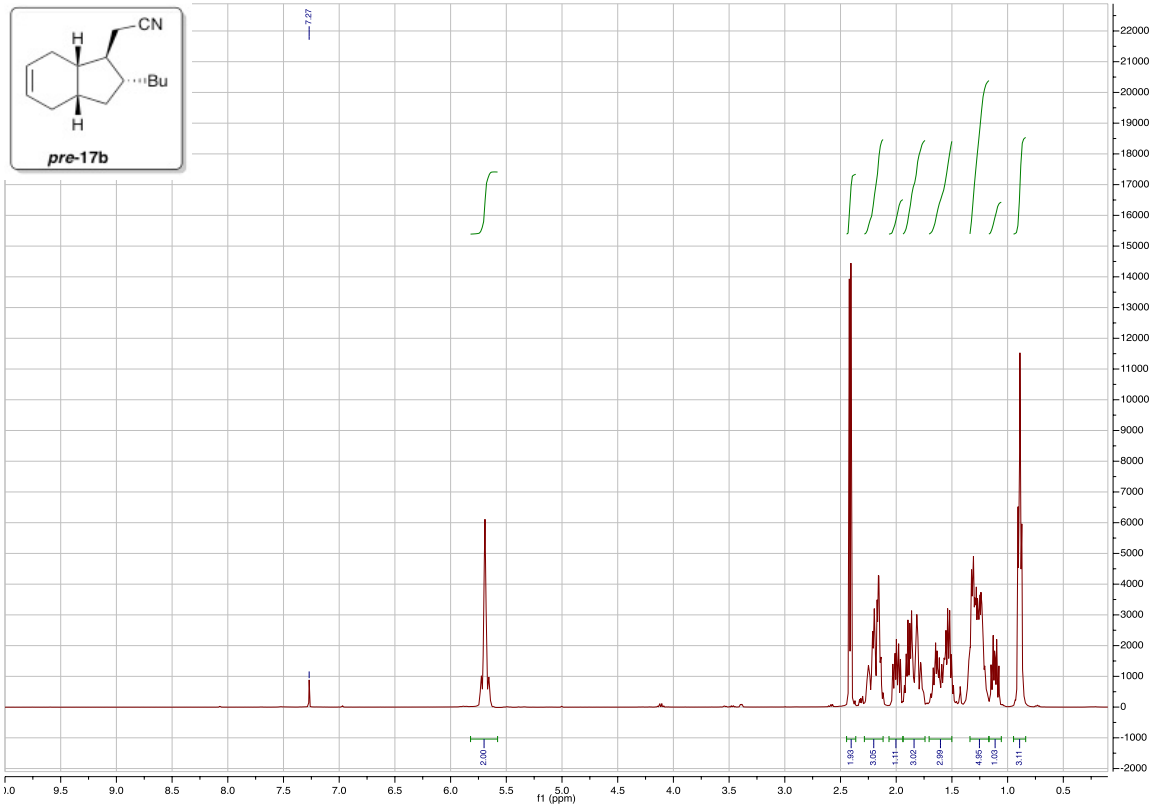


Figure S-25 ^{1}H-NMR spectrum of compound ***pre*-17b**.

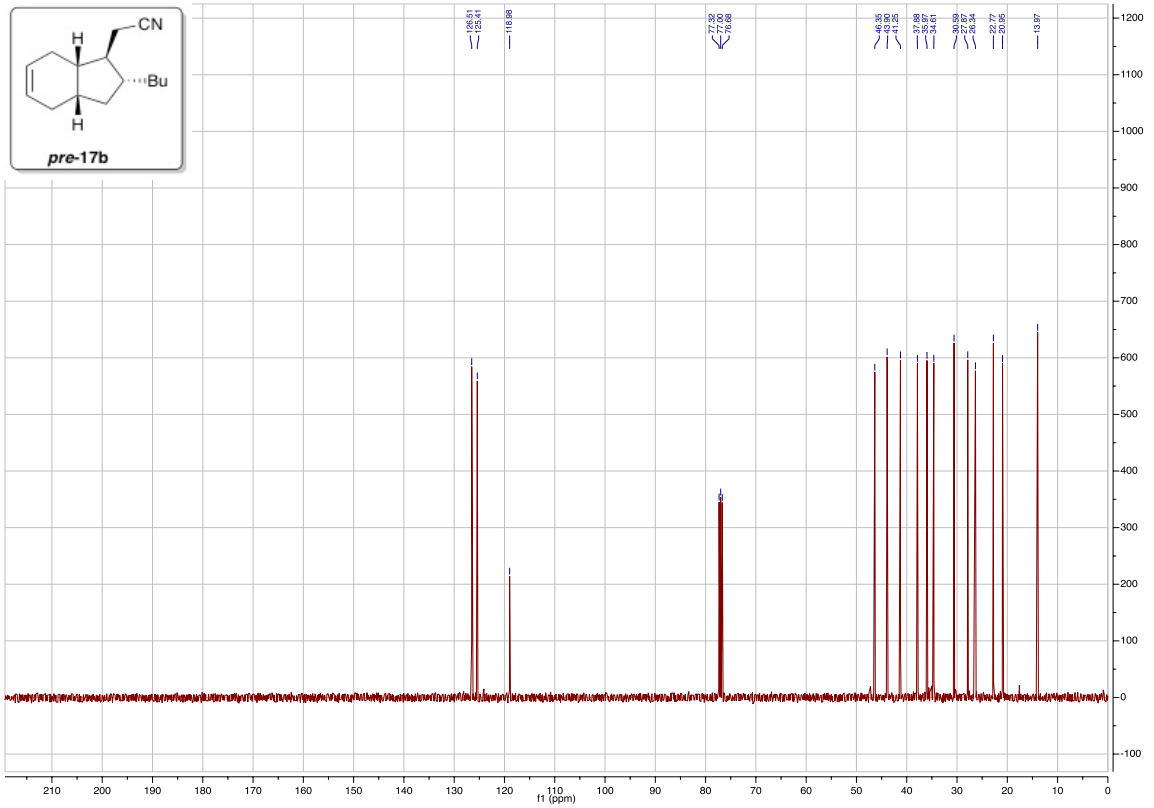


Figure S-26 ^{13}C-NMR spectrum of compound ***pre*-17b**.

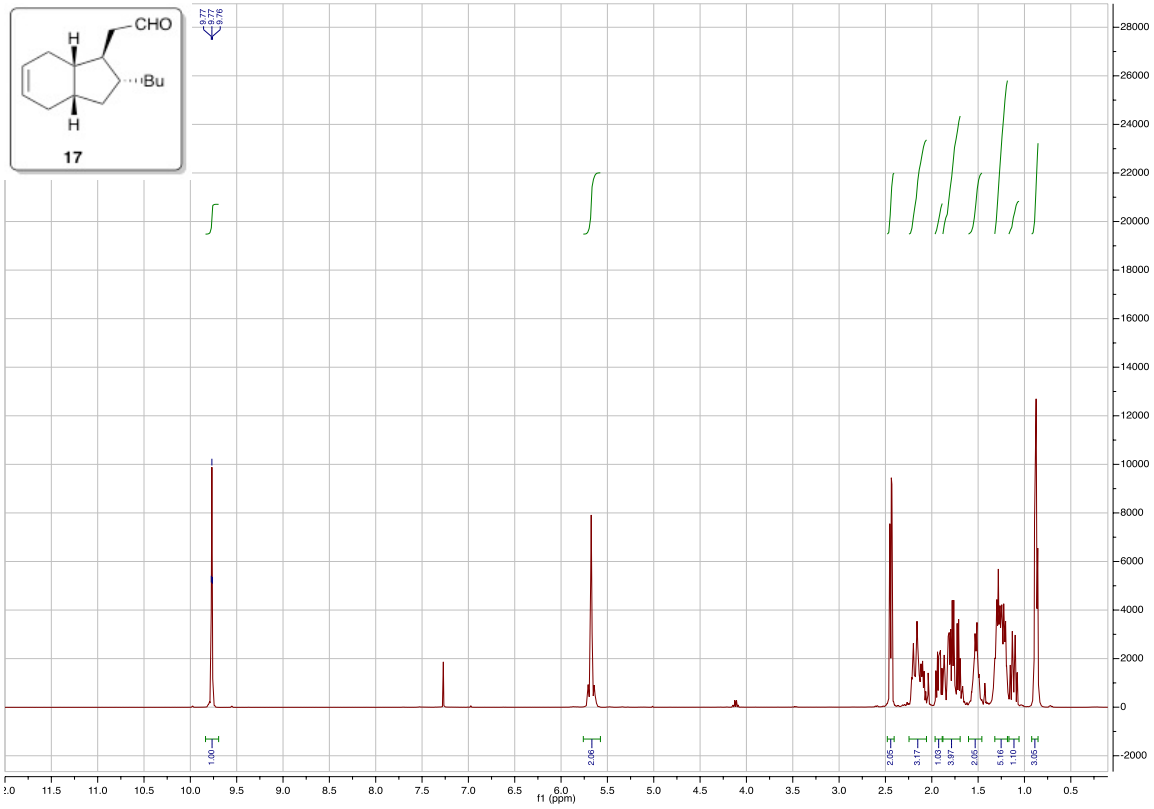


Figure S-27 ^{1}H-NMR spectrum of compound **17**.

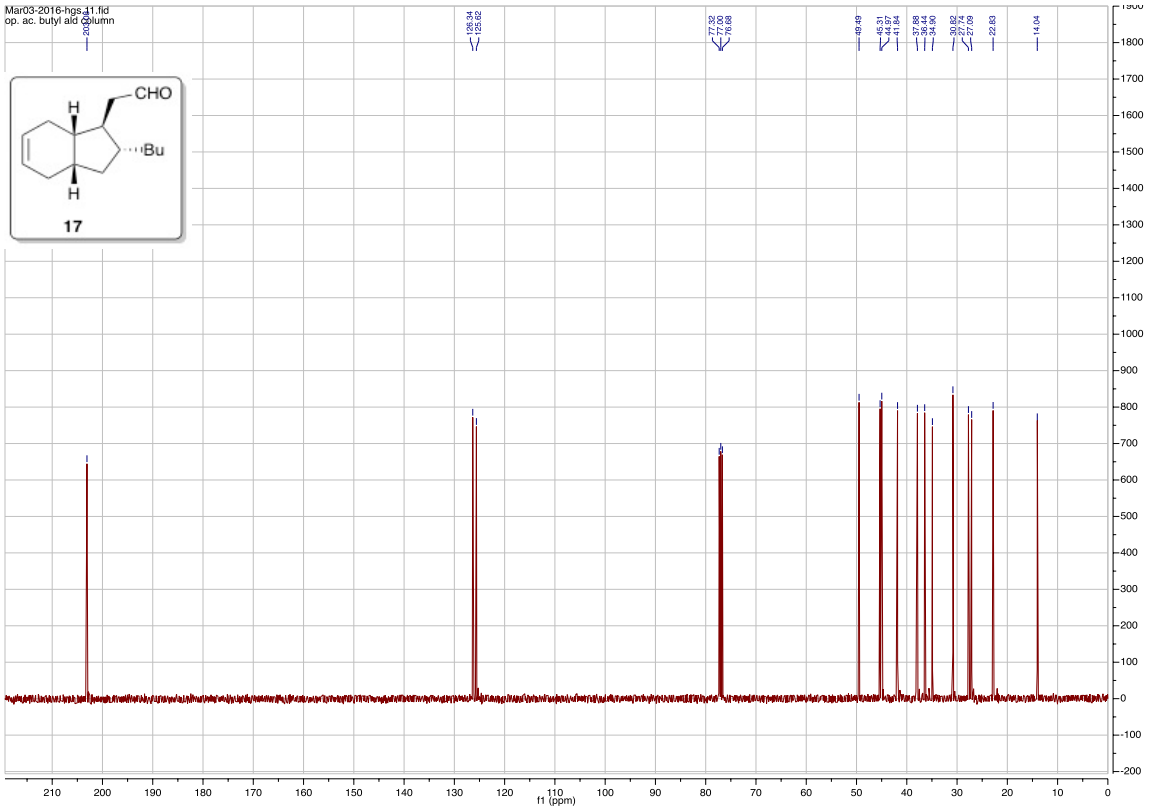


Figure S-28 ^{13}C-NMR spectrum of compound **17**.

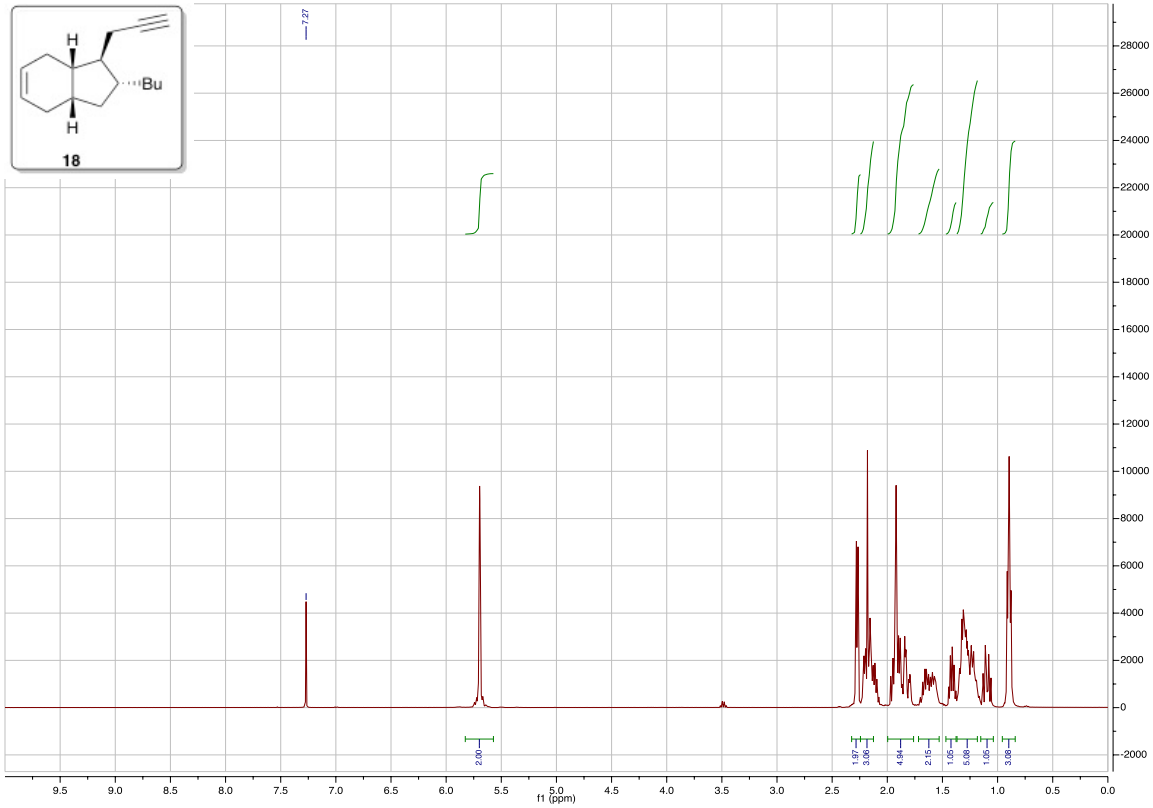


Figure S-29 ^{1}H-NMR spectrum of compound **18**.

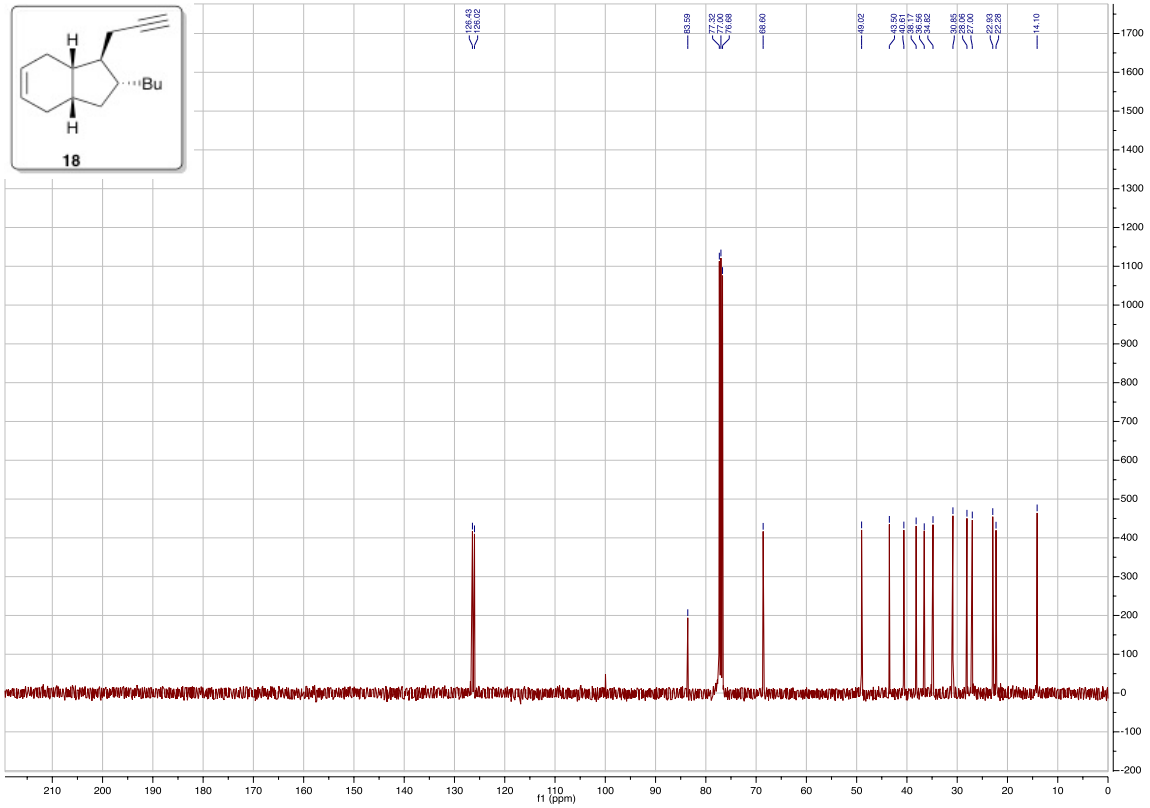


Figure S-30 ^{13}C-NMR spectrum of compound **18**.

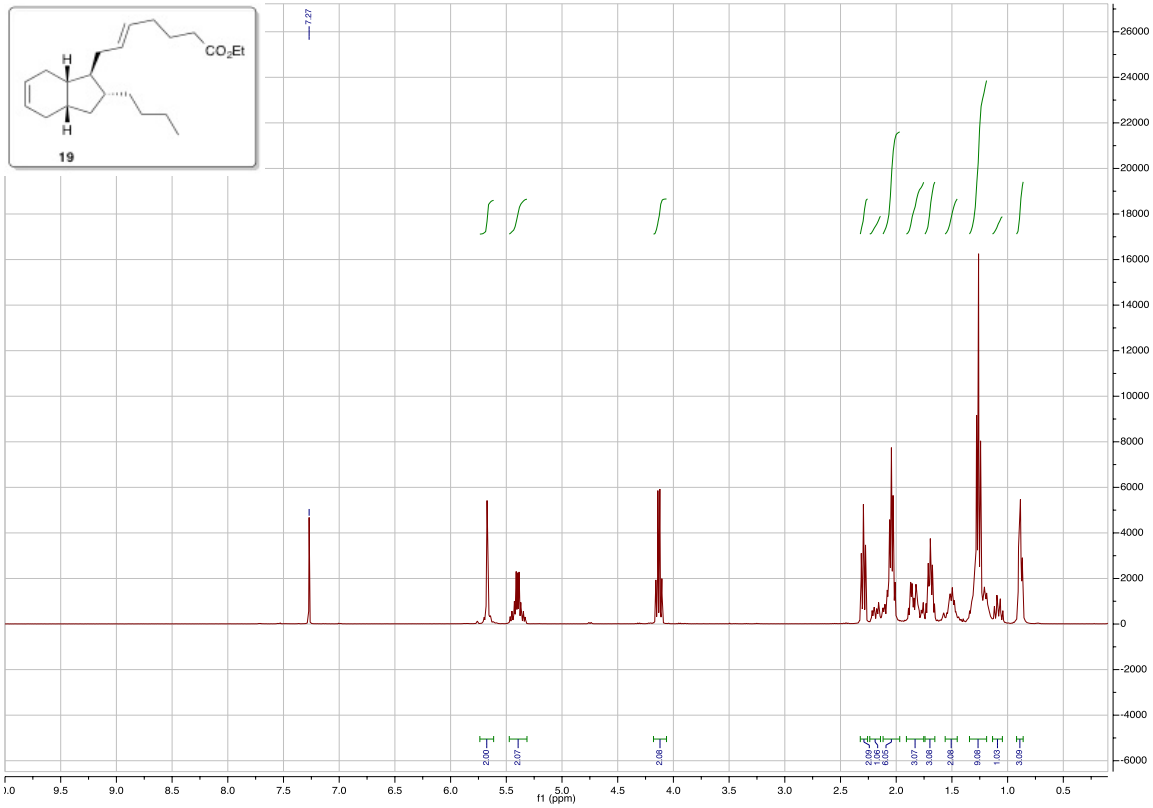


Figure S-31 ^{1}H-NMR spectrum of compound **19**.

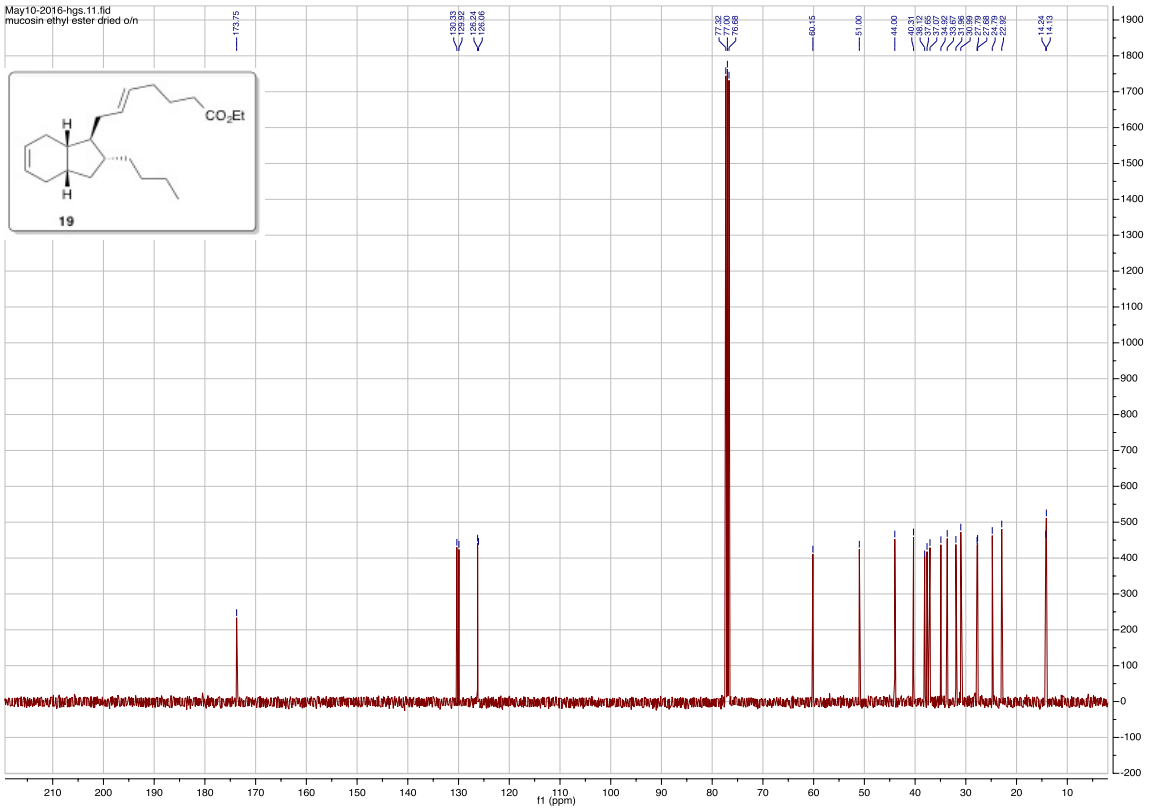


Figure S-32 ^{13}C-NMR spectrum of compound **19**.

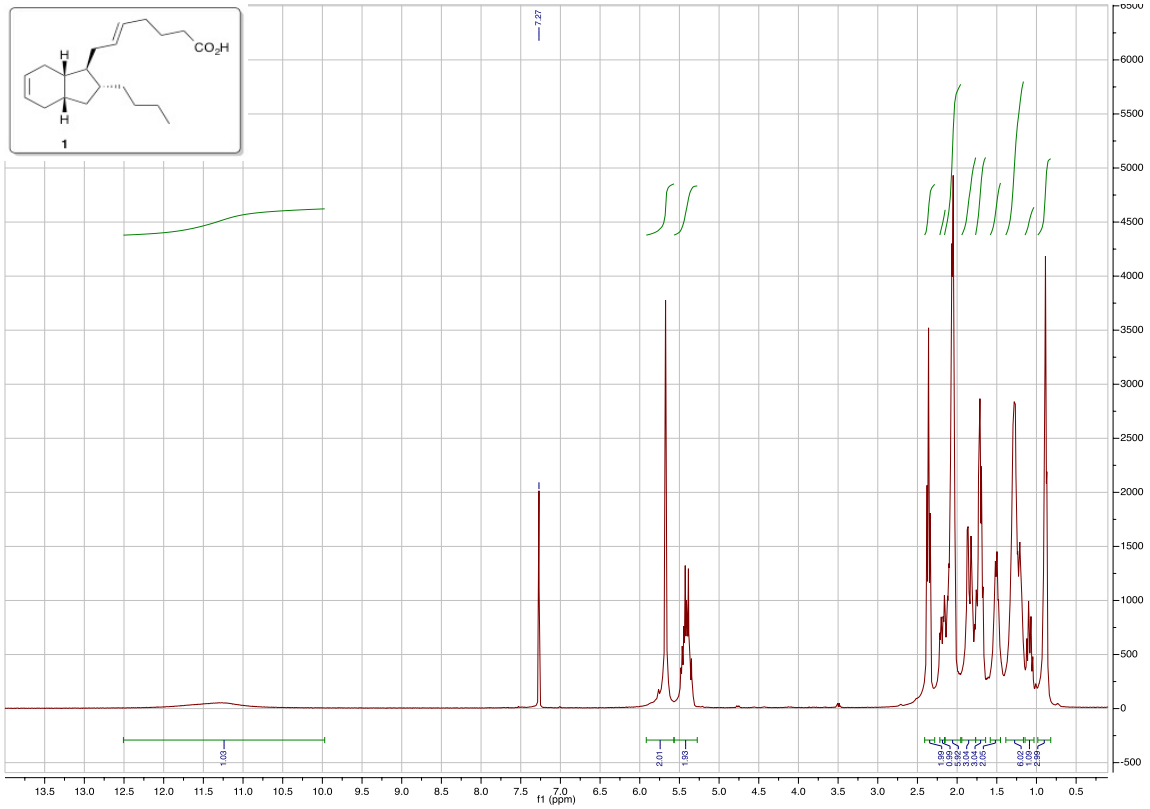


Figure S-33 ^{1}H-NMR spectrum of compound **1**.

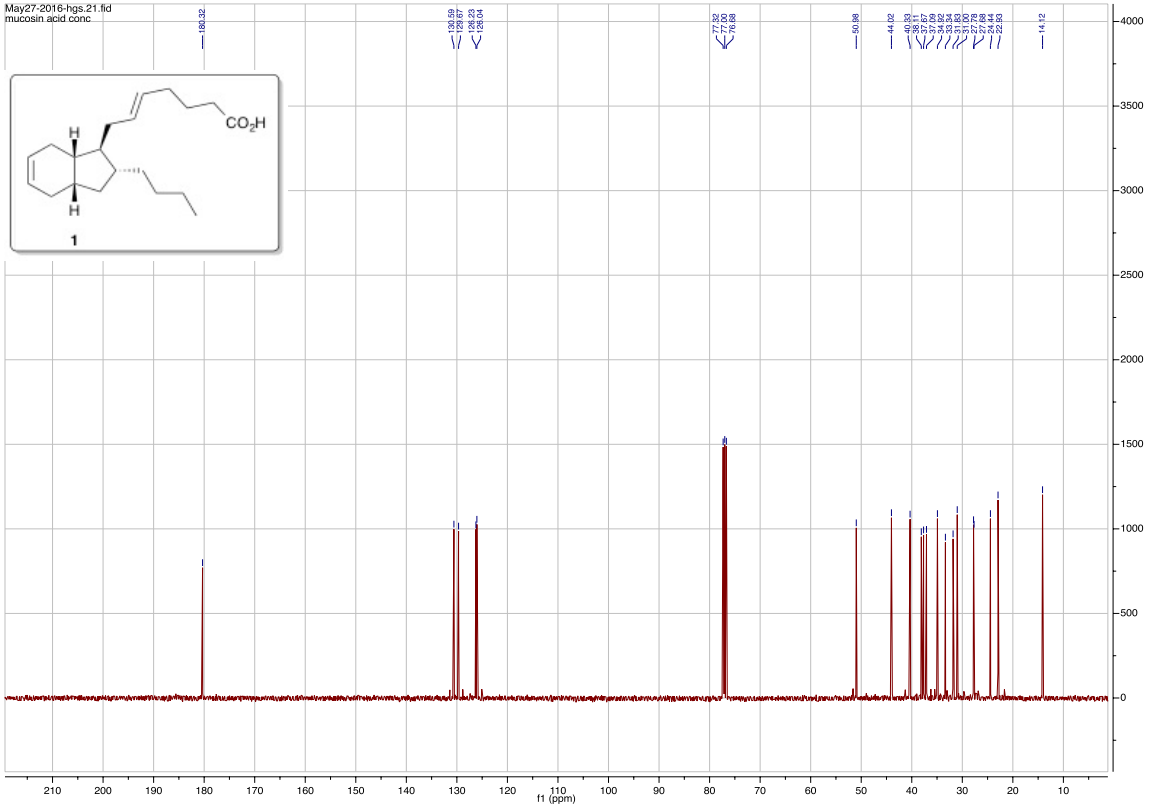


Figure S-34 ^{13}C-NMR spectrum of compound **1**.

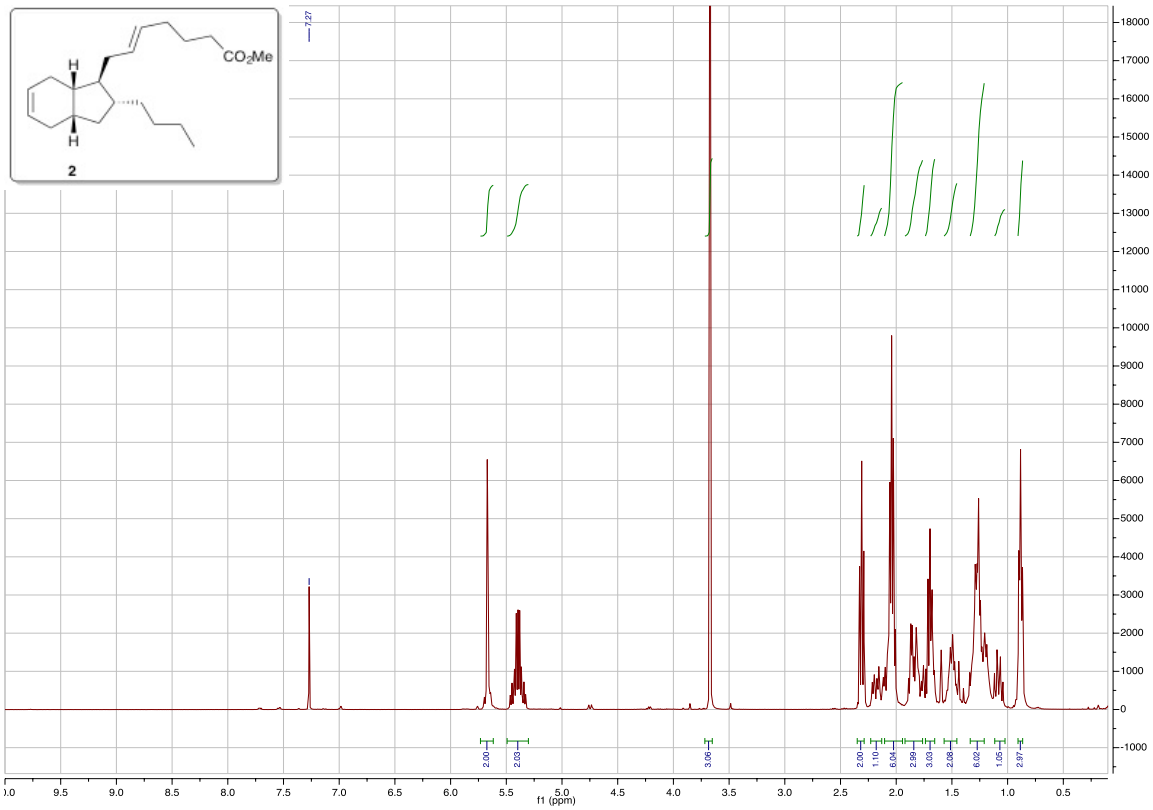


Figure S-35 ^{1}H-NMR spectrum of compound **2**.

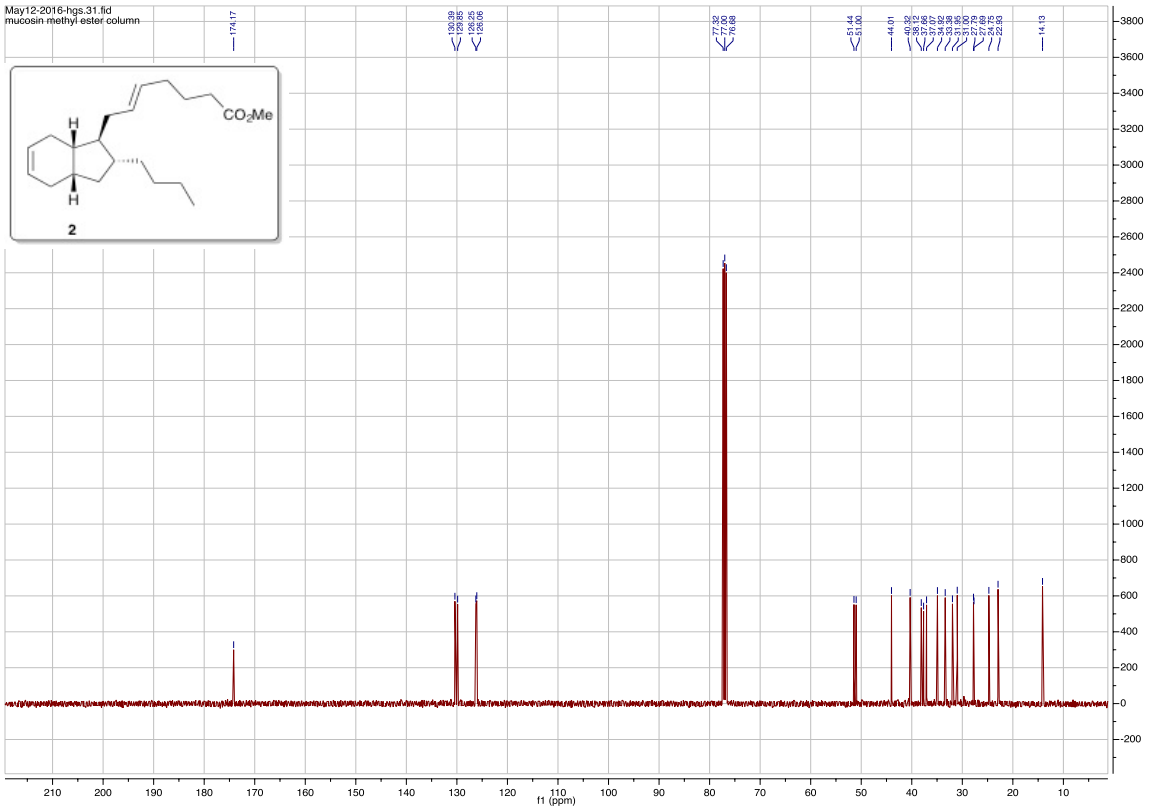


Figure S-36 ^{13}C-NMR spectrum of compound **2**.

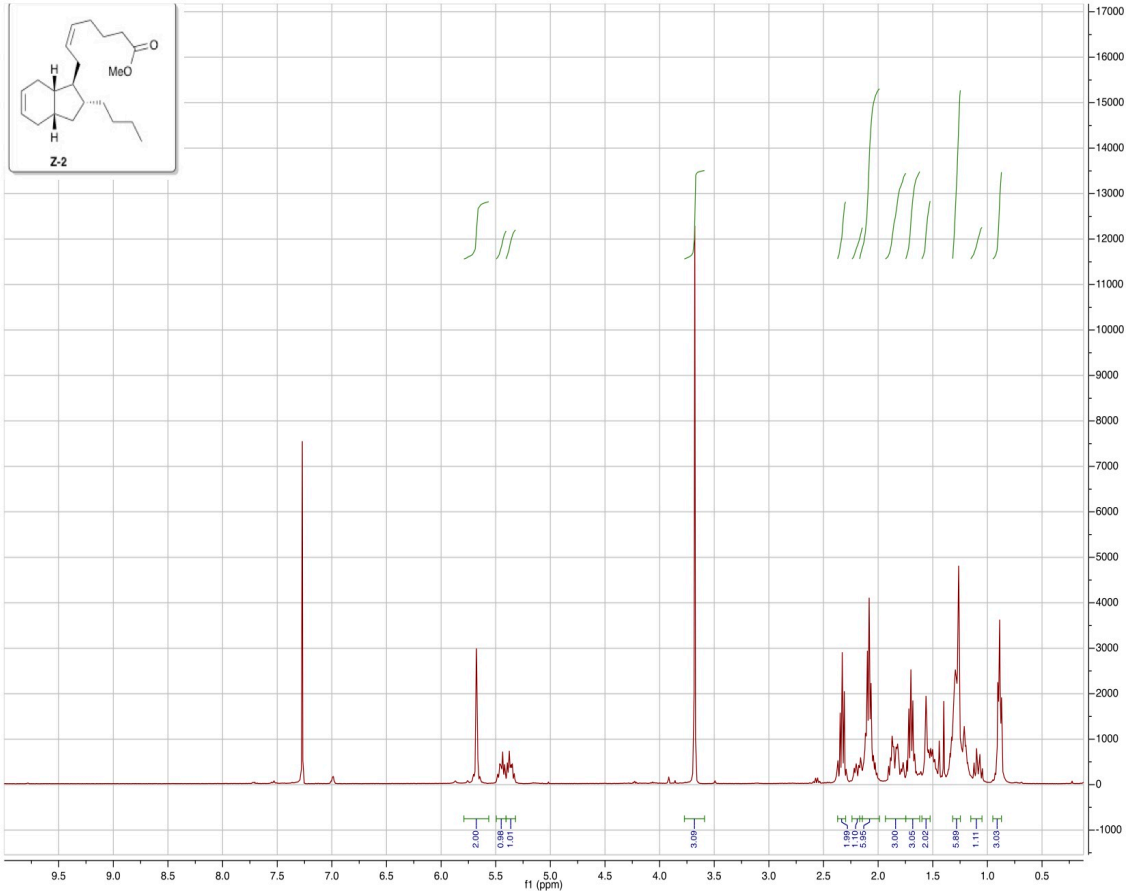
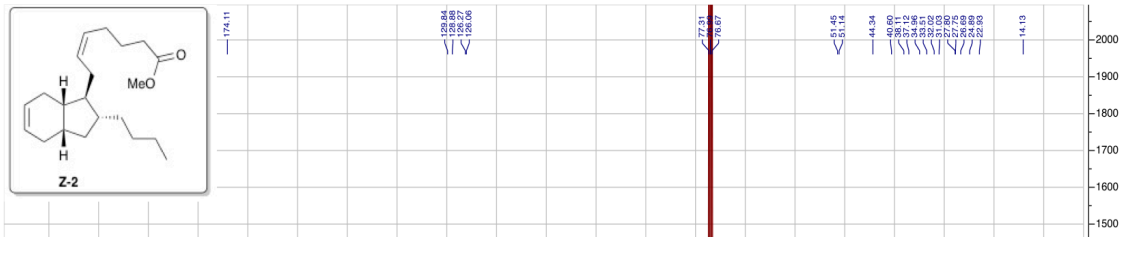
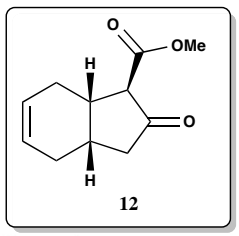


Figure S-37 ^{1}H-NMR spectrum of compound **2*Z*** for comparison with **2.**



Elemental Composition Report Page 1



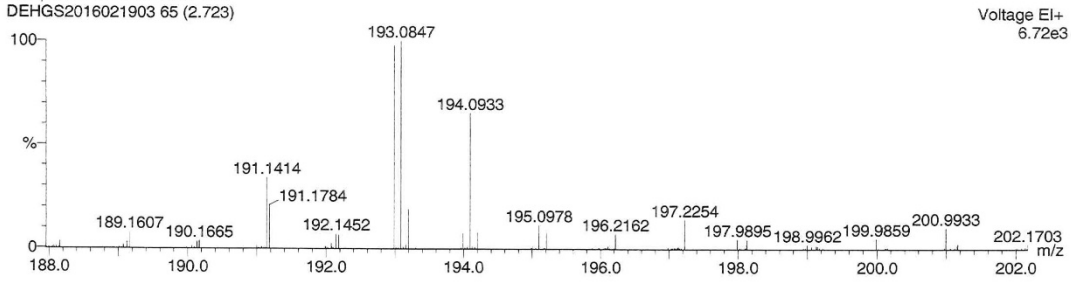
12

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
47 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

Sample 3 C11H14O3 MW 194
DEHGS2016021903 65 (2.723)

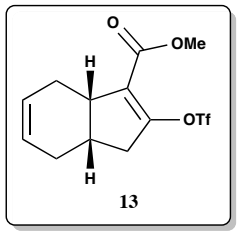
Voltage EI+
6.72e3



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
194.0933	194.0943	-1.0	-5.1	5.0	1	C11 H14 O3

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

Elemental Composition Report Page 1



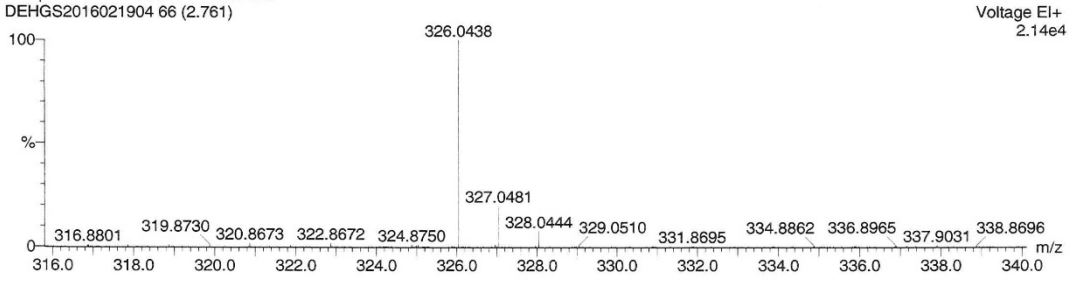
13

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
287 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)

Sample 4 C12H13O5SF3 MW 326
DEHGS2016021904 66 (2.761)

Voltage EI+
2.14e4



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
326.0438	326.0436	0.2	0.7	5.0	3	C12 H13 O5 S F3
	326.0424	1.4	4.2	9.0	2	C15 H12 O4 S F2
	326.0413	2.5	7.7	13.0	1	C18 H11 O3 S F

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

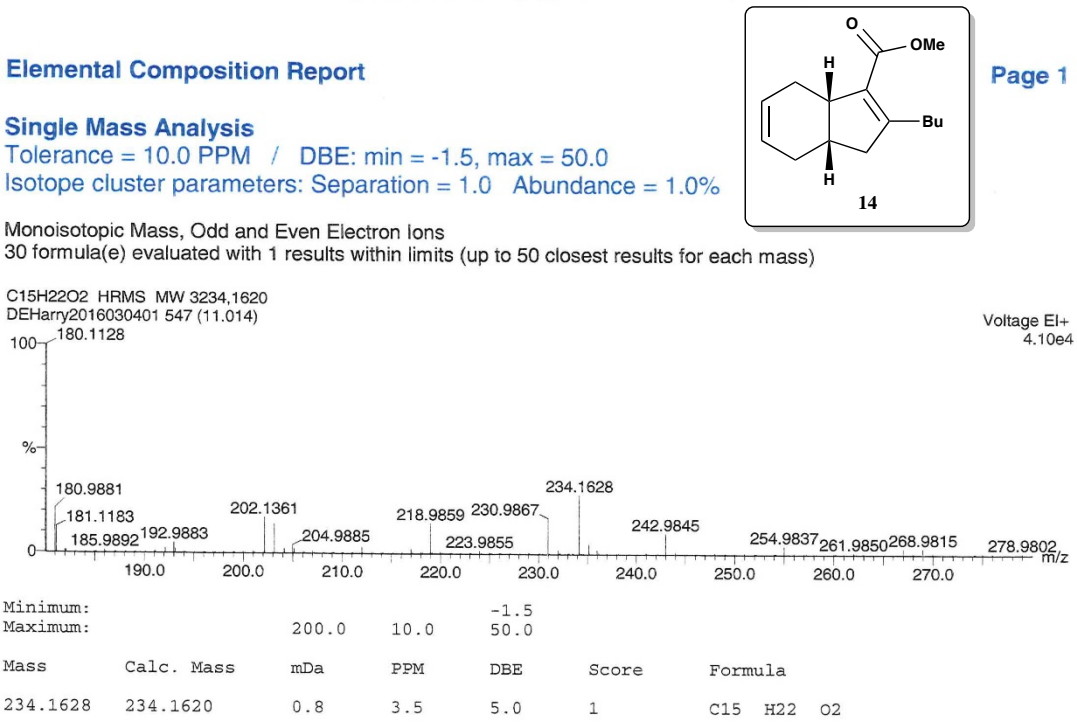


Figure S-41 HRMS of compound **14**.

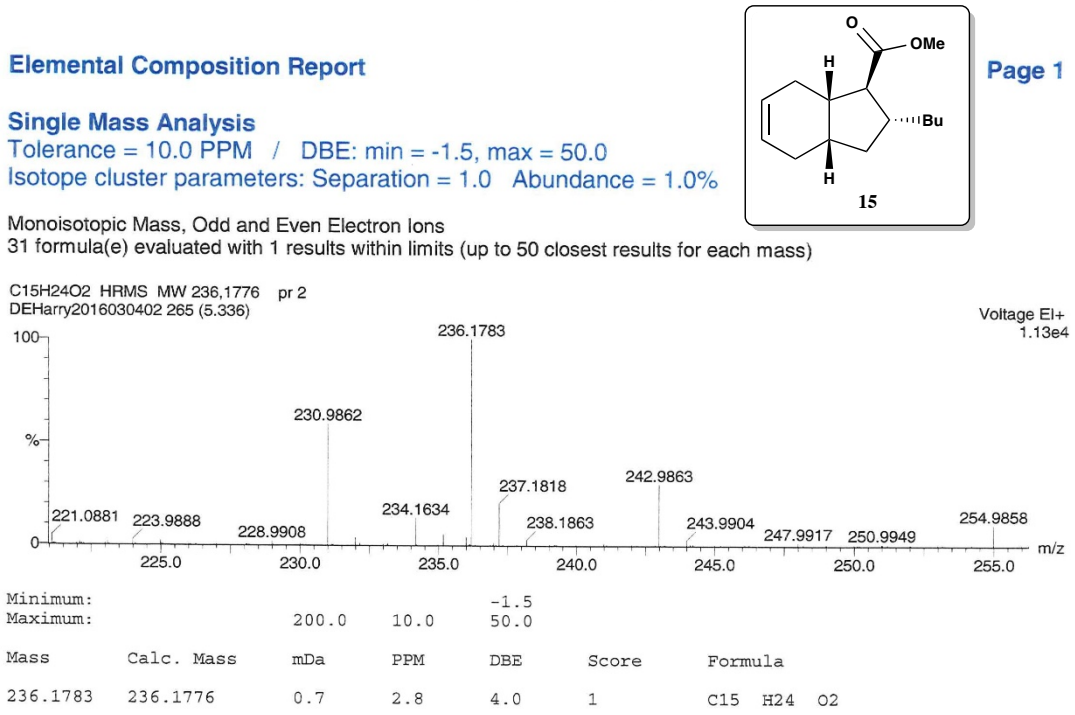


Figure S-42 HRMS of compound **15**.

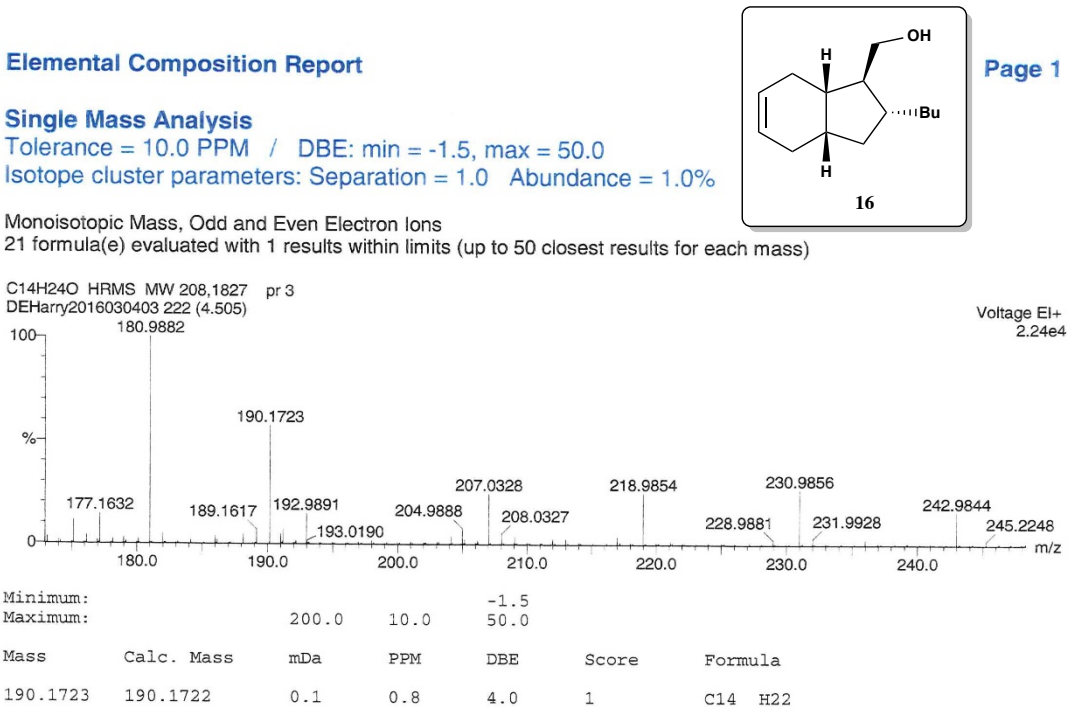


Figure S-43 HRMS of compound **16**.

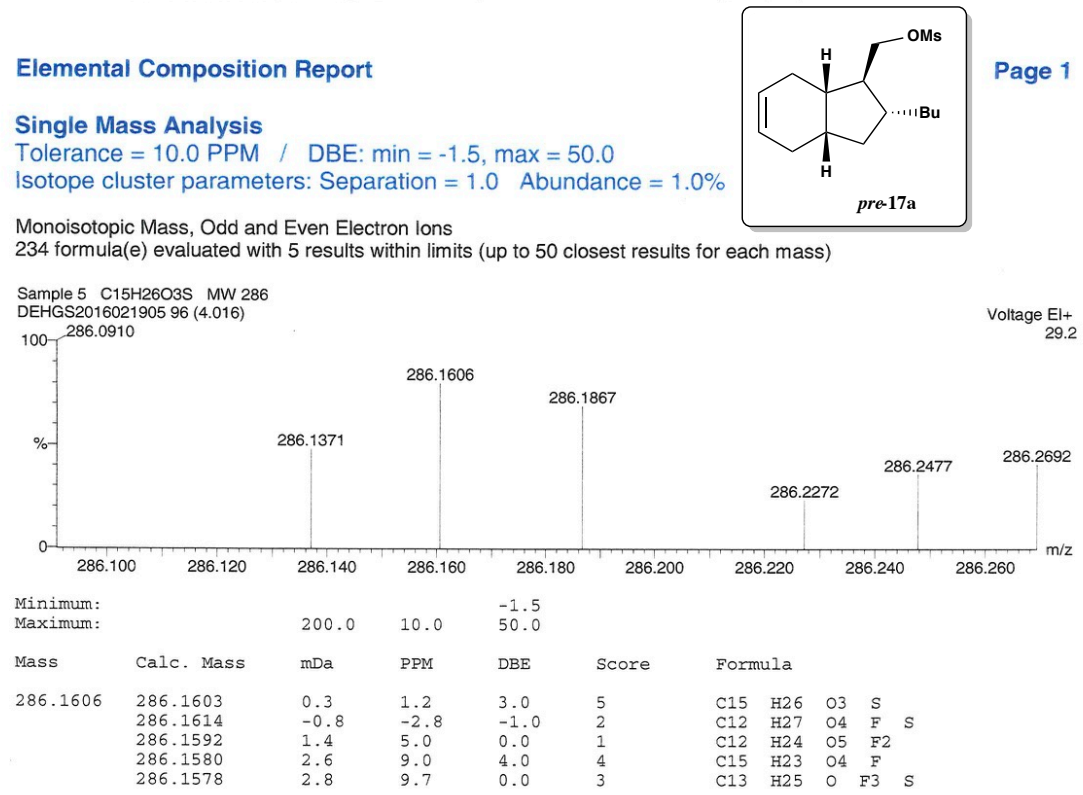


Figure S-44 HRMS of compound ***pre*-17a**.

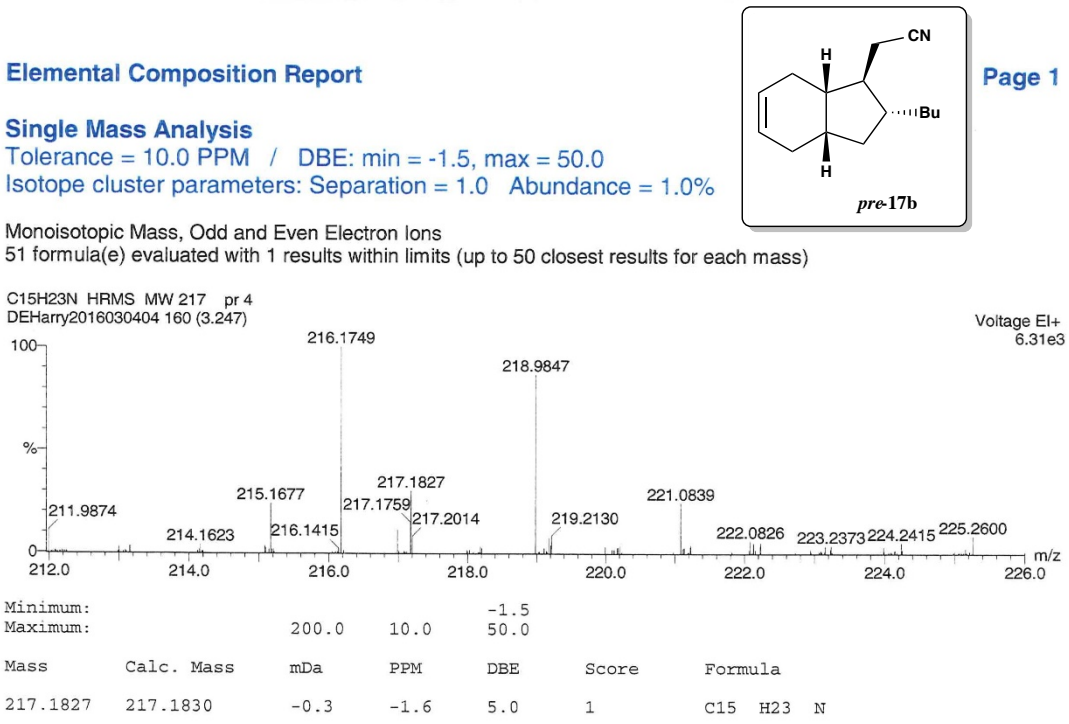


Figure S-45 HRMS of compound ***pre*-17b**.

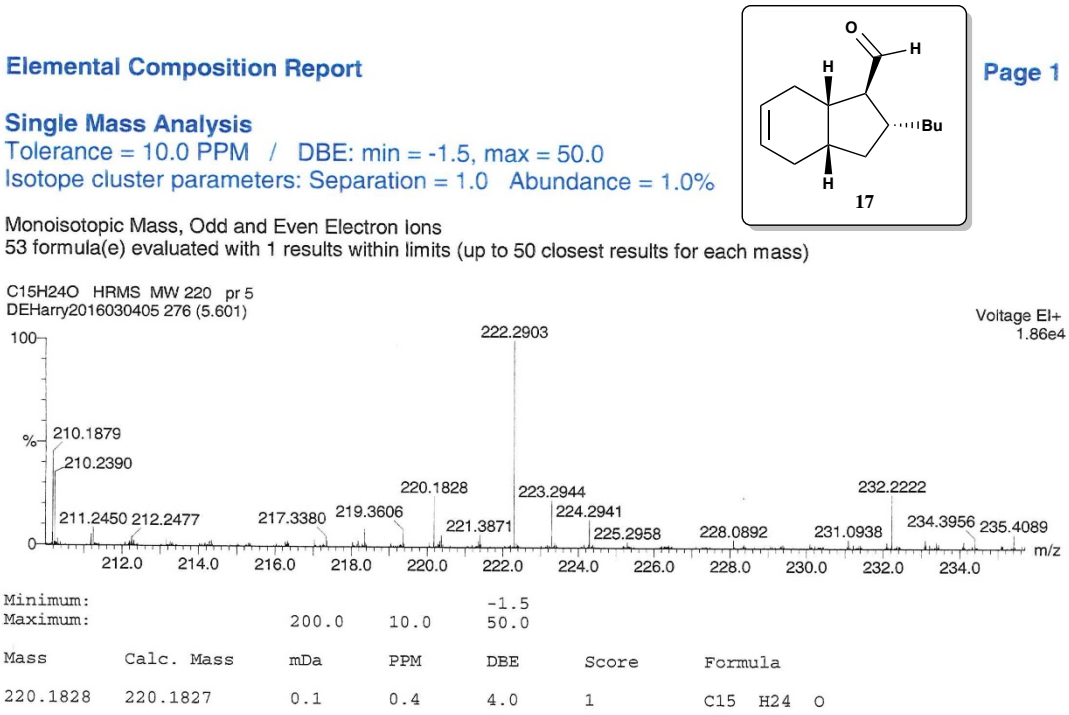


Figure S-46 HRMS of compound **17**.

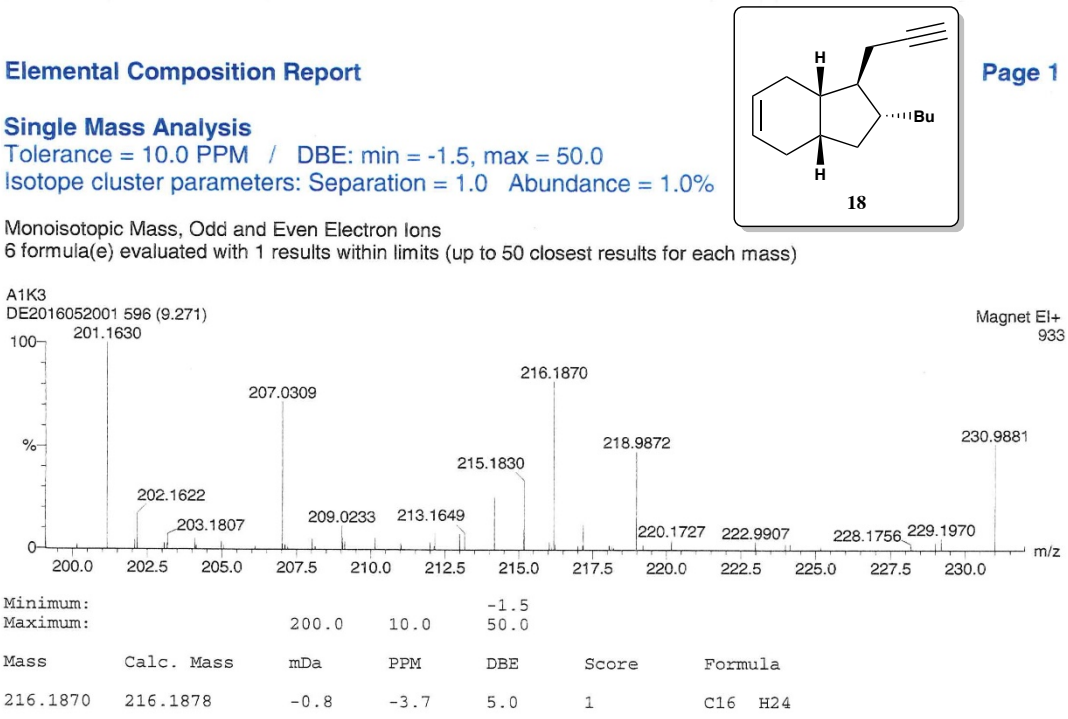


Figure S-47 HRMS of compound **18**.

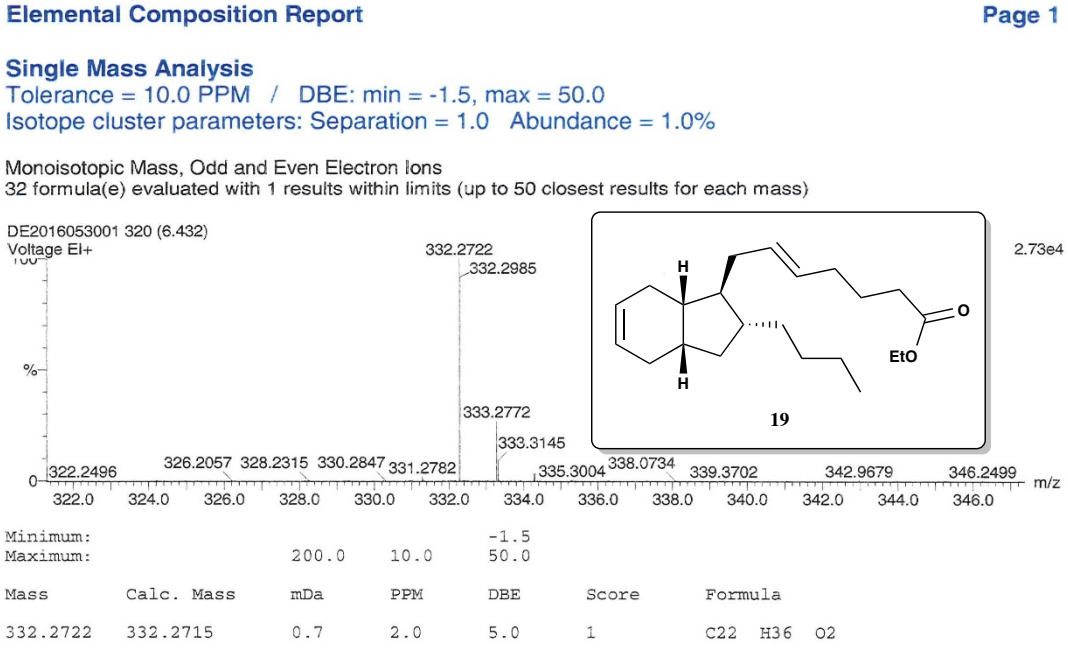
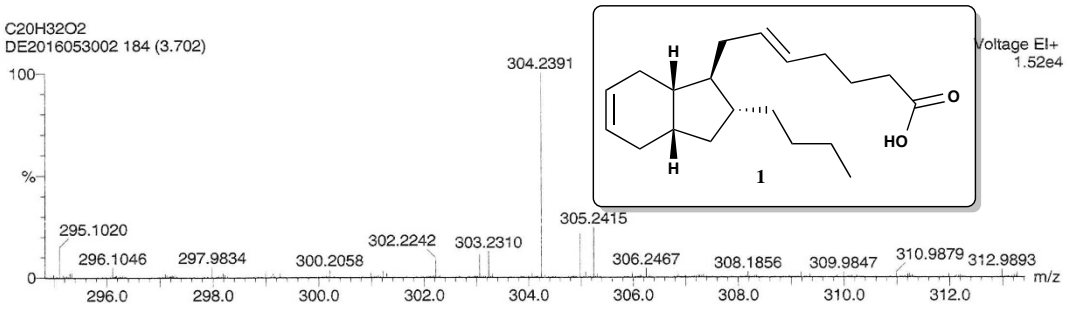


Figure S-48 HRMS of compound **19**.

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
29 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)



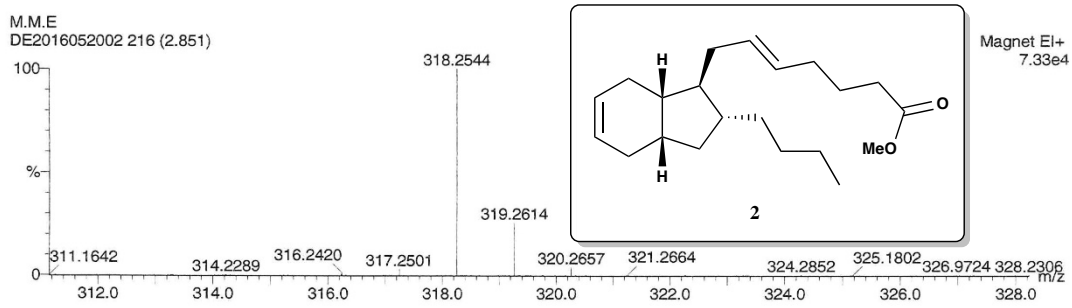
Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
304.2391	304.2402	-1.1	-3.7	5.0	1	C20 H32 O2

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

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
31 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
318.2544	318.2559	-1.5	-4.7	5.0	1	C21 H34 O2

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

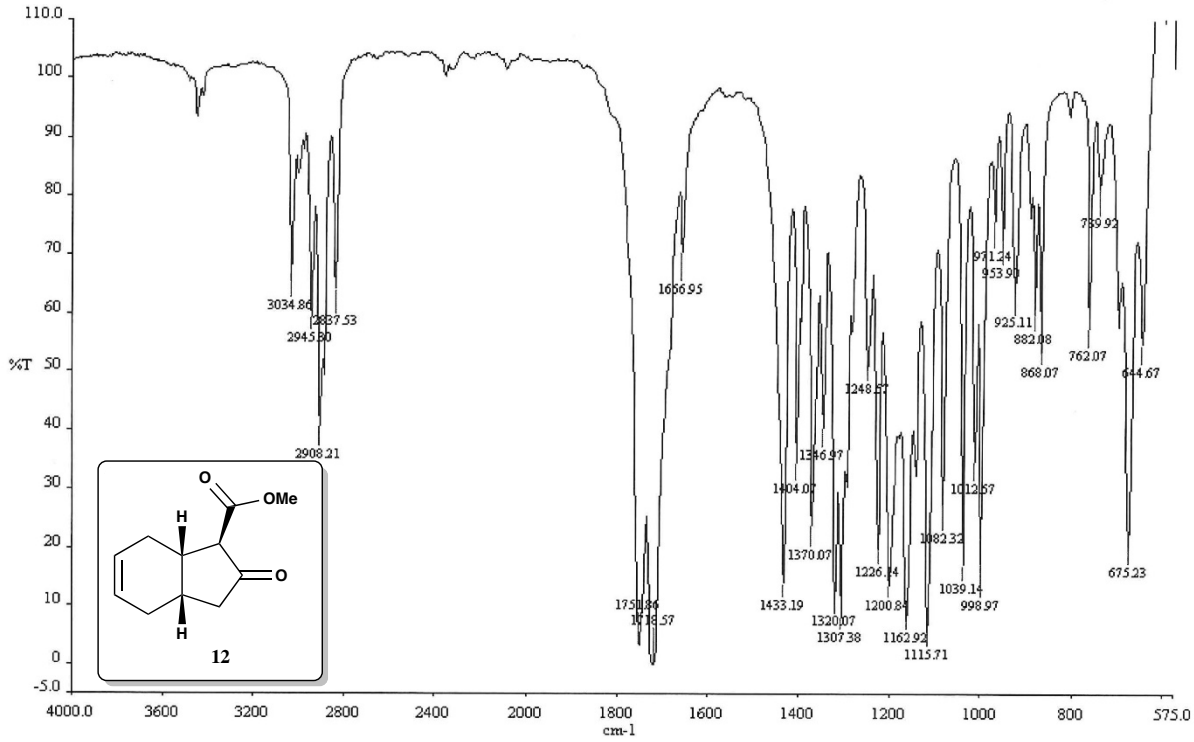


Figure S-51 IR of compound **12**.

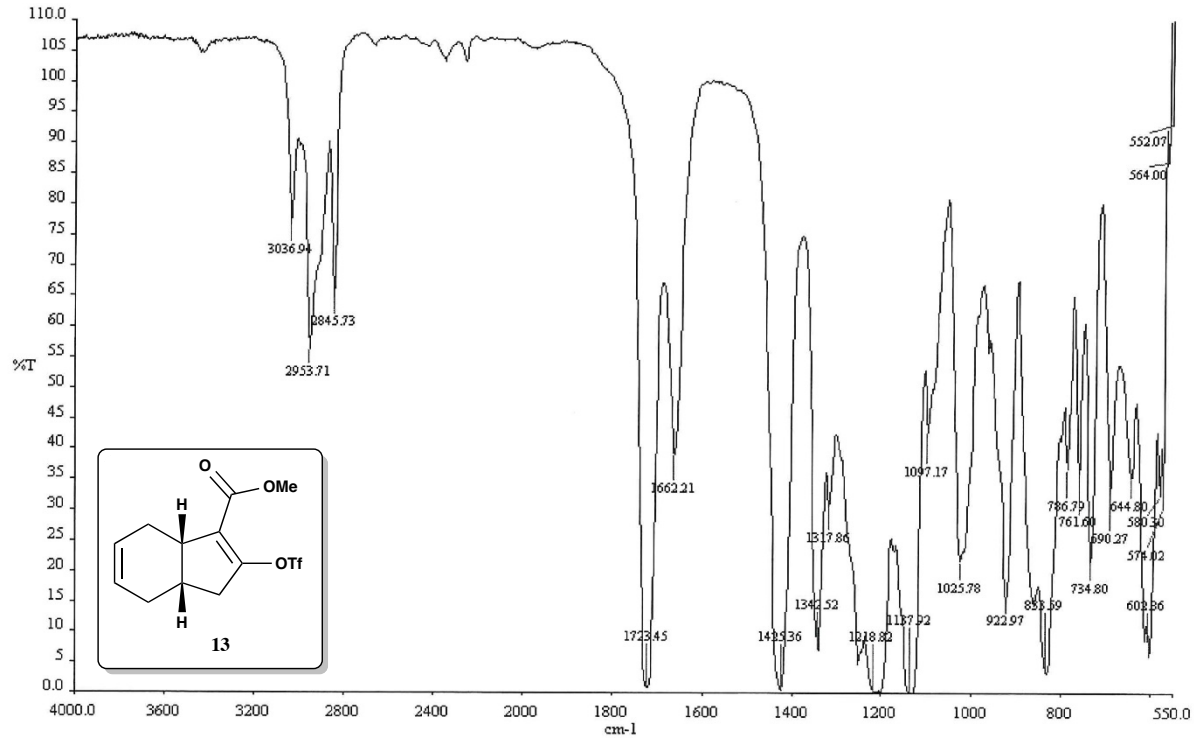


Figure S-52 IR of compound **13**.

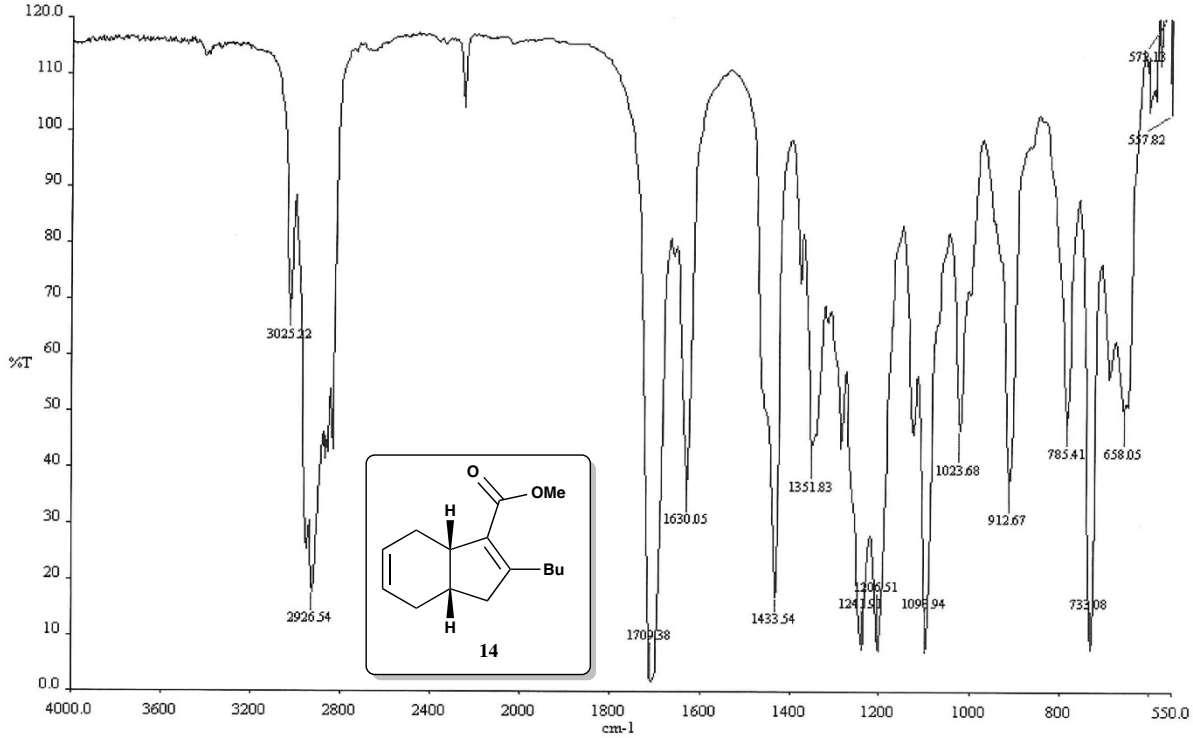


Figure S-53 IR of compound **14**.

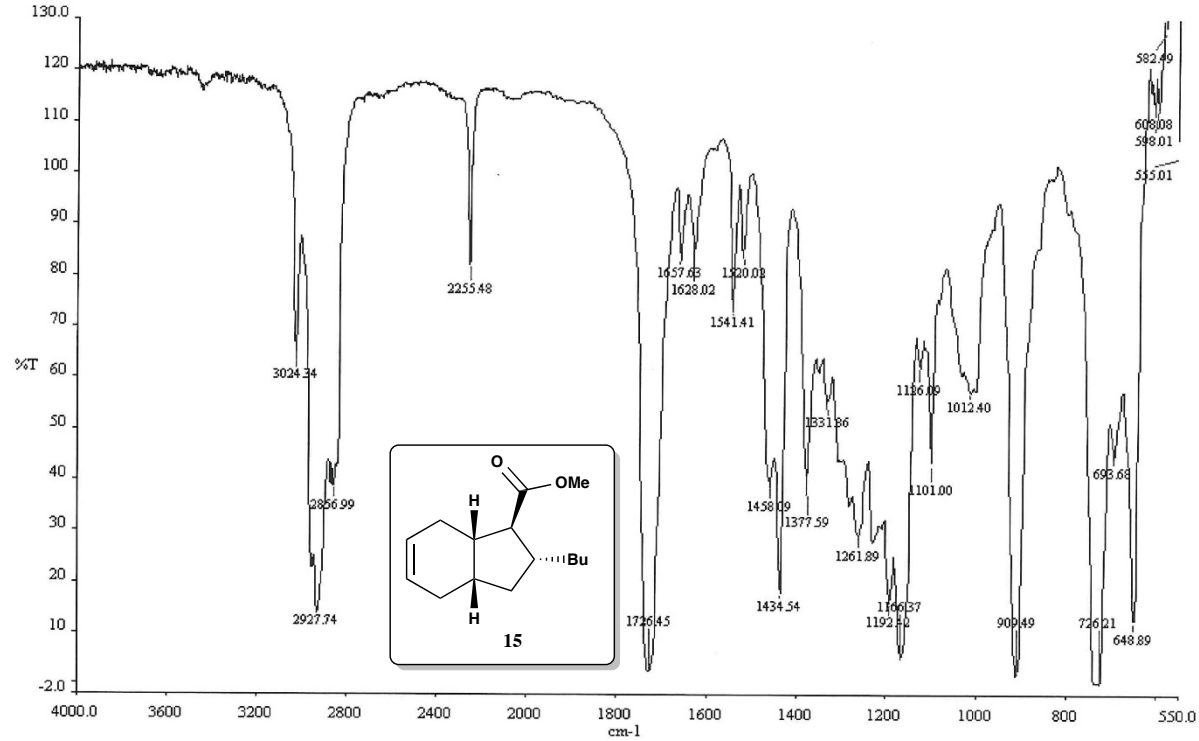


Figure S-54 IR of compound **15**.

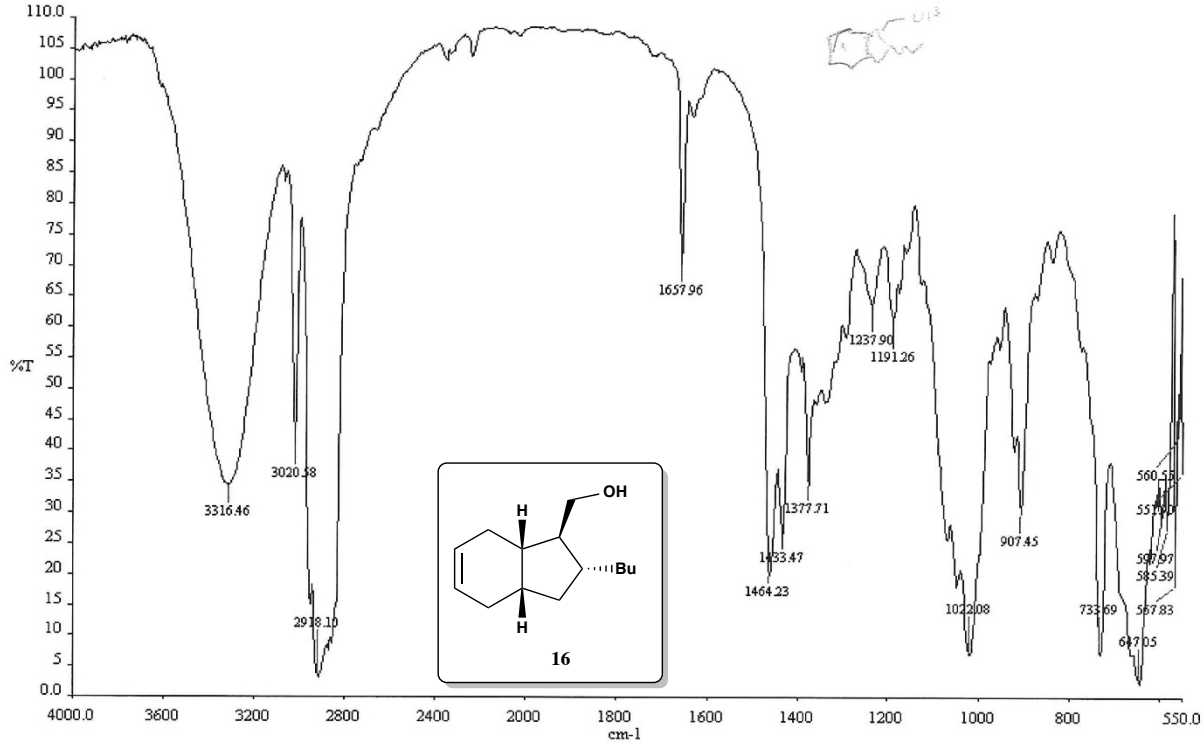


Figure S-55 IR of compound **16**.

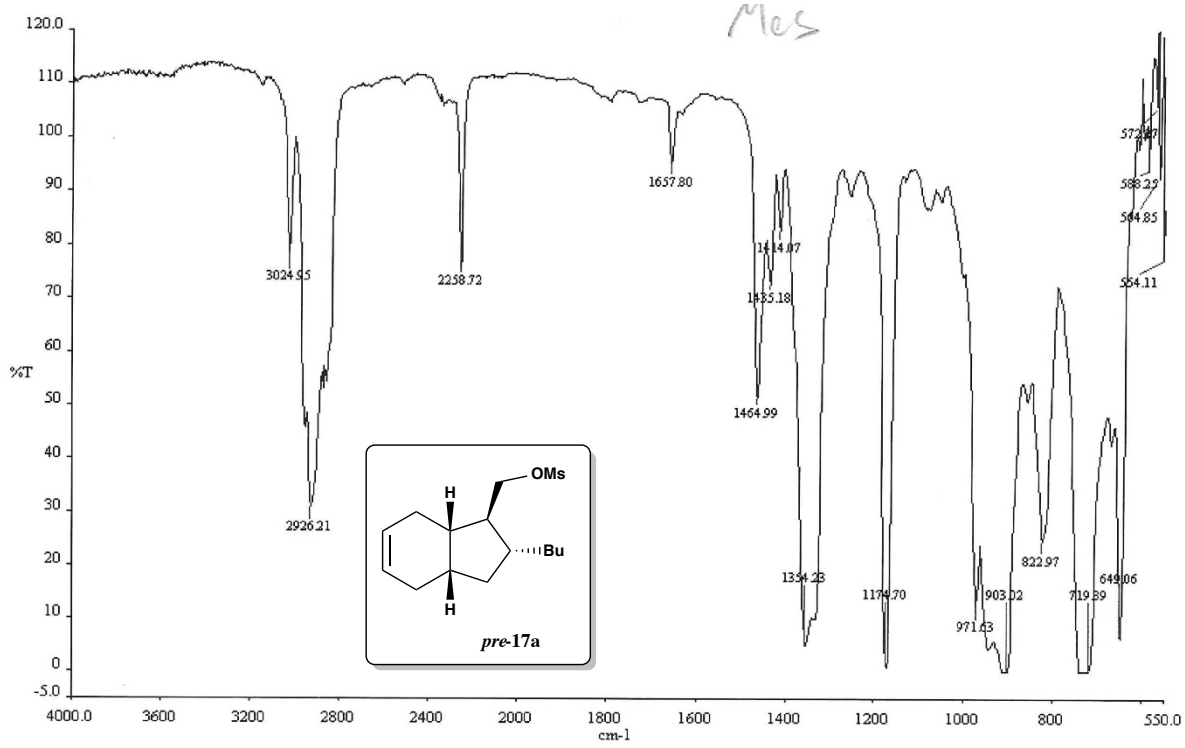


Figure S-56 IR of compound ***pre*-17a**.

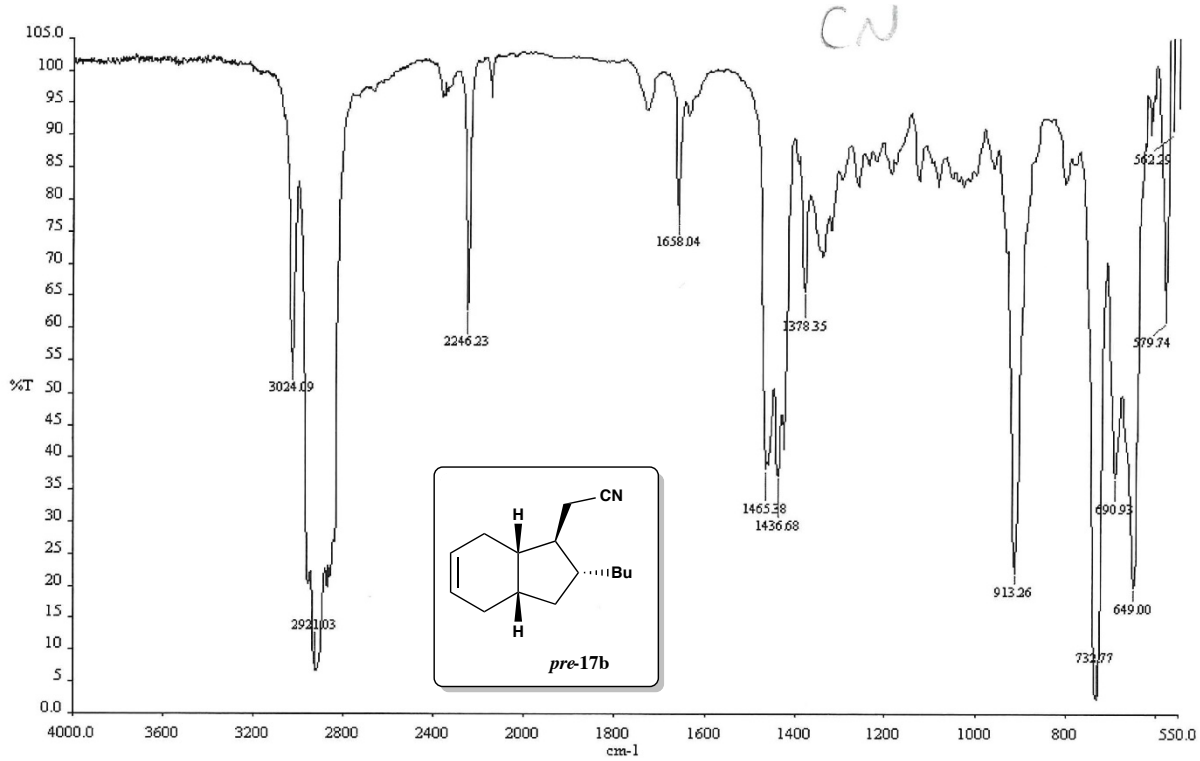


Figure S-57 IR of compound ***pre*-17b**.

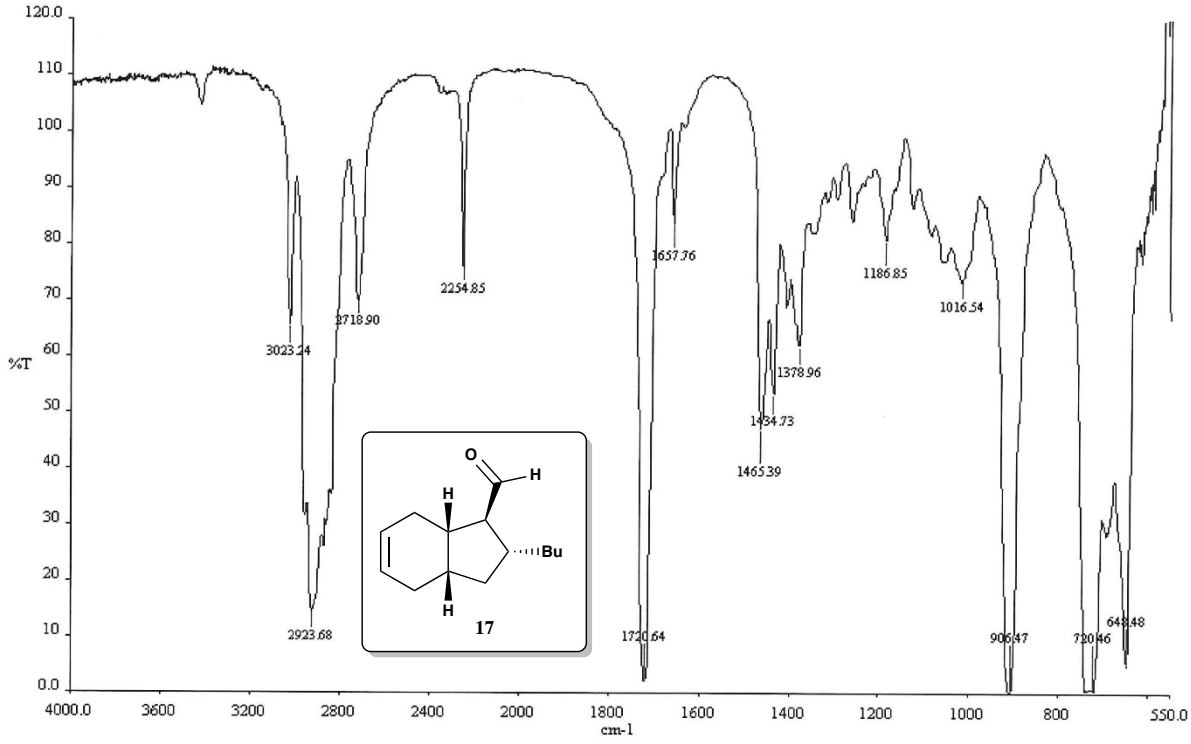


Figure S-58 IR of compound **17**.

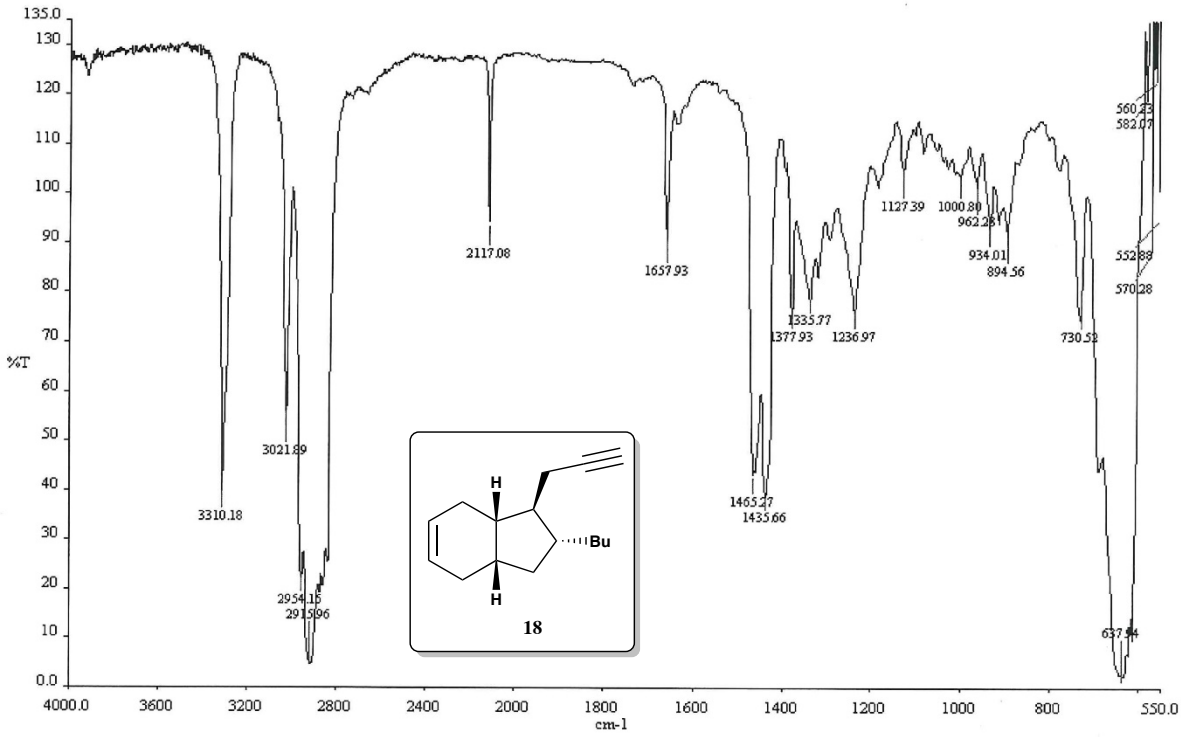


Figure S-59 IR of compound **18**.

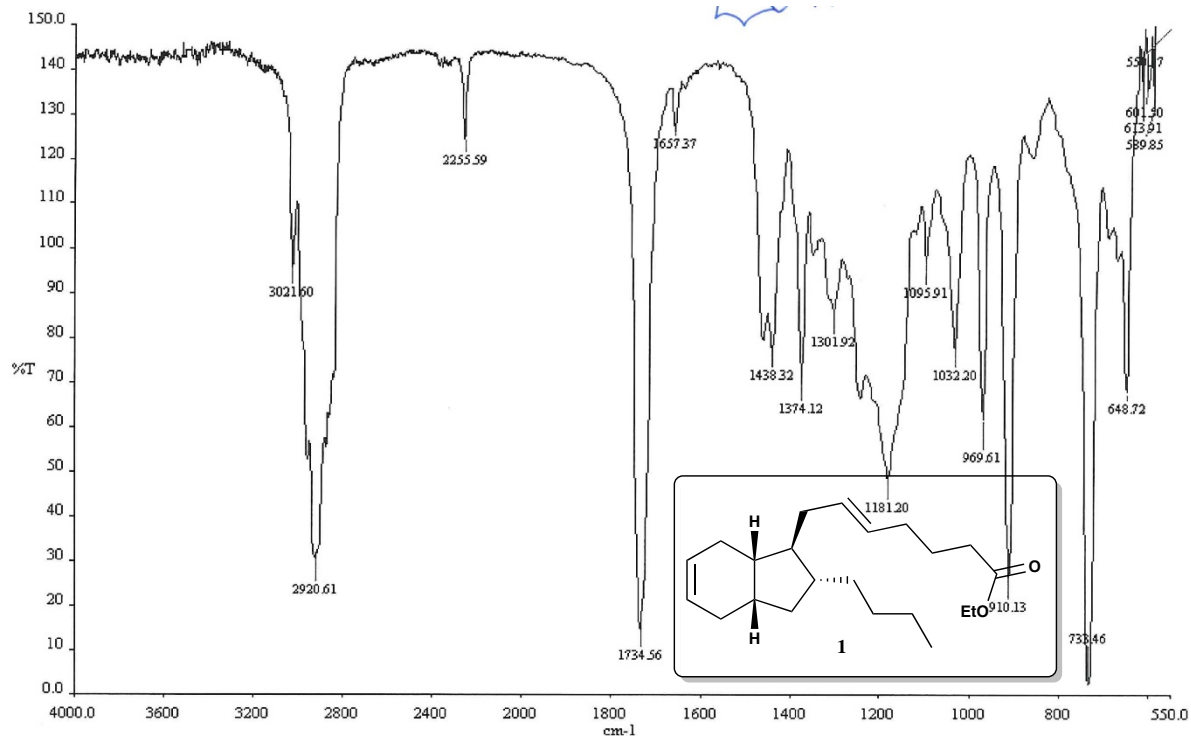


Figure S-60 IR of compound **19**.

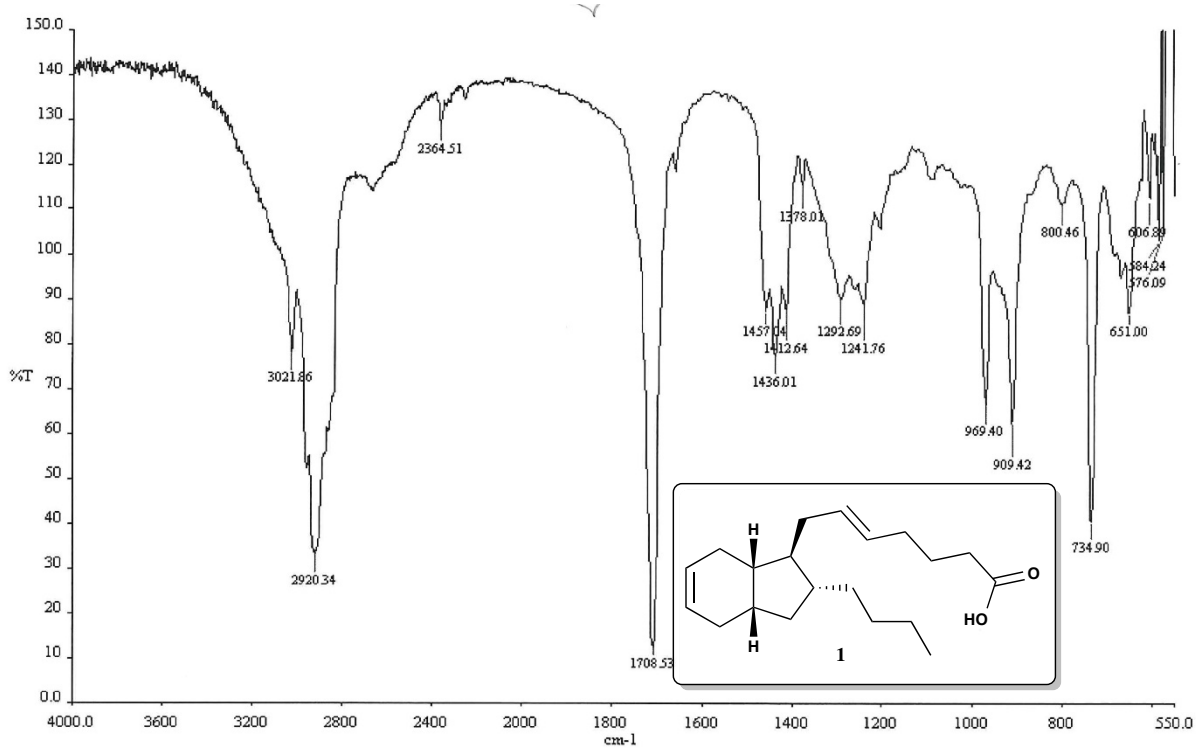


Figure S-61 IR of compound **1**.

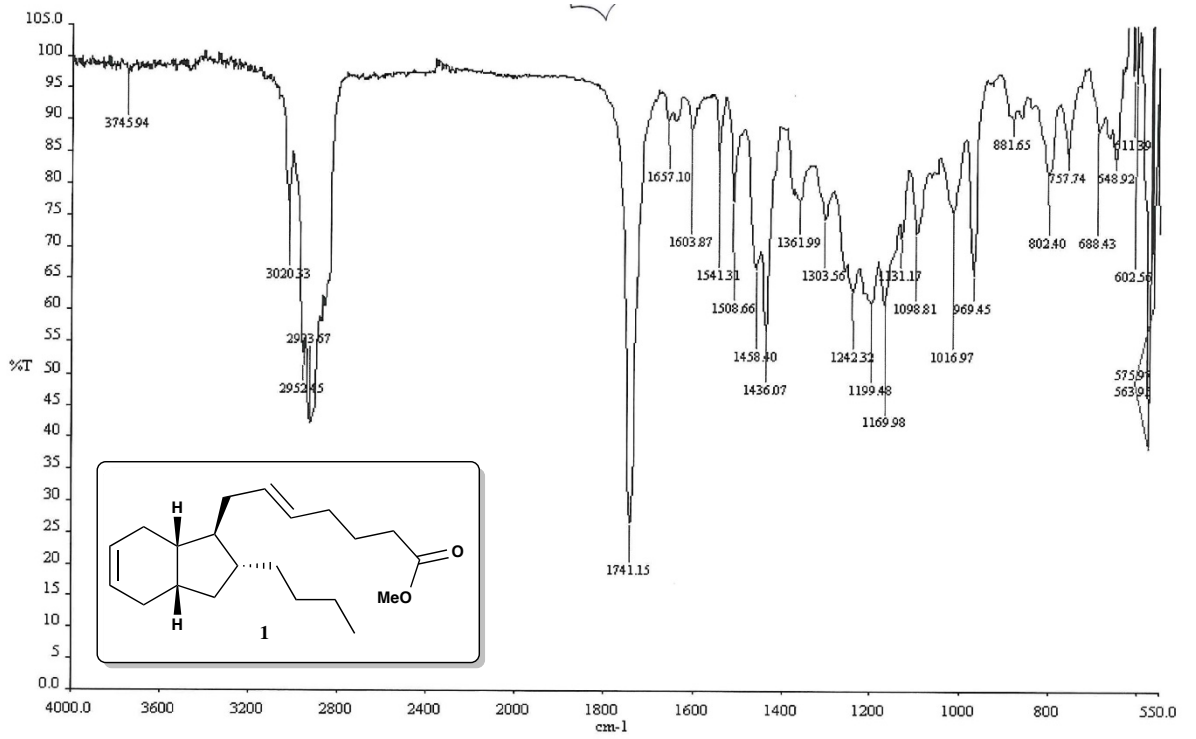
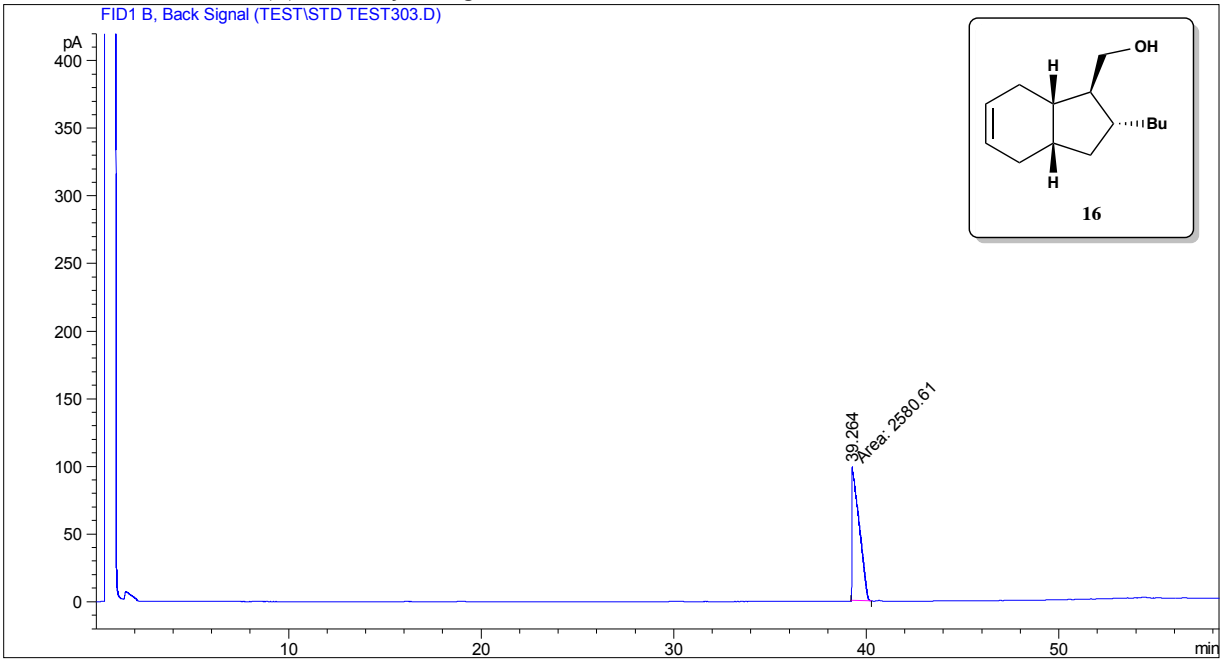


Figure S-62 IR of compound **2**.

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

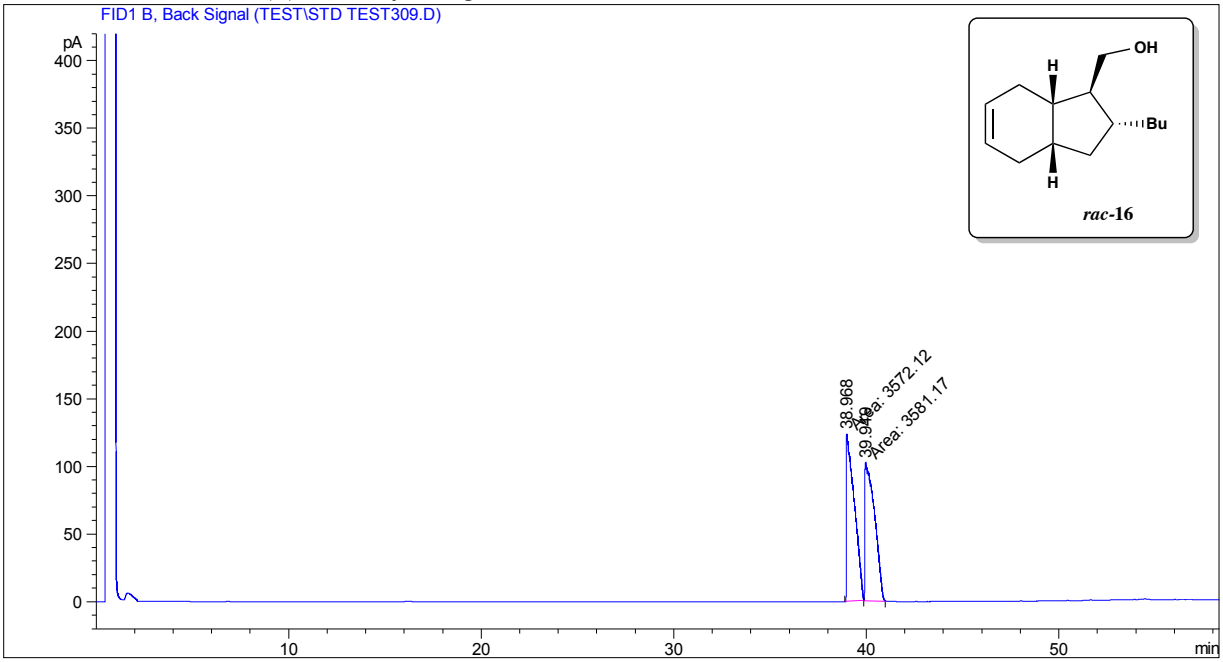
==========
*** End of Report ***

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

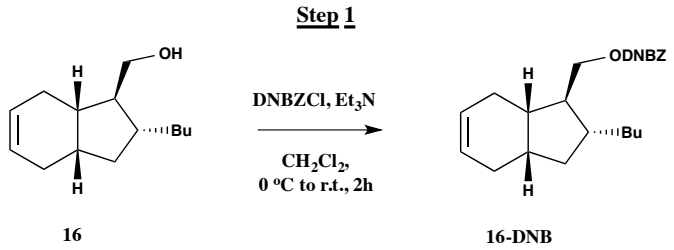
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

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

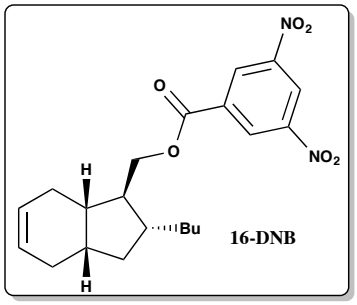
Figure S-66 Report from chiral GLC of compound *rac*-**16**.

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



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.

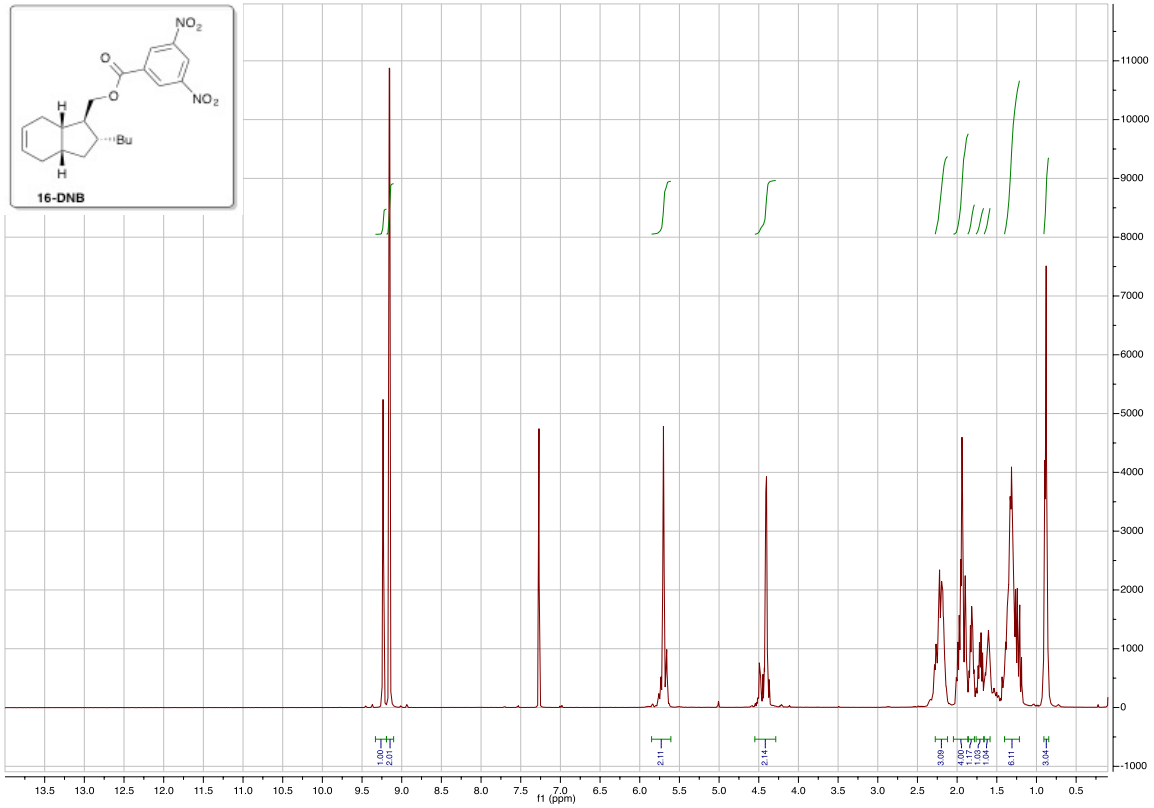


Figure S-67 ^{1}H-NMR spectrum of compound **16-DNB**.

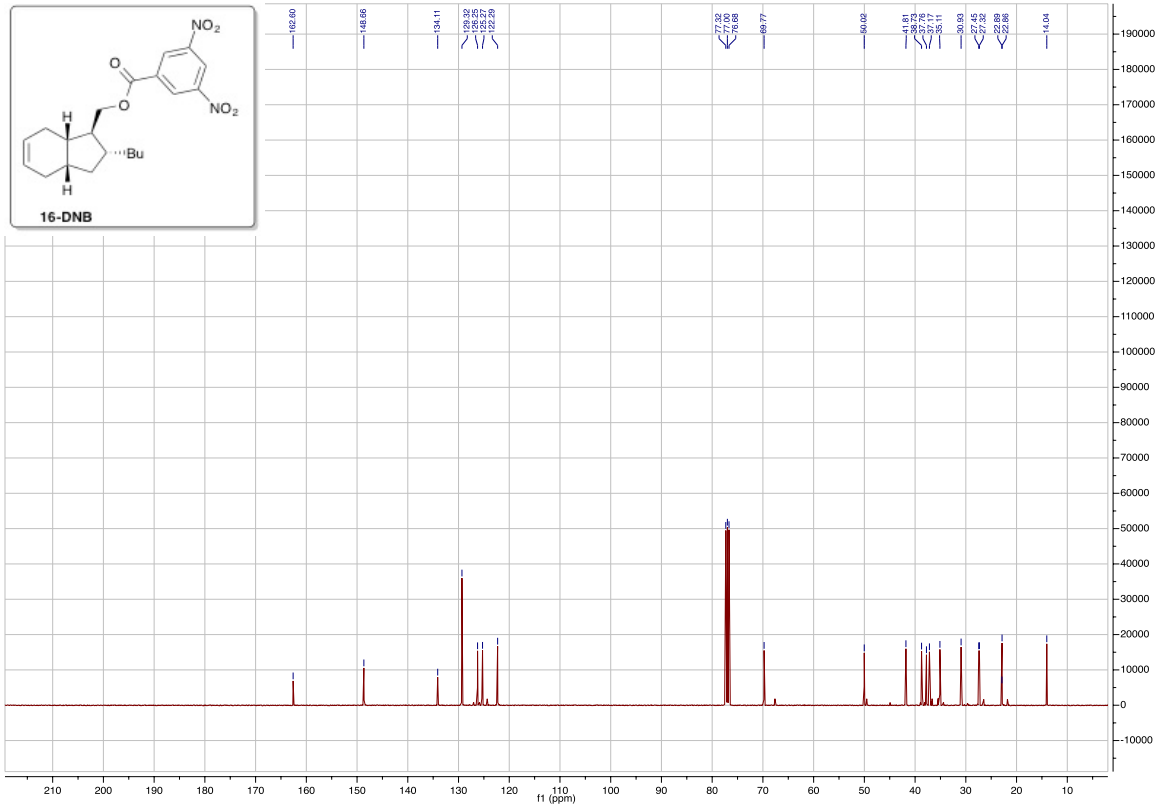


Figure S-68 ^{13}C-NMR spectrum of compound **16-DNB**.

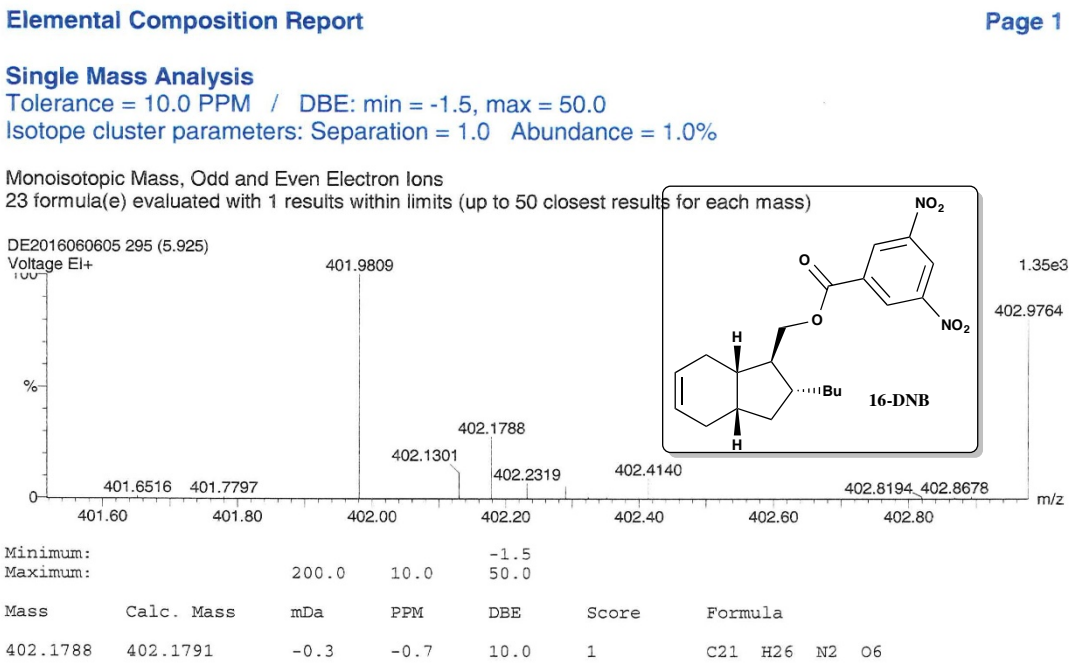


Figure S-69 HRMS spectrum of compound **16-DNB**.

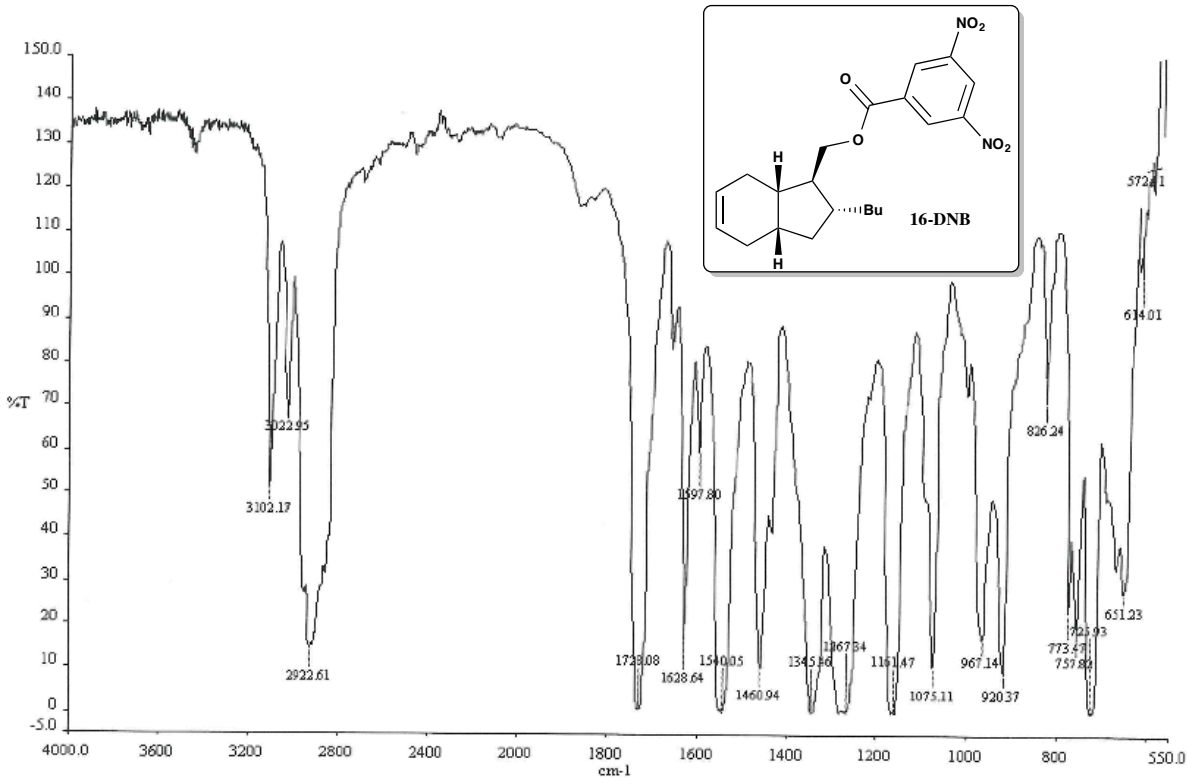


Figure S-70 IR spectrum of compound **16-DNB**.

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

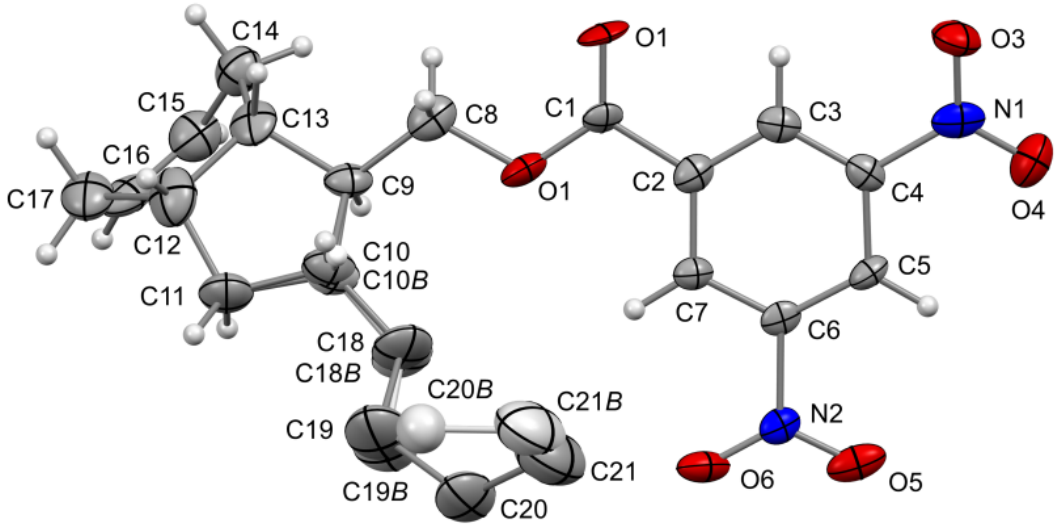


Figure S-71 Single crystal X-ray structure of the 3,5-dinitrobenzoate of alcohol **16** at 110 K. The disordered ^{n}butyl group (H-atoms omitted) has a major orientation [occupancy 0.74(3)] with *trans,trans,gauche*+ torsion angles along C9-C10-C18-C19-C20-C21, while the minor orientation (atoms in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

$(\Delta/\sigma)_{max} < 0.001$
$\Delta\rho_{max} = 0.30$ 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 (Å²)

	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

Pursuing the true structure of marine natural product mucosin: Stereopermutation on the bicyclo[4.3.0]non-3-ene scaffold.

Simen Gjelseth Antonsen, Harrison Gallantree-Smith, Carl Henrik Görbitz, Trond Vidar Hansen, Jens Mortansson Jelstrup Nolsøe and Yngve Stenstrøm. Manuscript.

Pursuing the true structure of the marine natural product mucosin. Part 2

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Recently we reported on the first total synthesis of mucosin according to its claimed structure. Having demonstrated the erroneous assignment, we now pursue the true identity of the marine natural product. A stereodivergent strategy has been devised, which takes advantage of a topological bias displayed by the featured bicyclo[4.3.0]non-3-ene scaffold.

Certain metabolites derived from polyunsaturated fatty acids (PUFAs) play a key role in mammalian physiology, where they orchestrate both inflammatory response as well as the return to homeostasis.[1] By combining total synthesis with chemical biology and molecular pharmacology, a number of distinct eicosanoids and docosanoids have been identified, which are active in the cascade elicited by noxious stimuli.[2,3] As a result, novel natural products with an underlying PUFA motif are of great interest as potential immunomodulators.

Since antiquity, sea dwelling organisms have proven to be a particularly abundant source of new chemical entities, set apart from those found in the terrestrial environment.[4] Thus, the ancient Phoenicians were renowned for their trading with Tyrian purple from the *Murex* sea snail.[5] Rising above mere prospecting, modern-day discovery, enabled by the advent of powerful analytical instruments and methods, has found a wealth of bioactive compounds in the marine environment.[6,7]

Ostensibly, mucosin (**1**) is a natural product that was isolated from the Mediterranean sponge *Reniera mucosa* as methyl ester **2**.[8] Formally classified as an eicosanoid, it has been conjectured to originate from arachidonic acid (**3**), based on the C_{20}-architechture (Fig. 1). While sharing some noticeable features with the prostane scaffold, the compound differs by having an unusual bicyclic core. Clearly, in the

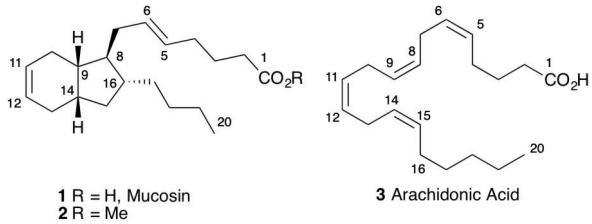

Fig. 1 Suggested structure of mucosin and its relation to arachidonic acid.

structure proposed for mucosin (**1**), the characteristic cyclopentane ring is integrated in a *cis*-fused bicyclo[4.3.0]non-3-ene system. However, turning to the elucidation of methyl ester **2**, the assignment of topology poses a challenge. While only a small molecule, the structure is compact in terms of the four contiguous stereocentres. In NMR, pertaining to both 1D- and 2D- techniques, the distinguishing resonances/correlations are ensconced in a crowded aliphatic region. Consequently, the assignment published by Casapullo *et al.*,[8] based on work performed at moderate magnetic field strengths, does not convince on its own.

Fascinated by the structure and the prostanoid motif, we devised a practical, divergent and synthetically unambiguous strategy to establish the proposed stereochemistry. At the end of the campaign, capitalizing on X-ray crystallography to pinpoint the relative arrangement, it was concluded that mucosin (**1**) does not represent the portrayed compound.[9] In a pursuit to identify the natural product isolated from *Reniera mucosa*, our intent is to achieve the goal by manipulation of the bicyclo[4.3.0]non-3-ene system. We herein detail synthesis of the mucosin diastereomer **1***, demonstrating aspects of the chosen route with regard to stereochemical control (Fig. 2).

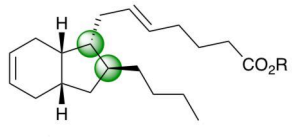

Fig. 2 Stereopermutation on the *cis*-bicyclo[4.3.0]non-3-ene scaffold.

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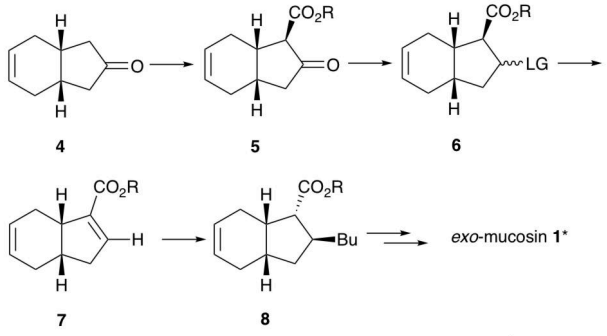
† Electronic Supplementary Information (ESI) available: Experimental procedures and characterisation data for all new compounds; crystal data and refinement details are included to give evidence of the relative stereochemistry of a late stage intermediate. See DOI: 10.1039/x0xx00000x

A central feature in our divergent strategy (Scheme 1) was to take advantage of the efficient desymmetrization of *meso*-ketone **4**.[9] After a chiral foothold had been established, it would then be a matter of introducing a functional pattern amenable for subsequent stereoiteration. Ideally, in order to uncover the topologically deviant point(s), the prerequisite *cis*-fused keto ester **5** should also be interconvertible with the *trans*-fused system if need be. However, we first chose to examine the configuration at the appended positions. Thus, along these lines and having previously established a diastereochemical bias, addition of some suitable nucleophile to conjugate ester **7** was judged to follow the precognised trend. By completing the sequence, a new compound **1*** with the topology inverted at C8 and C16 would result. Aptly, this could then be named *exo*-mucosin **1***, since the bulky group added during the stereodifferentiating step was projected to occupy the *exo* face of the bicycle (Fig. 3). Once *exo*-mucosin **1*** had been made, the physical data could be compared against those published for the natural product.

By the developed protocol, our synthesis commenced with the desymmetrization of *meso*-ketone **4**,[9] using Mander's reagent in combination with the lithium amide of (+)-bis[(*R*)-phenyethyl]amine (Scheme 2). This chiral amide is sometimes also referred to as Simpkins' base.[10] Then, with asymmetric keto ester **9** in hand, conjugated ester **10** was prepared by a three-step procedure, involving sequential manipulation of the keto moiety. Accordingly, the ketone in **9** was reduced, whereupon the corresponding alcohol was turned into a mesylate. Finally, the intermediate mesylate was subjected to base-induced elimination, whereby the Michael acceptor motif was produced.



Scheme 1 Key strategic points towards synthesis of *exo*-mucosin **1***.

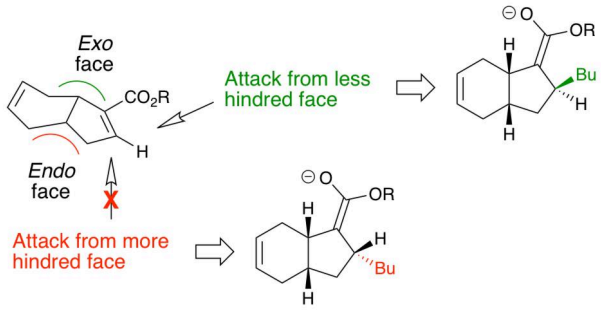
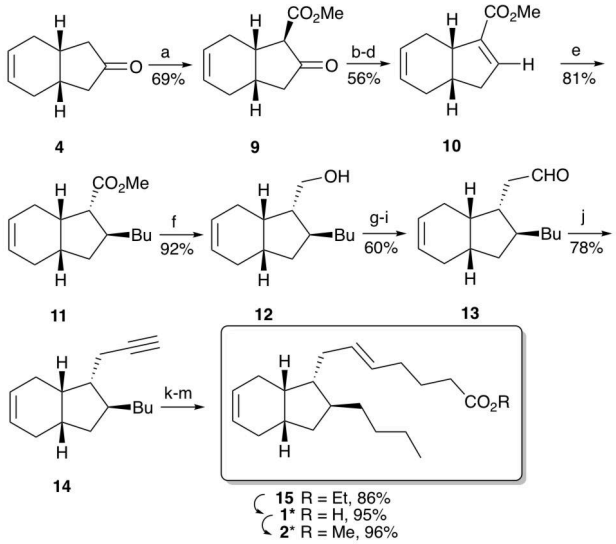


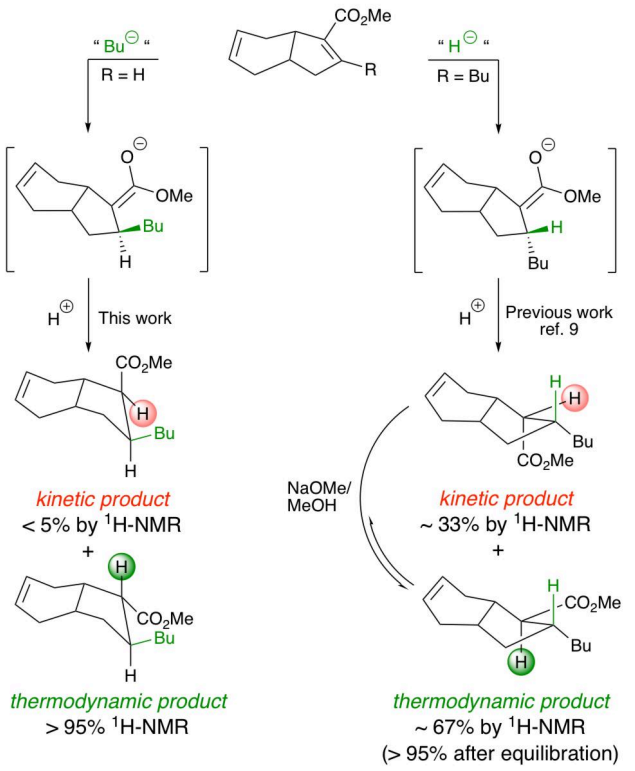
Fig. 3 Projected diastereotopic bias in the conjugate addition.



Scheme 2 The synthesis of *exo*-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 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) $NaBH_4$ in MeOH at 0 °C, 1 h, then dilute aq. HCl (1 M); (c)) Et_3N in CH_2Cl_2, followed by MsCl, briefly 0 °C then rt, overnight; (d) DBU in toluene, dropwise addition, rt, overnight; (e) CuI and TMSCl added to **10** in THF, -35 °C, 5 min, then dropwise addition of BuMgCl, -35 °C, 2h, then NH_4Cl (aq., satd.); (f); DIBAL-H in hexane, 0 °C to rt, 1 h, then Rochelle´s salt (aq., satd.), 0 °C; (g) Et_3N in CH_2Cl_2, followed by MsCl, briefly 0 °C then rt, overnight; (h) KCN in DMSO, 70 °C, 2 h; (i) DIBAL-H in hexane, -78 °C, 0.33 h, then Rochelle´s salt (aq., satd.) in the cold; (j) Ohira-Bestmann reagent and K_2CO_3 in MeOH, 0 °C to rt, 1 h; (k) 1) DIBAL-H added to Cp_2ZrCl_2 in THF, 0 °C, 1 h, then **14**, 0 °C, 1 h. 2) I_2 added in cold, 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, overnight; (m) $TMSCHN_2$ in toluene/MeOH, rt, 1 h.

Having carried out the delineated transformation, the key stereoiterative concept could be tested in the elaboration of conjugated ester **10**. While addition to the less hindered *exo*-face seemed inevitable, the resulting stereochemistry at the ester-appended chiral centre was somewhat uncertain *a priori*. Simplistically, depending on whether the supervening ester enolate is intercepted by H^+ at the equatorial or axial position of C8, the protonated species will correspond to the kinetic and the thermodynamic product, respectively. Reflecting the ambivalent stereochemical nature of the C8-carbanion, and based on our previous experience,[9] conjugate addition to **10** could consequently lead to a mixture of epimers.

In reality, with Cu(I)-catalysed conjugate addition, using BuMgCl as nucleophile in the presence of TMSCl, the reaction gave ester **11** as the sole compound (Scheme 3). Presumably, the Lewis acid takes on dual roles: Not only does TMSCl lower the LUMO of the Michael acceptor, but also stabilizes the ester enolate.[11] Hence, ester **11** ought to be the conjectured thermodynamic product. Subsequent reduction provided the corresponding carbinol **12**, which could also be readily derivatized for the purpose of X-ray analysis. By obtaining suitable crystals of the dinitrobenzoate **12-DNB**, the relative configuration of the four contiguous stereocentres could be established (Fig. 4). This also confirmed the *exo*-facial and



Scheme 3 The observed divergent diastereoselectivity in conjugate addition to the *cis*-bicyclo[4.3.0]non-3-ene system based on ^{1}H-NMR ($CDCl_3$).

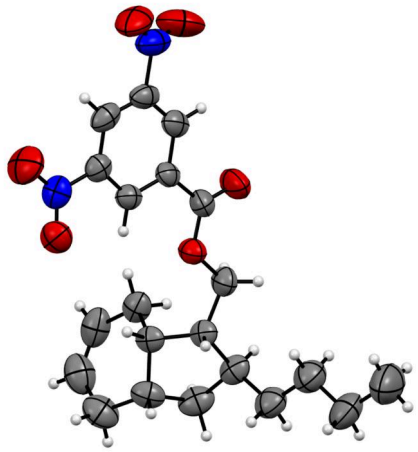


Fig 4 Single crystal X-ray structure obtained from the 3,5-dinitrobenzoate of alcohol **12** at 298 K.

thermodynamic preference in the reaction of **10**, using the specified conditions.

With the intended topological pattern confirmed, carbinol **12** was taken through a course of four steps to install an alkyne handle by the Ohira-Bestmann protocol.[12] Although ^{1}H-NMR of the natural product clearly indicates the presence of an *E*-alkene,[8] the *en route* aldehyde **13** could also serve as a relay point for *Z*-selective olefination. However, with the cited observation in mind, alkyne **14** was transformed accordingly to provide the featured *E*-configured alkenyl ester motif. This was achieved by performing three consecutive reactions in one-pot. Thus, by means of stereospecific hydrometallation[13] and halodemetallation,[14] alkyne **14** rendered the corresponding *E*-vinyl halide as substrate for Pd-catalysed cross-coupling with a commercial zinc reagent.[15] The target molecule, *exo*-mucosin **1***, was then obtained after hydrolysis of ester **15**. Finally, re-esterification gave methyl ester **2***, to be compared with the data published by Casapullo *et al.*[8]

As pointed out in our preceding communication,[9] the *cis*-fused bicyclo[4.3.0]non-3-ene system is not often encountered in nature. Adhering to the supposition that arachidonic acid (**3**) is the biogenetic origin of mucosin,[16] the geometry proposed for the core structure invokes a formal disrotatory ring-closure.[17] At a more profound level, the machinery leading to the natural product may traverse any number of pericyclic pathways.[9] Of particular interest, though, is the mounting evidence found to implicate enzyme-catalysed Diels-Alder reactions in biological systems.[18] The preceding biosynthetic transformation of **3**, into a suitable conjugated precursor for cycloaddition, is known to take place in several marine species.[16,19,20] However, in all the cases where a Diels-Alderase has provided the transformative impetus,[21-25] the authors of this paper have found no example of *cis*-fusion.

Cycloaddition via a non-enzymatic pathway is also possible. Thus, Gerwick has proposed allylic carbocations as conceptual intermediates in the biogenesis of marine carbocyclic oxylipins, such as prostaglandin A2 (PGA_2).[26] In this sense, arachidonic acid (**3**) provides a link between mucosin and the prostanoid scaffold, pointing towards a possible mechanism. Yet, for the majority of examples found, the annulation provides a *trans*-1,2-disubstituted cyclopentane ring.[27]

At the outset, given the erroneous assignment, it was avoided to form a clear-cut opinion as to the true nature of the bicyclic buttress, which provided the backbone of mucosin. Although being an uncommon structural feature, it would be premature to conclude that the *cis*-fused bicyclo[4.3.0]non-3-ene system was incongruous. Nevertheless, when recordings were made on methyl ester **2***, the data did not match those reported for the compound isolated from *Reniera mucosa*. This was most convincingly demonstrated by comparing the ^{13}C-NMR spectra (Table 1): Out of the 20 resonances that are observable for the carbon framework, excluding the methoxy group, 16 display deviating shifts. Furthermore, the optical rotation of **2*** did not only differ in magnitude, but also in sign: While the naturally occurring material and its purported structure **2** have values of $[\alpha]_D^{26} = -35.5°$ and $-9.8°$ ($c = 0.8$, hexane), respectively,[8,9] the diastereomer **2*** had an $[\alpha]_D^{26} = +64°$ ($c = 0.8$, hexane).

By achieving a rational synthesis of *exo*-mucosin **2***, the target selection has been narrowed down. Yet, in terms of the *cis*-fused bicycle, there are permutants still unaccounted for. However, given the obvious sterical encumbrance of the two remaining *syn*-diastereomers, they seemed unlikely candidates considering the biogenesis of marine carbocyclic oxylipins.[27] Hence, it was inferred that the natural product named mucosin has a *trans*-fused bicyclo[4.3.0]non-3-ene ring system.

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

Casapullo *et al.*:[8]	Previous work.:[9]	This work:
174.2	174.2	174.2
130.0	130.4	***131.2***
129.8	129.9	***129.0***
127.0	***126.3***	***125.3***
127.0	***126.1***	***125.1***
52.1	***51.4***	***51.6***
51.4	51.0	51.4
47.1	***44.0***	***41.3***
42.1	***40.3***	***37.2***
39.9	***38.1***	***36.2***
36.7	***37.7***	***35.5***
36.5	***37.1***	***35.4***
36.4	***34.9***	***33.4***
33.2	33.4	33.0
32.0	31.9	31.9
31.7	***31.0***	***31.0***
31.5	***27.8***	***26.9***
30.7	***27.7***	***24.7***
24.5	24.8	***23.0***
22.6	22.9	***21.7***
13.8	14.1	14.1

Albeit that the outlined synthesis did not yield the ultimate target, the sequence provided an answer to a central question: namely, the question about the geometry of the fused bicycle. Moreover, taken together with what we have detailed before,[9] the current findings have demonstrated a fascinating chemical aspect of the *cis*-fused bicyclo[4.3.0]non-3-ene scaffold, that unfolds when a Michael acceptor motif is incorporated. Thus, swapping the functional group at the β-position of the Michael acceptor with the functional group of the Michael donor, a complete inversion of diastereoselectivity was observed. The transformation proved to be doubly orthogonal, as even the stereochemistry at the α-position was inverted in the process. In summary, the conjugate system shown in **Scheme 3** displays a remarkable diastereotopic preference, enabling excellent control over the reactive manifold.

To recapitulate our findings, we have investigated the *anti*-diastereomer **2*** of the proposed structure **2**. Through the execution of 13 discrete linear steps, the target molecule was obtained in an overall yield of 10.6%. By incorporating a Michael acceptor motif, we have revealed an innate topological bias of the *cis*-fused bicyclo[4.3.0]non-3-ene system. Combined with our previously developed three step one-pot alkyne iteration, we have shown that *exo*-mucosin **1*** is not identical to the natural product. In view of the biogenetic cues in literature,[21-26] we suggest that the assigned configuration at the bridgeheads is the main issue.

Having committed to achieve full structural elucidation of mucosin and to provide samples for biological testing, we are currently turning the developed strategy towards elaboration of the *trans*-fused bicyclo[4.3.0]non-3-ene scaffold.

Acknowledgements

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Pursuing the true structure of the marine natural product mucosin. Part 2

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Supporting Information

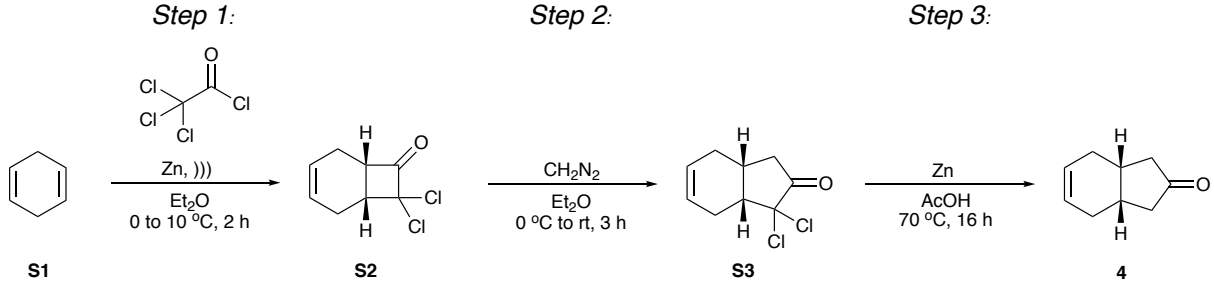
Table of Contents:

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% *ee* 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} aluminum-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 and DMSO-d_6). The following abbreviation, appt, has been used to designate an apparent triplet. 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

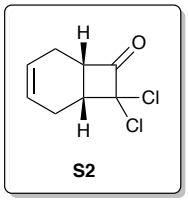
conditions stated. X-ray crystallography was performed on a Bruker D8 Vantage single-crystal CCD diffractometer instrument with a fine-focus sealed tube as the radiation source, using a graphite monochromator at the stated

Preparation of *meso*-ketone (4):



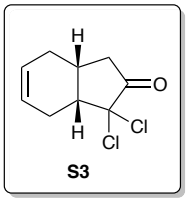
Scheme S-1 Synthetic route to *meso*-ketone **4**.

***rac*-(1*R*,6*S*)-8,8-Dichlorobicyclo[4.2.0]oct-3-en-7-one (S2).[1]**



1,4-Cyclohexadiene (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 (22.8 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 completed 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 (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.[1] Yield: 8.67 g (73%); ^{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.; 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 (hexane/EtOAc 4:1, $KMnO_4$ stain): R_f = 0.65.

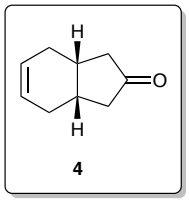
***rac*-(1*R*,6*R*)-7,7-Dichlorobicyclo[4.3.0]non-3-en-8-one (S3).**



To a stirring solution of *rac*-(1*R*,6*S*)-8,8-dichlorobicyclo[4.2.0]oct-3-en-7-one **S3** (2 g, 10.47 mmol, 1.0 equiv.) in dry Et_2O (50 mL), at 0 °C, was added diazomethane (2.47g, 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 (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 (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).[2]**



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[4.3.0]non-3-en-8-one **S3** (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 (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.[2] 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 (hexane/EtOAc 4:1, $KMnO_4$ stain): R_f = 0.51.

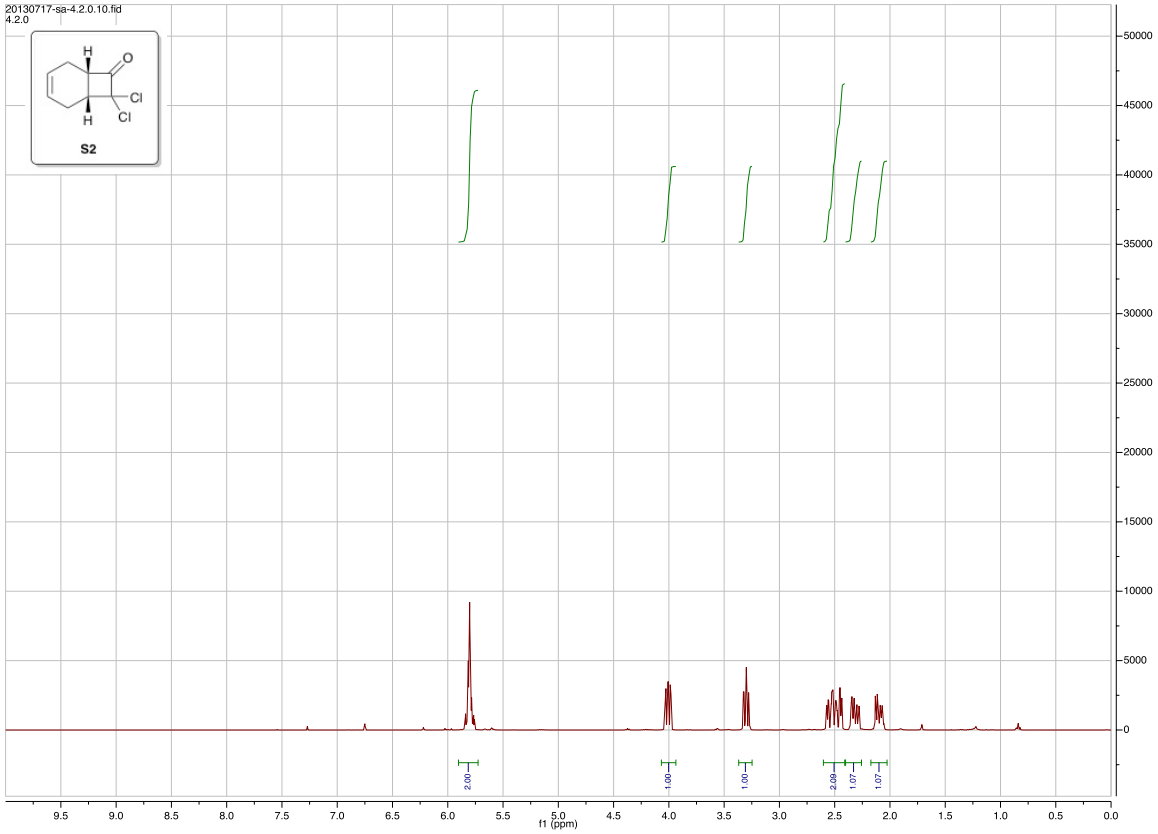


Figure S-1 ^{1}H-NMR spectrum of compound **S2**.

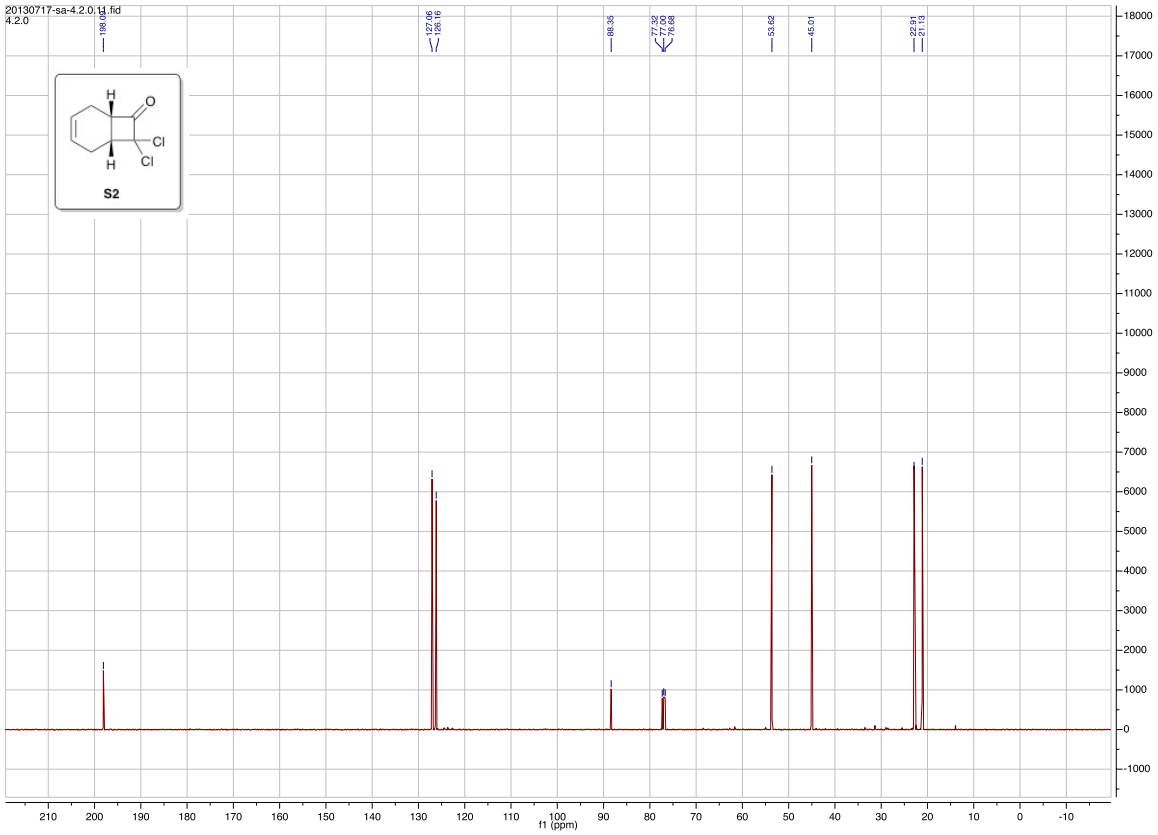


Figure S-2 ^{13}C-NMR spectrum of compound **S2**.

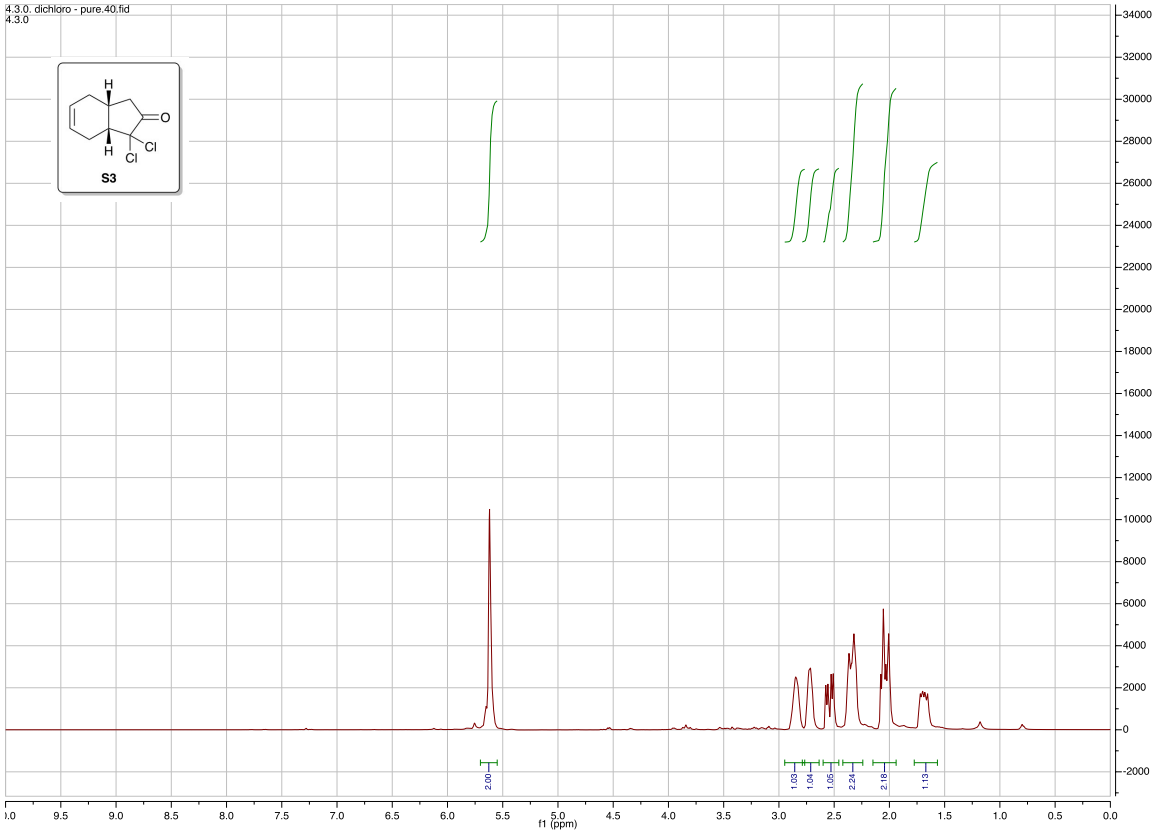


Figure S-3 ^{1}H-NMR spectrum of compound **S3**.

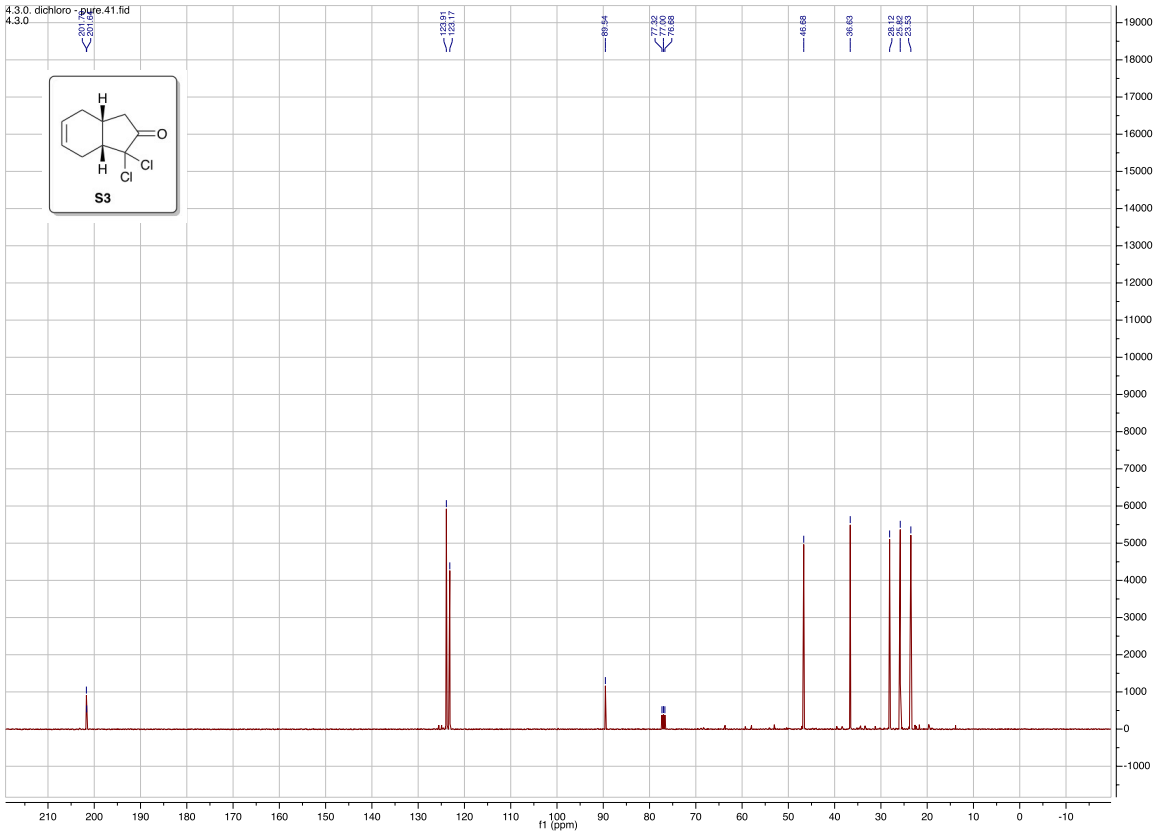


Figure S-4 ^{13}C-NMR spectrum of compound **S3**.

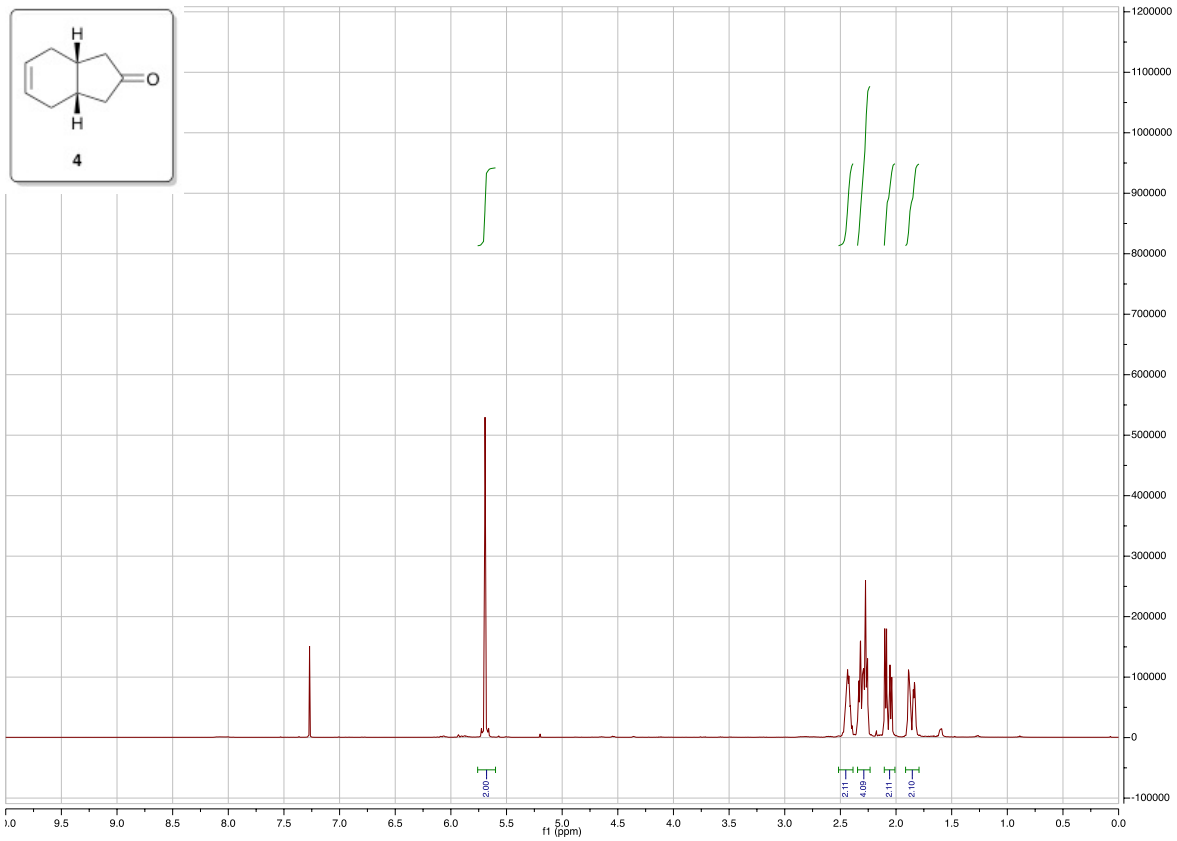


Figure S-5 ^{1}H-NMR spectrum of compound **4**.

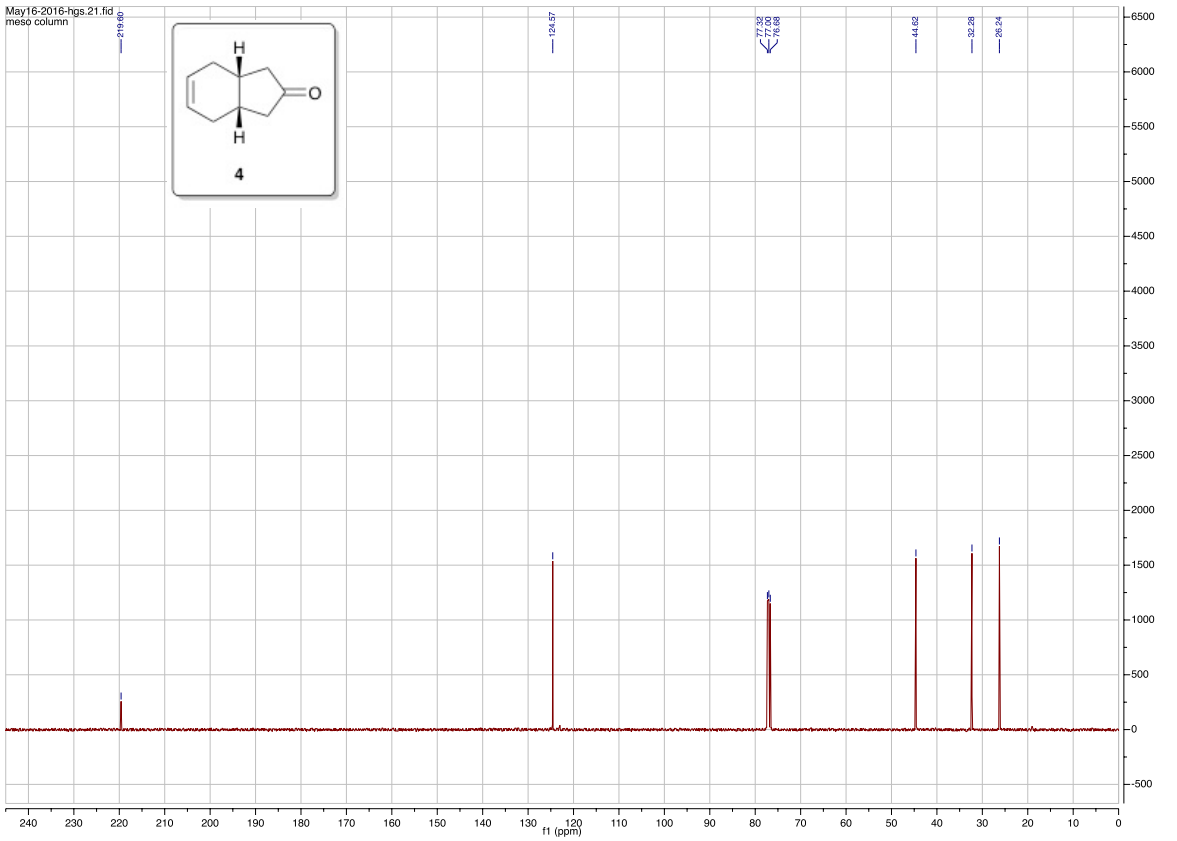


Figure S-6 ^{13}C-NMR spectrum of compound **4**.

Elemental Composition Report Page 1

Single Mass Analysis
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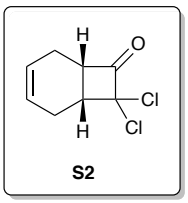
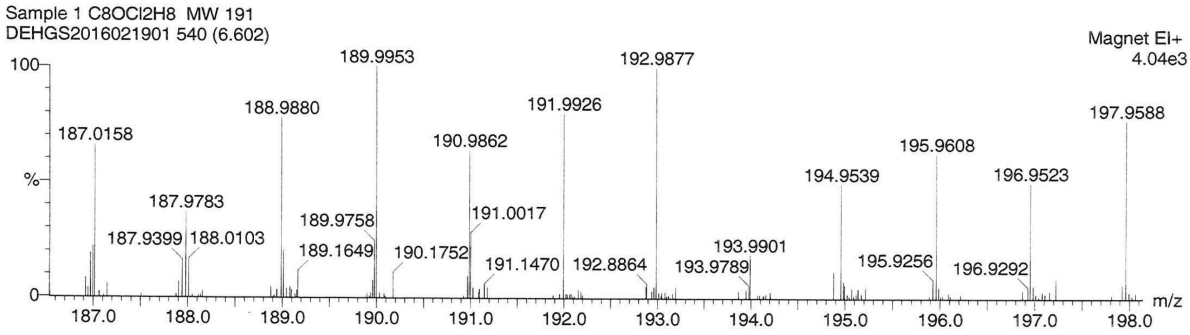
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%



S2

Monoisotopic Mass, Odd and Even Electron Ions
44 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

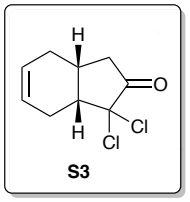


Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
189.9953	189.9952	0.1	0.4	4.0	1	C8 H8 O Cl2

Figure S-7 HRMS of compound **S2**.

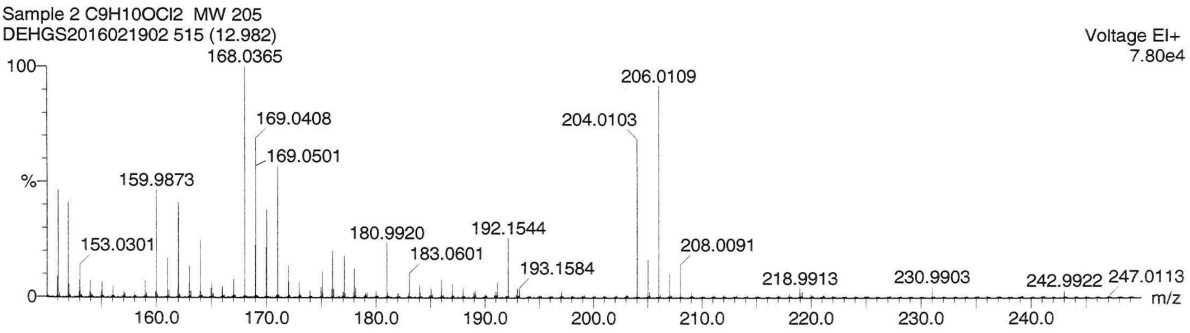
Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%



S3

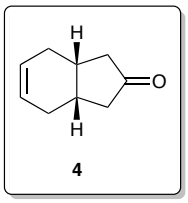
Monoisotopic Mass, Odd and Even Electron Ions
48 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
204.0103	204.0109	-0.6	-2.8	4.0	1	C9 H10 O Cl2

Figure S-8 HRMS of compound **S3**.

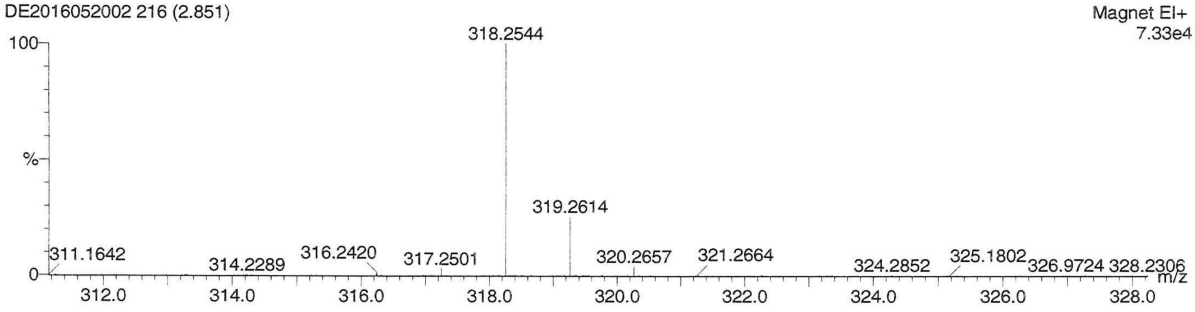
Elemental Composition Report **Page 1**



Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
31 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

M.M.E
DE2016052002 216 (2.851)



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
318.2544	318.2559	-1.5	-4.7	5.0	1	C21 H34 O2

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

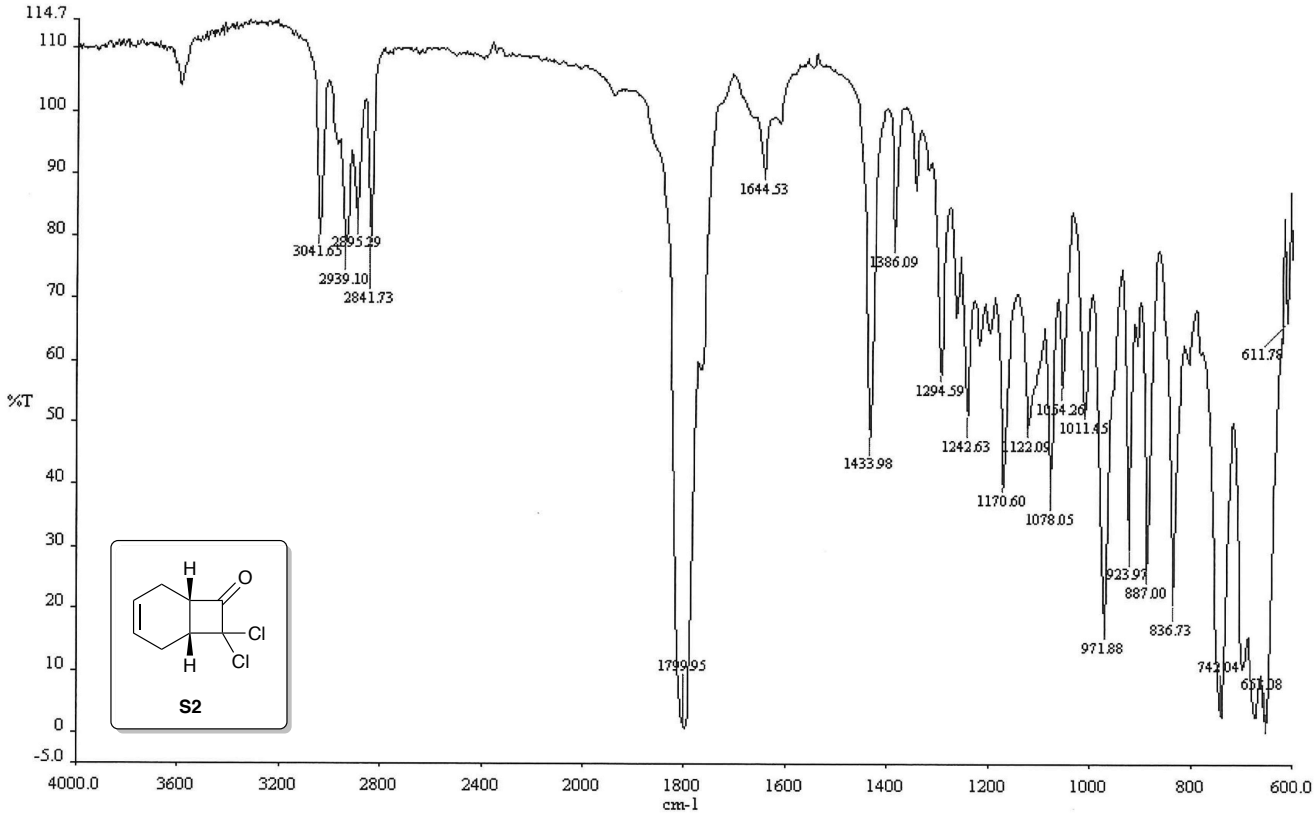


Figure S-10 IR of compound **S2**.

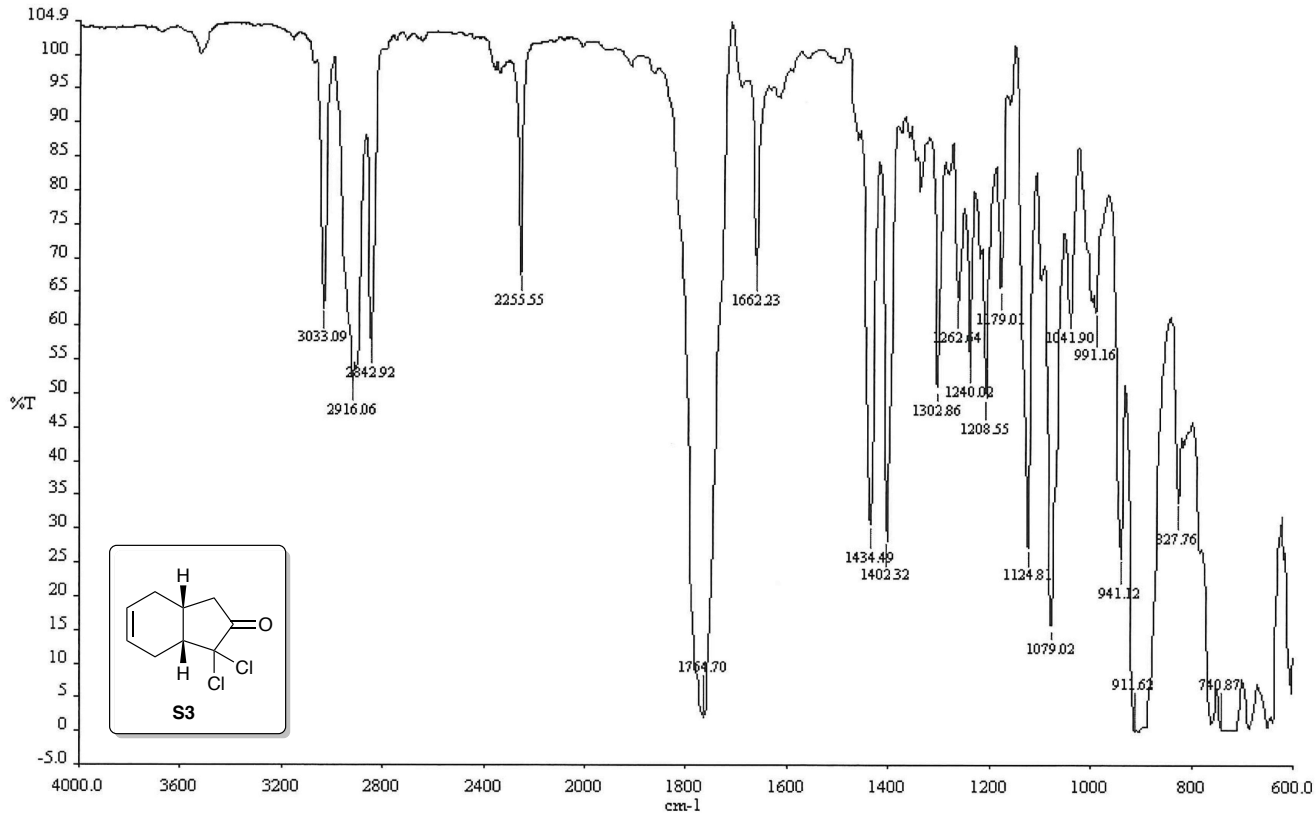


Figure S-11 IR of compound **S3**.

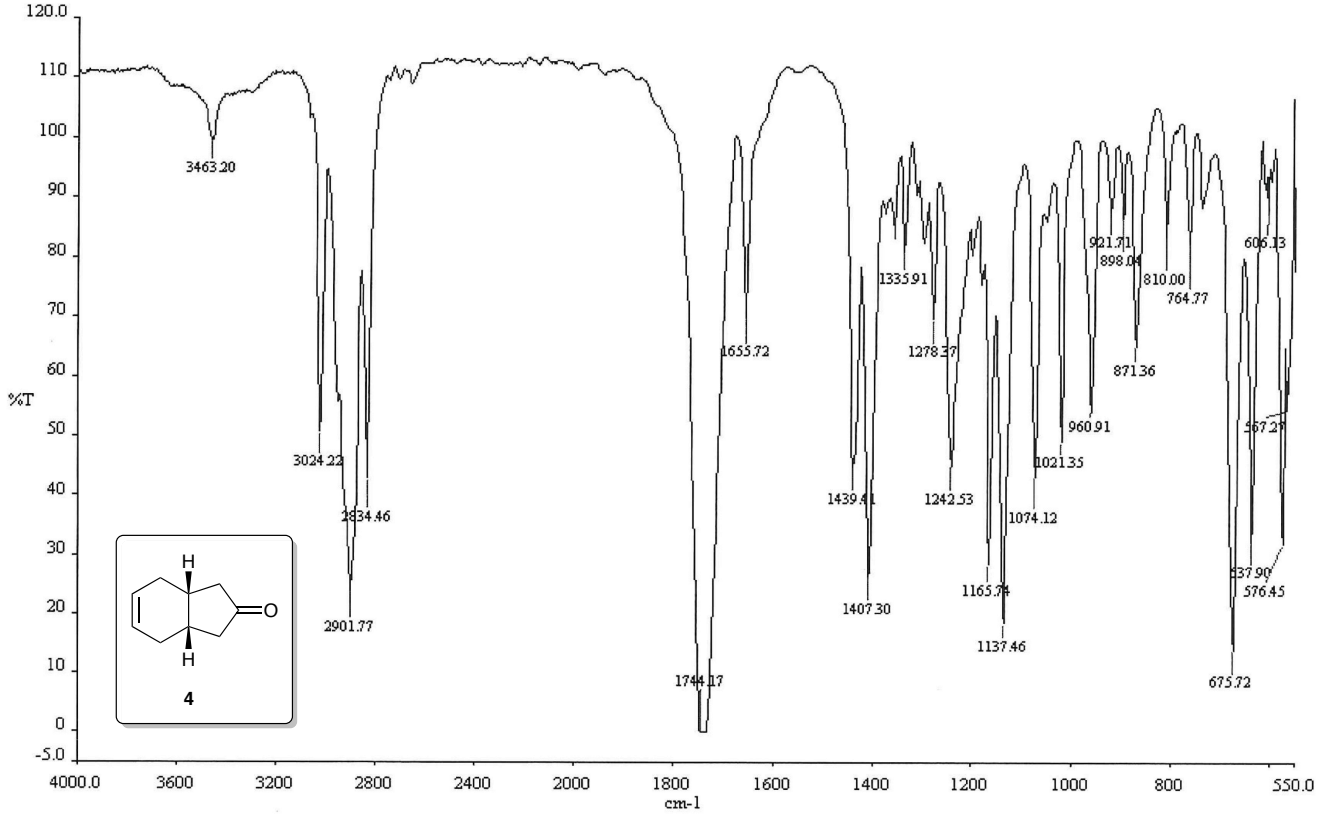
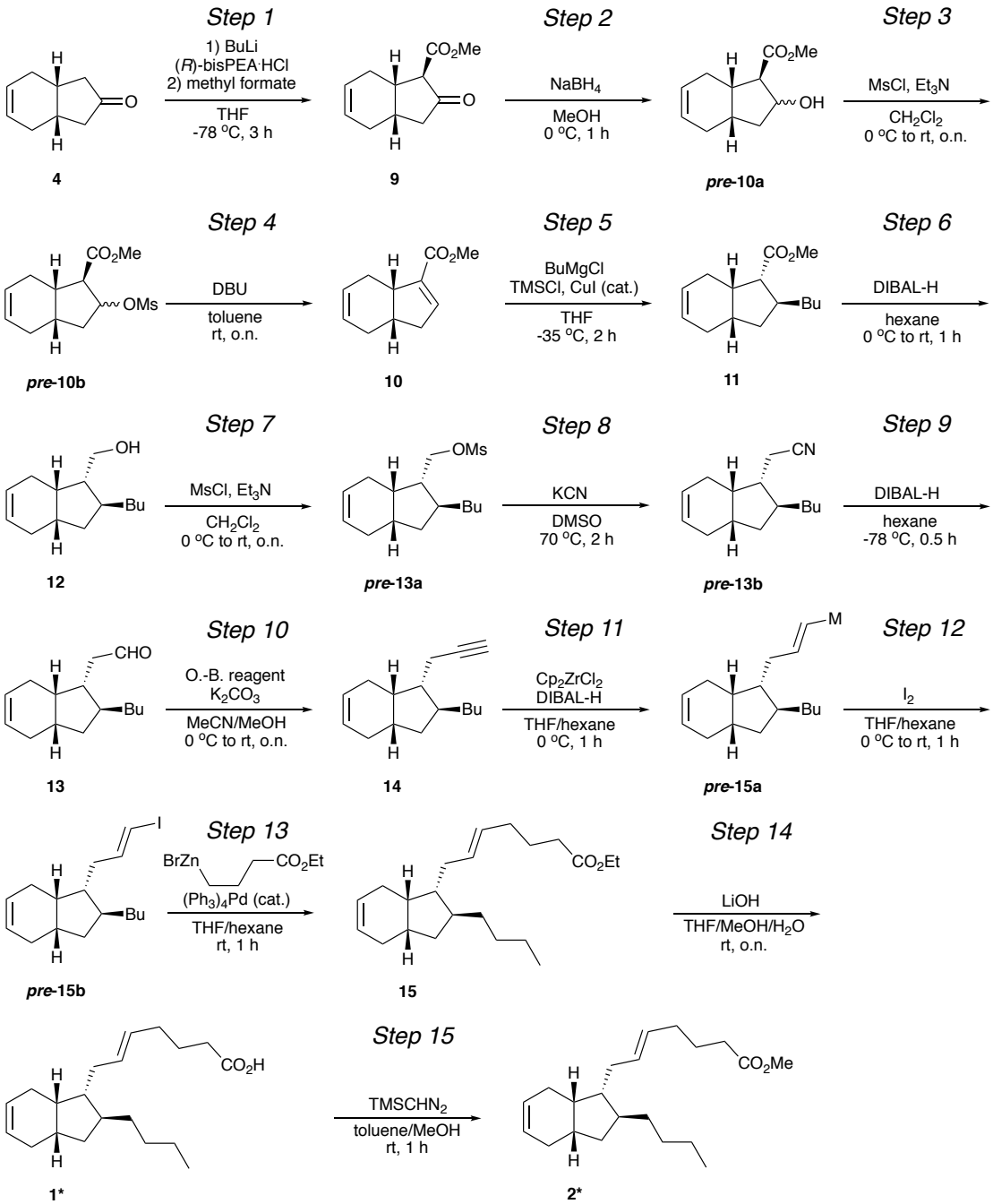


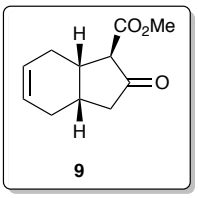
Figure S-12 IR of compound **4**.

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



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

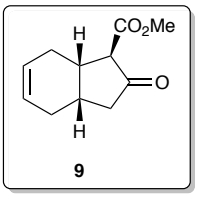
Methyl (1*S*,6*S*,7*R*)-8-oxobicyclo[4.3.0]non-3-ene-7-carboxylate (9).[3]



9

(+)-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 BuLi (2.5M in 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** (0.826 g, 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 (hexane/EtOAc 5:1) to form a colourless oil. This oil was then recrystallised from hexane at 0°C, filtered and air dried to obtaine the title compound as white crystals. All spectroscopic and physical data were in full agreement with those reported in the literature.[3] Yield: 0.812 g (69%); $[\alpha]_D^{26} - 161$ ($c = 0.1$, $CHCl_3$); ^{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 (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 (9).**

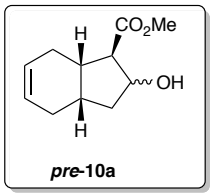


9

LDA (1M in THF/Hexanes) (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** (0.150 g, 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 (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: 0.166 g, (78%).

The material was used in the preparation of racemic reference material for chiral GLC analysis.
Methyl (1*S*,6*S,*7*R*,8*RS*)-8-hydroxybicyclo[4.3.0]non-3-ene-7-carboxylate (*pre*-10a).

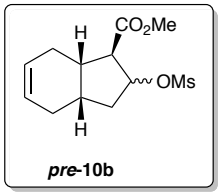


***pre*-10a**

To stirring solution of methyl (1*S*,6*S,*7*R*)-8-oxobicyclo[4.3.0]non-3-ene-7-carboxylate **9** (1.40 g, 7.21 mmol, 1.0 equiv.) in MeOH (50 mL) at 0 ^{o}C was added $NaBH_4$ (0,410 g, 10.8 mmol, 1.5 equiv.). The reaction was monitored by TLC and was deemed complete after 1 h. Then, dilute aq. HCl (10 mL, 1 M) was added drowise at 0 ^{o}C. The quenched reaction was concentrated *in vacuo* to afford a crude mixture. This was poured over Et_2O (50 mL), whereupon water (50 mL) was added. The organic layer was separated and the aqueous layer was extracted with Et_2O (2 x 50 mL). The organic layers were combined, washed with brine (1 x 100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a colourless, oily, residue. The crude was purified by column chromatography on silica (hexane/EtOAc 7:3) to afford the C8-epimeric title compound as colourless oil. Yield: 1.07 g (76%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81-5.71 (m, 2H), 4.57-4.39 (m, 1H), 3.73 (s, 3H), 2.65-2.50 (m, 1H), 2.38-2.10 (m, 6H), 2.10-1.84 (m, 2H), 1.52-1.42 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.5 (major), 174.8 (minor), 127.5 (minor), 126.7 (minor), 126.6 (major), 125.6 (major), 75.3 (major), 73.0 (minor), 57.3 (major), 53.9 (minor), 51.8 (major), 51.7 (minor), 42.1 (minor), 40.9 (major), 39.0 (major), 38.0 (minor), 34.0 (minor), 33.6 (major), 27.9 (minor), 27.7 (major), 26.3 (major), 26.2 (minor); IR (neat, cm^{-1}) 3439 (br), 3026 (w), 2914 (w), 2841 (w), 1712 (s), 1438 (m); HRMS (EI+): Exact mass calculated for $C_{11}H_{16}O_3$ $[M]^+$: 196.1099, found 196.1087; TLC (hexane/EtOAc 4:1, $KMnO_4$ stain): R_f = 0.30.

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

Methyl (1*S*,6*S,*7*R*,8*RS*)-8-((methylsulfonyl)oxy)bicyclo[4.3.0]non-3-ene-7-carboxylate (*pre*-10b).



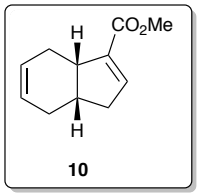
***pre*-10b**

To stirring solution of methyl (1*S*,6*S,*7*R*,8*RS*)-8-hydroxybicyclo[4.3.0]non-3-ene-7-carboxylate ***pre*-10a** (1.07 g, 5.45 mmol, 1.0 equiv.) in dry CH_2Cl_2 (50 mL) at ambient temperature was added Et_3N (1.14 ml, 8.18 mmol, 1.5 equiv.) in a dropwise manner. The resulting mixture was left stirring for 5 min. and then cooled to 0 ^{o}C. Subsequently, methanesulfonyl chloride (0.51 mL, 6.59 mmol, 1.2 equiv.) was added in a dropwise manner and the reaction mixture was left stirring for 10 min. with continued cooling. Then, the cooling was discontinued and the reaction mixture was allowed to attain ambient temperature over night. At this point the reaction mixture had turned from colourless to yellow. Brine (20 mL) was added in a dropwise manner and the volatiles were removed *in vacuo*. The resulting yellow liquid was poured over EtOAc (50 mL) and satd. aq. $NaHCO_3$ (50 mL) was added. The organic layer was separated and the aqueous layer was extracted with EtOAc (2 x 50 mL). The organic layers were combined and washed with brine (1 x 100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a yellow, oily, residue. The crude was purified by

column chromatography on silica (hexane/EtOAc 4:1) to afford the C8-epimeric title compound as a yellow oil. Yield: 1.16 g (77%); ^{1}H NMR (400 MHz, $CDCl_3$) δ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 127.3 (minor), 126.8 (minor), 125.6 (major), 124.4 (major), 83.9 (major), 83.8 (minor), 54.6 (major), 53.6 (minor), 52.2 (major), 51.9 (minor), 40.9 (minor), 39.8 (major), 39.1 (major), 38.3 (minor), 37.9 (major), 36.4 (minor), 33.9 (major), 33.5 (minor), 27.6 (minor), 26.6 (major), 26.0 (minor), 25.4 (major); IR (neat, cm^{-1}) 3031 (w), 2942 (w), 2847 (w), 1734 (s), 1438 (w), 1354 (s); HRMS (EI+): Exact mass calculated for $C_{12}H_{18}O_5S$ $[M]^+$: 274.0875, found 274.0865; TLC (hexane/EtOAc 4:1, $KMnO_4$ stain): R_f = 0.45.

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

Methyl (1*S*,6*S*)-bicyclo[4.3.0]non-3,7-diene-7-carboxylate (10).

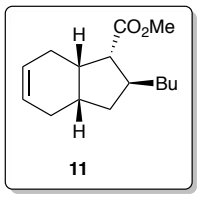


10

To a stirring solution of methyl (1*S*,6*S,7R*,8*RS*)-8-((methylsulfonyl)oxy)bicyclo[4.3.0]non-3-ene-7-carboxylate ***pre*-10b** (1.40 g, 5.10 mmol, 1.0 equiv.) in dry toluene (30 mL) at ambient temperature was added DBU (1.73 mL, 11.6 mmol, 2.3 equiv.) in a dropwise manner over 5 min. The reaction mixture was stirred over night at the stated conditions. Having deemed the reaction complete by TLC, water (10 mL) and dilute aq. HCl (10 ml, 0.5 M) was added. The resulting mixture was poured over Et_2O (20 mL) and the organic layer was separated. The aqueous layer was extracted with Et_2O (2 x 30 mL). The organic layers were combined, washed in succession with water (1 x 50 mL) and brine (1 x 50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to obtain a oily residue. The crude was purified by column chromatography on silica (hexane/EtOAc 9:1) to afford the title compound as colourless oil. Yield: 0.866 g, (95%); $[\alpha]_D^{26} + 180$ ($c = 0.8$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80-6.69 (m, 1H), 5.95-5.84 (m, 1H), 5.84-5.74 (m, 1H), 3.74 (s, 3H), 3.04-3.92 (m, 1H), 2.67-2.51 (m, 2H), 2.50-2.36 (m, 1H), 2.34-2.12 (m, 2H), 2.00-1.82 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6, 143.5, 140.8, 128.3, 127.0, 51.2, 41.2, 39.4, 36.0, 27.8, 26.6; IR (neat, cm^{-1}) 3031 (w), 2931 (w), 2841 (w), 1712 (s), 1628 (w), 1438 (m); HRMS (EI+): Exact mass calculated for $C_{11}H_{14}O_2$ $[M]^+$: 178.0994, found 178.1000; TLC (hexane/EtOAc 4:1, $KMnO_4$ stain): R_f = 0.60.

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

Methyl (1*S*,6*S*,7*R*,8*S*)-8-butylbicyclo[4.3.0]non-3-ene-7-carboxylate (11).



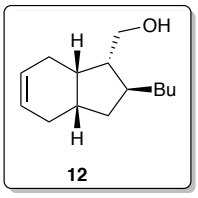
11

To a solution of methyl (1S,6S)-bicyclo[4.3.0]non-3,7-diene-7-carboxylate **10** (0.421 g, 2.36 mmol, 1.0 equiv.) in dry THF (20 mL) at -35 °C was added in succession CuI (0.045 g, 0.24 mmol, 0.1 equiv.) and TMSCl (0.641 g, 0.75 mL, 5.90 mmol, 2.5 equiv.). The resulting slightly heterogenous mixture caused by suspended CuI was stirred for 5 min, whereupon BuMgCl (2.0 M in THF) (2.36 mL, 4.72 mmol, 2.0 equiv.) was added in a dropwise manner during the course of 2h, maintaining the temperature between at -35 °C. Initial colours cycled between clear and yellow, but gradually took a

transient purple hue while reverting to clear. Upon completing the addition, the purple colour persisted (cloudy amethyst). At this point TLC revealed that the starting material had been consumed. The reaction was treated with aq. satd. NH_4Cl (5 mL) and diluted with Et_2O/water (30 mL, 2:1). The phases were separated and the aq. phase was extracted with Et_2O (3 x 20 mL). The combined org. phases were washed with brine (15 ml), dried over $MgSO_4$, filtered and the solvent was evaporated *in vacuo*. The residue was purified by column chromatography on silica (hexane/EtOAc 90:10) to afford the title compound as a colourless oil. Yield: 0.496 g (81%); $[\alpha]_D^{26} + 63°$ ($c = 0.8$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.66-5.56 (m, 2H), 3.67 (s, 3H), 2.58-2.46 (m, 2H), 2.36-2.16 (m, 3H), 1,95-1.68 (m, 4H), 1.45-1.35 (m, 2H), 1.35-1.15 (m, 5H), 0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.8, 124.7, 124.6, 56.0, 51.3, 38.9, 37.4, 36.8, 36.0, 35.3, 30.4, 26.5, 22.8, 22.6, 14.1; IR (neat, cm^{-1}) 3020 (w), 2925 (m), 1734 (s); HRMS (EI+): Exact mass calculated for $C_{15}H_{24}O_2$ $[M]^+$: 236.1776, found 236.1763; TLC (hexanes/EtOAc 80:20, $KMnO_4$ stain): R_f = 0.70.

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

(1*S*,6*S*,7*R*,8*S*)-8-Butyl-7-(hydroxymethyl)bicyclo[4.3.0]non-3-ene (12).

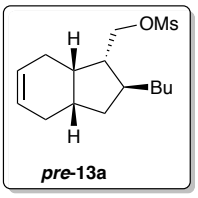


12

Methyl (1*S*,6*S*,7*R*,8*S*)-8-butylbicyclo[4.3.0]non-3-ene-7-carboxylate **11** (0.496 g, 2.10 mmol, 1.0 equiv.) was dissolved in hexane (10 mL) at room temperature and stirred for 5 min. The solution was then cooled to 0 °C and DIBAL-H (1M in hexane) (4.2 mL, 4.20 mmol, 2.0 equiv.) was added dropwise over 5 min. The reaction was then left to warm to r.t. After 1 h the reaction was cooled back to 0 °C and quenched with sat. aq. NH_4Cl (5 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 (20 mL) and the organic layer separated. The aqueous layer was extracted with EtOAc (2 x 50 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 (hexane/EtOAc 95:5) to afford the title compound as a colourless oil. Yield: 0.400 g, (92%); $[\alpha]_D^{26} + 104$ ($c = 0.8$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 5.77-5.51 (m, 2H), 3.77-3.58 (m, 2H), 2.38-2.22 (m, 1H), 2.22-2-05 (m, 2H), 2.05-1.71 (m, 4H) 1.71-1.52 (m, 2H), 1.52-1.35 (2H) 1.35-1.14 (m, 6H), 0.88 (t, *J* = 7. Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 125.3, 124.9, 63.3, 53.7, 38.1, 36.8, 36.3, 35.5, 35.4, 30.8, 26.6, 22.9, 21.6, 14.1; IR (neat, cm^{-1}) 3328 (br.), 3020 (w), 2925 (s); HRMS (EI+): Exact mass calculated for $C_{14}H_{24}O$ $[M]^+$: 208.1827, found 208.1832; TLC (hexane/EtOAc 4:1, $KMnO_4$ stain): R_f = 0.40.

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

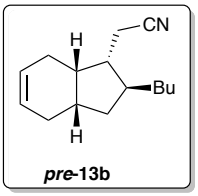
(1*S*,6*S*,7*R*,8*S*)-8-Butyl-7-((methylsulfonyl)oxymethyl)bicyclo[4.3.0]non-3-ene (*pre*-13a).



***pre*-13a**

To a stirring solution of (1*S*,6*S*,7*R*,8*S*)-8-butyl-7-(hydroxymethyl)bicyclo[4.3.0]non-3-ene **12** (0.400 g, 1.92 mmol, 1.0 equiv.) in dry CH_2Cl_2 (5 mL) at room temperature, was added Et_3N (0.54 mL, 3.84 mmol, 2.0 equiv.) dropwise. This solution was left stirring for 5 min then cooled to 0 °C. Then methanesulfonyl chloride (0.45 mL, 5.76 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 over night. The reaction mixture turned colourless to yellow. Then, brine (10 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 (hexane/EtOAc 95:5) to afford the title compound as a colourless oil. Yield: 0.497 g, (90%); $[\alpha]_D^{26} + 79$ ($c = 0.8$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.60-5.50 (m, 2H), 4.21-4.12 (m, 2H), 2.94 (s, 3H), 2.25-2.11 (m, 1H), 2.11-1.99 (m, 2H), 1.98-1.83 (m, 2H), 1.83-1.76 (m, 1H), 1.71-1.55 (m, 3H), 1.40-1.30 (m, 2H), 1.28- 1.10 (m, 5H), 0.82 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 125.3, 124.4, 70.3, 50.0, 38.1, 37.4, 36.6, 36.4, 35.4, 35.3, 30.7, 26.4, 22.8, 21.4, 14.1; IR (neat, cm^{-1}) 3020 (w), 2925 (m), 1354 (s); HRMS (EI+): Exact mass calculated for $C_{15}H_{26}O_3S_2$ $[M]^+$: 286.1603, found 286.1627; TLC (hexane/EtOAc 4:1, $KMnO_4$ stain): R_f = 0.50.

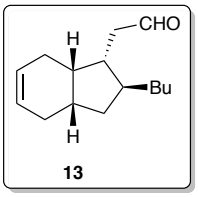
(1*S*,6*S*,7*R*,8*S*)-8-Butyl-7-(cyanomethyl)bicyclo[4.3.0]non-3-ene (*pre*-13b).



***pre*-13b**

To a stirring solution of (1*S*,6*S*,7*R*,8*S*)-8-butyl-7-((methylsulfonyl)oxymethyl)bicyclo[4.3.0]non-3-ene ***pre*-13a** (0.497 g, 1.74 mmol, 1.0 equiv.) in dry DMSO (30 mL) was added solid KCN (0.675 g, 10.4 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. Then, 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 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 (hexane/EtOAc 98:2) to give the title compound as a colourless oil. Yield: 0.325, (86%); $[\alpha]_D^{26} + 111$ ($c = 0.8$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82-5.47 (m, 2H), 2.42-2.28 (m, 2H), 2.18-2.14 (m, 2H), 2.04-1.85 (m, 3H), 1.74-1.65 (m, 3H), 1.51-1.35 (m, 2H), 1.33-1.19 (m, 5H), 0.89 (t, *J* = 7.0 Hz, 3H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 125.4, 124.2, 119.6, 47.3, 41.4, 37.9, 35.8, 35.7, 35.1, 30.6, 26.5, 22.8, 21.5, 17.7, 14.1; IR (neat, cm^{-1}) 3026 (w), 2919 (s), 2248 (w), 1465 (w) 1436 (w); HRMS (EI+): Exact mass calculated for $C_{15}H_{23}N$ $[M]^+$: 217.1830, found 217.1845; TLC (hexane/EtOAc 4:1, $KMnO_4$ stain): R_f = 0.80.

(1*S*,6*S*,7*R*,8*S*)-8-Butyl-7-(formylmethyl)bicyclo[4.3.0]non-3-ene (13).

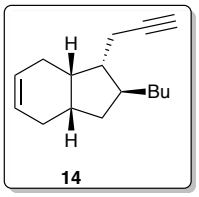


13

A stirring solution of (1*S*,6*S*,7*R*,8*S*)-8-butyl-7-(cyanomethyl)bicyclo[4.3.0]non-3-ene ***pre*-13b** (0.322 g, 1.48 mmol, 1.0 equiv.) in hexane (10 mL) was cooled to -78 °C. Then DIBAL-H (1M in hexane) (2.20

mL, 2.22 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 (hexane/EtOAc, 95:5) to afford the title compound as a colourless oil. Yield: 0.253 mg, (78%); $[\alpha]_D^{26} + 101$ ($c = 0.8$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.79 (t, *J* = 2.3 Hz, 1H), 5.68-5.51 (m, 2H), 2.49-2.44 (m, 2H), 2.35-2.23 (m, 1H), 2.22-2.12 (m, 1H), 2.10-1.98 (m, 2H), 1.92-1.78 (m, 2H), 1.74-1.60 (m, 3H), 1.47-1.35 (m, 2H), 1.35-1.15 (m, 5H), 0.88 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.9, 125.3, 124.7, 45.2, 44.7, 41.4, 37.5, 35.7, 35.6, 34.9, 30.8, 26.7, 22.9, 22.1, 14.1; IR (neat, cm^{-1}) 3020 (w), 2919 (m), 2712 (w), 1723 (s); HRMS (EI+): Exact mass calculated for $C_{15}H_{24}O$ $[M]^+$: 220.1827, found 220.1824; TLC (hexane/EtOAc 4:1, $KMnO_4$ stain): R_f = 0.80.

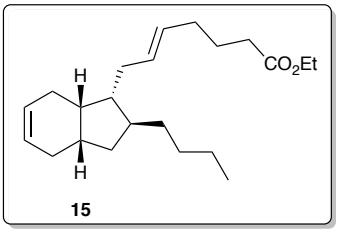
(1*S*,6*S*,7*R*,8*S*)-8-Butyl-7-(prop-2'-yn-1'-yl)bicyclo[4.3.0]non-3-ene (14).



14

To a stirring solution of (1*S*,6*S*,7*R*,8*S*)-8-butyl-7-(formylmethyl)bicyclo[4.3.0]non-3-ene **13** (0.253 g, 1.15 mmol, 1.0 equiv.) in dry MeOH (6 mL) at 0 °C was added solid K_2CO_3 (0.381 mg, 2.76 mmol, 2.4 equiv.) in one portion and Ohira-Bestmann reagent (10% w/w in MeCN) (3.9 mL, 3.32 g, 1.73 mmol, 1.5 equiv.). The suspension was then warmed to room temperature and left stirring 1 h. 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 (hexane/EtOAc, 95:5) to afford title compound as a colourless oil. Yield: 0.193 g, (78%); $[\alpha]_D^{26} + 119$ ($c = 0.8$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.72-5.58 (m, 2H), 2.35-2.24 (m, 2H), 2.18-2.09 (m, 3H), 2.07-1.98 (m, 1H), 1.91 (t, *J* = 2.7 Hz, 1H), 1.89-1.83 (m, 1H), 1.83-1.75 (m, 1H), 1.75-1.55 (m, 3H), 1.55-1.45 (m, 1H), 1.45-1.36 (m, 1H), 1.36-1.13 (m, 5H), 0.89 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ; 125.2, 125.0, 84.4, 67.9, 50.2, 41.2, 37.7, 36.1, 35.9, 35.2, 30.8, 26.8, 22.9, 21.4, 18.9, 14.1; IR (neat, cm^{-1}) 3311 (m), 3020 (w), 2919 (s), 1432 (m); HRMS (EI+): Exact mass calculated for $C_{16}H_{24}$ $[M]^+$: 216.1878, found 216.1867; TLC (hexane, $KMnO_4$ stain and anisaldehyde dip): R_f = 0.25.

(1*S*,6*S*,7*R*,8*S*)-8-Butyl-7-((*E*)-7'-ethoxy-7'-oxohept-2'-enyl)bicyclo[4.3.0]non-3-ene (15).

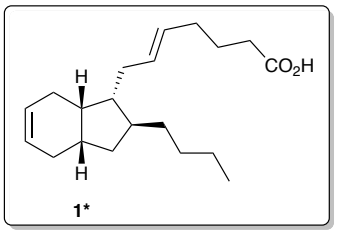


15

To a stirring solution of Cp_2ZrCl_2 (0.400 g, 1.37 mmol, 2.0 equiv.) in dry THF (8 ml) at 0 °C was added DIBAL-H (1M in hexane) (1.37 mL, 1.37 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*R*,8*S*)-8-butyl-7-(prop-2'-yn-1'-yl)bicyclo[4.3.0]non-3-ene **14** (0.148 g, 0.684 mmol, 1.0 equiv.) dissolved in dry THF (4 mL) was

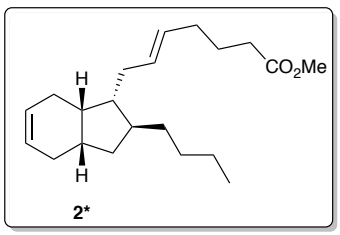
added dropwise to the reaction mixture at 0 °C. After 1 h at 0 °C iodine (0.260 g, 1.02 mmol, 1.5 equiv.) was added in one portion to the homogeneous yellow reaction mixture. The reaction mixture was then warmed to room temperature and stirred for 1 h. To the preformed vinyl iodide was successively added 4-ethoxy-4-oxobutylzinc bromide solution (0.5M in THF) (2.7 mL, 1.37 mmol, 2.0 equiv.) dropwise and $(Ph_3P)_4Pd$ (0.079 g, 0.068 mmol, 0.1 equiv.) in one portion. The resulting light 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 50 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 (hexane/EtOAc, 95:5) to afford the title compound as a colourless oil. Yield: 0.196 g, (86%); $[\alpha]_D^{26} + 67$ (c = 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.69-5.56 (m, 2H), 5.48-5.33 (m, 2H), 4.12 (q, *J* = 7.1 Hz, 2 H), 2.31-2.21 (appt, *J* = 7.5 Hz, 3H), 2.16-1.82 (m, 8H), 1.75-1.50 (m, 6H), 1.48-1.41 (m, 1H), 1.39-1.22 (m, 9H), 0.88 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8, 131.2, 129.1, 125.3, 125.1, 60.2, 51.6, 41.3, 37.3, 36.2, 35.6, 35.5, 33.7, 33.0, 31.9 31.0, 26.9, 24.8, 23.0, 21.7, 14.2, 14.1; IR (neat, cm^{-1}) 3020 (w), 2925 (m), 1734 (s); HRMS (EI+): Exact mass calculated for $C_{22}H_{36}O_2$ $[M]^+$: 332.2715, found 332.2709; TLC (hexane/EtOAc 95:5, $KMnO_4$ stain): R_f = 0.65.

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



To a stirring solution of the (1*S*,6*S*,7*R*,8*S*)-8-butyl-7-((*E*)-7'-ethoxy-7'-oxohept-2'-enyl)bicyclo[4.3.0]non-3-ene **15** (0.177 g, 0.533 mmol, 1.0 equiv.) in THF/MeOH/H_2O (2:2:1) (5 mL) at room temperature was added lithium hydroxide monohydrate (0.783 mg, 18.7 mmol, 35.0 equiv.) in one portion. The reaction mixture was left stirring and monitored by TLC. Left over night, 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 combined and washed with brine (1 x 20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to provide a colourless oil. This was purified by column chromatography on silica (hexane/EtOAc, 3:2) to afford the title compound as a colourless oil. Yield: 0.154 g, (95%); $[\alpha]_D^{26} + 77$ ($c = 0.8$, hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.63 (br, 1H), 5.67-5.56 (m, 2H), 5.50-5.34 (m, 2H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.33-2.22 (m, 1H), 2.15-2.12 (m, 8H), 1.77-1.49 (m, 6H), 1.48-1.40 (m,1H), 1.40-1.08 (m, 6H), 0.88 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.3, 131.4, 128.9, 125.3, 125.1, 51.6, 41.3, 37.3, 36.2, 35.6, 35.5, 33.4, 33.0, 31.8, 31.0, 26.9, 24.5, 23.0, 21.7, 14.2; IR (neat, cm^{-1}) 3020 (w), 2925 (m), 1712 (s); HRMS (EI+): Exact mass calculated for $C_{20}H_{32}O_2$ $[M]^+$: 304.2402, found 304.2400; TLC (hexane/EtOAc 3:2, $KMnO_4$ stain): R_f = 0.40.

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



To a stirring solution of (1*S*,6*S*,7*R*,8*S*)-8-butyl-7-((*E*)-7'-hydroxy-7'-oxohept-2'-enyl)bicyclo[4.3.0]non-3-ene **1*** (0.023 g, 0.076 mmol, 1.0 equiv.) in toluene/MeOH (3:2) (5 mL) at room temperature was added TMS diazomethane solution (2M in hexane) (0.06 mL, 0.113 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 (hexane/EtOAc, 95:5) to afford the title compound as a colourless oil. Yield: 23 mg, (96%); $[\alpha]_D^{26} + 64$ (c = 0.8, hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.69-5.56 (m, 2H), 5.49-5.32 (m, 2H), 3.66 (s, 3H), 2.29 (t, *J* = 7.5 Hz, 2H), 2.26-2.21 (m, 1H), 2.13-1.82 (m, 8H), 1.75-1.63 (m, 3H), 1.63-1.50 (m, 3H), 1.40-1.08 (m, 7H), 0.88 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ; 174.2, 131.2, 129.0, 125.3, 125.1, 51.6, 51.4, 41.3, 37.2, 36.2, 35.5, 35.4, 33.4, 33.0, 31.9, 31.0, 26.9, 24.7, 23.0, 21.7, 14.1; IR (neat, cm^{-1}) 3020 (w), 2925 (s), 1745 (s); HRMS (EI+): Exact mass calculated for $C_{21}H_{34}O_2$ $[M]^+$: 318.2559, found 318.2552; TLC (hexane/EtOAc 95:5, $KMnO_4$ stain): R_f =0.65.

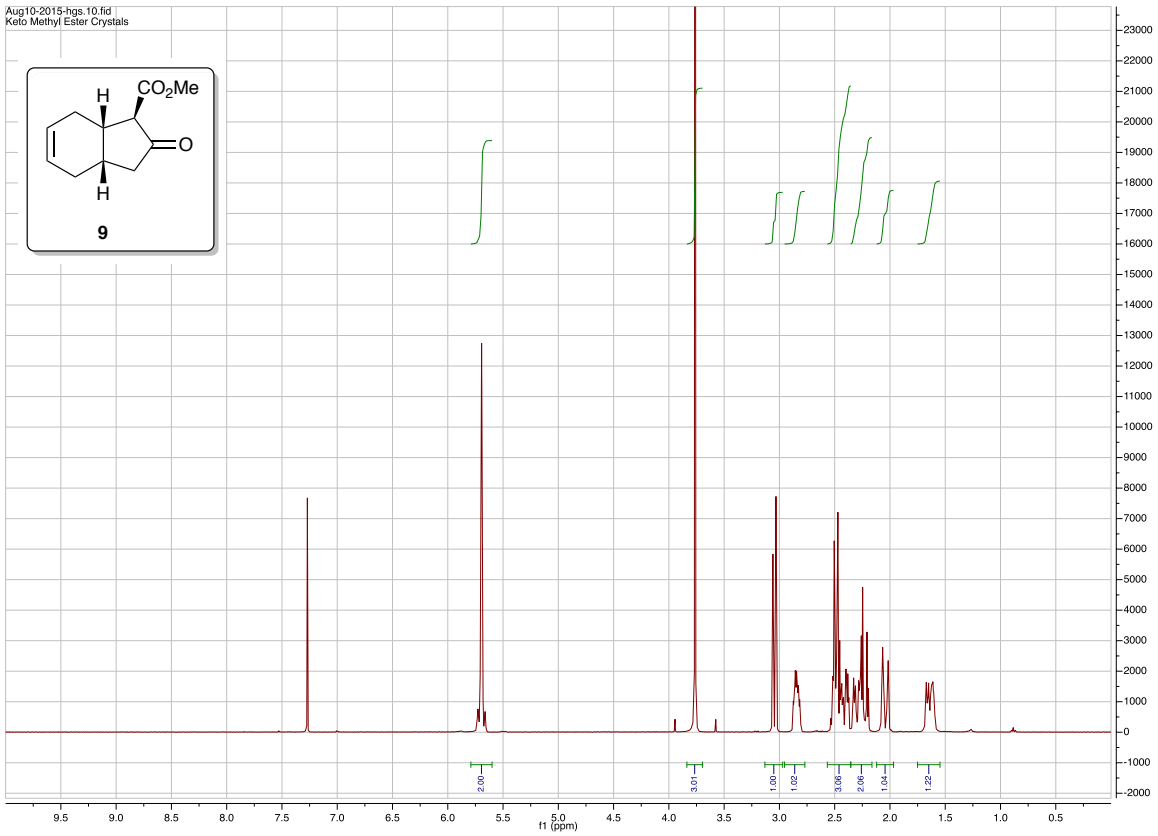


Figure S-13 ^{1}H-NMR spectrum of compound **9**.

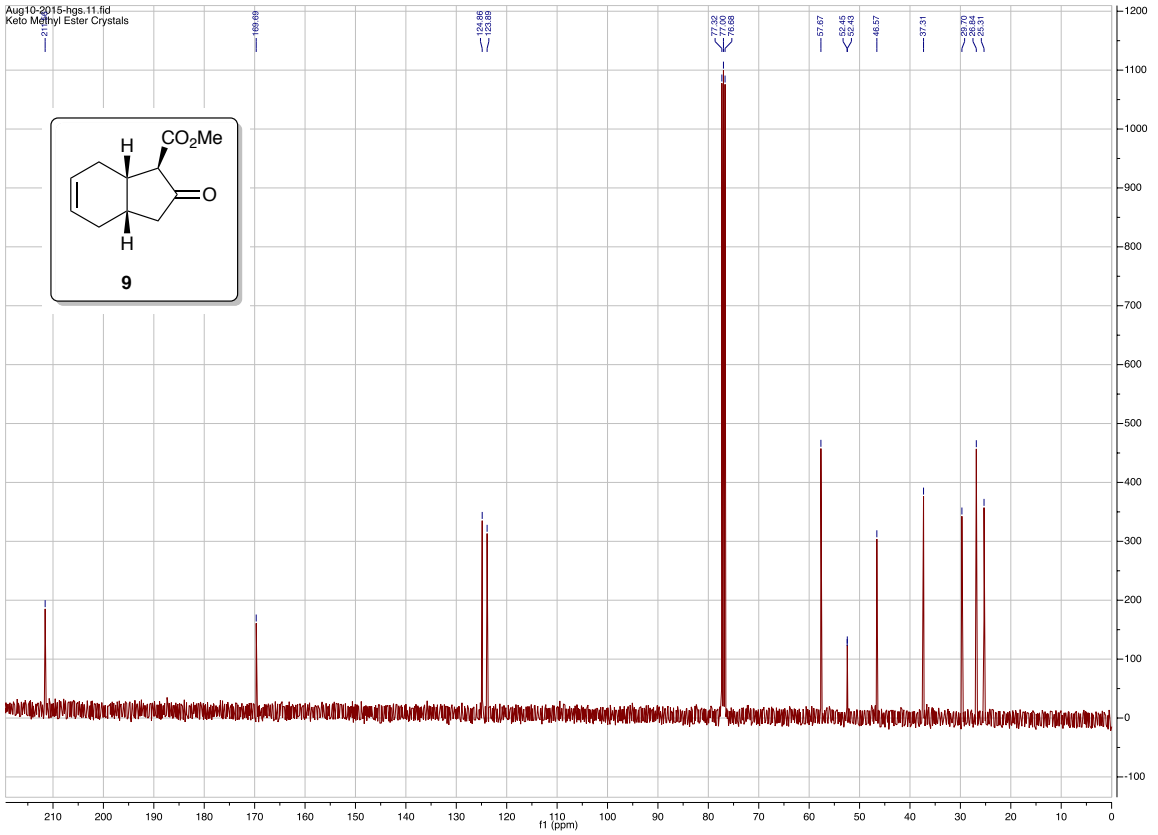


Figure S-14 ^{13}C-NMR spectrum of compound **9**.

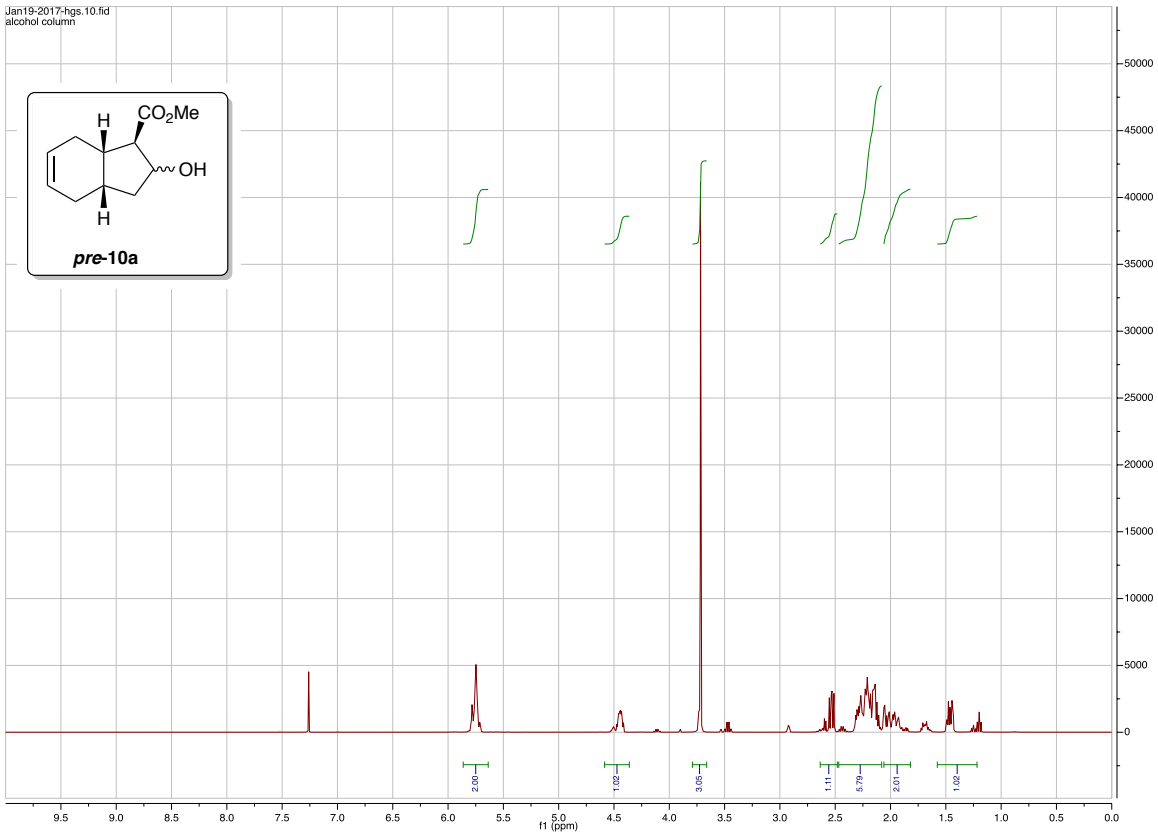


Figure S-15 ^{1}H-NMR spectrum of compound ***pre*-10a**.

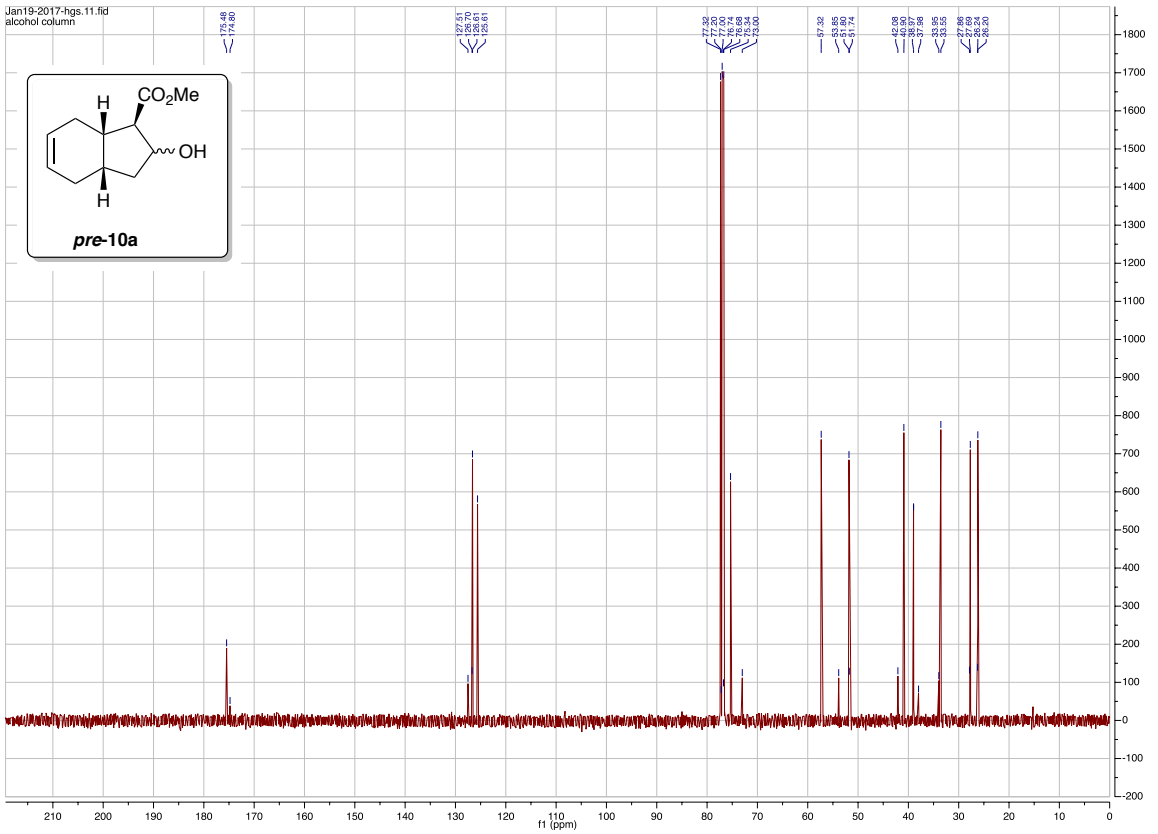


Figure S-16 ^{13}C-NMR spectrum of compound ***pre*-10a**.

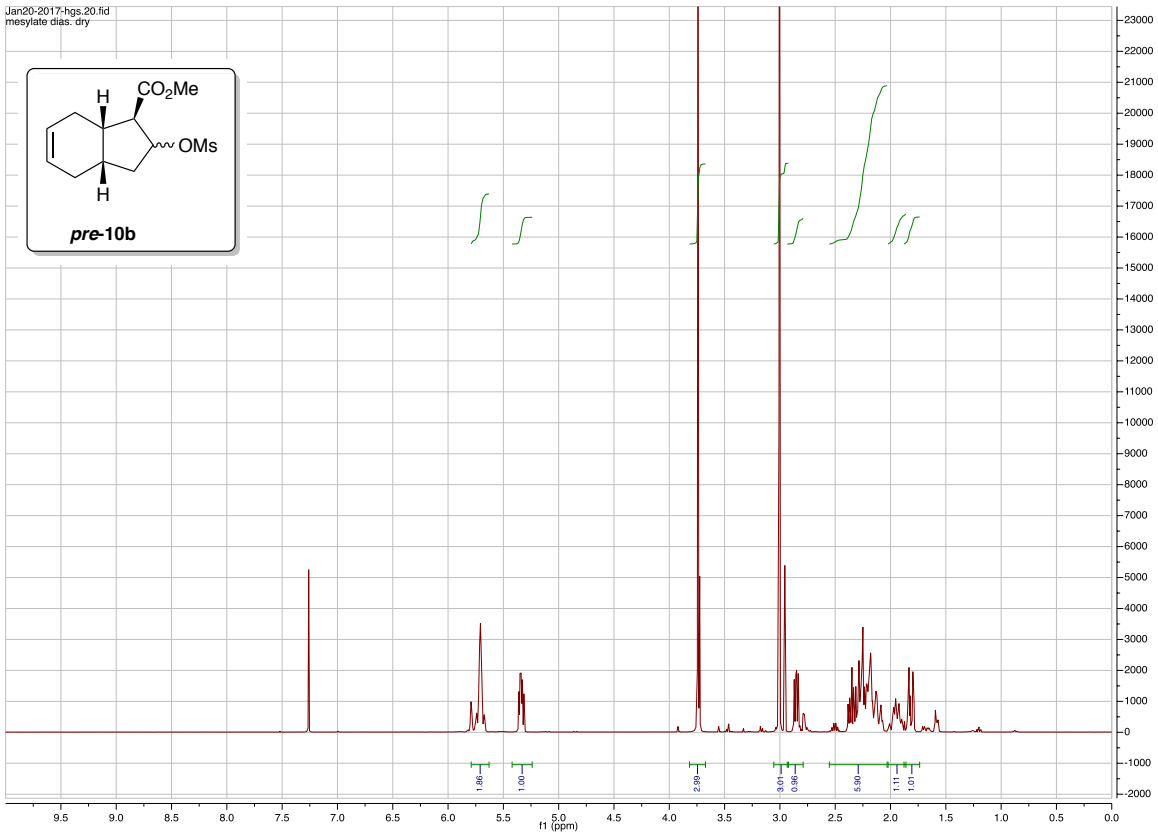


Figure S-17 ^{1}H-NMR spectrum of compound ***pre*-10b**.

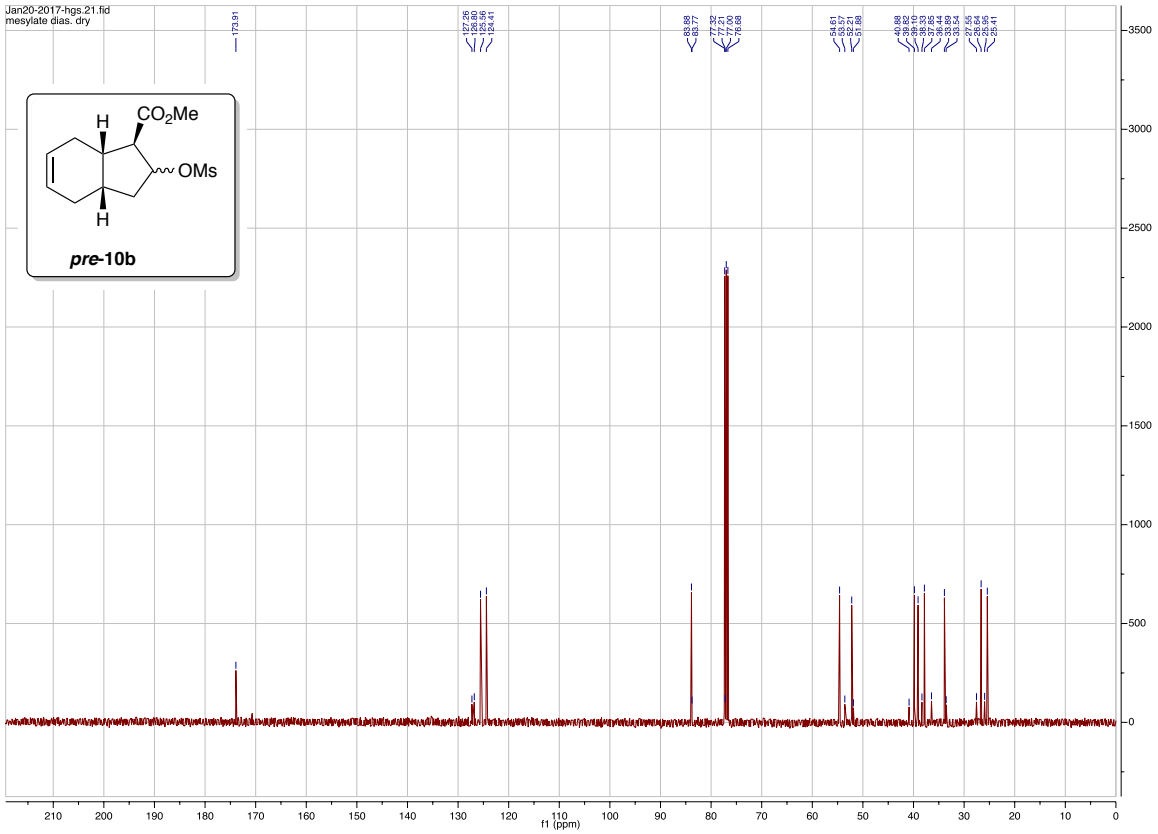


Figure S-18 ^{13}C-NMR spectrum of compound ***pre*-10b**.

Figure S-19 ^{1}H-NMR spectrum of compound **10**.

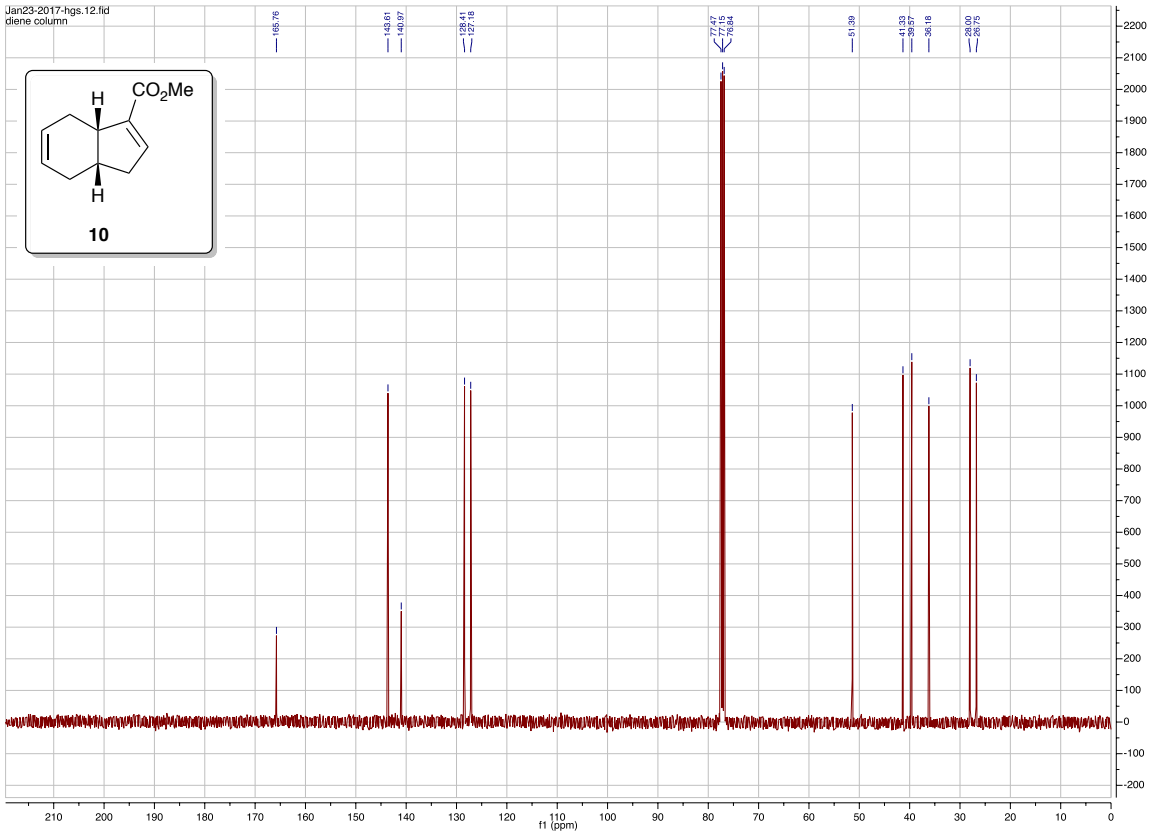


Figure S-20 ^{13}C-NMR spectrum of compound **10**.

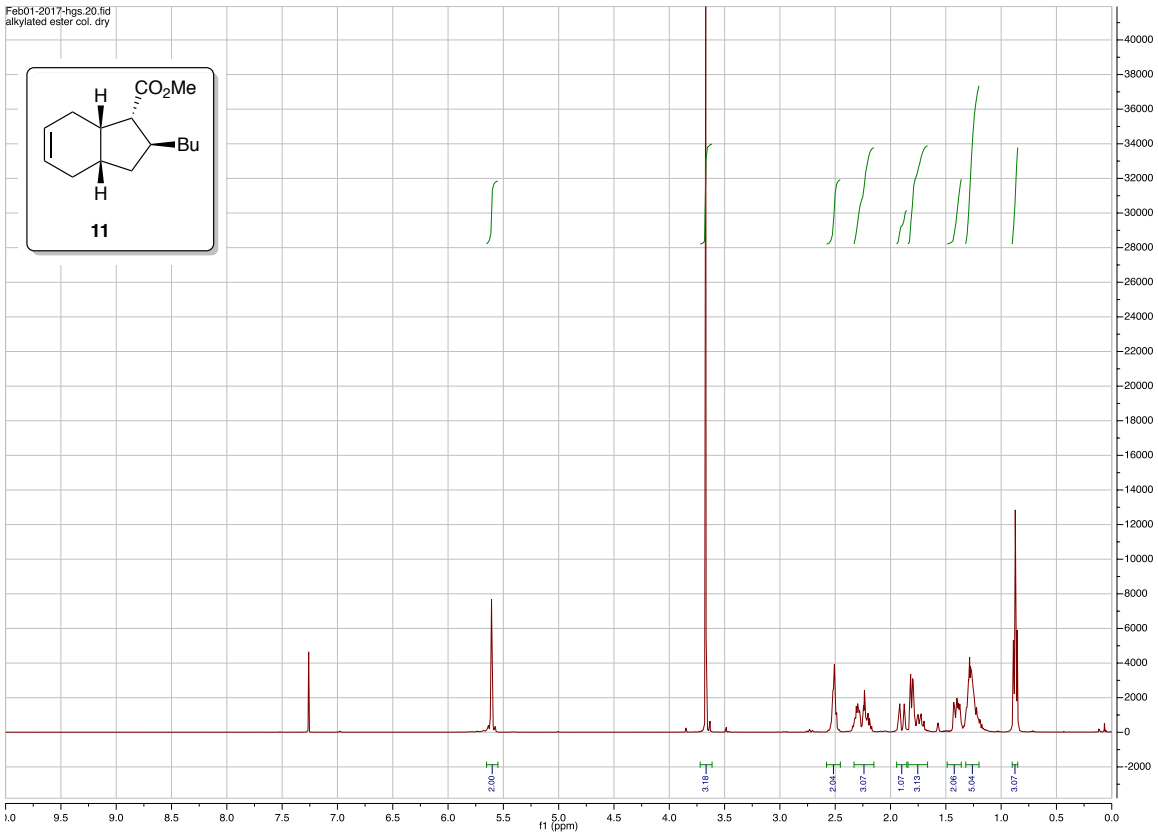


Figure S-21 ^{1}H-NMR spectrum of compound **11**.

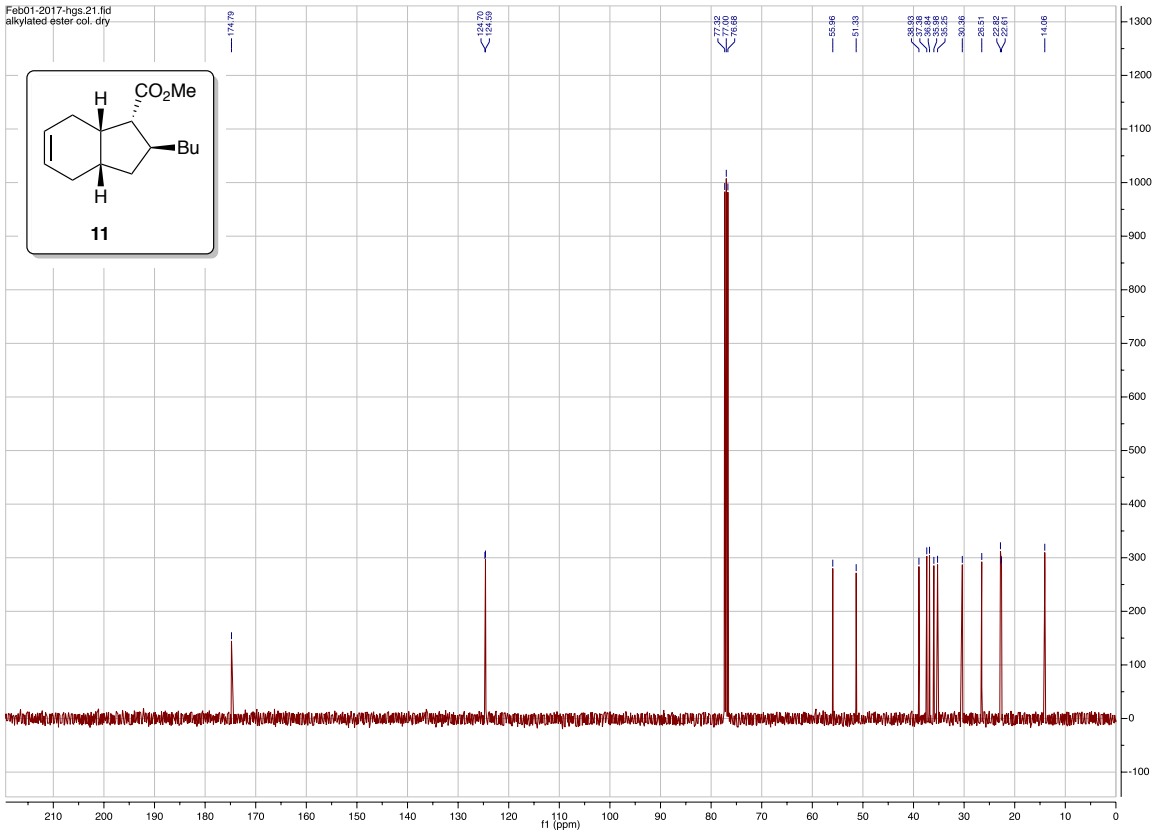


Figure S-22 ^{13}C-NMR spectrum of compound **11**.

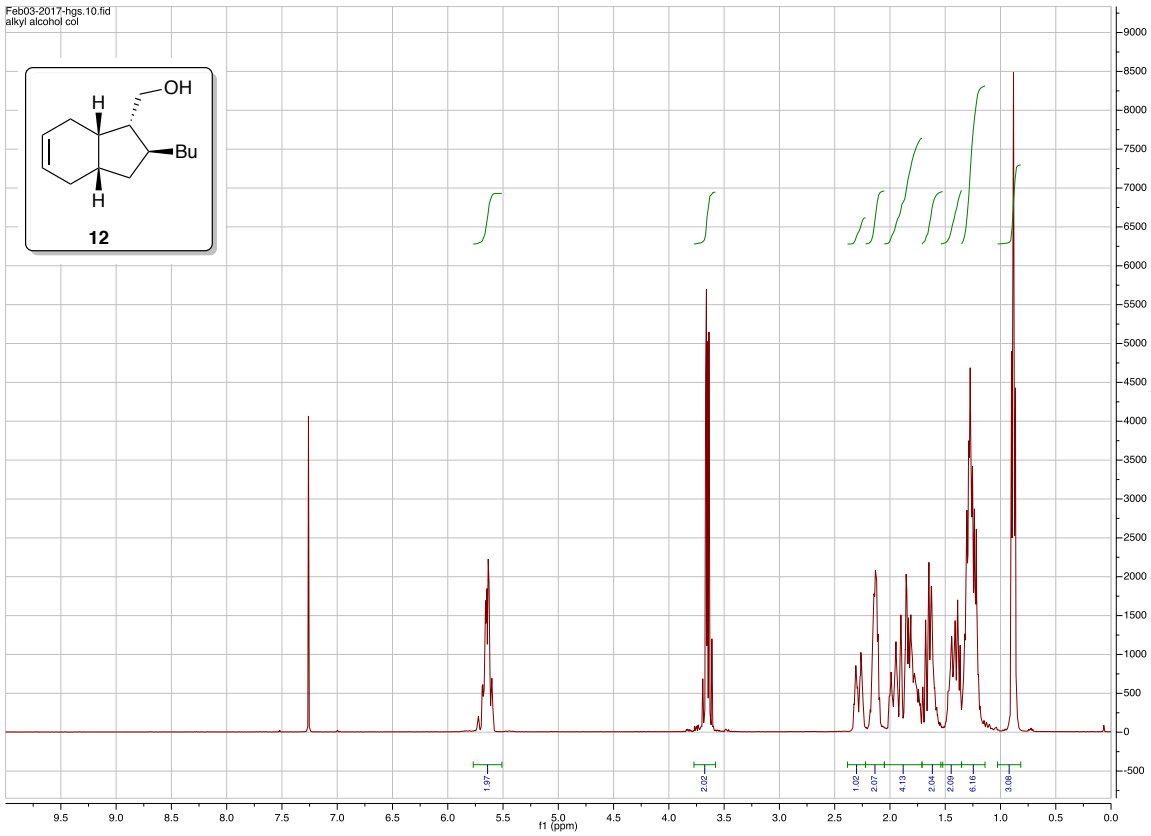


Figure S-23 ^{1}H-NMR spectrum of compound **12**.

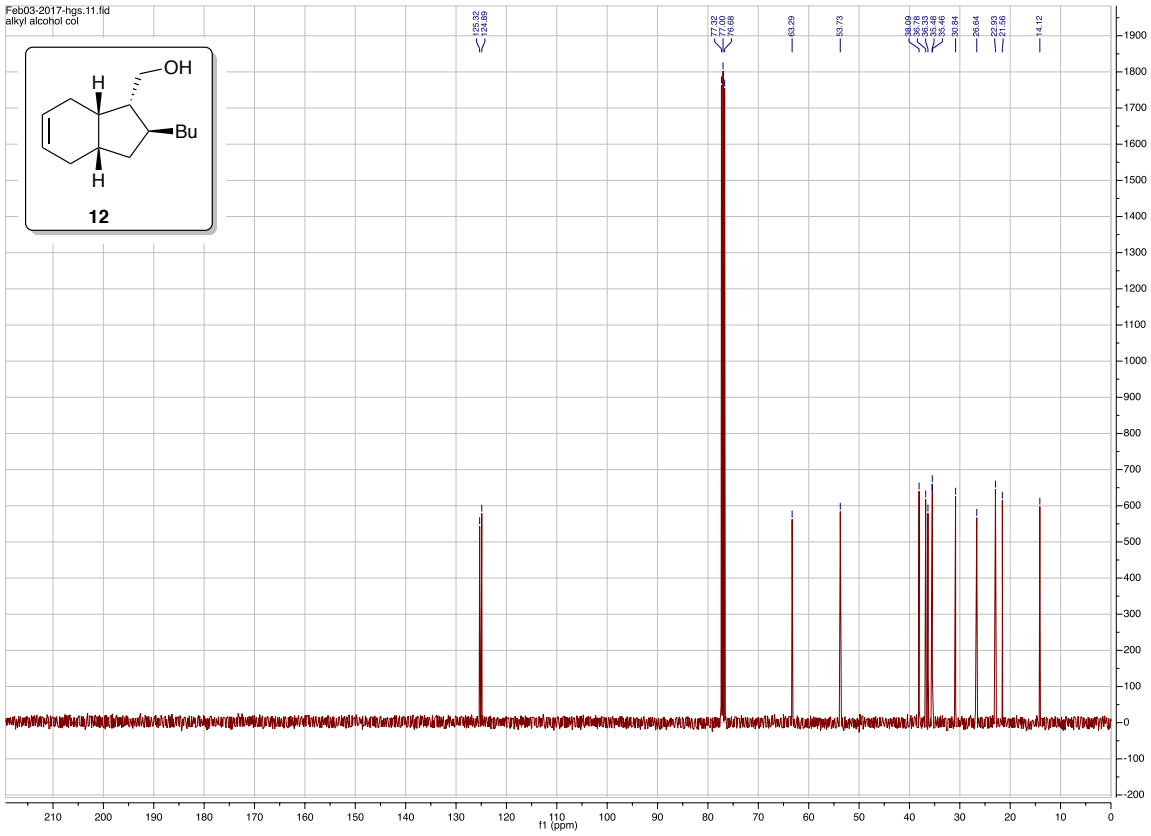


Figure S-24 ^{13}C-NMR spectrum of compound **12**.

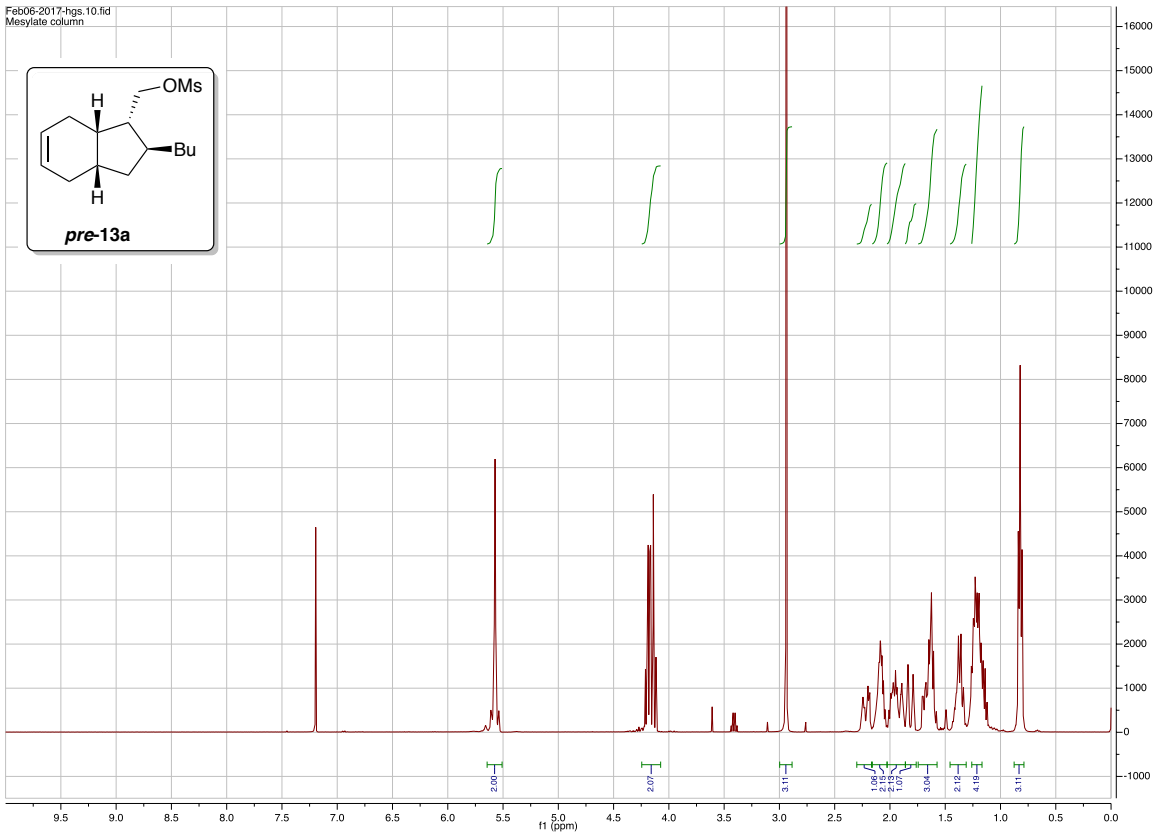


Figure S-25 ^{1}H-NMR spectrum of compound ***pre*-13a**.

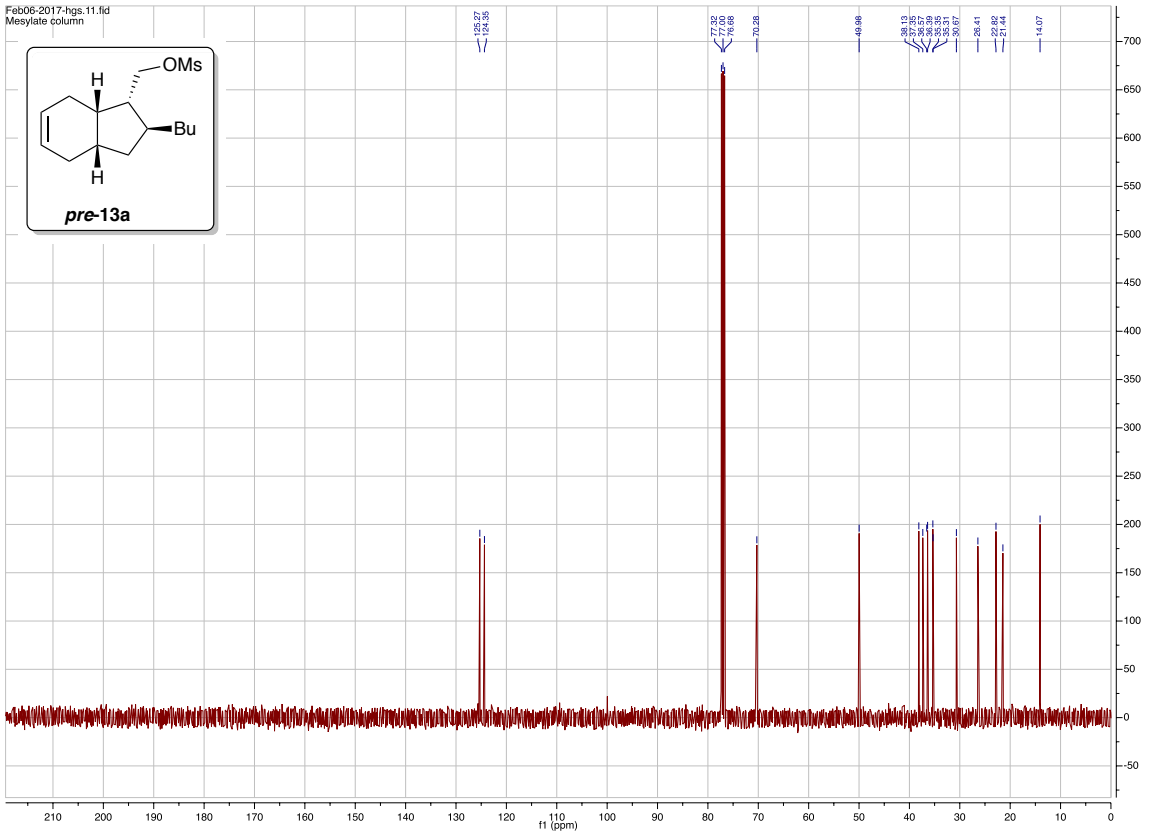


Figure S-26 ^{13}C-NMR spectrum of compound ***pre*-13a**.

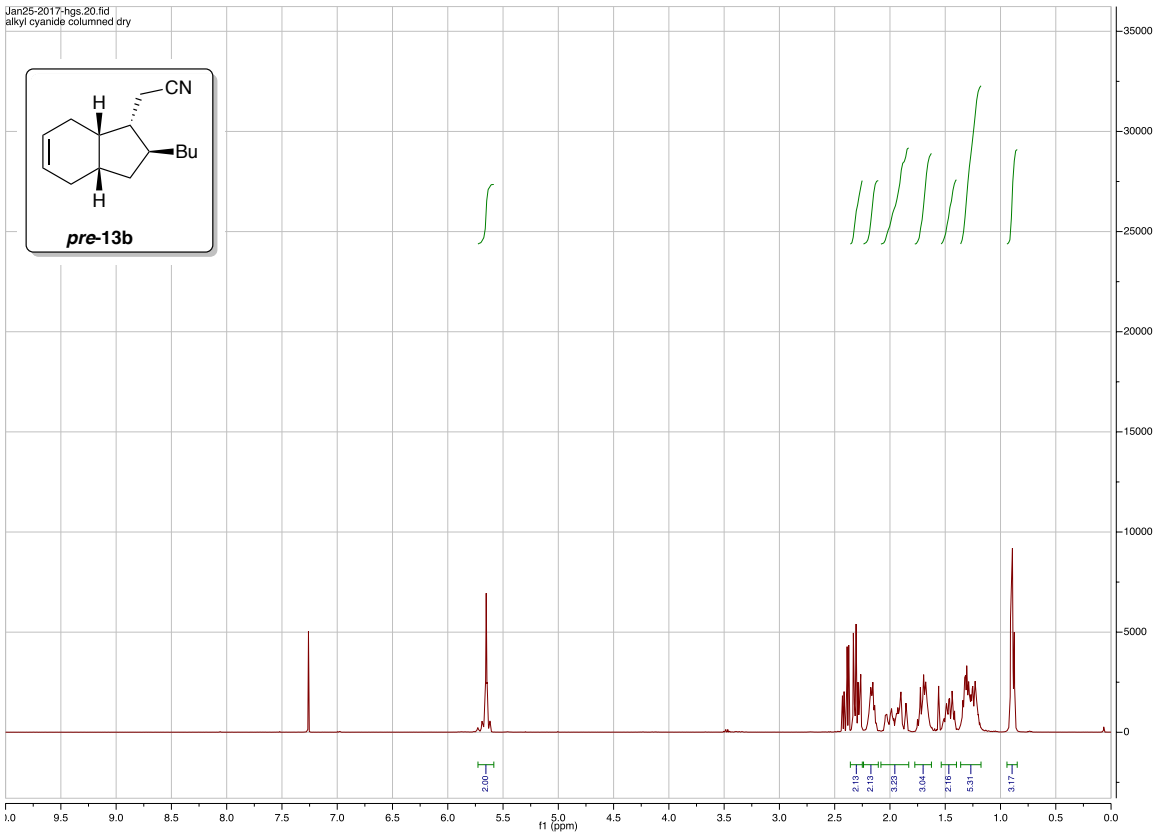


Figure S-27 ^{1}H-NMR spectrum of compound ***pre*-13b**.

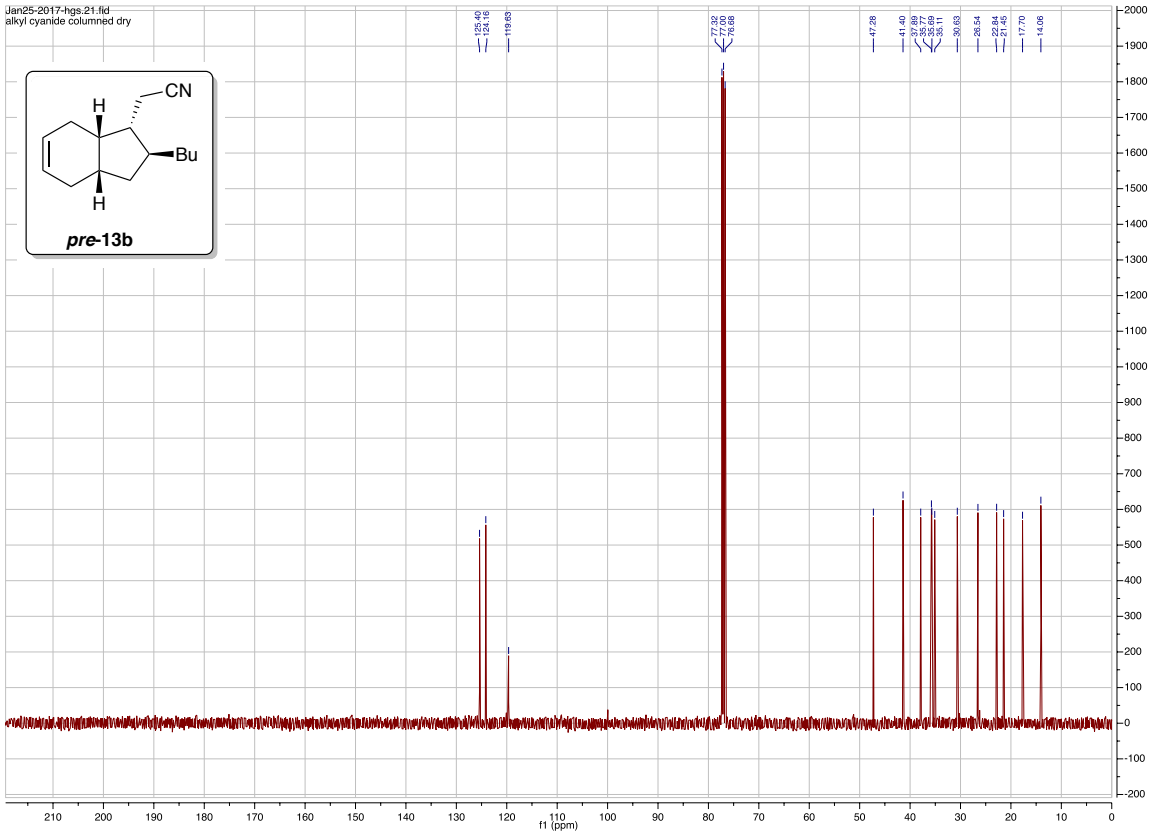


Figure S-28 ^{13}C-NMR spectrum of compound ***pre*-13b**.

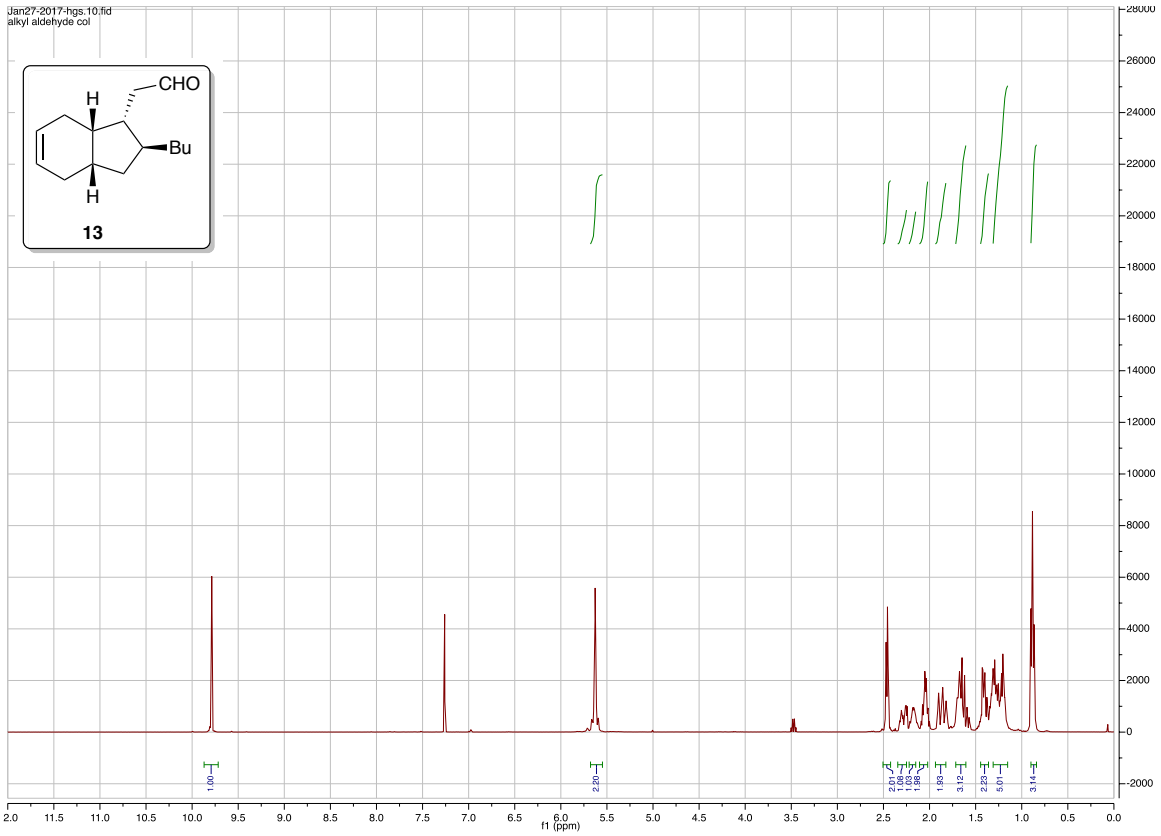


Figure S-29 ^{1}H-NMR spectrum of compound **13**.

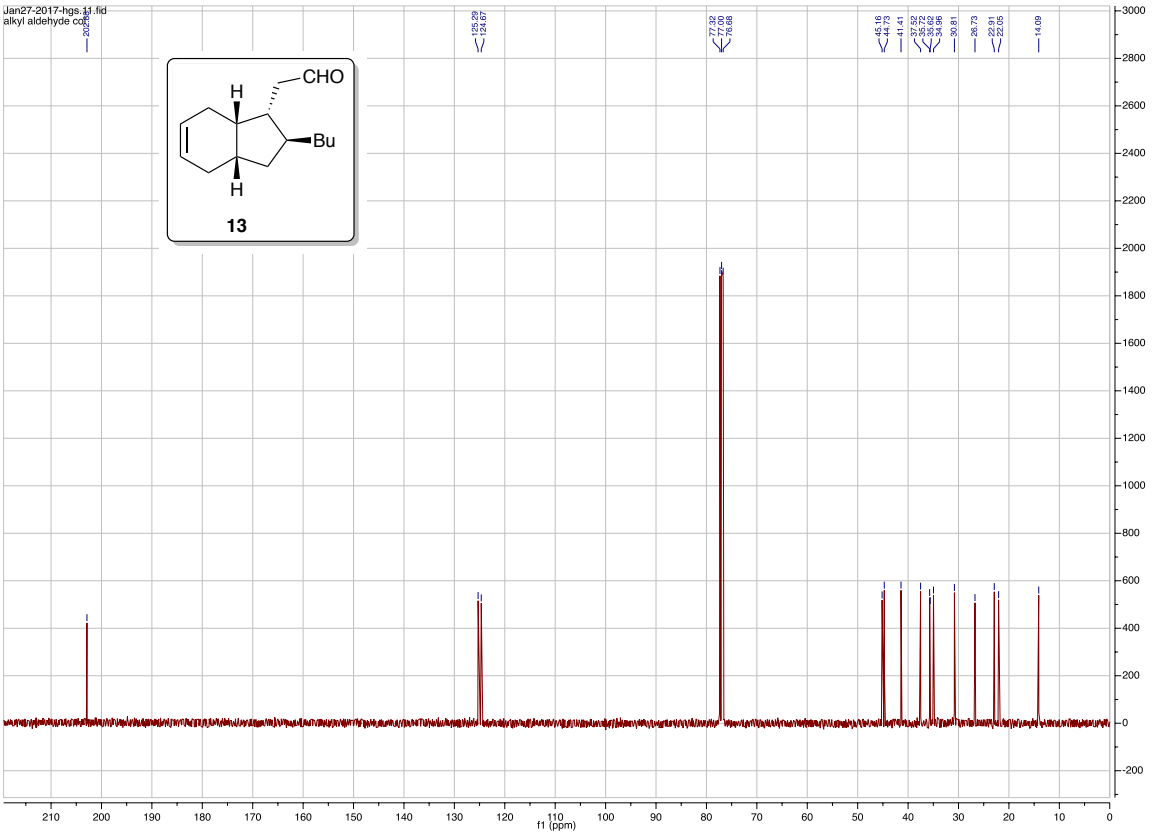


Figure S-30 ^{13}C-NMR spectrum of compound **13**.

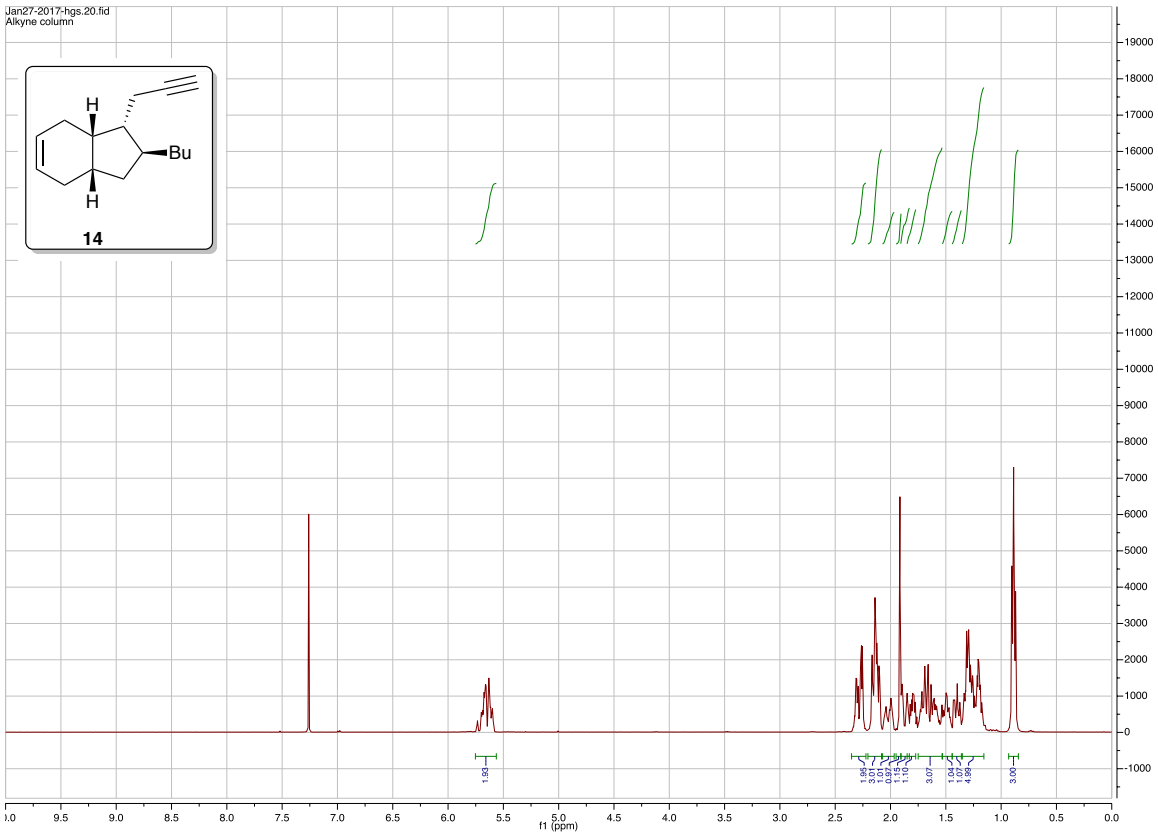


Figure S-31 ^{1}H-NMR spectrum of compound **14**.

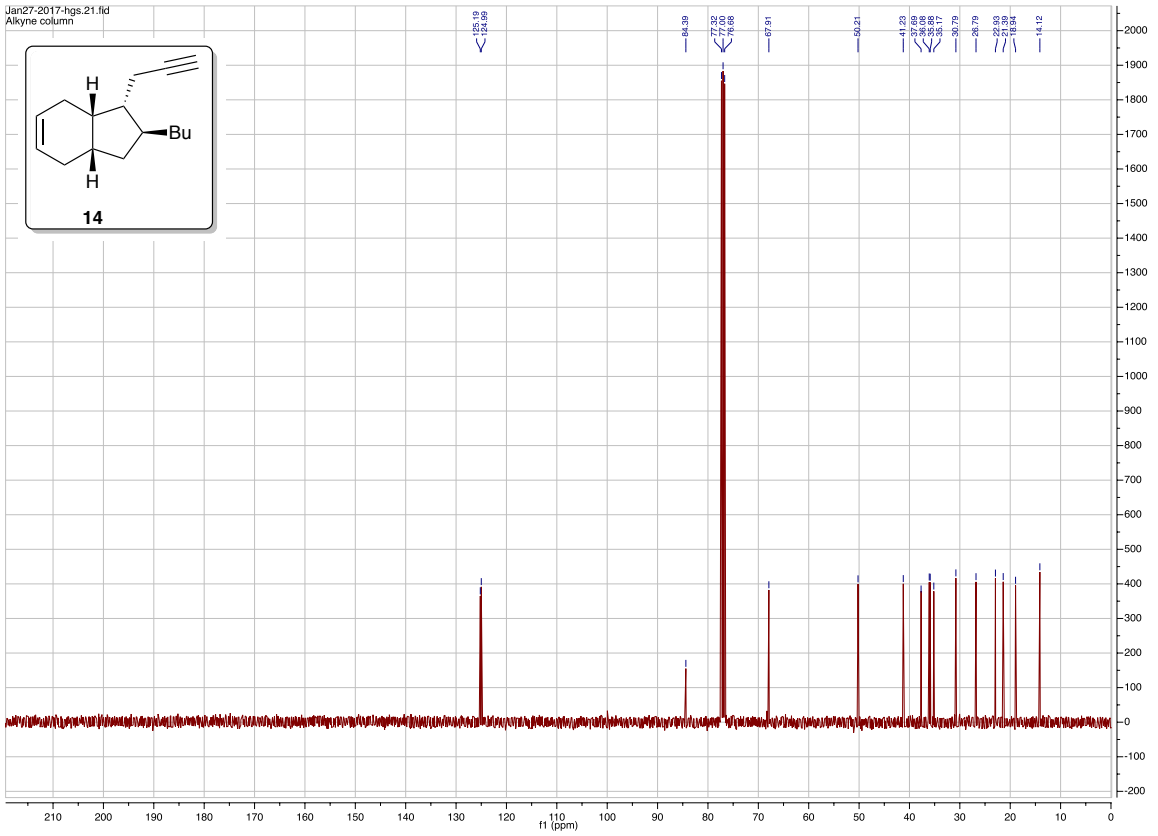


Figure S-32 ^{13}C-NMR spectrum of compound **14**.

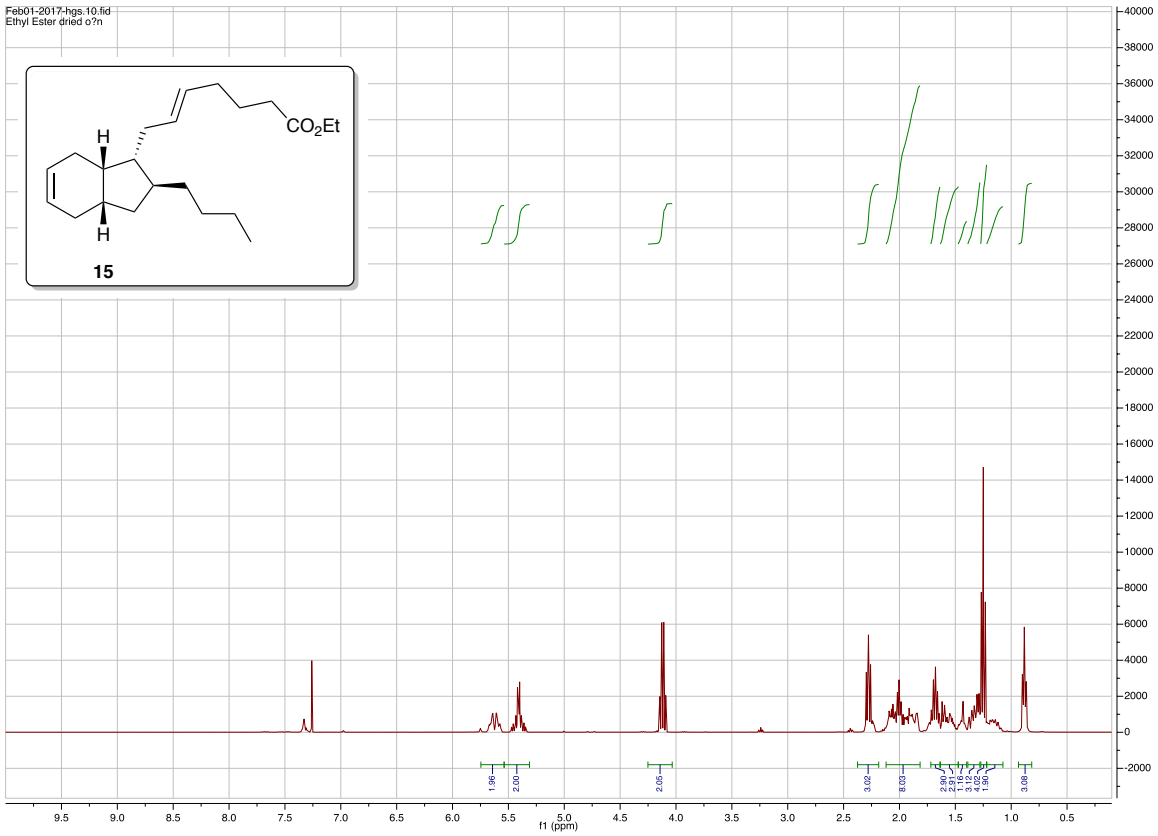


Figure S-33 ^{1}H-NMR spectrum of compound **15**.

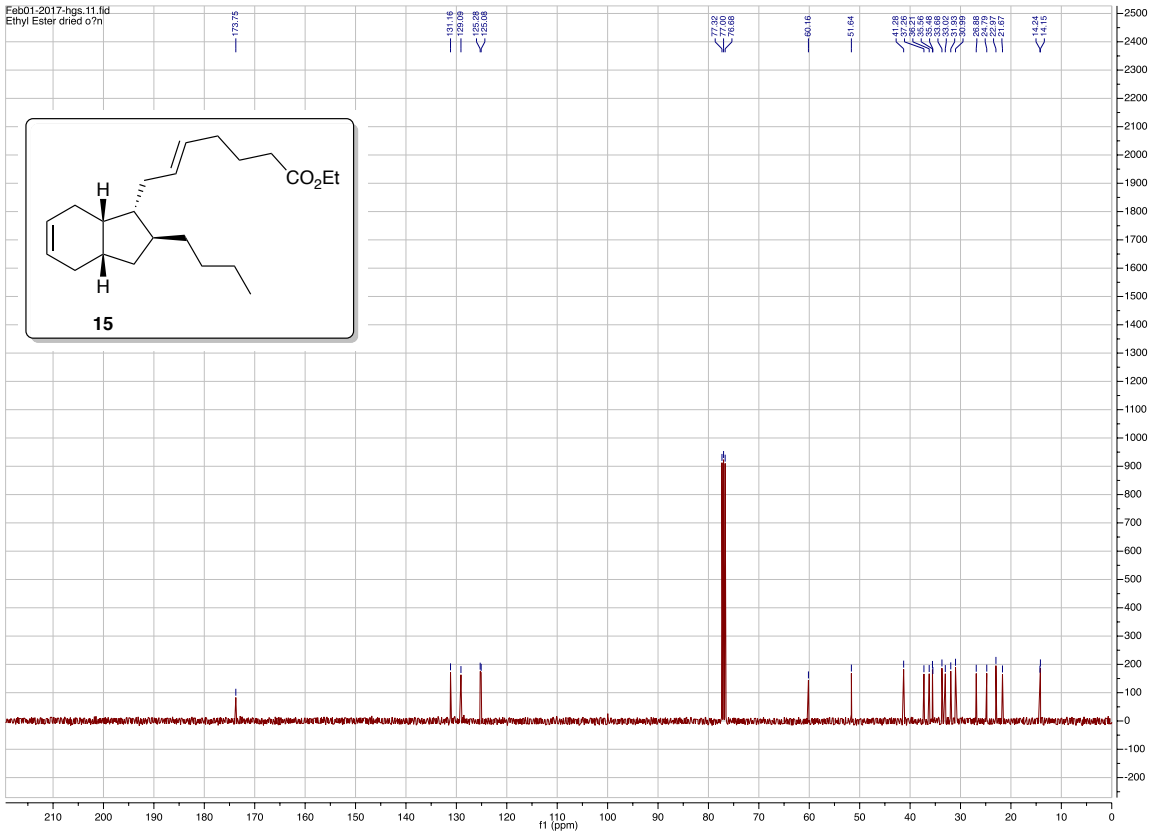


Figure S-34 ^{13}C-NMR spectrum of compound **15**.

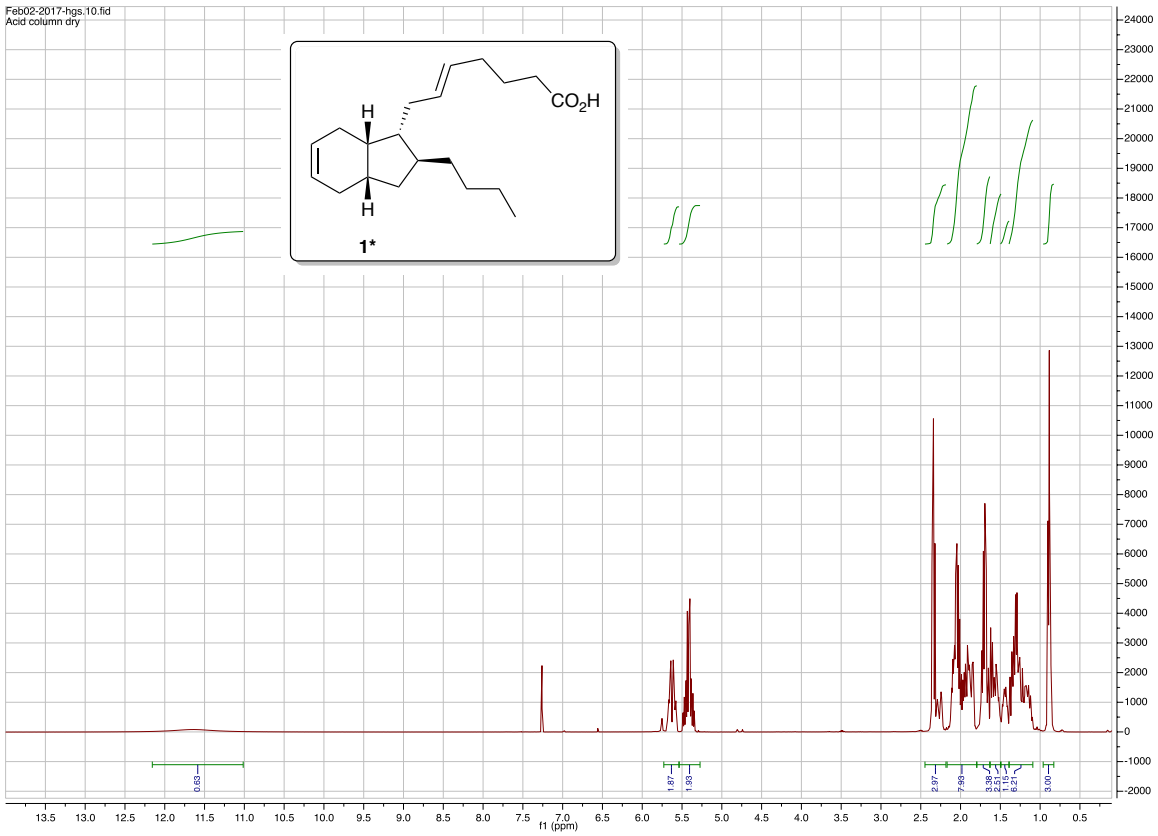


Figure S-35 ^{1}H-NMR spectrum of compound **1***.

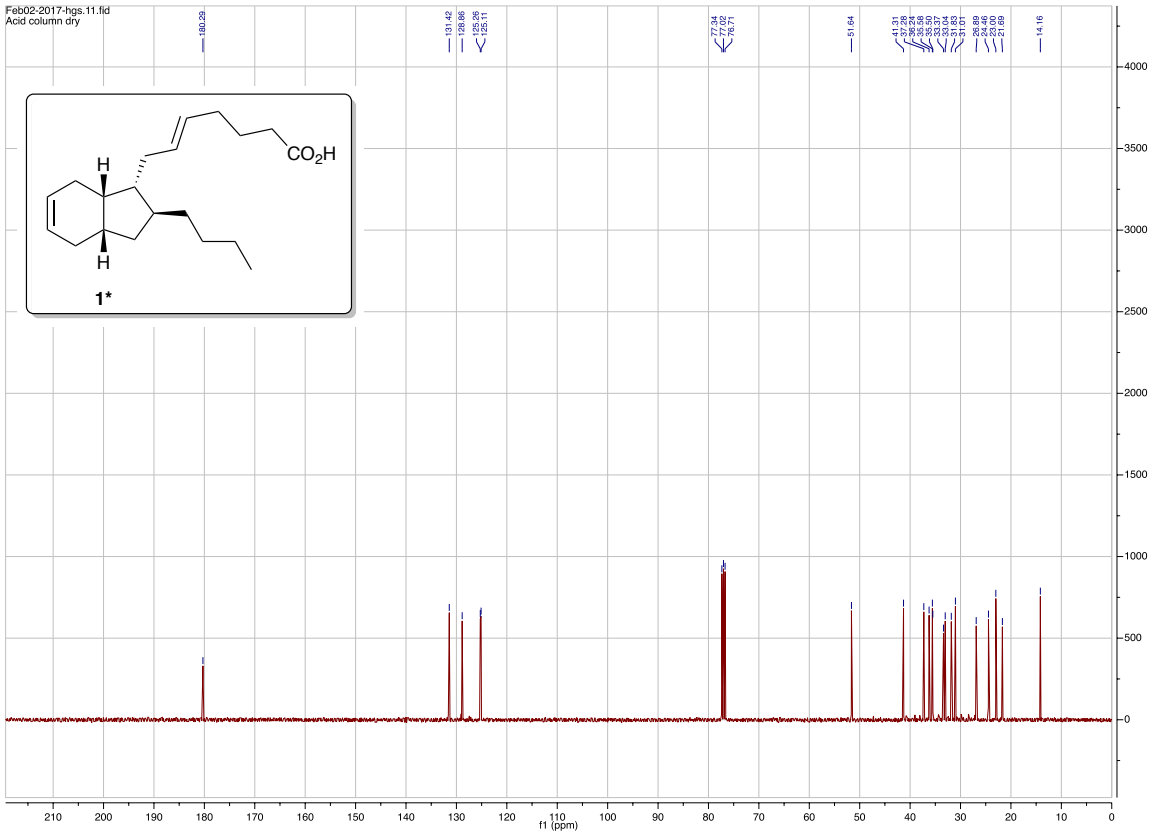


Figure S-36 ^{13}C-NMR spectrum of compound **1***.

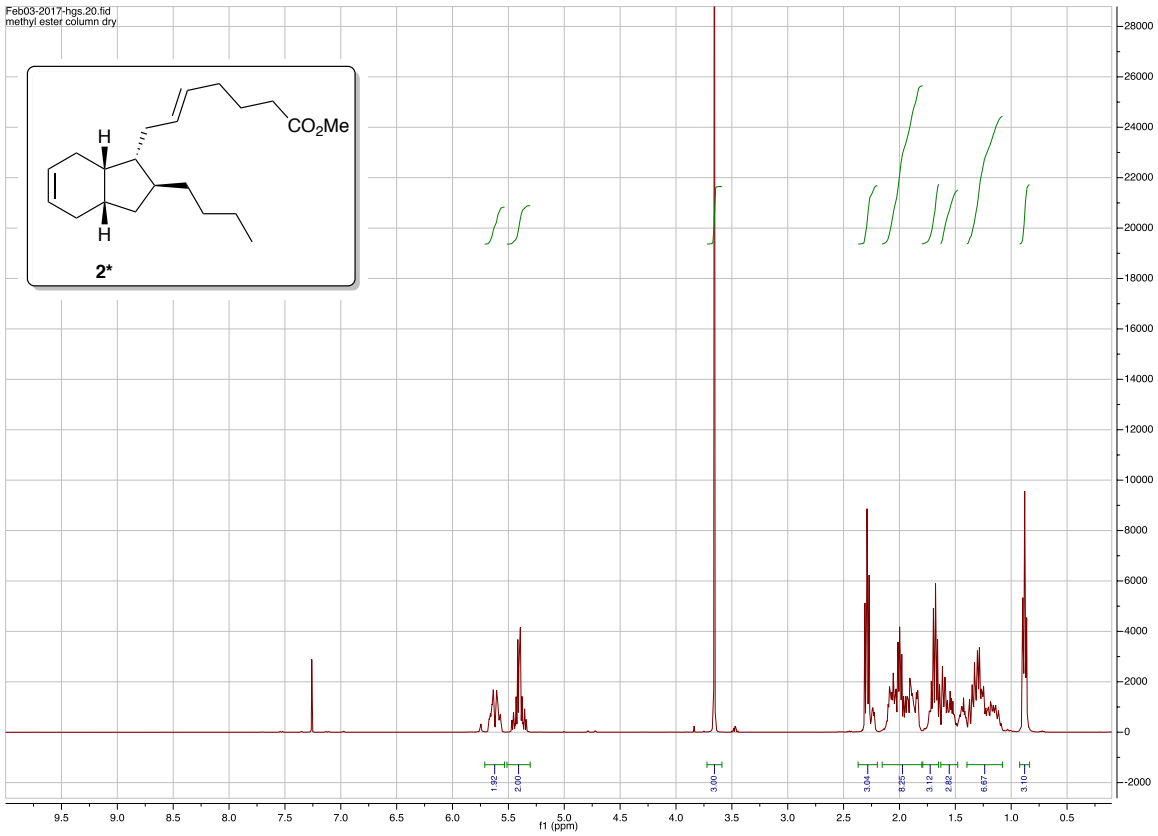


Figure S-37 ^{1}H-NMR spectrum of compound **2***.

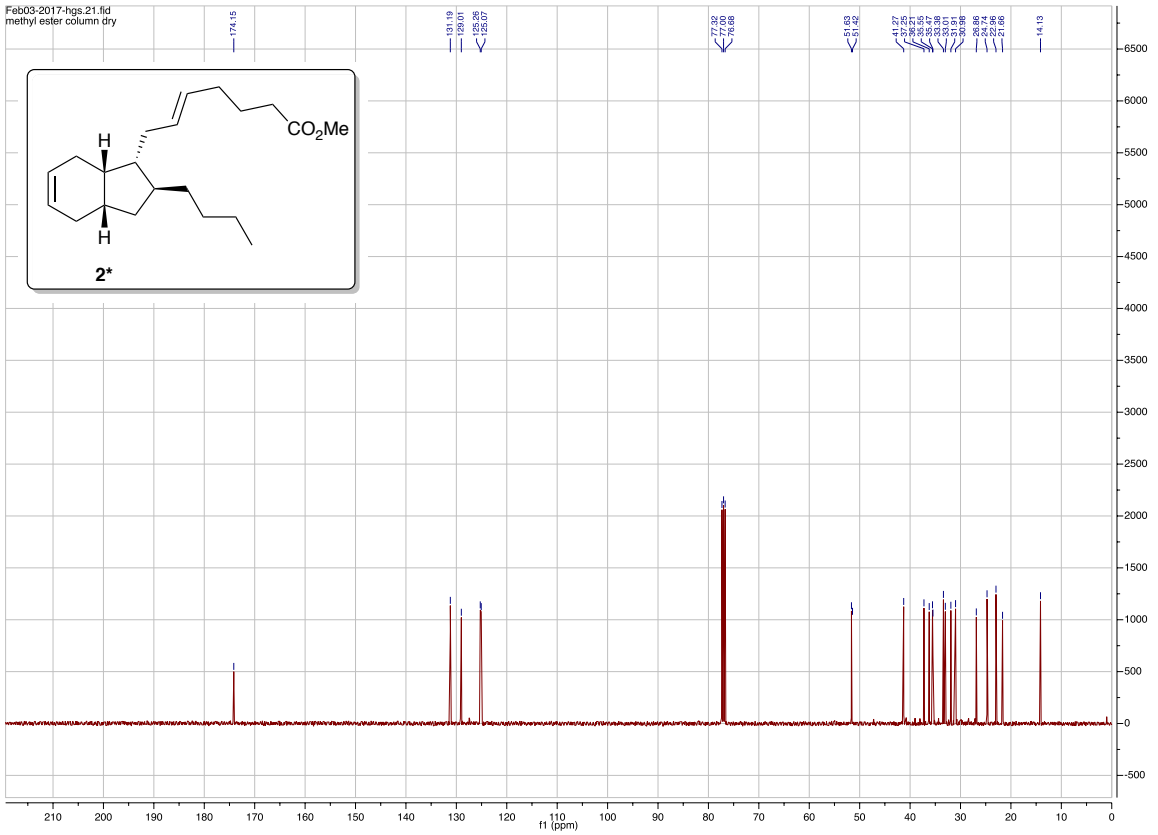


Figure S-38 ^{13}C-NMR spectrum of compound **2***.

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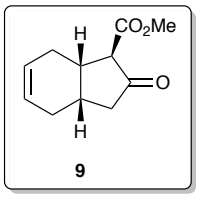
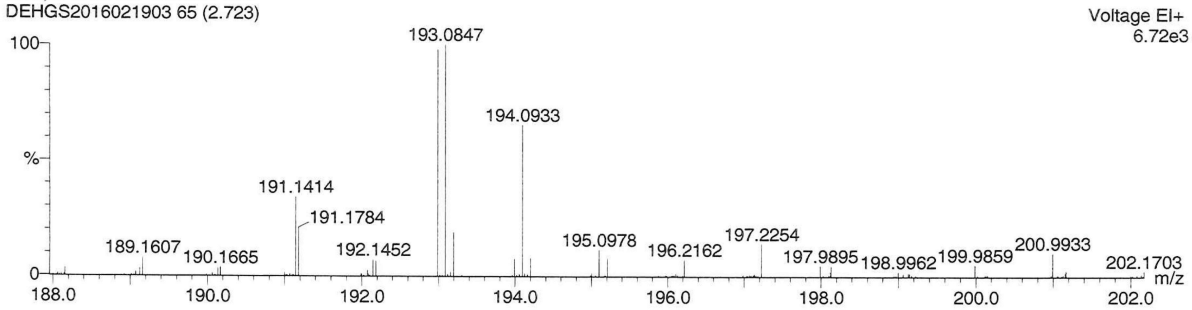


Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
47 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

Sample 3 C11H14O3 MW 194
DEHGS2016021903 65 (2.723)



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
194.0933	194.0943	-1.0	-5.1	5.0	1	C11 H14 O3

Figure S-39 HRMS of compound **9**.

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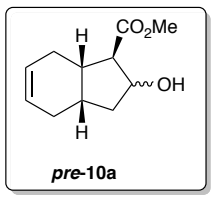
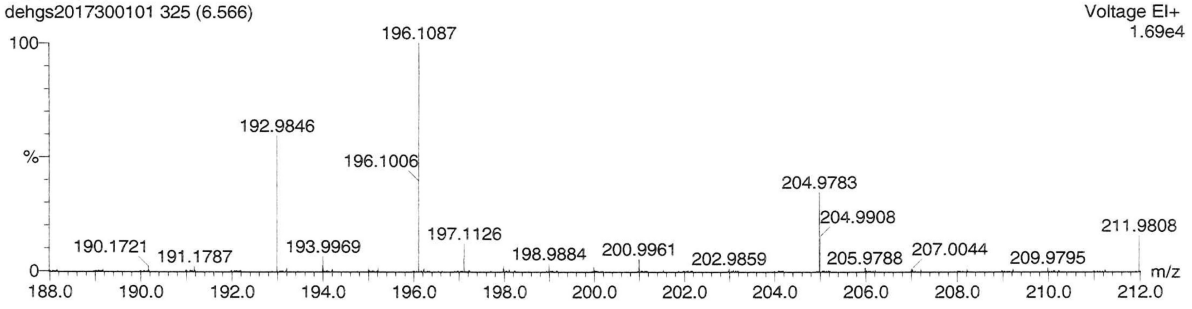


Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
18 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

1 C11H16O3
dehgs2017300101 325 (6.566)



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
196.1087	196.1099	-1.2	-6.3	4.0	1	C11 H16 O3

Figure S-40 HRMS of compound ***pre*-10a**.

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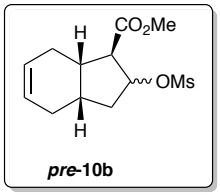
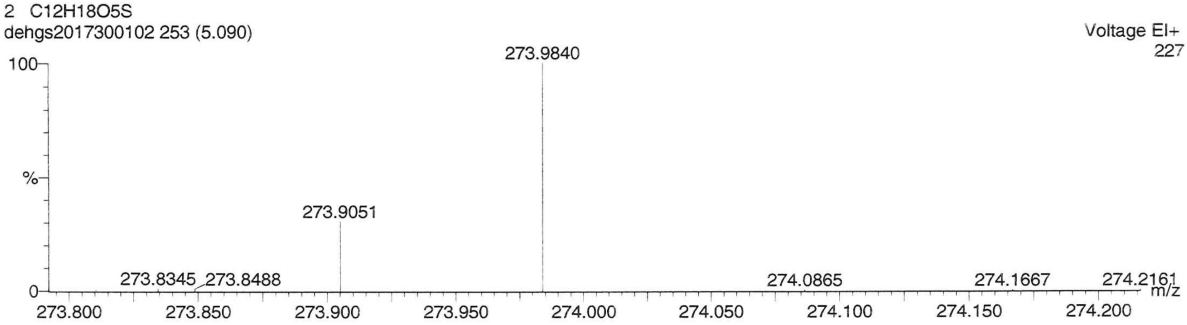


Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
31 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

2 C12H18O5S
dehgs2017300102 253 (5.090)



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
274.0865	274.0875	-1.0	-3.6	4.0	1	C12 H18 O5 S

Figure S-41 HRMS of compound ***pre*-10b**.

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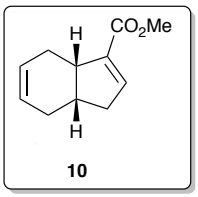
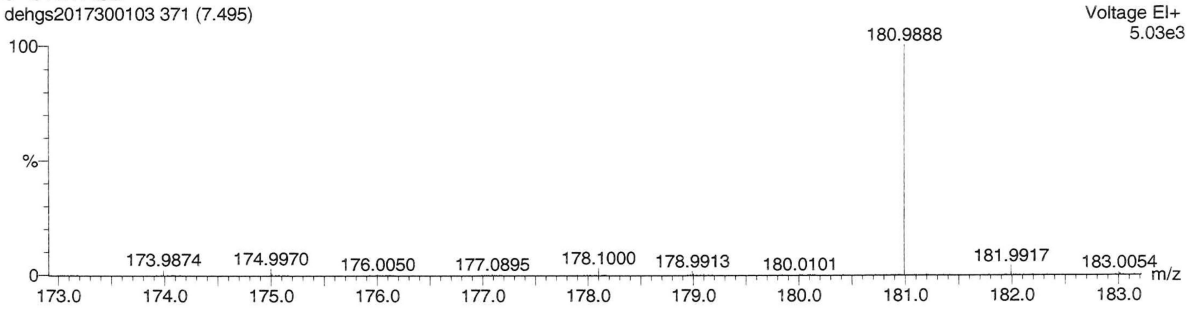


Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
21 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

3 C11H14O2
dehgs2017300103 371 (7.495)



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
178.1000	178.0994	0.6	3.5	5.0	1	C11 H14 O2

Figure S-42 HRMS of compound **10**.

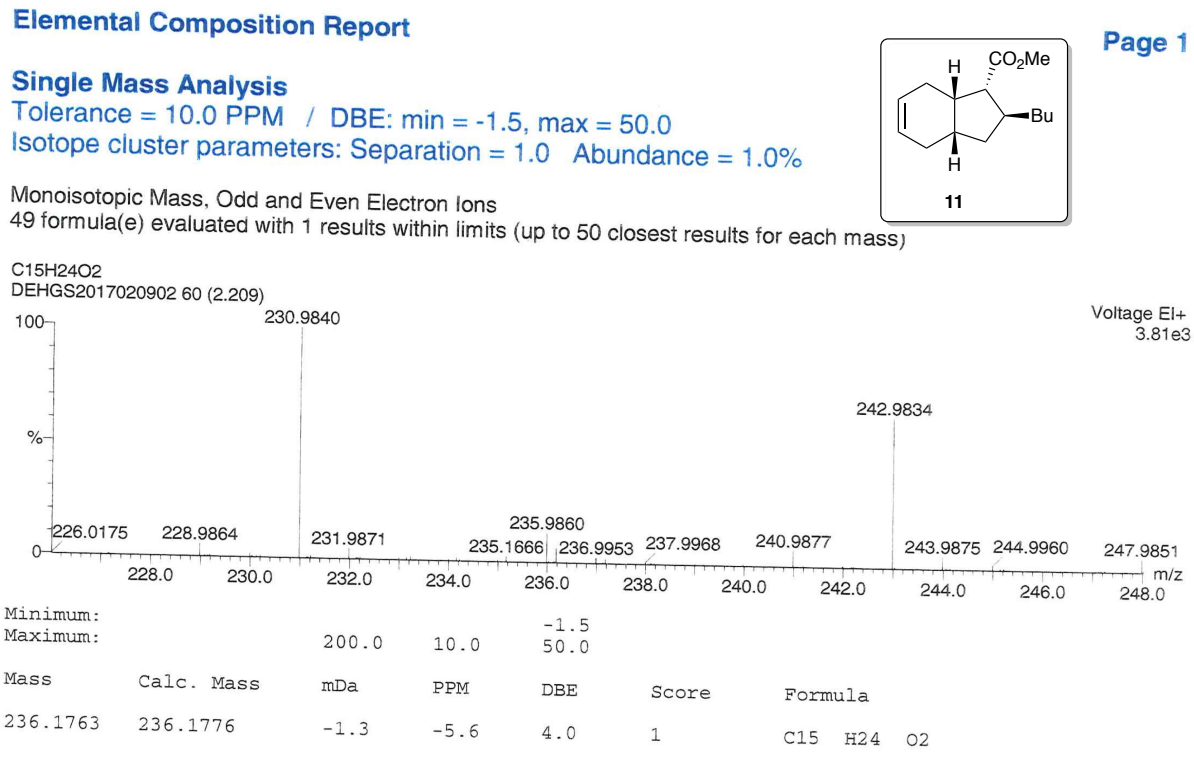


Figure S-43 HRMS of compound **11**.

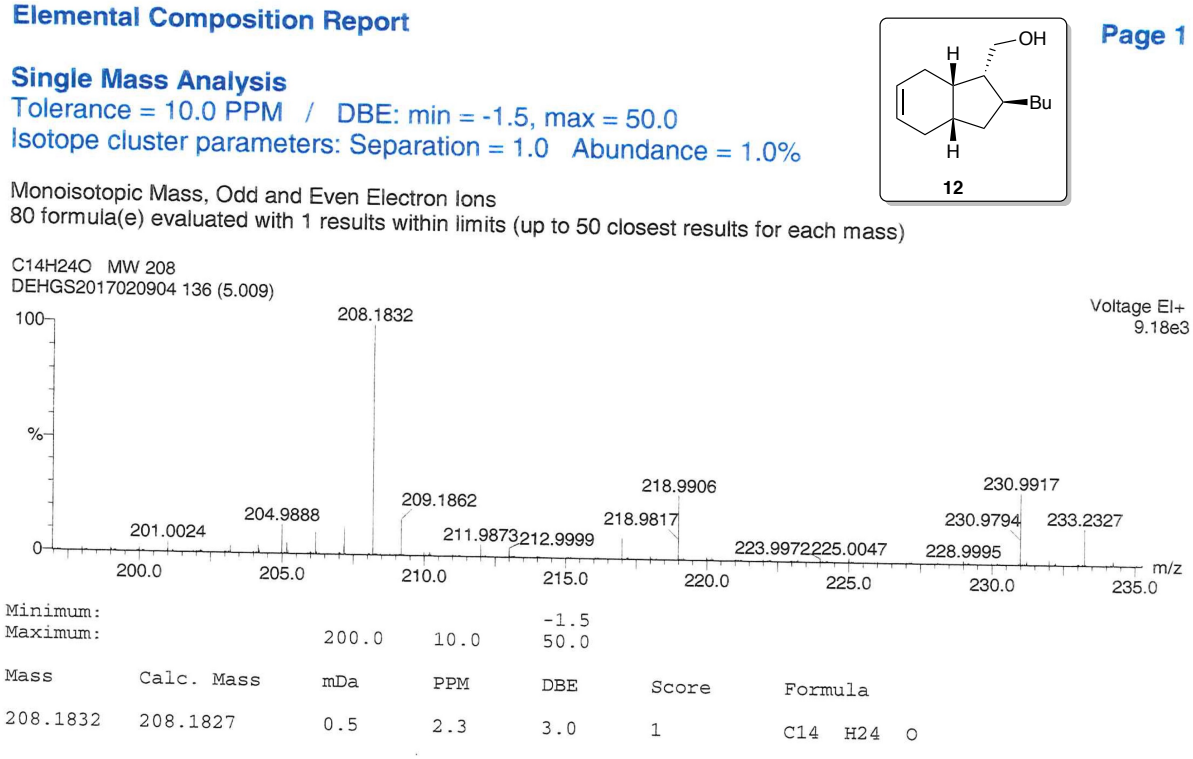


Figure S-44 HRMS of compound **12**.

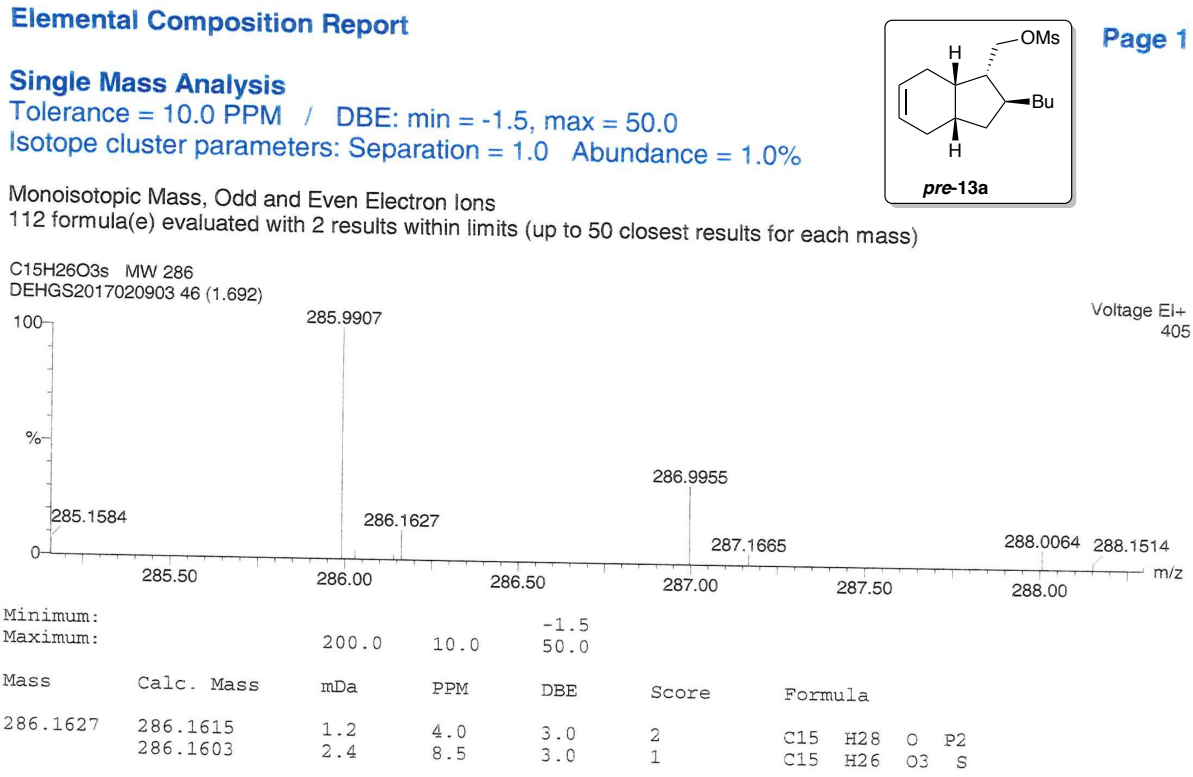


Figure S-45 HRMS of compound ***pre*-13a**.

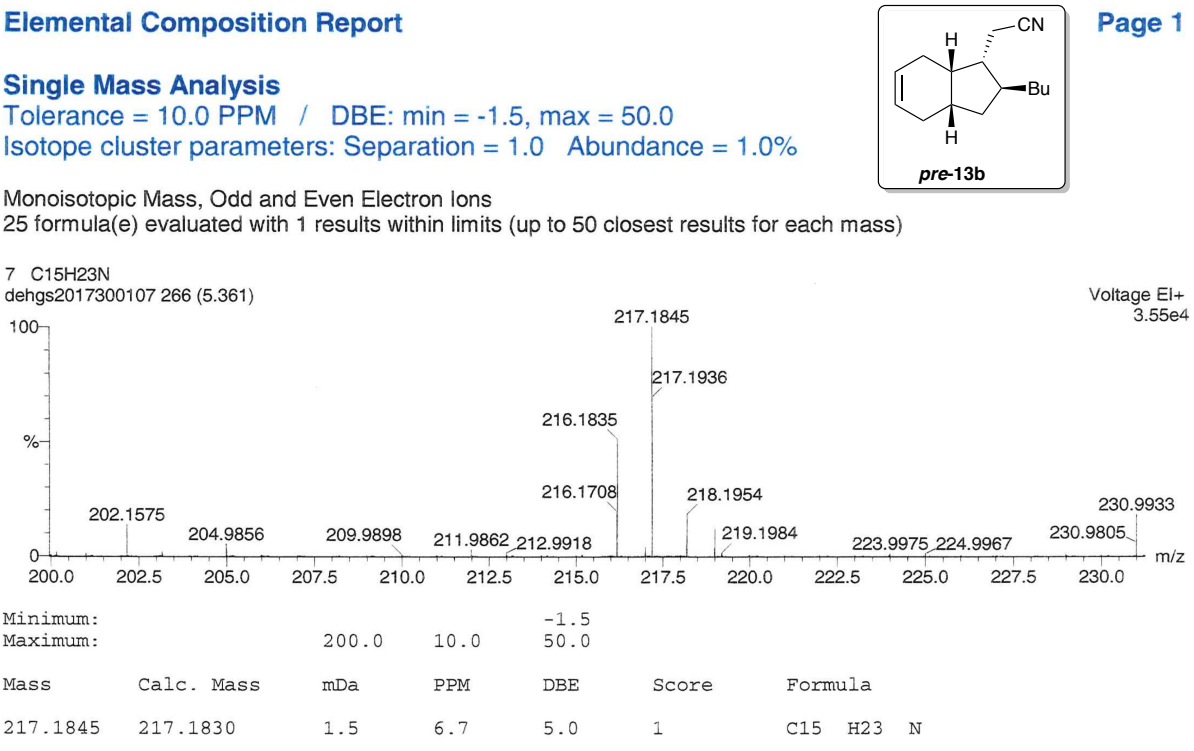


Figure S-46 HRMS of compound ***pre*-13b**.

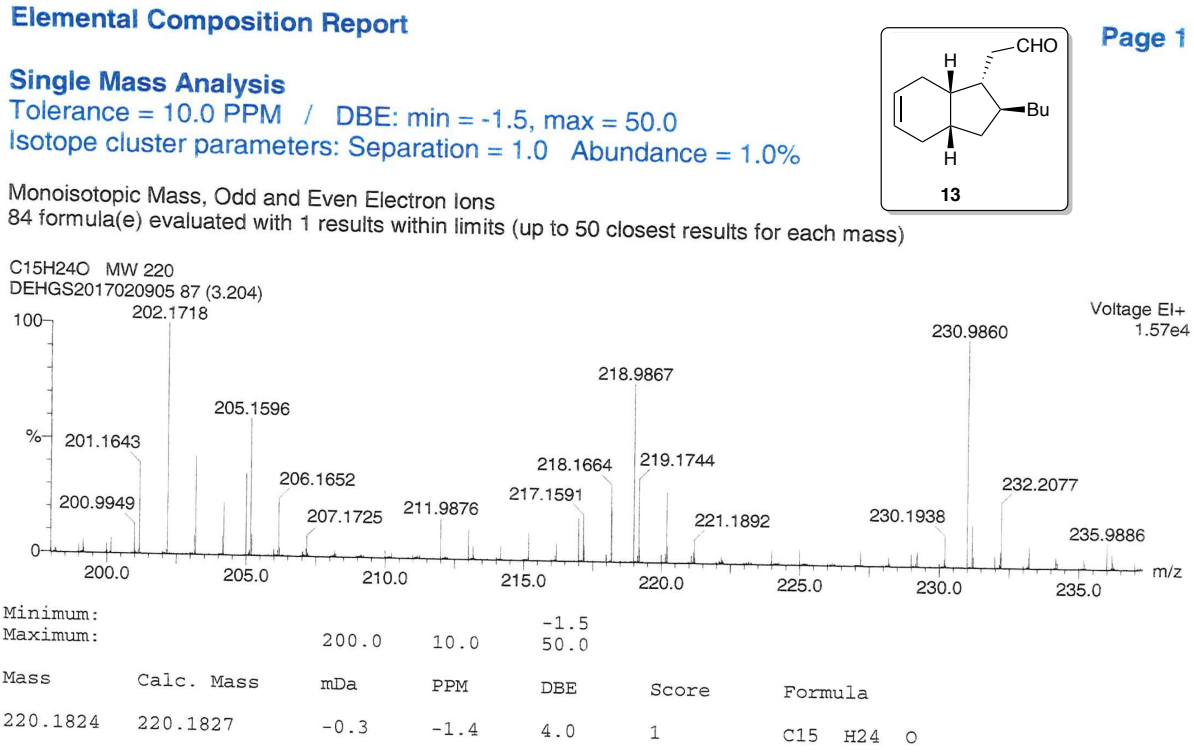


Figure S-47 HRMS of compound **13**.

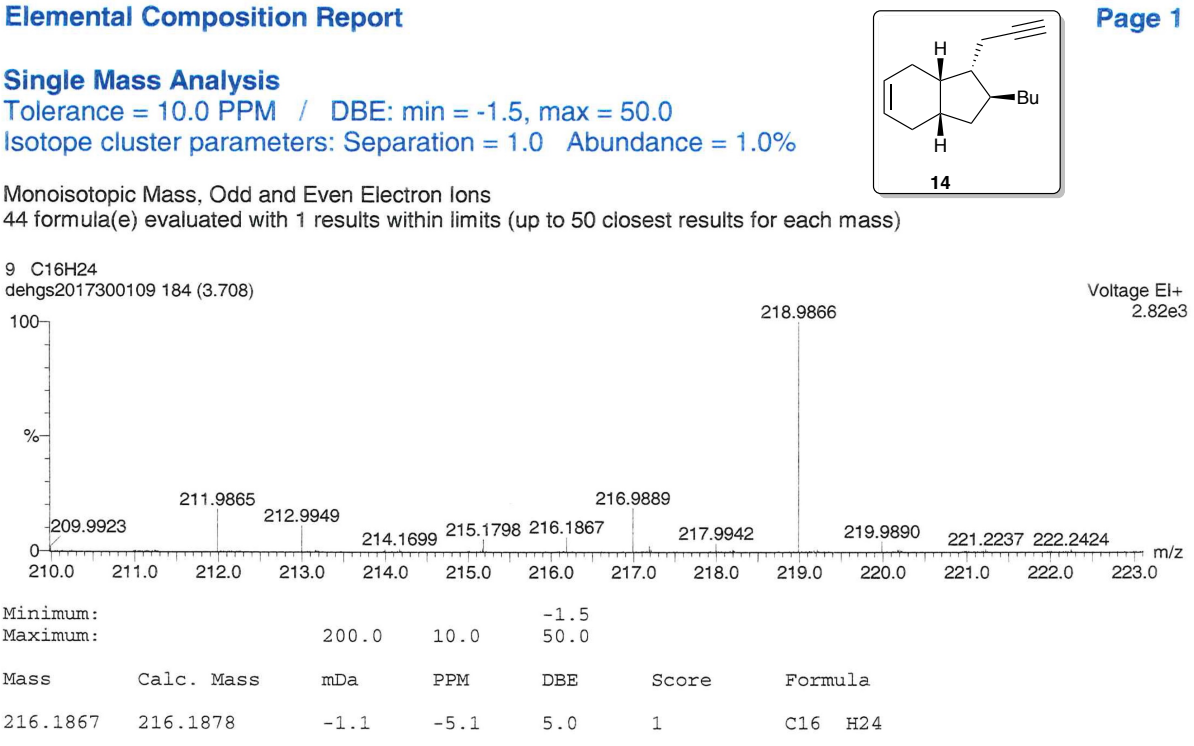


Figure S-48 HRMS of compound **14**.

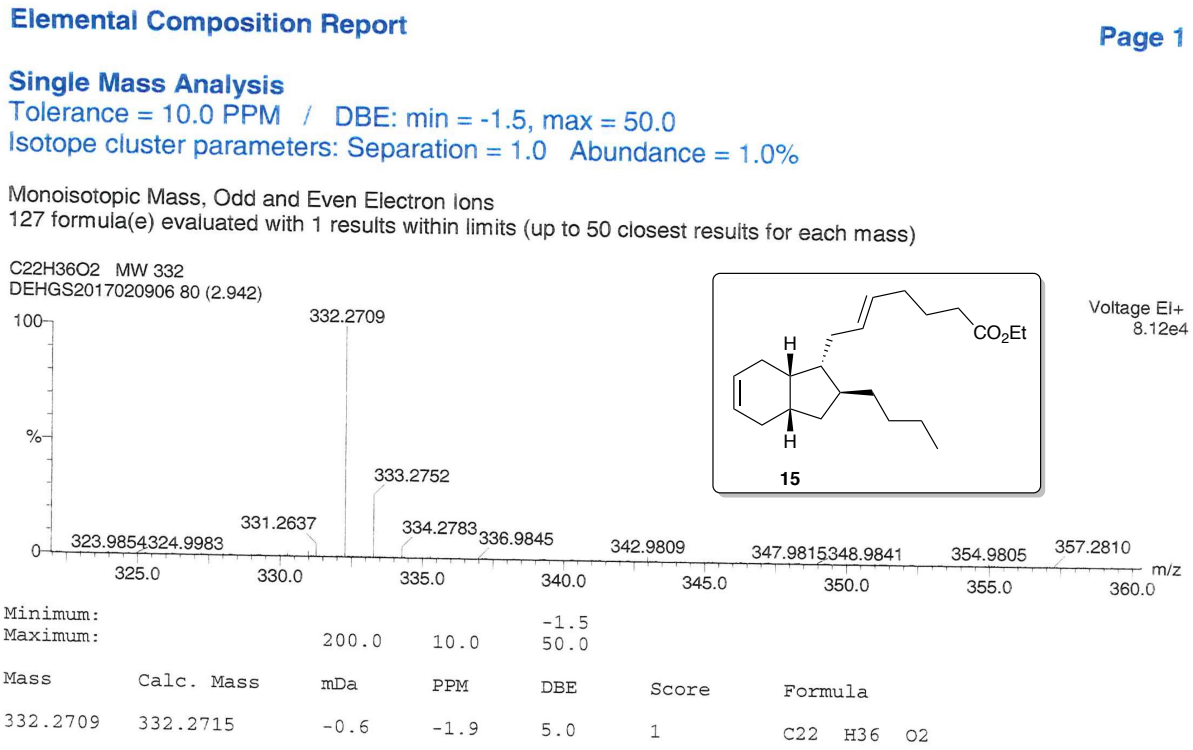


Figure S-49 HRMS of compound **15**.

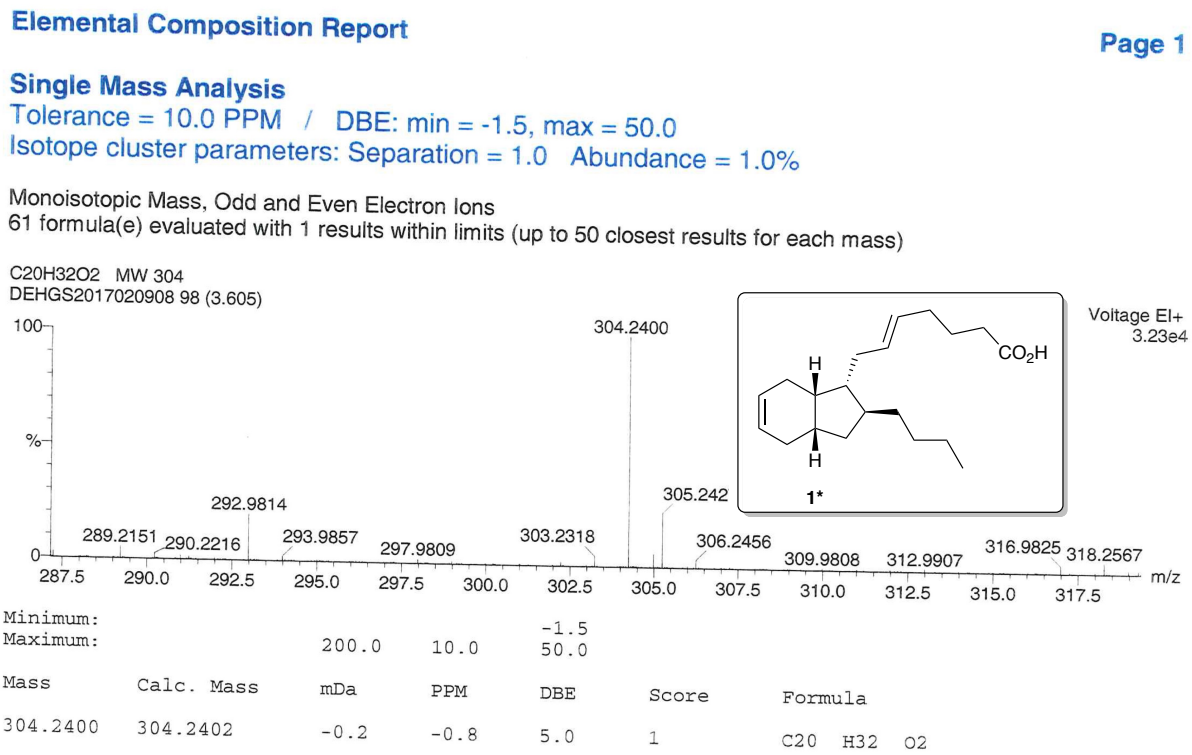


Figure S-50 HRMS of compound **1***.

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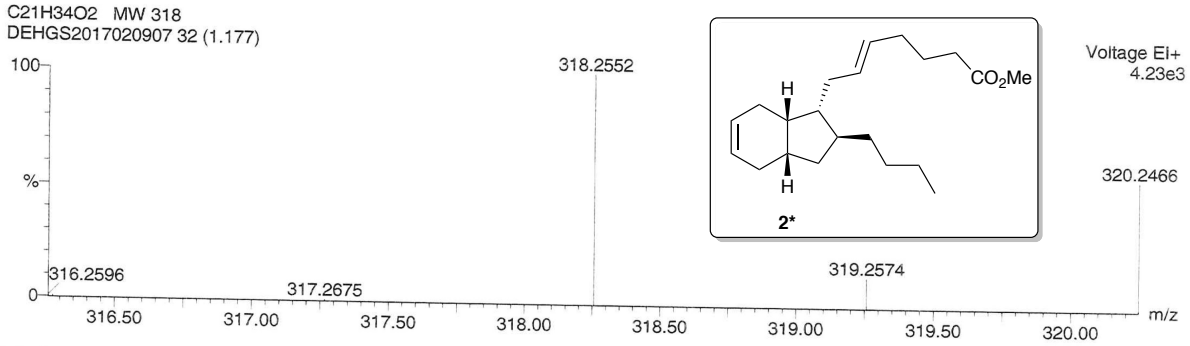
Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
64 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

C21H34O2 MW 318
DEHGS2017020907 32 (1.177)



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
318.2552	318.2559	-0.7	-2.1	5.0	1	C21 H34 O2

Figure S-51 HRMS of compound **2***.

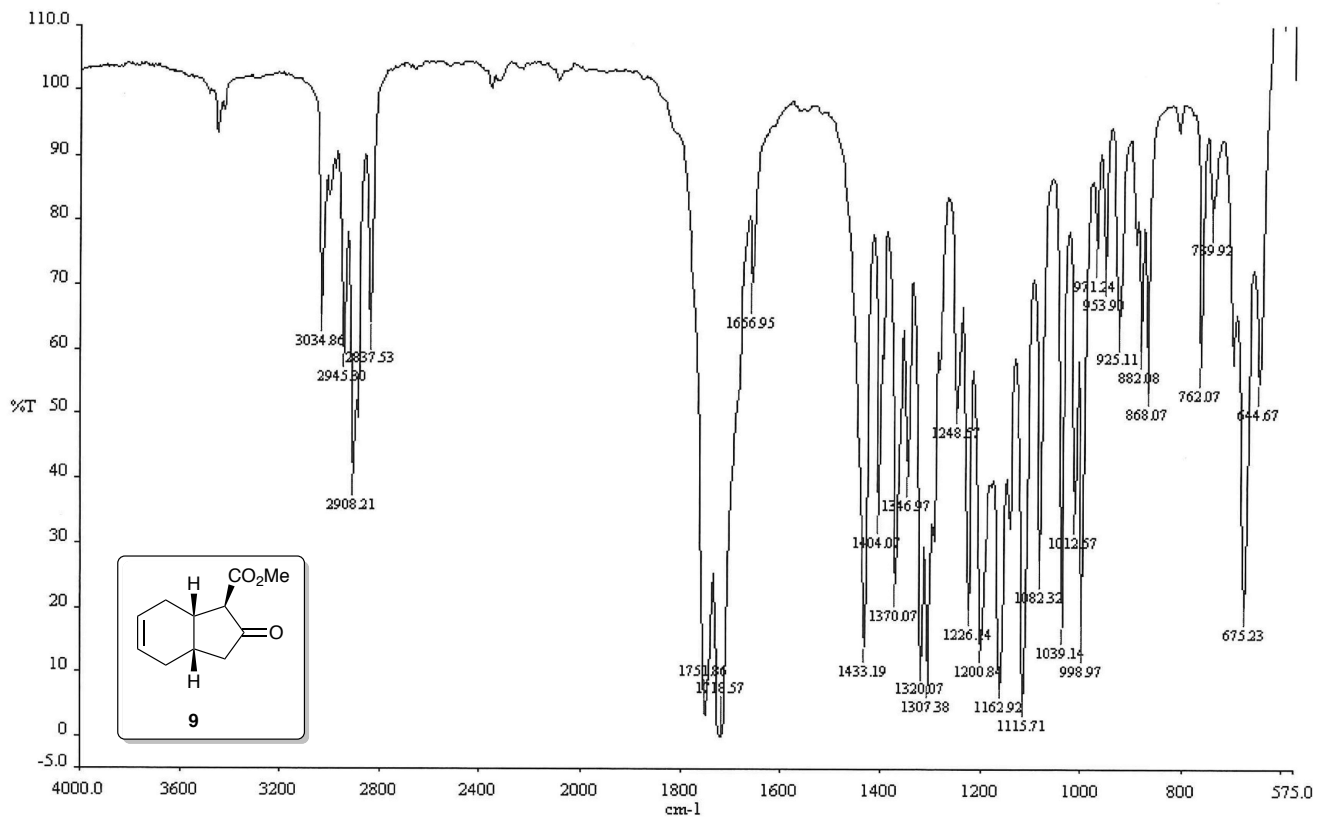


Figure S-52 IR of compound **9**.

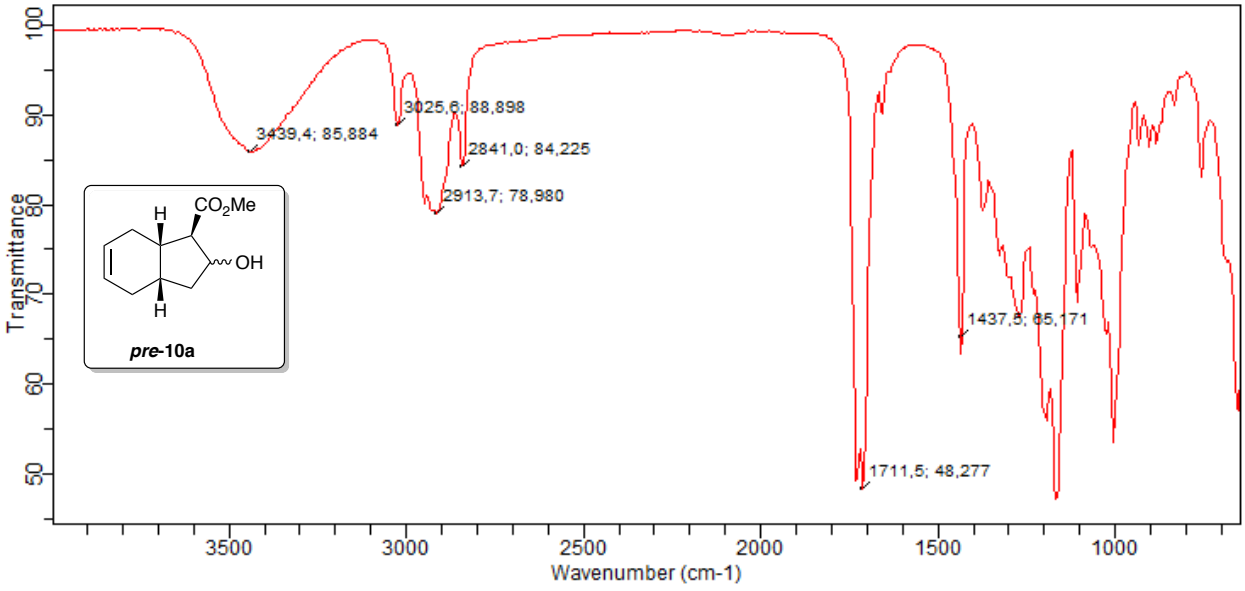


Figure S-53 IR of compound ***pre*-10a**.

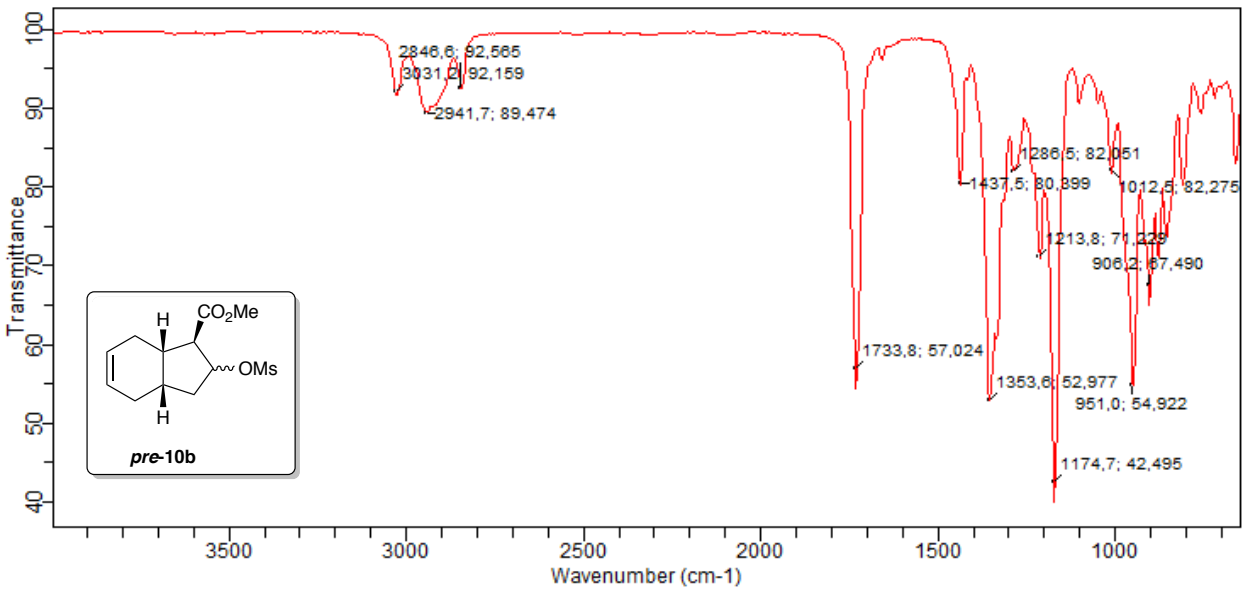


Figure S-54 IR of compound ***pre*-10b**.

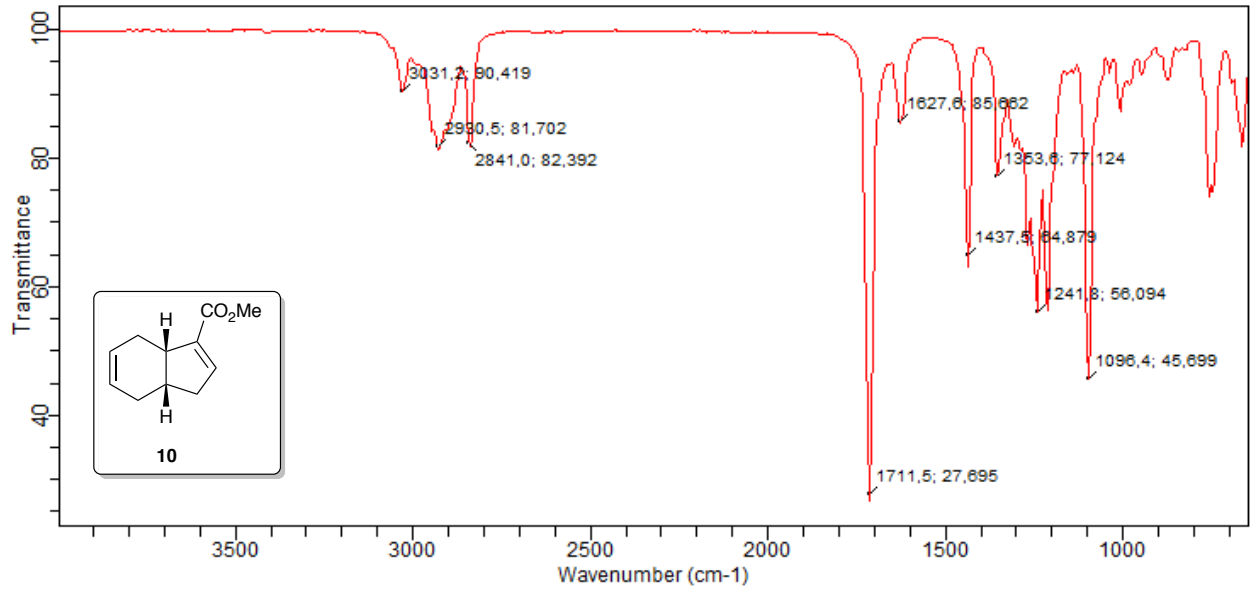


Figure S-55 IR of compound **10**.

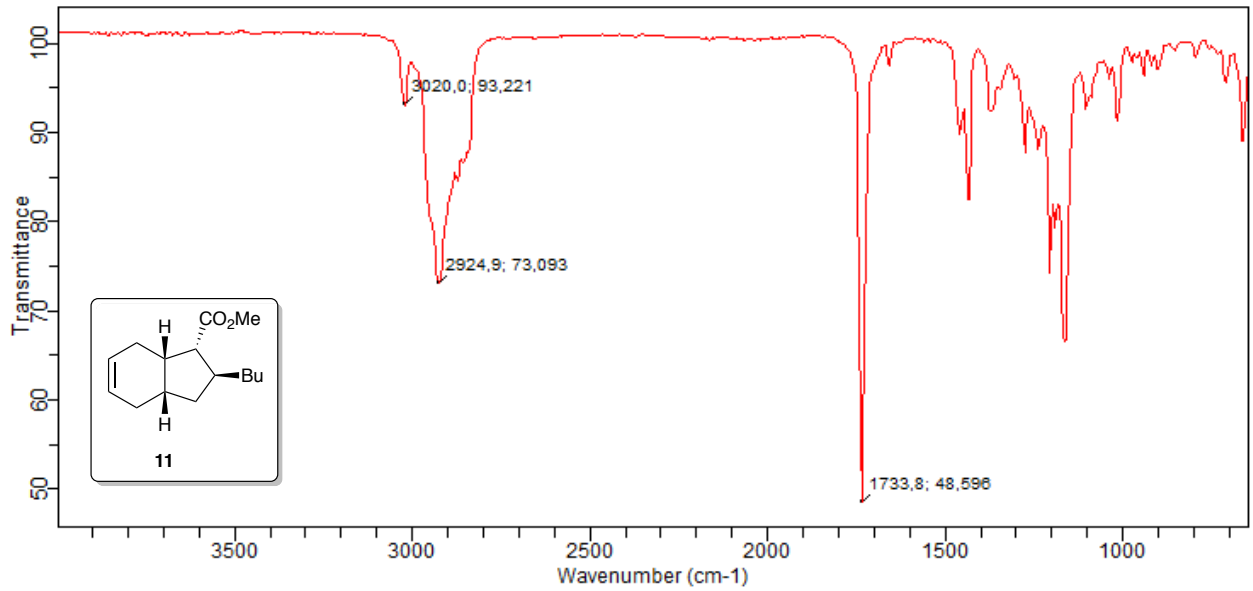


Figure S-56 IR of compound **11**.

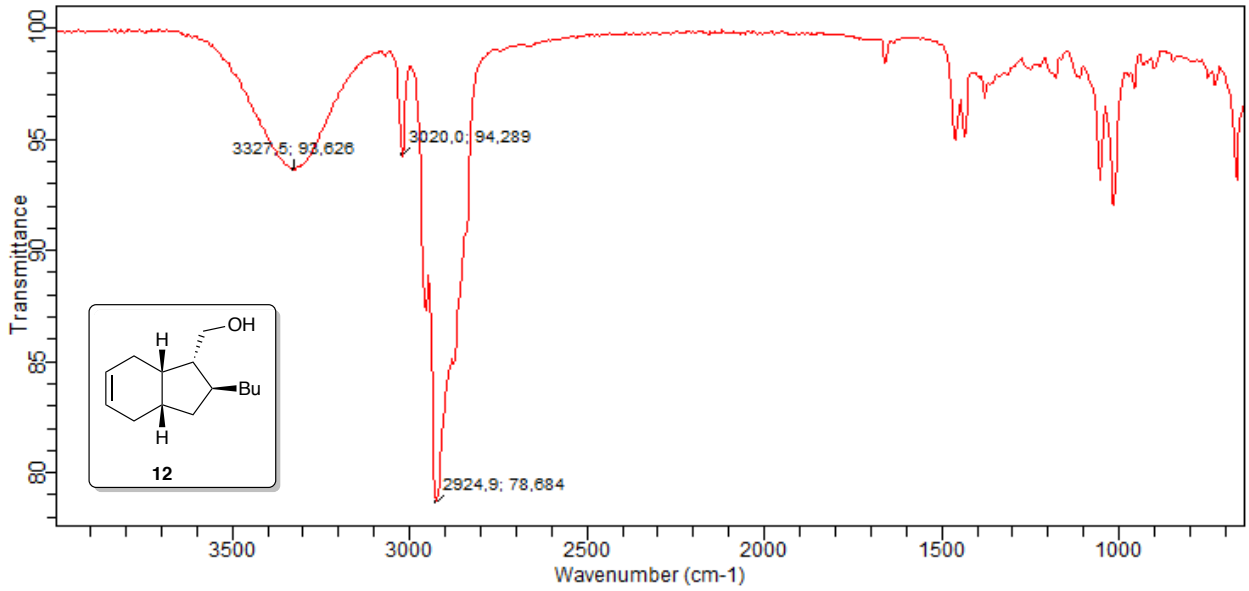


Figure S-57 IR of compound **12**.

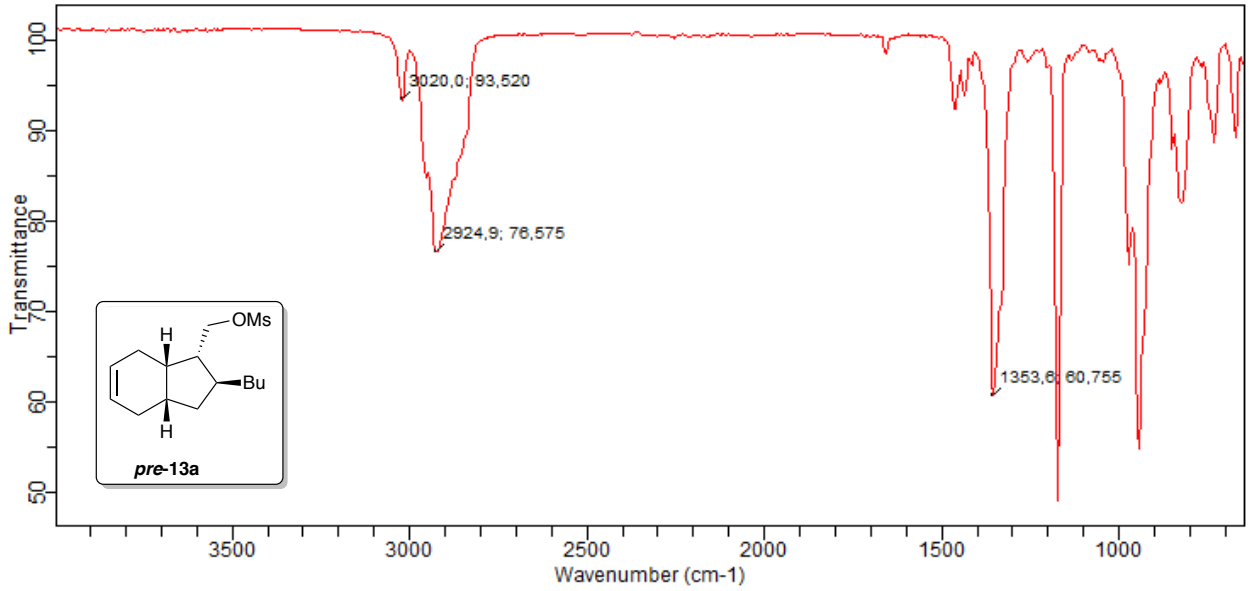


Figure S-58 IR of compound ***pre*-13a**.

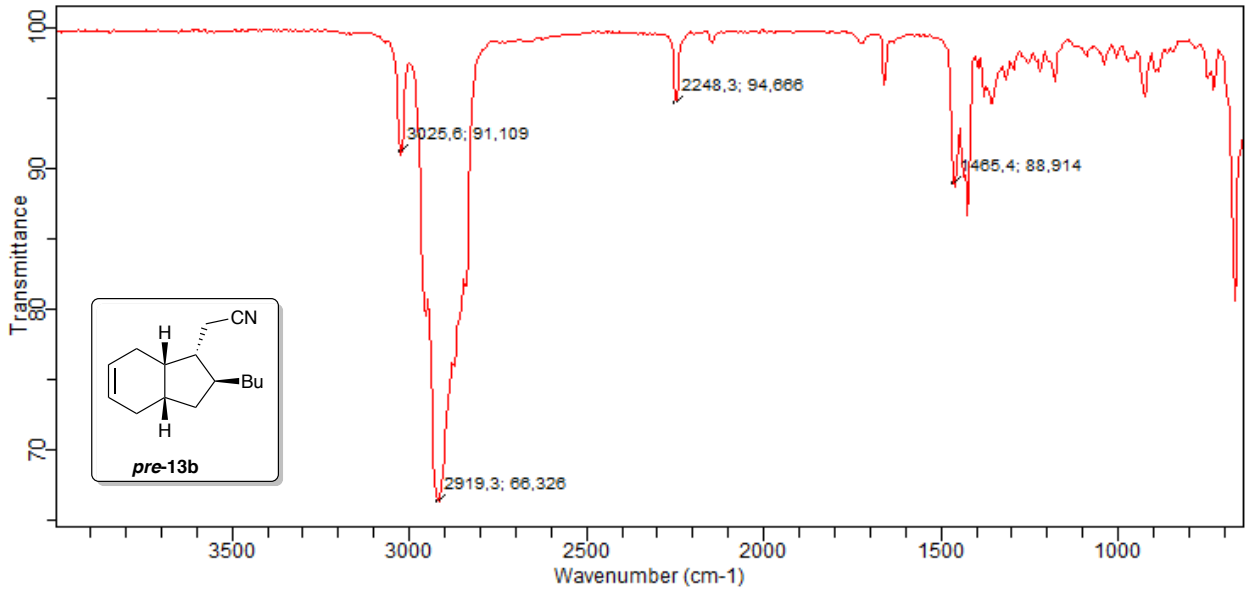


Figure S-59 IR of compound ***pre*-13b**.

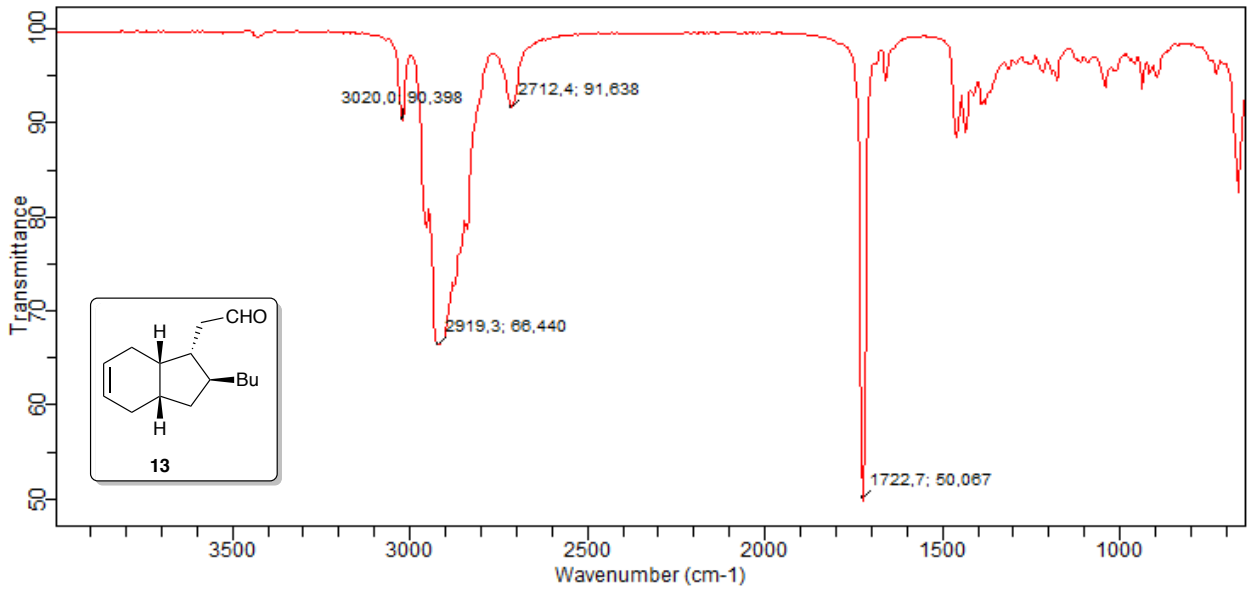


Figure S-60 IR of compound **13**.

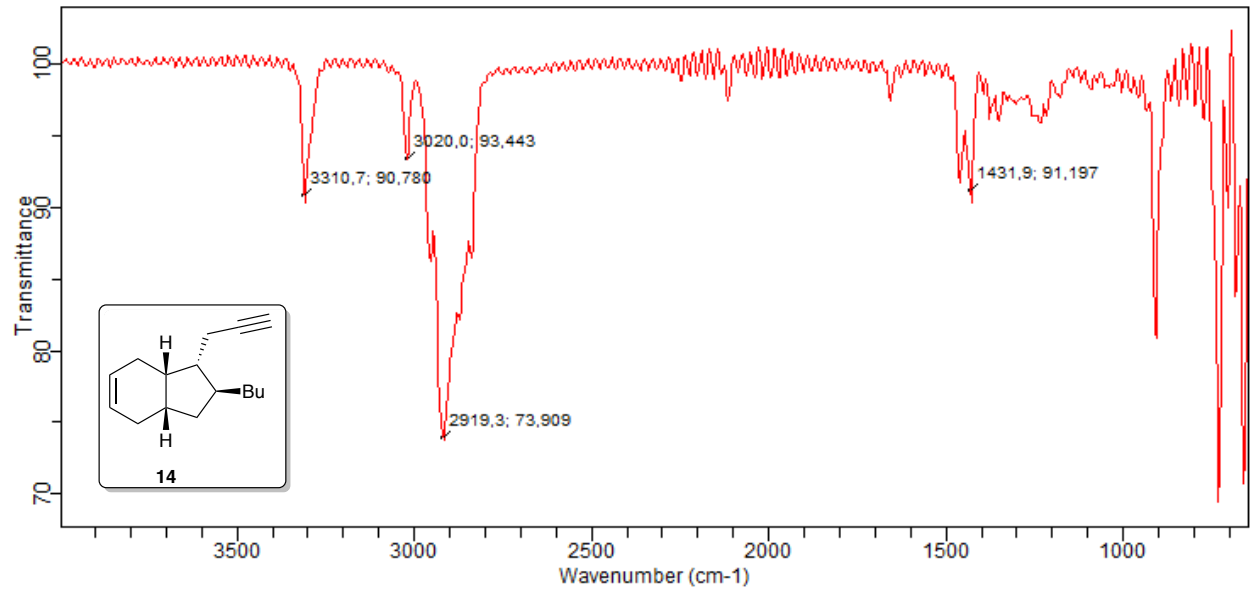


Figure S-61 IR of compound **14**.

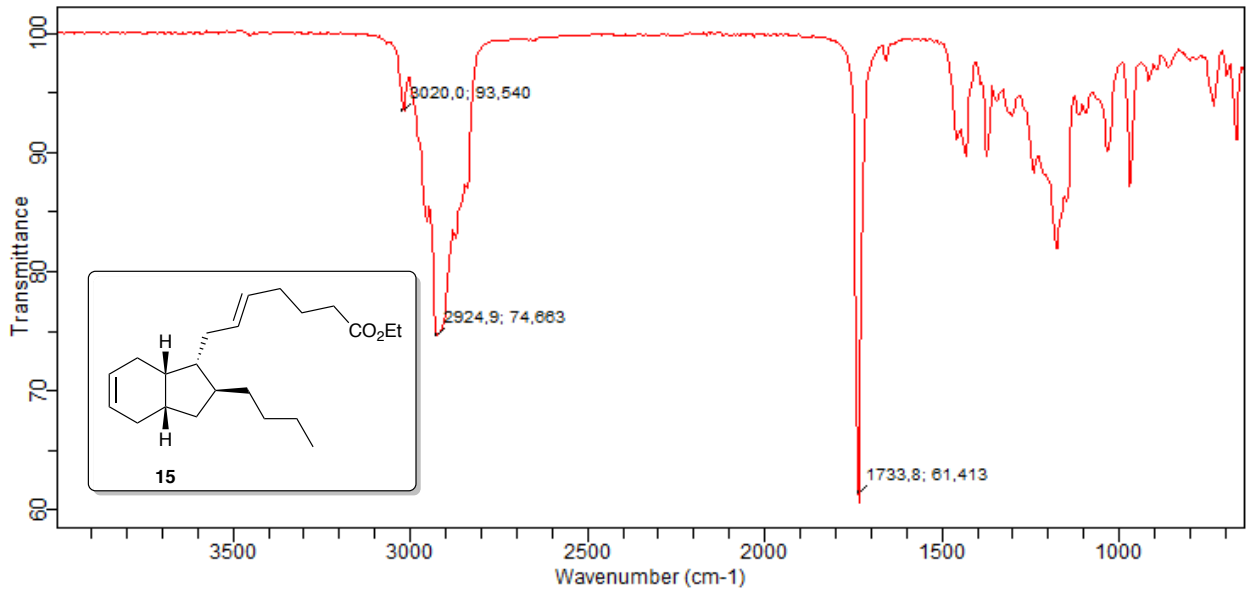


Figure S-62 IR of compound **15**.

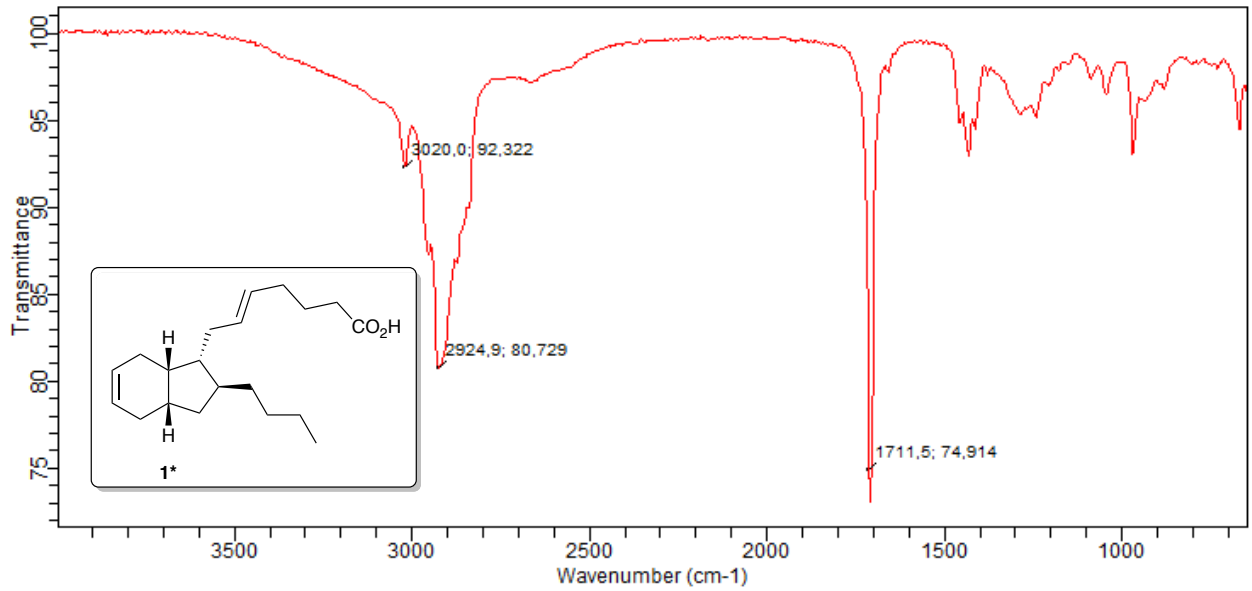


Figure S-63 IR of compound **1***.

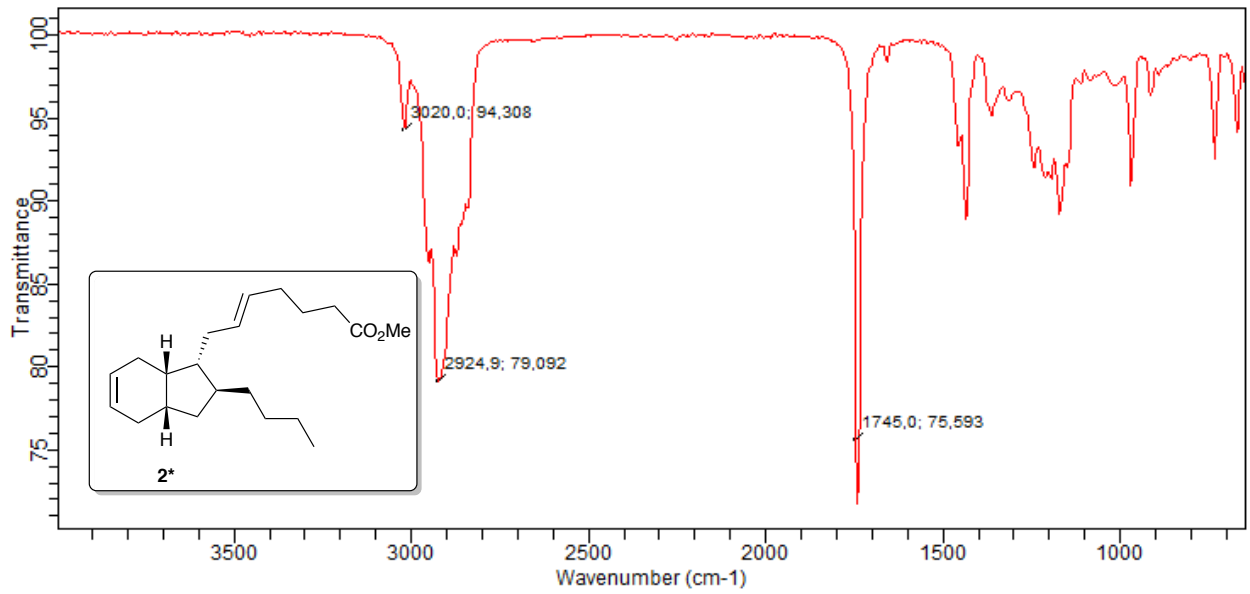
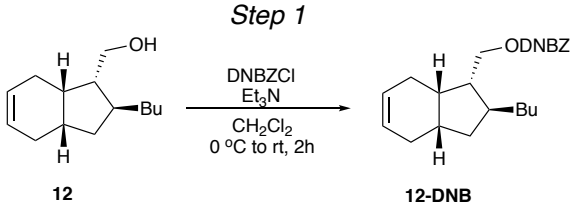


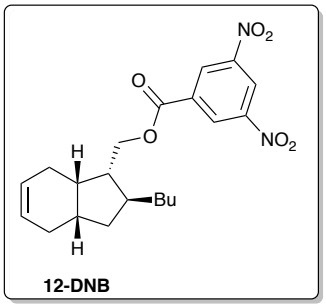
Figure S-64 IR of compound **2***.

Preparation 3,5-dinitrobenzoate derivative of (12).



Scheme S-3 Derivatization of advanced intermediate **12** to 3,5-dinitrobenzoate **12-DNB**.

(1*S*,6*S*,7*R*,8*S*)-8-Butyl-7-((3,5-dinitrobenzoyl)oxymethyl)bicyclo[4.3.0]non-3-ene (12-DNB).



A stirring solution of (1*S*,6*S*,7*S*,8*R*)-8-butyl-7-(hydroxymethyl)bicyclo[4.3.0]non-3-ene **12** (0.129 g, 0.619 mmol, 1.0 equiv.) in dry DCM (20 mL) was added Et_3N (0.26 mL, 1.86 mmol, 3.0 equiv.) dropwise. The solution was then cooled to 0 °C and 3,5-dinitrobenzoyl chloride (0.214 g, 0.929 mmol, 1.5 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 (hexane/EtOAc, 95:5) to afford the title compound as a white powder. Yield: 0.222 g (89%), $[\alpha]_D^{26} + 42$ ($c = 0.8$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.23 (t, $J = 2.2$ Hz, 1H), 9.14 (d, $J = 2.2$ Hz, 2H), 5.70-5.61 (m, 2H), 4.49 (s, 1H), 4.47 (d, $J = 1.9$ Hz, 1H), 2.37-2.10 (m, 4H), 2.02-1.88 (m, 2H), 1.88-1.68 (m, 3H), 1.58-1.43 (m, 2H), 1.40-1.23 (m, 5H), 0.89 (t, $J = 6.7$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.5, 148.7, 134.1, 129.3, 125.4, 124.3, 122.3, 67.7, 49.5, 38.6, 36.9, 36.7, 35.5, 35.4, 30.8, 26.5, 22.9, 21.8, 14.1; IR (neat, cm^{-1}) 3098 (w), 3020 (w), 2931 (m), 1723 (s), 1538 (s); HRMS (EI+): Exact mass calculated for $C_{21}H_{26}N_2O_6$ $[M]^+$: 402.1791, found 402.1797; m.p.: 115-117 °C; TLC (hexane/EtOAc 4:1, $KMnO_4$ stain): R_f =0.75.

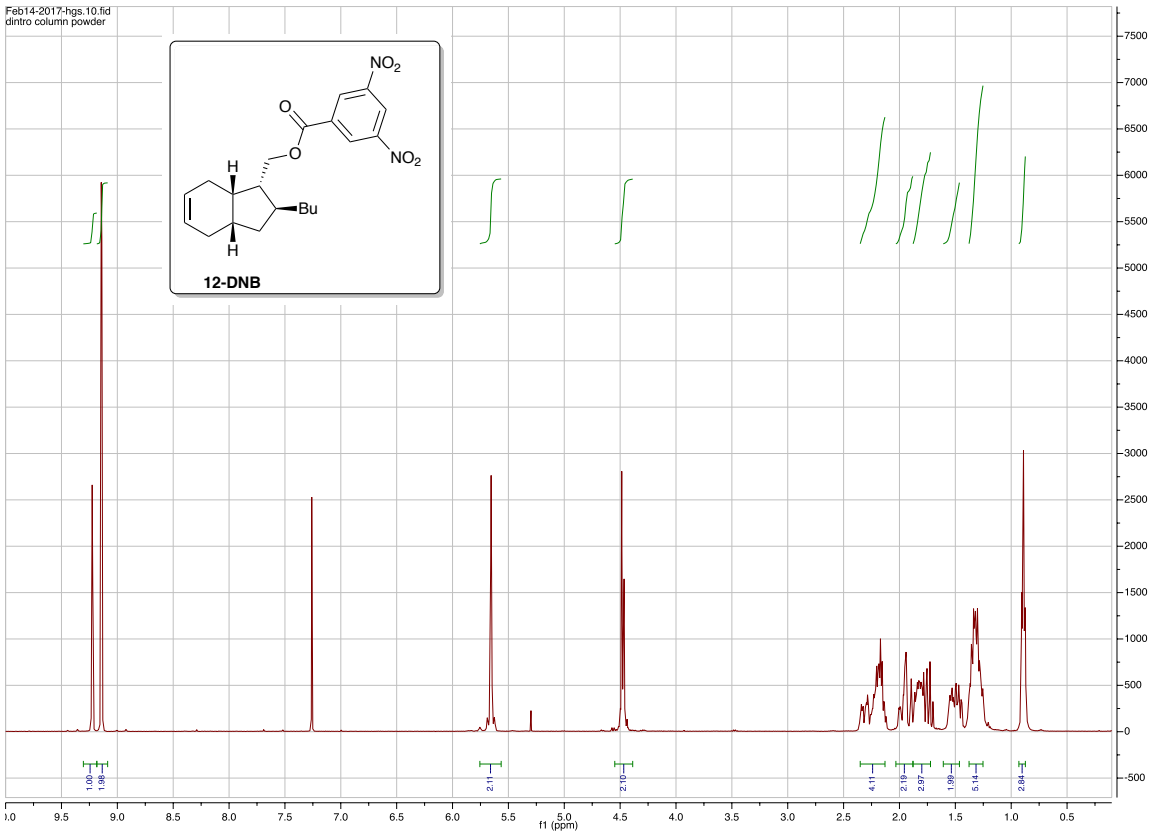


Figure S-65 ^{1}H-NMR spectrum of compound **12-DNB**.

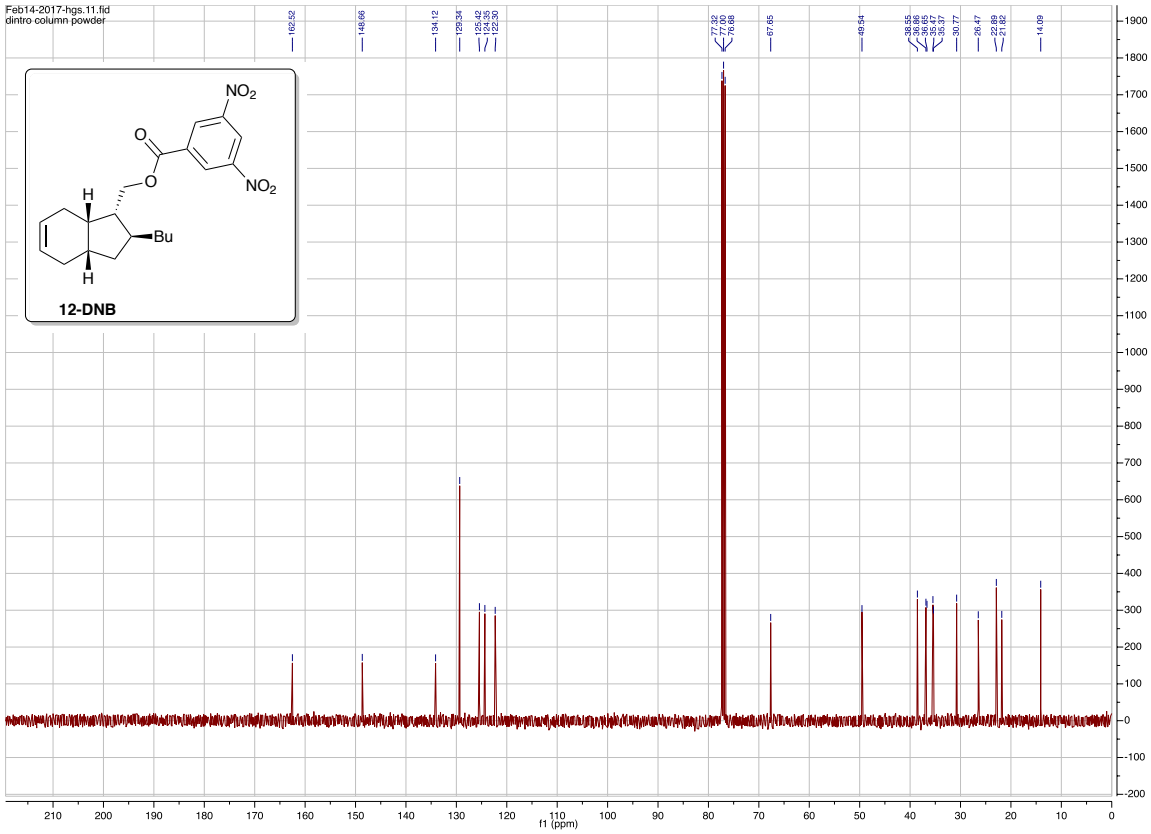


Figure S-66 ^{13}C-NMR spectrum of compound **12-DNB**.

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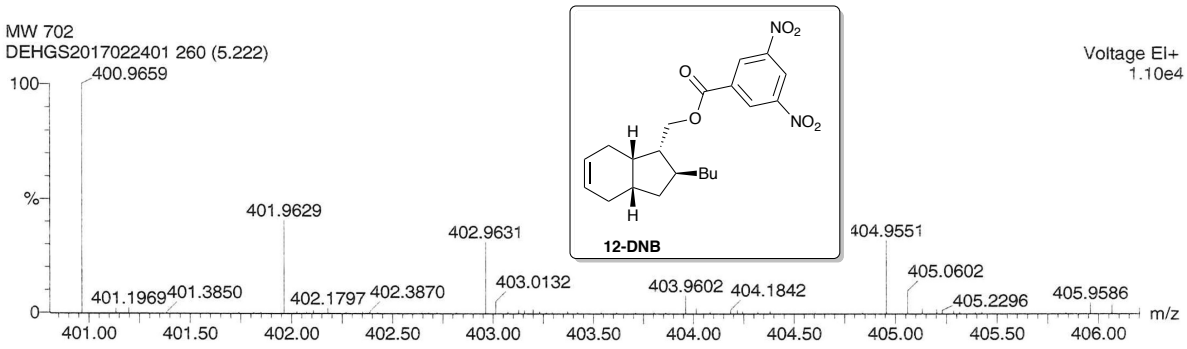
Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
107 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
402.1797	402.1791	0.6	1.5	10.0	2	C21 H26 N2 O6
	402.1831	-3.4	-8.5	14.0	1	C26 H26 O4

Figure S-67 HRMS spectrum of compound **12-DNB**.

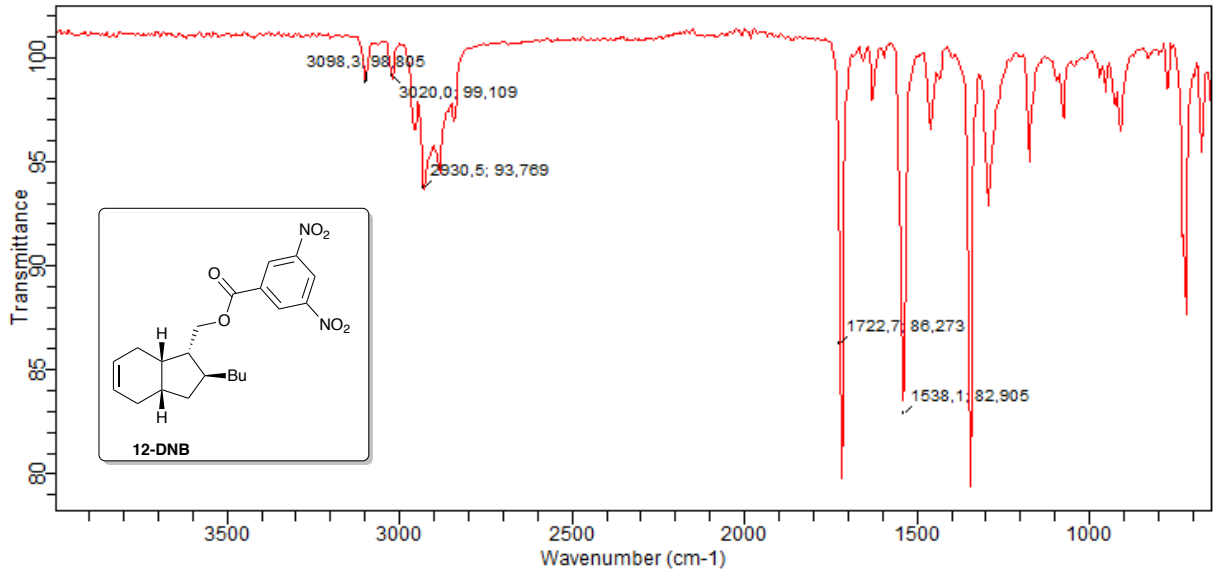


Figure S-68 IR spectrum of compound **12-DNB**.

X-ray crystallography on compound (12-DNB):

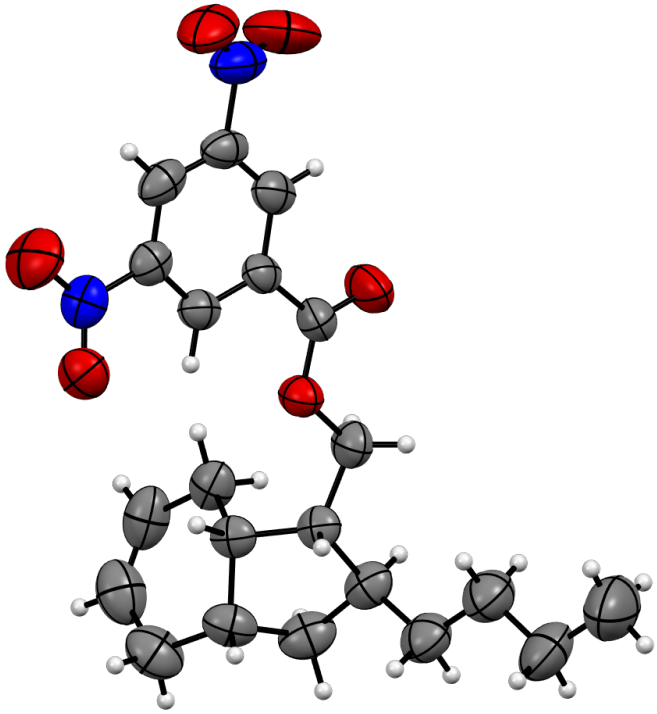


Figure S-69 ORTEP plot of compound **12-DNB** (top perspective).

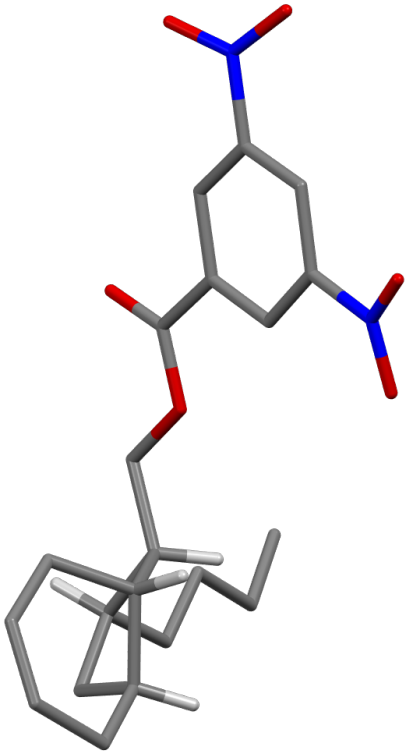


Figure S-70 Capped-sticks presentation of compound **12-DNB** (top perspective).

Pursuing the true structure of marine natural product mucosin. Part 2.

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Abstract

Recently we reported on the first total synthesis of mucosin according to its claimed structure. Having demonstrated the erroneous assignment, we now pursue the true identity of the marine natural product. A stereodivergent strategy has been devised, which takes advantage of a topological bias displayed by the featured bicyclo[4.3.0]non-3-ene scaffold.

1. Comment

Systematic name is (1*S*,6S,7*R*,8S)-8-Butyl-7-((3,5- dinitrobenzoyl)oxymethyl)bicyclo[4.3.0]non-3-ene, formula $C_{21}H_{26}N_2O_6$.

2. Synthesis and crystallization

How you prepared the crystal (include text).

3. Refinement

Isotropic, constrained refinement of all H atoms; The H atoms were included in calculated positions and treated as riding: CH = 0.93–0.98 Å with U_{iso}(H)= 1.2U_{eq} of the carrier atom, or 1.5U_{eq} for the methyl group.

Table 1

Experimental details

Crystal data	
Chemical formula	$C_{21}H_{26}N_2O_6$
M_r	402.44
Crystal system, space group	Orthorhombic, $P2_12_12_1$
Temperature (K)	297
a, b, c (Å)	5.0886 (5), 11.5288 (11), 35.602 (3)
V (Å^3)	2088.6 (3)
Z	4
Radiation type	Mo $K\alpha$
μ (mm^{-1})	0.09
Crystal size (mm)	0.81 × 0.23 × 0.03
Data collection	
Diffractometer	Bruker D8 Venture with a Photon 100 CMOS detector
Absorption correction	Multi-scan *SADABS* (Bruker, 2016)
T_{min}, T_{max}	0.906, 1.000

No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	9297, 3950, 2611
R_{int}	0.030
$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.610
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.053, 0.124, 1.05
No. of reflections	3950
No. of parameters	263
H-atom treatment	H-atom parameters constrained
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.14, −0.16
Absolute structure	Flack x determined using 803 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).
Absolute structure parameter	−0.9 (6)

Computer programs: *APEX3* (Bruker, 2016), *SHELXT* (Sheldrick, 2015a), *Mercury* (Macrae *et al.*, 2008), *SHELXL2014* (Sheldrick, 2015b).

Acknowledgements

Funding information

References

Bruker (2016). *APEX3*, *SAINT*+ and *SADABS*. Bruker AXS, Inc., Madison, Wisconsin, USA.

Macrae, C. F., Bruno, I. J., Chisholm, J. A., Edgington, P. R., McCabe, P., Pidcock, E., Rodriguez-Monge, L., Taylor, R., van de Streek, J. & Wood, P. A. (2008). *J. Appl. Cryst.* **41**, 466–470.

Sheldrick, G. M. (2015*a*). *Acta Cryst.* A**71**, 3–8.

Sheldrick, G. M. (2015*b*). *Acta Cryst.* C**71**, 3–8.

Figure 1

The molecular structure of (I) with atomic numbering indicated.

supporting information

Pursuing the true structure of marine natural product mucosin. Part 2.

Computing details

Data collection: *APEX3* (Bruker, 2016); cell refinement: *APEX3* (Bruker, 2016); data reduction: *APEX3* (Bruker, 2016); program(s) used to solve structure: *APEX3* (Bruker, 2016); program(s) used to refine structure: *SHELXT* (Sheldrick, 2015a); molecular graphics: *Mercury* (Macrae *et al.*, 2008); software used to prepare material for publication: *SHELXL2014* (Sheldrick, 2015b).

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

Crystal data

$C_{21}H_{26}N_2O_6$
$M_r = 402.44$
Orthorhombic, $P2_12_12_1$
$a = 5.0886$ (5) Å
$b = 11.5288$ (11) Å
$c = 35.602$ (3) Å
$V = 2088.6$ (3) Å^3
$Z = 4$
$F(000) = 856$
$D_x = 1.280$ Mg m^{-3}
Mo $K\alpha$ radiation, $\lambda = 0.71073$ Å
Cell parameters from 3545 reflections
$\theta = 2.5$–$23.1°$
$\mu = 0.09$ mm^{-1}
$T = 297$ K
Flat needle, colorless
0.81 × 0.23 × 0.03 mm

Data collection

Bruker D8 Venture with a Photon 100 CMOS detector diffractometer
Radiation source: fine-focus sealed tube
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, 2016)
$T_{min} = 0.906$, $T_{max} = 1.000$
9297 measured reflections
3950 independent reflections
2611 reflections with $I > 2\sigma(I)$
$R_{int} = 0.030$
$\theta_{max} = 25.7°$, $\theta_{min} = 2.5°$
$h = -4 \rightarrow 6$
$k = -14 \rightarrow 14$
$l = -43 \rightarrow 33$

Refinement

Refinement on F^2
Least-squares matrix: full
$R[F^2 > 2\sigma(F^2)] = 0.053$
$wR(F^2) = 0.124$
$S = 1.05$
3950 reflections
263 parameters
0 restraints
Hydrogen site location: inferred from neighbouring sites
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.0508P)^2 + 0.326P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max} < 0.001$
$\Delta\rho_{max} = 0.14$ e Å^{-3}
$\Delta\rho_{min} = -0.16$ e Å^{-3}
Absolute structure: Flack x determined using 803 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).
Absolute structure parameter: −0.9 (6)

Special details

Geometry. All e.s.d.'s (except the e.s.d. in the dihedral angle between two l.s. planes) are estimated using the full covariance matrix. The cell e.s.d.'s are taken into account individually in the estimation of e.s.d.'s in distances, angles and torsion angles; correlations between e.s.d.'s in cell parameters are only used when they are defined by crystal symmetry. An approximate (isotropic) treatment of cell e.s.d.'s is used for estimating e.s.d.'s involving l.s. planes.

Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (Å²)

	x	*y*	*z*	U_{iso}*/U_{eq}
C1	0.1952 (8)	0.3211 (3)	0.32542 (10)	0.0657 (10)
H1	0.3773	0.3354	0.3177	0.079*
C2	0.1407 (10)	0.1930 (4)	0.32050 (13)	0.0889 (14)
H21	0.0939	0.1785	0.2945	0.107*
H22	0.2999	0.1499	0.3259	0.107*
C3	−0.0722 (10)	0.1499 (4)	0.34495 (15)	0.0912 (14)
H3	−0.1344	0.0751	0.3409	0.109*
C4	−0.1797 (9)	0.2110 (4)	0.37227 (14)	0.0814 (12)
H4	−0.3115	0.1762	0.3864	0.098*
C5	−0.1037 (7)	0.3314 (3)	0.38181 (10)	0.0645 (10)
H51	−0.0969	0.3392	0.4089	0.077*
H52	−0.2379	0.3839	0.3726	0.077*
C6	0.1622 (6)	0.3673 (3)	0.36535 (9)	0.0520 (9)
H6	0.3051	0.3388	0.3813	0.062*
C9	0.0196 (8)	0.4038 (4)	0.30336 (10)	0.0751 (12)
H91	0.0678	0.4039	0.2770	0.090*
H92	−0.1632	0.3810	0.3056	0.090*
C7	0.1905 (7)	0.4977 (3)	0.35926 (8)	0.0533 (9)
H7	0.3786	0.5146	0.3570	0.064*
C8	0.0627 (8)	0.5224 (3)	0.32047 (9)	0.0625 (10)
H8	−0.1077	0.5602	0.3243	0.075*
C10	0.0829 (8)	0.5738 (3)	0.38977 (9)	0.0633 (10)
H101	0.1131	0.6548	0.3838	0.076*
H102	−0.1048	0.5616	0.3924	0.076*
C11	0.2342 (10)	0.5985 (4)	0.29485 (10)	0.0817 (13)
H111	0.3996	0.5586	0.2907	0.098*
H112	0.1473	0.6051	0.2707	0.098*
C12	0.2924 (12)	0.7161 (4)	0.30846 (12)	0.0915 (14)
H121	0.3886	0.7103	0.3319	0.110*
H122	0.1280	0.7556	0.3136	0.110*
C13	0.4519 (12)	0.7890 (4)	0.28093 (12)	0.1029 (16)
H131	0.6242	0.7543	0.2778	0.124*
H132	0.3652	0.7887	0.2567	0.124*
C14	0.4825 (19)	0.9093 (5)	0.29375 (17)	0.153 (3)
H141	0.5852	0.9518	0.2758	0.229*
H142	0.5699	0.9101	0.3177	0.229*
H143	0.3126	0.9447	0.2962	0.229*
O1′	0.2180 (5)	0.54315 (19)	0.42458 (6)	0.0607 (7)
O2′	−0.0468 (7)	0.6630 (3)	0.45574 (8)	0.0954 (10)
O3′	0.0468 (10)	0.6832 (4)	0.59179 (10)	0.1279 (16)
O4′	0.4052 (10)	0.6242 (3)	0.61725 (9)	0.1141 (14)
O5′	0.8863 (5)	0.3287 (2)	0.49051 (9)	0.0784 (8)
O6′	0.8736 (7)	0.3167 (3)	0.55058 (9)	0.1096 (12)
N1′	0.2532 (12)	0.6308 (3)	0.59142 (11)	0.0878 (13)
N2′	0.8003 (7)	0.3561 (3)	0.52080 (11)	0.0683 (9)
C1′	0.2723 (7)	0.5574 (3)	0.48966 (9)	0.0509 (8)
C2′	0.2014 (8)	0.6073 (3)	0.52338 (10)	0.0599 (9)
H2′	0.0676	0.6622	0.5242	0.072*
C3′	0.3294 (9)	0.5755 (3)	0.55553 (10)	0.0622 (10)

C4′	0.5238 (9)	0.4942 (3)	0.55576 (10)	0.0650 (11)
H4′	0.6082	0.4733	0.5779	0.078*
C5′	0.5905 (7)	0.4444 (3)	0.52200 (10)	0.0551 (9)
C6′	0.4711 (7)	0.4746 (3)	0.48884 (9)	0.0518 (9)
H6′	0.5221	0.4404	0.4663	0.062*
C7′	0.1300 (8)	0.5941 (3)	0.45514 (10)	0.0601 (10)

Atomic displacement parameters (Å²)

	U^{11}	U^{22}	U^{33}	U^{12}	U^{13}	U^{23}
C1	0.051 (2)	0.078 (2)	0.068 (2)	−0.005 (2)	0.003 (2)	−0.016 (2)
C2	0.086 (3)	0.083 (3)	0.097 (3)	−0.009 (3)	−0.009 (3)	−0.028 (3)
C3	0.084 (3)	0.069 (3)	0.120 (4)	−0.008 (3)	−0.026 (3)	−0.005 (3)
C4	0.059 (3)	0.079 (3)	0.106 (3)	−0.010 (2)	−0.010 (3)	0.028 (3)
C5	0.047 (2)	0.073 (2)	0.074 (2)	0.003 (2)	0.0025 (19)	0.012 (2)
C6	0.0348 (18)	0.067 (2)	0.054 (2)	0.0045 (17)	−0.0045 (16)	−0.0017 (17)
C9	0.063 (3)	0.102 (3)	0.060 (2)	−0.014 (2)	−0.009 (2)	−0.002 (2)
C7	0.0433 (19)	0.063 (2)	0.054 (2)	0.0036 (18)	−0.0063 (17)	0.0010 (16)
C8	0.049 (2)	0.080 (3)	0.058 (2)	0.002 (2)	−0.0079 (19)	0.007 (2)
C10	0.067 (2)	0.062 (2)	0.061 (2)	0.014 (2)	−0.008 (2)	0.0048 (18)
C11	0.094 (3)	0.088 (3)	0.063 (2)	−0.001 (3)	−0.010 (2)	0.008 (2)
C12	0.113 (4)	0.088 (3)	0.074 (3)	−0.004 (3)	−0.003 (3)	0.006 (2)
C13	0.127 (4)	0.105 (4)	0.076 (3)	−0.014 (3)	−0.010 (3)	0.028 (3)
C14	0.249 (9)	0.093 (4)	0.117 (4)	−0.019 (5)	0.014 (5)	0.012 (3)
O1′	0.0680 (17)	0.0637 (14)	0.0505 (13)	0.0126 (14)	−0.0048 (13)	−0.0066 (11)
O2′	0.120 (3)	0.089 (2)	0.0774 (17)	0.054 (2)	−0.0023 (18)	−0.0060 (16)
O3′	0.126 (3)	0.166 (4)	0.092 (2)	0.003 (3)	0.039 (3)	−0.042 (2)
O4′	0.186 (4)	0.104 (2)	0.0523 (17)	−0.020 (3)	−0.008 (2)	−0.0130 (16)
O5′	0.0633 (18)	0.0866 (19)	0.085 (2)	0.0119 (16)	0.0027 (16)	0.0022 (16)
O6′	0.109 (3)	0.136 (3)	0.084 (2)	0.029 (3)	−0.029 (2)	0.020 (2)
N1′	0.124 (4)	0.079 (2)	0.060 (3)	−0.032 (3)	0.021 (3)	−0.013 (2)
N2′	0.058 (2)	0.072 (2)	0.075 (2)	−0.0055 (18)	−0.011 (2)	0.0087 (19)
C1′	0.062 (2)	0.0384 (16)	0.053 (2)	−0.0085 (17)	0.0074 (18)	−0.0072 (15)
C2′	0.069 (2)	0.0455 (18)	0.066 (2)	−0.0153 (18)	0.012 (2)	−0.0062 (17)
C3′	0.077 (3)	0.058 (2)	0.052 (2)	−0.021 (2)	0.015 (2)	−0.0077 (18)
C4′	0.076 (3)	0.068 (2)	0.052 (2)	−0.029 (2)	−0.004 (2)	0.006 (2)
C5′	0.052 (2)	0.055 (2)	0.058 (2)	−0.0128 (19)	0.0001 (19)	0.0012 (18)
C6′	0.056 (2)	0.0494 (19)	0.050 (2)	−0.0092 (18)	0.0047 (18)	−0.0042 (16)
C7′	0.072 (3)	0.0462 (19)	0.062 (2)	0.008 (2)	0.000 (2)	−0.0016 (18)

Geometric parameters (Å, º)

C1—C2	1.513 (6)	C11—H112	0.9700
C1—C9	1.524 (5)	C12—C13	1.525 (7)
C1—C6	1.527 (4)	C12—H121	0.9700
C1—H1	0.9800	C12—H122	0.9700
C2—C3	1.476 (7)	C13—C14	1.469 (7)
C2—H21	0.9700	C13—H131	0.9700
C2—H22	0.9700	C13—H132	0.9700
C3—C4	1.319 (6)	C14—H141	0.9600
C3—H3	0.9300	C14—H142	0.9600
C4—C5	1.480 (6)	C14—H143	0.9600

C4—H4	0.9300	O1′—C7′	1.315 (4)
C5—C6	1.532 (5)	O2′—C7′	1.200 (4)
C5—H51	0.9700	O3′—N1′	1.212 (6)
C5—H52	0.9700	O4′—N1′	1.204 (5)
C6—C7	1.526 (4)	O5′—N2′	1.206 (4)
C6—H6	0.9800	O6′—N2′	1.212 (4)
C9—C8	1.513 (5)	N1′—C3′	1.479 (5)
C9—H91	0.9700	N2′—C5′	1.476 (5)
C9—H92	0.9700	C1′—C2′	1.380 (4)
C7—C10	1.500 (5)	C1′—C6′	1.391 (5)
C7—C8	1.553 (4)	C1′—C7′	1.488 (5)
C7—H7	0.9800	C2′—C3′	1.367 (5)
C8—C11	1.538 (5)	C2′—H2′	0.9300
C8—H8	0.9800	C3′—C4′	1.363 (5)
C10—O1′	1.461 (4)	C4′—C5′	1.375 (5)
C10—H101	0.9700	C4′—H4′	0.9300
C10—H102	0.9700	C5′—C6′	1.372 (5)
C11—C12	1.470 (6)	C6′—H6′	0.9300
C11—H111	0.9700		

C2—C1—C9	116.3 (3)	H101—C10—H102	108.5
C2—C1—C6	115.3 (3)	C12—C11—C8	116.4 (4)
C9—C1—C6	101.4 (3)	C12—C11—H111	108.2
C2—C1—H1	107.8	C8—C11—H111	108.2
C9—C1—H1	107.8	C12—C11—H112	108.2
C6—C1—H1	107.8	C8—C11—H112	108.2
C3—C2—C1	113.3 (4)	H111—C11—H112	107.3
C3—C2—H21	108.9	C11—C12—C13	113.8 (4)
C1—C2—H21	108.9	C11—C12—H121	108.8
C3—C2—H22	108.9	C13—C12—H121	108.8
C1—C2—H22	108.9	C11—C12—H122	108.8
H21—C2—H22	107.7	C13—C12—H122	108.8
C4—C3—C2	124.0 (4)	H121—C12—H122	107.7
C4—C3—H3	118.0	C14—C13—C12	112.2 (5)
C2—C3—H3	118.0	C14—C13—H131	109.2
C3—C4—C5	124.1 (4)	C12—C13—H131	109.2
C3—C4—H4	117.9	C14—C13—H132	109.2
C5—C4—H4	117.9	C12—C13—H132	109.2
C4—C5—C6	113.3 (3)	H131—C13—H132	107.9
C4—C5—H51	108.9	C13—C14—H141	109.5
C6—C5—H51	108.9	C13—C14—H142	109.5
C4—C5—H52	108.9	H141—C14—H142	109.5
C6—C5—H52	108.9	C13—C14—H143	109.5
H51—C5—H52	107.7	H141—C14—H143	109.5
C7—C6—C1	101.6 (3)	H142—C14—H143	109.5
C7—C6—C5	113.8 (3)	C7′—O1′—C10	115.7 (3)
C1—C6—C5	111.0 (3)	O4′—N1′—O3′	125.4 (4)
C7—C6—H6	110.0	O4′—N1′—C3′	117.6 (5)
C1—C6—H6	110.0	O3′—N1′—C3′	116.8 (5)
C5—C6—H6	110.0	O5′—N2′—O6′	124.9 (4)
C8—C9—C1	105.8 (3)	O5′—N2′—C5′	118.0 (3)
C8—C9—H91	110.6	O6′—N2′—C5′	117.1 (4)

C1—C9—H91	110.6	C2′—C1′—C6′	119.6 (3)
C8—C9—H92	110.6	C2′—C1′—C7′	118.2 (3)
C1—C9—H92	110.6	C6′—C1′—C7′	122.2 (3)
H91—C9—H92	108.7	C3′—C2′—C1′	119.5 (4)
C10—C7—C6	116.0 (3)	C3′—C2′—H2′	120.3
C10—C7—C8	112.6 (3)	C1′—C2′—H2′	120.3
C6—C7—C8	105.5 (3)	C4′—C3′—C2′	122.4 (3)
C10—C7—H7	107.4	C4′—C3′—N1′	118.8 (4)
C6—C7—H7	107.4	C2′—C3′—N1′	118.9 (4)
C8—C7—H7	107.4	C3′—C4′—C5′	117.5 (4)
C9—C8—C11	111.1 (3)	C3′—C4′—H4′	121.3
C9—C8—C7	104.7 (3)	C5′—C4′—H4′	121.3
C11—C8—C7	113.2 (3)	C6′—C5′—C4′	122.5 (4)
C9—C8—H8	109.2	C6′—C5′—N2′	118.0 (3)
C11—C8—H8	109.2	C4′—C5′—N2′	119.5 (3)
C7—C8—H8	109.2	C5′—C6′—C1′	118.6 (3)
O1′—C10—C7	107.5 (3)	C5′—C6′—H6′	120.7
O1′—C10—H101	110.2	C1′—C6′—H6′	120.7
C7—C10—H101	110.2	O2′—C7′—O1′	124.5 (4)
O1′—C10—H102	110.2	O2′—C7′—C1′	122.6 (3)
C7—C10—H102	110.2	O1′—C7′—C1′	113.0 (3)

C9—C1—C2—C3	−83.7 (5)	C11—C12—C13—C14	174.1 (6)
C6—C1—C2—C3	34.9 (5)	C7—C10—O1′—C7′	−173.8 (3)
C1—C2—C3—C4	−9.4 (7)	C6′—C1′—C2′—C3′	0.6 (5)
C2—C3—C4—C5	0.8 (7)	C7′—C1′—C2′—C3′	−179.8 (3)
C3—C4—C5—C6	−17.1 (6)	C1′—C2′—C3′—C4′	−1.0 (5)
C2—C1—C6—C7	−172.0 (4)	C1′—C2′—C3′—N1′	179.3 (3)
C9—C1—C6—C7	−45.5 (3)	O4′—N1′—C3′—C4′	18.2 (5)
C2—C1—C6—C5	−50.6 (4)	O3′—N1′—C3′—C4′	−164.8 (4)
C9—C1—C6—C5	75.9 (4)	O4′—N1′—C3′—C2′	−162.1 (4)
C4—C5—C6—C7	154.2 (3)	O3′—N1′—C3′—C2′	14.8 (6)
C4—C5—C6—C1	40.3 (4)	C2′—C3′—C4′—C5′	0.4 (5)
C2—C1—C9—C8	165.4 (4)	N1′—C3′—C4′—C5′	−180.0 (3)
C6—C1—C9—C8	39.5 (4)	C3′—C4′—C5′—C6′	0.7 (5)
C1—C6—C7—C10	160.6 (3)	C3′—C4′—C5′—N2′	179.9 (3)
C5—C6—C7—C10	41.1 (4)	O5′—N2′—C5′—C6′	11.9 (5)
C1—C6—C7—C8	35.2 (3)	O6′—N2′—C5′—C6′	−168.3 (3)
C5—C6—C7—C8	−84.2 (3)	O5′—N2′—C5′—C4′	−167.3 (3)
C1—C9—C8—C11	105.0 (4)	O6′—N2′—C5′—C4′	12.5 (5)
C1—C9—C8—C7	−17.6 (4)	C4′—C5′—C6′—C1′	−1.1 (5)
C10—C7—C8—C9	−138.6 (3)	N2′—C5′—C6′—C1′	179.7 (3)
C6—C7—C8—C9	−11.1 (4)	C2′—C1′—C6′—C5′	0.4 (5)
C10—C7—C8—C11	100.3 (4)	C7′—C1′—C6′—C5′	−179.2 (3)
C6—C7—C8—C11	−132.2 (3)	C10—O1′—C7′—O2′	−0.9 (5)
C6—C7—C10—O1′	58.6 (4)	C10—O1′—C7′—C1′	178.8 (3)
C8—C7—C10—O1′	−179.7 (3)	C2′—C1′—C7′—O2′	−1.8 (5)
C9—C8—C11—C12	−179.6 (4)	C6′—C1′—C7′—O2′	177.8 (4)
C7—C8—C11—C12	−62.2 (5)	C2′—C1′—C7′—O1′	178.4 (3)
C8—C11—C12—C13	−177.2 (4)	C6′—C1′—C7′—O1′	−2.0 (5)

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PAPER III

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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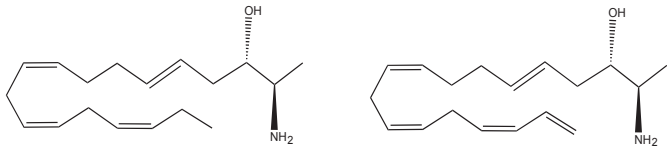
ABSTRACT

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.

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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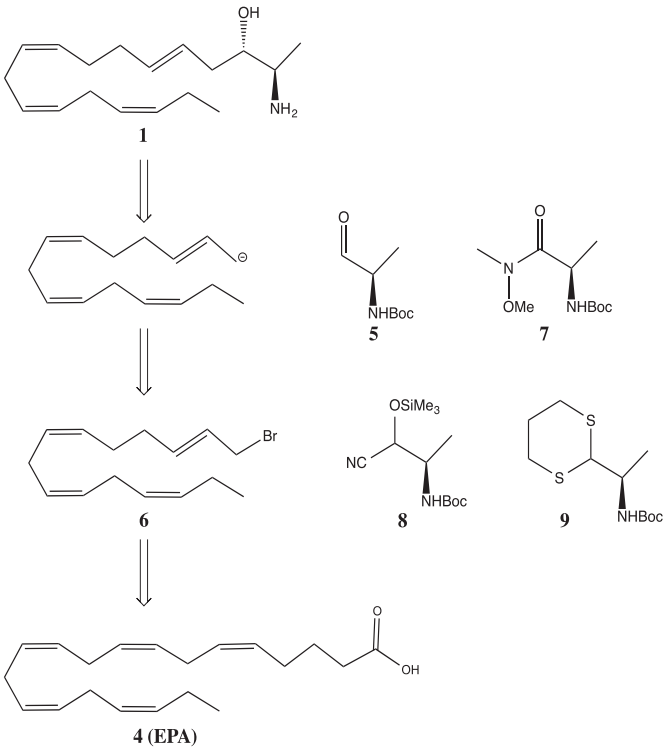
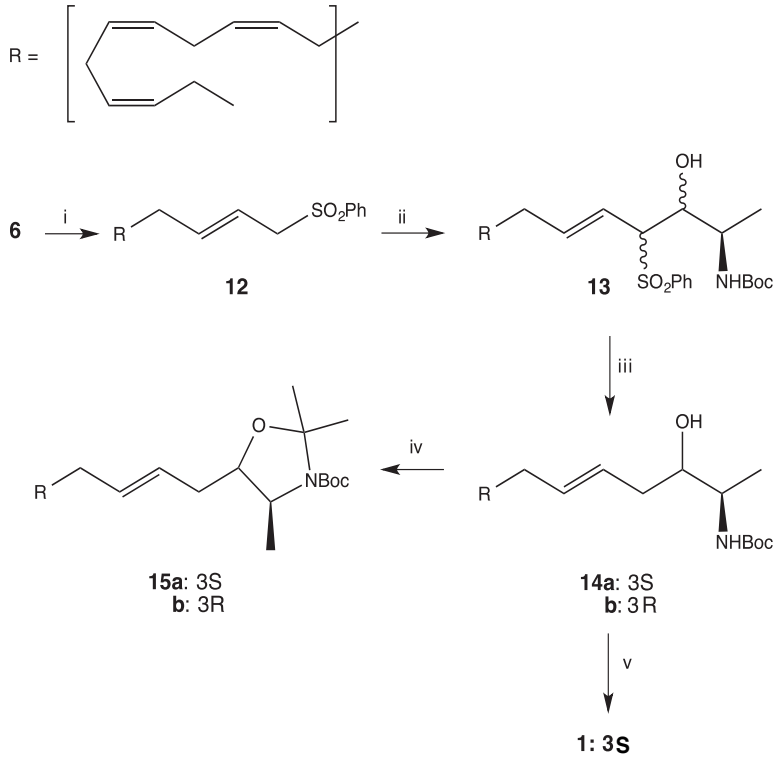


Scheme 1. Retrosynthetic analysis for the synthesis of **1**.

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.

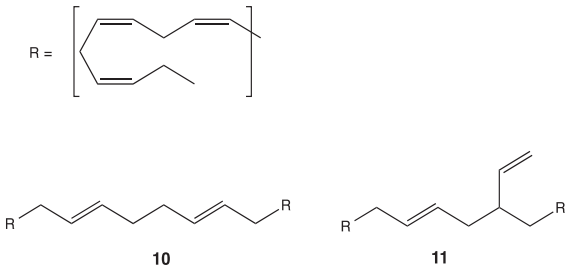


Fig. 2. Dimeric structures from Grignard reaction.

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.

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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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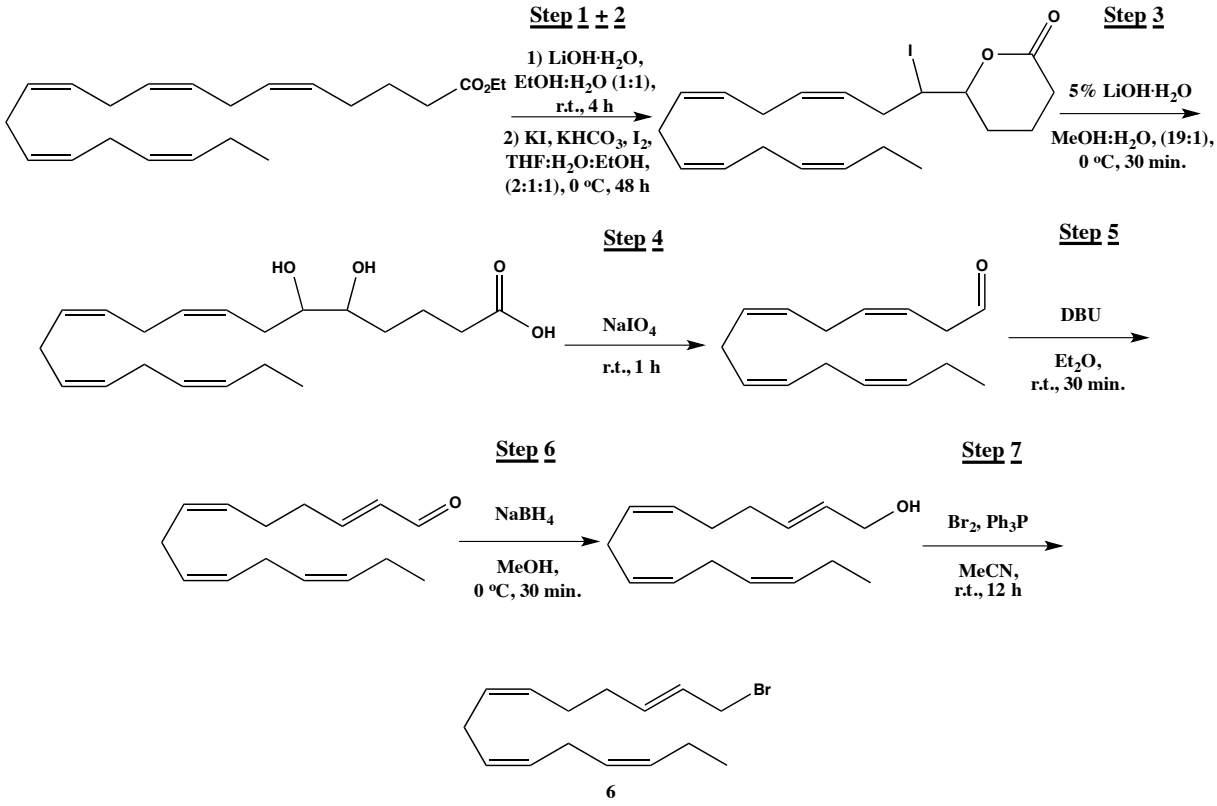
Table of Contents:

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**.

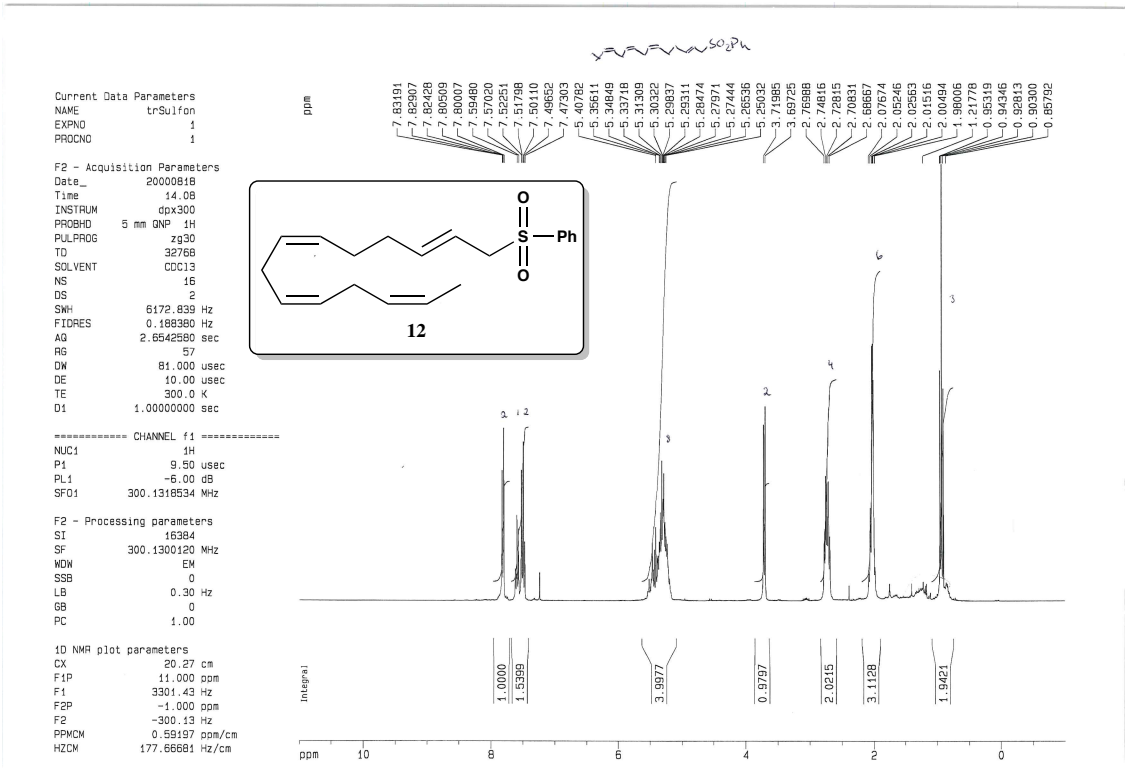


Figure S-1 ^{1}H-NMR spectrum of compound **12**.

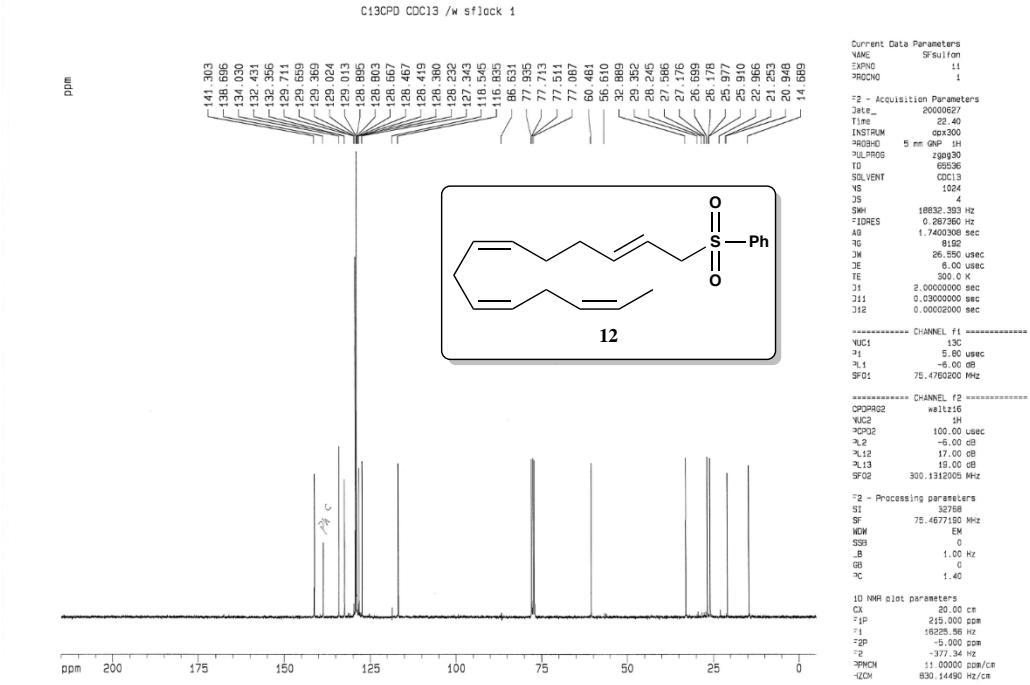


Figure S-2 ^{13}C-NMR spectrum of compound **12.**

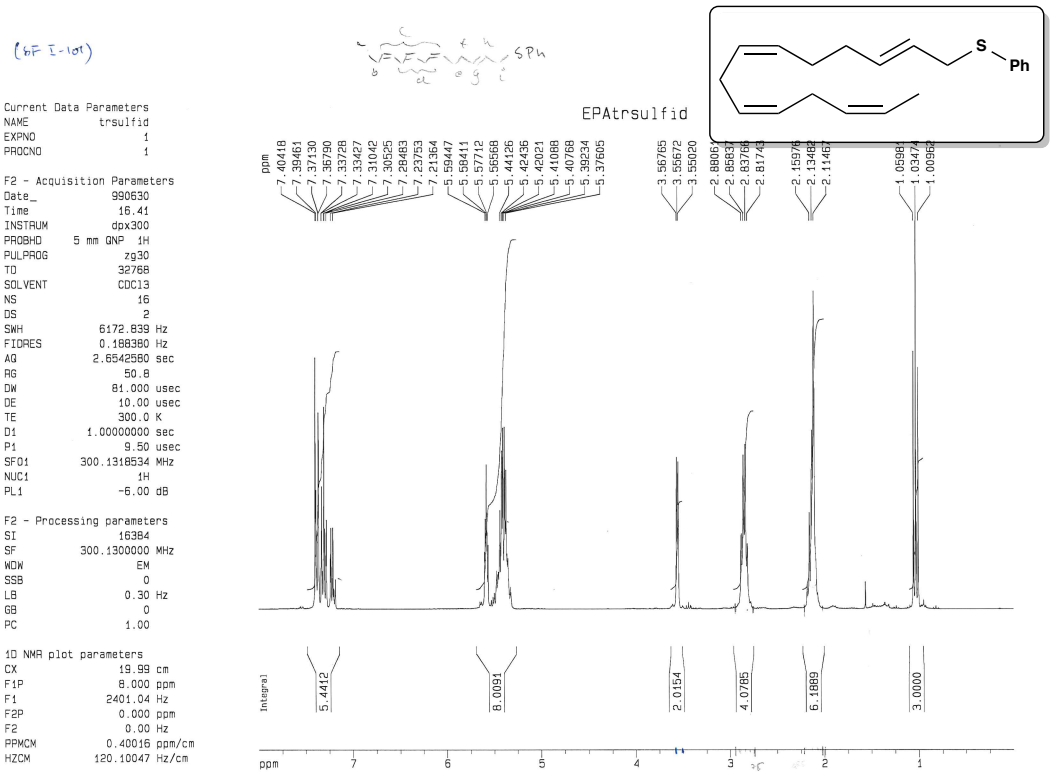


Figure S-3 ^{1}H-NMR spectrum of intermediate sulfide.

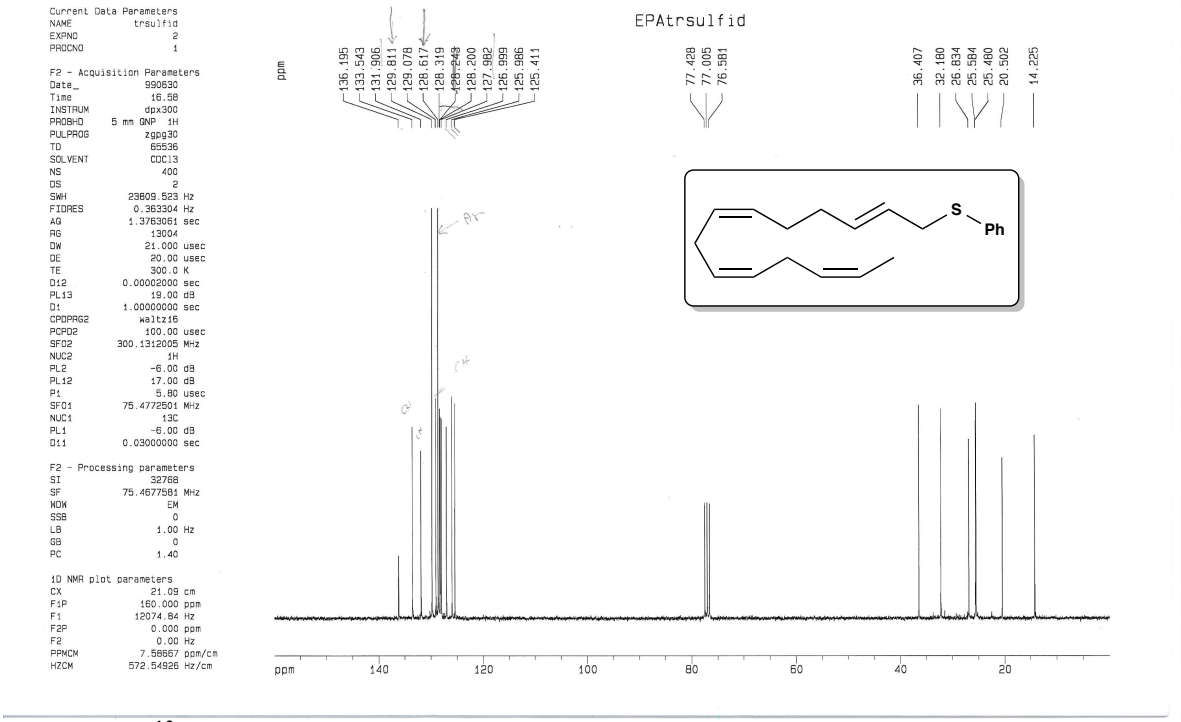


Figure S-4 ^{13}C-NMR spectrum of intermediate sulfide.

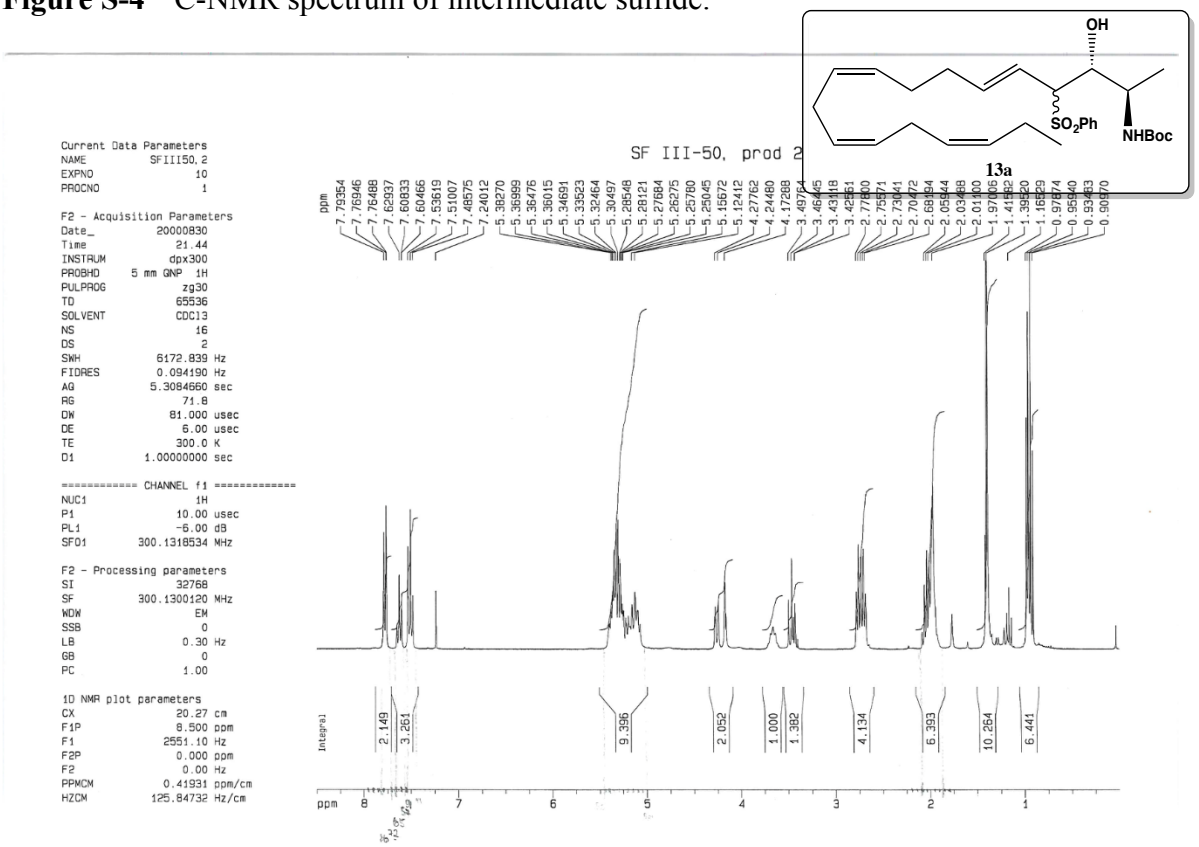


Figure S-5 ^{1}H-NMR spectrum of compound **13a**.

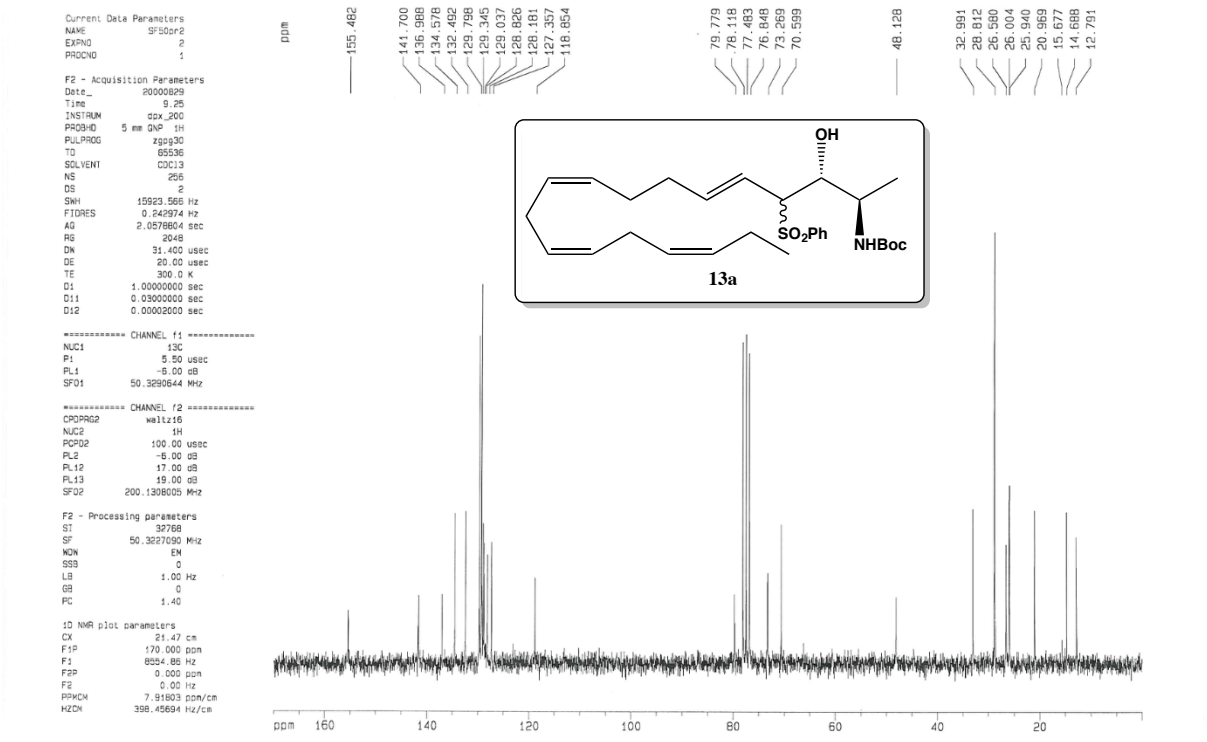


Figure S-6 ^{13}C-NMR spectrum of compound **13a**.

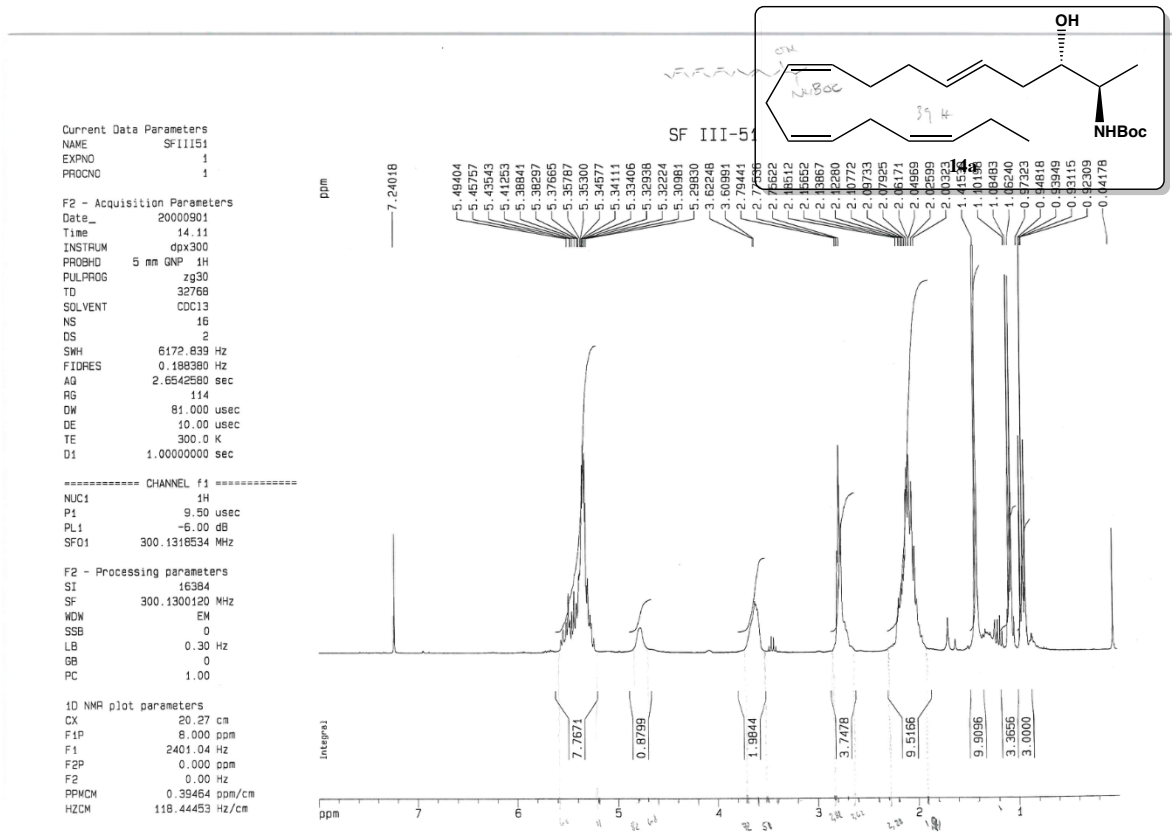


Figure S-7 ^{1}H-NMR spectrum of compound **14a**.

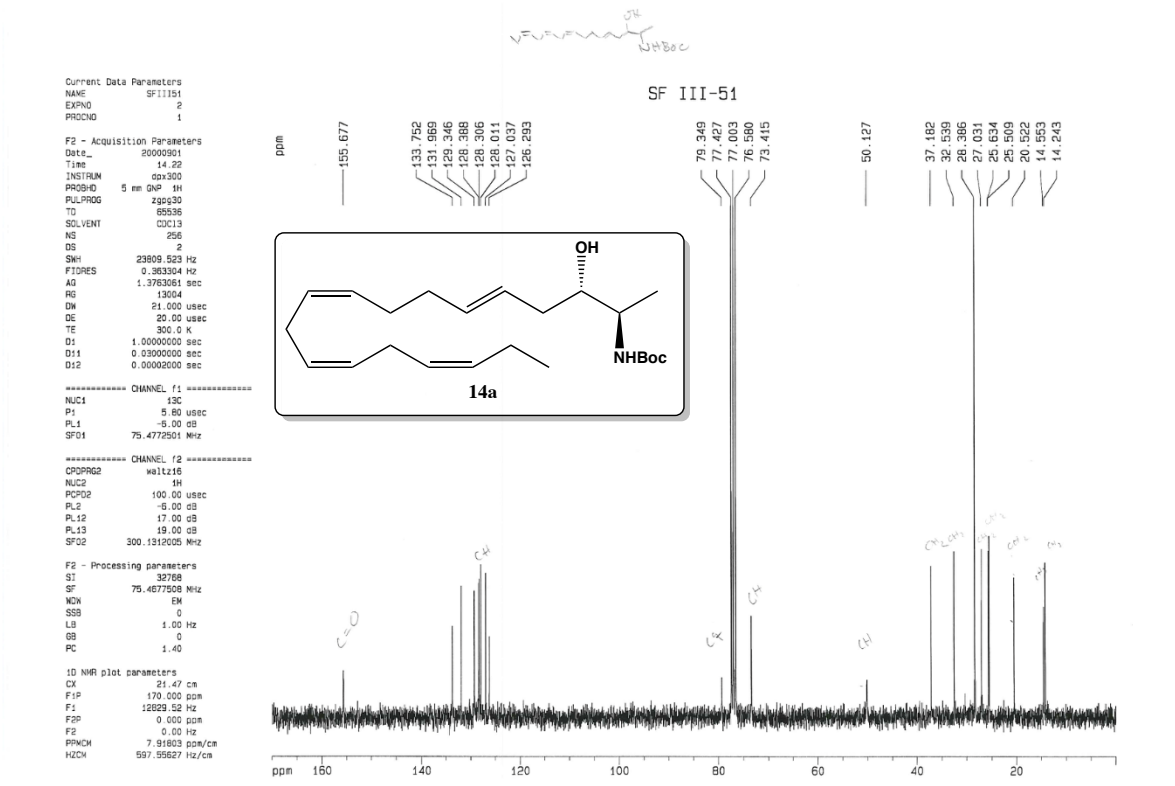


Figure S-8 ^{13}C-NMR spectrum of compound **14a**.

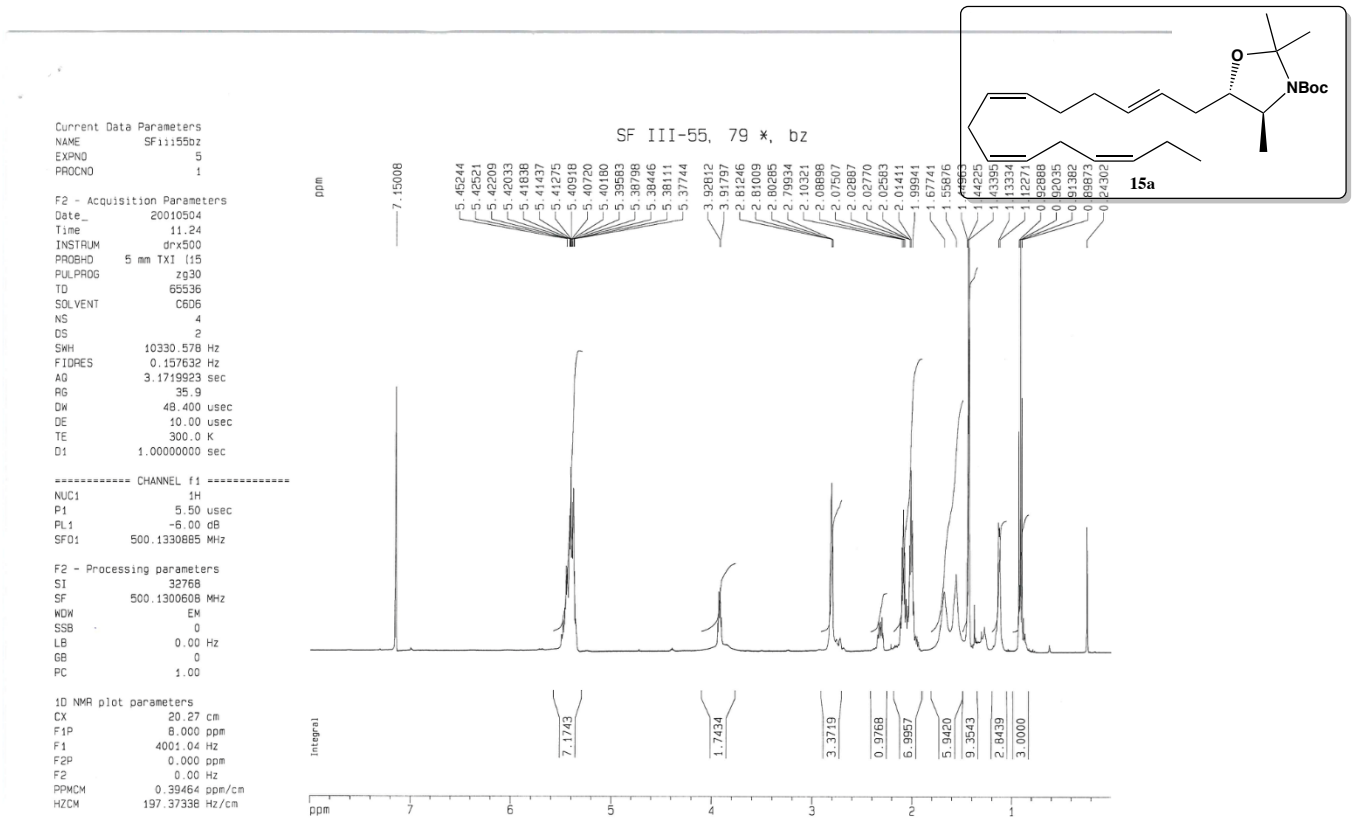


Figure S-9 ^{1}H-NMR spectrum of compound **15a**.

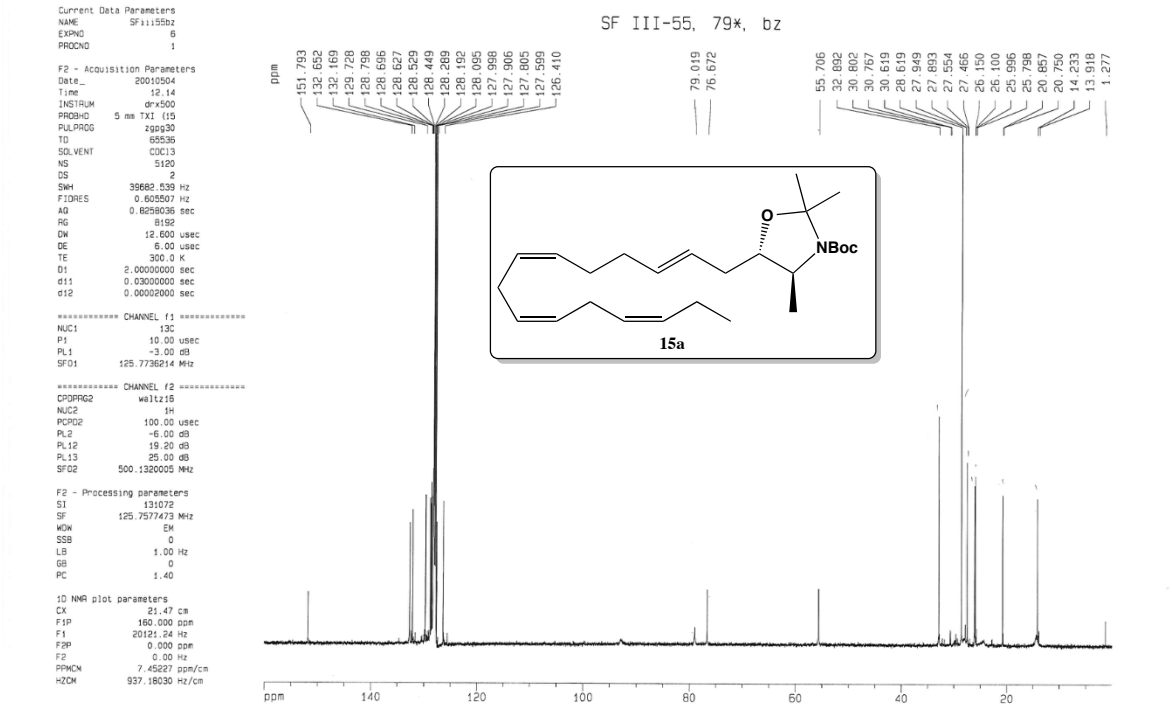


Figure S-10 ^{13}C-NMR spectrum of compound **15a**.

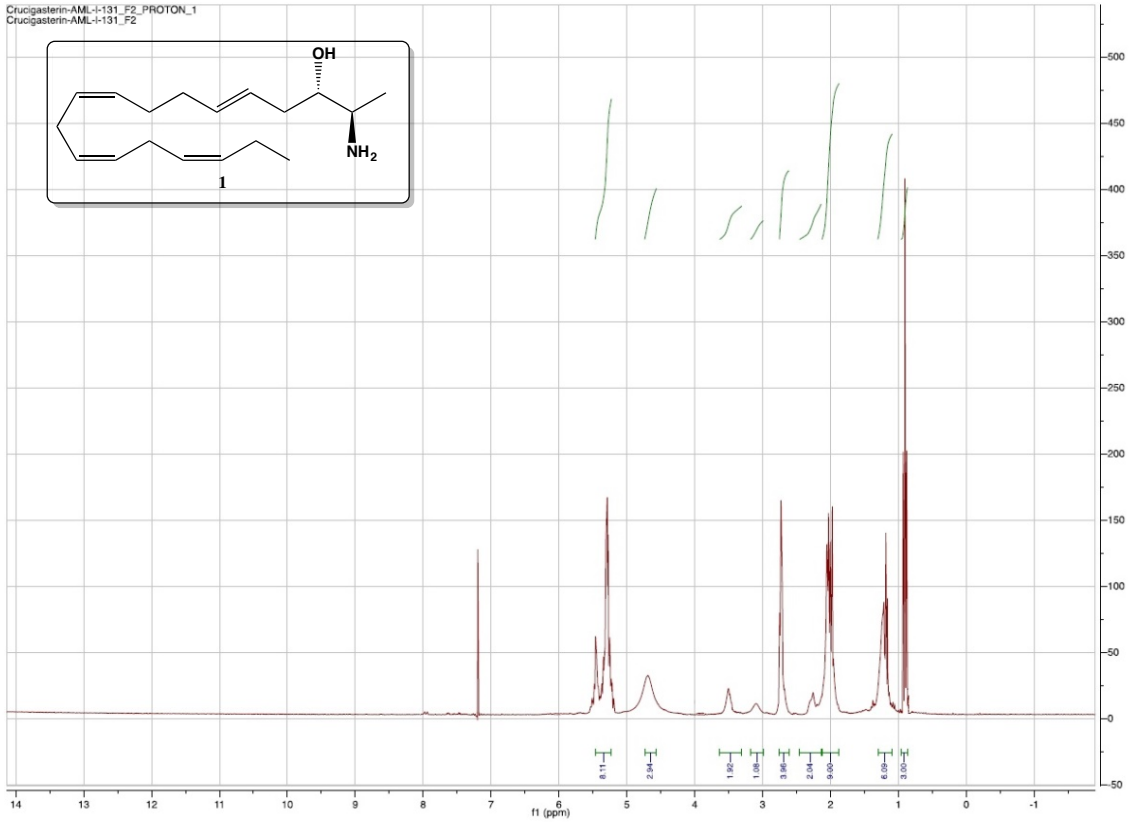


Figure S-11 ^{1}H-NMR spectrum of compound **1**.

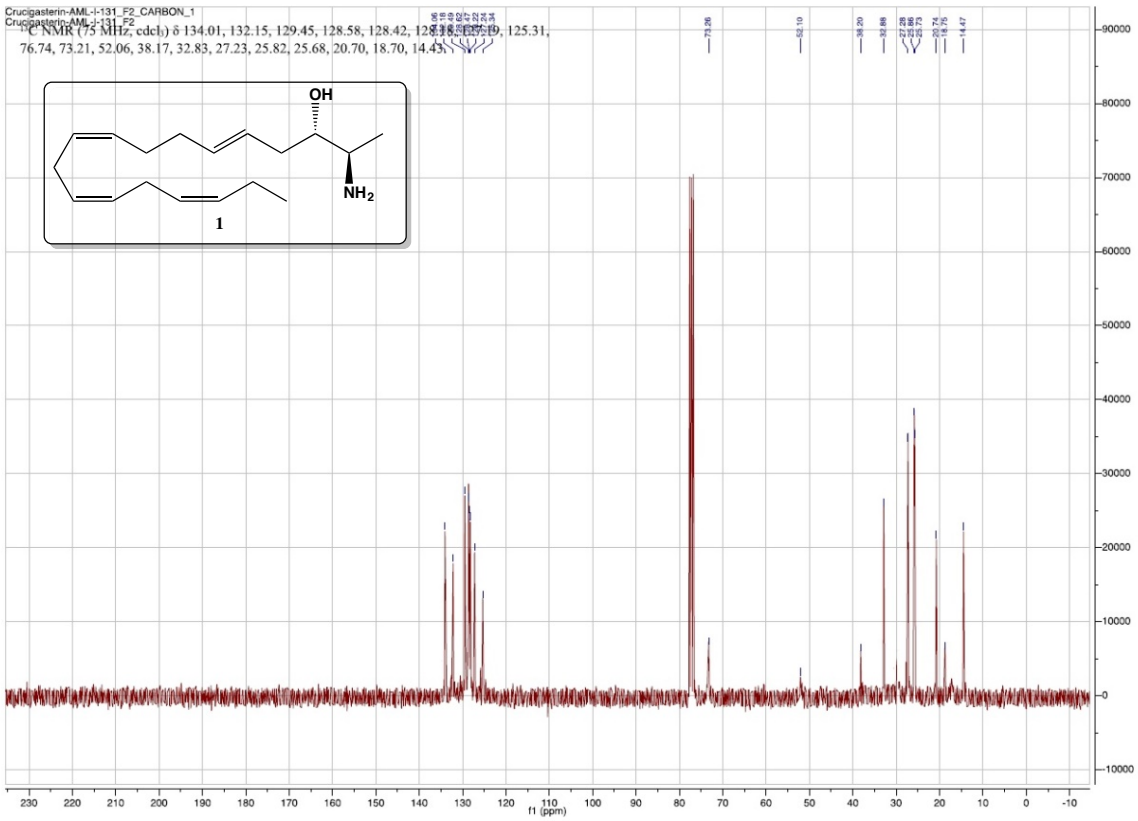


Figure S-12 ^{13}C-NMR spectrum of compound **1**.

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PAPER IV

Synthesis of Obscuraminol A using an organocatalyzed enantioselective Henry reaction.

Liudmila Filippova, Simen Gjelseth Antonsen, Yngve; Hansen Stenstrøm and Trond Vidar Hansen. *Tetrahedron*, **2016**, 72, 6572-6577.

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Synthesis of obscuraminol A using an organocatalyzed enantioselective Henry reaction

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ABSTRACT

The first synthesis of the polyunsaturated amino alcohol natural product obscuraminol A is reported. This stereoselective synthesis was based on an *anti*- and enantioselective organocatalyzed Henry reaction followed by a chemoselective SmI_2-mediated reduction that affected only the nitro-group of the Henry product. These efforts yielded obscuraminol A where the configuration of the all-*Z* skipped double bonds was conserved from the starting material, *i.e.* the ethyl ester of (all-*Z*)-eicosa-5,8,11,14,17-pentaenoic acid. Our synthesis confirmed the reported structure of obscuraminol A.

1. Introduction

Sphingosines are common biomembrane constituents as the backbone of sphingolipids. Sphingolipids are involved in many biological functions.[1] The sphingolipid class of natural products derived from long-chain vicinal amino alcohols with the 1-deoxygenated sphingosines base are widely distributed among marine invertebrates, especially in marine ascidian.[1] The wide spectrum of bioactivities reported for these substances, such as cytotoxicity, antimicrobial and antifungal effects, makes them interesting targets in marine bioprospecting efforts towards the development of novel pharmaceuticals.[2–7] For example, (2*S*,3*R*)-2-amino-3-octadecanol (**1**), isolated from the mollusk *Spisula polinima*, also known as spisulosine (Fig. 1),[8] showed activity against several tumor cell lines. The structural simple saturated natural product **1** has been subjected to clinical trial development programs against solid tumors.[9] However, the related compounds with a polyunsaturated *Z*-skipped side chain have been less explored. Examples of this class of the sphingoids are obscuraminol A (**2**) and crucigasterin 277 (**3**) (Fig. 1).

The vicinal *anti*-amino alcohol **2** was assigned the same (2*S*,3*R*)-configuration as spisulosine (**1**) by application of Mosher's method.[10] However, this has not yet been established by matching data obtained from total synthesis efforts, which is very often necessary for determination of absolute configurations of vicinal amino alcohols. Obscuraminol A (**2**) was isolated by Garrido et al. in 2001 from the chloroform extracts of the marine ascidian *Pseudodistoma obscurum*.[4] Shortly after, Clark et al. reported the isolation of the *N*-acetyl derivative of **2** from the sponge *Haliclona* sp. 1031, and named it halaminol D.[3] Structurally, compound **2** is related to the cytotoxic and antimicrobial polyunsaturated amino alcohol crucigasterin 277 (**3**), the latter has been isolated from the Mediterranean tunicate *Pseudodistoma crucigaster*.[5] Recently we reported a total synthesis of **3** that proved its structure and afforded sufficient amount of material for biological studies.[11] This synthesis, as well as those of several other polyunsaturated natural products with a *Z*-skipped moiety, utilized the commercially available polyunsaturated fatty acid (all-*Z*)-eicosa-5,8,11,14,17-pentaenoic acid (EPA, **4**), Scheme 1, as a convenient starting material.[12,13] For most of these synthetic studies, four of the five *Z*-skipped double bonds in **4** were conserved in the final targets. Therefore, the formation of multiple skipped Z-double bonds from either repeated, steroselective reduction reaction of internal alkynes,[13,14] or using multiple *Z*-selective Wittig reactions,[13,15] is omitted. These advantages were also taken into consideration in our retrosynthetic analysis of obscuraminol A (**2**). To date, there are no reports on the synthesis of this highly unsaturated *anti*-amino alcohol. Herein, we report our efforts towards a stereoselective synthesis of obscuraminol A (**2**), employing **4a** as a convenient starting material.

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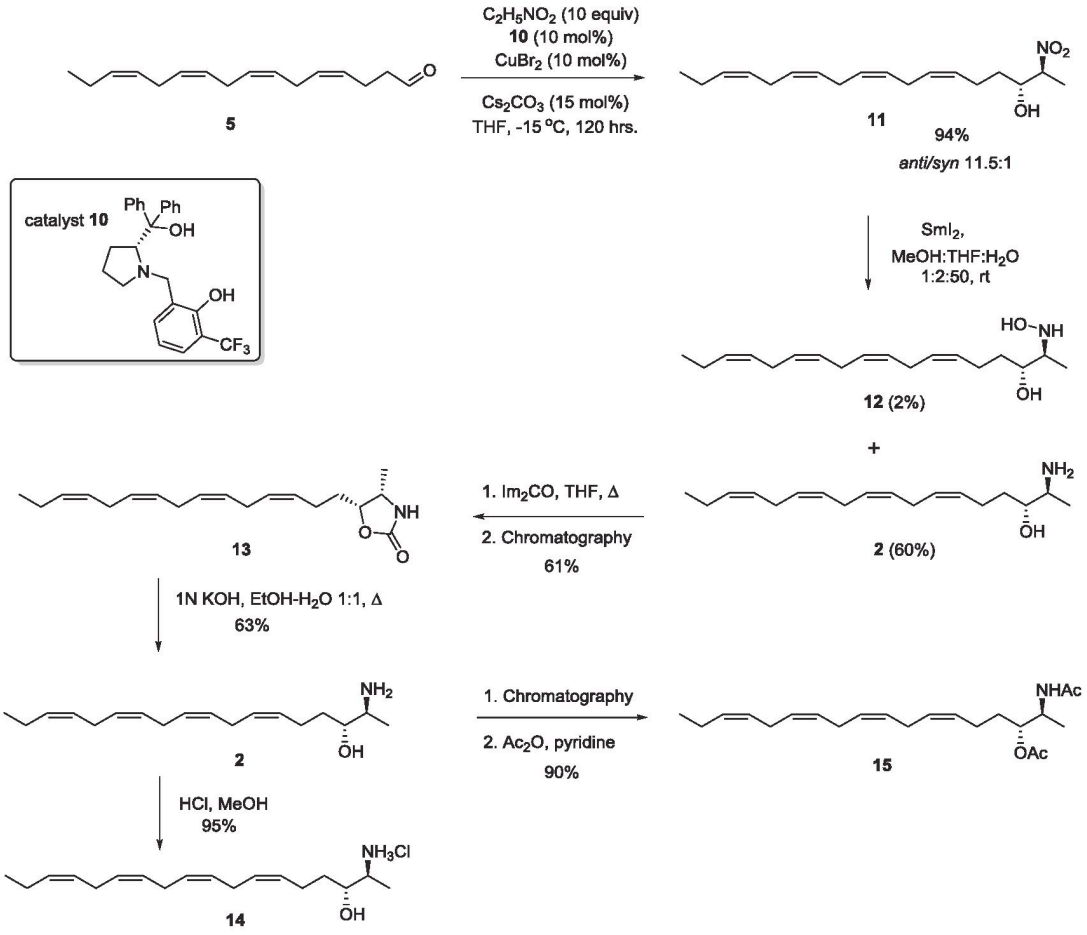
of the Henry reaction have appeared using metal-based catalysts,[17] enzymatic protocols[18] or organocatalysts.[19] Organocatalysts for both *syn*- and *anti*-diastereoselective variations of the Henry reaction have been developed as well.[20] However, most of the *anti*-diastereoselective catalysts reported give high selectivity only towards aromatic aldehydes.[20] Recently, we reported an asymmetric Henry reaction using a pinane-derived ligand copper (II) complex that worked reasonably well for aliphatic aldehydes.[21] Unfortunately, when this system was applied for the reaction between **5** and nitroethane, the expected nitroaldol product was obtained in very low yields and with poor selectivity towards the undesired *syn*-diastereomer. So we turned our attention to literature protocols for the generation of aliphatic *anti*-nitro alcohols. The phenol-proline derived ligand **10** reported by Wang and co-workers[20e] seemed the most promising one, and was prepared as previously described (See Supplementary data).[20e] Applying **10** to the nitroaldol reaction using the original conditions (THF, −15 °C) resulted in a sluggish reaction. However, after a prolonged reaction time, the (2*S*,3*R*)-nitroaldol adduct **11** was isolated in 94% yield and in good diastereoselectivity (*anti*/*syn* 11.5:1) as determined by ^{1}H NMR analysis (Scheme 3). The enantiomeric excess was determined to be *ee*=83% by HPLC analyses (See Supplementary data). The absolute configuration of the isolated major product of the nitroalcohol **11** was not determined at this stage. However, based on NMR data and by comparison with analogs reported by Wang and co-workers, the relative configuration was tentatively assigned as *anti*.[20e] Raising the reaction temperature to 8 °C significantly reduced the reaction time, but resulted in a diminished diastereoselectivity (*anti*/*syn* 5.7:1). Lowering the temperature to −20 °C did not improve the selectivity.

The most common way for the reduction of vicinal nitroaldols into their corresponding amino alcohols with retention of configuration is by hydrogenation using Pd/C.[20b,c,e] Obviously, this method is not applicable towards substrates with a high degree of unsaturation, such as **11**. Transfer hydrogenation protocols have also been reported,[22] but in our hands only complex reaction mixtures were returned. The conventional reduction of the nitro-group using zinc powder in acidic media (HCl/AcOH)[20a,23] afforded the reduced product, but in addition to a low yield of the reaction, these attempts were accompanied by partial elimination of the hydroxyl group. Attempts to protect the hydroxyl functionality as its TBDPS-ether[24] gave a complex mixture of products. Reduction protocols employing nickel boride, which has previously been utilized in nitroaldol reduction reactions,[25] affected the double bonds. When using the $NaBH_4$–$ZnCl_2$ reductive system, the retro-nitroaldol reaction was observed to take place.[26] Finally, we tried a previously reported SmI_2-mediated reduction protocol[27] on the nitroaldol product **11**. Gratifyingly, this resulted in the isolation of the desired amino-alcohol **2** in 60% yield. In addition, the hydroxylamine **12** was detected as a by-product (Scheme 3). The structure of **12** was assigned based on NMR- and MS-analyses of mixed fractions with unreacted **11**, **2** and **12**. Full conversion to the target molecule **2** was not achieved even when increasing the molar equivalents of SmI_2 to 10 equiv or using prolonged reaction times.



Scheme 3. Synthesis of obscuraminol A (**2**) and derivatives.

After purification by column chromatography, this reduction protocol afforded the target amino alcohol **2** with a lower diastereoselectivity than expected from the starting material. The diastereomeric ratio was determined to be 5.2:1 in favor of the desired *anti*-isomer (See Supplementary data). The origin of the lowered diastereomeric ratio is currently under investigation. Unfortunately, the resulting mixture of the two diastereomers of the target the amino-alcohol **2** was inseparable at this stage. In order to enhance the separation of these isomers, as well as to remove compound **12**, the diastereomeric mixture of **2** and with minor amounts of **12** present, was treated with 1,1′-carbonyldiimidazole to afford two cyclic oxazolidinones where the major product was the *cis*-isomer **13** that was isolated after chromatography in 61% yield. The configuration of **13** was confirmed by NOESY and ROESY experiments (See Supplementary data), which showed a clear correlation for the protons of C3 and C4. Similar observations have been reported by Garrido et al.[4] Moreover, these experiments provided evidence for the initial assignment of the relative configuration of **11** also as *anti*.

The isolated oxazolidinone **13** was hydrolyzed[28] under alkaline conditions that provided access to the target amino alcohol **2** (dr>20:1 determined by ^{1}H NMR). Unfortunately, there was a discrepancy in the specific optical rotation values and the NMR data of the synthetic product with data reported for naturally occurring obscuraminol A (**2**) (See Supplementary data).[4] Examination of the literature data revealed that **2** was isolated as its hydrochloride salt. So, the amino-alcohol was converted to its hydrochloride salt **14** after treatment with excess hydrogen chloride in MeOH. The spectral data for **14** were in agreement with those reported earlier.[4] Moreover, the specific optical rotation data of synthetic **14** ($[\alpha]_D^{20}$+2.0, *c*=0.14, MeOH) was dextrorotary, as previously reported ($[\alpha]_D^{20}$+4.8, *c*=0.14, MeOH).[4] These data supported that the absolute configuration of **2** is 2*S*,3*R*, but a significant difference in the rather low figures for the specific optical rotation data was observed. In order to build more confidence in establishing the absolute configuration at C2 and C3, the diacetate **15** was also prepared (See Supplementary data).[4] To our delight, the specific optical rotation data of the synthetic diacetate of **2** showed similar values as reported in the literature,[4] $[\alpha]_D^{20}$+23.8 (*c*=0.65, $CHCl_3$) and $[\alpha]_D^{20}$+23.3 (*c*=0.65, $CHCl_3$), respectively.[29] Hence, the absolute configuration of the stereogenic centers in **2** is indeed 2*S*,3*R* as originally assigned by Garrido et al. The enantiomeric excess of **15** was determined by GLC-analyses to be *ee*=88% (see Supplementary data).

3. Conclusions

To summarize, the first synthesis of obscuraminol A (**2**) has been presented using an organocatalyzed *anti*- and enantioselective Henry reaction using the Wang catalyst **10**. Several synthetic strategies have been used for the stereoselective synthesis of vicinal amino-alcohols,[31] but organocatalysis offers many advantages.[32] An important part for advancing this important field of environmentally benign asymmetric synthesis is to apply such methodology in the total synthesis of natural products, now extended to polyunsaturated sphingolipids. Of note, the challenging chemoselective reduction of the nitro group in **11** was achieved by employing a mild and rather underutilized SmI_2-mediated reduction protocol.[27] These efforts yielded the naturally occurring obscuraminol A (**2**) in 6% overall yield over 11 steps from the ethyl ester of eicosapentaenoic acid (**4**). Noteworthy, the stereochemistry of the four *Z*-skipped double bonds has been conserved from the starting material **4a**. The synthetic work presented confirmed the assigned structure of the natural product **2** and provided sufficient material for biological testing. However, a highly stereoselective preparation of obscuraminol A (**2**) is still elusive. Such efforts are ongoing and will be reported in due time.

4. Experimental section

4.1. General methods

All reactions were performed under a nitrogen atmosphere protected from light exposure. All reagents and solvents were commercial grade and used without further purification. Thin layer chromatography (TLC) was performed using aluminum backed silica gel 60 F_{254} plates and flash chromatography utilized silica gel 60 (40–63 μm) from Merck. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded in $CDCl_3$ on a Bruker Ascend™ 400 spectrometer. Chemical shifts are measured in ppm relative to residual solvent peak as internal standard set to δ 7.26 and 77.0. HRMS was performed using EI method of ionization. IR spectra (4000–600 cm^{-1}) were recorded on Perkin–Elmer Spectrum BX series FTIR spectrophotometer using a reflectance cell (HATR). Optical rotations were measured using a 1 mL cell with 1.0 dm path length on Perkin Elmer 341 polarimeter in dedicated solvent. HPLC analyses were performed on Agilent 1200 Series instrument using an AD-H column with a chiral stationary phase. The GC analyses were performed on an Agilent GC system using Agilent J1W HP-5 GC column (20 m, i.d.=0.18 mm) with FID detector or a CP Chirasil 7502 column with FID detector. Diastereomeric ratios or yields reported in this paper have not been validated by calibration, please see reference Hudlicky and Wernerova for discussions and guidelines.[33]

4.2. (4*Z*,7*Z*,10*Z*,13*Z*)-hexadeca-4,7,10,13-tetraenal (5)

The aldehyde **5** was prepared from **4a** as previously reported.[12l] The overall yield of **5** is 30%. All spectroscopic data were in full agreement with the literature.[12f,l] ^{1}H NMR (400 MHz, $CHCl_3$): δ 9.76 (t, *J*=1.5 Hz, 1H), 5.47–5.23 (m, 8H), 2.89–2.75 (m, 6H), 2.56–2.44 (m, 2H), 2.44–2.33 (m, 2H), 2.12–1.99 (m, 2H), 0.95 (t, *J*=7.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 201.9, 132.0, 129.4, 128.6, 128.4, 127.8, 127.8, 127.6, 127.0, 43.7, 25.6, 25.6, 25.5, 20.6, 20.0, 14.3.

4.3. (*R*)-2-((2-(hydroxydiphenylmethyl)pyrrolidin-1-yl) methyl)-6-(trifluoromethyl)phenol

Catalyst **10** was prepared according to the procedure by Wang et al.[20e] A white solid resulted (64%), mp: 125–127 °C, with spectroscopic data in agreement with the literature.[20a] ^{1}H NMR (400 MHz, $CHCl_3$) δ 7.59–7.46 (m, 4H), 7.34–7.18 (m, 6H), 7.12–7.08 (m, 1H), 6.92 (d, *J*=7.4 Hz, 1H), 6.68 (t, *J*=7.7 Hz, 1H), 5.20 (s, 1H), 3.91 (dd, *J*=9.5, 4.7 Hz, 1H), 3.47 (d, *J*=13.6 Hz, 1H), 3.30 (d, *J*=13.6 Hz, 1H), 2.96 (ddd, *J*=10.0, 6.3, 3.4 Hz, 1H), 2.34 (td, *J*=9.8, 6.5 Hz, 1H), 2.17–2.00 (m, 1H), 1.88 (ddt, *J*=12.6, 8.2, 4.3 Hz, 1H), 1.74–1.64 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.7, 144.9, 144.3, 130.6, 127.4, 127.3, 126.0, 125.9, 125.0, 124.8, 123.1, 116.8, 78.8, 71.2, 59.4, 54.5, 28.3, 23.1.

4.4. (2*S*,3*R*,6*Z*,9*Z*,12*Z*,15*Z*)-2-Nitrooctadeca-6,9,12,15-tetraen-3-ol (11)

To a solution of **10** (25 mg, 0.058 mmol), $CuBr_2$ (13 mg, 0.058 mmol) and Cs_2CO_3 (28 mg, 0.087 mmol) in 1.2 mL of THF, nitroethane (5.9 mmol, 440 μL) was added. The mixture was left stirring for the next 4 h at room temperature until a white precipitate appeared. The tube was centrifuged for 5 min (6000 rpm), and the supernatant was transferred to the test tube containing pre-cooled to −15 °C aldehyde **5** (137 mg, 0.59 mmol). The mixture was reacted at −15 °C until completion as monitored by TLC

(120 h). Then the volatiles were removed under reduced pressure and the residue was then directly subjected to silica gel column eluting with hexane:EtOAc (10:1) to afford 170 mg (94%) of the nitroaldol **11** as a colorless oil; $[\alpha]_D^{20}=-11.5$ (c=0.3, $CHCl_3$, *anti/syn* 11.5:1); ν_{max} (liquid film) 3665–3250 (br), 3011, 2963, 2933, 2874, 1547, 1453, 1392, 1020, 797, 705 cm^{-1}; *ee*=83% (HPLC, Chiracel AD-H column, hexane:isopropanol 98:2, 1.0 mL/min, 25 °C, 215 nm): t_R (*anti* minor)=10.98 min, t_R (*anti* major)=12.05 min, t_R (*syn* major)=13.93 min, t_R (*syn* minor)=14.47 min; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.49–5.23 (m, 8H), 4.47 (qd, J=6.9, 3.0 Hz, 1H), 4.16 (dq, J=7.5, 3.6 Hz, 1H anti), 2.86–2.75 (m, 6H), 2.32–2.12 (m, 3H), 2.05 (td, J=7.4, 1.3 Hz, 2H), 1.61–1.37 (m, 3H), 1.53 (d, J=7.5 Hz 3H), 0.95 (t, J=7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 132.1, 129.6, 128.7, 128.4, 128.3, 127.9, 127.8, 127.0, 86.4, 71.4, 32.7, 25.6, 25.6, 23.4, 20.6, 14.3, 12.6.

4.5. Obscuraminol A (2) obtained by SmI_2-mediated reduction

To a stirred 0.1 M solution of SmI_2 in THF (1.5 mmol, 15 mL) a solution of nitro-alcohol **11** as mixture of *syn*- and *anti*-isomer (62 mg, 0.2 mmol) in THF:MeOH 2:1 was added. The mixture was left stirring at ambient temperature until full conversion of **11** (TLC). After 6 h the reaction was quenched with 10% aqueous solution of $Na_2S_2O_3$ and extracted with EtOAc (3×10 mL). The combined organic phase was dried (Na_2SO_4) and evaporated. The residue was subjected to silica gel column chromatography eluting with $CHCl_3$:MeOH (9:1) to afford 33 mg (60%) of the amino-alcohol **2** as a colorless oil; R_f=0.13; dr (determined by ^{1}H NMR) 5.2:1 of *anti/syn*-isomers. The crude reaction product consisting of **2** and **12** was transformed into the corresponding oxazolidinone in the subsequent step.

4.6. *Cis*-(4*S*,5*S*)-4-methyl-5-((3*Z*,6*Z*,9*Z*,12*Z*)-pentadeca-3,6,9,12-tetraene-1-yl)oxazolidin-2-one (13)

To a solution of the amino-alcohol **2** (16 mg, 0.058 mmol) in dry THF (2 mL) was 1,1′-carbonyldiimidazole (14 mg, 0.087 mmol) added. The solution was refluxed for 12 h. The solvent was evaporated and the crude product was purified by silica gel chromatography eluting with hexane:EtOAc 7:3 to 1: 1 to obtain 10.3 mg (61%) of **13** as a colorless oil; R_f=0.48 (hexane:EtOAc 1:1, $KMnO_4$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.57–5.53 (br s, 1H), 5.49–5.24 (m, 8H), 4.56 (ddd, J=10.1, 7.4, 3.8 Hz, 1H), 3.89 (pentet, J=6.6 Hz, 1H), 2.89–2.73 (m, 6H), 2.34–2.14 (m, 2H), 2.10–2.01 (m, 2H), 1.82 (m, 1H), 1.53 (m, 1H), 1.15 (d, J=6.5 Hz, 3H), 0.95 (t, J=7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.3, 132.0, 129.5, 128.6, 128.4, 128.1, 127.9, 127.8, 127.0, 79.4, 51.0, 29.2, 25.6, 25.58, 25.5, 23.5, 20.5, 16.0, 14.3.

4.7. Obscuraminol A (2) obtained by hydrolysis of 13

A solution of the *cis*-carbamate **13** (6.9 mg, 0.023 mmol) in a 1N aqueous solution of KOH in EtOH-H_2O 1:1 (1 mL) was refluxed for 4 h. After cooling to room temperature, the mixture was diluted with water (1 mL) and was extracted with EtOAc (3×2.5 mL). The combined organic phase was dried ($MgSO_4$) and evaporated to afford 4 mg (63%) of **2** as a yellow oil; ν_{max} (liquid film) 3680–3200 (br), 3011, 1621, 1435, 1024, 801, 704 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.45–5.28 (m, 8H), 3.61–3.58 (m, 1H), 3.08–3.00 (m, 1H), 2.85–2.76 (m, 6H), 2.75 (br s, 4H), 2.28–2.24 (m, 1H), 2.19–2.16 (m, 1H), 2.07 (m, 2H), 1.51–1.40 (m, 2H), 1.09 (d, J=6.6 Hz, 2H), 0.97 (t, J=7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 132.0, 129.5, 128.6 (2×), 128.2 (2×), 127.9, 127.0, 73.1, 50.8, 32.3, 25.6, 25.5, 23.9, 20.6, 15.7, 14.3.

4.8. (2*R*,3*S*,6*Z*,9*Z*,12*Z*,15*Z*)-2-aminooctadeca-6,9,12,15-tetraene-3-ol hydrochloride (14)

The amino-alcohol **2**, obtained by hydrolysis of **13**, (5 mg, 0.017 mmol) in 0.5 mL of dry MeOH at 5 °C was immediately treated with a five-fold excess of HCl-gas in MeOH for 30 min. The solvent was removed by evaporation flushing with nitrogen to afford 5 mg (95%) its hydrochloride salt **14** as a pale yellow oil; $[\alpha]_D^{20}=+2.0$ (c=0.14, CH_3OH), lit.[4] $[\alpha]_D^{20}=+4.8$ (c=0.14, CH_3OH);[4] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (br s, 3H), 5.40–5.26 (m, 8H), 4.00 (m, 1H), 3.43 (m, 1H), 2.81–2.76 (m, 6H), 2.27–2.20 (m, 1H), 2.15–2.00 (m, 3H), 1.59–1.53 (m, 1H), 1.42–1.40 (m, 1H), 1.30 (d, J=6.7 Hz, 3H), 0.95 (t, J=7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 132.0, 129.0, 128.6, 128.4, 128.1, 127.8, 127.0, 70.1, 52.0, 32.8, 25.6, 25.5, 23.6, 20.6, 14.3, 12.0; HRMS (EI^+): Exact mass calculated for $C_{18}H_{32}NO$ $(M-Cl)^+$: 278.2474, found 278.2484.

4.9. (2*S*,3*R*,6*Z*,9*Z*,12*Z*,15*Z*)-2-acetamidooctadeca-6,9,12,15-tetraen-3-yl acetate (15, diacetate of obscuraminol A)

The amino-alcohol **2** (13 mg, 0.047 mmol) was dissolved in pyridine (0.2 mL) and Ac_2O (510 mg, 0.47 mmol was added at ambient temperature. The mixture was left stirring for 2 h, then the solvents were removed by evaporation flushing with nitrogen and the residue was purified using column chromatography eluting with hexane:EtOAc 1:1 to obtain 15 mg (90%) of **15** as a yellow oil in a 14:1 diastereomeric ratio; $[\alpha]_D^{20}=23.8$ (c=0.65, $CHCl_3$), lit.[4] $[\alpha]_D^{20}=23.3$ (c=0.65, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.84 (d, J=8.6 Hz, 1H), 5.43–5.22 (m, 8H), 4.83 (ddd, J=9.3, 4.3, 3.2 Hz, 1H), 4.14 (dqd, J=10.1, 8.4, 6.9, 3.2 Hz, 1H), 2.88–2.69 (m, 5H), 2.14–1.98 (m, 8H), 1.93 (s, 3 H), 1.71–1.48 (m, 2H), 1.07 (d, J=6.8 Hz, 3H), 0.95 (t, J=7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6, 169.3, 132.0, 129.0, 128.6, 128.4, 128.3, 128.0, 127.8, 127.0, 76.4, 47.7, 31.2, 25.6, 25.6, 25.5, 23.5, 23.4, 21.1, 20.6, 14.9, 14.3; HRMS (EI^+): Exact mass calculated for $C_{22}H_{35}NO_3$: 361.2617, found 361.2637; *ee*=88% (GLC, CP Chirasil 7502, 25 m, film 0.25 mm, i.d. 0.25 mm, 80 °C (45 min), then 2 °C/min, 150 °C (10 min)).

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Supplementary data

Supplementary data (Experimental procedures and characterization data of starting materials for making catalyst **10**, copies of IR-spectra, ^{1}H- and ^{13}C NMR spectra, as well as copies of chromatograms of GLC and HPLC analyses.) associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2016.08.070.

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Supporting information for

Synthesis of Obscuraminol A Using an Organocatalyzed Enantioselective Henry Reaction

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General information

All reactions were performed under a nitrogen atmosphere protected from light exposure. All reagents and solvents were commercial grade and used without further purification. The EPA ethyl ester **4a** was obtained as a gift from Pronova BioPharma AS, Sandefjord, Norway. Thin layer chromatography (TLC) was performed using aluminium backed silica gel 60 F_{254} plates and flash chromatography utilized silica gel 60 (40-63 μm) from Merck. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded in $CDCl_3$ on a Bruker Ascend^{TM} 400 spectrometer. Chemical shifts are measured in ppm relative to residual solvent peak as internal standard set to δ 7.26 and 77.0. HRMS was performed using EI method of ionization. IR spectra (4000-600 cm^{-1}) were recorded on Perkin-Elmer Specrtum BX series FT-IR spectrophotometer using a reflectance cell (HATR). Optical rotations were measured using a 1 mL cell with 1.0 dm path length on Perkin Elmer 341 polarimeter in dedicated solvent. HPLC analyzes were performed on Agilent 1200 Series instrument using an AD-H column with a chiral stationary phase. The GC analyses were performed on an Agilent GC system using Agilent J1W HP-5 GC column (20 m, i.d. = 0.18 mm) with FID detector or a CP Chirasil 7502 column with FID detector. The alcohol **7**, the mesylate **8** and the nitrile **9** have been prepared before.[1]

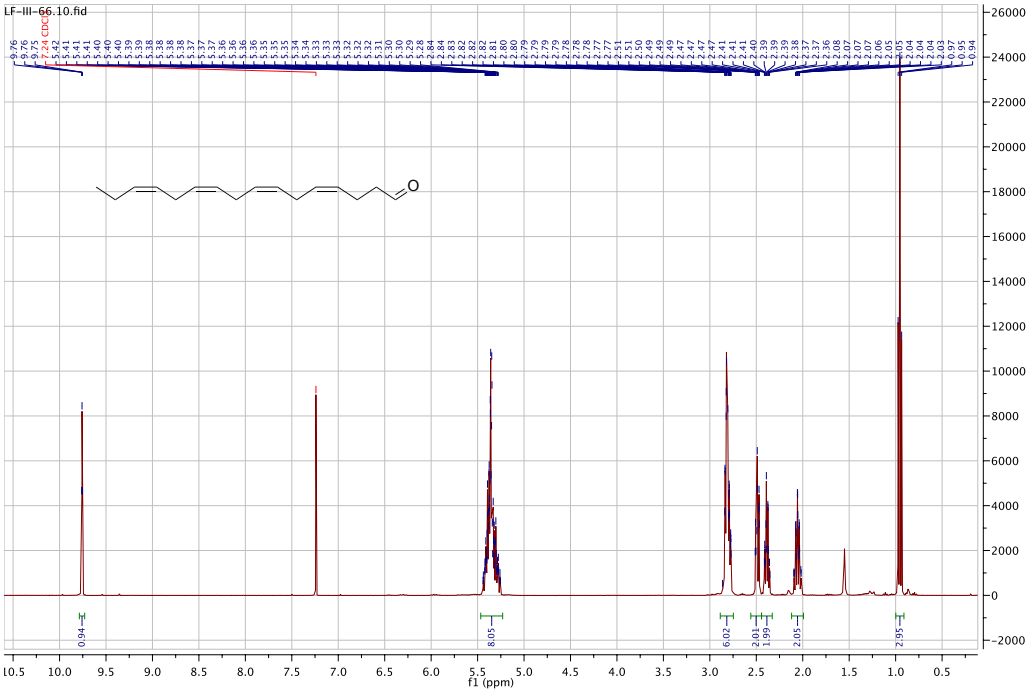


Figure S1. ^{1}H NMR spectrum of **5**

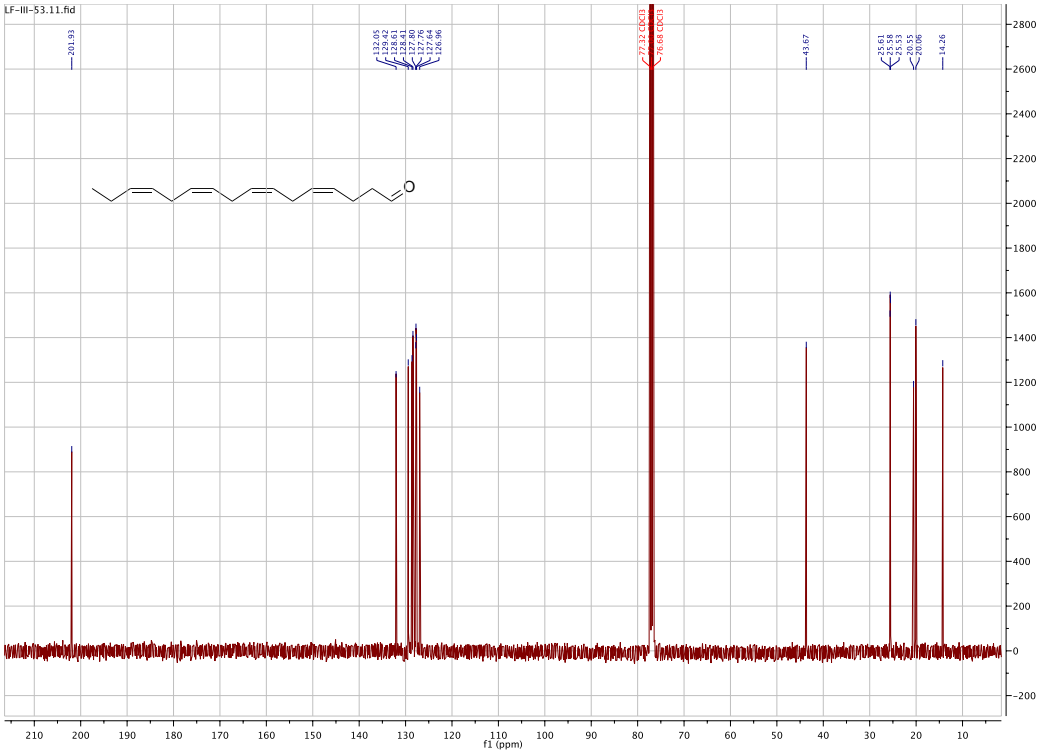


Figure S2. ^{13}C NMR spectrum of **5**

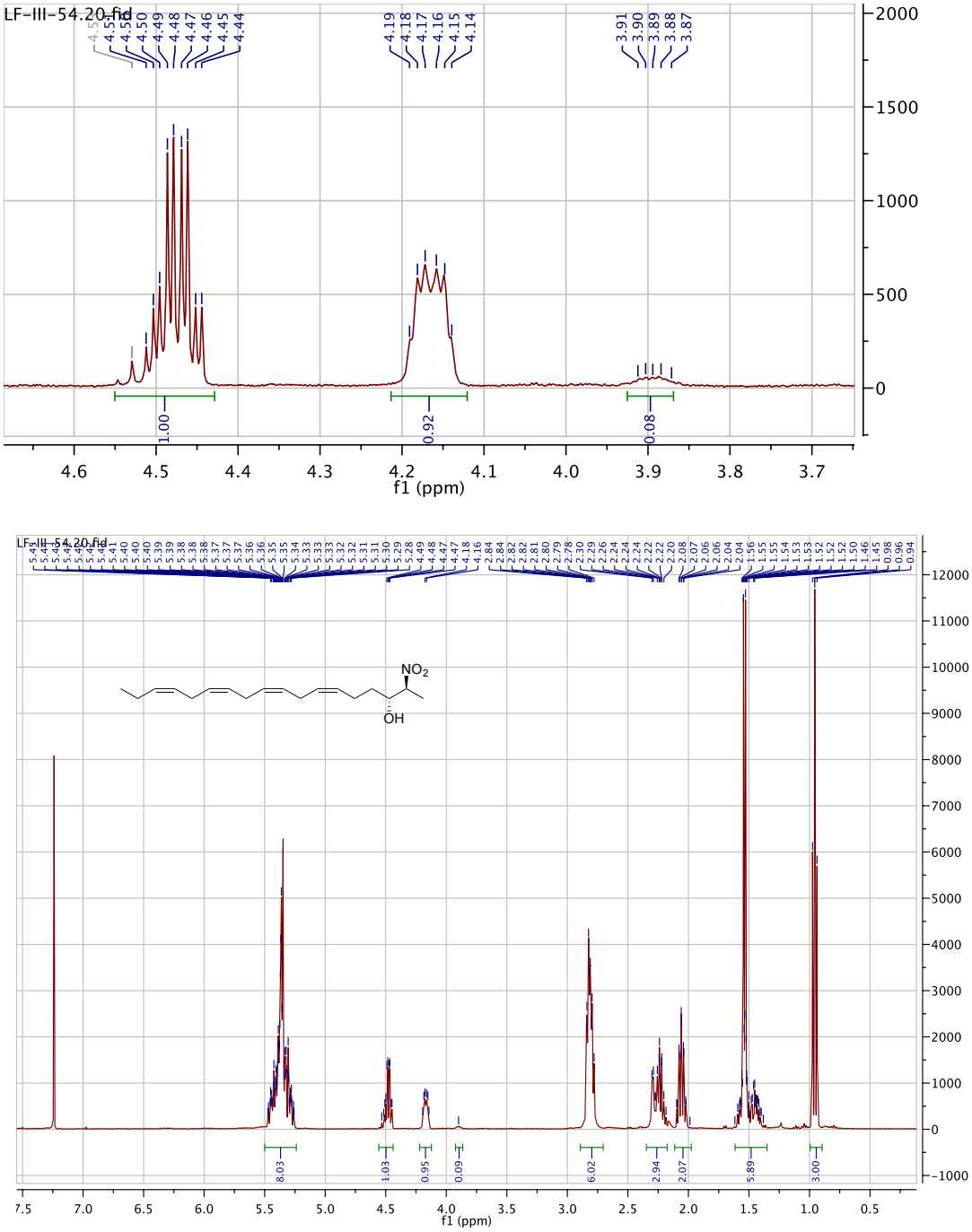


Figure S3. ^{1}H NMR spectrum of **11**

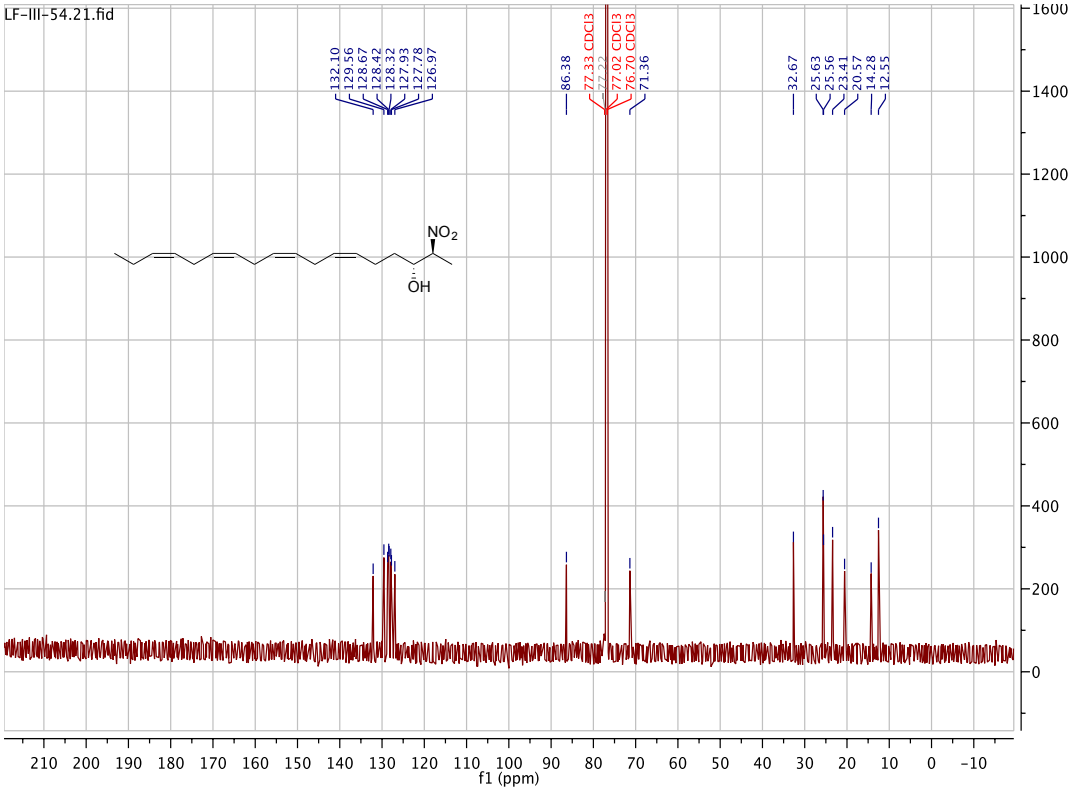


Figure S4. ^{13}C NMR spectrum of **11**

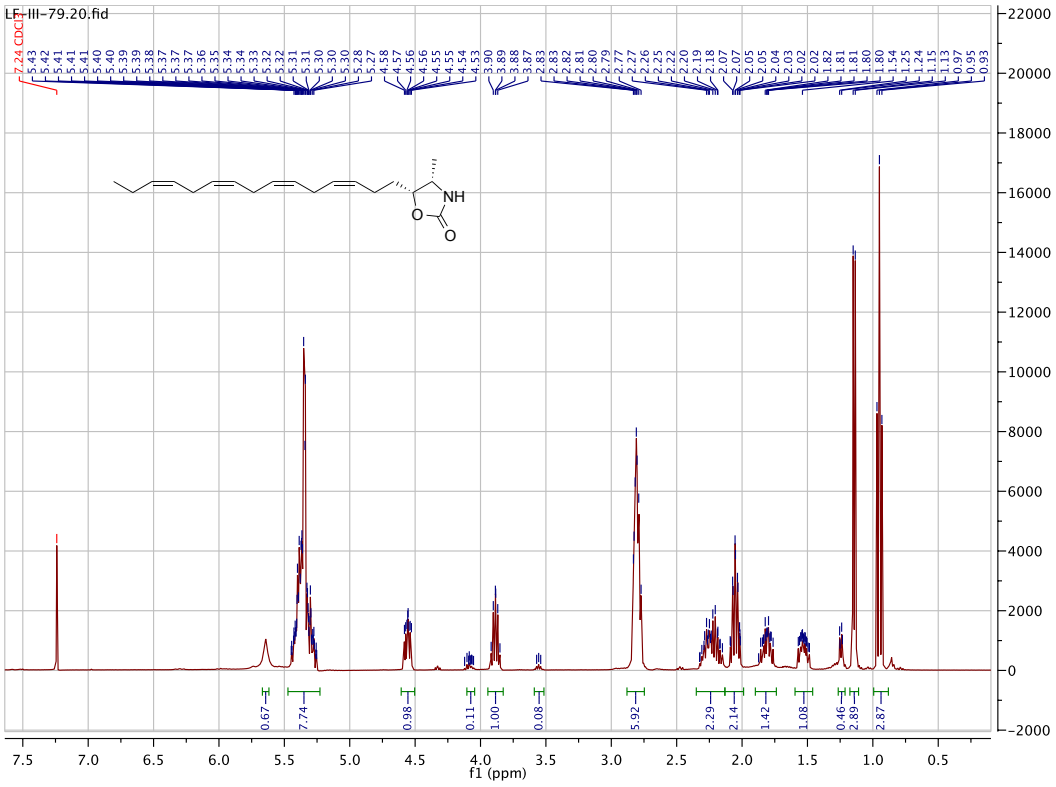


Figure S5. ^{1}H NMR spectrum of **13**

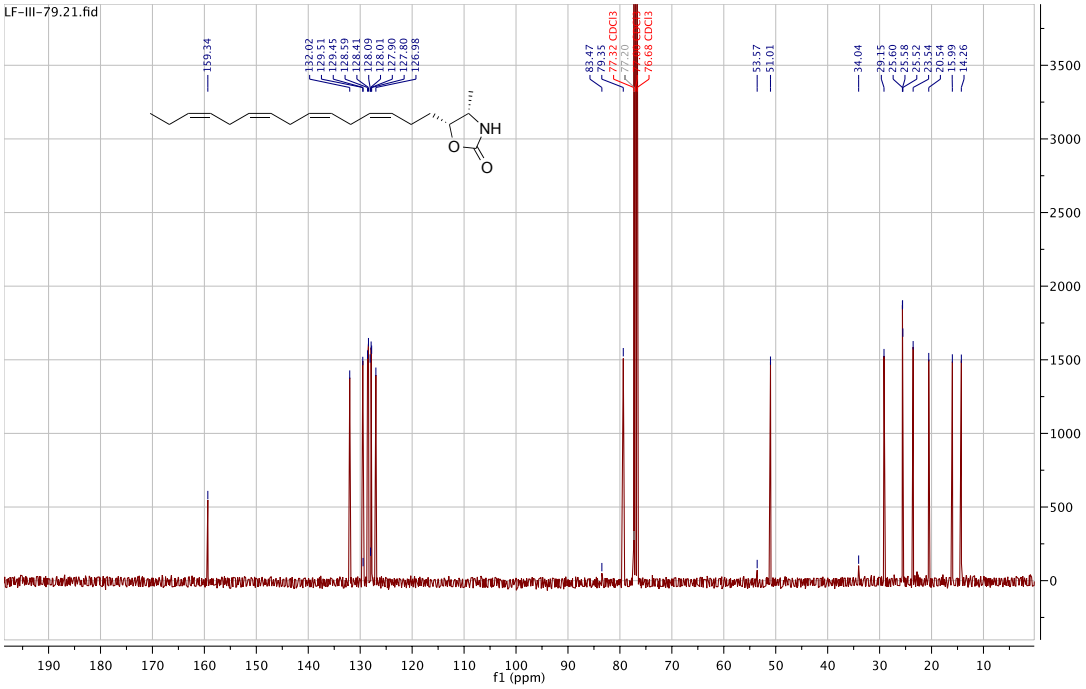


Figure S6. ^{13}C NMR spectrum of **13**

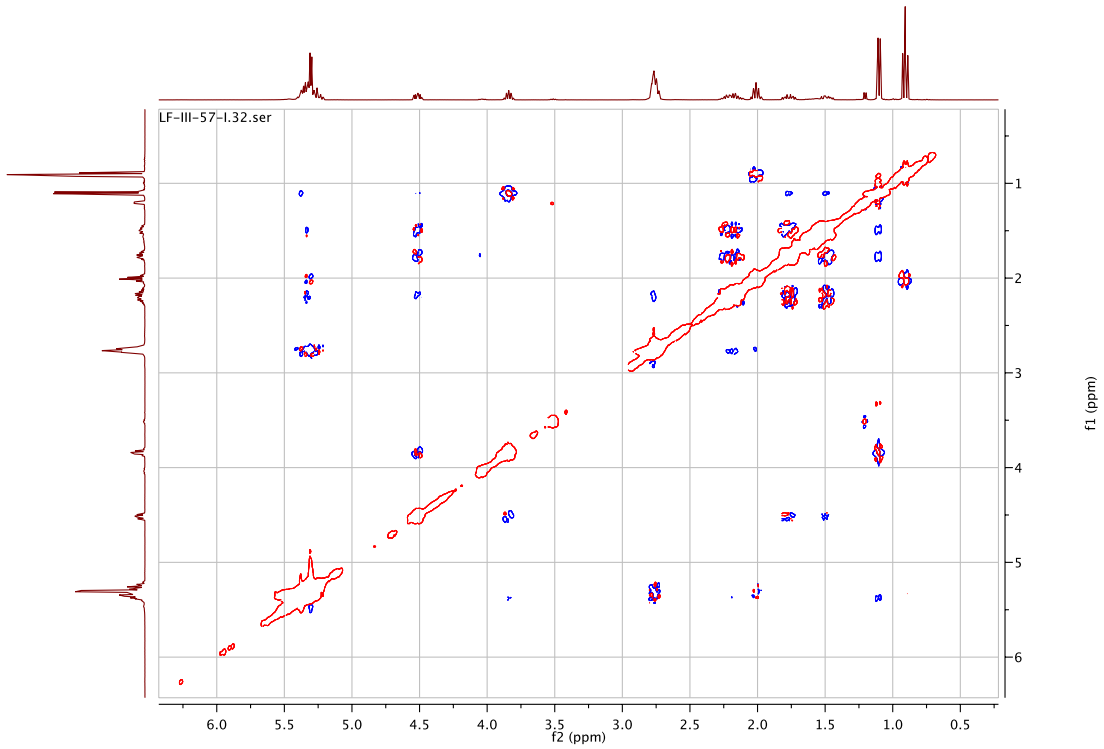


Figure S7. NOESY spectrum of **13**

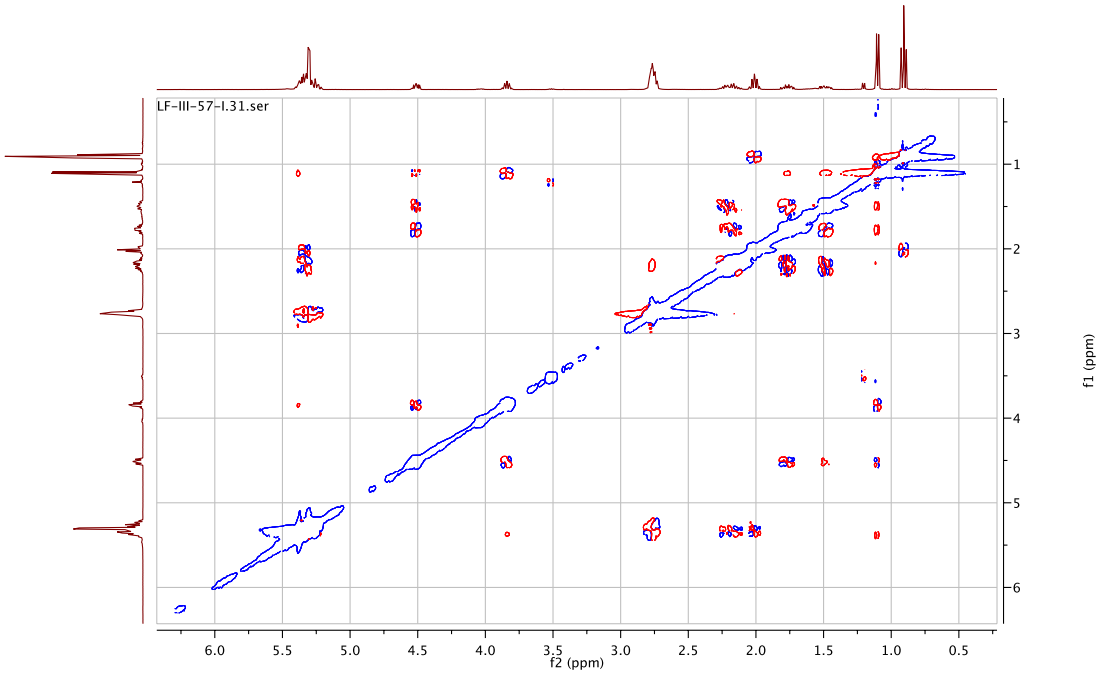


Figure S8. ROESY spectrum of **13**

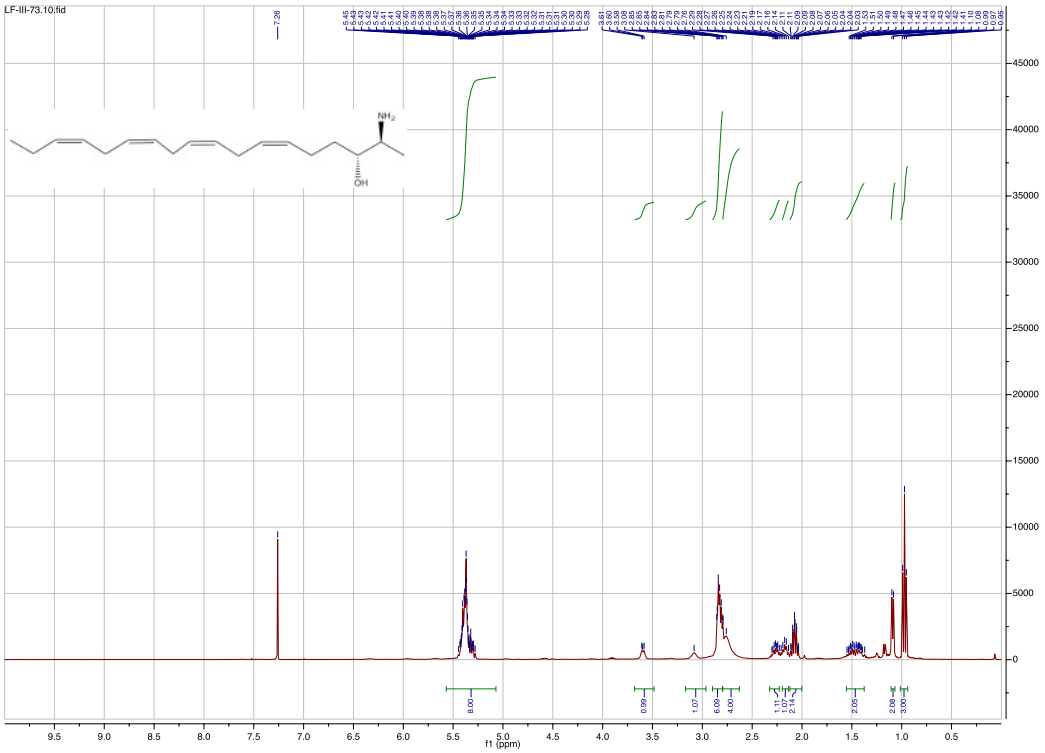


Figure S9. ^{1}H NMR spectrum of obscuraminol A (**2**)

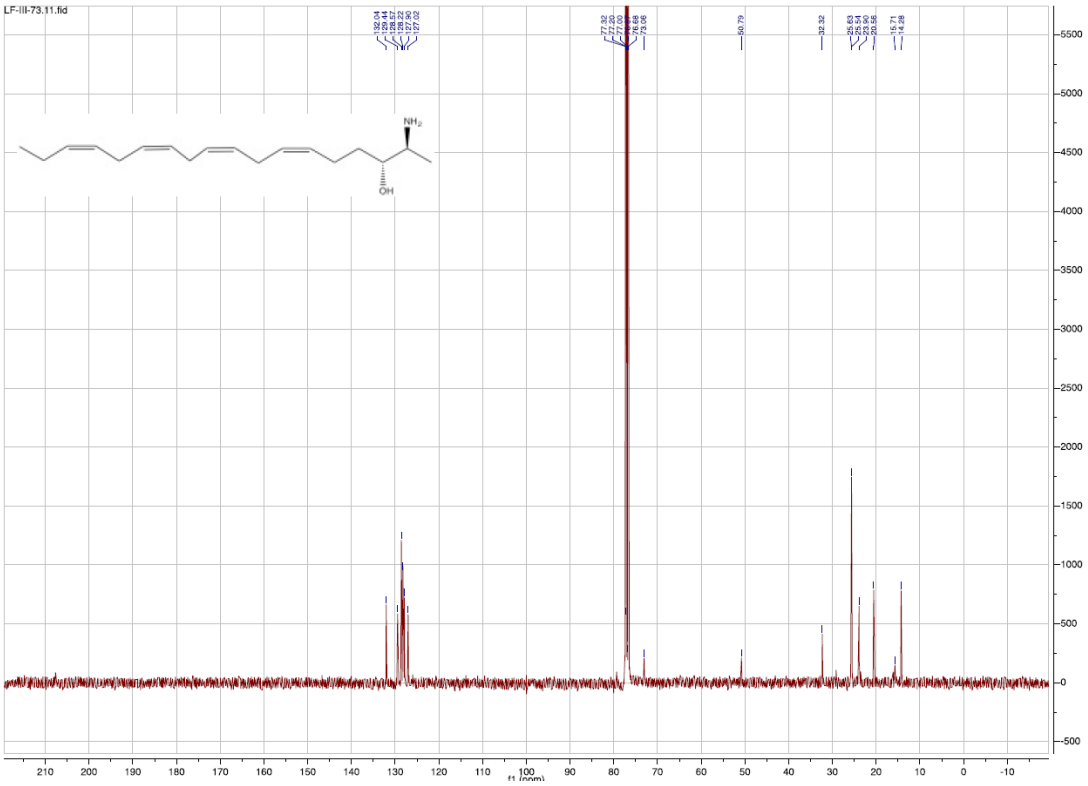


Figure S10. ^{13}C NMR spectrum of obscuraminol A (**2**)

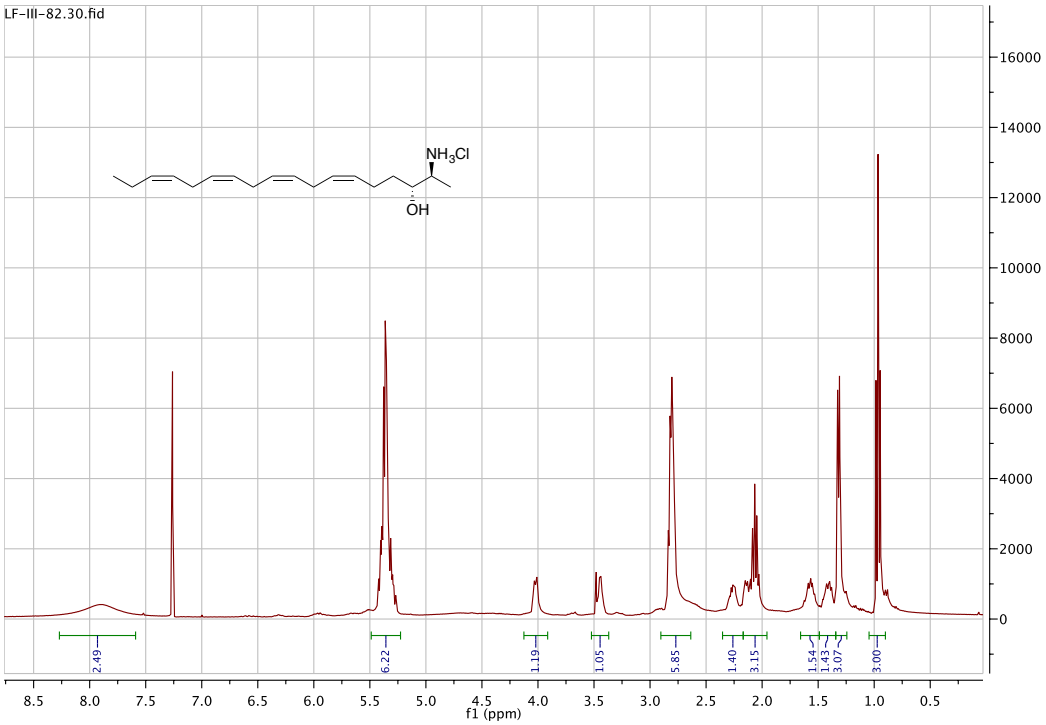


Figure S11. ^{1}H NMR spectrum of HCl-salt **14**

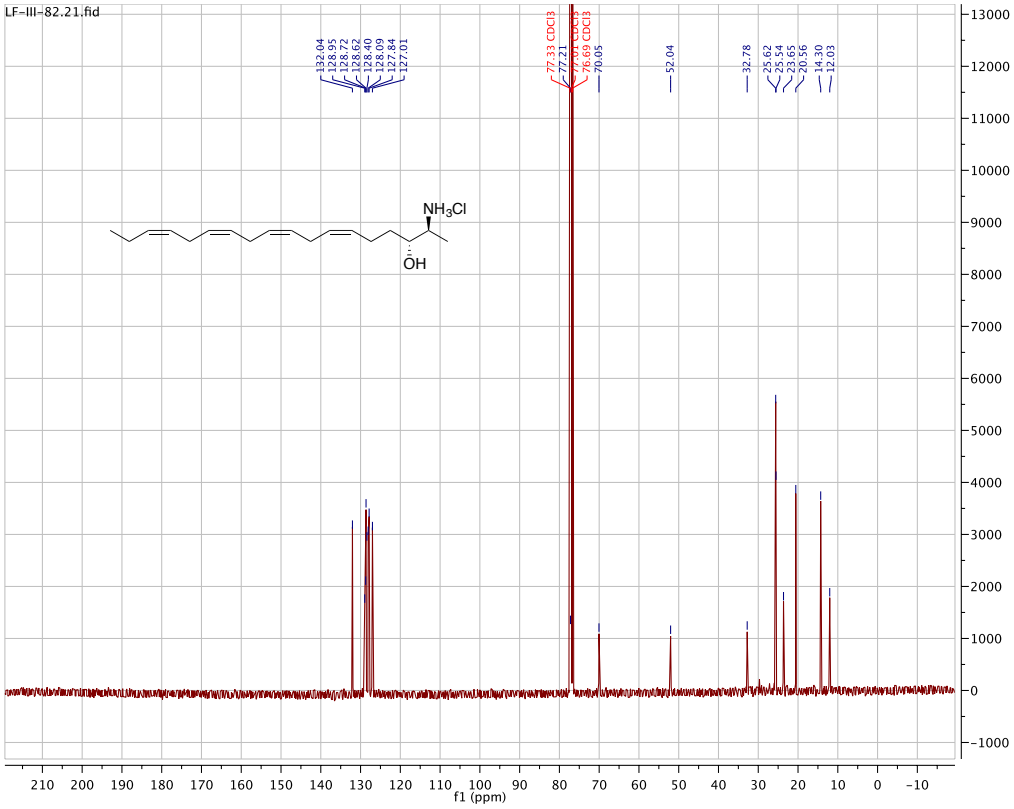


Figure S12. ^{13}C NMR spectrum of HCl-salt **14**

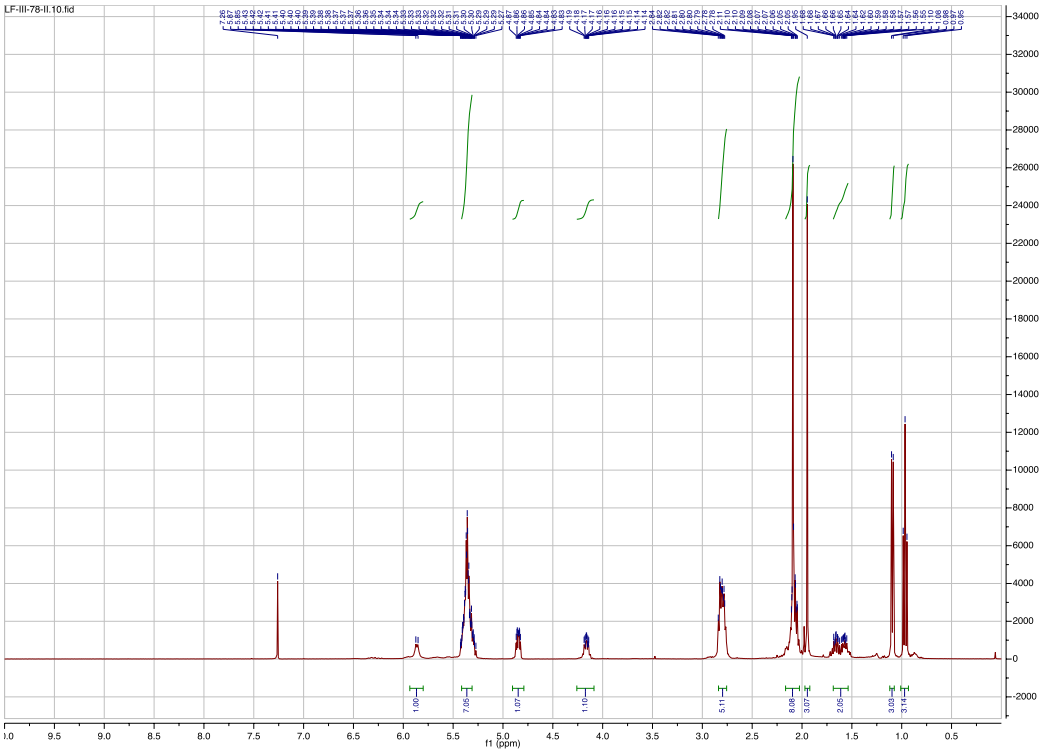


Figure S13. ^{1}H NMR spectrum of **15**

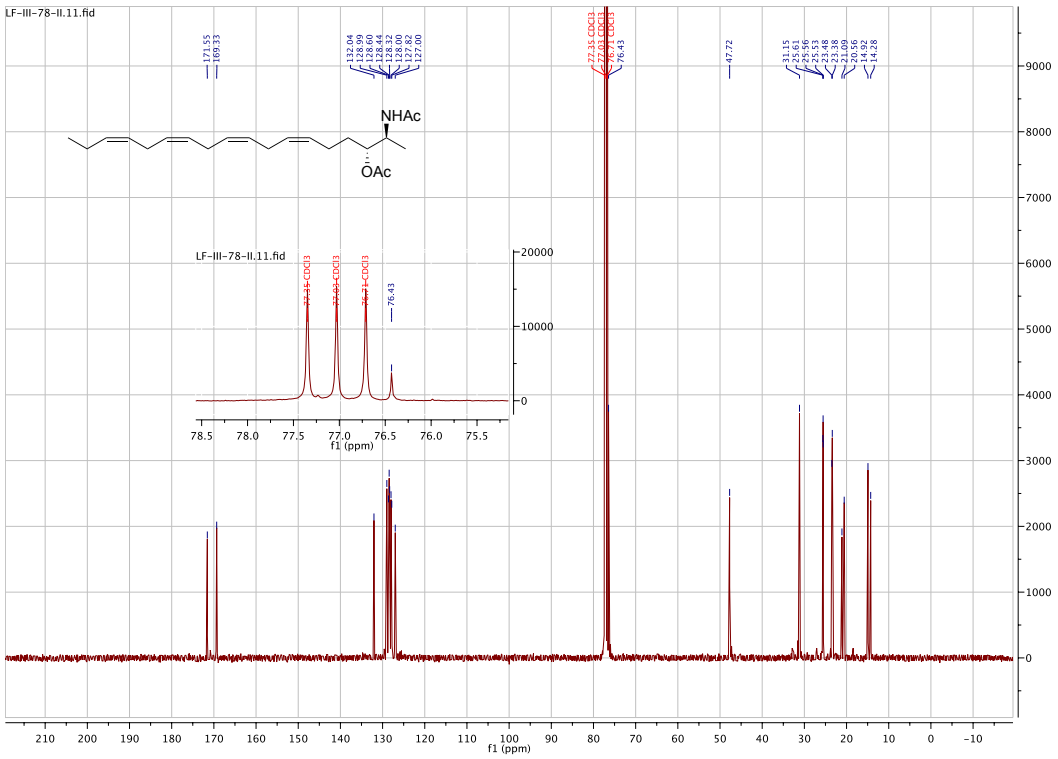


Figure S14. ^{13}C NMR spectrum of **15**

Data File C:\CHEM32\1\DATA\TEST\STD TEST329.D
Sample Name: obscuraminol diacetate LF-2015

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [ng/ul]	Grp	Name
Totals :				0.00000		

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	39.133	BB	0.1047	2.00266	0.21260	?
6	44.297	BV	0.1665	887.37659	94.20363	?
7	45.062	VB	0.1072	51.65311	5.48348	?
8	59.747	BBA	0.1917	9.44710e-1	0.10029	?
Totals :				941.97707		

1 Warnings or Errors :

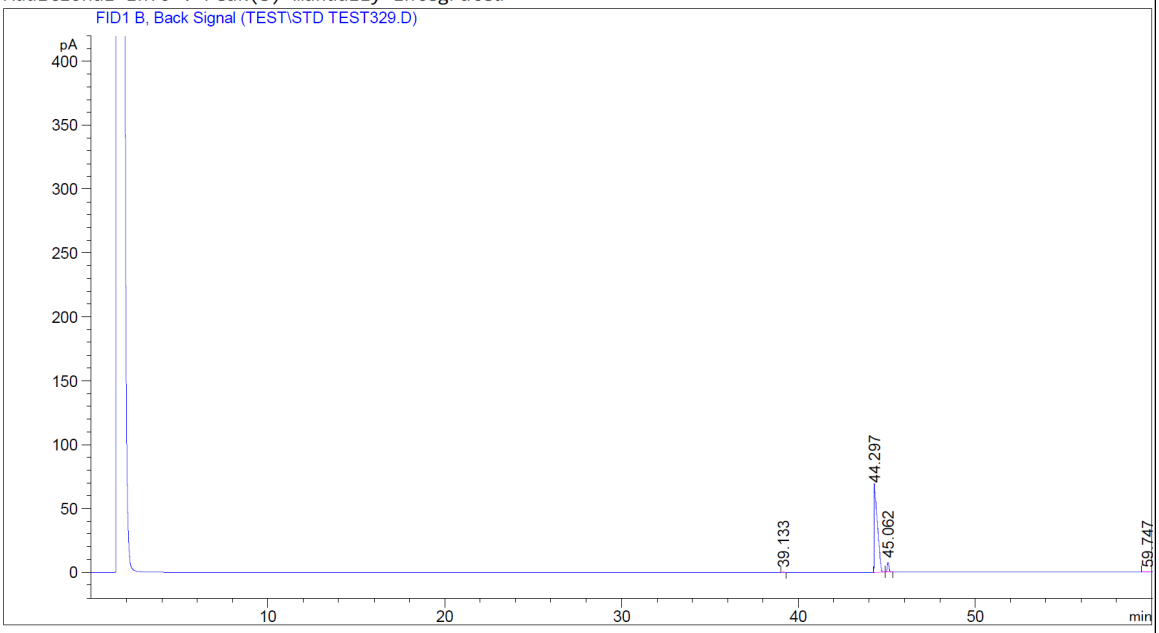
Warning : Calibrated compound(s) not found

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

Figure S15. GC chromatogram of **15** using a CP7502 Chirasil column

Data File C:\CHEM32\1\DATA\TEST\STD TEST329.D
Sample Name: obscuraminol diacetate LF-2015

```
======================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : 7820 GC                       Location : Vial 1
Injection Date  : 5/25/2016 15:39:59
                                                Inj Volume : Manually
Acq. Method     : C:\CHEM32\1\METHODS\CP7502\CP7502.M
Last changed    : 5/25/2016 15:38:27 by SYSTEM
Analysis Method : C:\CHEM32\1\METHODS\CP7502\CP7502.M
Last changed    : 5/26/2016 07:55:45 by SYSTEM
                  (modified after loading)
Sample Info     : 80 grader 45 min, 1 grader/min til 160 grader, 5 min hold time

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

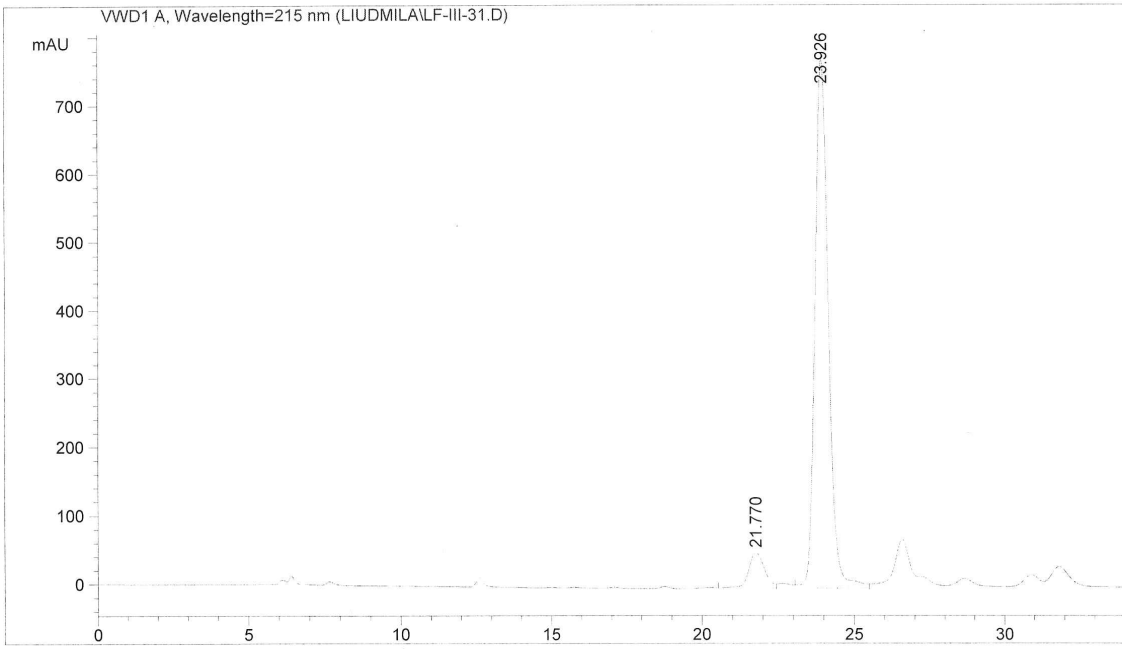



```
======================================================================
                    External Standard Report
======================================================================

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
```


Figure S16. HPLC chromatogram of **11** after reaction with nitroethane and **5** in the presence of catalyst **10**

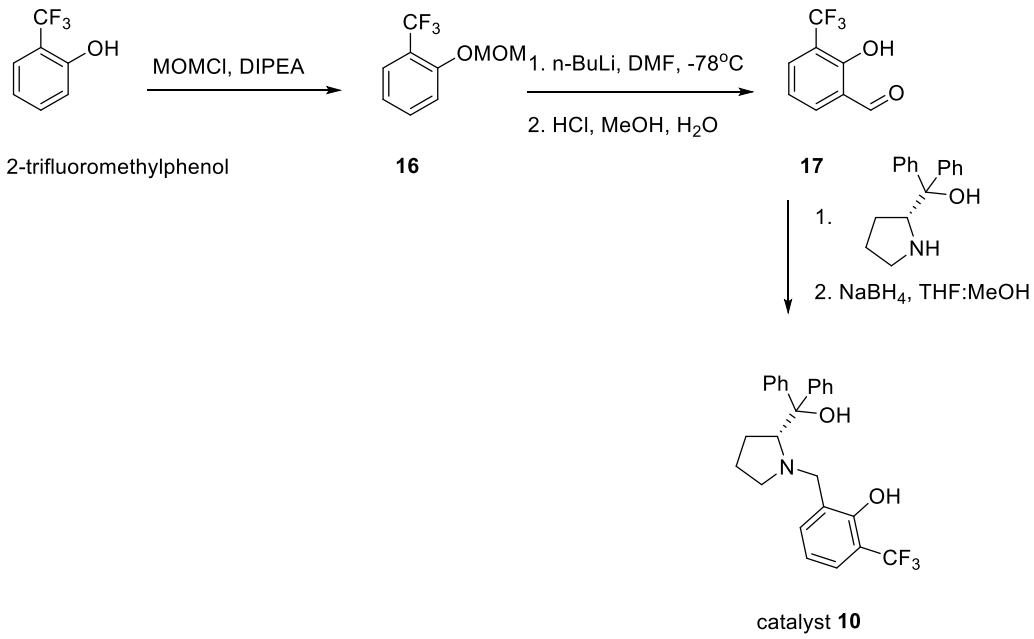
Data File C:\CHEM32\1\DATA\LIUDMILA\LF-III-31.D
Sample Name: LF-III-31

```
==========================================================================
Acq. Operator    : LIUDMILA
Acq. Instrument  : Instrument 1                    Location : Vial 1
Injection Date   : 16.03.2015 13:53:04
                                                   Inj Volume : 5 µl
Acq. Method      : C:\CHEM32\1\DATA\ANNE\C18 ISOKRATISK.M
Last changed     : 16.03.2015 13:51:08  by LIUDMILA
                   (modified after loading)
Analysis Method  : C:\CHEM32\1\DATA\ANNE\C18 ISOKRATISK.M
Last changed     : 16.03.2015 14:28:30  by LIUDMILA
                   (modified after loading)
Sample Info      : AD-H, hexane/iPrOH 98/2 0.5ml/min 220
```




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

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount         :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=215 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s   [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1  21.770 VV    0.5744 1958.79834   52.75241   7.6751
   2  23.926 VB    0.4692 2.35625e4   773.04370  92.3249

Totals :                  2.55213e4   825.79611
```

Synthesis of catalyst 10



Scheme S1. Synthesis of catalyst **10**.

The catalyst **10** was prepared according to Wang and co-workers[2] as outlined in Scheme S1.

1-Methoxymethoxy-2-trifluorobenzene (16) was prepared from commercial available 2-trifluoromethylphenol according to a procedure of Xia *et al.*[3] Yield: 86%. ^{1}H NMR (400 MHz, $CHCl_3$) δ 7.57 (d, J = 7.8 Hz. 1H), 7.45 (dd, J_1 = 8.3 Hz, J_2 = 7.5 Hz, 1H), 7.21 (d, J = 8.4 Hz, 1H), 7.04 (dd, $J_1 = J_2$ = 7.6 Hz, 1H),5.25 (s, 2H), 3.52 ppm (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.0, 133.2, 127.0 (q, J_{CF} = 5.3 Hz), 123.7 (q, J_{CF} = 272 Hz), 121.1, 119.6 (q, J_{CF} = 5.3 Hz), 115.2, 94.2, 56.3.

2-Hydroxy-3-(trifluoromethyl)benzaldehyde (17) was prepared according to the two step procedure of Daly *et al.*[4] Total yield after 2 steps: 43%. ^{1}H NMR (400 MHz, $CHCl_3$) δ 11.71 (s, 1H), 9.93 (s, 1H), 7.81 (d, J = 7.5 Hz, 1H), 7.75 (dd, J_1 = 7.8 Hz, J_2 = 1.7 Hz, 1H). 7.10 (ddd, $J_1 = J_2$ = 7.8 Hz, J_3 = 1.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 196.2, 159.7, 137.3, 134.0 (q, J_{CF} = 4.9 Hz), 122.9 (q, J_{CF} = 272 Hz), 121.1, 119.2, 118.9 (q, J_{CF} = 31.8 Hz).

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