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Maleidride Natural Products

Structural and Synthetic Studies

Maleidride Natural Products:

Structural and Synthetic Studies

David M. Heard

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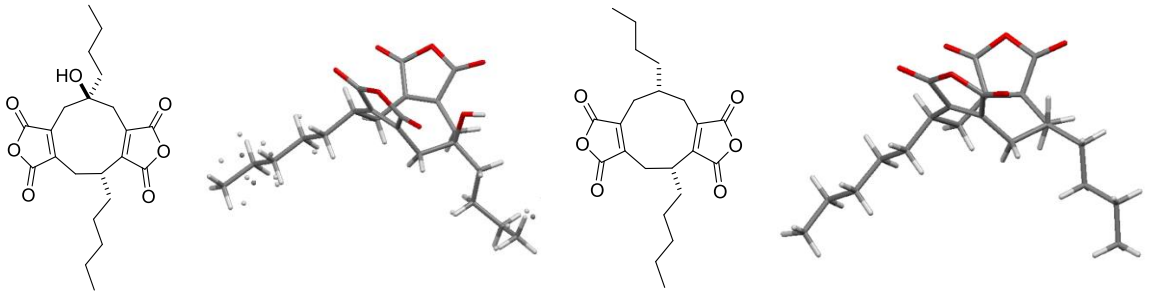
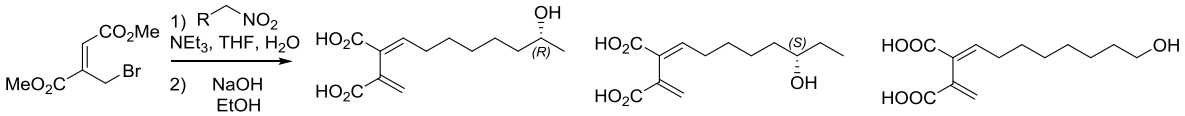
Abstract

The maleidrides are a class of polyketide natural products which possess a broad range of herbicidal and fungicidal properties. An introduction to their bioactivity and biosynthesis is described in **chapter one**, before the isolation and structural determination of two members of this family, scytalidin and deoxyscytalidin is described in **chapter two**. The relative stereochemistry of both natural products was determined by X-ray crystallography, and the absolute stereochemistry proposed on the basis of biosynthetic studies.



Scytalidin **Deoxyscytalidin**

In **chapter three**, the total synthesis of fulgenic diacids is described. The approach utilised a substitution-elimination reaction of nitroalkanes with an allylic bromide, in which two alkenes are formed concomitantly, including exclusive formation of an (*E*)-trisubstituted alkene moiety. This methodology is exemplified with the first total syntheses of the tricladic acids A-C, fungal natural products with interesting fungicidal and anti-cancer properties.

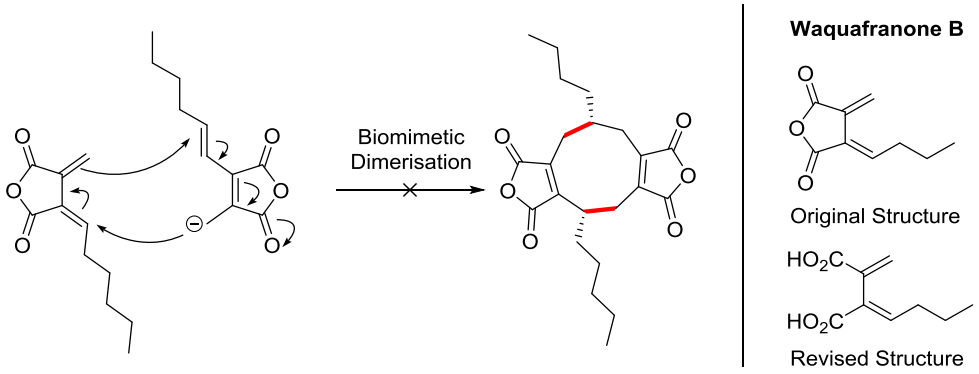


(*R*)-Tricladic Acid A
6 steps
16% overall yield

(*S*)-Tricladic Acid B
6 steps
22% overall yield

Tricladic Acid C
4 steps
20% overall yield

This methodology underpinned the investigation of a biomimetic synthesis of deoxyscytalidin in **chapter four**. The desired cyclisation could not be achieved, however as a result of this investigation the structure of the natural product waquafranone B has been revised.



Finally, a *de novo* approach to the synthesis of scytalidin is described in **chapter five**. A strategy utilising two-carbon ring expansion of cyclic β-ketoesters was devised, with which the nine-membered carbocylic ring of scytalidin would be formed. The synthesis of late-stage intermediates is described.

Acknowledgements

Firstly, I would like to thank Chris for her guidance, encouragement and optimism throughout my PhD. I have had the freedom to develop and pursue my own ideas, with the support and advice needed to enable me to learn all that I have. I'm also exceedingly grateful for the opportunity to work in a wide range of environments, and attend a range of conferences.

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A special thanks to the maleidrides team: Katherine, Kate, Agnes, Claudio, Catherine, Song and Dao for all their patience, help with culturing fungi and for teaching me a range of microbiology techniques I had never come across before. The interdisciplinary nature of this project has been extremely rewarding. Luoyi, Alex, Dan, Hanim, Tom A., Emma, Kun, Edith, Abi, Joe, Jawaher, Sbu, Hannah, Marija, Andrew, Bin and Dr Knowles – thanks to all of you for enriching my time in Bristol.

Thanks to Russell Cox for hosting me for an invaluable spell at LUH Hannover. I learnt a great deal from working in a completely different research environment. Thanks to Steffen Friedrich and Hailli Zhang for all your help in the lab, with successfully navigating the Mensa, and for showing me Hannover. Thanks to Kevin Doyle and Andrew Sharpe for all their advice whilst I was at Charles River Laboratories.

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Thanks to all the analytical and technical staff at the School of Chemistry for providing and maintaining a world-class research environment. In particular: Craig Butts, Paul Lawrence, Tom Leman and Paul Egan.

Thanks to my family for their unfaltering support in everything I do. And finally, a huge thank you to my best friend, Beth. Your patience, support, optimism, boundless enthusiasm and humour have kept me sane and smiling. Thanks for always being exactly what I need, I would not have achieved half of what I have without you.

Author's Declaration

The work described in this thesis was carried out in the School of Chemistry, University of Bristol under the supervision of Professor C. L. Willis between September 2014 and December 2018. The work is original, except where indicated by reference in the text, and has not been submitted for any other degrees. The views expressed in the thesis are those of the author and in no way represent of the University of Bristol.

David Michael Heard

March 2019

Abbreviations

δ	chemical shift
$[\alpha]_D$	specific rotation
Ac	acetyl
ACDH	alkylcitrate dehydratase
ACP	acyl carrier protein
ACS	alkylcitrate synthase
AIBN	2,2'-azobisisobutyronitrile
aq.	aqueous
Ar	aryl
AT	acyltransferase
Aux	auxiliary
BGC	biosynthetic gene cluster
Bn	benzyl
br.	broad
c	concentration
cat.	catalytic
CoA	coenzyme A
conc.	concentrated
d	doublet
d.r.	diastereomeric ratio
DABCO	1,4-diazabicyclo[2.2.2]octane
DAD	diode array detector
DBU	1,8-diazabicycloundec-7-ene
DCC	*N*,*N*-dicyclohexylcarbodiimide
DCM	dichloromethane
DDT	dichlorodiphenyltrichloroethane
DFT	density functional theory
DH	dehydratase
DMAD	dimethyl acetylenedicarboxylate
DMAP	4-dimethylaminopyridine
DME	1,2-dimethoxyethane
DMF	*N*,*N*-dimethylformamide

DMSO	dimethylsulfoxide
EI	electron-impact
Equiv.	equivalents
ER	enoyl reductase
ESI	electrospray ionisation
FAS	fatty acid synthase
G2	second generation grubbs' catalyst
h	hour
HIV	human immunodeficiency virus
HMBC	heteronuclear multiple-bond correlation spectroscopy
HMDS	hexamethyldisilazane
hr	highly reducing
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum correlation experiment
HWE	Horner-Wadsworth-Emmons
Hz	Hertz
IR	infrared
J	coupling constants
KI	ketosteroid isomerase
KR	ketoreductase
KS	ketosynthase
LCMS	liquid chromatography mass spectrometry
LDA	lithium diisopropylamide
Lit.	literature
M	molar
M^+	molecular ion
m	multiplet
m/z	mass: charge ratio
MAT	malonyl transferase
*m*CPBA	*meta*-chloroperoxybenzoic acid
MEA	malt extract agar
MEB	malt extract broth
MHz	megaHertz
MMFF94	Merck molecular force field 94

mp	melting point
MT	methyl transferase
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NSAE	nitronate S_N2' addition elimination
p-	*para*
PCM	polarisable continuum model
PDA	potato dextrose agar
PDB	potato dextrose broth
PEBP	phosphatidylethanolamine-binding protein
PKS	polyketide synthase
PM3	parametric method 3
ppm	parts per million
*p*TSA	*para*-toluenesulfonic acid
Py.	pyridine
q	quartet
s	singlet
SNAC	*N*-acetylcysteamine
sp.	unspecified strain of the genus
t	triplet
TBAI	tetra-*n*-butylammonium iodide
TBS	*tert*-butyldimethylsilyl
TE	thioesterase
TEMPO	2,2,6,6-tetramethylpiperidinyloxyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
TMSE	(trimethylsilyl)ethyl
UAMH	University of Alberta Microherbarium

Table of Contents

CHAPTER 1: Introduction

1. Introduction

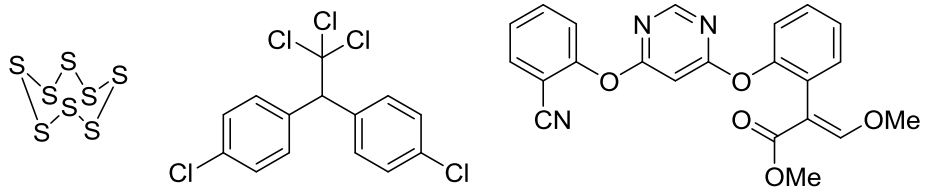
This thesis describes studies on the fungal secondary metabolites scytalidin and deoxyscytalidin, members of the maleidride family of polyketide natural products. The maleidrides have attracted considerable attention due to their potential application as selective agrichemicals. After an introduction to the maleidrides in **chapter one**, this thesis describes the isolation and structural elucidation of scytalidin and deoxyscytalidin in **chapter two**. In **chapters three** and **four**, the synthesis of building blocks and their subsequent role in a biomimetic synthesis of deoxyscytalidin is described, whilst **chapter five** reports a *de novo* approach to the total synthesis of scytalidin. Literature pertinent to each section is introduced at the start of each chapter.

1.1 History of Agrichemicals and Agrichemical Resistance

Antimicrobial resistance has recently gained considerable attention due to the threat posed to global public health by resistant species of bacteria, virus and fungi.[1,2] A similar, perhaps less well reported issue facing the world today is resistance to agrichemicals.

During the 20th century, the global population increased more than threefold, yet in the same time the amount of land used to grow crops per person decreased from 0.75 ha person^{-1} to just 0.35 ha person^{-1}.[3] This has been possible due to technological advancement in agriculture. As the global population continues to rise, and with a finite amount of arable land available, reliance on herbicides and pesticides to improve crop yields will continue to grow.[4]

The concept of using chemical means to prevent crop damage has developed from the use of "pest-averting" sulfur **1** in pre-Roman times,[5] to the broad spectrum biocides of the 1950s such as DDT (dichlorodiphenyltrichloroethane) **2**, through to today's modern herbicides, pesticides and fungicides such as Azoxystrobin **3**, which target specific weaknesses in undesired organisms, whilst displaying greatly reduced toxicity to the cultivated plant and humans.[4]



1 **2** **3**

Figure 1. Sulfur **1**, DDT **2**, and azoxystrobin **3** have all been used as pesticides.

However, in much the same way that widespread antibiotic use has led to antibiotic resistant bacteria, modern agrichemical usage has led to resistant weeds and pests. Early examples of resistance to insecticides was reported in the early 1900s, and became more prominent in the 1950s and 1960s as a variety of insects was reported to be resistant to a broad range of pesticides.[6]

Agrichemical-resistant weed strains took slightly longer to develop, but as can be seen in Figure **2**, the number of weed strains reported to exhibit resistance to herbicides have risen dramatically over the past thirty years.

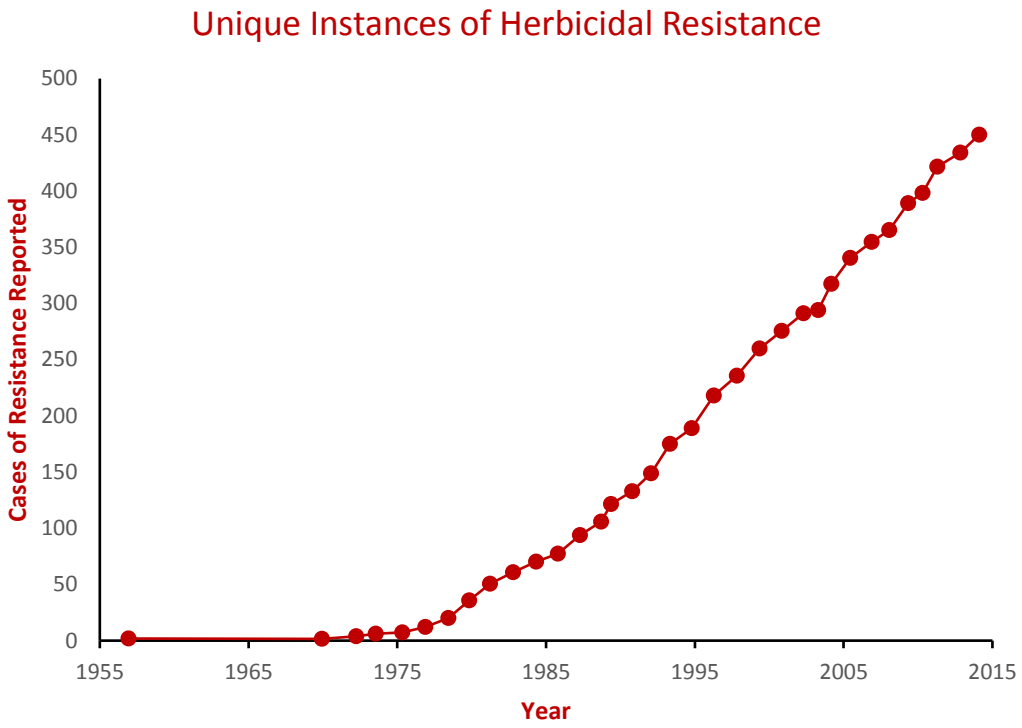


Figure 2. Unique instances of herbicidal resistance reported globally each year.[7]

This is not limited to outdated or niche agrichemicals, resistance to compounds with global use and importance has also been reported. Glyphosate is the world's most commonly used agrichemical, and is used predominantly for broad-spectrum weed control.[8] First introduced in 1974, no cases of glyphosate-resistant weed strains had been reported by 1994. Since then, the number of instances of glyphosate-resistant weeds has increased significantly.[9] In addition to these cases of resistance to single agrichemicals, weed strains are increasingly exhibiting multiple resistances. Nearly one hundred weed strains with resistance to two important classes of herbicide have been reported, and resistance to as many as ten classes of herbicide has been observed in the same weed strain.[7] To maintain our current ability to cheaply produce a wide variety of crops in high yield and feed a burgeoning world population, new herbicides with novel modes of action are a necessity. Concerningly, the commercialisation of new target sites has been a rarity over the last twenty-five years. Instead most new herbicides entering the market during that period were new molecules but of established classes of herbicide, resulting in a reduction of the number of tools available to tackle resistant weed strains.[10]

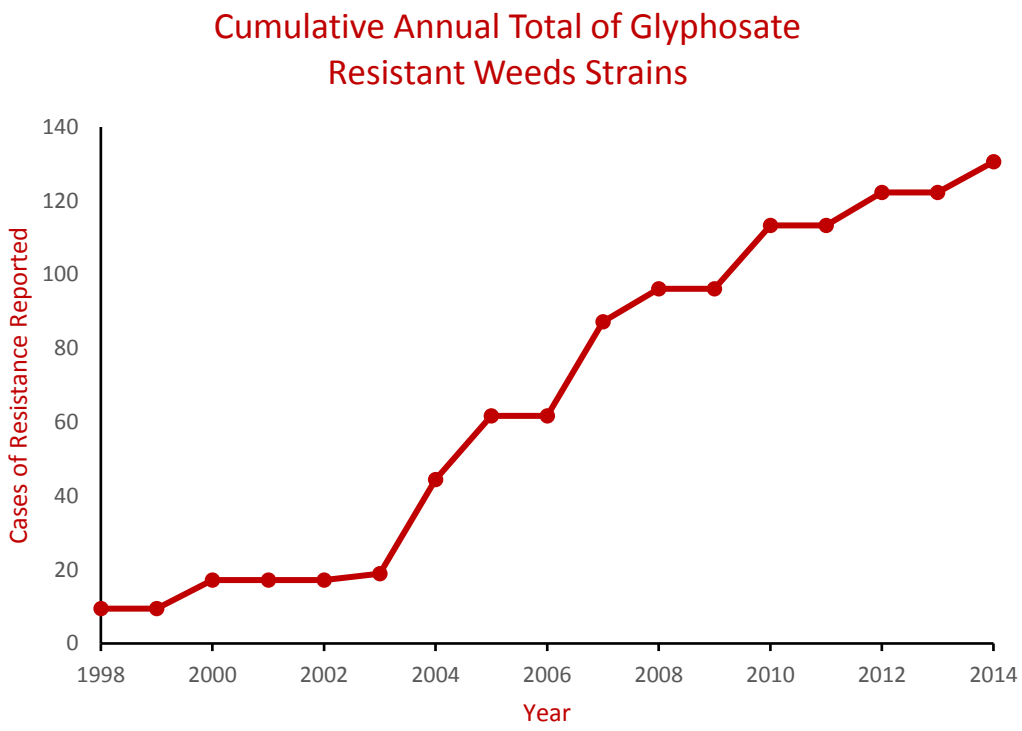
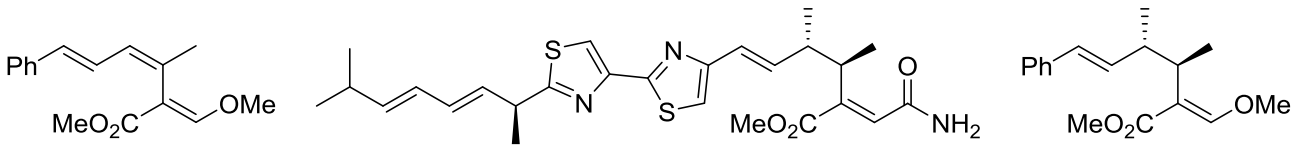


Figure 3. Reported resistance to glyphosate 1998-2014.[9]

1.2 Modern Agrichemical Development

The process of developing new herbicides in many ways mirrors modern drug discovery, with two main approaches to the problem. If a potential weakness can be identified in a weed strain, such as a key enzyme to inhibit, then screening against this can identify a lead fragment for further elaboration into a herbicide. Alternatively, bioactive natural products can be identified, which allows development without necessarily the need to understand the precise mode of action. Where drug discovery and agrichemical research differ however, is how widely they make use of natural product leads. Whilst nearly 80% of today's pharmaceuticals were derived from natural products, in 2004 just 11% of agricultural pesticides (by global sales) were developed in a similar fashion. This points to untapped potential in natural products to inspire new herbicides and pesticides.[10]

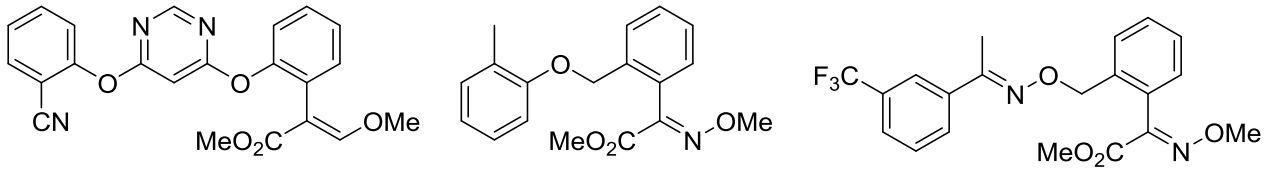
The strobilurin class of fungicides is one example of the discovery of a natural product leading to highly successful agrichemicals.[11] Strobilurin A, myxothiazol A and oudemansin A are naturally occurring β-methoxyacrylates produced by a range of fungi, particularly *Basidiomycete* wood-rotting strains. It was determined that their fungicidal activity originates from the binding of the strobilurins to the Q_o site of cytochrome b – therefore inhibiting mitochondrial respiration. Inhibition in this manner was previously unreported, and importantly toxicity testing for strobilurin A indicated it was significantly less toxic towards mice than fungi.[11] Therefore, the strobilurins appeared to be a class of compound offering novel, selective fungicidal activity.



Strobilurin A **Myxothiazol A** **Oudemansin A**

Figure 4. Naturally occurring strobilurins[11]

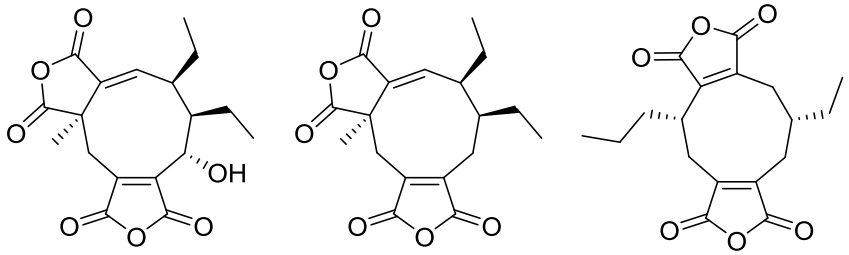
Researchers at Zeneca, Novartis and BASF separately identified strobilurin A and oudemansin A as promising candidates for development into a commercial fungicide.[12] Strobilurin A in particular was not directly suitable for agricultural use, due to both volatility and photolability.[13] Therefore, structures which increased stability, potency, and lending themselves to facile synthesis were desired. Structure-activity relationships were used to optimise the lead structures. Quickly, the β-methoxyacrylate moiety, found in each of the natural products, was identified as the key pharmacophore. Each group pursued a slightly different activity profile, and therefore arrived at different final compounds. This has led to several commercial strobilurin fungicides, sales of which in 1999 totalled $620m.[11]



Azoxystrobin (Zeneca) **Kresoxim-methyl (BASF)** **Trifloxystrobin (Novartis)**

Figure 5. Commercially available strobilurin fungicides[14]

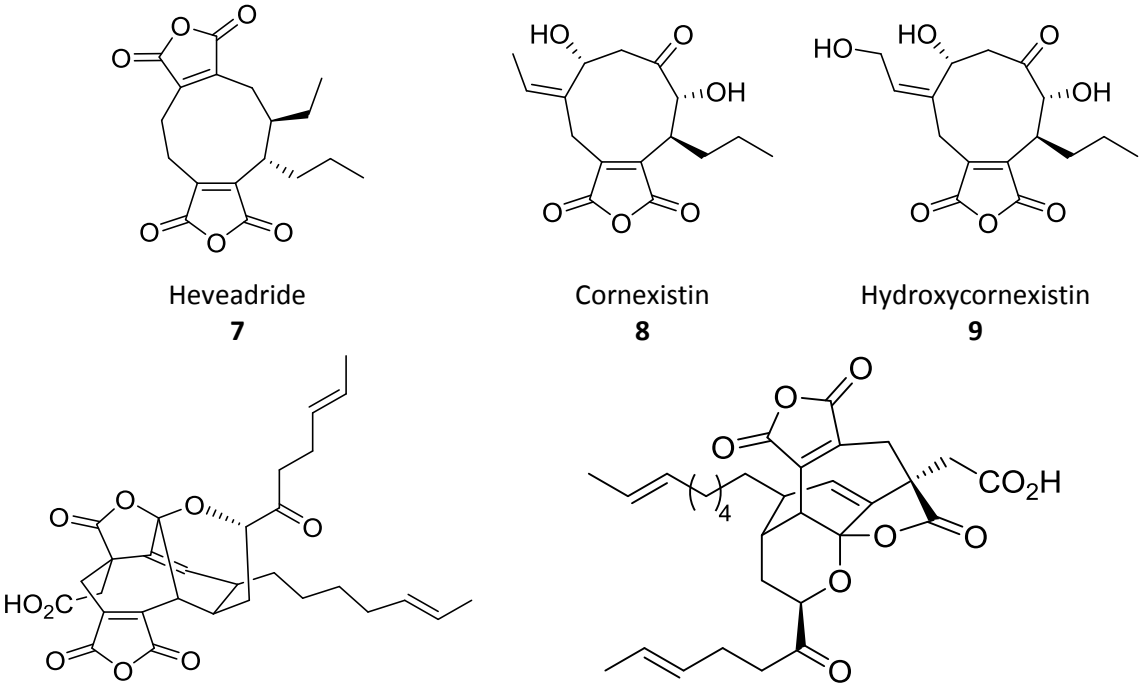
1.3 The Maleidrides



Glauconic acid **4** | Glaucanic acid **5** | Byssochlamic acid **6**

Figure 6. Structures of the earliest reported nonadrides.[15]

One class of fungal metabolite that shows promising biological activity is that of the maleidrides, which are characterised by a C_7-C_9 carbocyclic ring, fused to one or more cyclic anhydride moieties.[16] The first natural products of this class were isolated in 1931 by Wijkman and coworkers from *Penicillium glaucum*, and thus named glauconic acid **4** and glaucanic acid **5** (Figure 6).[17] The isolation of byssochlamic acid **6** from *Byssochlamys fulva* by Raistrick *et al.*[18] followed, with Barton and Sutherland coining the term "nonadrides" for these natural products, due to their common C_9 carbocyclic ring.[19]



Heveadride **7** | Cornexistin **8** | Hydroxycornexistin **9**

Rubratoxin B **10** | Phomoidride B **11**

Figure 7. Structures of maleidride natural products with medically and agrichemically useful bioactivity.[16]

Many other nonadrides have since been isolated from fungi, and have frequently shown interesting biological activity, of both pharmaceutical and agrichemical importance. One such example is heveadride **7**.[20] (Figure 7), and its dihydro- variant, which shows antifungal activity against a variety of human pathogens, such as *Aspergillus fumigatus*.[15,21] Aspergillosis is a leading cause of death in immunocompromised patients, such as those with HIV or recipients of organ transplants. Azole antifungal agents are the most common treatment, but resistant *Aspergillus* strains are on the rise.[22] Therefore, there is a clear need for new antifungal agents in order to treat these infections. Other examples of medically-relevant nonadrides include the structurally complex rubratoxins A-C, which have been investigated as potential anti-tumour agents,[23,24] and the phomoidrides A-D, which inhibit the enzyme Ras farnesyltransferase and have been considered as lead compounds for the development of anticancer agents.[25–27]

In addition to the potential pharmaceutical applications of the phomoidrides and rubratoxins, the maleidrides have generated significant interest due to their potential applications in agriculture. Like the strobilurin family of natural products, several maleidrides show broad anti-fungal or herbicidal activity and appear to achieve this without damaging valuable crop plants.

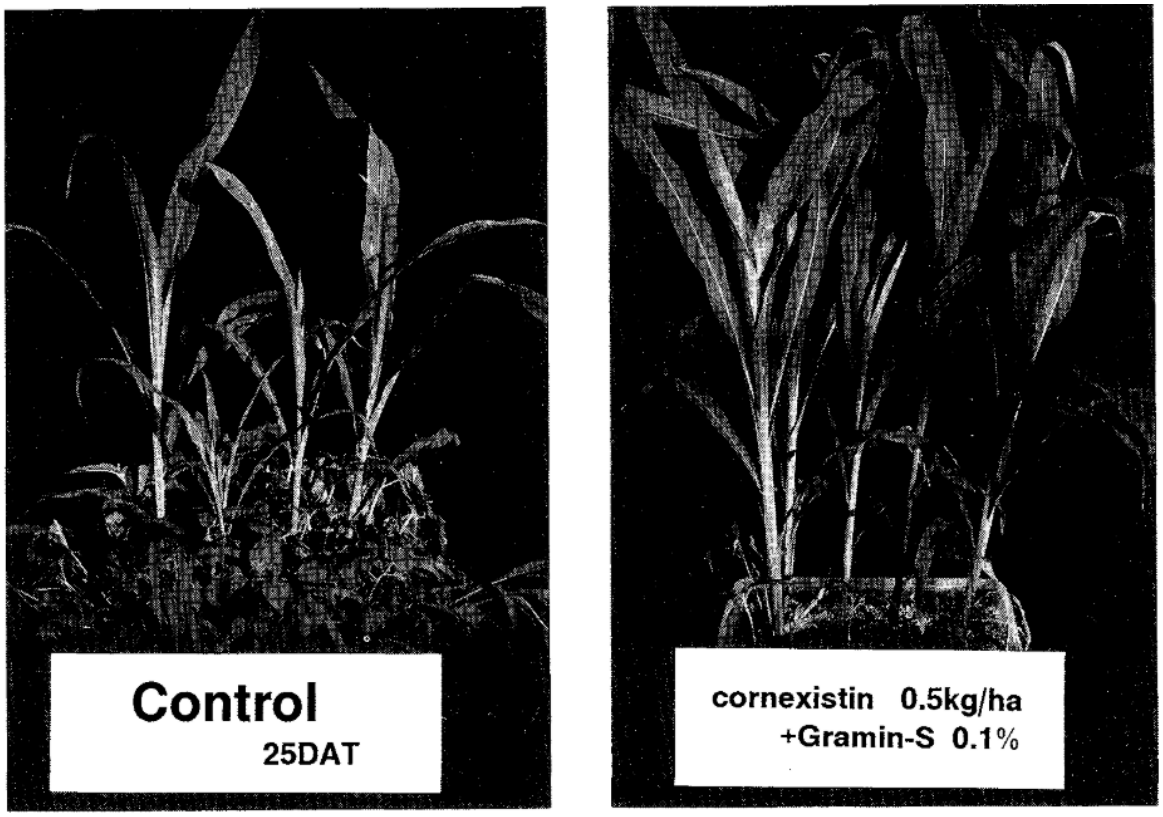
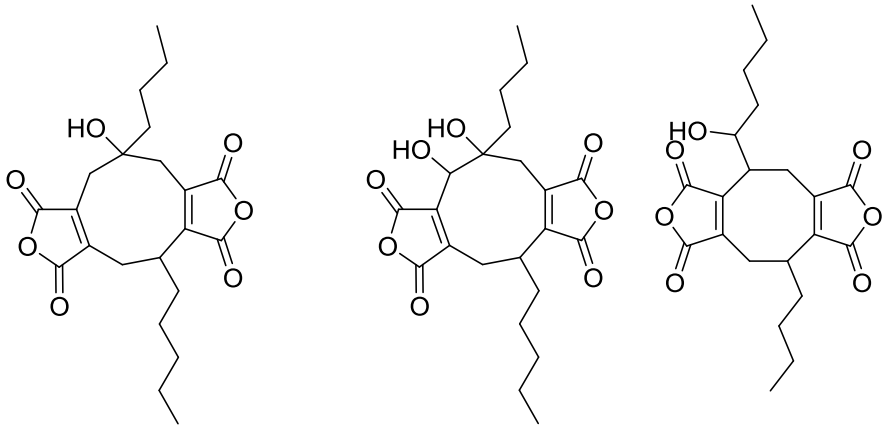


Figure 8. Growth comparison of maize plants treated with cornexistin **8** and untreated plants.[28]

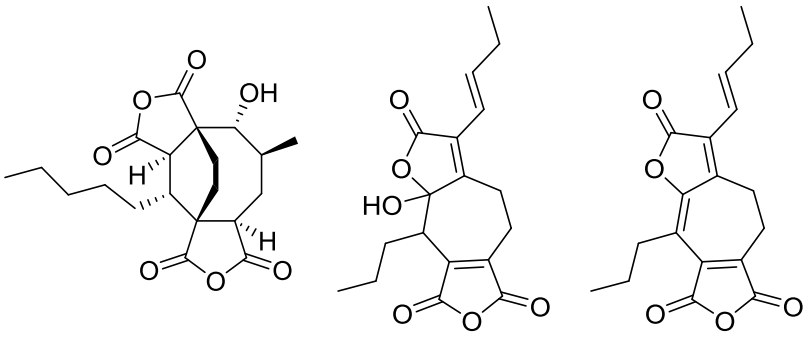
Cornexistin **8** and hydroxycornexistin **9**, isolated from *Paecilomyces variotii* SANK 21086 are two maleidrides that have been investigated further because of this activity profile.[28] Both compounds show potential as post-emergence herbicides, due to a broad spectrum of activity against both broadleaf and grass weeds, yet corn strains appear to be tolerant of both compounds. An example of this can be seen in Figure **8**. Hydroxycornexistin **9**, has been shown to be active at low concentrations against *Xanthium stumarium*, *Chenopodium album*, *Ipornoea hederaceae* and *Polygonum convolvulus*, all highly aggressive weed strains.[15] Additionally, cornexistin appears to show low toxicity in mice, with an oral LD_{50} of more than 1 g/kg. This selectivity has generated interest from the agrichemical companies Syngenta and BASF, with the latter patenting biosynthetic methods to improve titres of cornexistin and hydroxycornexistin.[29] Other maleidrides showing interesting biological activity (Figure 9) include scytalidin **12**,[30] (the subject of **Chapters Two**, **Three** and **Five**) and castaneolide **13**, a phytotoxic compound linked to chestnut black rot disease.[31]



Scytalidin **12** | Castaneolide **13** | Zopfiellin **14**

Figure 9. Structures of agrichemically-relevant maleidride natural products.[15]

Whilst the nonadrides have been known since the 1930s, their smaller ringed cousins the octadrides (containing a C_8 carbocyclic ring) were not isolated until 1996 when Nakajima and coworkers at Nissan Chemical Industries isolated zopfiellin **14** from *Zopfiella curvata*.[32] The octadride zopfiellin **14** also shows broad antimicrobial activity, and is especially effective against the plant pathogen *Botrytis cinerea*.[33] The isolation of the antifungal octadride viburspiran **15** (Figure 10) from *Cryptosporiopsis sp.* followed in 2011.[34] Following reinvestigation of the metabolite profile of *Byssochlamys fulva*, the first heptadride natural products (agnestradrides A **16** and B **17**) were reported by Simpson *et al.* in 2015. They therefore proposed the name maleidride for the larger family of natural products to encompass all these biosynthetically-related natural products.[16]

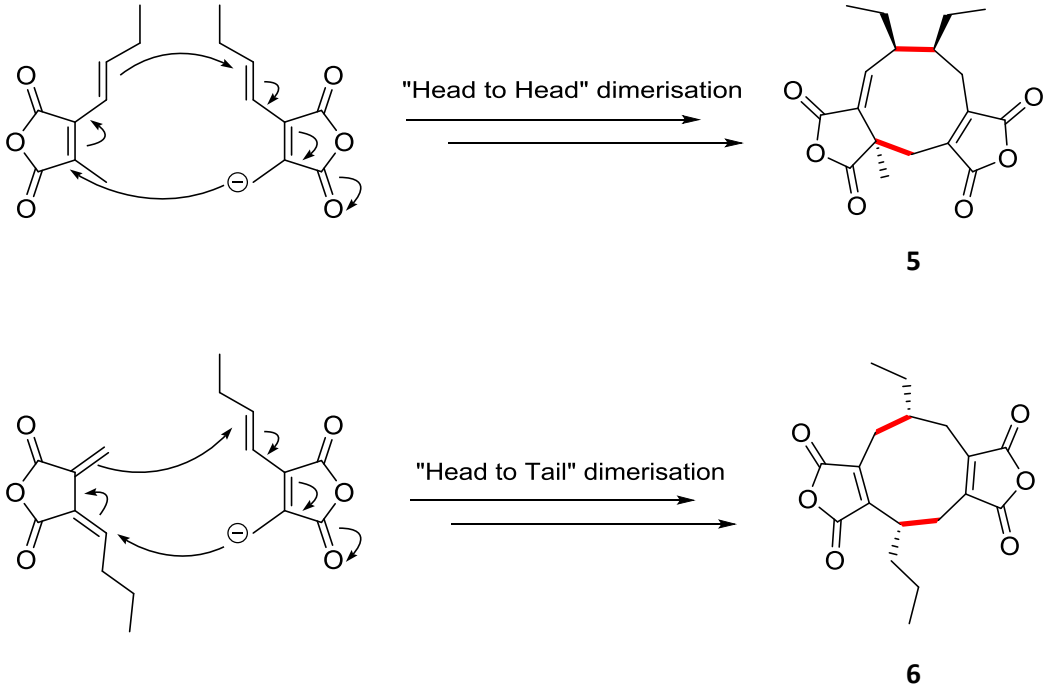


Viburspiran **15** | Agnestradride A **16** | Agnestradride B **17**

Figure 10. Structures of octadride and heptadride natural products.[16,34–36]

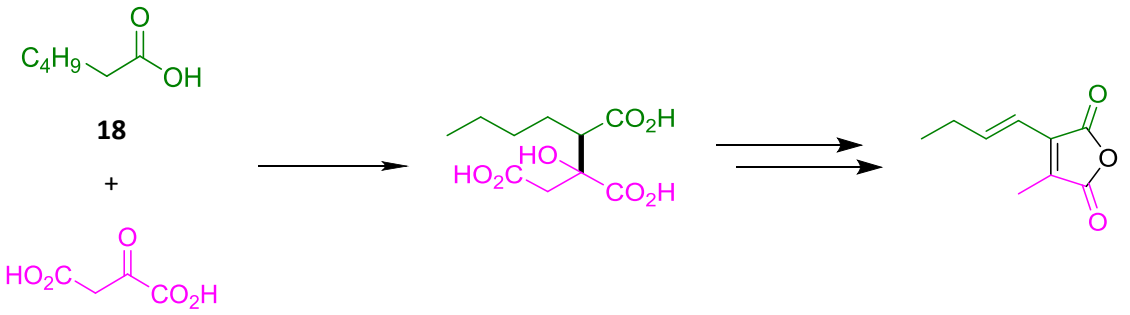
1.3.1 Maleidride Biosynthesis

Almost as soon as the first maleidrides had been isolated, work began on determining their biosynthetic origins. Barton and Sutherland proposed that glaucanic acid **5** and byssochlamic acid **6** could be formed from an anionic dimerisation of two identical C_9 fragments, shown in Scheme **1**. A "head-to-head" dimerisation would furnish glaucanic acid **5**, with byssochlamic acid **6** the result of a "head-to-tail" dimerisation involving a fulgenic anhydride isomer. The hydroxyl group in glauconic acid **4** would be the result of a pre- or post-dimerisation oxidation.[37]



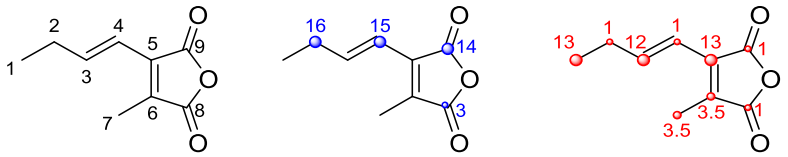
Scheme 1. Barton and Sutherland's proposed dimerisation of C_9 units to give nonadrides.[38]

Barton and Sutherland also considered the biosynthetic origins of the C_9 units, shown in Scheme **2**. They postulated that hexanoic acid **18** could condense with oxaloacetic acid **19** to yield a substituted citric acid **20**. Dehydration, decarboxylation and anhydride formation would then yield anhydride **21**, the proposed dimerisation precursor.



Scheme 2. Barton and Sutherland's hypothesised origin for the C_9 unit.[37]

Sutherland subsequently provided the first evidence in support of this hypothesis, investigating the biosynthesis of glauconic acid in *Penicillium purpurogenum*.[39] When fed with either [1-^{14}C] or [2-^{14}C]-acetate, formation of radiolabelled glauconic acid **4** was observed. Similar feeding experiments also gave the radiolabelled biosynthetic precursor **21**. The radiolabelling pattern was determined by degradation studies, using the same procedures used to first elucidate the structure of glauconic acid **4**, and other classical techniques[40–42]

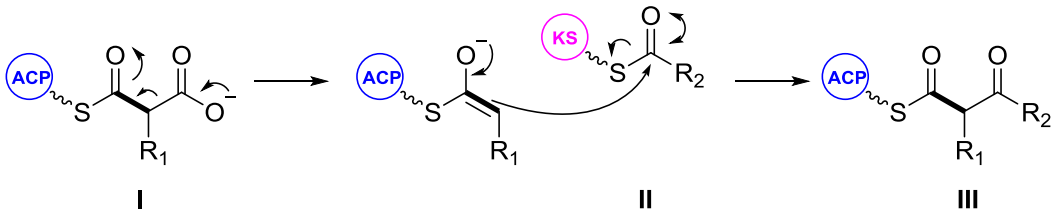


21 [1-^{14}C]-acetate fed **21** [2-^{14}C]-acetate fed **21**

Figure 11. Atom numbering for precursor **21**, and distribution of radioactivity in **21** from feeding experiments with [1-^{14}C]-acetate and [2-^{14}C]-acetate into *P. purpurogenum*.[43]

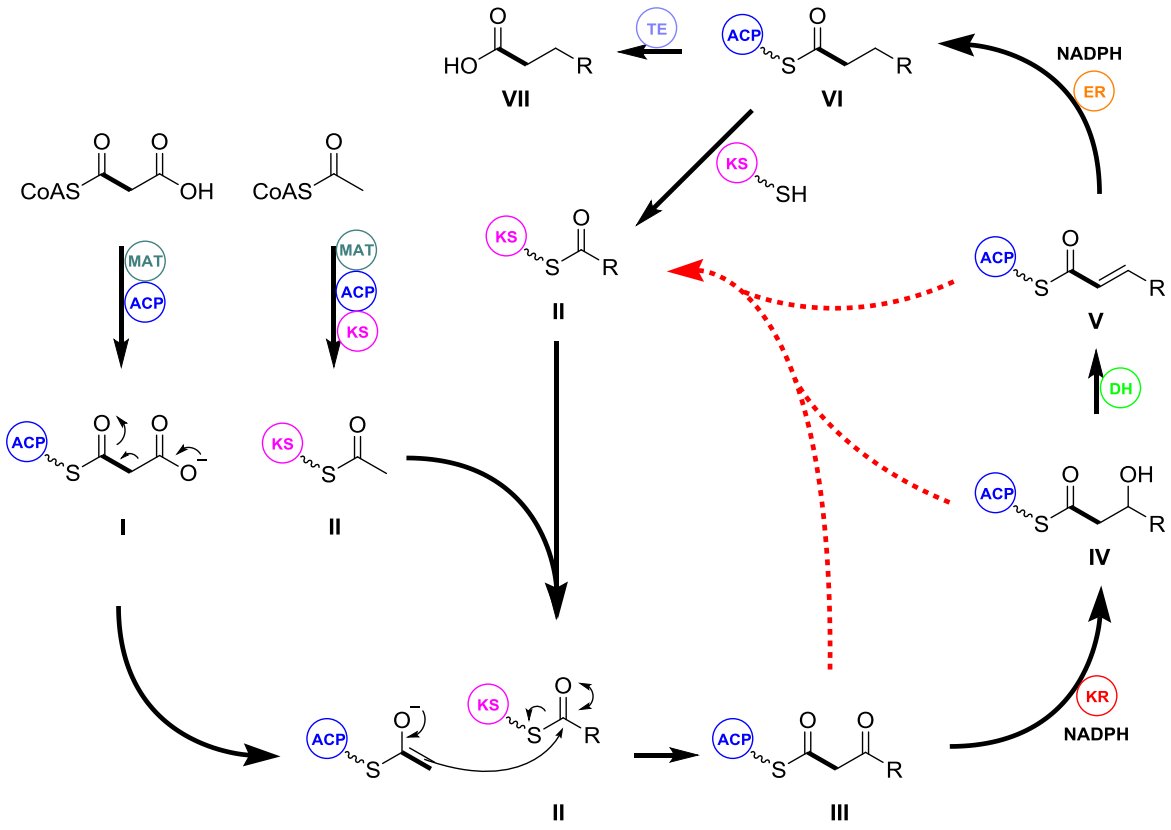
These labelling patterns, shown in Figure **11**, are consistent with the hypothesis for assembly of biosynthetic intermediate **21**, shown in Scheme **2**. Both feeding experiments support the idea that a hexanoate fragment **18** (green), constructed from intact acetates, is combined with a separate molecule (pink) for which acetate is not the primary building block. Studies into maleidride biosynthesis can now be subdivided into three parts: i) The origin of the hexanoate fragment, ii) Coupling of the hexanoate with the C_3 unit derived from oxaloacetic acid, iii) Proof of the proposed dimerisation process.

i) Hexanoate Fragment – Fatty Acid or Polyketide Origin?



Scheme 3. Decarboxylative Claisen condensation between a malonyl unit bound to an ACP and KS-bound acyl unit results in a two-carbon extension of the growing molecule. These two carbons are highlighted in bold.

The construction of the hexanoate moiety from intact acetates can be rationalised by either fatty acid or polyketide biosynthesis. Both processes utilise an enzyme-catalysed decarboxylative Claisen condensation between a malonyl unit and an acyl unit to extend a growing chain in C_2 units (Scheme **3**).[44,45] A series of reductive enzymes then act on the 1,3-dicarbonyl formed (Scheme 4). A ketoreductase (KR) catalyses the reduction of the β-ketone to a β-hydroxy group (**IV**), then dehydration by a dehydratase (DH) occurs, yielding an α-β-unsaturated thioester (**V**). This can then be further reduced by an enoyl reductase (ER), giving the fully saturated alkyl chain (**VI**). Throughout this process, the molecule remains bound to the acyl carrier protein (ACP).



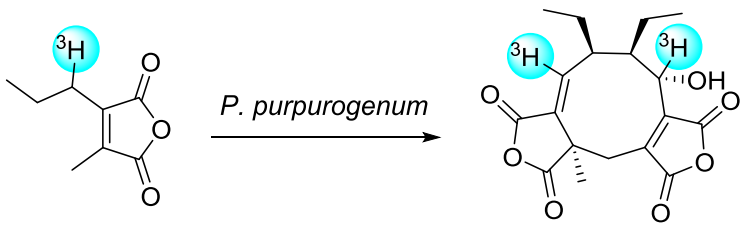
Scheme 4. Fatty acid biosynthesis (*black*) and additional steps possible in polyketide biosynthesis (*red*).

This saturated chain is then transferred from the ACP to KS (**II**), and can then undergo successive cycles of elongation and reduction until the required chain length is reached. Finally, the chain is cleaved by a thioesterase (TE), releasing the free acid (**VII**).

In fatty acid biosynthesis, each enzyme is always active, resulting in fully saturated *n*-alkyl carboxylic acids. A polyketide synthase however can bypass reductive steps so that a ketone, alcohol, alkene or methylene remain in the growing chain. In addition, polyketide synthases make use of a wider array of starter and extender units, enabling a great variety of substituents to be introduced with each round of chain elongation.

In the case of maleidride biosynthesis, it can now be seen that should the C_6 fragment arise from fatty acid biosynthesis, an additional reductive tailoring enzyme would be necessary to install the unsaturation seen in **21**. However if a polyketide synthase were to produce this C_6 fragment, the unsaturation could be formed in this process.

Early evidence favouring a polyketide synthase origin also came from feeding studies in *P. purpurogenum*. Very low levels of ^{14}C incorporation were reported when *P. purpurogenum* was fed with [1-^{14}C]-hexanoate. This would suggest that this is not a true precursor to glauconic acid, and can only be incorporated after the hexanoate is metabolised to give labelled acetate. This was further supported by studies with the tritiated substrate **22**. The glauconic acid isolated from cultures fed with **22** showed the expected radiolabelling pattern to support the proposed dimerisation, however the low levels of radioactivity suggested it may not be a true substrate for the dimerisation.



22 [4,13-$^3H_2$]-**4**

Scheme 5. Early evidence for a dimerisation comes from the feeding of a tritiated substrate by Sutherland.[39]

Definitive proof finally came when the genomic data became available. In 2015, Oikawa and coworkers identified a biosynthetic gene cluster in *ATCC 74256* responsible for phomoidride A production (Figure **12**).[46] The gene cluster contained a highly-reducing iterative polyketide synthase (hrPKS) containing the standard KS, AT, DH, *C*-methyl transferase (MT, considered inactive), ER, KR and ACP domains.[47] This was shown to be responsible for producing the fatty acyl chain, rather than a fatty acid synthase. Similarly, the biosynthetic gene cluster responsible for byssochlamic acid **6** production in *B. fulva* also contained a polyketide synthase.[48]

Figure 12. Biosynthetic gene cluster identified in *ATCC 74256* responsible for phomoidride A biosynthesis.[46] The PKS, ACDH and ACS domains are highly conserved in maleidride biosynthesis.

ii) C_3 Unit Derived From Oxaloacetic Acid

Returning to the results from labelling studies in *P. purpurogenum* shown in Figure **11**, the low level, broadly equal degree of incorporation shown at C-6 and C-7 supports the theory that oxaloacetate is the source of the three-carbon fragment. Through the citric acid cycle, there is constant conversion within the fungal culture of acetate to oxaloacetate (Figure **13**).

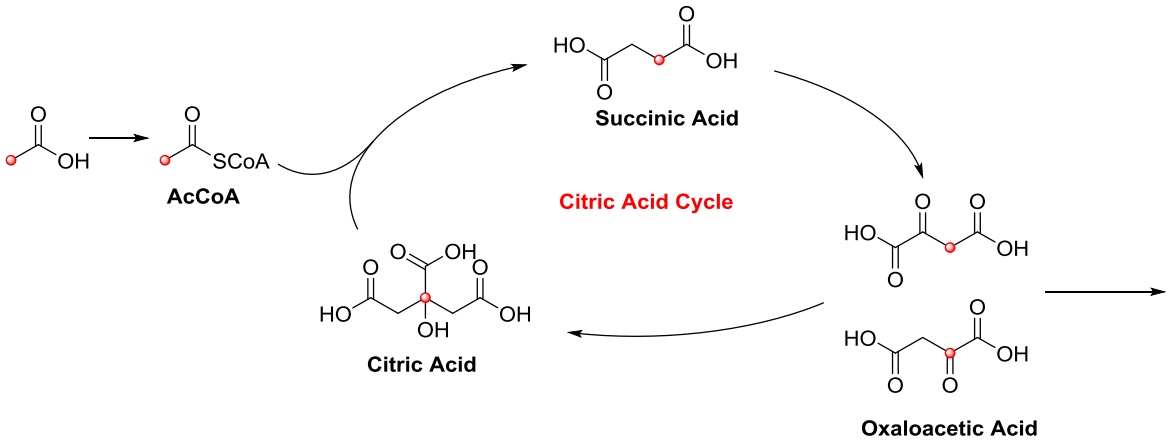
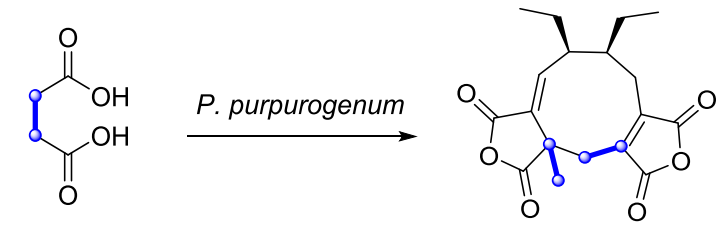


Figure 13. The citric acid (Krebs) cycle converts labelled acetate into two labelled oxaloacetic acids. (A simplified citric acid cycle is shown, highlighting key intermediates only)

When [^{14}C]-labelled acetate is added to the fermentation broth, this will be converted to [^{14}C]-oxaloacetic acid. This process proceeds via C_2-symmetric succinic acid, therefore the ^{14}C label can be positioned on either the methylene carbon or the carbonyl carbon of oxaloacetic acid. These carbons are the origins of C-6 and C-7 in **21**. Therefore, it is completely consistent with the hypothesis that equal levels of incorporation at C-6 and C-7 would be observed, and that the ability of the fungus to contribute non-labelled intermediates (such as pyruvate and succinate) to the citric acid cycle would result in lower overall incorporation. This was further supported by follow-up feeding experiments with [2-^{14}C]glucose (a pyruvate source) and [2,3-$^{14}C_2$]-succinate.



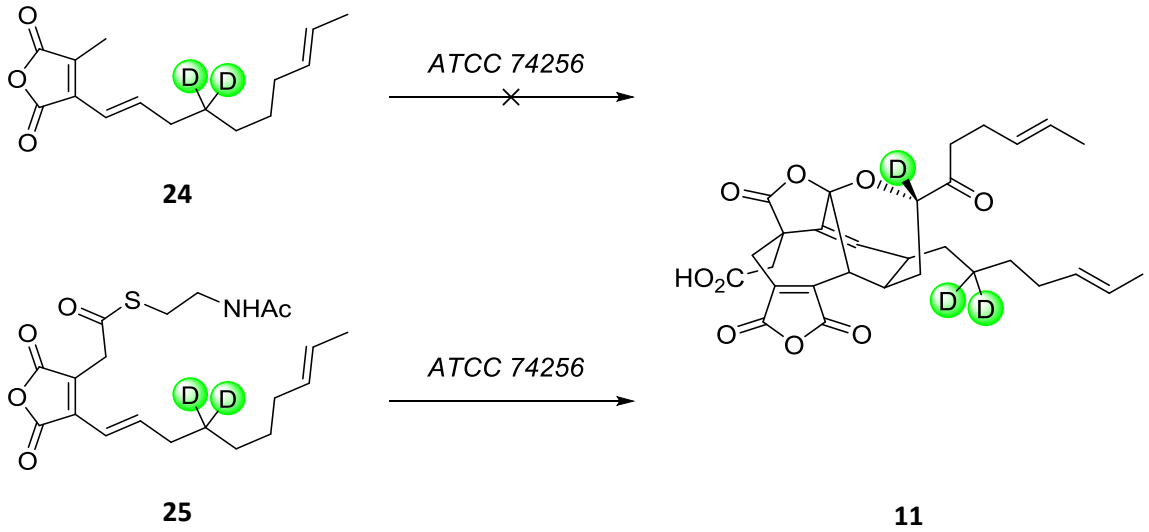
[2,3-$^{13}C_2$]-**23** [6,7,15,16-$^{13}C_4$]-**5**

Scheme 6. Incorporation of ^{13}C labelled succinate into glaucanic acid.[49]

Whilst radiolabelling had provided persuasive evidence that Barton and Sutherland's first biosynthetic hypothesis was broadly correct, further proof came from NMR studies from Holker.[49] Feeding [2,3-$^{13}C_2$]succinate **23** to *P. purpurogenum* resulted in incorporation of two intact [2,3-$^{13}C_2$] units, as predicted by the proposed biosynthesis (Scheme **2**). This further demonstrated that the C-6 and C-7 carbons of the dimerisation precursor **21** originate from oxaloacetate, and also confirm that a dimerisation is taking place. Further support for this general hypothesis for maleidride biosynthesis comes from the work of Tamm and coworkers with the rubratoxins, whereby further consistent results were obtained from labelling experiments.[50]

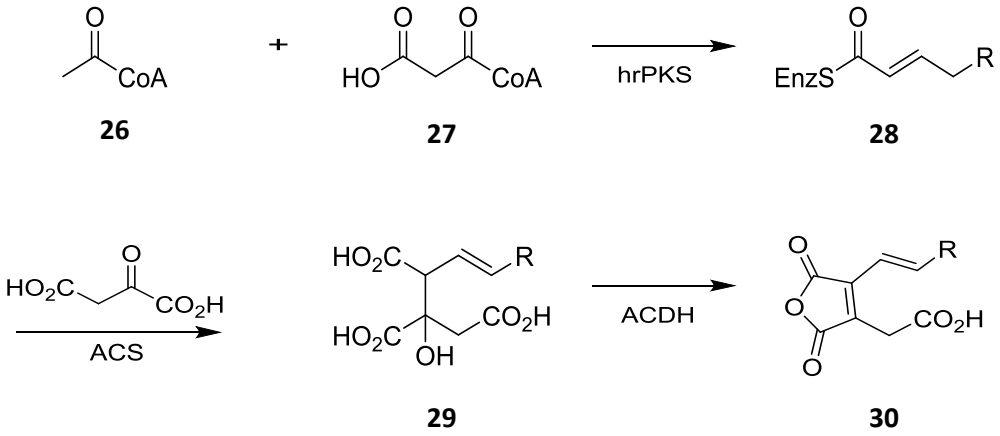
iii) Support for the Dimerisation Hypothesis

Studies towards the biosynthesis of the phomoidrides gave further insight into the exact nature of the dimerisation precursor. The labelled substrate **24** was fed to the phomoidride producing fungus *ATCC 74256* by Sulikowski and coworkers.[51] Interestingly, and in contrast to early results from Sutherland in *P. purpurogenum*, the vinylmethyl substrate (**24,** Scheme **7**) failed to produce any labelled phomoidride B **11**. Instead, when the SNAC (*N*-acetylcysteamine) derivative (**25**) was used, labelled phomoidride B **11** was produced. The labelling pattern was consistent with a dimerisation, but this experiment also strongly suggested that a carboxyl functionality was required for the dimerisation step. The SNAC derivative was necessary as the parent carboxylic acid spontaneously decarboxylates.[16,52] The timing of the decarboxylation necessary to produce the phomoidrides could not however be determined in this labelling study.



Scheme 7. Labelling studies in *ATCC 74256* demonstrate dimerisation to form phomoidrides requires a carboxylated substrate.[25]

More recently, Oikawa and coworkers reported the hrPKS responsible for phomidride production, as well as identifying an alkylcitrate synthase (ACS) enzyme, and an alkylcitrate dehydratase (ACDH) in *ATCC 74256* (Figure **12**).[46] Heterologous expression in *Aspergillus oryzae* of the hrPKS, ACS and ACDH was then shown to result in production of the decarboxylated dimerisation precursor **21**. These genes were also found to be highly conserved in the byssochlamic acid **6** producer *Byssochlamys fulva*, allowing a general biogenesis of the dimerisation precursor for maleidride natural products to be proposed.[48] Shown in Scheme **8**, a hrPKS catalyses the decarboxylative Claisen condensation between an acetyl-CoA **26** starter unit and sufficient malonyl-CoA extender units **27** to produce the appropriate enzyme-bound polyketide unit **28**. This species then undergoes condensation with oxaloacetic acid to yield **29** (there is some discrepancy as to whether a thioesterase cleaves the polyketide from the ACP first or not), before the dehydratase generates **30**.



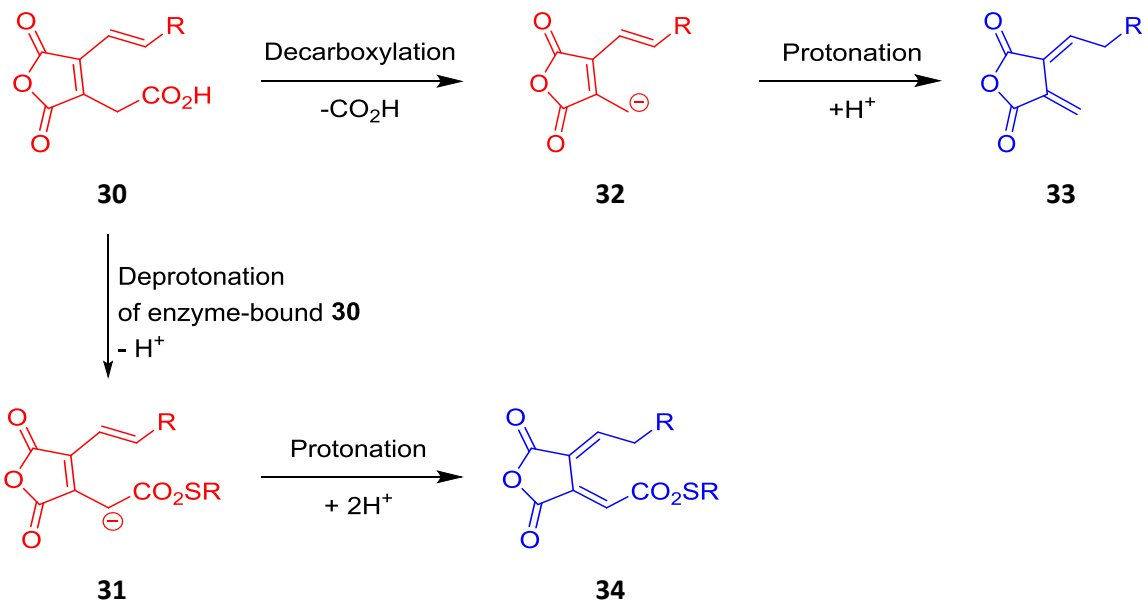
Scheme 8. Generalised biosynthesis of the nonadride dimerisation precursor

It has been shown that carboxylic acid **30** can undergo spontaneous decarboxylation, to yield the *exo*-methylene intermediate **32** (Scheme **9**) required for the dimerisation.[16] However, it is not yet

clear if the ACDH acts on the open diacid or succinic anhydride form of **29**, or whether decarboxylation of **30** occurs pre-dimerisation (thus generating the necessary anion), or post-dimerisation (with a thioester serving the purpose of reducing the pKa so deprotonation is more easily achieved).

In 2016, the genes responsible for the dimerisation step were identified by our group, who then showed that maleidride biosynthesis could be recreated in its entirety in a heterologous host, *Aspergillus oryzae*.[48] Four genes in *Byssochlamys fulva* that play essential roles in dimerisation were identified, putatively as ketosteroid isomerase-like proteins and phosphatidylethanolamine-binding proteins. Further research is required to establish their individual roles in this process.

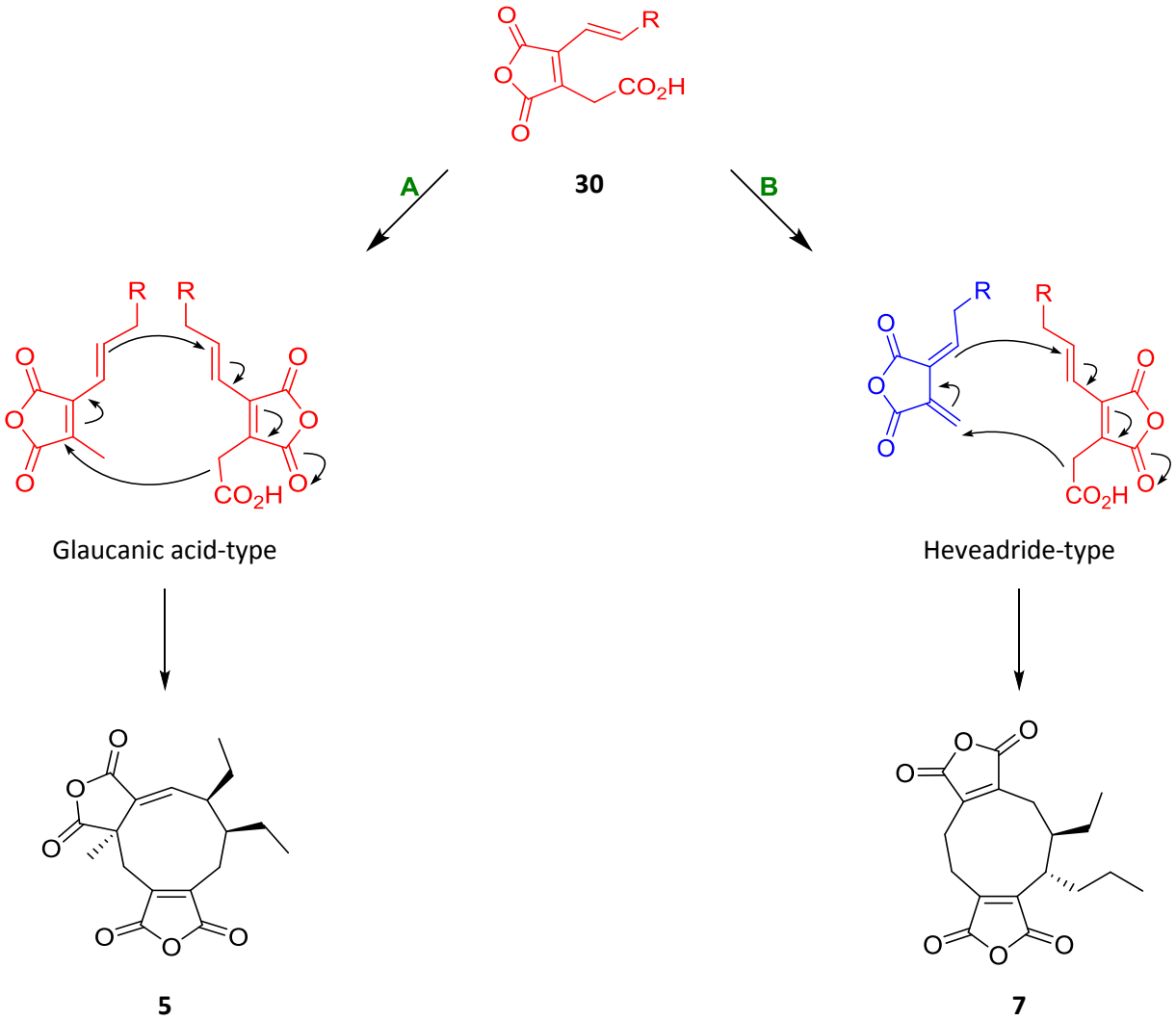
It is proposed that anhydride **30** is a common intermediate in the synthesis of all maleidride natural products. As previously stated, this is either deprotonated to yield **31**, or decarboxylates to **32** (Scheme **9**).[46,48] Either of these structures can be re-protonated to give the alkylfulgide moiety (**33** or **34**), the dimerisation partner required in the synthesis of maleidrides such as byssochlamic acid **6** or heveadride **7**.



Scheme 9. Possible route from anhydride **30** to the two intermediates necessary for all four dimerisation pathways. It is unclear as to which pathway predominates, and at which stage enzymes are involved.

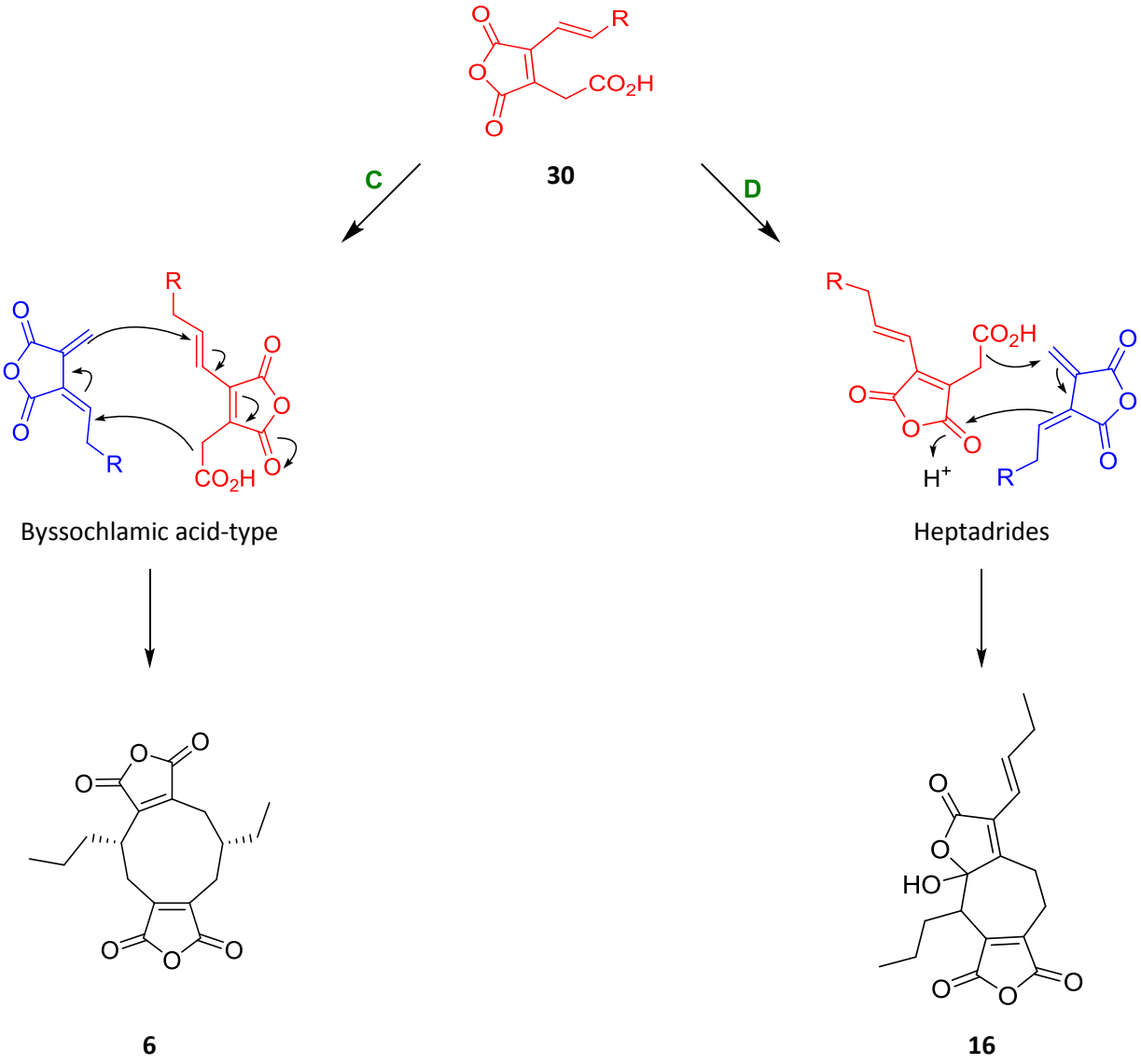
Generalised dimerisation pathways have now been proposed by both our group[48] and Oikawa[46], to account for the formation of maleidrides resembling glauconic acid **4**, byssochlamic acid **6**, heveadride **7** and the heptadride natural products (Schemes **10** and **11**). Each arise from the combination of maleic anhydride **30** and the alkylfulgide **33** in different fashions. In pathway **A**, the combination of two maleic anhydride **30** units in a "head-to-head" fashion gives glaucanic acid-type

natural products. In pathway **B**, a similar "head-to-head" arrangement, but of a maleic anhydride **30** and alkylfulgide **33**, gives rise to hevadride-type natural products.



Scheme 10. Dimerisation pathways leading to glaucanic acid **5** and heveadride **7**.

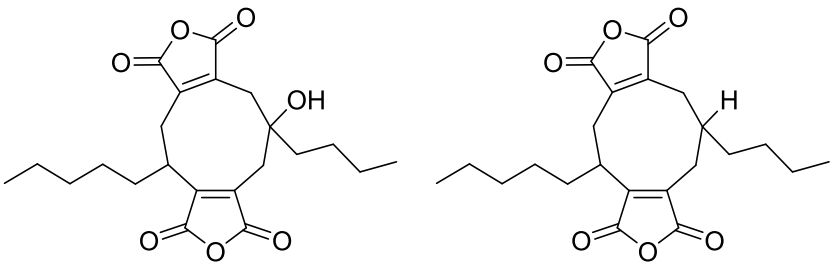
The same combination of monomers, arranged in a "head-to-tail" fashion (pathway **C,** Scheme 11) gives byssochlamic acid **6**-type maleidrides. Finally, the recently isolated agnestradrides (**16** and **17**), also produced by *Byssochlamys fulva*, require a different proposal.[16] Pathway **D** shows a "side-on" dimerisation from which these heptadrides arise.



Scheme 11. Proposed dimerisation pathways for maleidride sub-classes.[48]

1.4 Aims

Further work on the biosynthesis of maleidrides is currently ongoing in the Willis and Cox groups, in particular in relation to the maleidrides arising from a "head-to-tail" dimerisation, namely byssochlamic acid **6**, and the natural products isolated from *Scytalidium album*, scytalidin **12** and deoxyscytalidin **35**.[30]

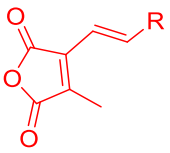


Scytalidin **12**

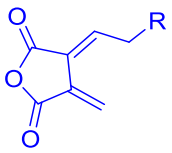
Deoxyscytalidin **35**

Figure 14. Scytalidin **12** and deoxyscytalidin **35**, key natural products investigated in this research programme.

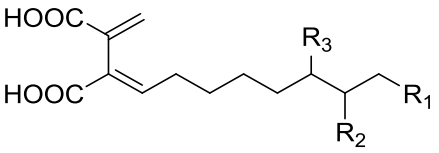
In order to explore the biosynthesis of the scytalidins, it was first necessary to establish culture conditions in which *S. album* produces these natural products. At the start of the project, there was no information available on the stereochemistry of scytalidin **12** and deoxyscytalidin **35**. Therefore, an early aim of this project was to isolate both natural products and determine their relative and absolute stereochemistry through a combination of NMR spectroscopy and X-ray crystallography. This work is detailed in **Chapter Two**.



Maleic Anhydride Monomer **32**



Alkylfulgide Monomer **33**



Tricladic acid A **36**, R_2 = OH, R_1 = R_3 = H

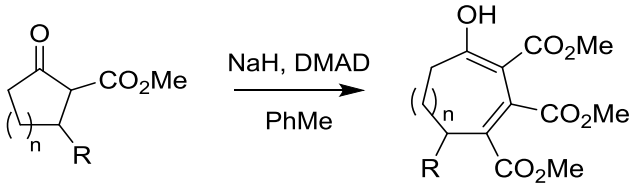
Tricladic acid B **37**, R_3 = OH, R_1 = R_2 = H

Tricladic acid C **38**, R_1 = OH, R_2 = R_3 = H

Figure 15. Structures of the monomers required for the study of the dimerisation process, and tricladic acids A-C **36**-**38**.[53]

Secondly, it was proposed to investigate the dimerisation process leading to deoxyscytalidin **35** *in vitro*, to establish whether a biomimetic synthesis of deoxyscytalidin **35** can be achieved. To do so, synthetic routes to the maleic anhydride **32** and alkylfulgide **33** were required. En route, this methodology was to be applied to the synthesis of the tricladic acids A-C **36**-**38**, fungicidal natural products produced by *Tricladium castaneicola*.[53] This work is detailed in **Chapters Three and Four**.

Finally, the dimerisation approach investigated would not be appropriate for the synthesis of scytalidin **12**, owing to the additional tertiary alcohol. Currently, no total synthesis of this natural product has been published, and so a further aim of this project was to establish a *de novo* synthesis of scytalidin **12**, using the ring expansion of β-ketoesters. This is described in **Chapter Five**.



39 **40**

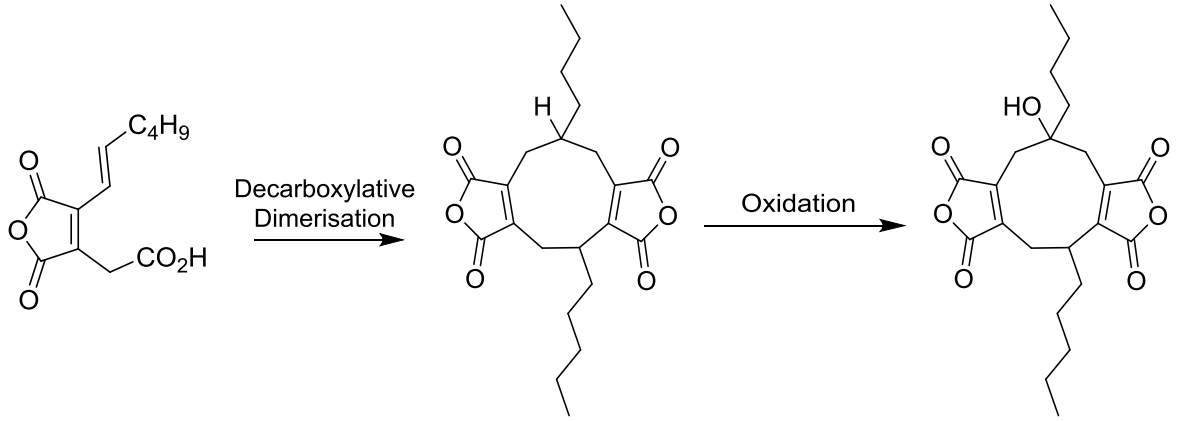
Scheme 12. Key ring expansion reaction in the proposed synthesis of scytalidin **12**.

CHAPTER 2: Scytalidin and Deoxyscytalidin: Isolation and Structure Elucidation

2. Scytalidin and Deoxyscytalidin: Isolation and Structural Elucidation

2.1 Introduction

First isolated by Stillwell and coworkers in 1972, scytalidin **12** is a nonadride natural product produced by *Scytalidium album*.[30,54] Whilst there have been no previous studies on the biosynthesis of scytalidin, based on the structural similarity to byssochlamic acid **6**, it is not unreasonable to assume a similar biosynthesis. Therefore, it was hypothesised (Scheme **13**) that scytalidin **12** arises from a "head-to-tail", byssochlamic acid-like decarboxylative dimerisation of two C_{12} anhydride units **41**, followed by an oxidation step.[51] The isolation of the deoxy analogue of scytalidin **35** by Ayer and coworkers provides some support in favour of this proposal.[55] Neither the relative nor absolute stereochemistry of scytalidin **12** or deoxyscytalidin **35** have previously been determined, and no total synthesis has been reported.[15]



Scheme 13. Proposed biosynthesis of scytalidin

Interest in scytalidin as a fungicide stems from the observation by Ricard and Bollen that *Scytalidium* species demonstrate marked *in vitro* inhibition of *Poria carbonica* growth.[56] This result was replicated using the isolated natural product by Stillwell, who also studied the effect of scytalidin **12** against 61 fungal strains, with activity found against 52 of these (Table **1**).[54] These fungal strains are predominantly associated with decay and deterioration of coniferous trees and wood products. Of particular interest is the activity displayed against *C. ulmi*, a causative agent of Dutch Elm Disease.[57] The reference compound nystatin is a broad spectrum fungicide, widely used in humans and present on the World Health Organisation's model list of essential medication.[58]

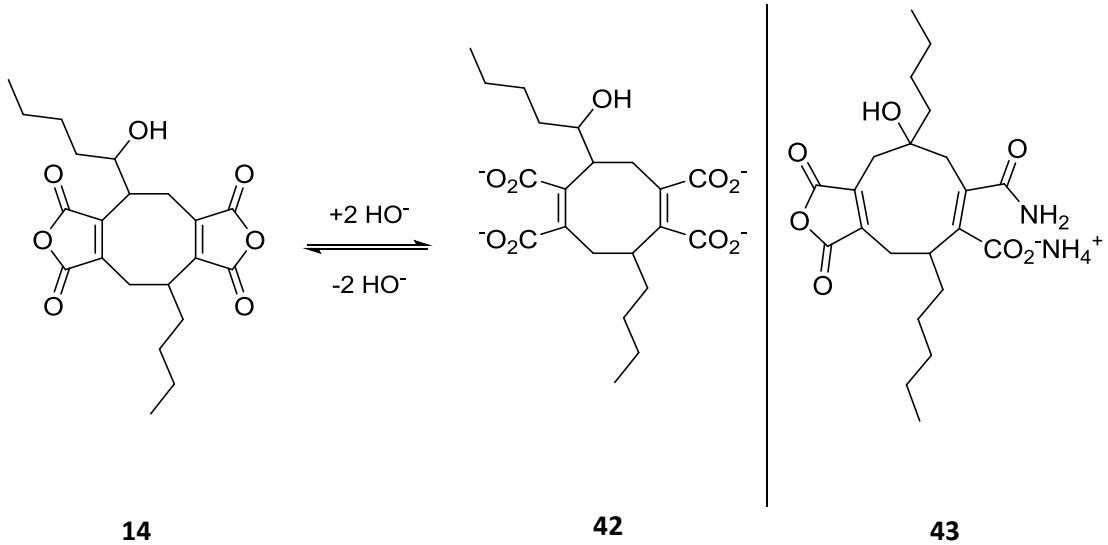
Of equal importance to the broad antifungal profile reported, was the observation by Strunz and Stillwell that even at 1000 ppm concentrations, scytalidin **12** had no detectable inhibition of seed germination in a variety of higher plants. This is a favourable trait in a prospective agrichemical, and

mirrors the findings in studies on the cornexistins (**8** and **9**). This led to the suggestion that scytalidin **12** may be an appropriate seed-treatment agent, to prevent fungal infection prior to planting.

Test organism	Width of inhibition zone / mm		
	Nystatin 100 μg/disc	Scytalidin 100 μg/disc	Scytalidin 50 μg/disc
Coniophora puteana	7	10	10
Fomes pini	5	11	10
Peniophora gigantea	20	16	15
Polyporus balsameus	17	15	12
Poria carbonica	9	14	11
Cephaloascus fragrans	12	21	18
Ceratocystis ulmi	8	9	9
Phytophthora infestans	9	11	10
Phytophthora lateralis	8	15	12
Chrysosporium sp.	3	12	9

Table 1. Antifungal activity screen of scytalidin **12**, in comparison to nystatin.[30]

It was also observed that the inhibition of *C. ulmi* by scytalidin **12** was strongly pH dependent, with over a hundredfold increase in the required concentration to inhibit growth at pH 8.5 compared to pH 3.5. This is in agreement with observations from Futagawa *et al*. that the octadride zopfiellin **14** shows considerably lower potency at higher pH, with this likely to be related to the ring opening of the anhydride moieties to the tetracarboxylic acid form.[33]



14 **42** **43**

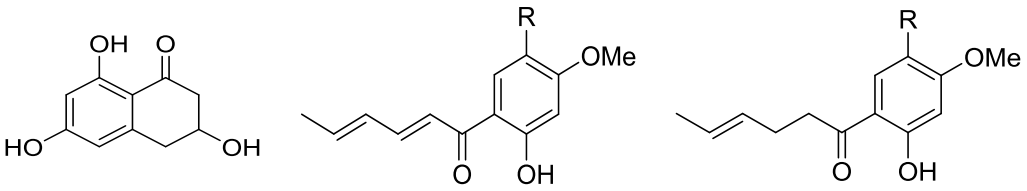
Scheme 14. Bis(anhydride) **14** and tetracarboxylate **42** forms of zopfiellin, as reported by Futagawa.[33] Monoammonium scytalidimate **43** (all four possible isomers are reported).[59]

This pH effect, combined with low water solubility led Strunz and coworkers to investigate a modification that improved solubility whilst retaining the fungitoxic activity of scytalidin. They achieved this by the reaction of scytalidin with liquid ammonia, yielding crudely defined "monoammonium scytalidamates".[59] Unfortunately, the planned biological evaluation of **43** has not been reported in the literature.

Prior to undertaking further investigation into both the biosynthesis and biological activity of scytalidin **12** and deoxyscytalidin **35**, the first goal was to determine the relative and absolute stereochemistry of both natural products. Do the side chains have the *cis*-arrangement observed in byssochlamic acid **6**? Do the side chains in scytalidin and deoxyscytalidin have the same stereochemistry? Knowledge of the stereochemistry of the natural products would be important in the design of a route for a total synthesis.

2.2 Results and Discussion

As well as scytalidin and deoxyscytalidin, several other natural products from *S. album* have been isolated and characterised. Findlay and coworkers isolated scytalone **44**, which appears to be an unrelated polyketide natural product, whilst Oberlies *et al*. reported eleven new compounds from a strain of *S. album*.[60,61] The structures include eight sorbicillinoids (of type **45** and **46**), two phthalides (**47** and **48**) and isosclerone **49**.



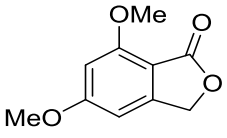
44

45

A: R= CHO
B: R= COOH
C: R= CH_2OH
D: R= CH_3

46

A: R= CHO
B: R= COOMe
C: R= CH_2OH
D: R= CH_3



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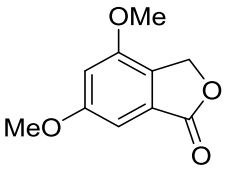
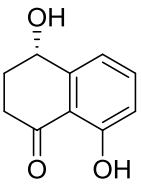


48



49

Figure 16. Additional natural products isolated from *S. album*.

Ayers reported that scytalidin was not isolated when *S. album* was grown on solid media, and all eleven natural products reported by Oberlies come from the growth of *S. album* on solid media.

Therefore, when surveying conditions for optimal titres of scytalidin, it was clear that surveying only liquid media would be prudent.

2.2.1 Optimisation of *S. album* Growth Conditions

The two strains of *S. album* used by Strunz and Stillwell in their initial isolation work were UAMH 3620 (ATCC 16675, FY strain) and UAMH 3611 (ATCC 22476, ex-type).[30,55] These were both purchased from the University of Alberta Microfungus collection and Herbarium (UAMH). The samples supplied were heavily contaminated with bacteria, but treatment with broad-spectrum antibacterial agents by Dr Zhongsu Song in our group enabled clean samples of the fungi to be obtained.

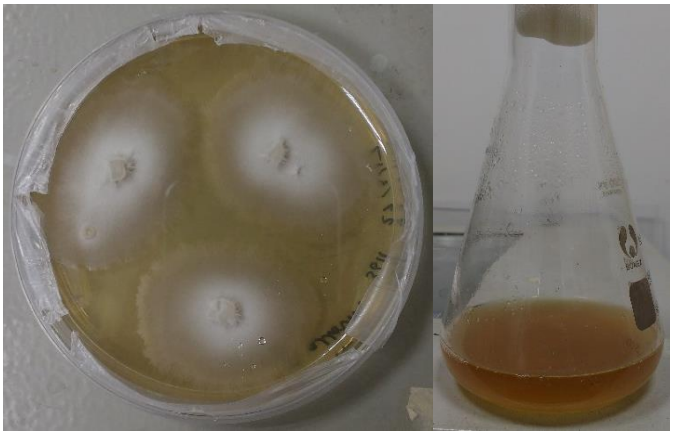


Figure 17. *S. album* UAMH 3611 strain grown on MEA (*left*) and in MEB (*right*).

The UAMH 3611 and UAMH 3620 samples were first grown upon plates of Malt Extract Agar (MEA). It was found that UAMH 3611 sporulated when grown on MEA, and so this gave three cell sources for inoculation into liquid media. Initially, 4 cm^3 portions of the agar plate were transferred into Malt Extract Broth (MEB) or Potato Dextrose Broth (PDB), and grown for 17 days. Then, the mycelia and the broth were separated, to see whether the natural product accumulates in the mycelia, or whether it is excreted by the fungus. The aqueous media was directly extracted with ethyl acetate, whilst the mycelia were blended in acetone, concentrated *in vacuo* and then a similar separation performed with ethyl acetate and water.

Syngenta provided a 150 µg sample of scytalidin **12** for use as an HPLC standard which assisted identification of the natural product within crude extracts.

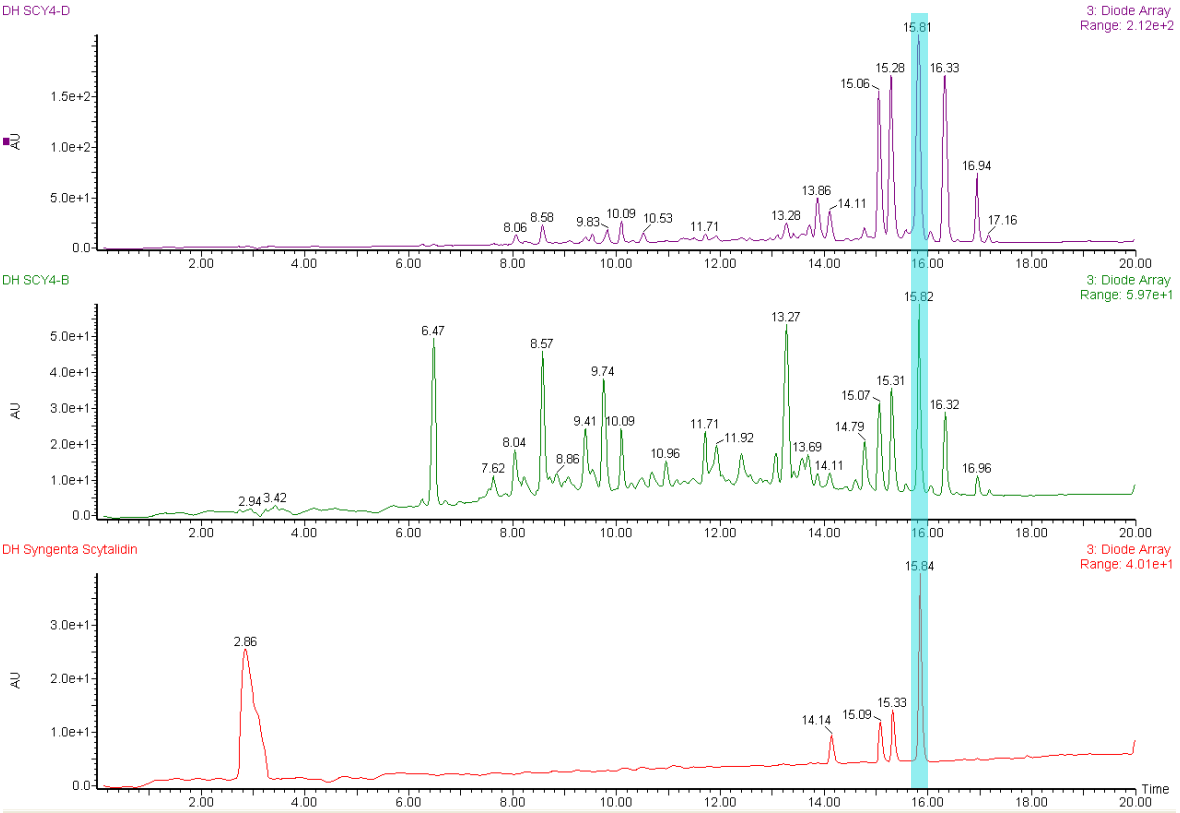


Figure 18. HPLC traces for UAMH3620 grown on PDB. Mycelial extract (*top*), aqueous media extract (*middle*), and scytalidin **12** standard provided by Syngenta (*bottom*). Scytalidin **12** peak highlighted.

Production of scytalidin was first observed in UAMH 3620 grown on PDB. As can be seen in Figure **18**, the mycelial extract gives a much better signal:noise ratio for the peak at 15.8 min than the aqueous media extracts. This peak was identified as scytalidin by comparison to the standard provided, as well as by in-line mass spectrometry (both ESI+ and ESI-). We therefore used mycelial extracts for all further experiments.

Next, the effect of baffled versus non-baffled flasks on the metabolite profile was studied. A baffled flask helps oxygenate the culture medium, and in some cases can lead to improved growth, and higher titres of highly oxygenated metabolites.[62] In this case, limited differences were observed by LCMS in the metabolite profile when UAMH 3620 was grown in baffled versus non-baffled flasks, as seen in Figure **19**.

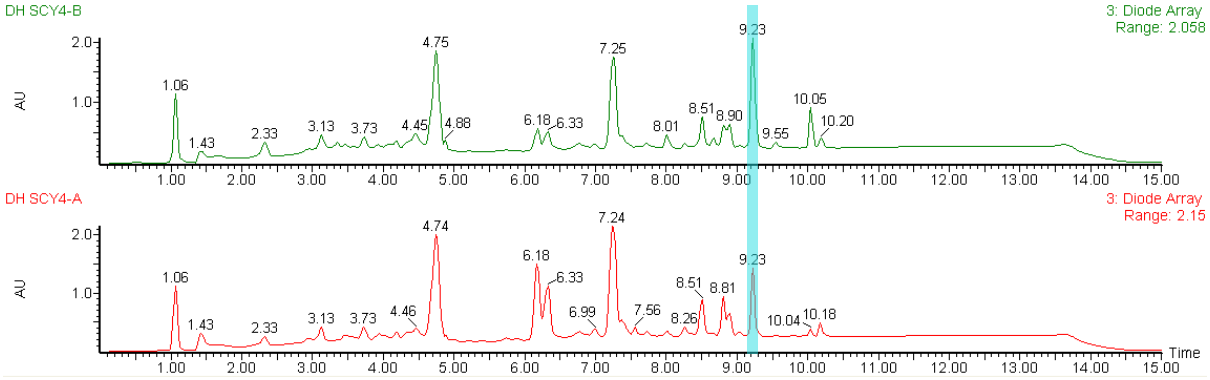


Figure 19. HPLC traces for the growth of UAMH 3620 on PDB in baffled (*top*) and non-baffled flasks (*bottom*). Scytalidin **12** peak highlighted. Note: Retention time of scytalidin is 9.23 min, as a different gradient to Figure **18** was applied.

It was observed that *S. album* appeared to grow slowly in liquid media when the mycelia established on agar was first transferred. Therefore, a liquid pre-culture was used instead. The agar was blended with media, this pre-culture then established over four days, then split across flasks of fresh media to grow for one month. Under these conditions, *S. album* appeared to grow faster in MEB, giving a greater mass of mycelia. This method was therefore used for all subsequent work.

Scytalidin could be obtained directly from the crude mycelial extract via trituration with diethyl ether, three rounds of this process yielding 10 mg/L of scytalidin in high purity. The ^{1}H NMR data (Table **2**) previously reported give wide ranges for peaks.[30] For example, all protons on the carbocylic ring of scytalidin are reported as a multiplet from 2.5-3.2 ppm. These data were insufficient for positive confirmation that our isolated material was indeed scytalidin.

Chemical Shift / ppm	Assignment
0.88	3H, t, *J*=6, CH_3
0.97	3H, t, *J*=7, CH_3
1.1-1.9	14H, m
2.5-3.2	6H, m
3.31-3.53	1H, OH

Table 2. ^{1}H NMR data for scytalidin **12** in $CDCl_3$, as reported by Strunz and Stillwell.[30]

To obtain a ^{1}H NMR spectrum of authentic scytalidin, the sample provided by Syngenta was used. With Dr Chris Williams, a ^{1}H NMR spectrum was obtained on an 80 µg aliquot of the scytalidin, using the BrisSynBio Bruker 700 microcryo instrument. A ^{13}C NMR spectrum could not be obtained owing to the small quantity of sample available. As can be seen in Figure **20**, the ^{1}H spectrum was in good agreement with that obtained from our own isolated scytalidin, and both match the chemical shifts reported.[30,63] ^{13}C NMR data also correlated well with the literature, as well as specific rotation. An specific rotation value of -57.8 (*c* 0.47, EtOAc) was obtained for our isolated scytalidin, compared to

a literature value of -66.6 (*c* 0.4745, EtOAc)].[30] This indicated the same enantiomer of scytalidin had been obtained.

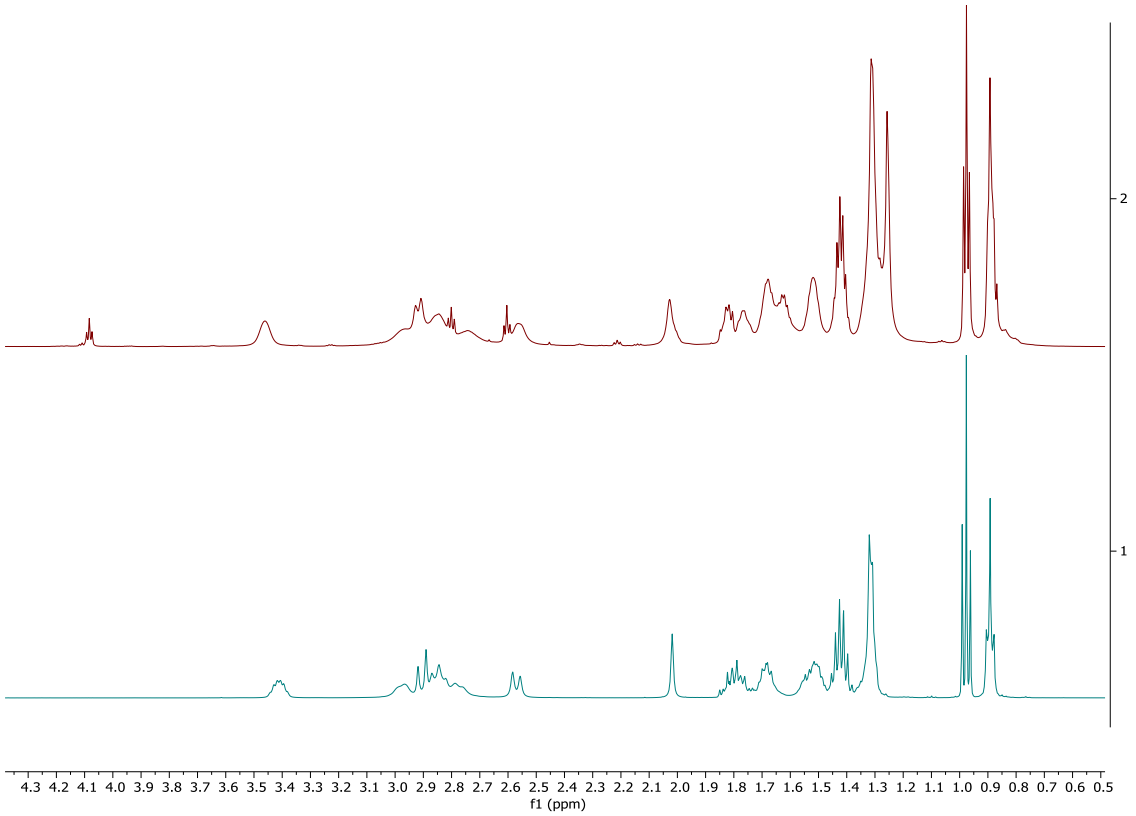


Figure 20. 700 MHz ^{1}H NMR spectrum of scytalidin **12** (*top*, sample provided by Syngenta) compared against the 400 MHz ^{1}H NMR spectrum of the matching HPLC peak in our own sample isolated from *S. album* UAMH 3620 (*bottom*).

The related maleidride deoxyscytalidin **35** was also produced by *S. album*,[55] and this was purified by Dr Claudio Greco through preparative HPLC, from the same extracts of *S. album*. As with scytalidin **12**, the NMR data obtained are consistent with published data.[55] A specific rotation value of -52.5 (*c* 0.13, $CHCl_3$) was obtained for the isolated deoxyscytalidin **35** (lit. $[\alpha]_D$ -82.2 (*c* 0.135, $CHCl_3$)).[55] The ^{1}H NMR for deoxyscytalidin **35** is very similar to the spectrum for (-)-byssochlamic acid **(–)-6**, which differs only in the length of the alkyl substituents – and for which the relative and absolute stereochemistry is known for both (-)-byssochlamic acid **(–)-6** and (+)-byssochlamic acid **(+)-6**.[64,65] This suggests that deoxyscytalidin **35** might have the same *cis*-arrangement of side chains, however this needed confirmation. No further stereochemical information could be determined from the NMR spectra, therefore X-ray crystallography was required.

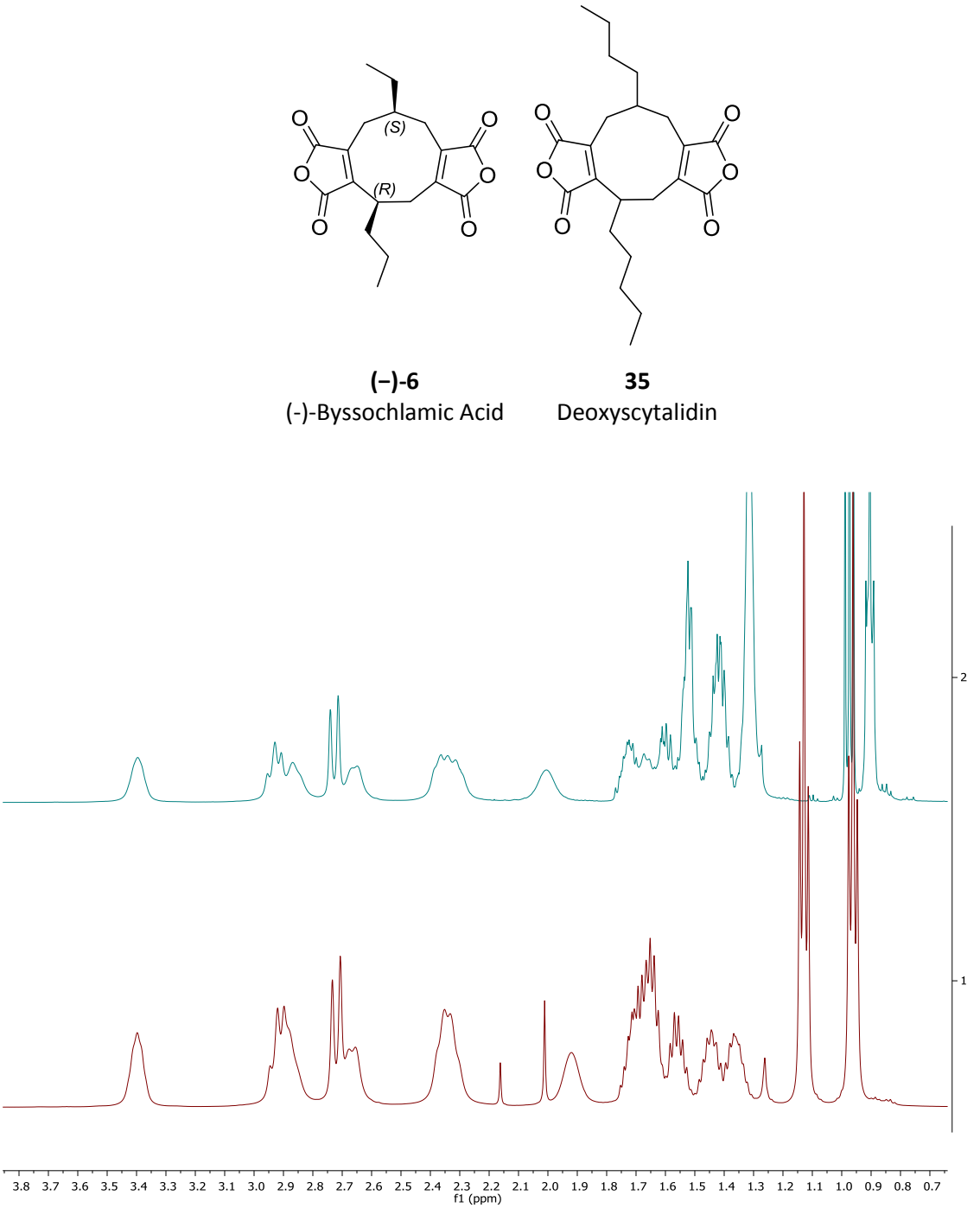
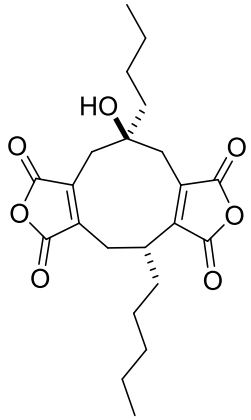
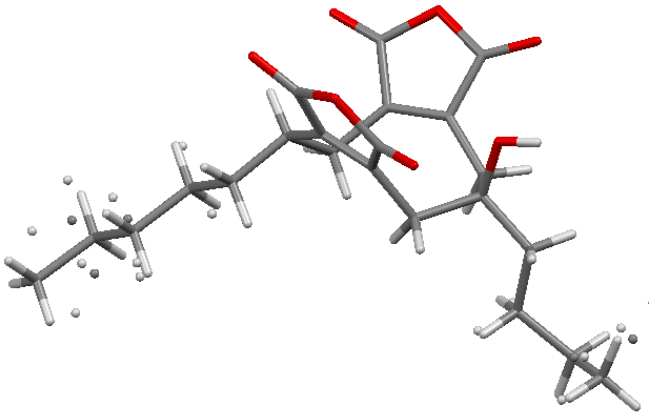


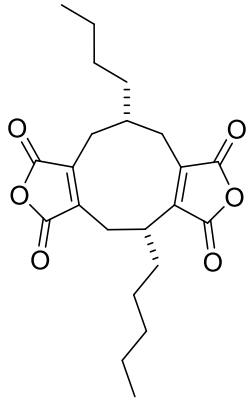
Figure 21. Structures of byssochlamic acid **(–)-6** and deoxyscytalidin **35**. ^{1}H NMR spectrum of deoxyscytalidin (*top*) and byssochlamic acid (*bottom*). Byssochlamic acid sample obtained by Agnieszka Szwalbe in our group.

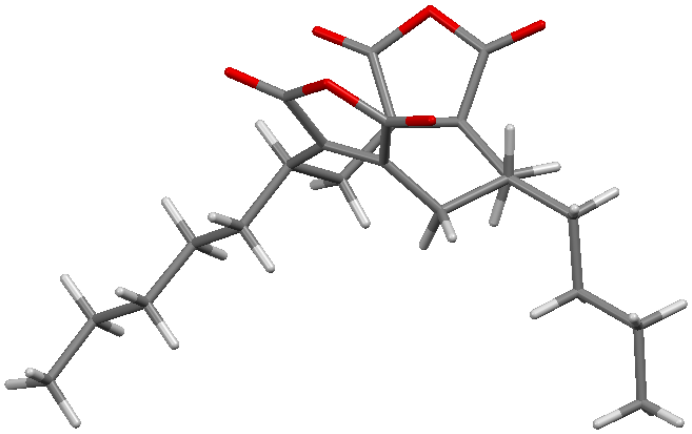
2.2.2 **X-Ray Crystallography**





12
Scytalidin

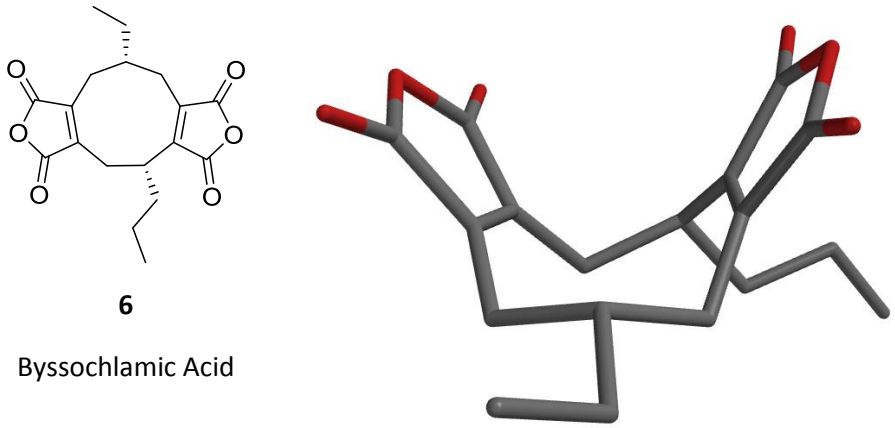




35
Deoxyscytalidin

Figure 22. Crystal structures of scytalidin **12** and deoxyscytalidin **35**, confirming their relative stereochemistries.

Crystals of scytalidin **12** and deoxyscytalidin **35** were obtained by recrystallisation from diethyl ether, from which the relative stereochemistry of both compounds was revealed by X-ray crystallography. The crystals obtained were not of sufficient quality to determine absolute stereochemistry. Scytalidin **12** and deoxyscytalidin **35** have the side chains on the same face of the ring, which adopts a bowl-shaped structure. This is fully consistent with the reported crystal structure of (-)-byssochlamic acid **(−)-6** (Figure **23**).[65]



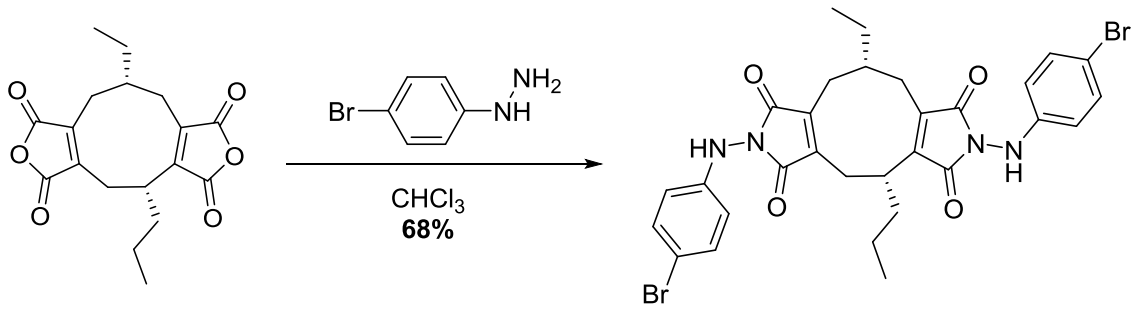
6

Byssochlamic Acid

Figure 23. Structure and crystal structure of (-)-byssochlamic acid **6**.[65]

2.2.3 Synthesis of Crystalline Derivatives

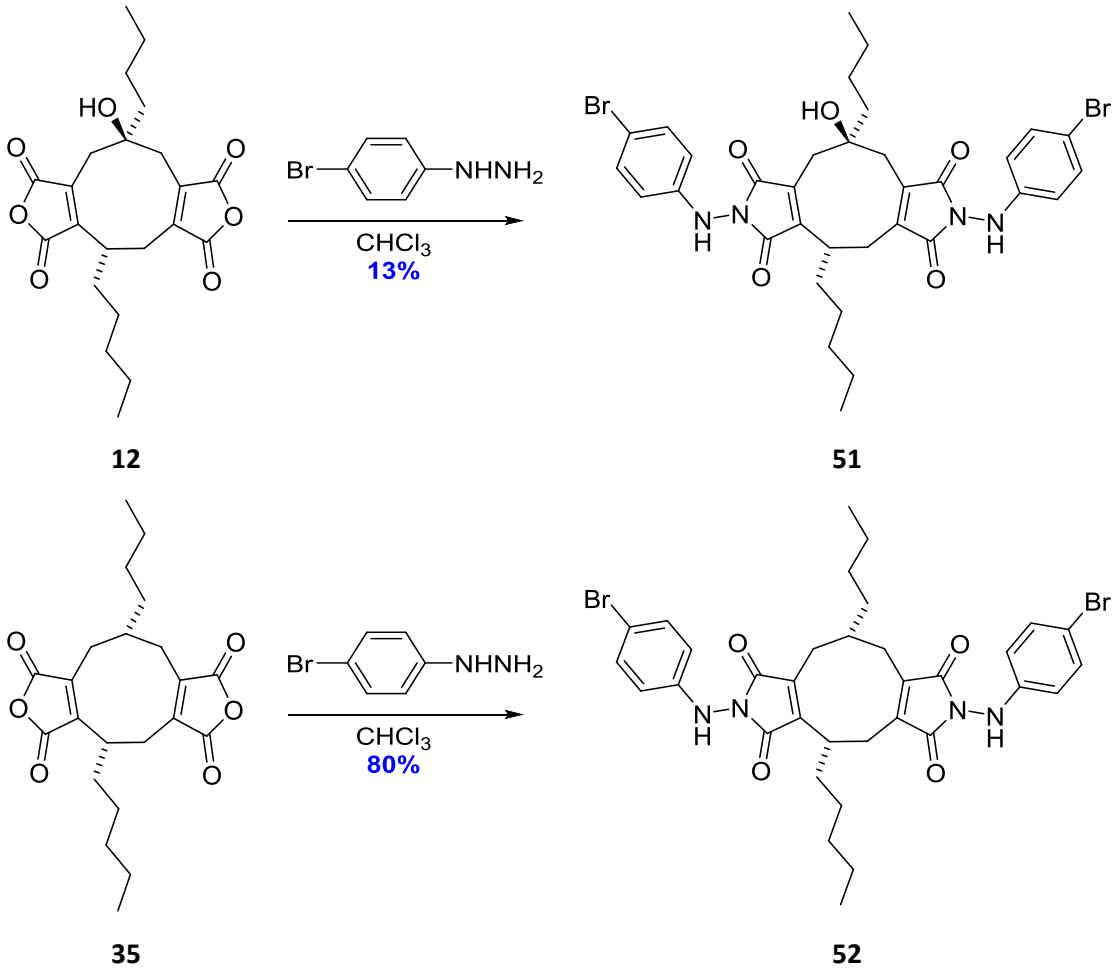
In order to investigate the absolute configurations of scytalidin **12** and deoxyscytalidin **35**, a series of derivatives was prepared, and crystallisation studies undertaken. This strategy has been previously applied to maleidride natural products, as Barton and Sutherland synthesised the bis(*p*-bromophenylhydrazyl)malemide derivative of byssochlamic acid **6** to aid the elucidation of the absolute stereochemistry by X-ray crystallography.[66] This gave a crystalline sample, with the incorporation of a heavy atom aiding X-ray crystallography.[67,68] This approach was also used by Sim to obtain absolute stereochemistry of (-)-byssochlamic acid.[65]



6 **50**

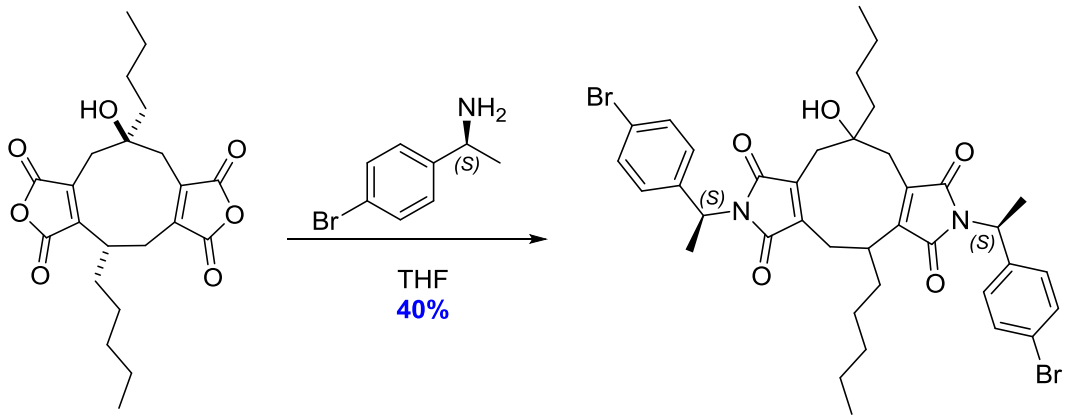
Scheme 15. Sim's **s**ynthesis of a crystalline derivative of (-)-byssochlamic acid in order to determine absolute stereochemistry.[65]

The equivalent *bis*(*p*-bromophenylhydrazyl)malemides were synthesised from scytalidin **12** and deoxyscytalidin **35** by treatment with 4-bromophenylhydrazine in $CHCl_3$, yielding **51** and **52** in 13% and 80% yield respectively (Scheme **16**). Unfortunately, in both cases the product was a brown oil, and crystallisation was not possible.



Scheme 16. Synthesis of *bis*(4-bromophenylhydrazide) derivatives of scytalidin **12** and deoxyscytalidin **35**.

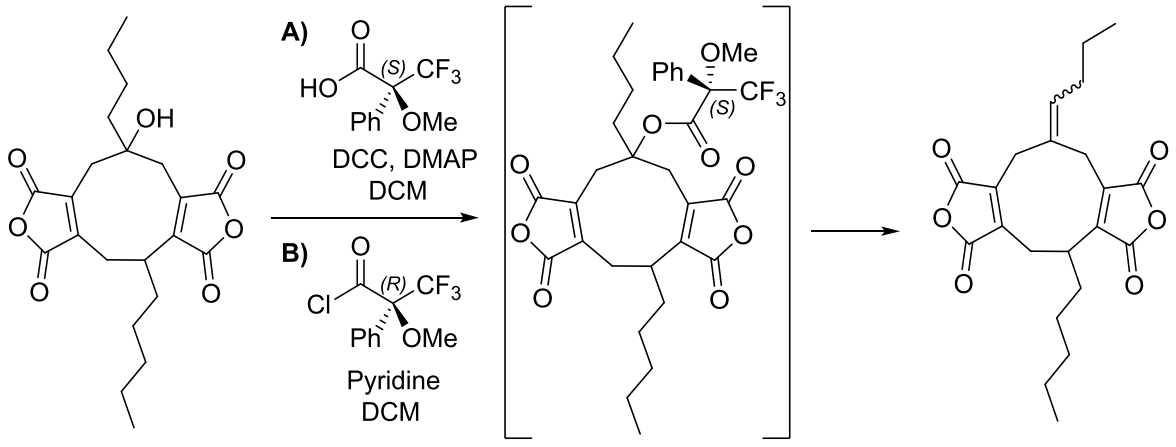
Next, introduction of an asymmetric centre of known absolute stereochemistry was investigated. Determination of absolute stereochemistry by X-ray crystallography requires high-quality crystals, which could not be obtained for either natural product. A derivative with a known stereocentre means absolute stereochemistry can be inferred without the need for such high-quality crystals. To this end, scytalidin **12** was derivatised with (*S*)-4-bromo-α-methylbenzylamine, yielding **53** in 40% yield (Scheme **17**). Unfortunately, once again this derivative was not crystalline.



12 **53**

Scheme 17. Synthesis of the maleimide **53** from scytalidin **12** in order to introduce a known stereocentre.

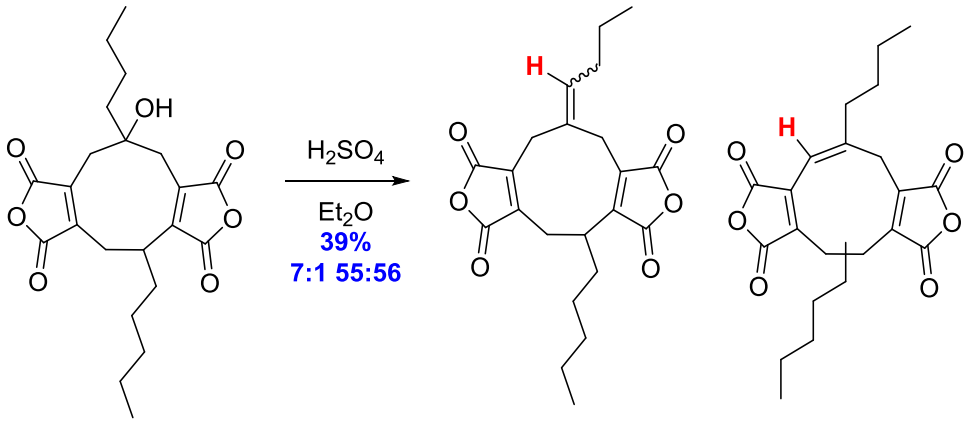
It was hoped that a known stereocentre could be introduced by derivatisation of the tertiary alcohol of scytalidin **12**. Treatment of scytalidin **12** with Mosher's acid and DCC/DMAP failed to form the desired ester **53**, instead a ^{1}H NMR spectrum of the crude reaction mixture suggested elimination of the alcohol had occurred to give a mixture of alkenes. This was confirmed by deliberate elimination of the alcohol with H_2SO_4/Et_2O, which yielded a complex mixture of alkenes in 39% yield (Scheme **18**). This mixture contained both the exocyclic alkene **55** and endocyclic alkene **56** in a 7:1 ratio, with both possible isomers of each also present. The ratio of products formed was determined through integration of the trisubstituted alkene proton, highlighted in red in Scheme **19**. Two triplets at 5.89 and 5.83 ppm (*J* 7.5 Hz) were indicative of the exocyclic alkene **55**, whilst singlets at 6.27 and 6.13 ppm result from the mixture of endocyclic alkene **56**. The elimination reaction could not be avoided when esterification of scytalidin **12** with Mosher's acid chloride was carried out.



12 **54**

Scheme 18. The Mosher's ester derivative **54** could not be synthesised from scytalidin **12**, instead elimination to a mixture of alkenes resulted.

If steric congestion was assisting the elimination reaction, it was hoped that a smaller chiral carboxylic acid might give a more favourable result. Therefore, the esterification of scytalidin **12** with (*S*)-2-bromopropanoic acid was investigated. Once again, Steglich esterification with DCC/DMAP resulted in elimination, as did esterification via the acid chloride.



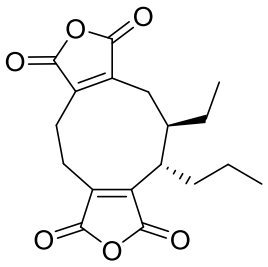
12 | **55** 2:1 ratio isomers | **56** 1:1.2 ratio regioisomers

Scheme 19. Elimination of scytalidin **12** to alkenes **55** and **56**, protons highlighted in red were used to determine the ratio of alkene products formed.

With only limited amounts of the natural products available, and no derivatives appearing more promising for crystallisation than the natural products themselves, it was decided that growing higher quality crystals of scytalidin **12** and deoxyscytalidin **35** was a better course of action. Unfortunately, the higher quality crystals necessary to determine the absolute stereochemistry could not be obtained, despite investigating a variety of crystallisation techniques (use of anti-solvents, seeding, slow evaporation, solvent layering, *vial-in-vial* techniques).[69] Promising crystals could be obtained from a DCM:Et_2O system at -18 °C – however these degraded rapidly when the temperature was raised, making X-ray diffraction unfeasible. Therefore, the absolute stereochemistry of scytalidin **12** and deoxyscytalidin **35** was investigated by other means.

2.2.4 Computational Investigations of Stereochemistry

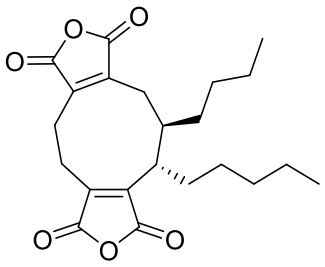
Given the structural similarity of byssochlamic acid **6** and deoxyscytalidin **35** (differing only by an additional ethylene unit in each side chain), it could be speculated that the structural change should have limited effect on the specific rotation of the natural products, and therefore from the known stereochemistry of byssochlamic acid, it would be possible to predict the absolute stereochemistry of deoxyscytalidin by a simple comparison of specific rotation values. Such an approach has previously been used to tentatively assign the absolute stereochemistry of homoheveadride **57** by comparison to the specific rotation of hevadride **7**.[70]



Heveadride

7

$[\alpha]_D^{22}$ +351 (*c*. 1.19 in CH_2Cl_2)



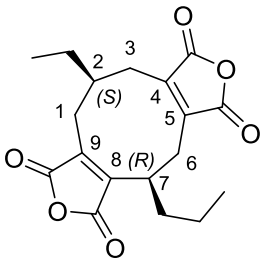
Homoheveadride

57

$[\alpha]_D^{22}$ + 118 (*c*. 0.50 in CH_2Cl_2)

Scheme 20. Comparison of structures and specific rotation values for heveadride **7** and homoheaveadride **57**.

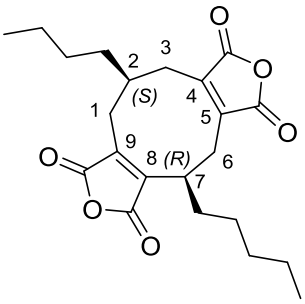
(−)-Byssochlamic acid **6** has a specific rotation of $[\alpha]_D^{25}$= -104 (*c*. 0.1, $CHCl_3$), and has been assigned (*R*)-propyl and (*S*)-ethyl by a combination of total synthesis, chemical degradation and X-ray crystallography.[64,65] Simple comparison would mean the (−)-deoxyscytalidin **35** isolated ($[\alpha]_D^{25}$= -53 (*c.* 0.13, $CHCl_3$)) would have (*R*)-pentyl and (*S*)-butyl side chains.



(−)-Byssochlamic acid

(–)-6

$[\alpha]_D^{25}$= −104 (*c*. 0.1, $CHCl_3$)



(−)-Deoxyscytalidin (proposed stereochemistry)

35

$[\alpha]_D^{25}$= −53 (*c.* 0.13, $CHCl_3$)

Figure 24. Structure and specific rotation of (-)-byssochlamic acid **(–)-6** and predicted stereochemistry of (−)-deoxyscytalidin **35**, based on a comparison of specific rotation values

Calculation of specific rotation by computational methods presented an alternate, though less convincing method with which to determine the absolute stereochemistry of deoxyscytalidin **35** (and therefore scytalidin **12**).

To provide computational evidence to indicate absolute stereochemistry for deoxyscytalidin, it is logical to first test the method on a related molecule with known stereochemistry, in order to illustrate the validity of this method. (−)-Byssochlamic acid **(–)-6** was selected, and the procedure reported by Autschbach applied.[71]

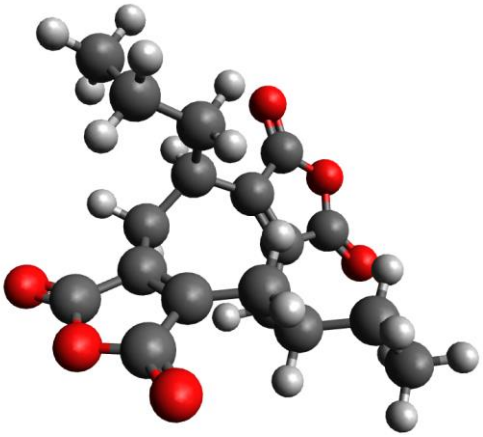


Figure 25. (2*S*,7*R*)-Byssochlamic acid **6** major conformer, minimised using molecular mechanics (MMFF94) in Avogadro.

Using the Avogadro molecular modelling software, a conformer search using the Merck Molecular Force Field (MMFF94) was used to generate a library of conformers of (-)-byssochlamic acid **(–)-6** in a vacuum. Thirteen conformers were found, **A-M**, in Table **3**. Using a Boltzmann distribution, the percentage contribution of each conformer to the overall state of (-)-byssochlamic acid **(–)-6** was determined. As can be seen in Table **3**, only six conformers contributed >1% to the overall state, and so the remainder were discounted for further analysis.

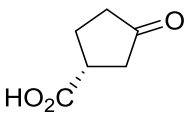
The remaining six conformers were then further optimised in Gaussian 09. Density Functional Theory (DFT), using the B3LYP/aug-cc-pVDZ basis set and a polarisable continuum model (PCM) for solvation in chloroform. Conformers **A** and **B** remained the dominant conformers, and the relative contribution of each conformer at this higher level of theory was broadly equivalent to those obtained from simple molecular mechanics.

With the coordinates obtained for these conformers at this higher level of theory, the specific rotation could then be calculated at a wavelength of 589 nm, the sodium D line used for practical measurement of specific rotation.[72] A Boltzmann average is then applied to these computed values, to give an indicative specific rotation for the molecule in solution. Unfortunately, as can be seen in Table **3**, the computed value was $[\alpha]_D^{25}$= +304. This differs significantly in both sign and magnitude when compared to the literature value ($[\alpha]_D^{25}$= -104). When the same methodology was applied to (2*S*,7*R*)-deoxyscytalidin **35**, a predicted specific rotation of $[\alpha]_D^{25}$= +191, also in complete disagreement with our prediction based on the direct comparison of experimentally determined specific rotation values. This indicates that this is a poor method for the determination of absolute stereochemistry in maleidrides.

Conformer	Energy (MMFF) / kJmol^{-1}	Boltzmann Percentage /%	Energy (DFT) / kJmol^{-1}	Boltzmann Percentage /%	$[\alpha]_D^{25}$ /deg cm^2 g^{-1}
A	-217.43	32.15	-3017996.2	42.51	334.06
B	-217.40	31.76	-3017995.7	34.84	270.48
C	-215.83	16.87	-3017992.8	10.92	326.22
D	-214.74	10.87	-3017992.5	9.64	255.91
E	-212.74	4.84	-3017987.3	1.18	384.92
F	-211.19	2.59	-3017986.6	0.91	317.5
G	-205.32	0.24			
H	-204.41	0.17	Boltzmann averaged specific rotation $[\alpha]_D^{25}$		+304
I	-204.18	0.15			
J	-203.41	0.11			
K	-203.04	0.10			
L	-202.96	0.09			
M	-201.14	0.04			

Table 3. Results of computational determination of the specific rotation of (-)-byssochlamic acid **(−)-6**, using Molecular Mechanics (MMFF) and DFT (B3LYP-aug-cc-pVDZ) levels of theory

Whilst disappointing, the calculation of the specific rotation of highly flexible molecules is known to be challenging. Paraconic acid **58**, studied by Marchesan *et al.*, is a good example of this challenge.[72] In their study, six major conformers of paraconic acid were identified, and when the specific rotation of each conformer was computed, values with both a positive and negative sign were obtained. Applying a Boltzmann average to these values could give the correct sign of the specific rotation when compared to experimental values, however small deviations in the relative energy of each conformer could lead to the opposite sign for the averaged specific rotation.

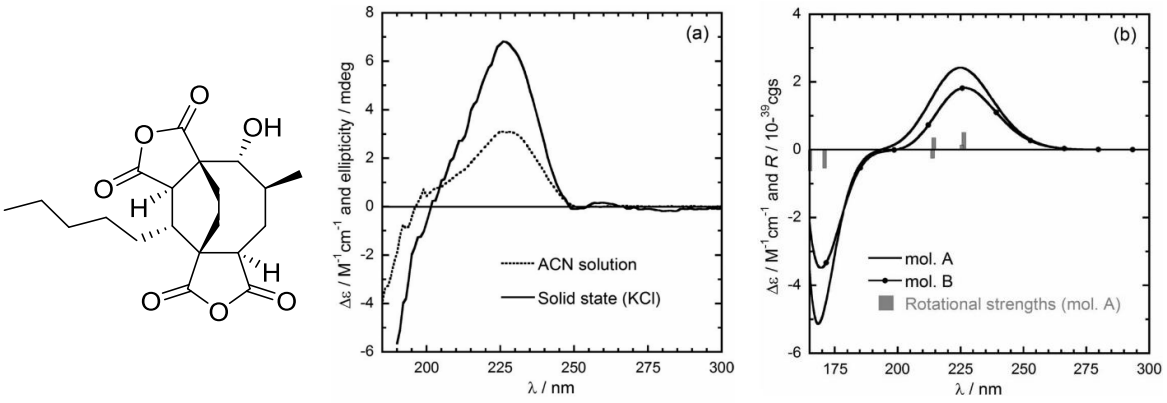


58

Figure 26. Structure of paraconic acid[72]

Further studies on flexible molecules supports the idea that they can be particularly unreliable, especially for the calculation of specific rotation at a single wavelength.[73,74] A better approach is to calculate the specific rotation at different wavelengths (optical rotation dispersion), or instead to compute the circular dichroism spectra of the molecule.[75] In this way, the accuracy of a single point of calculation is less important, as the overall picture is more illuminating.[76] This approach has been

successfully applied to viburspiran **15**, another member of the maleidride family of natural products.[34] Unfortunately, it was not possible to determine experimental solid state CD spectra during this project, and so synthesis of crystalline derivatives was the preferred approach.



Viburspiran **15** | Experimental CD Spectra | Computed CD Spectra

Figure 27. Absolute stereochemistry of viburspiran **15**, as determined by the comparison of computed solid state CD with that determined experimentally.[34]

2.2.5 **Inference of Absolute Stereochemistry by Biosynthetic Analysis**

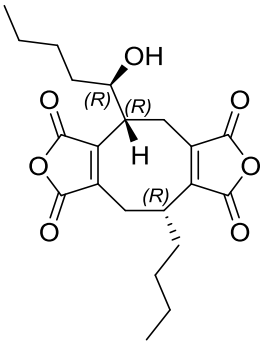
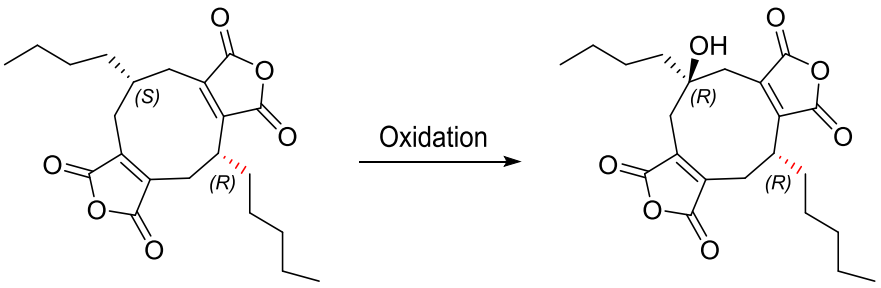


Figure 28. Absolute stereochemistry of (−)-(1*R*, 5*R*, 1'*R)*-zopfiellin **14**, determined by Dr Daniel O'Flynn in our group.

A final method by which the absolute stereochemistry of the scytalidin natural products can be inferred is through analysis of the biosynthetic pathways that give rise to these and related natural products. Recent biosynthetic studies within the Willis Group by Catherine Spencer and Dr Trong Tuan Dao have established a link between the biosynthesis of (−)-deoxyscytalidin **35** and zopfiellin **14** in *Diffractella curvata*, the natural producer of zopfiellin **14**. The absolute stereochemistry of zopfiellin **14** has previously been determined within the group by Dr Daniel O'Flynn through a combination of Mosher's ester analysis and X-ray crystallography.[1] The absolute stereochemistry of

[1] Daniel O'Flynn, *PhD Thesis*, University of Bristol, **2018**.

(−)-(1*R*, 5*R*, 1'*R)*-zopfiellin is shown in figure **28**. Due to confidentiality issues with Syngenta, the proposed biosynthetic pathway cannot be shown. However, it strongly suggests that (-)-deoxyscytalidin possesses (*S*)-butyl and (*R*)-pentyl side chains. This is consistent with the stereochemistry expected by simple comparison of the specific rotation of (−)-byssochlamic acid **(−)-6** and (−)-deoxyscytalidin **35**.



35 **12**

Scheme 21. Proposed final hydroxylation in scytalidin **12** biosynthesis.

In a similar fashion, it is then possible to infer the stereochemistry of (-)-scytalidin from (-)-deoxyscytalidin. There is only one biosynthetic gene cluster in *S. album* containing the enzymes required for maleidride biosynthesis. Therefore, it is highly likely that scytalidin is formed through hydroxylation of deoxyscytalidin, and not through a separate pathway. Whilst the mechanism of this hydroxylation is unknown, it is reasonable to assume that the stereochemistry of the (*R*)-pentyl chain, being distal to the required oxidation, is highly unlikely to change. The relative stereochemistry of the side-chains in both natural products is the same (through the X-ray crystallography data obtained), so we can therefore propose the absolute stereochemistry shown in Scheme **21** for (−)-deoxyscytalidin **35** and (−)-scytalidin **12**.

2.3 Conclusions and Future Work

It has been shown that *S. album* strain UAMH 3620 reliably produces both scytalidin **12** and deoxyscytalidin **35**. The strain was established on MEA, then a liquid pre-culture prepared by blending this agar with malt extract broth, for inoculation into multiple flasks for a 17 day growth phase. Analysis by HPLC confirmed the natural products were localised in the mycelia of the fungi, rather than the aqueous media.

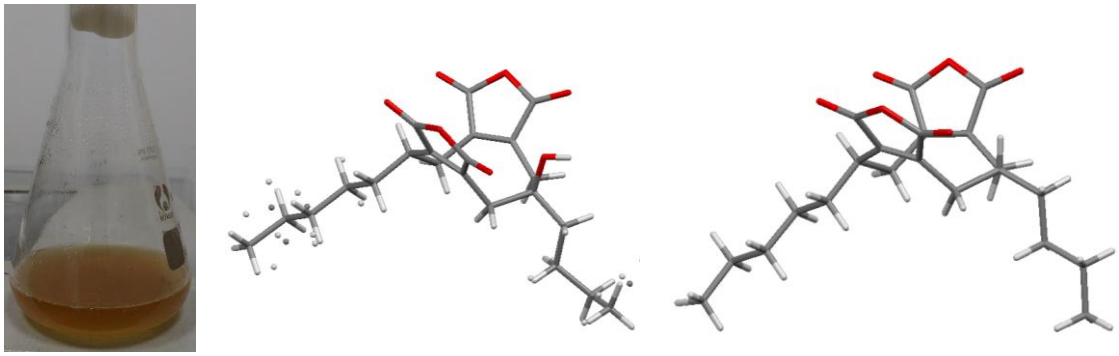
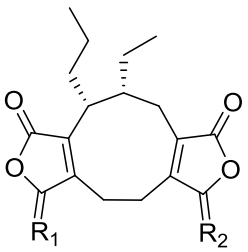


Figure 29. *S. album* liquid culture (*left*), crystal structure of scytalidin **12** (*centre*) and deoxyscytalidin **35** (*right*)

Scytalidin **12** was isolated in a titre of 10 mg/L by trituration of the mycelial extract, and deoxyscytalidin **35** was isolated by preparative HPLC in a similar titre. The natural products were then crystallised from diethyl ether, and X-ray structures were obtained. This allowed the relative stereochemistries to be determined. The absolute stereochemistry was then inferred by comparison of specific rotation to that of similar analogues, and through insights into the biosynthesis of zopfiellin **14** in *D. curvata*.

2.3.1 **Future Work**

As can be seen from the HPLC chromatograms obtained from the mycelial extracts (Figure **18**), *S. album* produces a wide variety of natural products, which may include previously undocumented maleidrides. Heveadride **7** analogues with different degrees of oxidation have been previously reported as minor metabolites from *Wicklowia aquatica*, shown in figure **30**.[77] Further analysis of *S. album* extracts may therefore lead to identification of similar metabolites. This is of interest because the oxidation state appears to have a significant impact on the antifungal activity of the maleidrides. Heveadride **7** and two analogues were found to be active against *Fusarium verticillioides*, commonly found to infect maize, whilst three further analogues showed no activity.[21,77,78]



58

A) R_1 = H OH, R_2= H OH
B) R_1 = H H, R_2= H H
C) R_1 = H OH, R_2= H H
D) R_1 = O, R_2= O
E) R_1 = O, R_2= H OH
F) R_1 = O, R_2= H H

Figure 30. Structures of heveadride analogues **58**, all isolated from *W. aquatica*.[77]

Whilst the absolute stereochemistry of scytalidin **12** and deoxyscytalidin **35** has been inferred by indirect means, it would be ideal to have higher quality crystallographic evidence with which to determine the absolute configuration unequivocally. This might be achieved through synthesis of further derivatives, or an increasingly extensive survey of solvents for crystallography.

Alternatively, solid state vibrational circular dichroism (CD) could be used to determine absolute stereochemistry. The crystallographic data already obtained provides input coordinates for computation of the CD spectrum, which can be compared to an experimentally determined spectrum. This has previously been applied to viburspiran **15**.[34]

To date, no studies on the anti-fungal properties of deoxyscytalidin **35** have been reported. Therefore it would be valuable to study the effect of the isolated deoxyscytalidin **35** and scytalidin **12** on similar fungi, in order to compare their potency. Both natural products are produced under the same conditions, but it is expected that an oxidative enzyme is responsible for conversion of deoxyscytalidin **35** to scytalidin **12**. The anti-fungal screening may show that one natural product is more active than the other, in which case identification (by genomic means) and manipulation of this oxidative enzyme might prove interesting. Overexpression of the oxidative enzyme may allow production of scytalidin **12** to be increased, whilst performing a gene knockout experiment to remove the oxidative enzyme could bias production towards deoxyscytalidin **35**.

CHAPTER 3: Total Synthesis of the Tricladic Acids

3. Total Synthesis of the Tricladic Acids

3.1 Introduction

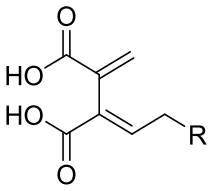
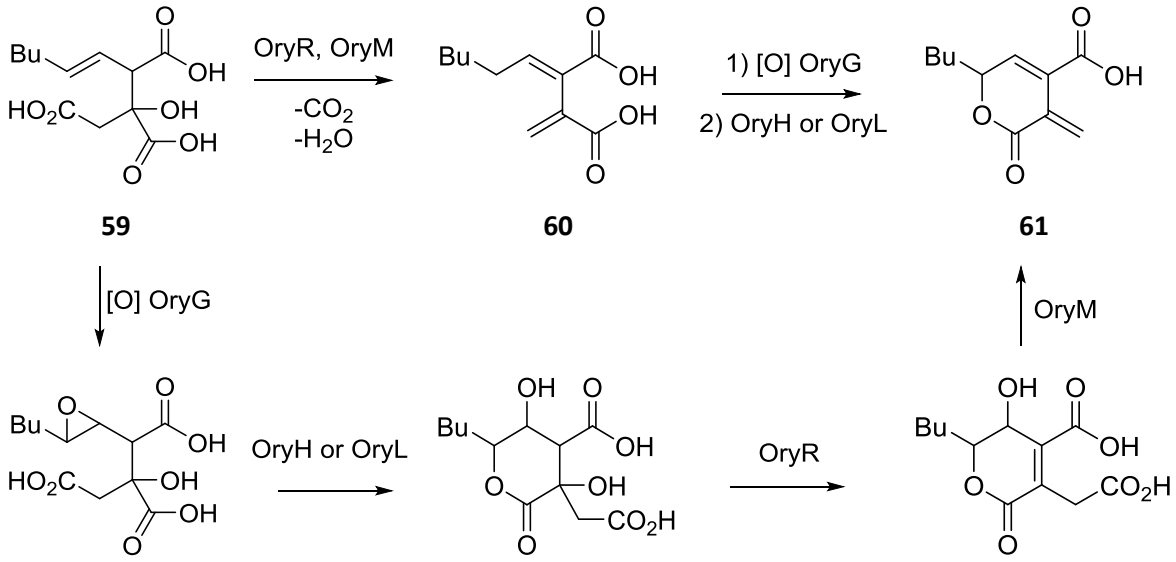


Figure 31. (*E*)-2-Alkylidene-3-methylenesuccinic acids, herein termed alkylfulgenic acids

Dialkylidenesuccinic acids, otherwise known as fulgenic acids, have been reported as bioactive natural products and proposed as intermediates in the biosynthesis of several fungal secondary metabolites.[53,79–82] However a lack of synthetic routes to alkylfulgenic acids has made further investigation difficult. There is only one previously reported synthesis of an alkylfulgenic acid, but this gives poor control over the trisubstituted alkene geometry.[83] In this chapter, an alternative synthetic approach to these structures is reported, and exemplified with the first total syntheses of the tricladic acids A-C **36**-**38**. This chemistry then underpins investigation of a biomimetic synthesis of the nonadrides, described in **Chapter 4**.

3.1.1 **Alkylfulgenic Acids in Oryzine Biosynthesis**

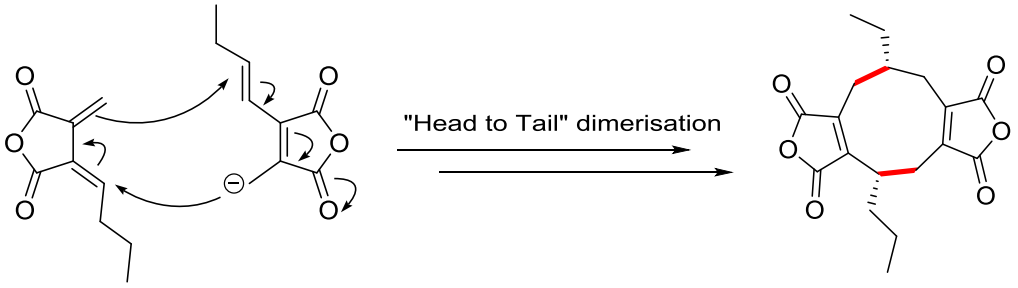
Alkyfulgenic acids are proposed intermediates in oryzine biosynthesis. The biosynthesis of oryzine B and C, isolated from *Aspergillus oryzae*, has yet to be experimentally verified, but two proposals have been made based upon a putative biosynthetic gene cluster identified by Cox *et al*.[79] Citrate **59** is thought to arise through condensation of octenyl CoA with oxaloacetic acid **19** (Scheme **22**). At this point, two possibilities exist. Decarboxylation and dehydration by OryM could form the alkylfulgenic acid **60**, which could undergo hydroxylation (OryG) and lactonisation (OryH or OryL) would then lead to oryzine B **61**. An alternative pathway involving epoxidation, ring closure, dehydration and decarboxylation is also possible, using these same enzymes. It is not possible to distinguish these pathways without access to biosynthetic intermediates, of which the alkylfulgenic acid **60** would be key. The lack of synthetic routes to these compounds therefore hinders further investigation.



Scheme 22. Proposed biosynthetic pathways to oryzine B **61**

3.1.2 **Alkylfulgenic Acids in Maleidride Biosynthesis**

In the context of our interest in maleidride biosynthesis, the alkylfulgenic acid structures become especially intriguing. The dimerisation step in the biosynthesis of the nonadrides is thought to proceed between a maleic anhydride structure, and an alkylfulgide – the anhydride form of alkylfulgenic acids (Scheme **23**).[84] If both compounds were synthesised, a base-catalysed dimerisation could be investigated (for further details see Section **4.2.3**), thus yielding nonadride structures.



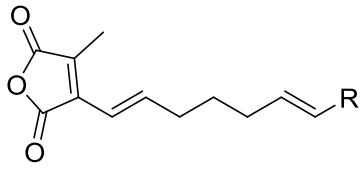
61 **21** Byssochlamic Acid **6**

Scheme **23**. Barton and Sutherland's proposed dimerisation mechanism between an alkylfulgide intermediate (**61**) and a maleic anhydride intermediate (**21**).[38]

3.1.3 **The Tricladic Acids**

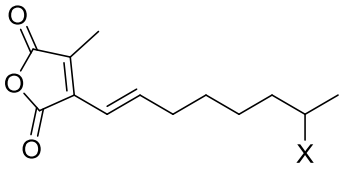
Seven new natural products were isolated in 2015 from the fungus *Tricladium castaneicola*. These consisted of four tricladolides, bearing a maleic anhydride core, and three tricladic acids which have

a fulgenic acid moiety.[53] It is interesting that *T. castaneicola* appears to produce both components necessary for the synthesis of maleidrides, but none were isolated.



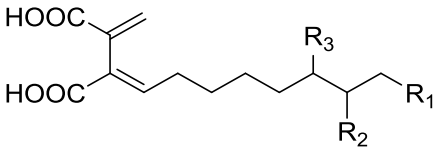
Tricladolide A, R = CO_2H

Tricladolide B, R = CH_2OH



Tricladolide C, X = OH

Tricladolide D, X = H



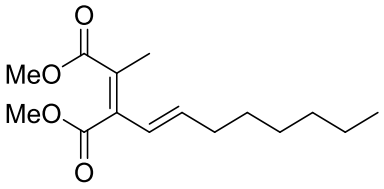
Tricladic acid A **36**, R_2 = OH, R_1 = R_3 = H

Tricladic acid B **37**, R_3 = OH, R_1 = R_2 = H

Tricladic acid C **38**, R_1 = OH, R_2 = R_3 = H

Figure 32. Structures of the tricladolides and tricladic acids.[53]

The tricladium natural products show potential as selective fungicides. Initial testing using samples isolated from *T. castaneicola* showed the compounds had the ability to inhibit growth of *Phytophthora capsici*, a common vegetable pathogen, but were inactive against bacteria and yeast. In particular, tricladolide D showed activity on par with that of Metalaxyl, a commercial anti-*Phytophthora* fungicide for which resistance is a growing concern.[85,86] In addition, all seven compounds were tested against the B16 mouse melanotic melanoma cell line. Again all compounds showed appreciable activity, with tricladolide D showing similar levels of activity to paclitaxel, a commercial breast cancer drug. Interestingly, the dimethyl ester of tricladolide D **62** showed even higher levels of activity.[53]



62

Figure 33. Structure of tricladolide D dimethyl ester.

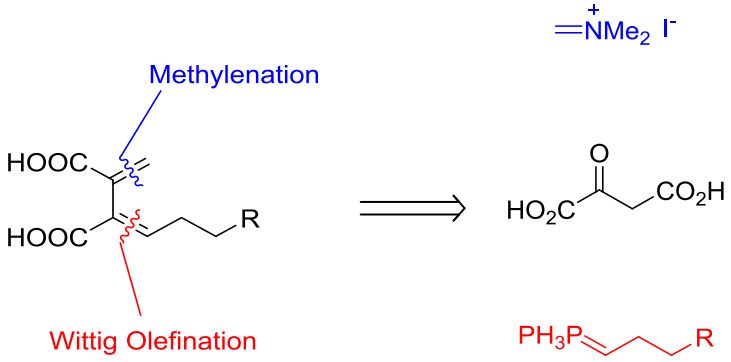
Further investigations into the biological properties of this family of natural products have been hindered by the low titres of the natural products, and the difficulty in culturing *Tricladium castaneicola*. The strain requires 20 days of growth on agar before transferring to 1 L of liquid culture for an additional 20 days. This 40 days of growth only yields 2 mg/L of tricladolide D, whilst the tricladic acids required 72 L of culture media to isolate between 3 and 7 mg. Through total synthesis, the stereochemistry of **36** and **37** can be confirmed. The stereochemistry of tricladic acid A **36** has previously been assigned as near-racemic due to the small value for the specific rotation recorded, whilst tricladic acid B **37** has been assigned as a 3:1 *S*:*R* ratio by Mosher's ester analysis.[53] This can

be confirmed by synthesis, and the structure-activity relationship of these compounds with respect to their anti-cancer and fungicidal properties then more fully investigated.

3.2 Results and Discussion

The interesting biological activity of the tricladic acids, and the relevance of alkylfulgenic acids to maleidride biosynthesis therefore made them an interesting target for synthesis. In designing synthetic routes to the tricladic acids, key considerations were to ensure the alkene geometry was set correctly; that different side chains could be introduced late in the route; and that each dimethyl ester derivative was synthesised en route, as each of them would be valuable for SAR studies.[53]

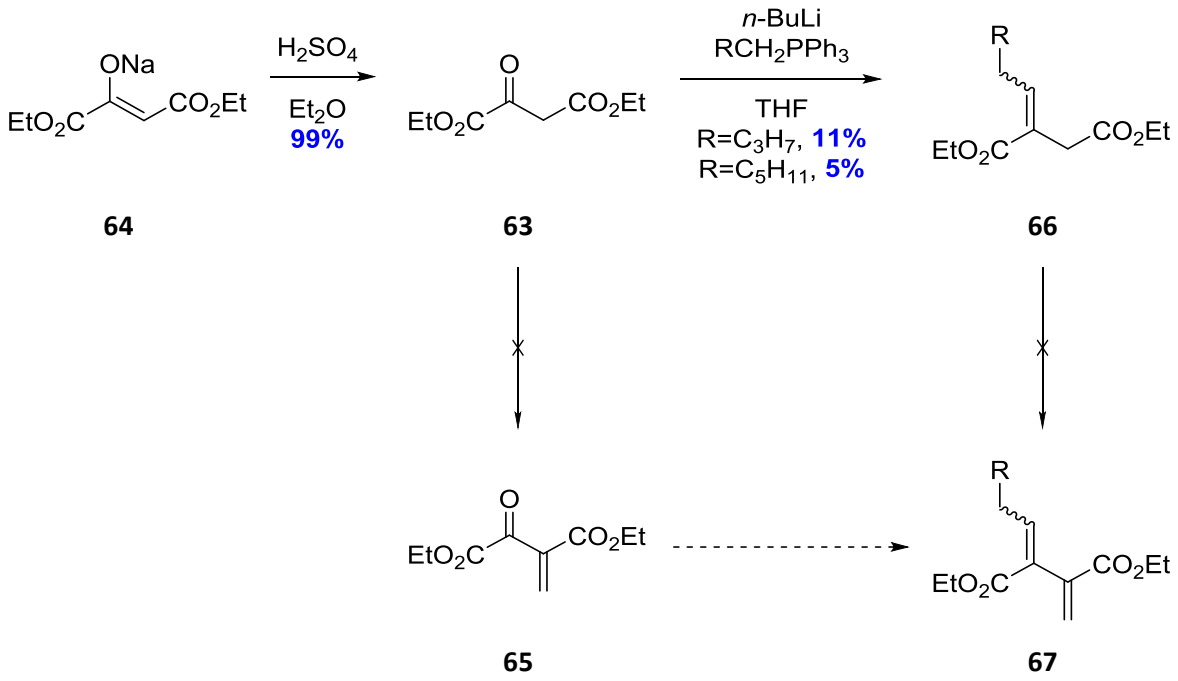
3.2.1 Initial Retrosynthesis and Initial Routes Investigated



Scheme 24. General retrosynthetic analysis of a tricladic acid

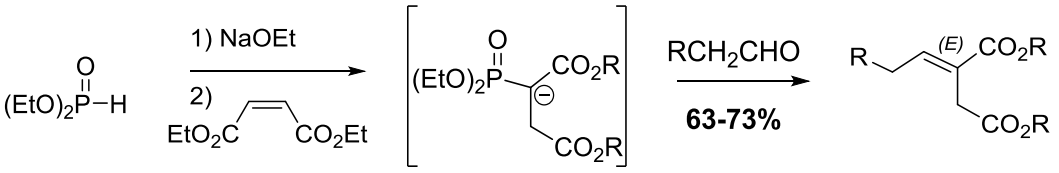
Retrosynthetic analysis (Scheme **24**) suggested disconnection of both alkenes would allow synthesis of the tricladic acid diene structure from oxaloacetate **63**. One alkene could be introduced by Wittig olefination, and the other via an Eschenmoser methylenation reaction.[87]

Diethyl oxaloacetate **63** was obtained by protonation of the commercially available sodium salt **64** in 99% yield, from which both the Wittig reaction and methylenation reaction could be explored (Scheme **25**). Attempts to α-methylenate oxaloacetate **63** were unsuccessful, with no product detected when using formaldehyde, trioxane, formalin or Eschemoser's salt as the C_1 source. Given this reaction would form the highly activated enoate **65**, which has been previously reported as unstable,[88] it is likely that this species was too reactive under the reaction conditions to be isolated.



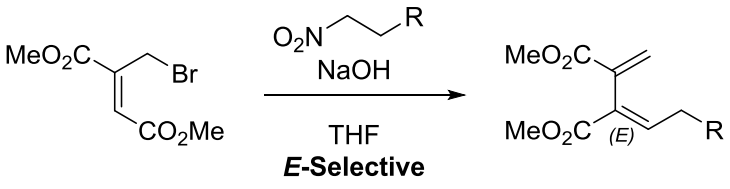
Scheme 25. Attempted synthesis of the tricladic acid diene structure **67** from diethyl oxaloacetate.

Performing the Wittig reaction first means there is no intermediate in which an alkene is activated by two carbonyls, and so it was hoped this would circumvent this issue. The Wittig reaction of **63** was carried out with *n*-BuLi and the alkyl Wittig salt, but gave very poor yields of the alkene **66**, in a 3.5:1 *Z*:*E* ratio. Surprisingly, the yields were significantly different between a butenyl (11%) and hexenyl (5%) side chain, but in neither case were the stereoisomers separable.



Scheme 26. Shen's *E*-selective synthesis of 3-alkoxycarbonyl-β,γ-unsaturated esters.[89]

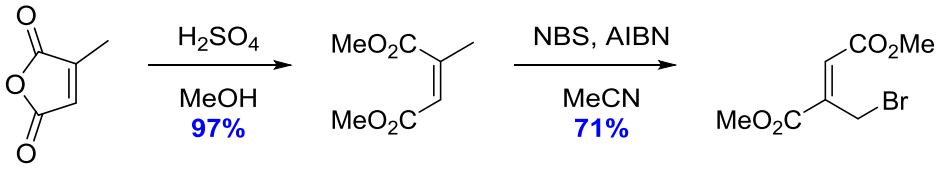
Disappointingly, the α-methylenation reaction of **66** still failed under all conditions investigated.[89] Shen has reported an alternative *E*-selective synthesis of the 3-alkoxycarbonyl-β,γ-unsaturated ester moiety (Scheme **26**). However, as the α-methylenation step could not be achieved, this option was not pursued further. Instead, an approach reported by Amri and coworkers was investigated (Scheme **27**).[90]



68 **69**

Scheme 27. Amri's synthesis of (*E*)-1,3 dienes.[90]

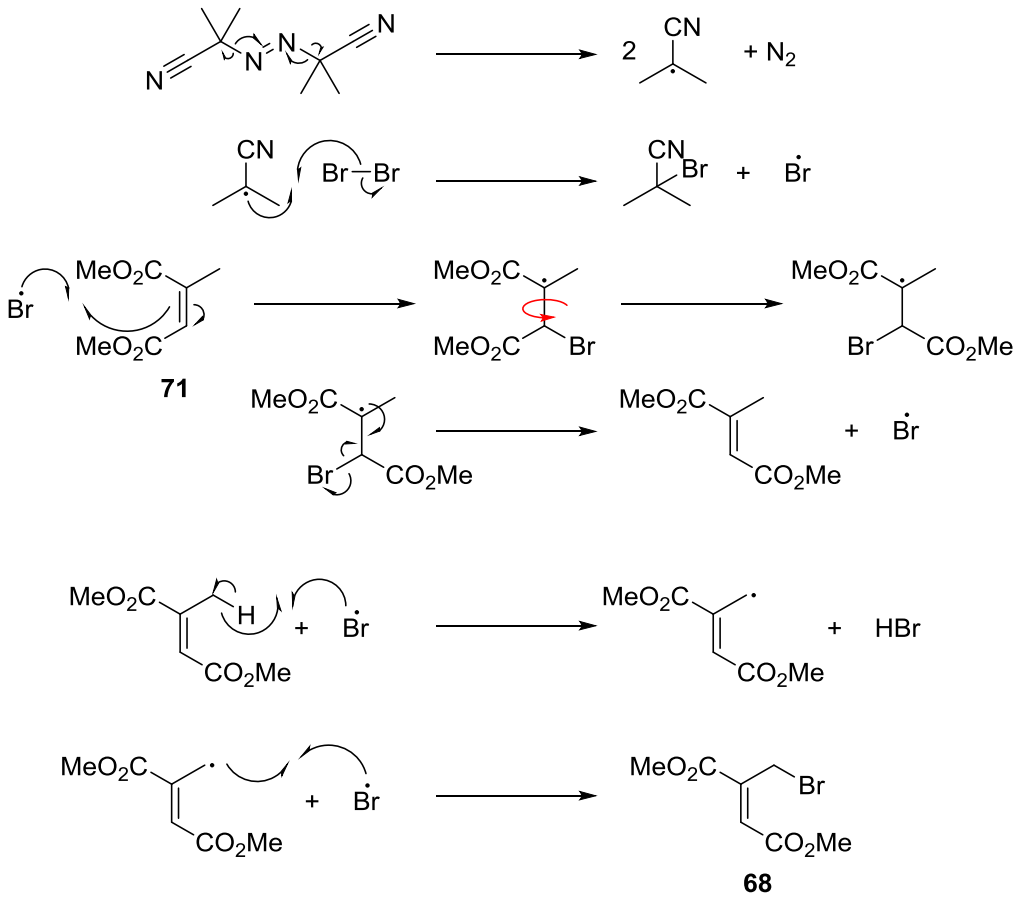
Firstly, dimethyl (α-bromomethyl)fumarate **68** was synthesised in two steps from citraconic anhydride **70** (Scheme **28**). Opening the anhydride with acidic methanol proceeded cleanly to yield dimethyl ester **71** in 97% yield, then Wohl-Ziegler bromination gave bromide **68** in 71% yield. Whilst it is common to replace carbon tetrachloride in radical bromination reactions with cyclohexane,[91] the yield in this case was very poor (19%). Solvent selection for these reactions is usually dictated by a desire to keep a low concentration of reagents in the reaction to prevent side reactions, however in this case acetonitrile, in which all reagents are completely soluble, was found to give a much higher yield (71%). Alkene geometry was assigned by comparison to literature ^{1}H NMR data.[92]



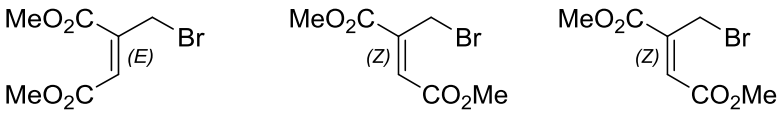
70 **71** **68**

Scheme 28. Synthesis of bromofumarate **68** from citraconic anhydride.

The observed alkene isomerisation is well known in the literature, and occurs due to addition-elimination of the bromine radical to the alkene.[93] When this reaction is stopped prematurely, dimethyl fumarate can be isolated, indicating this addition-elimination process is faster than the allylic bromination. A mechanistic explanation for this is shown in Scheme **29**. Fortunately, the alkene geometry obtained matches that previously used by Amri and coworkers,[90] and this alkene geometry is irrelevant to the final product desired.



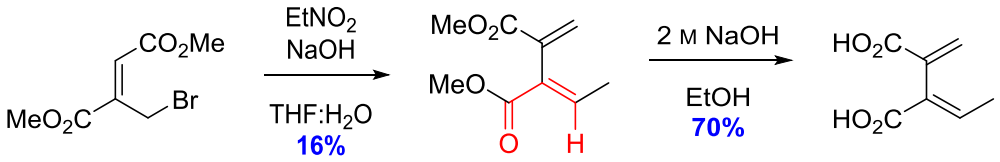
Scheme 29. Synthesis of bromide **68** from alkene **71**, highlighting the alkene isomerisation. In accordance with the reports of Goldfinger, a bromine radical rather than a succinimidyl radical propagator is depicted.[93–96] (Regeneration of the bromine radical not shown)



(*E*)-68	**(*Z*)-68**	**(*Z*)-68**
Literature Data	**Literature Data**	**This work**
δ_H (400 MHz, $CDCl_3$)	**δ_H (400 MHz, $CDCl_3$)**	**δ_H (400 MHz, $CDCl_3$)**
6.24 (1H, t, *J* 1.0, HC=C)	6.83 (1H, s, HC=C)	6.77 (1H, s, CH)
4.13 (2H, d, *J* 1.0, CH_2Br)	4.72 (2H, s, CH_2Br)	4.67 (2H, s, CH_2Br)
3.85 (3H, s, CO_2Me)	3.88 (3H, s, CO_2Me)	3.83 (3H, s, OCH_3)
3.77 (3 H, s, CO_2Me).	3.83 (3H, s, CO_2Me)	3.78 (3H, s, OCH_3)

Table 4. Comparison of ^{1}H NMR shifts for (*E*)-**68** and (*Z*)-**68** with that prepared in this project.[97]

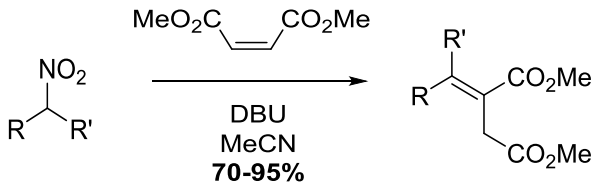
The exclusive formation of the (*E*)-alkene in one pot reported for the nitronate S_N2'-alkylation-elimination reaction (NSAE reaction) was then investigated. Replication of the reported reaction of bromide **68** with nitroethane in aqueous NaOH led to very poor yields, ranging from 4-16% of the desired product **72** (Scheme **30**). Nevertheless, exclusive formation of the (*E*)-alkene was observed as reported in the literature. The alkene geometry was confirmed by comparison of ^1H chemical shifts to literature,[53,83] NOESY NMR experiments, and also through an EXSIDE experiment.[98] EXSIDE NMR shows heteronuclear H-C coupling constants over three bonds – a longer range than is achieved by other experiments. In this case, a $^3J_{(CH)}$ of 7.5 Hz between H-4 and the ester carbonyl was observed, indicative of a *cis* relationship (see Scheme **30**).



68 **72** **73**

Scheme 30. Replication of Amri's reaction conditions gave poor yields of **72**. The *cis*-relationship observed by an EXSIDE NMR experiment is highlighted.

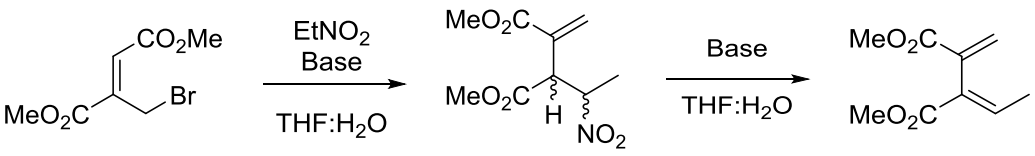
Hydrolysis of the diester **72** with sodium hydroxide in aqueous ethanol gave diacid **73** in 70% yield. Whilst a viable route had been found to the tricladic acids, the poor yielding NSAE reaction limited its use. Optimisation of the Amri conditions for the formation of diene **72** were therefore sought.



74 **75**

Scheme 31. Michael addition of nitroalkanes **74** to dimethyl maleate, reported by Ballini *et al*.[99]

To begin, different bases were investigated, including DABCO, DBU and NEt_3 in THF. In each case no reaction was observed. This was disappointing as Ballini *et al.* have previously reported a similar reaction of nitroalkane **74** using DBU (Scheme **31**).[99]



68 **76** **72**

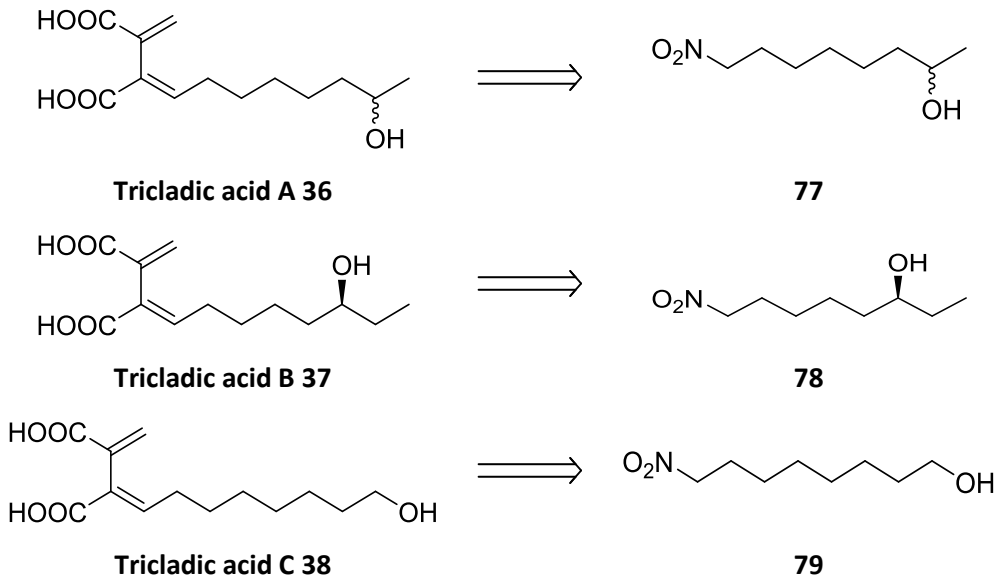
Scheme 32. Synthesis of diene **72** via intermediate nitroalkanol **76**.

This base screen (DABCO, DBU, NEt_3) was then repeated in a THF:water solvent mixture. With DBU a complex mixture was produced, and DABCO gave predominantly the intermediate nitroalkane **76** as a mixture of diastereomers, as well as both (*E*) and (*Z*)-**72** (Scheme **32**). These intermediate structures, from which HNO_2 has not been eliminated, have previously been reported under phase-transfer conditions.[100] Triethylamine gave the desired product **72** in 24% yield. Water appears to be essential to the reaction, the use of methanol as an alternative protic solvent led to formation of significant quantities of (*Z*)-**72**.

Entry	Solvent	Equiv. NEt_3	Addition time bromide **68** / min	NMR Yield of **72** / %
1	THF:H_2O	4	5	15
2	THF:H_2O	4	20	56
3	THF:H_2O	4	120	63
4	THF	4	120	0
5	THF:H_2O	1	120	0
6	THF:H_2O	2	120	21

Table 5. Optimisation of addition time and equivalents of base, performed by Emyr Tayler

The rate of addition of the bromide **68** to a preformed nitronate solution had a significant impact on the yield of the diene **72**. As can be seen in Table **5**, a slower addition gave a marked improvement in yield. With yields for the NSAE reaction now reliably 50-60%, synthesis of the nitroalkanes (**77**-**79**) necessary for the synthesis of tricladic acid A-C (**36**-**38**) was next explored.

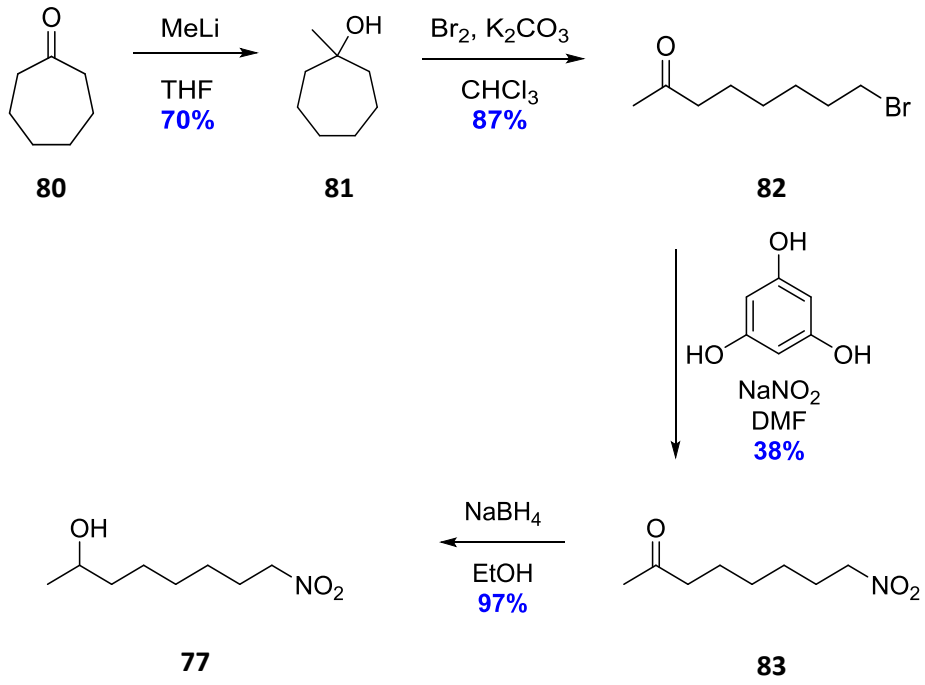


Scheme 33. Retrosynthetic analysis of each tricladic acid, using the NSAE reaction.

3.2.2 First Generation Synthesis of Tricladic Acids A & B

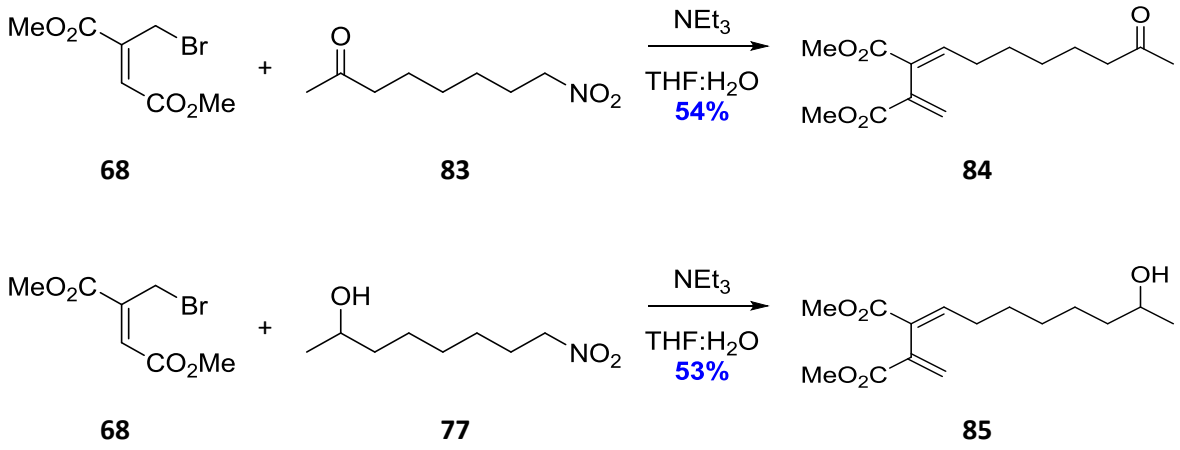
Tricladic Acid A

For tricladic acid A, the required nitroalkane **77** was synthesised in 4 steps (Scheme **34**). Addition of methyllithium to cycloheptanone **80** gave tertiary alcohol **81** in 70% yield. This alcohol **81** was treated with bromine and potassium carbonate to induce a retro-Barbier fragmentation,[101] giving the *ω*-bromoketone **82** in 87% yield. Displacement of the bromide with sodium nitrite in dimethylsulfoxide (DMSO) gave the *ω*-nitroketone **83** in only 38% yield due to the bidentate nature of the nitrite nucleophile. *N*-alkylation and *O*-alkylation are both possible, leading to either the desired nitroalkane or a nitrite ester by-product.[102,103] Addition of phloroglucinol as a nitrite scavenger was used in an attempt to facilitate separation of these products, however this did not improve the yield of **83**.[104–107] The *ω*-nitroketone **83** thus obtained was then reduced with sodium borohydride to the corresponding alcohol **77**. Whilst trying to develop the route, a stereoselective reduction of the ketone was not deemed necessary.



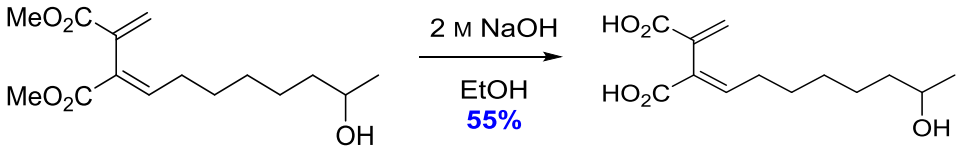
Scheme 34. Preparation of nitroalcohol **77**, required for the synthesis of tricladic acid A **36.**

Interestingly, the NSAE reaction could be performed with either ketone **83** or alcohol **77**, yielding the desired dienes **84** and **85** in 54% and 53% yield respectively (Scheme **35**). Hence an enantioselective reduction of the ketone could be performed either before or after the nitro-aldol reaction.



Scheme 35. NSAE reactions yield the desired dienes with either a ketone or alcohol moiety

Treatment of diester **85** with ethanolic sodium hydroxide afforded the racemic natural product **36** in 55% yield. The product partially degraded during purification by silica chromatography. NMR data for the synthetic diacid **36** were in good agreement with those obtained from isolated tricladic acid A, thus completing the first total synthesis of a tricladic acid natural product.



85 **36**

Scheme 36. Deprotection of diester **85** to yield racemic tricladic acid A **36**.

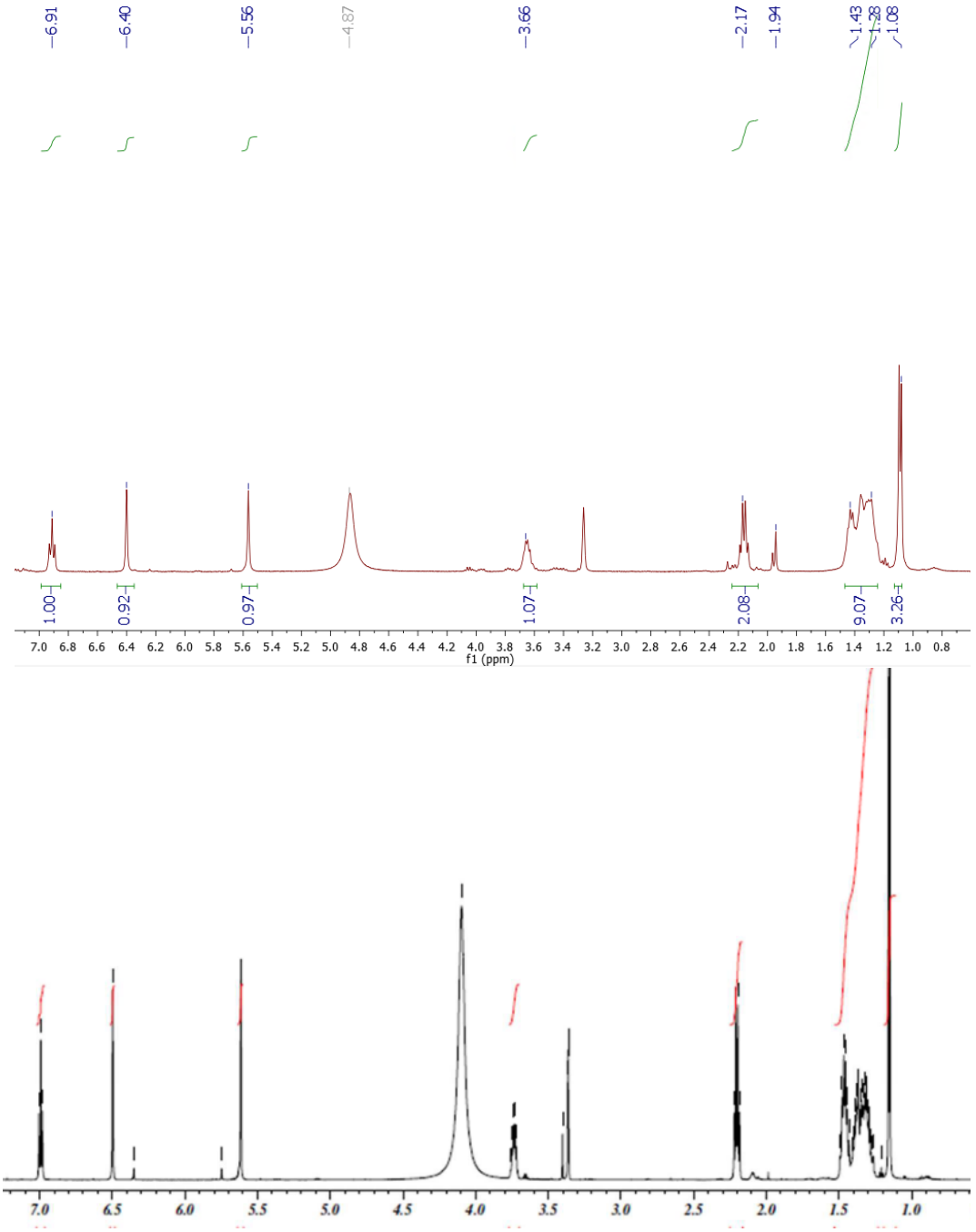
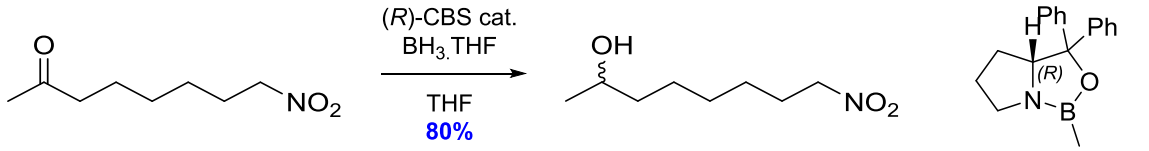


Figure 34. Comparison of ^{1}H NMR spectra (500 MHz in 3:1 $CDCl_3$:CD_3OD) for synthetic tricladic acid A (*top*) and the natural product isolated from *T. castaneicola* (*bottom*).[53]

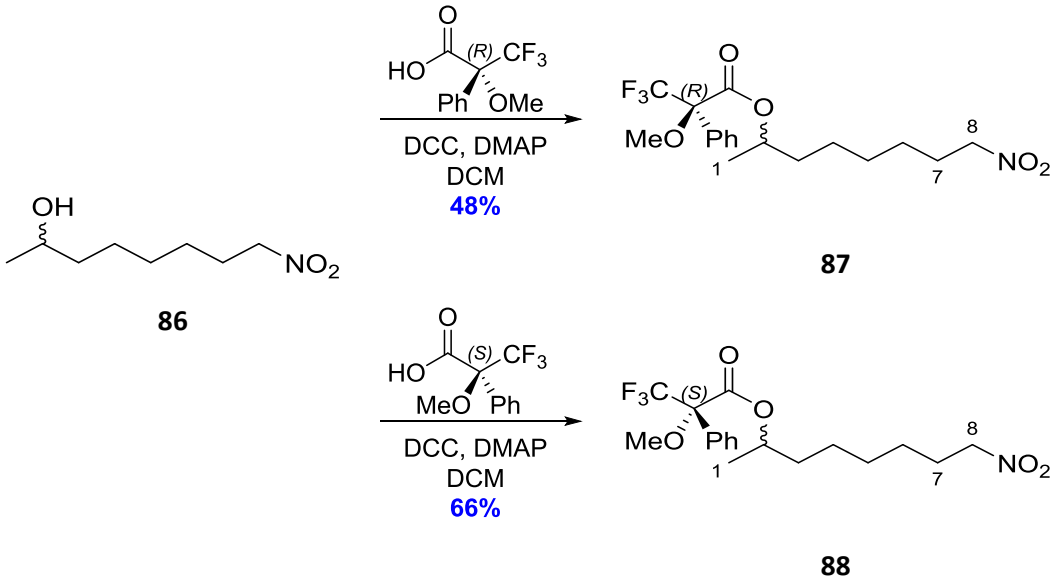
An enantioselective reduction of nitroketone **83** was next explored (Scheme **37**). The Corey-Bakshi-Shibata (CBS) reduction was chosen for this purpose.[108] Nitroketone **83** and the oxazaborolidine catalyst were pre-mixed at 0 °C, then the BH_3·THF complex added. The reaction was complete by TLC within 15 minutes, and an 80% yield of nitroalcohol **86** obtained.



83 **86** **(*R*)-CBS oxazaborolidine**

Scheme 37. CBS reduction of nitroketone **83**

Mosher's ester analysis was used to determine the enantiopurity of the product by ^{1}H NMR analysis.[109–111] The esters required were synthesised via the DCC/DMAP coupling of nitroalcohol **86** with (*R*)- and (*S*)- α-methoxy-α-trifluoromethylphenylacetic acid (MTPA-OH) (Scheme **38**).



Scheme 38. Synthesis of (*R*)- and (*S*)- MTPA esters of nitroalcohol **86**. Numbering shown is for Mosher's ester analysis in Table **6**.

The ^{1}H NMR spectra of the esters **87** and **88** were analysed by comparing the chemical shift of related protons. It can be seen in Table **6** that the chemical shift for 1-H_3 in (*S*)-MTPA **88** has an upfield shift with respect to 1-H_3 in (*R*)-MTPA **87**, indicating an eclipsing, shielding interaction with the phenyl substituent of the ester in (*S*)-MTPA **88**, but not in (*R*)-MTPA **87**. This would indicate that the methyl substituent is R_2 in the projections shown. Similarly, the 7-H_2 and 8-H_2 peaks show the opposite relationship, experiencing a greater upfield, shielding interaction in (*R*)-MTPA **87** than (*S*)-MTPA **88**. This is consistent with these groups being part of the R_1 substituent. The magnitude of the shielding

effect is likely decreased as both methylenes are further away from the ester substituent than the methyl group.

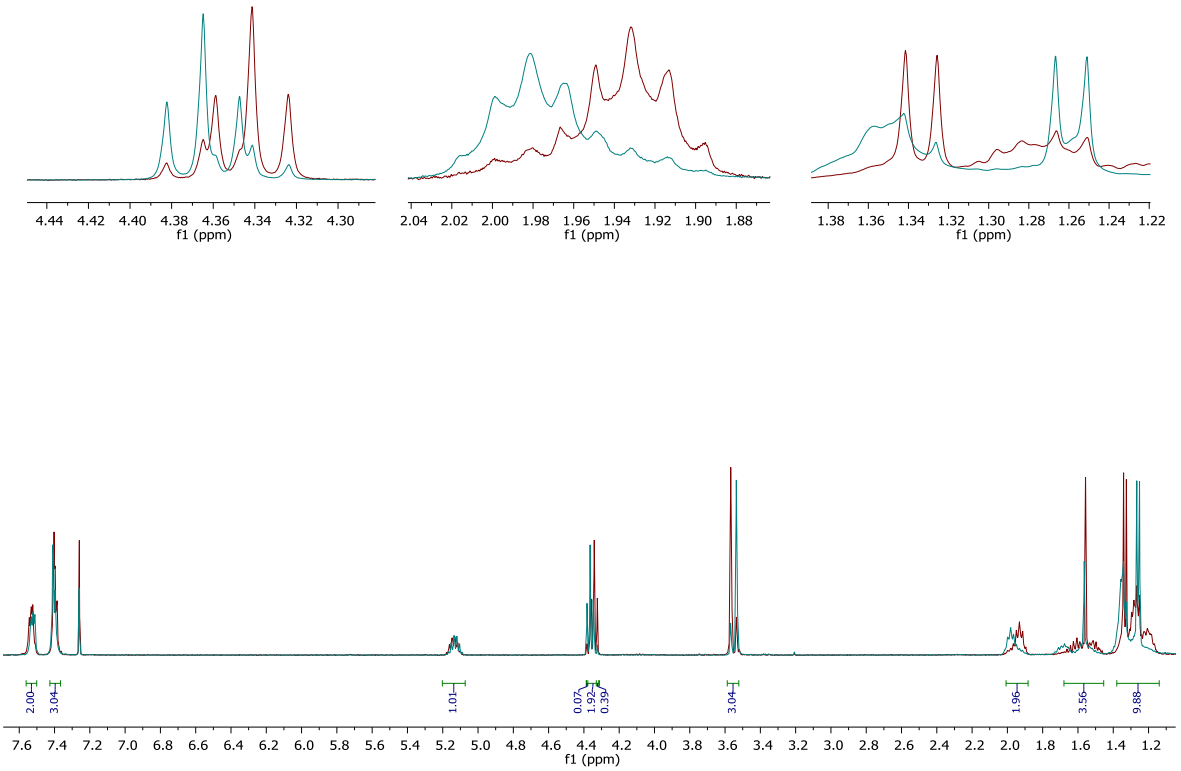
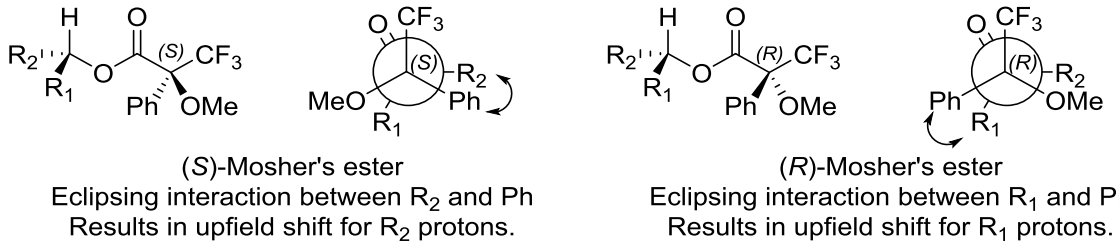


Figure 35. Overlay of ^{1}H NMR spectra of Mosher's ester derivatives (*R*)-MTPA **87** (red) and (*S*)-MTPA **88** (blue). Expansions are shown of the key signals used in the analysis.



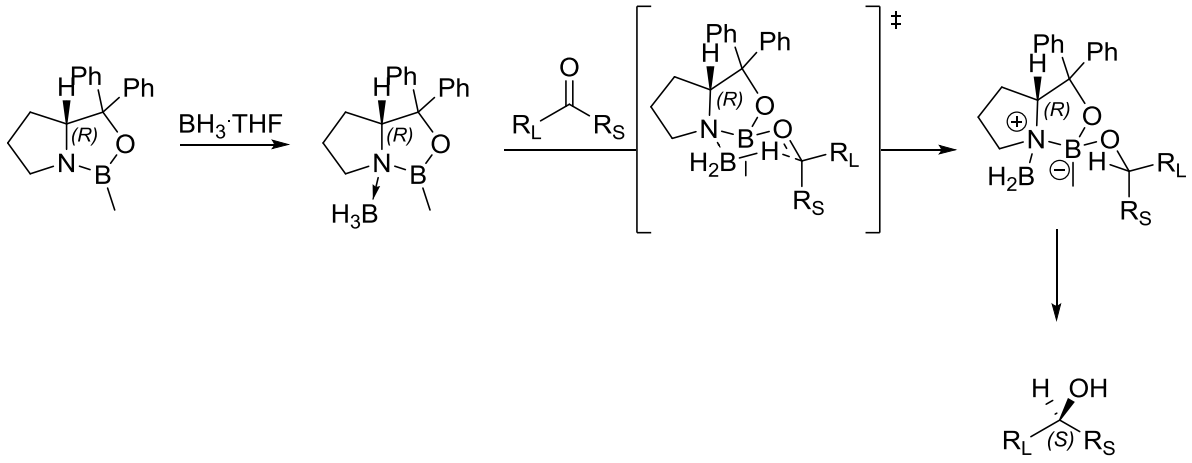
(*S*)-Mosher's ester
Eclipsing interaction between R_2 and Ph
Results in upfield shift for R_2 protons.

(*R*)-Mosher's ester
Eclipsing interaction between R_1 and Ph
Results in upfield shift for R_1 protons.

Assignment	δ_S-88	δ_R -87	δ_S-δ_R
1-CH_3	1.26	1.335	-0.075
7-CH_2	1.98	1.93	+0.05
8-CH_2	4.36	4.34	+0.02

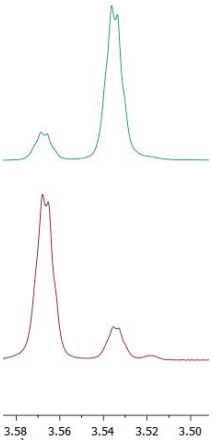
Table 6. Dominant spectroscopic conformers for Mosher's ester analysis, highlighting the relevant eclipsing interactions. Table of ^{1}H NMR chemical shifts for assignable protons in each Mosher ester **87** and **88**.

The change in chemical shift (δ_S-δ_R) indicate R_1 is the alkylchain bearing the 7-H_2 and 8-H_2, whilst the 1-H_3 group behaves as expected for the R_2 substituent. Nitroalcohol **86** must therefore have an (*S*)-configuration. This is consistent with the stereochemistry predicted by the mechanism for the CBS reduction, shown in Scheme **39**.[112–114] In the chair transition state, the steric interaction between the larger ketone substituent (R_L) and the catalyst is minimised through a pseudo-equatorial arrangement, thus dictating the face of the ketone to which the hydride is delivered. The (*R*)-oxazaborolidine catalyst results in an (*S*)-alcohol forming, in agreement with the results obtained from the Mosher's analysis of nitroalcohol **86**.



Scheme 39. Generalised mechanism of the CBS-reduction.

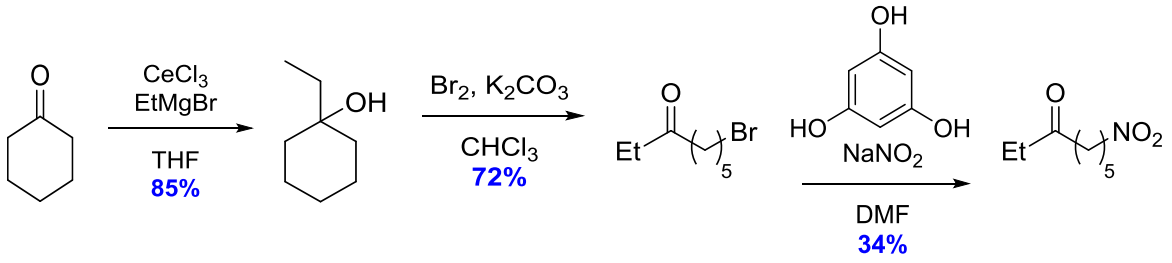
The MTPA-esters **87** and **88** gave resolved peaks in the ^{1}H NMR spectrum corresponding to the methoxy group of the MTPA ester into two signals - the (*R**,*R**) and (*R**,*S**) diastereomers. For **87** and **88**, a 1:0.19 integration ratio was apparent, corresponding to an e.e. of 68%. Given the magnitude of the specific rotation value of tricladic acid A was small, this was a disappointing result. A high e.e. is desirable to give the best possible confirmation that tricladic acid A is indeed produced by *T. castaneicola* in a near-racemic mixture. Whilst alternative enantioselective reductions could have been explored, instead an approach using a chiral starting material was favoured. (See section **3.2.3**).



Scheme 40. Methoxy peaks in the ^{1}H NMR spectra for (*S*)-MTPA **88** (top) and (*R*)-MTPA **87** (bottom).

Tricladic Acid B

The synthesis of tricladic acid B followed a very similar route to tricladic acid A. Addition of ethylmagnesium bromide to cyclohexanone **89** gave tertiary alcohol **90.** Initially, alcohol **90** was isolated in only 34% yield. Imamoto *et al.* have noted that the propensity of cyclohexanone to enolise in the presence of Grignard reagents leads to competing aldol reactions, but that addition of $CeCl_3$ increased the yield of the 1,2-addition product.[115] They suggest the strong oxophilicity of cerium (III) chloride activated the carbonyl by coordination, although could not rule out the basicity of the Grignard reagent being attenuated by $CeCl_3$. Using EtMgBr/$CeCl_3$, an 85% yield of 1-ethylcyclohexan-1-ol **90** was obtained.

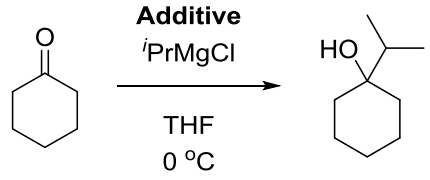


89 **90** **91** **92**

Scheme 41. Synthesis of nitroketone **92**

One drawback of this process was the time-consuming preparation of anhydrous $CeCl_3$ from commercially available heptahydrate. $CeCl_3.7H_2O$ was first heated to 90 °C under vacuum to form the monohydrate. If heated above 100 °C, hydrolysis to CeOCl predominates. The monohydrate was then heated to 140 °C to yield anhydrous $CeCl_3$.[116] However, it has been subsequently suggested that this material is rarely completely anhydrous, and an additional equivalent of the organometallic reagent is necessary to compensate for this.[117] This complex preparation, which must be repeated for each use of $CeCl_3$ due to its highly hygroscopic nature, made this reaction non-trivial and yields of alcohol **90** were highly variable. The use of soluble $LaCl_3{\cdot}2LiCl$ has been reported by Knochel to be

more reliable in the addition of Grignard reagents to carbonyls than $CeCl_3$.[118] The $LaCl_3$·2LiCl solution in THF is commercially available, gives higher yields, and can be used catalytically.[119] Therefore this additive offers significant improvements if this reaction were to be used in the future.

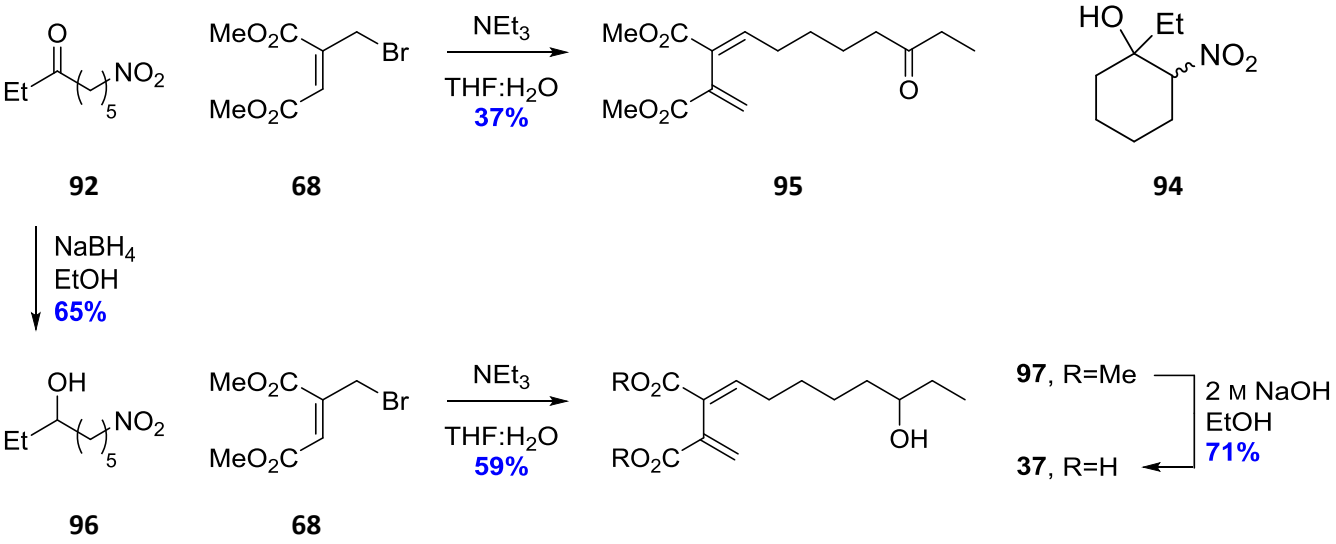


89 **93**

Additive	Yield /%
None	**30**
$CeCl_3$ (Imamoto)[115]	**80**
$LaCl_3$·2LiCl (Knochel)[119]	**97**

Scheme 42. The effect of different additives on the 1,2-addition of Grignard reagents to enolisable ketones.[118]

From alcohol **90**, bromide **91** was synthesised via a retro-Barbier fragmentation, in a 72% yield (Scheme **41**). Displacement of the bromide with sodium nitrite gave nitroketone **92** in 34% yield. In this case, the NSAE reaction between **92** and **68** was complicated by formation of two cyclic β-nitroalcohols **94**, reducing the yield of the desired diene **95** to 37% (Scheme **43**). Reduction of nitroketone **92** to alcohol **96** prior to the NSAE step removed this issue, producing diene **97** in 59% yield. It is interesting that this 6-*exo*-trig cyclisation of **92** was observed, whilst the 7-*exo*-trig cyclisation of **83** was not, despite both being favourable according to Baldwin's rules.[120] Hydrolysis of diester **97** gave (±)-tricladic acid B **37** in 71% yield, and the ^1H and ^{13}C NMR data were in good agreement with those reported for the natural product (Figure **36**).[53]



Scheme 43. Nitro-aldol reactions and synthesis of (±)-tricladic acid B **37**.

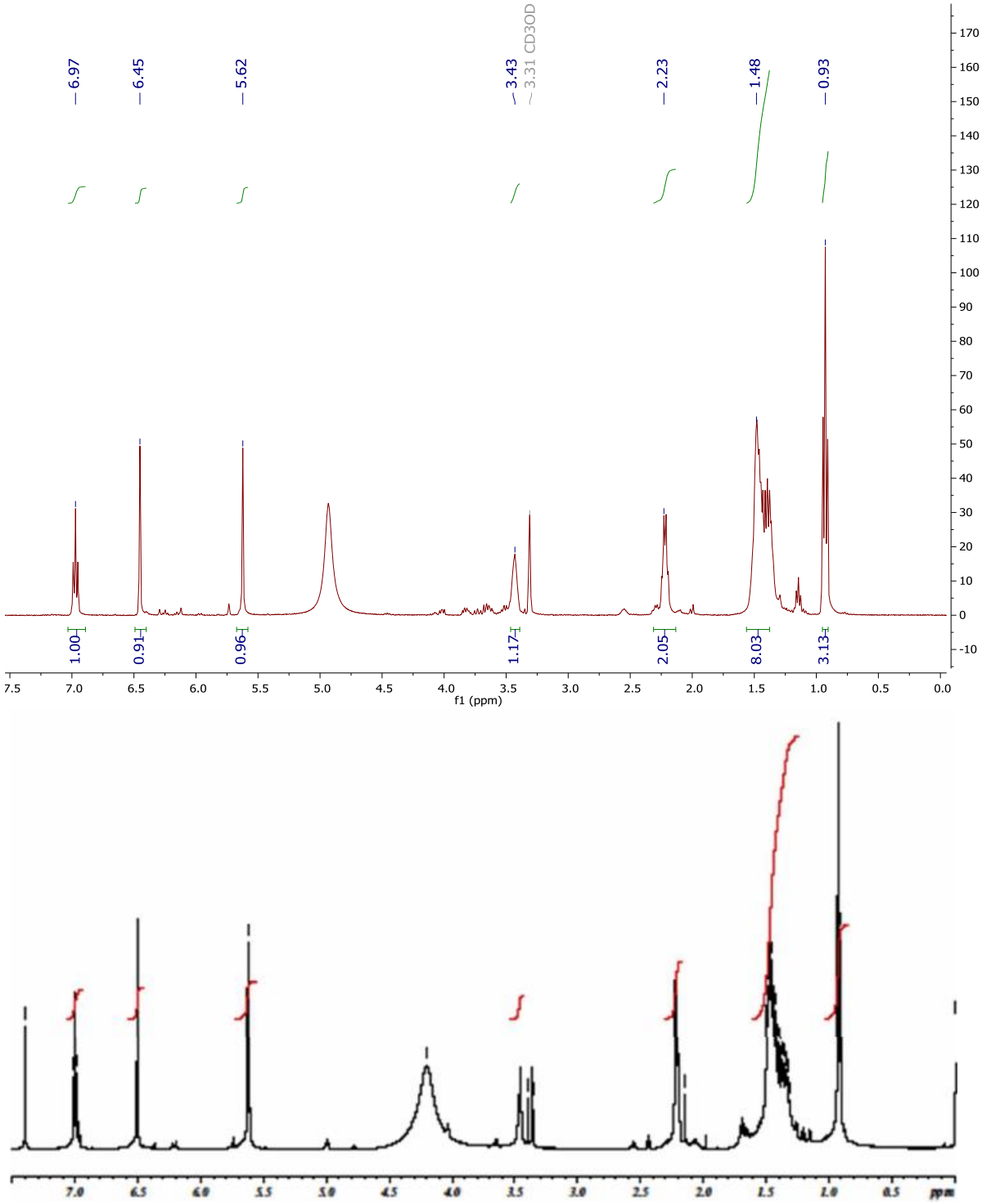


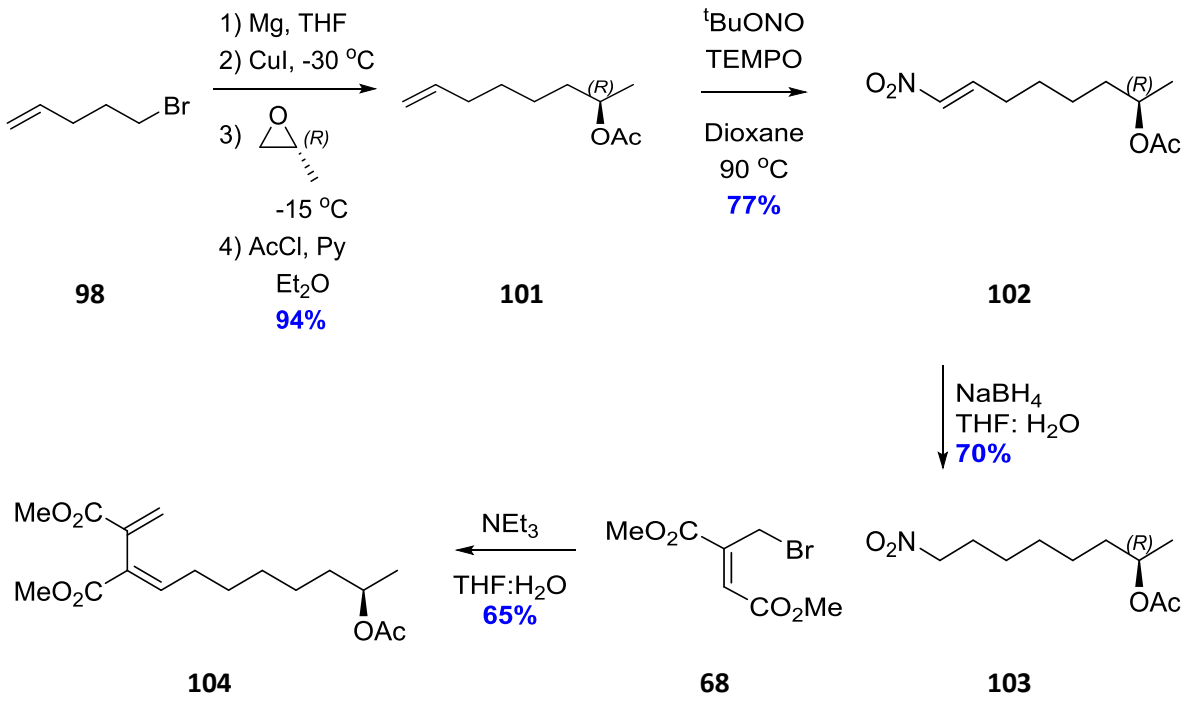
Figure 36. Comparison of ^{1}H NMR spectra (500 MHz in 3:1 $CDCl_3$:CD_3OD) for synthetic tricladic acid B (top) and the natural product isolated from *T. castaneicola* (bottom).[53]

This route however does not lend itself well to an enantioselective reduction to produce the required chiral secondary alcohol. CBS reduction gives poor enantioselectivity when the substituents are an ethyl group and a longer alkyl chain, due to the similar size of the substituents.[121,122] As the reported specific rotation for tricladic acid A and B are small, it was felt routes employing chiral starting materials would be preferable, making comparison to literature specific rotation data more straightforward.

3.2.3 Second Generation Syntheses of Tricladic Acids A & B

In redesigning the synthetic strategies, further improvements were sought. For example, conversion of the bromoalkane to the required nitroalkane with sodium nitrite was low yielding, due to formation of the alkyl nitrite through competitive *O*-alkylation rather than the desired *N*-alkylation. Any synthesis relying on an S_N2 reaction to install the nitro- functionality would suffer this issue. Alkene nitration appeared to offer one solution to this problem. Whilst traditionally this has been achieved with harsh conditions such as HNO_3/H_2SO_4,[123] or with highly toxic reagents such as $HgCl_2/NaNO_2$,[124] several recent publications utilise TEMPO in combination with a nitrate/nitrite source to achieve the desired transformation under milder conditions.[125–127] Given the ease with which nitroalkenes can be reduced to nitroalkanes with $NaBH_4$,[128,129] the synthesis shown in Scheme **44** was investigated.

Tricladic Acid A

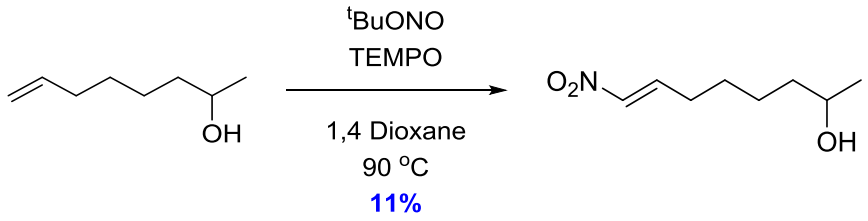


Scheme 44. Synthesis of (*R*)-tricladic acid dimethyl ester **104** from (*R*)-propylene oxide.

The organocuprate was formed from alkenyl bromide **98** via the Grignard reagent, then used to open the oxirane ring of (*R*)-propylene oxide. The organocuprate reagent was used, rather than the Grignard reagent directly, to suppress formation of the bromohydrin.[130,131] Exclusive opening of the epoxide at the least hindered carbon was confirmed by comparison to literature, in particular the ^{1}H NMR signal at 3.73 ppm showed both the correct chemical shift, and integration for the desired product.[132] ^{1}H NMR analysis of the crude reaction mixture showed no sign of the CH_2OH (literature

3.45 ppm) moiety formed as a result of ring-opening at the more hindered carbon, or of the formation of a bromohydrin.[133,134]

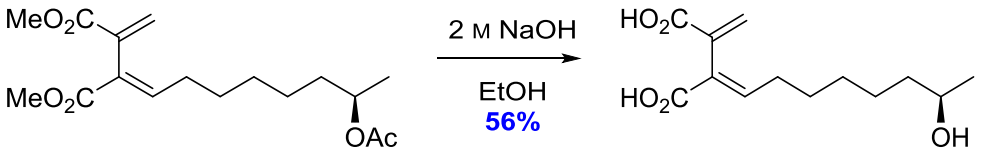
TEMPO is frequently employed in the oxidation of alcohols,[135–138] including the use of ^{t}BuONO/TEMPO.[139] It was therefore expected that protection of alcohol **99** would be necessary to achieve the desired alkene nitration. A trial reaction using racemic alcohol **99** demonstrated that this was indeed the case, as treatment of **99** with ^{t}BuONO/TEMPO gave nitroalcohol **100** in 11% yield (Scheme **45**).



99 **100**

Scheme 45. Trial alkene nitration with an unprotected alcohol **99**.

Therefore, the alcohol **99** was protected as the acetate (Scheme **44**). The crude material from the organocuprate addition was used directly, giving the acetate **101** in 94% yield over two steps. An acetate was chosen as it could be removed alongside the other ester protecting groups at the end of the synthesis. The alkene nitration proceeded smoothly using ^{t}BuONO/TEMPO to give nitroalkene **102** in 77% yield. The resultant nitroalkene **102** was reduced to the nitroalkane **103** with sodium borohydride. This reaction was sluggish in dioxane-ethanol but was complete in one hour in THF-methanol.



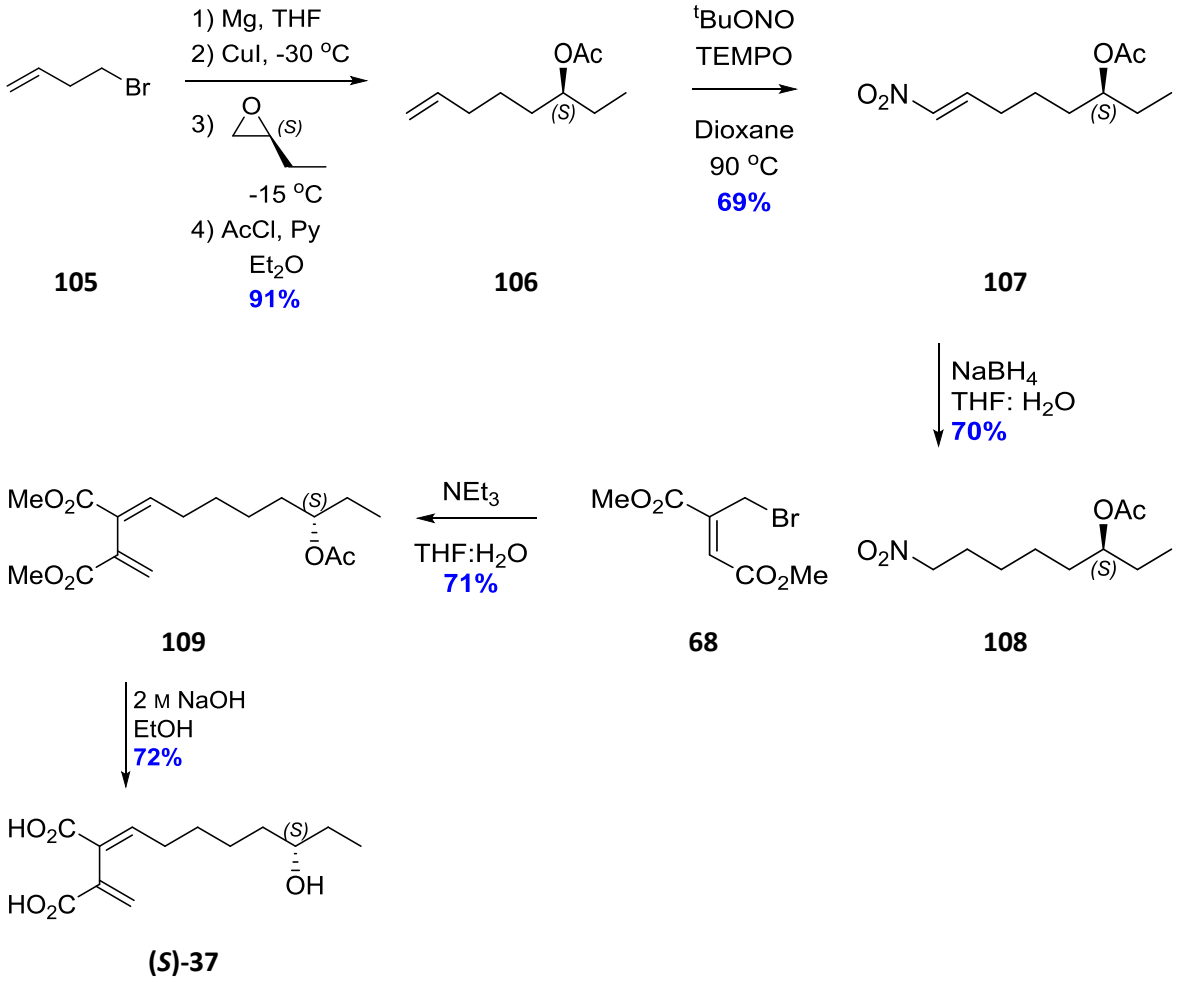
104 **(*R*)-36**

Scheme 46. Deprotection of triester **104** to yield (*R*)-tricladic acid A **(*R*)-36**

The synthesis of tricladic acid A was completed using the NSAE reaction to give triester **104**, which was hydrolysed with NaOH/EtOH:H_2O. NMR data for the (*R*)-tricladic acid A **36** correlated well with both the racemic material previously synthesised, and the natural product. Comparison of the specific rotation of (*R*)-tricladic acid A ($[\alpha]_D^{22}$ -5, *c* 0.91, MeOH) with that reported for the isolated tricladic acid A from *T. castaneicola* ($[\alpha]_D^{22}$ -0.4, *c* 0.35, MeOH) is in accord with the proposal that the fungus produces a near-racemic mixture of tricladic acid A.[53]

Tricladic Acid B

The synthesis of (*S*)-tricladic acid B was similarly achieved, starting with (*S*)-epoxybutane and 4-bromobut-1-ene **105** (Scheme **47**). With the exception of the initial starting materials, no other changes to the route were necessary. Ring-opening of the chiral epoxide and acetylation of the resulting alcohol were performed in one pot, having established that the protecting group was both necessary and appropriate during the synthesis of (*R*)-tricladic acid A **36**.



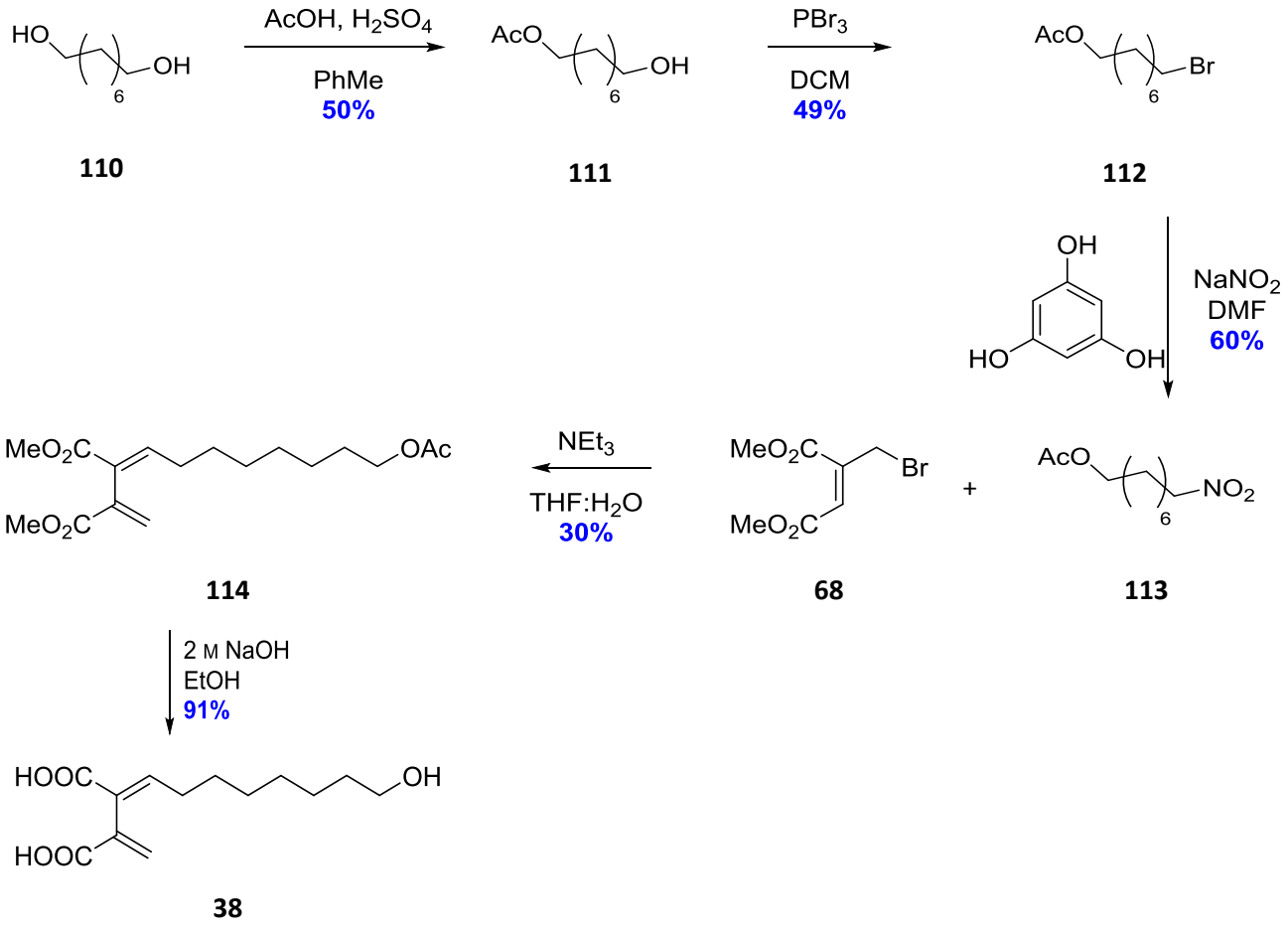
Scheme 47. Total synthesis of (*S*)-tricladic acid B **(*S*)-37**

The specific rotation of the (*S*)-tricladic acid **37** thus synthesised ($[\alpha]_D^{22}$ +4, *c* 1.70, MeOH) was in agreement with the proposal that the natural product consists of a 3:1 (*S*:*R*) ratio ($[\alpha]_D^{22}$ +2.0, *c* 0.34, MeOH), as assigned by Ojika and coworkers on the basis of a Mosher ester analysis.[53]

3.2.4 Total Synthesis of Tricladic Acid C

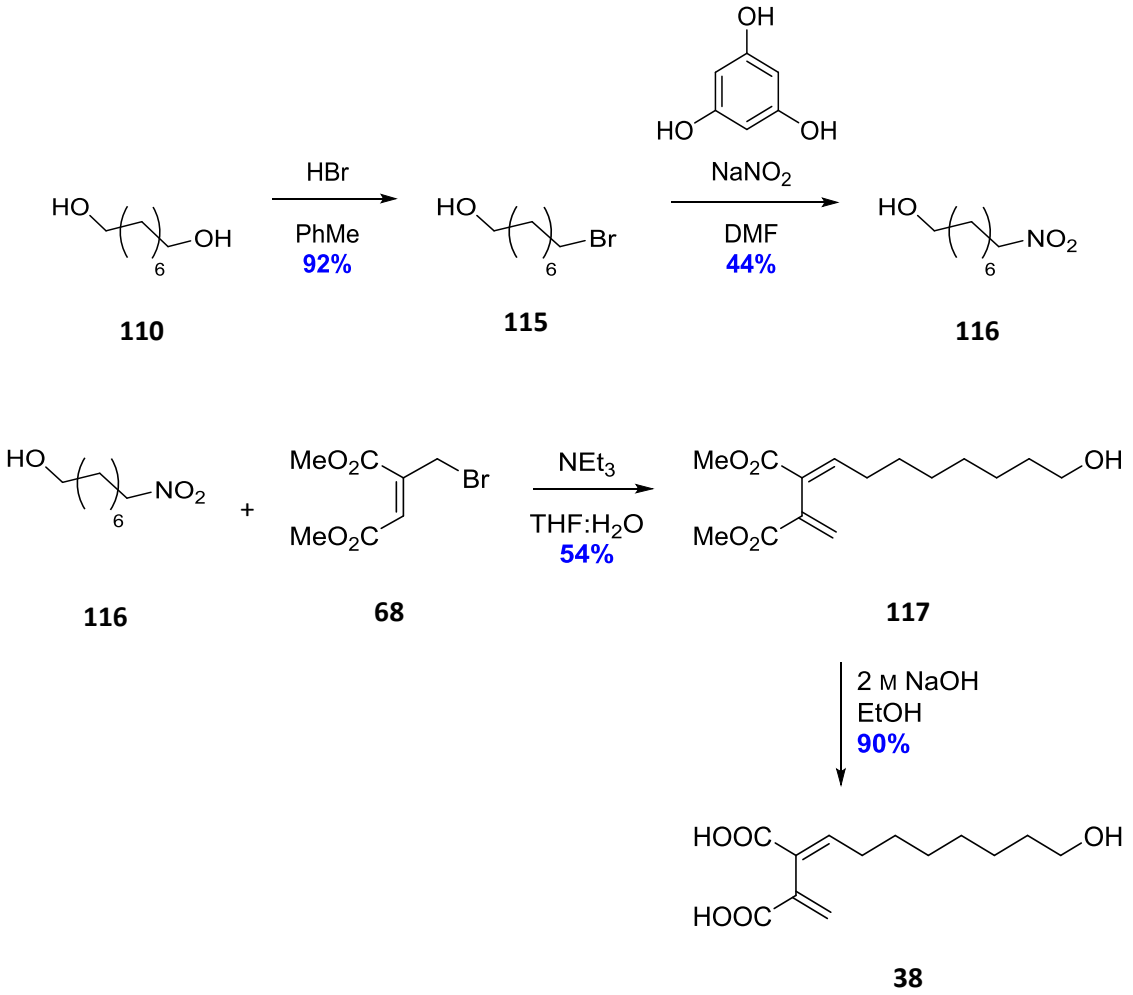
Synthesis of tricladic acid C was readily achieved. Starting with monoacetylation of 1,8-octanediol **110** (Scheme **48**), conversion of alcohol **111** to bromide **112** with PBr_3 in 49% yield, then displacement

of the bromide with sodium nitrite gave nitroalkane **113** for the NSAE reaction. Global deprotection of the resulting triester **114** by hydrolysis then yielded tricladic acid C **38**, with NMR data in good agreement with the literature.[53]



Scheme 48. Initial synthesis of tricladic acid C **38**.

This route however had significant issues. Acetylation of diol **110** gave a mixture of diol, monoacetate **111** and diacetate which required separation. The bromination of **111** with PBr_3 gave a disappointing 49% yield. Given the success of the previous route based on alkene nitration, a similar could have been used, but would still require alcohol protection. Instead, it was decided to instead pursue the route shown in Scheme **49**.



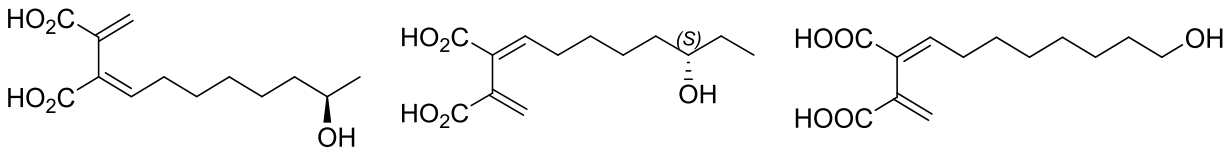
Scheme 49. Revised route to tricladic acid C **38**

Monobromination of diol **110** was achieved in 92% yield by refluxing with $HBr_{(aq)}$ in toluene, a significant improvement on the acetylation-bromination procedure previously employed in Scheme **48** (92% vs. a comparable 25% yield over two steps). Treatment of **115** with sodium nitrite yielded ω-nitroalcohol **116**, with which the NSAE reaction was performed to give diester **117** in 24% yield over two steps. Whilst this route does not avoid the *O* vs *N*-alkylation issues previously experienced when using the nitrite nucleophile, it gave the desired nitroalkane **116** in 40% yield over two steps, compared to 15% yield over three steps previously. Synthesis of tricladic acid C was completed by deprotection of diester **117**, to yield the natural product **38**.

3.3 Conclusions and Future Work

The tricladic acids are novel fulgenic acid natural products, with anti-fungal and anti-cancer properties. Further investigations of the natural products have not been possible as low titres are produced by *T. castaneicola*, and the culturing of the fungus is not straightforward.[140] Previously, production of 3.6 mg of tricladic acid A and 7.5 mg of tricladic acid B required 34 days and 72 flasks

of culture. The total syntheses reported herein have meant 115 mg of (*R*)-tricladic acid A and 124 mg of (*S*)-tricladic acid B were produced in six synthetic steps, in 16% and 22% overall yield respectively.



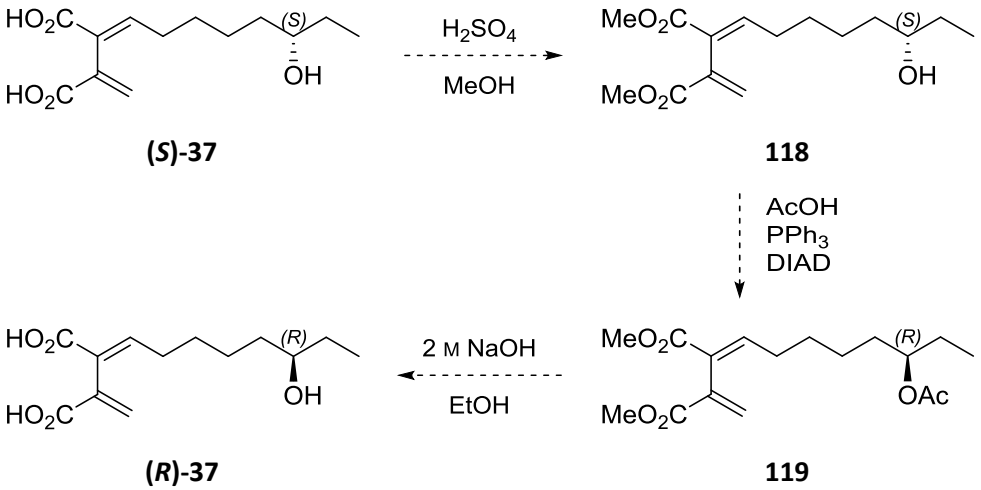
36 (*R*)-Tricladic Acid A **6 steps, 16% overall yield**

37 (*S*)-Tricladic Acid B **6 steps, 22% overall yield**

38 Tricladic Acid C **4 steps, 20% overall yield**

Scheme 50. Natural products synthesised and overall yields

As the tricladic acids can now be accessed in greater quantities, further testing of their biological activity will be possible. As well as testing the compounds against additional fungal strains and cancer cell lines, access to single enantiomers through total synthesis means the importance of stereochemistry on bioactivity can also be assessed. The synthesis of racemic tricladic acids, as well as a single enantiomers of tricladic acid A and B has been described herein. The opposite enantiomers could be prepared from the starting epoxides with the opposite stereochemistry, or alternatively a Mitsunobu reaction could be used to invert the stereochemistry of the alcohol.[141,142] Unfortunately, the diacid functionality would likely interfere with the Mitsunobu reaction, so a protection and deprotection step are still necessary (Scheme **51**). However, the intermediate **119** could also be used to introduce a variety of functionality to the tricladic acid scaffold, for example amines and halides.[143–145]



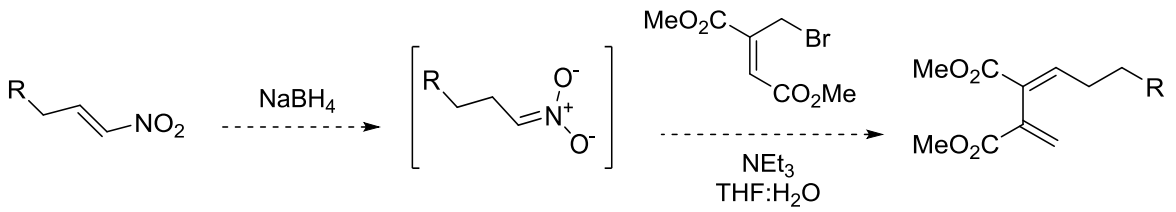
(*S*)-37 **118** **(*R*)-37** **119**

Scheme 51. Proposed Mitsunobu inversion of (*S*)-tricladic acid B to (*R*)-tricladic acid B

The synthetic route devised also means natural product analogues can be synthesised and tested. Okija and coworkers reported that hydroxylation of the side chains appeared to strongly reduce the

anti-*Phytophthora* activity of the tricladolides.[53] Therefore, synthesis of analogues of the tricladic acids with simple alkyl chains may be one way to improve activity, and will also allow direct comparison of the maleic diene and fulgenic diene moieties, to assess which is more potent. Total synthesis proceeds via the dimethyl ester, allowing the biological activity of these compounds to be tested. As has been shown for tricladolide D, these structures reportedly show higher cytotoxicity against B16 melanoma cells than Paclitaxel, an important chemotherapy drug.[146]

Further improvement of the synthetic route may also be possible. The reduction of the nitroalkene **120** to a nitroalkane and the following NSAE reaction both proceed via the nitronate anion **121**. Therefore, it might be possible to perform both of these steps in a one-pot reaction.



120 **121** **122**

Scheme 52. Proposed one-pot reduction and NSAE reaction of nitroalkene **120**

Within the wider goal of studying maleidride biosynthesis, synthesis of alkylfulgenic acids was necessary in order to explore the dimerisation step. The chemistry developed herein provides the basis for such work as described in Chapter **4**.

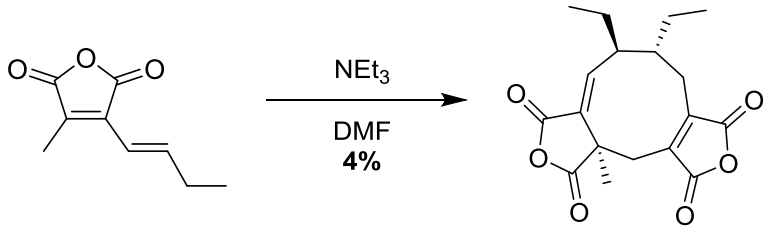
CHAPTER 4: Biomimetic Synthesis of Maleidrides

4. Biomimetic Synthesis of Maleidrides

4.1 Introduction

With routes to both the tricladic acids and tricladolides developed, the next step was to investigate the biomimetic synthesis of nonadrides through coupling these compounds. Previous studies mimicking this key dimerisation step in maleidride biosynthesis have been reported, but predominantly focus on the formation of glaucanic acid-type structures, resulting from the combination of two equivalents of the maleic anhydride (tricladolide) precursor.

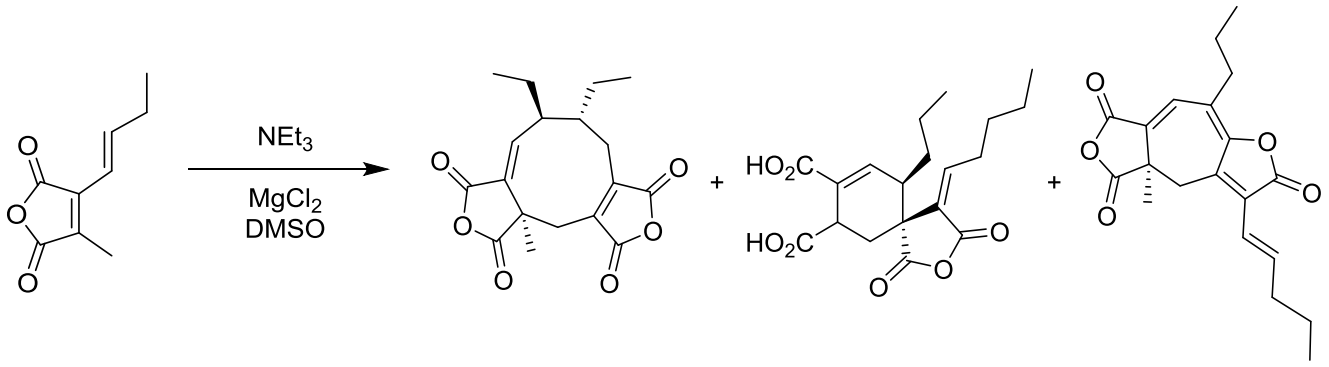
Early *in vitro* studies by Sutherland showed that it was possible to deprotonate methylmaleic anhydride precursor **21** by treatment with triethylamine in DMF, and thus trigger a dimerisation to isoglaucanic acid **123** in 4% yield.[147]



21 **123**

Scheme 53. *In vitro* dimerisation to produce *iso*glaucanic acid **123**.[147]

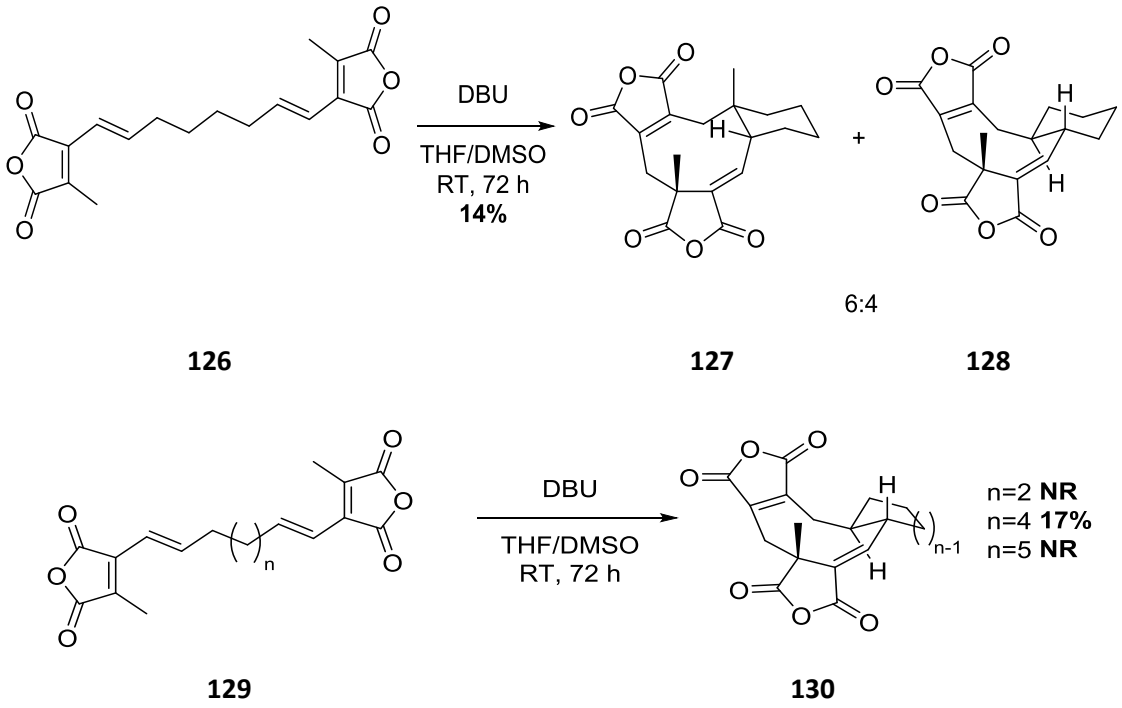
The biomimetic synthesis of *iso*glaucanic acid was further investigated by Baldwin and coworkers as part of investigations towards the bio-inspired synthesis of the phomoidrides.[148] Treatment of precursor **21** with NEt_3 and $MgCl_2$ in DMSO gave isoglaucanic acid **123** and two other products, spiro-compound **124** and heptadride-like structure **125**, shown in Scheme **54**. It was suggested that $MgCl_2$ coordinates to two equivalents of the starting material, thus aiding dimerisation. The isolation of these alternative products indicated that a stepwise Michael addition mechanism was more likely than a concerted 6π+4π cyclodimerisation. Reaction conditions for this dimerisation were more thoroughly investigated, with the choice of base, solvent, temperature and the presence of additives all shown to have an impact on the overall yield of dimers, and also the ratio in which they are formed. In all cases, polymerisation of the starting material predominated.



21 **123** **124** **125**

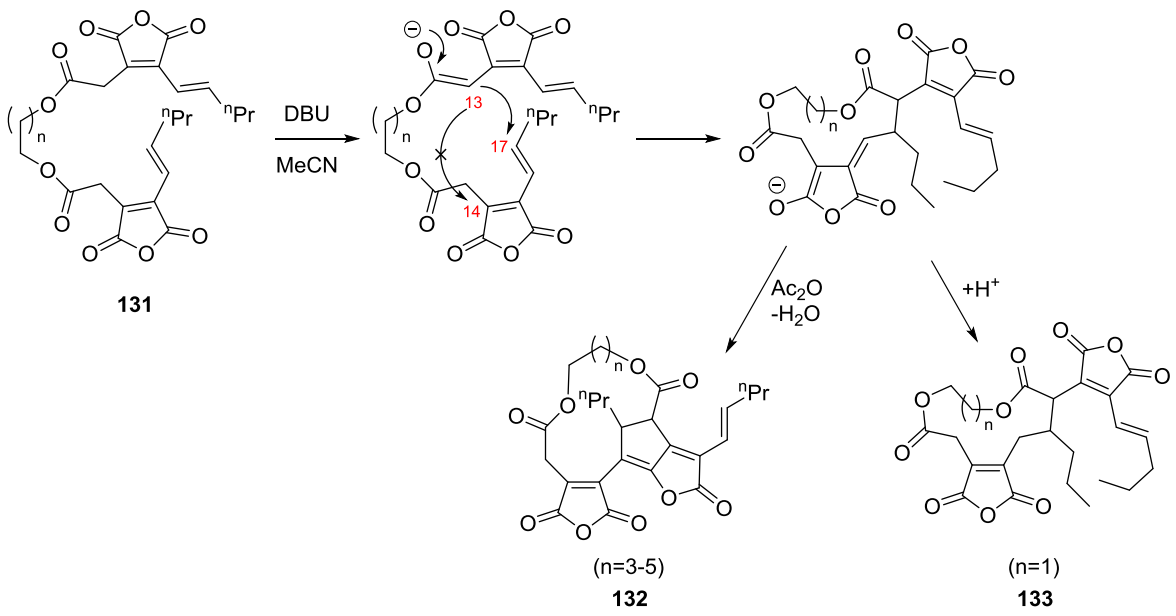
Scheme 54. Products formed in Baldwin's dimerisation study.[148]

The Baldwin group further investigated this dimerisation by considering the effect of tethering two of the maleic anhydride starting units together, using **126** (Scheme **55**).[149] In this way, they hoped to develop methodology applicable to the synthesis of the phomoidrides A and B **11**. These maleidride natural products have received significant synthetic attention due to their potent inhibition of squalene synthase and RAS-farnesyl transferase.[26,150] The tethering resulted in modest improvements to the yield of glaucanic acid analogues, resulting from a *head-to-head* dimerisation mode. Tethering meant re-optimisation of reaction conditions was necessary, the use of triethylamine with the tethered substrate failed to trigger a cyclisation, and there was no evidence that deprotonation was occurring, even at elevated temperatures. DBU however did lead to formation of the desired anion, and resulted in dimerisation to **127** and **128**. Cleaner reaction profiles were obtained in DMSO/THF mixtures, but the reaction could be performed in a variety of solvents. $MgCl_2$ or other additives had no impact on the reaction, presumably because the pre-organisation it was thought to promote is now instead achieved by the tether.



Scheme 55. Glaucanic acid analogues resulting from Baldwin's tethering experiments.[149] **NR** indicates no reaction.

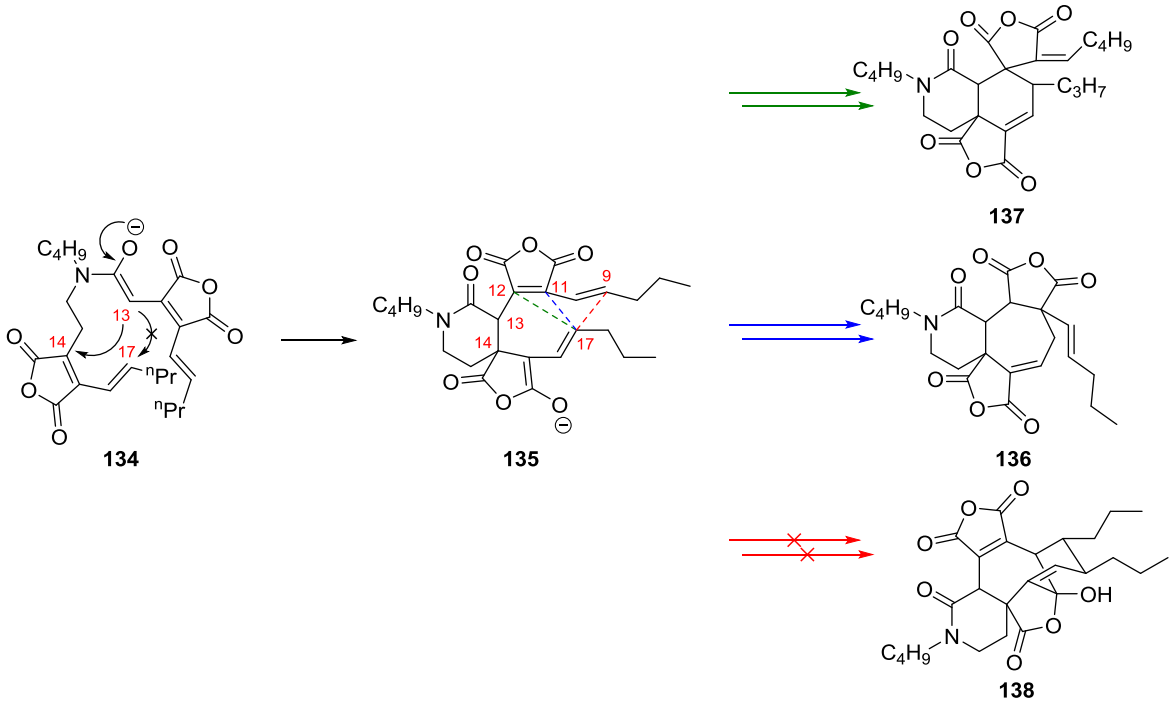
Further tethering experiments were performed by Sulikowski.[151] Whilst Baldwin tried to form the reactive anion by deprotonation of a vinylmethyl moiety with an amine base, studies on maleidride biosynthesis suggest the presence of a carboxyl substituent at this position. Whether the decarboxylation forms the anion required for dimerisation, or if the carboxyl group serves to lower the pKa of the vinylmethylene proton, then later decarboxylates, is unclear. Sulikowski and coworkers therefore chose to use this carboxyl functionality to attach their tether, forming bis-esters **131** with varying chain lengths (Scheme **56**). This pre-organises the molecule in a different fashion to that achieved by Baldwin *et al.*, but was hypothesised to be beneficial for *head-to-head* dimerisation.



Scheme 56. Sulikowski's bis-ester tethers lead to a C13-C17 bond formation, rather than the desired C13-C14 bond.[151]

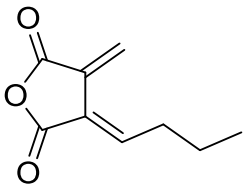
In all instances however, the enolate generated failed to form the desired C-13→C-14 bond, and instead the dominant reaction pathway was Michael addition of the C-13 enolate to C-17, giving **132** or **133**. It was suggested that either the Michael addition pathway was not occurring or was reversible, and therefore an equilibrium existed between C-13→C-14 and C-13→C-17 bond formation. If the C13-C17 bond was the thermodynamic endpoint of the reaction, perhaps the C-13→C-14 product may be a kinetically-produced intermediate. A trapping agent was added to the reaction mixture (acetic anhydride), with the aim of preventing this kinetic intermediate degrading, however no such product was isolated. Instead, the C-13→C-17 product underwent a further dehydration with the longer tethers, generating the lactones **132** and **133** shown.

Follow-up work by the Sulikowski group instead used an amide tether between the maleic anhydride units.[152] With this substrate **134**, the desired C-13→C-14 bond (the first bond made in maleimide biosynthesis) was successfully formed (**135**, Scheme **57**). At this point the resulting dienolate **135** could potentially form a C-C bond to C-9, C-11 or C-12 (shown in green, blue and red respectively in Scheme **57**). Products resulting from addition to C-11 (**136**) and C-12 (**137**) were observed, however no products resulting from the C-9 addition were detected. This was unfortunate as C-9 addition products (**138**) could lead to maleimide structures. The transition state for each bond forming process was modelled computationally, and suggested that the desired C-17→C-9 bond forming process was higher in energy than the pathways observed.



Scheme 57. Sulikowski's dimerisation efforts with an amide-tethered substrate.[152]

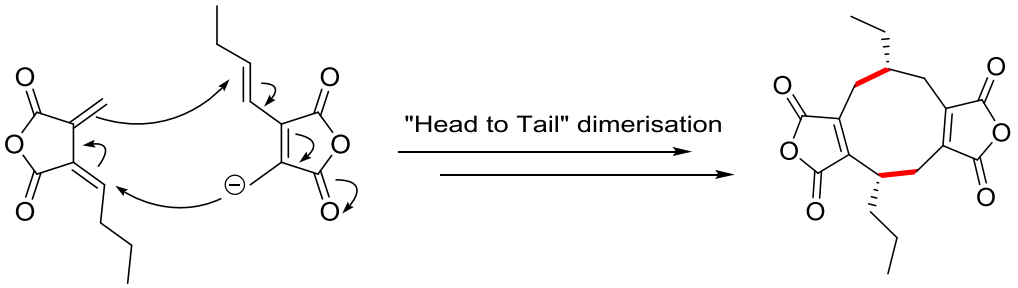
In all of these examples, the dimerisation process has relied on the maleic anhydride monomer structure, and the fulgenic anhydride postulated as a biosynthetic intermediate in the biosynthesis of deoxyscytalidin or byssochlamic acid was not explored. There is one early example of the synthesis of such a fulgenic anhydride, however it was deemed too reactive to explore further.[83] This is contrary to the observation by the Hosoe group that the natural product waquafranone B could be isolated from the freshwater fungus *Wicklowia aquatica*, and characterised without difficulty.[77] Therefore, with our chemistry to the tricladic acids developed, we aimed to investigate the structure and stability of waquafranone B **139**.



Waquafranone B, 139

4.2 Results and Discussion

Head-to-tail dimerisation to yield nonadrides of the byssochlamic acid/deoxyscytalidin-type structure requires the combination of a fulgenic anhydride **139** and maleic anhydride monomer **21** (Scheme **58**). Thus in order to investigate this it was necessary to prepare both fragments.



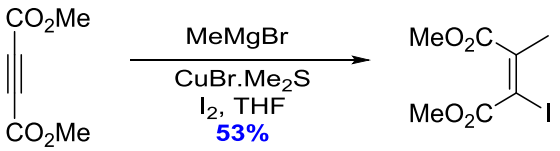
139 **21** **6**

Byssochlamic Acid

Scheme 58. Barton and Sutherland's proposed dimerisation mechanism between a fulgenic anhydride (**139**) and a maleic anhydride intermediate (**21**).[38]

4.2.1 Synthesis of Tricladolide D and the Scytalidin Monomer

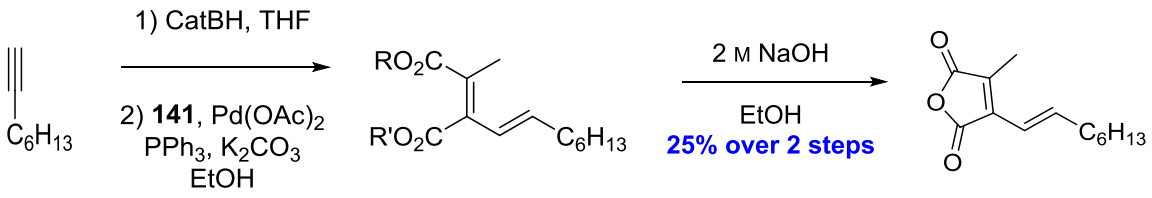
Synthesis of the maleic anhydride monomer **21** has previously been reported by Baldwin and coworkers, for their biomimetic syntheses of *iso*glaucanic acid,[148] and the same route was used herein. Conjugate addition of a methylcuprate reagent generated from MeMgBr and CuBr.Me_2S to dimethyl acetylenedicarboxylate **140**, followed by trapping with iodine, generated vinyl iodide **141** in 53% yield (Scheme **59**).[153] This reaction was very sensitive to temperature and generated a significant exotherm, and so slow addition of each reagent was necessary using a syringe pump, whilst maintaining an internal temperature below -67 °C.



140 **141**

Scheme 59. Synthesis of vinyl iodide **141** by 1,4-addition of an organocuprate, followed by trapping with iodine.[153]

Hydroboration of 1-octyne **142** with catecholborane yielded catecholboronic ester **143** (Scheme **60**). This reaction was performed with an excess of 1-octyne **142**, as it was found that unreacted alkyne could be much more easily removed from the catecholboronic ester **143** than catecholborane.

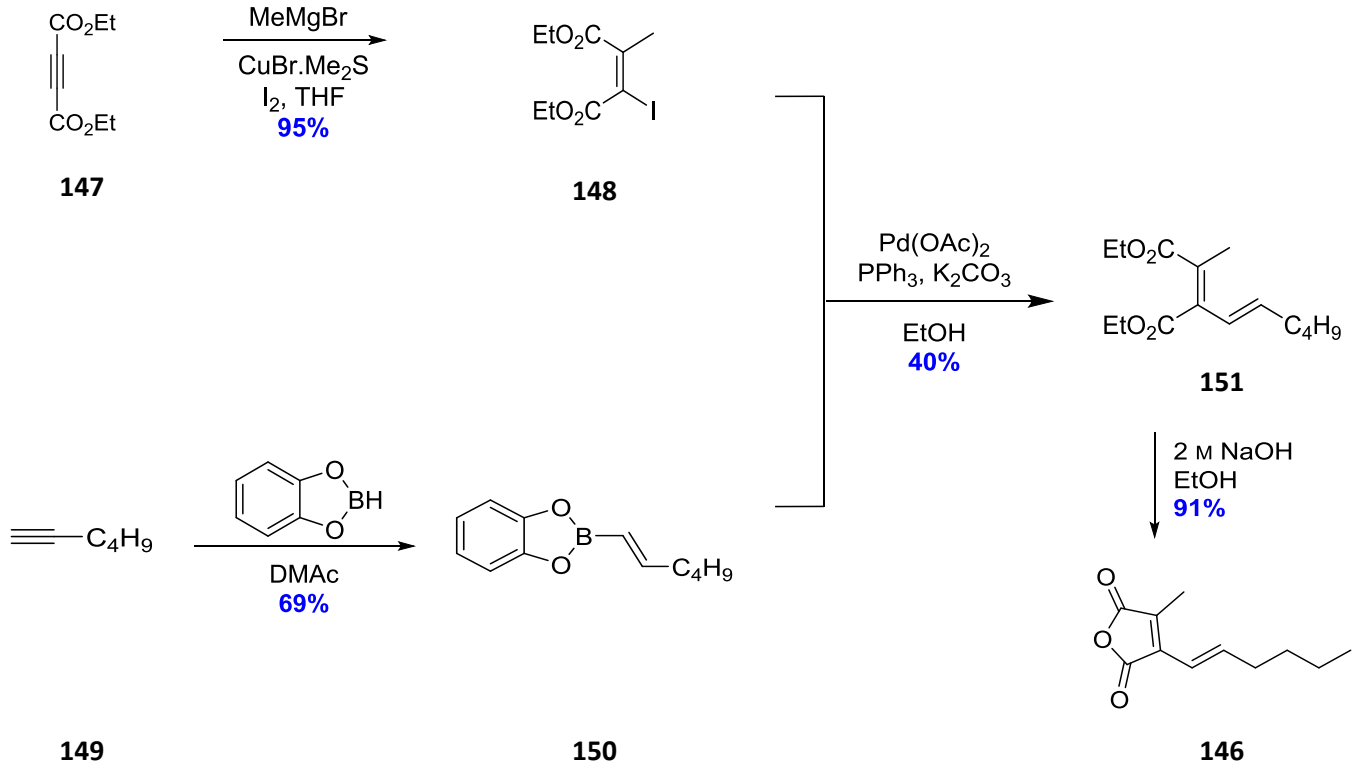


142 **144** **145**

Scheme 60. Synthesis of tricladolide D **145**. R=Me or Et, R'=Me or Et.

Suzuki coupling of vinyl iodide **141** and catecholboronic ester **143** was then performed using [$Pd(PPh_3)_4$] in ethanol. Whilst this did lead to the cross-coupled product **144**, transesterification of the dimethyl ester moiety with ethanol complicated purification, as the methylethyl, dimethyl and diethyl products were all formed. Nevertheless, this mixture could be hydrolysed to tricladolide D **145** in 25% yield over two steps. ^{1}H and ^{13}C NMR data were in agreement with the literature.[53]

Two improvements were therefore made when this route was subsequently used for the synthesis of the scytalidin monomer **146**, which differs only in the length of the alkyl chain. Firstly, whilst the hydroboration was possible using commercially available 1 M solutions of catecholborane in THF, both yield and purity were greatly improved when the reaction was performed solvent-free, with the addition of 0.1 equiv. of dimethylacetamide (DMAc).[154] This gave a catecholboronic ester which could be used without purification.

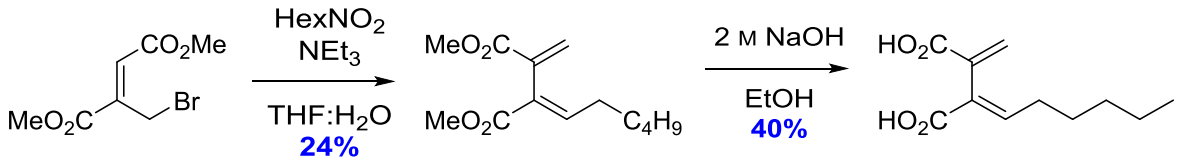


Scheme 61. Synthesis of the scytalidin monomer **146**

To avoid the transesterification issue, the solvent for the Suzuki coupling was matched to the esters present on the starting material. As the Suzuki coupling is reported to perform poorly in methanol, this synthetic route was instead performed with diethyl acetylenedicarboxylate **147**.[153] This made isolation of the Suzuki reaction product **151** significantly easier. Hydrolysis of the diester **151** to the anhydride **146** was then performed, yielding the required monomer.

4.2.2 Synthesis of Alkylfulgides

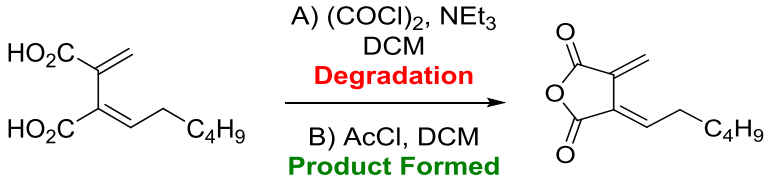
With the chemistry necessary for the synthesis of fulgenic diacids developed in order to synthesise the tricladic acids, the ring closure to a fulgenic anhydride (fulgide) was next explored. For this, a simple alkyl fulgenic diacid **152** was first synthesised (Scheme **62**). The NSAE reaction was employed again, using nitrohexane and dimethyl α-bromethylfumarate **68**. Hydrolysis of the resulting diester **153** yielded diacid **152**. Treatment of diacid **152** with oxalyl chloride/triethylamine (as previously reported[83]) was unsuccessful, leading to a complex mixture of products, one of which being the maleic anhydride structure **146**. Instead, the required anhydride **154** was synthesised by reaction of diacid **152** with acetyl chloride at -30 °C overnight (Scheme **63**).



68 **153** **152**

Scheme 62. Synthesis of diacid **152**.

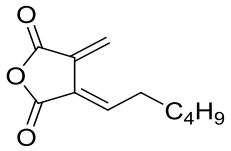
The anhydride **154** thus obtained is ostensibly a close homologue of waquafranone B **139**, differing only in the length of the alkyl chain.[77] However, the synthesised anhydride **154** showed remarkably different physical and spectroscopic properties compared to those reported for the natural product,[77] and correlated well with those previously reported for synthetic material (Table **7**).[83] Rather than being a stable compound as one could imagine for isolation from fungal cultures, the fulgide **154** degraded quickly at room temperature to a complex mixture of products.



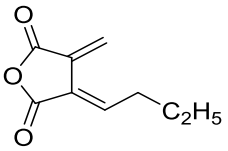
152 **154**

Scheme 63. Synthesis of anhydride **154** from diacid **152**

Significant differences in the ^{1}H NMR chemical shifts between synthetic **154** and waquafranone B **139** also confirmed suspicions that the natural product may have a different structure from that originally proposed. As can be seen in Table 7, the ^{1}H NMR chemical shifts for synthetic **154** are much more similar to those reported by previous synthetic efforts, rather than waquafranone B **139**. This discrepancy will be addressed in section **4.2.2**.



154



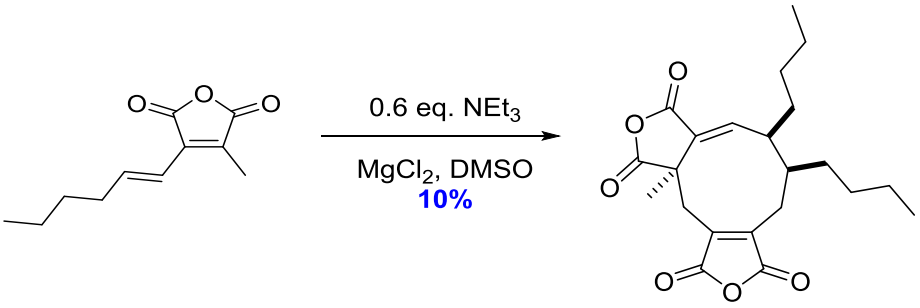
Waquafranone B, 139

Compound	Solvent	NMR δ_H
Waquafranone B **139**	$CDCl_3$	6.97, 6.45, 5.62
Anhydride **154**	$CDCl_3$	7.22, 6.57, 6.12
Huff Synthesis **139**	CCl_4	7.10, 6.50, 6.10
Anhydride **154**	CCl_4	7.10, 6.50, 6.03

Table 7. Comparison of olefinic ^{1}H NMR chemical shifts for waquafranone B **139**,[77] anhydride **154** synthesised in this work, and a prior synthetic report.[83]

4.2.3 **Cross-dimerisations**

With both monomers for the dimerisation study now synthesised, this step could now be investigated. Firstly, a repeat of the Baldwin synthesis of *iso*-glaucanic acid was carried out, to check the appropriate anion could be formed.[83] Following literature conditions, the analogue **155** was synthesised in 10% yield, the same yield as reported by Baldwin.



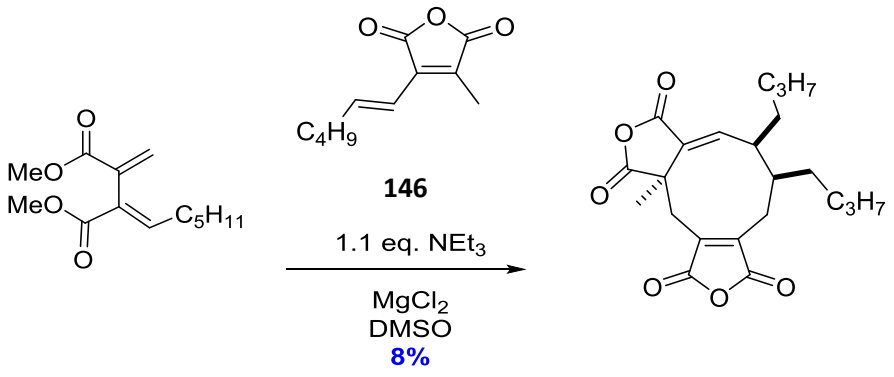
146 **155**

Scheme 64. Synthesis of an *iso*-glaucanic acid analogue **155** via dimerisation of **146**.

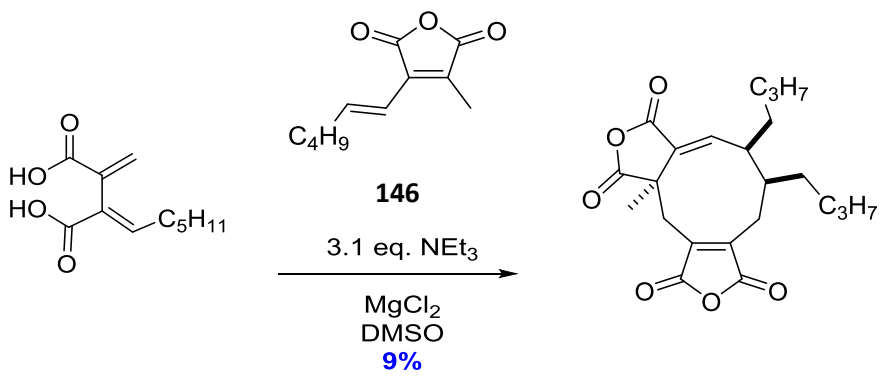
Knowing that fulgenic anhydride **154** was unstable at room temperature, the use of the stable diester **153** and diacid **152** in the cross-dimerisation reaction was attractive. Treatment of diester **153** or diacid **152** with **146**, NEt_3 and $MgCl_2$ gave only *iso*-glaucanic acid analogue **155**, with diester **153** and diacid **152** recovered unchanged from the respective reactions (Scheme **65**). This was the case even when the concentration of maleic anhydride **146** was kept low through a syringe pump addition over 8 h. Under all conditions investigated, diester **153** and diacid **152** were recovered unchanged.

Fulgenic anhydride **154** was then used. This was freshly prepared from diacid **152** in acetyl chloride at -30 °C, then the excess acetyl chloride and solvent removed *in vacuo*, whilst maintaining the temperature at -30 °C. The crude fulgenic anhydride **154** was then dissolved in DMF and the

temperature kept at -30 °C for each attempted dimerisation. The solvent swap to DMF was necessary, as a DMSO solution would freeze at the temperatures necessary, and DMF has successfully been used previously in dimerisation reactions.[148]



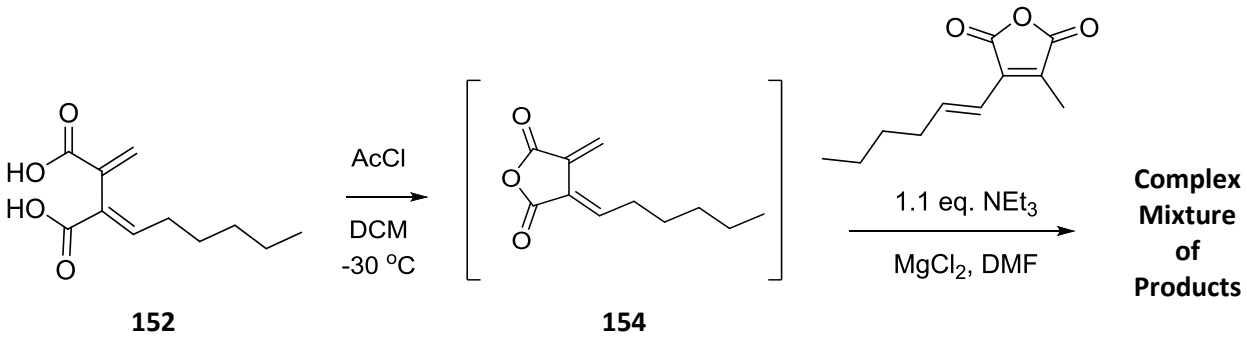
153 **155**
(**153** recovered unchanged)



152 **155**
(**152** recovered unchanged)

Scheme 65. Cross dimerisation of diester **153** or diacid **152** with maleic anhydride **146**.

LCMS analysis of the crude reaction mixture is shown in Figure **37**. Many products were formed, of which 4 peaks (9.20 min, 9.40 min, 9.70 min and 10.40 min) showed the correct mass (388) in the corresponding ES- trace for the dimer. Of these, the peak at 10.40 min showed the same retention time as a standard of deoxyscytalidin **35** (highlighted), and several common fragment peaks were observed in the ES- mass spectrum (388, 343, 193). Whilst high-resolution mass spectrometry confirmed the presence of a compound with a mass of 387.1808 ($C_{22}H_{27}O_6$), as expected for deoxyscytalidin **35**, neither ^{1}H or ^{13}C NMR analysis of the crude reaction mixture showed peaks matching deoxyscytalidin **35**, nor could any deoxyscytalidin **35** be obtained by preparative LCMS. Interestingly, the iso-glaucanic acid analogue **155** was no longer formed under these conditions. It must therefore be concluded that if any deoxyscytalidin **35** was formed, it was in insufficient quantity for this to be a practical synthesis.



152 **154**

Scheme 66. Attempted dimerisation of alkylfulgide **154** with anhydride **146**

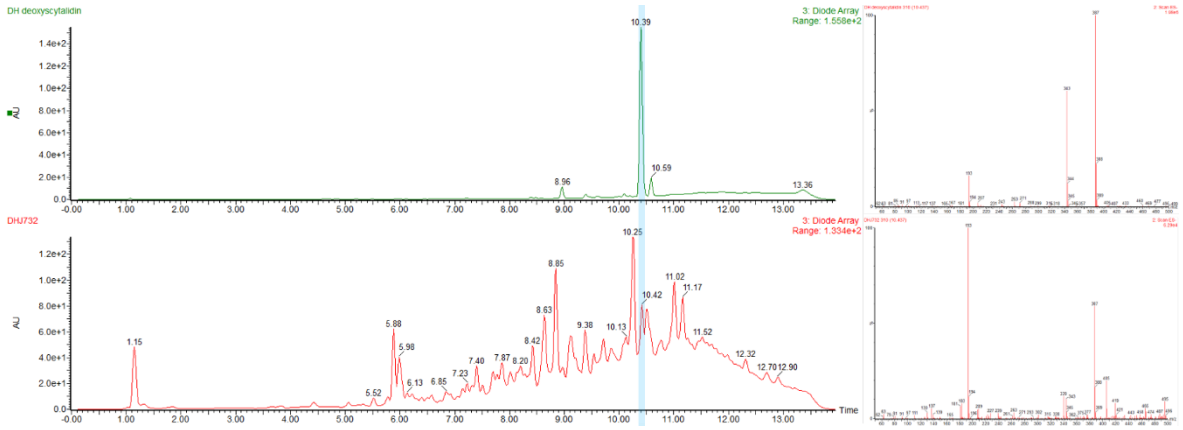
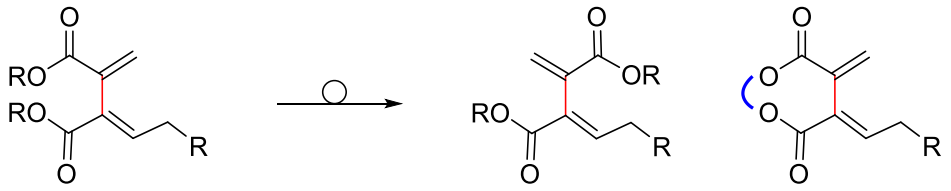


Figure 37. LCMS analysis of the dimerisation reaction between maleic anhydride **146** and fulgide **154**. Shown are diode array traces of a standard of deoxyscytalidin **35** (*top*), and crude reaction mixture (*bottom*). Respective ES- fragmentation patterns are shown for the peak at 10.4 min.

4.2.4 **Conformationally Locked Analogues**

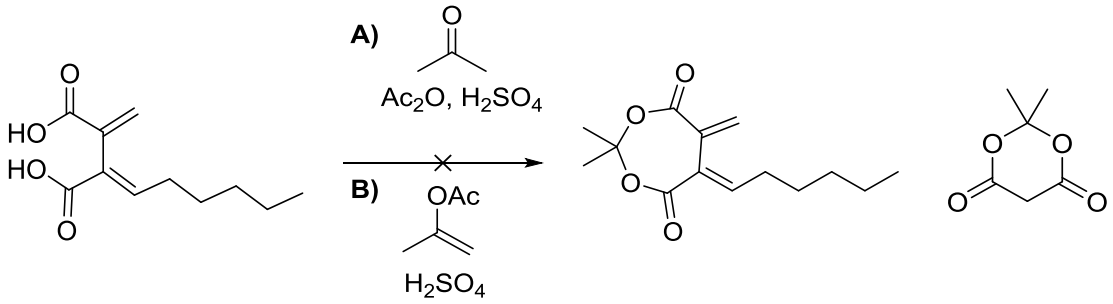
Given the fulgenic anhydride **154** was highly reactive, and the corresponding diester **153** and diacid **152** unreactive under the conditions for dimerisation, a compound with intermediate reactivity was desirable. Ideally, such a compound would be stable at room temperature and in the presence of base but would successfully perform the cross-dimerisation. It was hypothesised that the diene moiety of anhydride **154** adopts a coplanar configuration, giving good orbital overlap and facilitating the required reaction. In contrast, the free rotation around the C2-C3 bond (highlighted in Scheme **67**) meant diacid **152** and diester **153** do not adopt this reactive coplanarity for a significant proportion of the time. It was hoped that a conformationally locked diester, of the type **156** would force a greater degree of coplanarity in the diene moiety, without leading to the significant instability demonstrated for anhydride **154**.



152 (R=H), **153** (R=Me) **152** (R=H), **153** (R=Me) **156**

Scheme 67. Free rotation around the highlighted bond may prevent adoption of a reactive conformer for the dimerisation, this could be prevented through a tether.

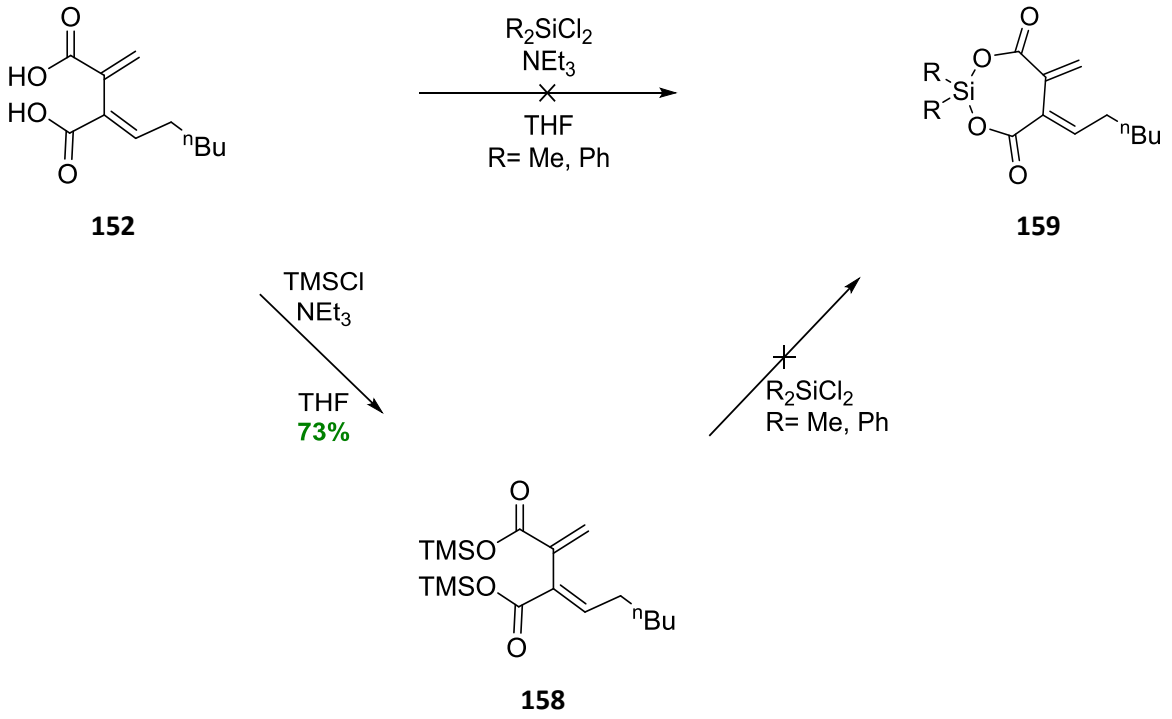
Synthesis of acetonide **157**, similar to Meldrum's acid, was initially explored. Following the original synthesis of Meldrum's acid, with acetic anhydride and sulfuric acid in acetone, gave a complex mixture of degradation products.[155] An updated, somewhat milder procedure using propenyl acetate also failed to give the desired acetonide.[156]



152 **157** **Meldrum's acid**

Scheme 68. Attempted synthesis of a Meldrum's acid-like acetonide from diacid **152**

A silyl linkage, using a dimethylsilyl or diphenylsilyl bridge was next explored. There is literature precedent for the formation of such linkages proceeds via the bis(TMS) ester.[157,158] This bis(TMS) ester **158** was successfully formed from diacid **152**, however the following transsilylation reaction could not be achieved with dimethyldichlorosilane or diphenyldischlorosilane, with a complex mixture of degradation products produced. Synthesis directly from diacid **152** was also unsuccessful.

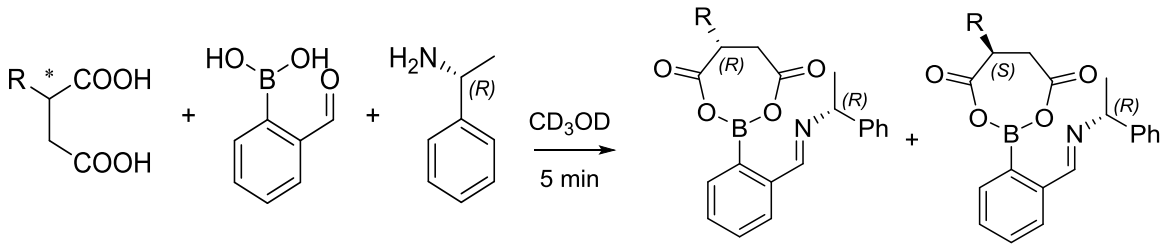


152 **159**

158

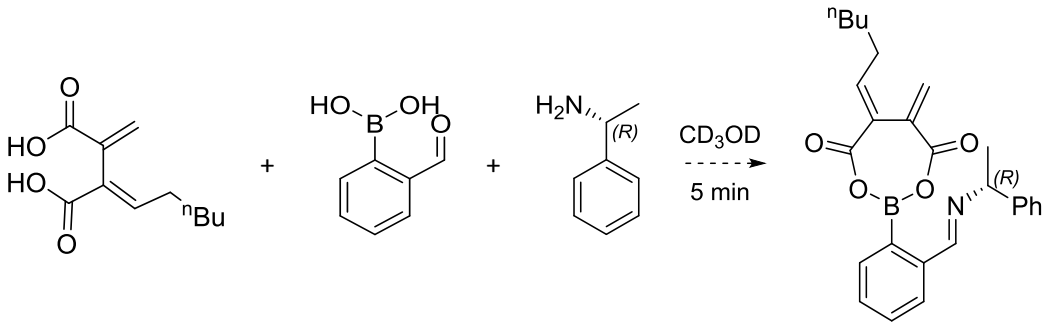
Scheme 69. Attempted synthesis of a silyl-bridged analogue **159**.

Finally, an approach utilising an imino-boronate ester complex was investigated. Originally reported for the spectroscopic differentiation of chiral succinic acids, it was hoped that it could be applied in this context to lock the conformation of **152**.[159]



Scheme 70. Suryaprakash's method for spectroscopic discrimination of chiral diacids through diastereomeric imino-boronate complexes.[159]

(*R*)-(+)-α-Methylbenzylamine, 2-formylphenylboronic acid and diacid **152** were combined in deuterated methanol (Scheme **71**), and the condensation reaction followed by ^{1}H NMR analysis. As can be seen in figure **38**, upon addition of 0.5 equivalents of the amine and boronic acid, a small upfield shift of the olefinic peaks was observed. The effect was much less pronounced on the methylene protons from the alkyl chain. Adding a further 0.5 equivalents of each reagent lead to an upfield shift of similar magnitude.



152 **160**

Scheme 71. Attempted condensation reaction between diacid **152**, 2-formylphenylboronic acid and (*R*)-(+)-α-methylbenzylamine.

The ^{1}H NMR spectrum in the presence of 0.5 equivalents of each reagent is the most illuminating. An equal mixture of the starting diacid **152** and product **160** would be expected to show separate peaks for each species. Whilst an averaged signal can be obtained if the reaction is fast on the NMR timescale, it seems unlikely that this reaction is sufficiently rapid.[160]

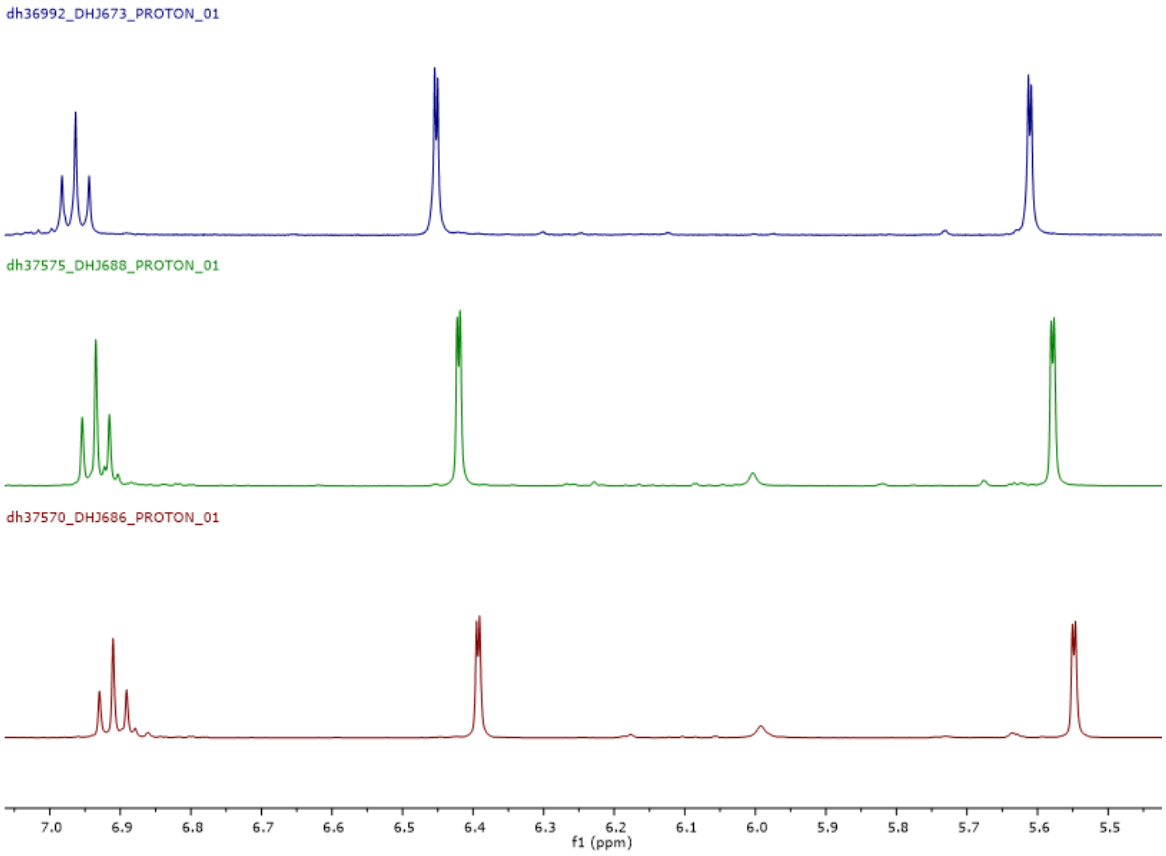


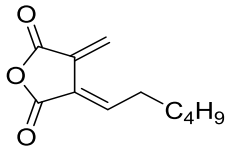
Figure 38. ^{1}H NMR of the olefinic region of the complexation of **152** with α-methylbenzylamine and 2-formylboronic acid. NMR of diacid **152** (*top*), upon addition of 0.5 equivalents of amine and boronic acid (*middle*), and 1.0 equivalents of amine and boronic acid (*bottom*).

Instead, this result suggests that the addition of an amine and boronic acid to the reaction mixture is changing the basicity of the reaction mixture, and the change in chemical shift of the olefinic

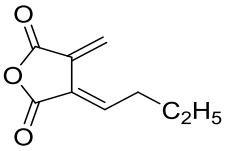
protons is a result of deprotonation of the carboxylic acid moieties, rather than formation of the boronate ester. This was confirmed by addition of an excess of triethylamine to the reaction mixture, leading to a much more dramatic shift in chemical shift for the protons of interest. It was therefore concluded that the condensation of the boronic acid with diacid **152** was not occurring, although the imine formation between the chiral benzylamine and formylphenylboronic acid was observed. Therefore, this is not an appropriate method with which to conformationally restrain diacid **152** for the desired dimerisation reaction.

4.2.5 Structural Reassignment of Waquafranone B

It was noted in Section **4.2.2** that the physical properties and NMR data obtained for anhydride **154** were markedly different to those reported for waquafranone B, a very similar homologue. This suggested either the additional ethylene unit had a marked effect on the properties of the anhydride **154**, or that the structure of waquafranone B had been mis-assigned in the literature.



154



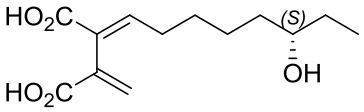
Waquafranone B, 139

Compound	Solvent	NMR δ_H
Waquafranone B **139**	$CDCl_3$	6.97, 6.45, 5.62
Anhydride **154**	$CDCl_3$	7.22, 6.57, 6.12
Huff Synthesis **139**	CCl_4	7.10, 6.50, 6.10
Anhydride **154**	CCl_4	7.10, 6.50, 6.03

Table 8. Comparison of olefinic ^{1}H NMR chemical shifts for waquafranone B,[77] the anhydride **154** synthesised in this work, and a prior synthetic report by Huff.[83]

The structure of our synthesised fulgide **154** was secure, due to good NMR agreement with other previous literature,[83] the extensive characterisation of the alkene geometry by EXSIDE and NOESY NMR carried out on the precursor, and the successful synthesis of the tricladic acids achieved using this methodology.

In order to assign a new structure to waquafranone B, the likely alternative structures were first considered. The NMR data were next compared to the tricladic acids, shown in table **9**.

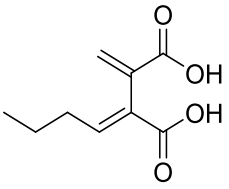


37

Compound	^{1}H NMR (olefinic region, $CDCl_3$)	^{13}C NMR (olefinic region, $CDCl_3$)
Tricladic Acid B **37**	6.96, 6.44, 5.61	145.4, 136.7, 131.0, 128.2
Waquafranone B **139**	6.97, 6.45, 5.62	146.8, 137.9, 132.4, 129.8

Table 9. Comparison of NMR data reported for waquafranone B **139** with that obtained for tricladic acid B **37**. Tricladic acid B was used for this comparison due to higher solubility in $CDCl_3$ than other members of this family of natural products.

As can be seen, ^{1}H and ^{13}C data are in much better agreement for this diacid than for the anhydride **154**, suggesting waquafranone B **139** has been previously assigned a fulgide structure, where it is more likely to be a fulgenic diacid. Literature IR data for waquafranone B report only a single carbonyl stretch at 1685 cm^{-1}, which is consistent with an unsaturated carboxylic acid. An unsaturated anhydride would be expected to show two bands in the IR spectrum, for the symmetric and asymmetric stretches at 1870 cm^{-1} and 1770 cm^{-1} respectively.[161] Whilst the reported MS peak at 166.0628 was assigned previously as the M^{+} fragment, this could equally be explained by the loss of water upon ionisation.[162]



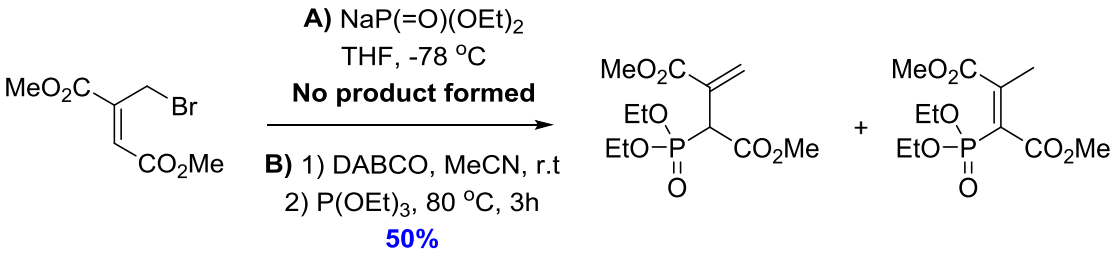
161

Figure 39. Proposed structure of waquafranone B

This analysis therefore suggested the structure shown in figure **39** as the true structure of waquafranone B, and thus a synthetic sample was required. A route based on a Horner-Wadsworth-Emmons (HWE) reaction was first explored. In comparison to the NSAE reaction investigated for the synthesis of the tricladic acids, this approach was previously reported to show poorer stereoselectivity in the formation of the trisubstituted alkene. However, when trying to prove the structure of waquafranone B, this would actually be advantageous.[90] Simultaneous synthesis of **(*Z*)-161** would allow us to prove that misassignment of alkene geometry was not responsible for the anomalous NMR data reported for the natural product.

Phosphonate **162** has previously been synthesised by Amri from bromide **68**, which was previously used in synthesis of the tricladic acids (Chapter **3**).[90] However, the reported conditions, using the diethyl phosphite anion in THF, could not be reproduced. Instead, conditions reported by Kim were

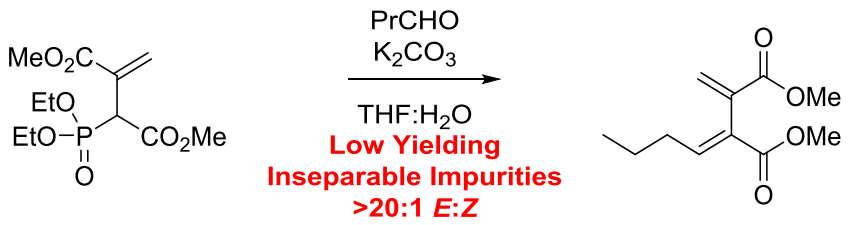
used (Scheme **68**).[163] This Michaelis-Arbuzov approach gave phosphonate **162** in 50% yield. This approach was not without difficulties, as extended reaction times resulted in isomerisation of the phosphonate, yielding **163**. In addition, purification of the desired phosphonate **162** was laborious, and so it was used crude.



68 **162** **163**

Scheme 72. A Michaelis-Arbuzov reaction was used for the synthesis of phosphonate **162**.

The HWE reaction of phosphonate **162** with K_2CO_3/butyraldehyde in THF:Water did lead to some product **164** formation, however this could not be separated from the large quantity of by-products formed in this reaction. In addition, integration of the olefinic peaks in the ^{1}H NMR spectrum suggested an *E*:*Z* ratio in excess of 20:1. The combination of purification difficulties and very low yields of **(*Z*)-164** meant this reaction possessed no advantages compared to the NSAE reaction previously applied in Chapter **3**. Alternative olefination reactions were therefore investigated, with the aim of increasing the yield of **(*Z*)-164**.

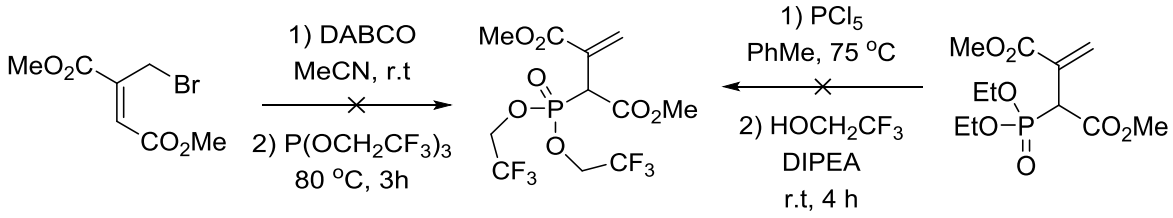


162 **164**

Scheme 73. HWE synthesis of diene **164**.

Given the Michaelis-Arbuzov approach could introduce the desired *exo*-methylene at the same time as the phosphonate, changing the phosphonate seemed worthy of investigation. To this end, a Still-Gennari reaction was considered.[164] This required the synthesis of bis(trifluoroethyl)phosphonester **165**, and it was hoped that a Michaelis-Arbuzov reaction between tris(trifluoroethyl)phosphite and bromide **68** could be achieved using the same conditions (DABCO/MeCN) used to successfully synthesise phosphonate **162**. Unfortunately, no formation of the desired phosphonate **165** was detected, a problem previously reported when using tris(trifluoroethyl)phosphite.[165] Still and Gennari have reported the synthesis of bis(trifluoroethyl)phosphonesters from the corresponding bis(ethyl)phosphonester via formation of the dichlorophosphonate. However, this also failed to give

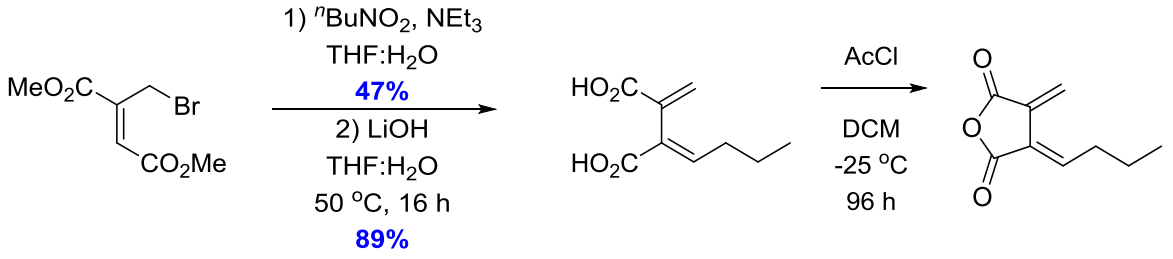
165, instead complete degradation of **162** was observed. Whilst this could have been explored further, for example using the KF/Alumina conditions reported by Busch-Petersen,[166] or through the use of Ando phosphonates,[167] time constraints meant the NSAE approach was instead investigated.



68 **165** **162**

Scheme 74. Routes investigated towards bis(trifluoroethyl)phosphonester **165**

The NSAE reaction of bromide **68** with 1-nitrobutane/triethylamine in THF:H_2O yielded diester **166** in 47% yield. However, deprotection of the diester **166** to diacid **167** under the conditions previously used (2 M NaOH/THF/H_2O) led to significant degradation of the diacid **167**. Column chromatography of diacid **167** also led to increased formation of degradation products.



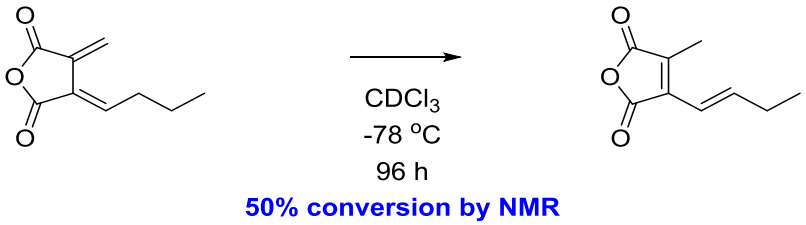
68 **167** **139**

Scheme 75. Synthesis of reported waquafranone B structure **139** from bromide **68**.

Hydrolysis of diester **166** under alternative conditions was investigated. Hydrolysis with bis(tributyltin) oxide/PhMe or sodium trimethysilanolate/DCM returned only starting materials,[168,169] whilst with ten equivalents of LiOH in THF/H_2O, hydrolysis of a single ester moiety was achieved. This reaction was remarkably clean compared to the previously used basic hydrolysis (NaOH/H_2O/THF), with only the mixture of half-esters present in the crude material by ^{1}H NMR analysis. Therefore, these conditions were further investigated. Prolonged reaction times (96 h) did not change the products formed, however heating to 50 °C for 16 h gave diacid **167** in 89% yield. Formation of the alkylfulgide **139** was achieved by treatment of diacid **167** with acetyl chloride in DCM at -25 °C for 96 h. The NMR data for **139** were then compared to those reported for waquafranone B, reported to have the same structure.

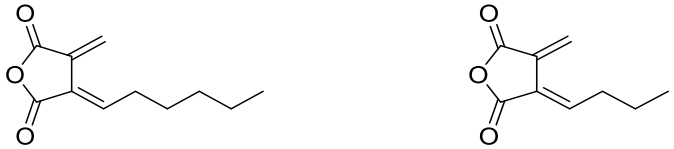
As can be seen in Table **10**, there are significant differences between the NMR data reported for the natural product waquafranone B, and the material thus synthesised. In particular, the 9-H (5.62 vs.

6.13 ppm), and C-2/C-3 (122.3/130.4 vs. 132.4/137.9 ppm). In contrast, greater correlation was seen when comparing ^{1}H NMR data for synthesised **139** with those reported by Huff, and the previously synthesised hexylidene derivative **154** (Table **10**). Additionally, alkylfulgide **139** exhibited the same instability reported by Huff.[83] A sample of **139** was maintained at -78 °C for 48 h, in which time 50% conversion to the maleic anhydride form was observed (Scheme **76**). Together, this would suggest that waquafranone B does not have an alkylfulgide structure.



139 **21**

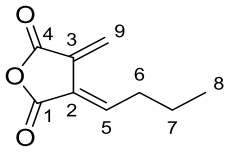
Scheme 76. Conversion of **139** to **21** at low temperature.



154 **139**

Compound	NMR δ_H / CCl_4
Anhydride 154	7.10, 6.50, 6.03
Huff Synthesis **139**	7.10, 6.50, 6.10
Anhydride **139**	7.10, 6.50, 6.02

Table 10. Comparison of olefinic ^{1}H NMR chemical shifts for anhydrides **154** and **139** (synthesised in this work) and a prior synthetic report.[83] In the absence of an internal standard or solvent peak, chemical shifts are referenced by the 7.10 ppm peak.



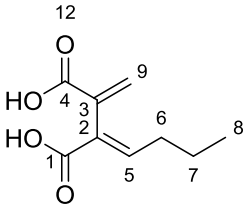
139 / Waquafranone B Original Structure

Position	Anhydride 139 δ_H / $CDCl_3$	Anhydride 139 δ_C / $CDCl_3$	Waquafranone B δ_H / $CDCl_3$	Waquafranone B δ_C / $CDCl_3$
1		163.9		169.7
2		122.3		132.4
3		130.4		137.9
4		163.8		169.4
5	7.24 (1H, t, *J* 7.5)	149.3	6.97 (1H, t, *J* 7.8)	146.8
6	2.52 (2H, q, *J* 7.5)	32.0	2.18 (2H, dd, *J* 7.8, 7.7)	32.6
7	1.68 (2H, q, *J* 7.5)	21.6	1.49 (2H, qd, *J* 7.6, 7.4)	23.0
8	1.04 (3H, t, *J* 7.5)	14.0	0.94 (3H, t, *J* 7.4)	14.2
9	6.13 (1H, s) 6.59 (1H, s)	125.3	5.62 (1H, d, *J* 1.7) 6.45 (1H, d, *J* 1.7)	129.8

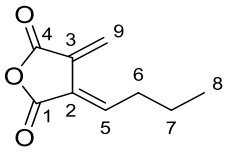
Table 11. Comparison of NMR data for waquafranone B,[77] and the anhydride **139** synthesised in this work.

It was therefore hypothesised that waquafranone B has a diacid structure, rather than an anhydride. The comparison of NMR data reported for waquafranone B and the synthesised diacid **167** are shown in Table **12**.

The data obtained for diacid **167** is in much closer agreement with that reported for waquafranone B, although does not match perfectly. In particular, the difference in chemical shift at the 5-H and 9-H positions was a concern. However, as was observed in Figure **38**, the NMR of alkylfulgenic diacids vary on addition of base to the sample. Therefore, it is likely that the difference between the NMR data arises from a difference in the pH. This was confirmed by addition of 0.5 equivalents of DABCO to a sample of diacid **167**. As can be seen in Figure **40**, this significantly changes the chemical shift of the olefinic protons, in both the ^{1}H and ^{13}C NMR spectra in the presence of DABCO in much better agreement with the literature data for waquafranone B. This strongly suggests that the structure of the natural product has been misasigned as anhydride **139**, when it is in fact diacid **167**.



167



Waquafranone B Original Structure

Position	Diacid 167 δ_H / $CDCl_3$	Diacid 167 δ_C / $CDCl_3$	Waquafranone B δ_H / $CDCl_3$	Waquafranone B δ_C / $CDCl_3$
1		171.9		169.7
2		131.5		132.4
3		134.8		137.9
4		171.7		169.4
5	7.12 (1H, t, *J* 7.8)	148.9	6.97 (1H, t, *J* 7.8)	146.8
6	2.21 (2H, q, *J* 7.5)	31.8	2.18 (2H, dd, *J* 7.8, 7.7)	32.6
7	1.50 (2H, q *J* 7.4)	22.0	1.49 (2H, qd, *J* 7.6, 7.4)	23.0
8	0.93 (3H, t, *J* 7.4)	14.0	0.94 (3H, t, *J* 7.4)	14.2
9	5.71 (1H, d, *J* 1.5) 6.61 (1H, d, *J* 1.5)	129.5	5.62 (1H, d, *J* 1.7) 6.45 (1H, d, *J* 1.7)	129.8

Table 12. ^{1}H and ^{13}C NMR data for diacid **167** and reported data for waquafranone B.

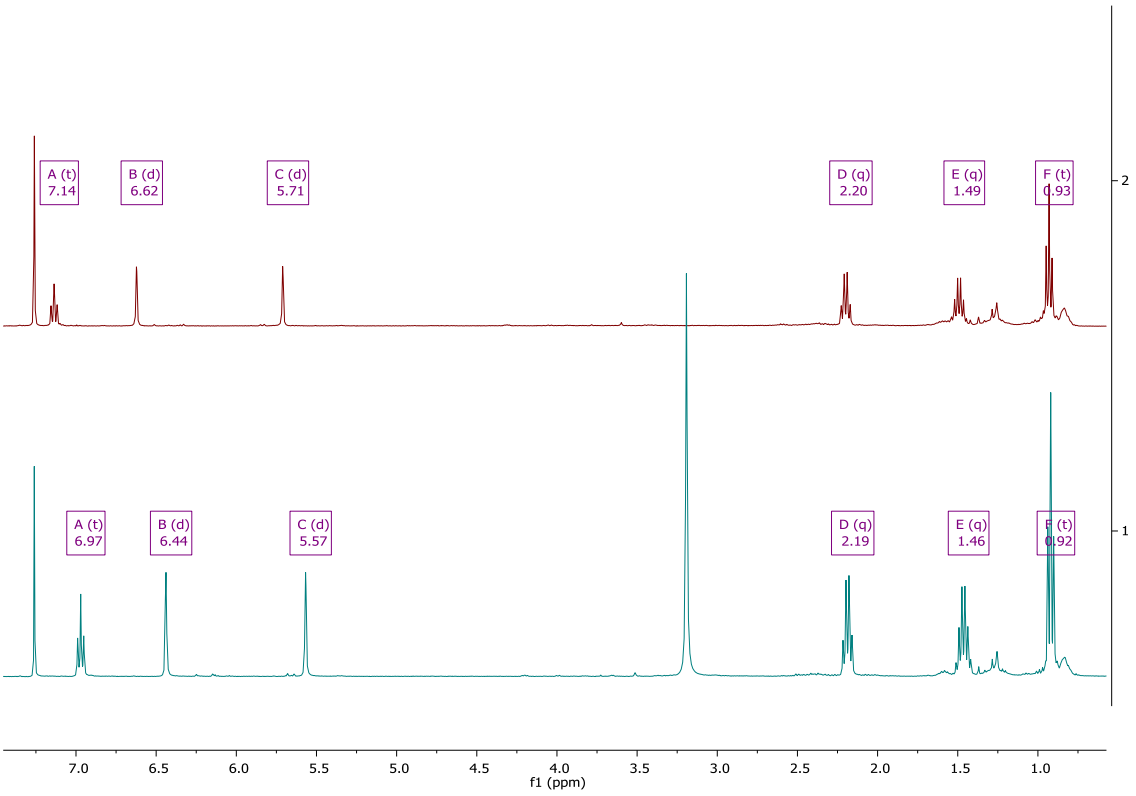
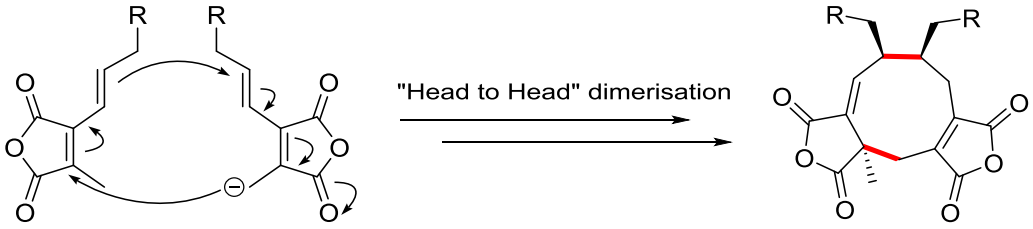


Figure 40. Comparison of ^{1}H NMR of diacid **167** (*top*), and diacid **167** with 0.5 equiv. DABCO (*bottom*). Note: Additional peak at 3.20 ppm in the bottom spectrum is the added DABCO.

Position	Diacid 167 + DABCO δ_H / $CDCl_3$	Diacid 167 + DABCO δ_C / $CDCl_3$	Waquafranone B δ_H / $CDCl_3$	Waquafranone B δ_C / $CDCl_3$
1		171.6		169.7
2		131.5		132.4
3		137.1		137.9
4		171.2		169.4
5	6.96 (1H, t, *J* 7.5)	146.7	6.97 (1H, t, *J* 7.8)	146.8
6	2.19 (2H, q, *J* 7.5)	31.7	2.18 (2H, dd, *J* 7.8, 7.7)	32.6
7	1.46 (2H, q *J* 7.4)	22.2	1.49 (2H, qd, *J* 7.6, 7.4)	23.0
8	0.92 (3H, t, *J* 7.4)	14.0	0.94 (3H, t, *J* 7.4)	14.2
9	5.56 (1H, d, *J* 1.5) 6.43 (1H, d, *J* 1.5)	129.2	5.62 (1H, d, *J* 1.7) 6.45 (1H, d, *J* 1.7)	129.8

Table 13. ^{1}H and ^{13}C NMR data for diacid **167** with 0.5 equiv. DABCO, reported data for waquafranone B.

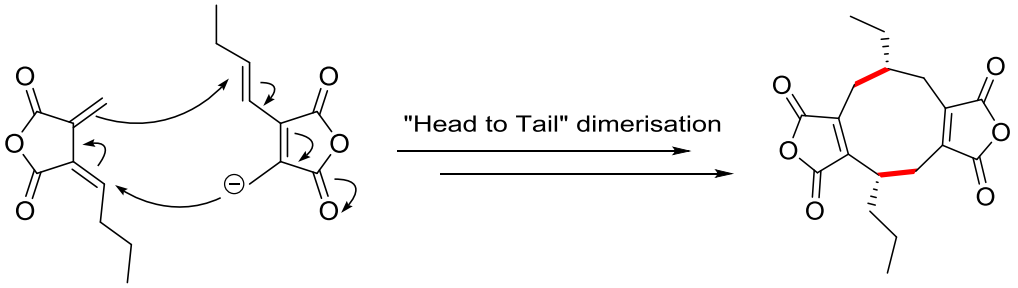
4.3 Conclusions and Future Work



168

Scheme 77. Previous biomimetic dimerisation studies by Barton, Baldwin and Sulikowski focus on "the head-to-head" dimerisation mode.[51,83,148,151]

Previous investigations into biomimetic synthesis of maleidrides have been limited to the "head-to-tail" dimerisation mode, giving rise to glaucanic acid derivatives **168** (Scheme **77**). The "head-to-tail" dimerisation mode (Scheme **78**) has not received significant attention, owing to the reported instability of alkylfulgide **139**. This instability was challenged in 2010 by the isolation of waquafranone B **139** from *W. aquatica*.

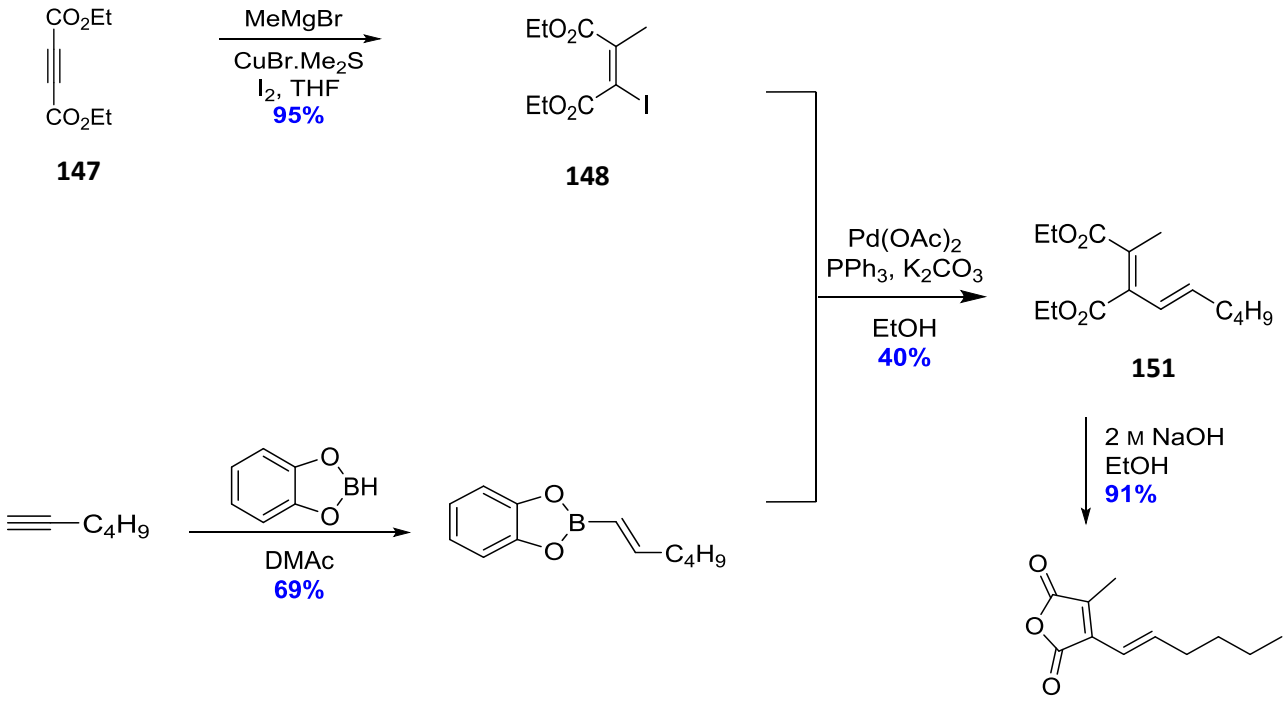


139 **21** **6**

Byssochlamic Acid

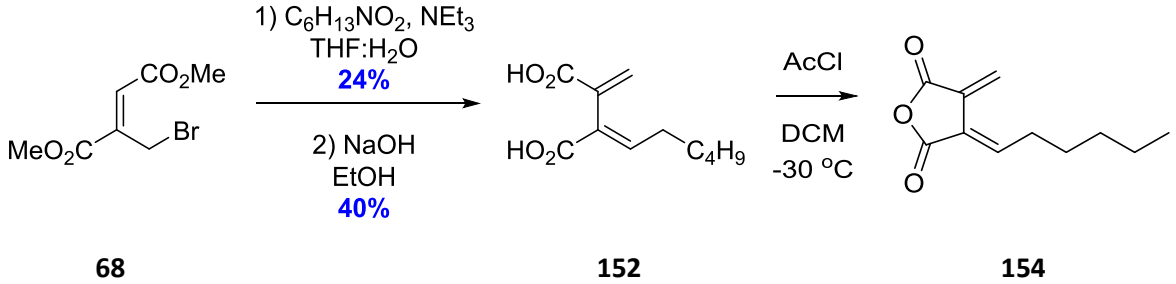
Scheme 78. Barton and Sutherland's proposed dimerisation mechanism between an alkylfulgide (**139**) and a maleic anhydride intermediate (**21**).[38]

In order to investigate the "head-to-tail" dimerisation towards deoxyscytalidin, the required starting materials were both synthesised. Baldwin's synthesis of disubstituted maleic anhydrides was modified to enable facile synthesis of anhydride **146**. Tranesterification during the Suzuki cross-coupling was overcome through the use of the diethyl acetylenedicarboxylate **147**, and improved yields of the catecholboronic ester **150** were achieved through addition of catalytic DMAc. This meant that anhydride **146** could be synthesised in three steps and 35% overall yield.[153]



Scheme 79. Synthesis of anhydride **146**.

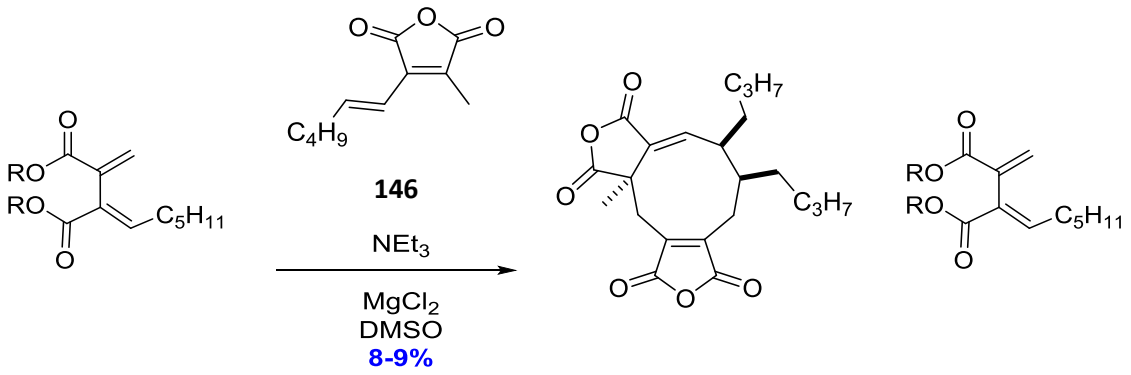
Building on the NSAE methodology developed in Chapter **3**, the diacid **152** was synthesised in two steps and 10% overall yield from bromide **68**. The diacid was converted to the anhydride **154** by treatment with acetyl chloride in DCM. It was found that this compound was highly unstable, making it difficult to use in studies of the "head-to-tail" dimerisation.



Scheme 80. Synthesis of alkylfulgide **154**.

Nevertheless, the dimerisation step was investigated, combining maleic anhydride **146** with either alkylfulgide **154**, diacid **152** or diester **153**. It was found that the diacid **152** and diester **153** were unreactive towards dimerisation, with the head-to-head dimerisation of maleic anhydride **146** giving rise to isoglaucanic acid analogues **155** in both cases. The head-to-head dimerisation of maleic anhydride **146** was inhibited by alkylfulgide **154**, with the reaction forming a complex mixture of products. High resolution mass spectrometry and LCMS analysis suggested formation of a product with the correct mass and retention time to be deoxyscytalidin **35**, however NMR analysis of the

crude reaction products did not support this conclusion, and deoxyscytalidin **35** could not be isolated from the reaction.

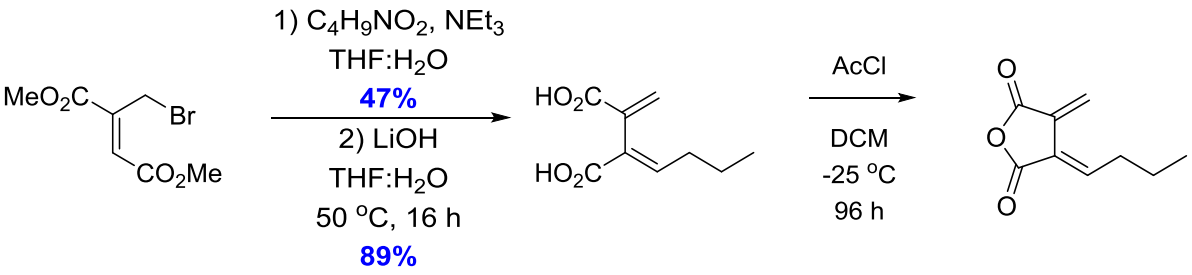


152 R=H **155** **152 R=H**

153 R=Me **153 R=Me**

Scheme 81. Investigation of the dimerisation of **152**/**153** and **146** yielded only the head-to-head dimer **155** and unreacted **152**/**153**.

The instability of anhydride **154** meant the structure of the natural product waquafranone B was doubted. The proposed structure **139** of Gloer *et al.* was synthesised in three steps from bromide **68**, and the NMR data obtained differed significantly from the literature. However, it was shown that the NMR of diacid **167** is highly sensitive to the pH of the sample, and after addition of base to an NMR sample of diacid **167**, the NMR data obtained were in agreement with literature data for waquafranone B. It is therefore strongly suggested that waquafranone B is in fact diacid **167**.



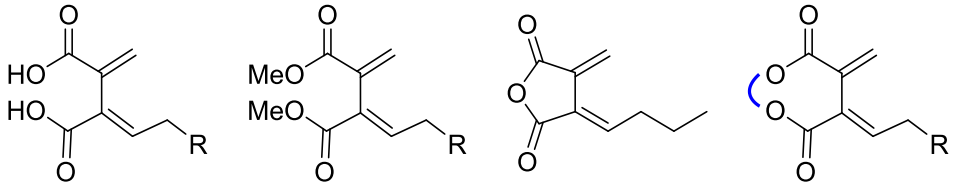
68 **167** **139**

Revised Structure of Waqafranone B **Original Structure of Waquafranone B**

Scheme 82. Synthesis of reported waquafranone B structure **139** from bromide **68**.

Future Work

Alkylfulgide **154** is too unstable for further studies of the "head-to-tail" dimerisation to be easily explored, whilst diacid **152** and diester **153** were recovered unchanged (Figure **41**). No comformationally locked analogues of alkylfulgide **154** were successfully synthesised during this project, however this remains an avenue worth pursuing.

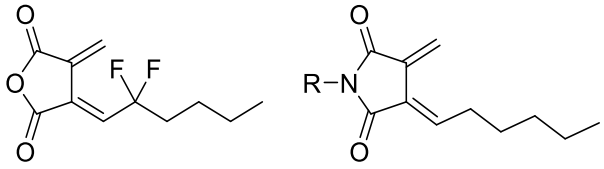


152 **153** **154** **156**

Unreactive **Unreactive** **Unstable** **Underexplored**

Figure 41. Possible coupling partners for the "head-to-tail" dimerisation.

Two possible analogues are shown in Figure **42**. It has been shown that one pathway for degradation of alkylfulgide **154** is conversion to the maleic anhydride form (Scheme **76**). Preventing removal of a proton from C-6 with a *gem*-difluoro group may result in a sufficiently stable alkylfulgide **169** with which dimerisation can be explored. To date, no computational modelling of this dimerisation reaction has been performed. Modelling of the HOMO of **146** or LUMO of **152**/**153**/**154** may show that the energy levels are inappropriate for a productive reaction, and allow rational design of an alkylfulgide analogue which is more suited to dimerisation. The succinimide **170** is one possible analogue in which the energy of the LUMO is likely to be significantly different.



169 **170**

Figure 42. Possible future analogues with which to investigate "head-to-tail" dimerisation.

CHAPTER 5: Total Synthesis of Scytalidin

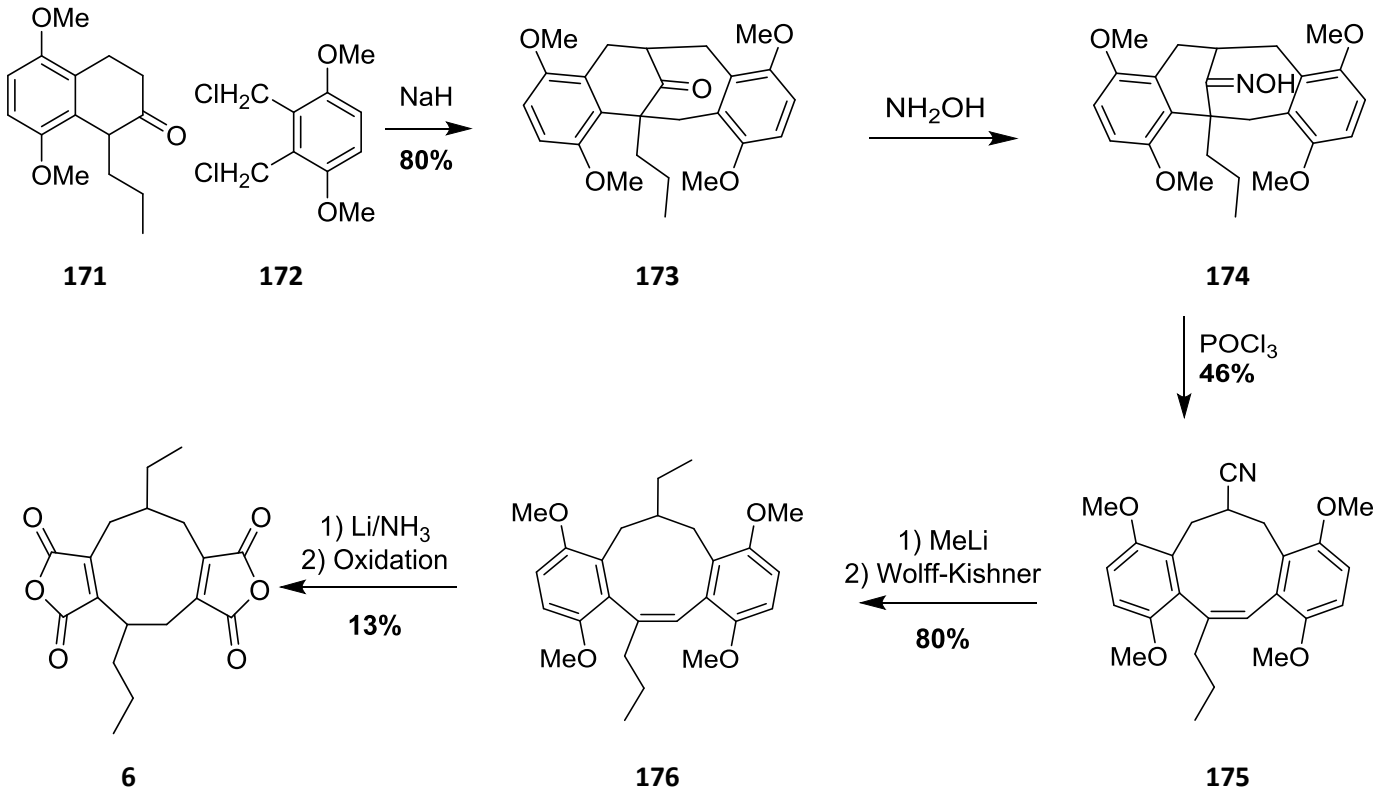
5. Total Synthesis of Scytalidin

5.1 Introduction

Whilst a significant body of research has been dedicated to the synthesis of cyclopentane and cyclohexane-derived cyclic molecules, the synthesis of larger carbocycles has been left comparatively unexplored.[170] The same wealth of techniques does not exist, and indeed methods such as cyclisation reactions are frequently considered inappropriate due to entropic and enthalpic barriers.[171] Therefore, previous approaches towards a total synthesis of highly oxygenated, carbocyclic natural products such as the maleidrides have frequently relied on particularly creative approaches.

5.2 Previous Total Syntheses of the Maleidrides

5.2.1 Stork's Total Synthesis of Byssochlamic Acid



171 **172** **173** **174** **175** **176** **6**

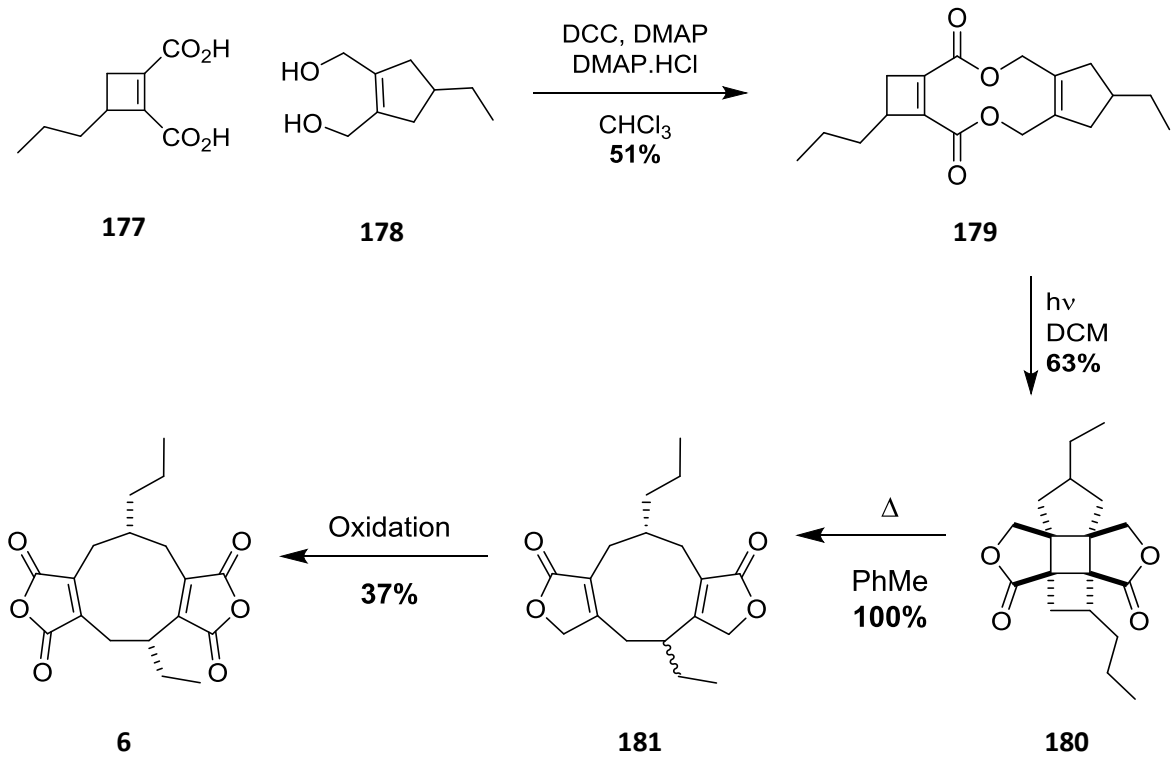
Scheme 83. Stork's synthesis of (±)-byssochlamic acid[172]

Given the unusual structural motifs and interesting biological activities, the maleidrides have attracted attention from synthetic chemists. Stork completed the first synthesis of (±)-byssochlamic acid **6** in 1972, shown in Scheme **83**.[172] The tetralone **171** and benzyl chloride **172** were prepared from commercial substrates in one and two steps respectively, before treatment with sodium hydride promoted a double alkylation to form **173**. Beckmann fragmentation via formation of the oxime **174**, then treatment with $POCl_3$ yielded the nitrile **175**. The ethyl side chain was installed by addition of methyllithium and *in-situ* Wolff-Kishner reduction of the resulting imine to give **176**,

before a dissolving metal reduction of the alkene set the side chains in a *cis*-relationship. Surprisingly, the dimethoxycatechol groups did not undergo Birch reduction simultaneously, despite the similarity in reaction conditions.[173,174] The lack of oxidation-sensitive functionality in the core of byssochlamic acid **6** meant that dimethoxycatechols could be used as synthons for the anhydride moieties, which were unveiled in the final step by harsh oxidation conditions (*i*: BBr_3, DCM, *ii*: $KMnO_4$, DME, *iii*: $Pb(OAc)_4$, AcOH).

5.2.2 **Drapela and White's Syntheses of Byssochlamic Acid**

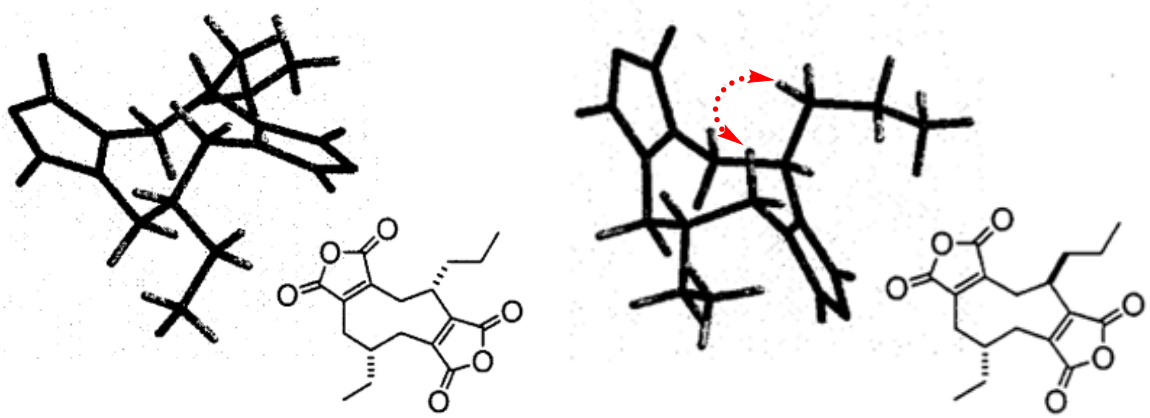
Stork's approach was followed by further investigations by White *et al.*, based upon a "photoaddition-cycloreversion metathesis" strategy to construct the central ring of byssochlamic acid **6**, shown in Scheme **84**.[175] After preparation of cyclobutene **177** and cyclopentene **178** fragments, Steglich esterification gave diester **179**. A photochemical [2+2] cycloaddition yielded three isomers of cyclobutene **180**, which upon refluxing in toluene, underwent cycloreversion to establish the nonadiene core **181**. A two-step oxidation then yielded the racemic natural product **6**. Interestingly, during this process the ethyl substituent was epimerised to give exclusively *cis* side chains.



Scheme 84. White's racemic byssochlamic acid synthesis.[175]

Preference for a *cis* configuration of side chains reported by White and coworkers was not an isolated phenomenon. Stork's alkene reduction under thermodynamic control yielded exclusively the *cis*-side chains of **6**. Additionally, computational work by the White group established a 2.6 kcal mol^{-1} energy

difference between the PM3 energy minimised *cis* and *trans* structures, and they also observed that the sodium enolate of **6** returned only the *cis*- and not *trans*- relationship upon protonation. This preference was rationalised as being the result of a pseudo-axial orientation of the propyl side chain in *trans*-**6**, which creates a transannular steric interaction with an *endo*-hydrogen in the methylene group across the ring (Figure **43**). White *et al.* therefore realised that the stereochemistry of the propyl side chain could be completely controlled from the ethyl substituent, and thus setting this stereocentre would allow an asymmetric synthesis to be completed.[64] Thus, the first enantioselective synthesis of a nonadride was duly completed by the White group in 2000, following a similar approach to that shown in Scheme **84**, but with a single enantiomer of **177**.



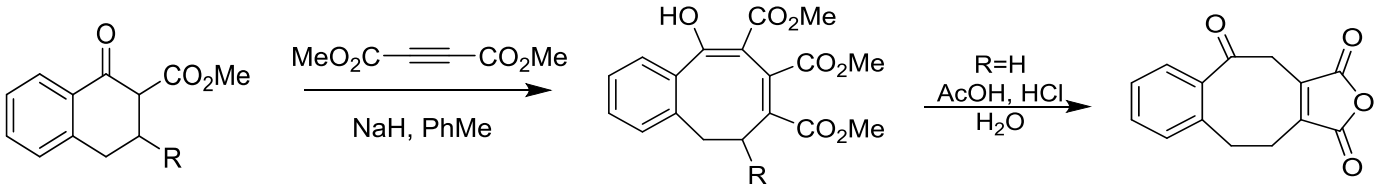
cis-Byssochlamic acid **6** *trans*-Byssochlamic acid **6**

Figure 43. Energy-minimised (PM3) comformers of *cis*- and *trans*-byssochlamic acid **6**, highlighting the transannular interaction which destabilises the *trans*- form.[64]

5.2.3 Musso's Synthesis of Zopfiellin Derivatives

Total synthesis of cornexistin **8** has been investigated by Clark and Taylor,[176–178] and the groups of Nicolaou, Shair, Danishefsky and Fukuyama have all reported total syntheses of the polycyclic phomoidrides. However, the additional complexity of these structures mean the routes adopted have little relevance to the synthesis of scytalidin or byssochlamic acid-like structures. However, the synthesis of analogues of the octadride zopfiellin **14** by Musso and coworkers is of particular interest when seeking to synthesise maleidrides with unbridged carbocyclic cores.[176,179–183]

Ring expansion of substituted 2-carboxymethyltetralones **182** with methodology developed by Proctor and Frew was used to both expand the ring to the desired cyclooctane structure and install the anhydride moiety.[184] In this manner, they were able to generate quickly sufficient quantities of each analogue for their biological activities to be examined. It was found that each of these structures showed broad anti-fungal activity comparable to commercial fungicides, including activity against the pathogens *Phytophthora infestans* and *Pythium ultimum*.



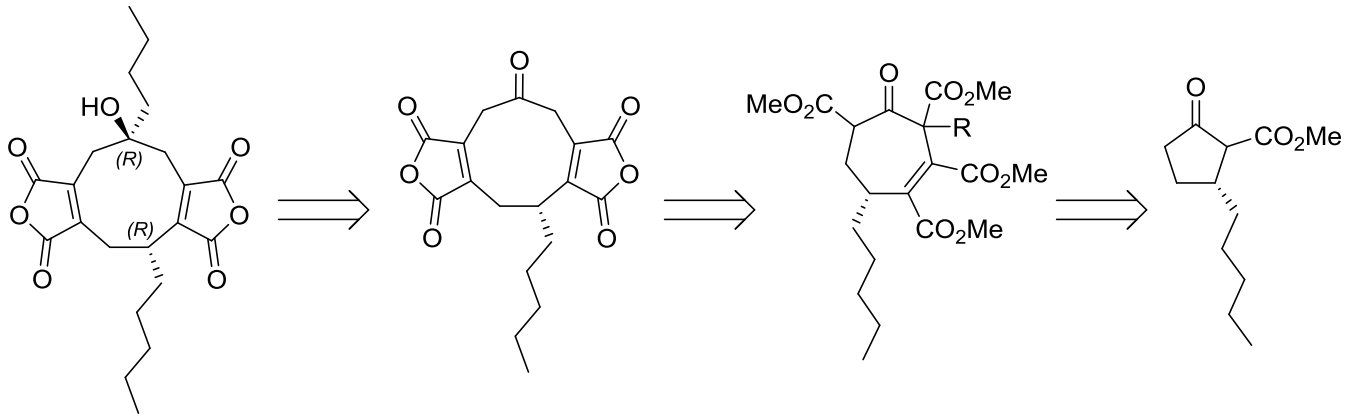
182 **183** **184**

Scheme 85. Musso and coworkers synthesis of zopfiellin derivatives **184**.[183]

Focusing on efficient synthesis of analogues to zopfiellin **14**, Musso's investigation did not install the second maleic anhydride moiety of zopfiellin **14**, instead using an aromatic substituent. Combining this approach with the dimethoxycatechol synthon strategy of Stork could have allowed total synthesis of the natural product, an idea that has previously been persued within the Willis group.[52] Therefore, the power of ring expansion methodology to produce medium ring carbocycles fused to maleic anhydrides in an efficient fashion has been well established.

5.3 Results and Discussion

5.3.1 Retrosynthetic Approach

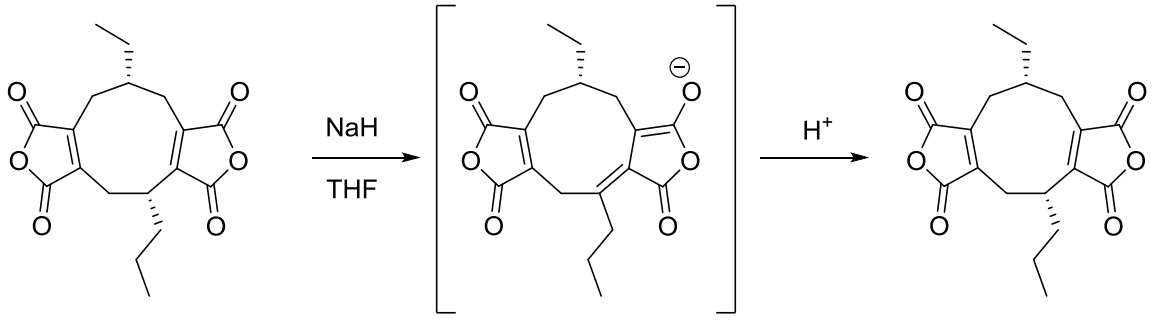


12 **185** **186** **187**

Scheme 86. Retrosynthetic strategy to scytalidin **12**

Retrosynthetic analysis of scytalidin **12** began with disconnection of the *n*-butyl side chain to give ketone **185**, as shown in Scheme **86**. A stereoselective addition of the butyl group would be required. Fortunately, if this was to prove impossible then previous investigations of byssochlamic acid **6** by White suggest a synthesis of a single diastereomer of (±)-scytalidin could still be achieved. It has been shown both experimentally and computationally that the *cis*-arrangement of side chains in byssochlamic acid is more stable than the *trans*-diastereomer.[175] Scytalidin **12** has a *cis* configuration, as established through X-ray crystallography in Chapter **2.2.2**.

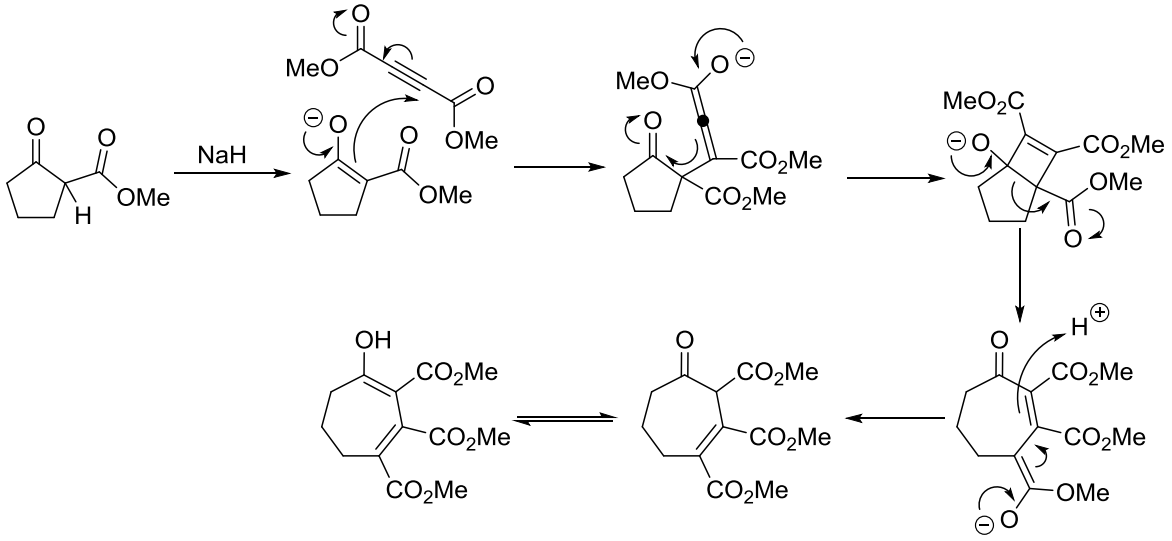
Computational modelling by White *et al.* showed byssochlamic acid **6** to be 2.6 kcal mol^{-1} more stable than the *trans*-diastereomer. Our own computational modelling of scytalidin **12** and deoxyscytalidin **35** indicated a similar energy difference. The hydroxyl group of scytalidin **12** did not change this.



Scheme 87. Deprotonation of (-)-byssochlamic acid **6**, followed by reprotonation, returns the natural product with no isomerisation.[175]

Experimental evidence comes from the allylic deprotonation of byssochlamic acid. As can be seen in Scheme **87**, forming an enolate from (-)-byssochlamic acid removes the stereocentre of the propyl side chain. Yet on addition of acid, protonation occurs only from a single face, to return (-)-byssochlamic acid exclusively, as shown by optical polarimetry.

Through control of the stereochemistry of the pentyl side chain earlier in the synthetic route, it was hoped that substrate-controlled stereoselective addition of the butyl side chain could be achieved later in the synthesis. Should this, or other approaches stereoselective addition prove unsuccessful, treatment of the resulting diastereomeric mixture with NaH/THF would allow us to epimerise the pentyl sidechain to give a single diastereomer of (±)-scytalidin.

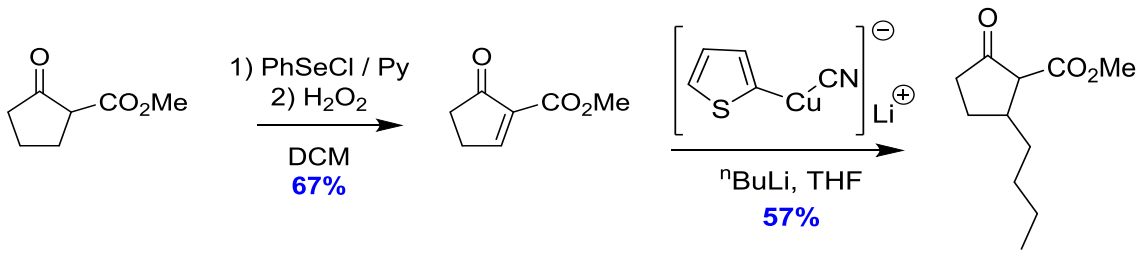


Scheme 88. Mechanism of ring expansion, as proposed by Frew and Proctor.[185]

The cyclononadiene core was to be constructed in a similar fashion to that employed by Musso and coworkers to synthesise zopfiellin analogues (Scheme **87**, **182→183**).[183] Through applying two rounds of this two carbon ring expansion methodology, we hoped to start from a cyclopentanone core **187** and expand first to a "heptadride"-like intermediate structure **186**, with the functionality installed for one maleic anhydride unit, then a second round of ring expansion would complete the cyclononadiene core **185**. It was anticipated that the β-ketoester functionality needed for the second ring expansion could be introduced as necessary, by alkoxycarbonylation of the ketone present.

5.3.2 Routes to Heptadridic Structures

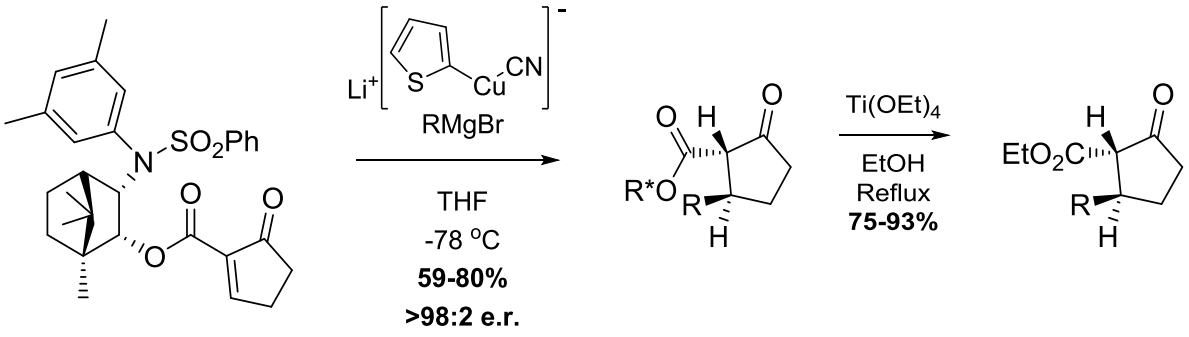
Our synthetic work commenced by investigating two routes to the 2-methoxycarbonyl, 3-alkyl cyclopentanones required for the first ring expansion step. Our first approach was to start from the commerically available cyclic β-ketoester **188**, which would be converted to enone **189**, then undergo a conjugate addition reaction to install the side chain, as shown in Scheme **89**. Other than a solitary example of the desired oxidation using $Pb(OAc)_4$ and $Cu(OAc)_2$ to obtain the unsaturated ketoester in poor yield,[186] the majority of literature employs a selenoxide elimination approach.[187]



188 **189** **193**

Scheme 89. Synthesis of 3-butyl-2-methoxycarbonylcyclopentanone

Following the method of Liotta *et al.*,[187] ketoester **188** was treated with phenylselenyl chloride and pyridine, resulting in formation of the selenide intermediate. The pyridine was removed, and then hydrogen peroxide added to generate a selenoxide, which eliminated to form the unsaturated ketoester **189** in 67% yield. This reaction was not without its drawbacks. The product and starting material are inseparable by silica chromatography, making it often necessary to use up to an additional equivalent of PhSeCl in order to ensure complete formation of the selenide. Alternative conditions were therefore sought. Several bases in conjunction with diphenyl disulfide were investigated to generate the intermediate thioether, as was the replacement of PhSeCl with PhSCl (generated *in-situ* from thiophenol and *N*-chlorosuccinimide) or diphenyl diselenide. Unfortunately in all cases no reaction was observed.

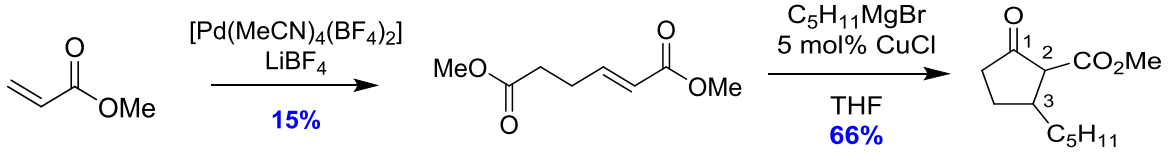


190 **191** **192**

Scheme 90. Use of chiral auxiliaries and Lipschultz cuprates to achieve highly diastereoselective conjugate addition to α,β-unsaturated enone **190**.[188]

A further issue with this approach was the instability of the unsaturated compound **189** (which has been noted in the literature before[88]). Nevertheless, the Liotta conditions gave sufficient quantities of the unsaturated β-ketoester **189** for the next step, the conjugate addition, to be explored. Helmchen and coworkers have reported an asymmetric approach to this transformation, based on the use of ester chiral auxiliaries **190** and Lipshultz cuprates.[188] As the total synthesis of scytalidin would rely on this step to set the stereochemistry of the pentyl side chain, it was desirable to use conditions similar to the asymmetric variant. A Lipschultz cuprate was generated from the addition of *n*-butyllithium[2] to 2-thienylcyanocuprate, which on addition of unsaturated β-ketoester **189** gave the 3-alkyl ketoester **193** in 57% yield. A 10:1 d.r was determined through ^{1}H NMR spectroscopy, by integration of the doublet arising from the signals assigned to 2-H in **193**.

Whilst this two-step approach did generate the required 3-alkyl β-ketoester **193**, it was felt that it was not amenable to scale up, based on both cost and the instability of **189**. Therefore, an alternative route based on a tandem conjugate addition-Dieckmann cyclisation was explored, as shown in Scheme **91**.



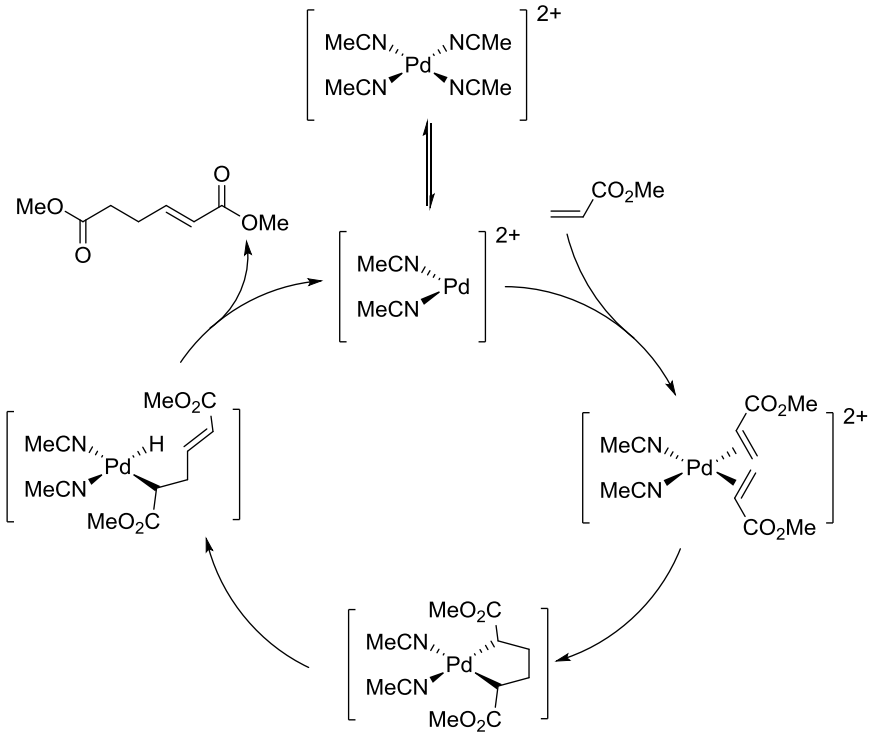
194 **195** **196**

Scheme 91. Conjugate addition - Dieckmann cyclisation approach to 3-alkyl cyclopentane ketoesters

Using a synthesis reported by Nugent and Hobbs,[189,190] a Pd-catalysed dimerisation of methyl acrylate **194** gave dimethyl 2-hexenedioate **195** as predominantly the *trans* geometry, albeit in only 15% yield, and contaminated with dimethyl 3-hexenedioate, which could not be separated by

[2] *n*-Butyllithium was used for the test reaction rather than *n*-pentyllithium due to its wide availability.

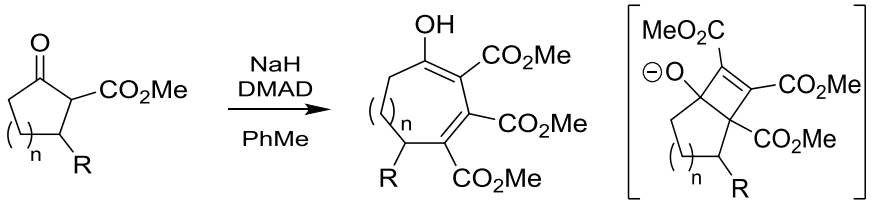
distillation or silica chromatography. This species could be the result of an altered catalytic cycle, or through alkene isomerisation – a phenomenon that has been reported with the $[Pd(MeCN)_4(BF_4)_2]$ catalyst used.[191,192] The reasons for this yield being much lower than the 81% reported are unclear. The methyl acrylate used contained the same stabilisers as reported,[189] with the catalyst purchased from the same supplier and fresh $LiBF_4$ used. Despite the low yield, the low loading of the catalyst (0.5 mol%) combined with the low cost of methyl acrylate made this an acceptable method with which to synthesise multi-gram quantities of unsaturated ester **195**.



Scheme 92. Proposed mechanism for the Pd-catalysed dimerisation of methyl acrylate.[193]

Following the dimerisation of methyl acrylate **194** to yield hexenedioate **195**, addition of pentylmagnesium bromide and Cu(I)Cl in THF gave 3-pentyl cyclic ketoester **196** in 66% yield. This reaction proceeds via conjugate addition of the organocuprate reagent to the unsaturated ester, with the resulting enolate undergoing a Dieckmann condensation with the second ester, to generate the desired β-ketoester.

With this much more promising route to substituted β-ketoesters developed, the ring expansion step was investigated. Treatment of **197** (R=H, n=1) with NaH then DMAD in toluene (conditions reported by Frew and Proctor) gave the ring expanded product **198** in 83% yield (Scheme **93**).



197 **198-201** **202**

	n	R	Yield / %
198	1	H	**83**
199	1	^{n}Bu	**46**
200	1	^{n}Pen	**61**
201	3	H	**68**

Scheme 93. Ring expansion of cyclic β-ketoesters.

This reaction proceeds through the intermediate [n+2.2.0] bicycle **202**, followed by a retro-aldol fragmentation. It has been shown previously that slow addition of DMAD is necessary to ensure formation of **202**.[52] This methodology was then applied to the previously synthesised 3-alkyl β-ketoesters, to give **199** and **200** in 46% and 61% yields respectively. Additionally, ring expansion of cycloheptanone β-ketoester **197** (R=H, n=3) was also possible, which gave the nine-membered ring **201**. This was an important demonstration that the ring expansion methodology investigated was appropriate for the synthesis of scytalidin **12**. The ring expansion products **198-201** exist exclusively in the enol form in solution. This was clear from the ^{1}H NMR spectrum, which showed a singlet between 12.5-13.1 ppm, and no evidence of an α-C-H peak.

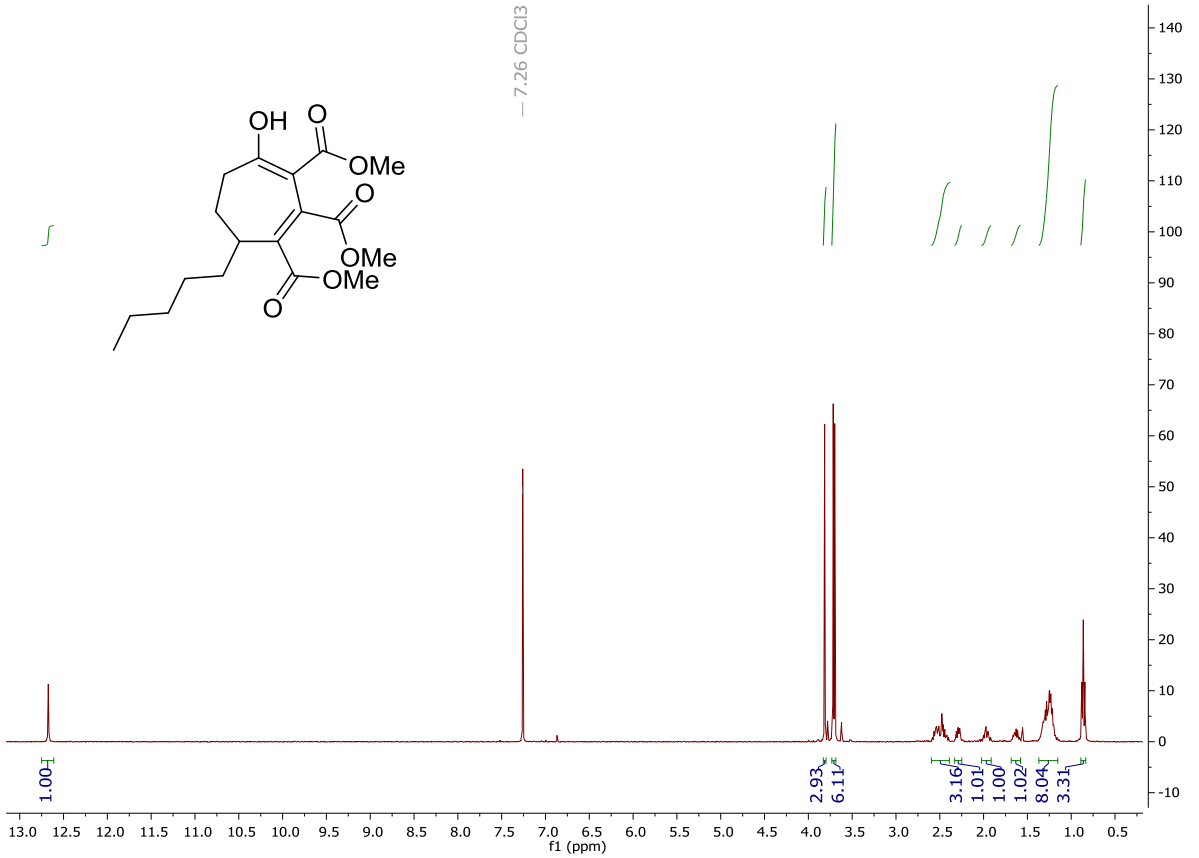
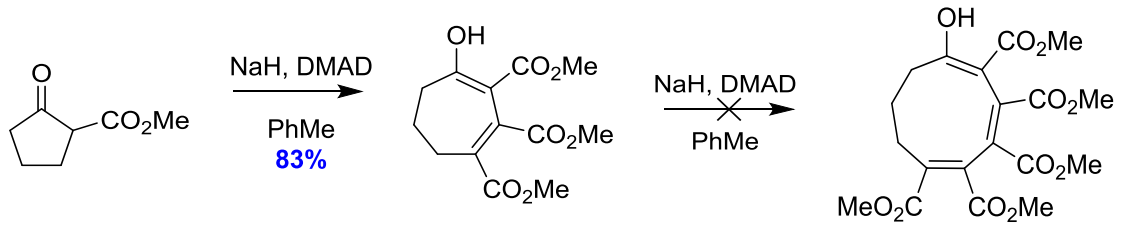


Figure 44. Structure and ^{1}H NMR spectrum of **200**.

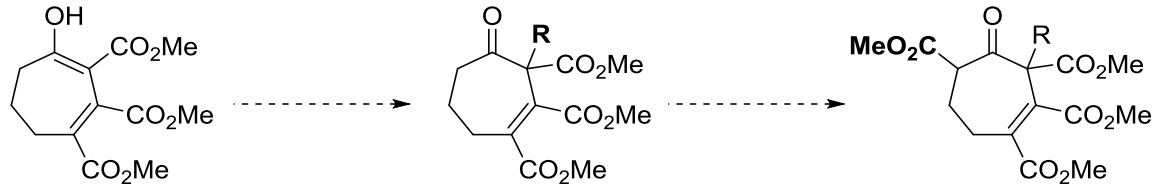
Given the product of this reaction is also a β-ketoester (albeit in the enol form), we explored whether this could undergo a second ring expansion reaction. Treatment of **198** with NaH/DMAD in toluene, even in excess, returned only starting material, and not the double-expansion product **203**.



197 **198** **203**

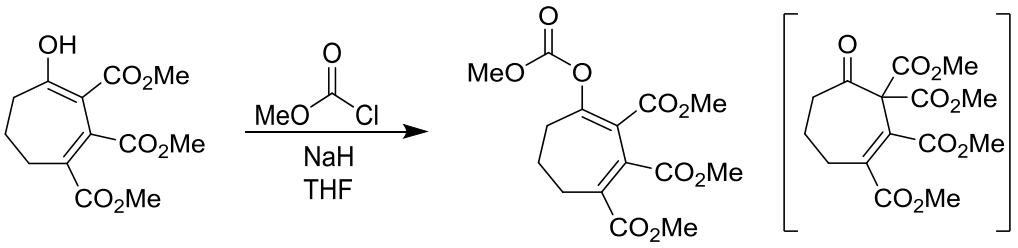
Scheme 94. Attempted iterative double ring expansion of **197**.

We hypothesised that this lack of reactivity of **198** may be due to the conjugated enolate form rendering the α-carbon less reactive. This was further evidenced by our attempts to install a substituent at the α-carbon (herein termed α-protection), in order to generate a ketone which could be α'-alkoxycarbonylated to yield a substrate for ring expansion on the other side of the ring. This strategy is shown in Scheme **95**.



Scheme 95. Strategy for the synthesis of a substrate for the second ring expansion step

Initial attempts to install an ester at the α-carbon by deprotonation of **198** with NaH in THF and treatment with methyl chloroformate led to exclusive formation of the *O*-substituted compound **204** in 97% yield, rather than the desired *C*-substituted **205**. *O*-acylation is a known issue with the acetylation of ketone enolates.[194,195] It has been separately reported that a solvent switch to diethyl ether, and the use of magnesium rather than sodium or lithium enolates can help promote *C*-acylation, however neither of these approaches (individually or together) yielded the desired *C*-substituted product **205**, with ^{1}H NMR analysis of the crude reaction product showing solely **204** in all cases.[196,197]



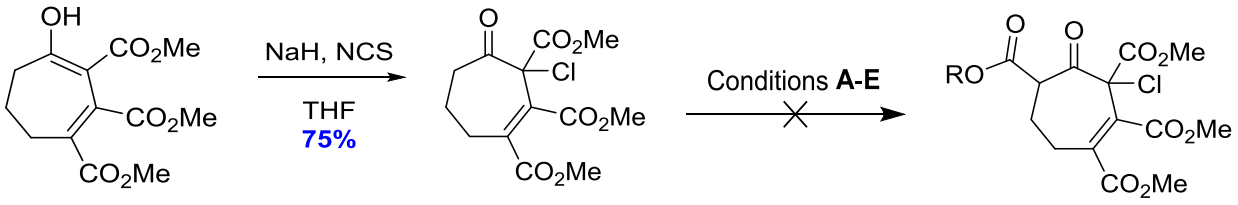
198 **204** **205**

97% **0%**

Scheme 96. Methoxycarbonylation of triester **198**.

Instead, it was proposed that the use of an electrophile with little or no affinity for reactions at oxygen would help promote formation of the α-protected ketone. A chlorine substituent would achieve this aim, and α-dechlorination can be effected with zinc powder in acetic acid, conditions which would allow either concomitant removal of the ester protecting groups if necessary, or allow orthogonal deprotection.[3] Thus triester **198** was treated with NaH and *N*-chlorosuccinimide (NCS), which yielded the α-chloro-β-ketoester **206** in 75% yield.

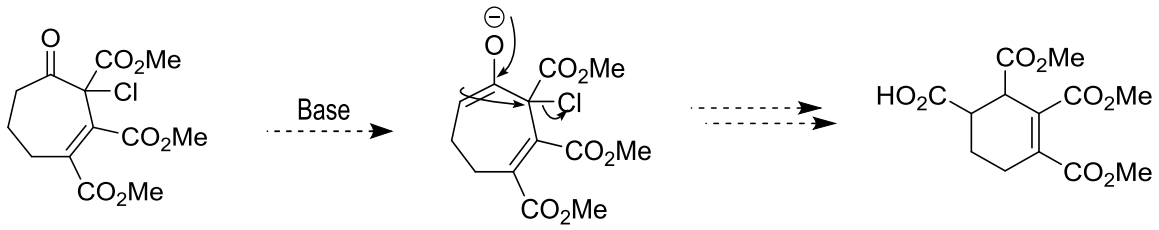
[3] The triester can be treated with refluxing acetic acid with hydrochloric acid, resulting in one-pot α-decarboxylation and anhydride formation.[185] This is reported later in this thesis.



198 **206**

Scheme 97. α-Chlorination of triester **198** and subsequent investigation of α'-functionalisation. Conditions investigated: **A)** NaH, MeOC(O)Cl, THF **B)** LiHMDS, MeOC(O)Cl, THF **C)** LiHMDS, MeOC(O)Cl **D)** NaH, $(MeO)_2CO$ **E)** LiHMDS, D_2O

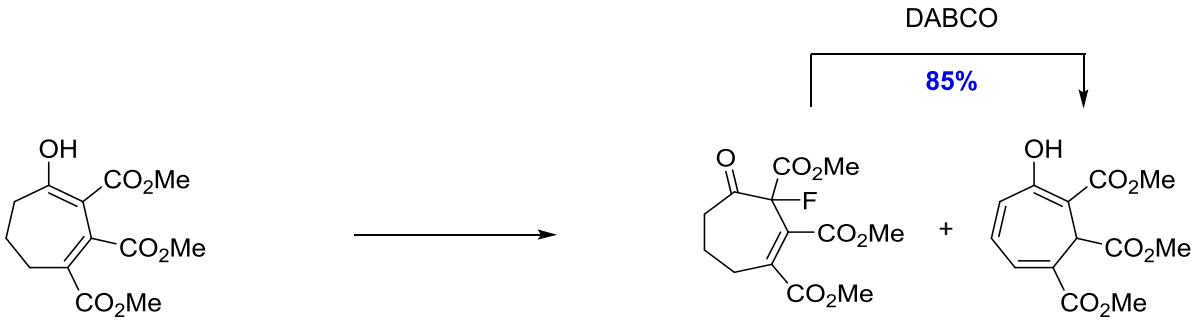
A variety of conditions (A-E, Scheme **97**) were then screened for the α'-deprotonation of β-ketoester **206**, however in all cases significant degradation of the starting material was observed. A Favorskii rearrangement could be one source of degradation, but no products were isolated to support this proposal.



206 **208**

Scheme 98. Potential degradation of **206** via a Favorskii rearrangement.

As α-chloro-β-ketoester **206** was found to be unstable under basic conditions, the α-fluoro derivative was explored, with the expectation that the strong C-F bond would confer increased stability. Triester **198** was initially α-fluorinated with the combination of sodium hydride and Selectfluor, which gave α-fluoro ketone **209** in 34% yield, as well as a 9% yield of an unexpected triene byproduct, **210**– the structure of which was confirmed by X-ray crystallography (Figure **45**).



198 **209** **210**

Method 1: NaH, Selectfluor, THF	34%	9%
Method 2: Selectfluor, MeCN	72%	0%

Scheme 99. Fluorination of triester **198** under basic and neutral conditions

In order to boost the yield of **209**, fluorination was investigated under neutral conditions, with Selectfluor in acetonitrile, giving **209** in a 72% yield, with none of the by-product **210** detected. This suggested that the α-fluoro-β-ketoester was unstable in base. However, after stirring **209** with NaH in THF for 24 hours, only a small amount of degradation was observed – but the triene **210** was not present.

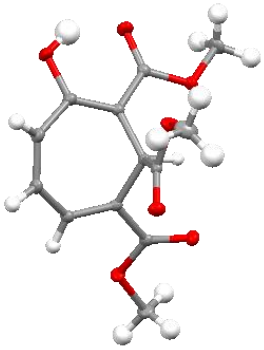


Figure 45. Crystal structure of triene **210**

Under the original NaH/Selectfluor conditions for α-fluorination, an equivalent of a DABCO-like base is generated as the Selectfluor is consumed. Therefore, ester **209** was treated with NaH and DABCO. This did lead to some formation of **210** (Figure **46**d). Interestingly, treatment of **209** with DABCO gave complete conversion to triene **210** (Figure **46**e). This reaction was then shown not to be unique to DABCO, with triethylamine and diisopropylamine also affording **210** cleanly. Additionally, the α-chloroketone **206** can be converted to **210** under similar conditions.

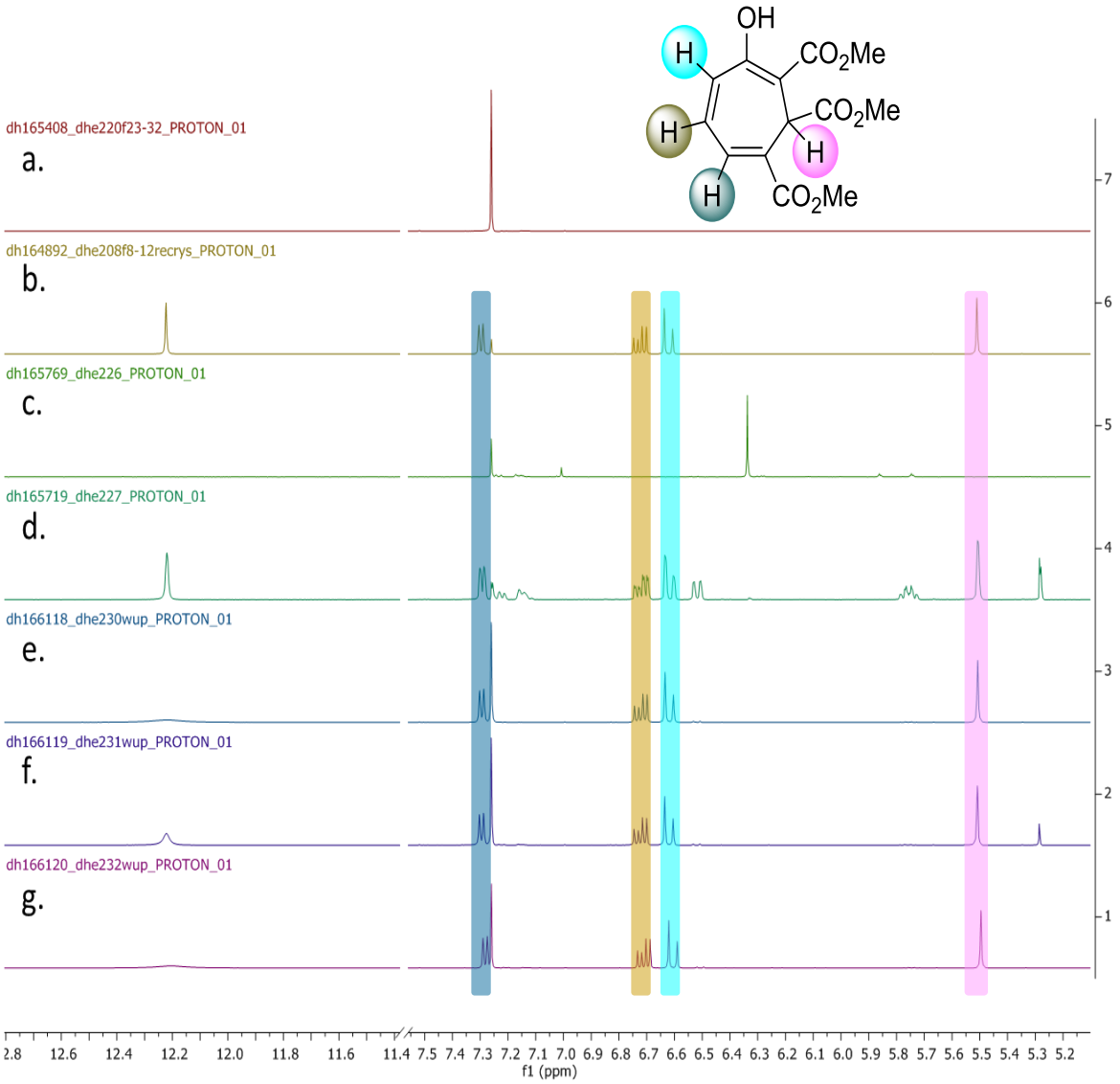
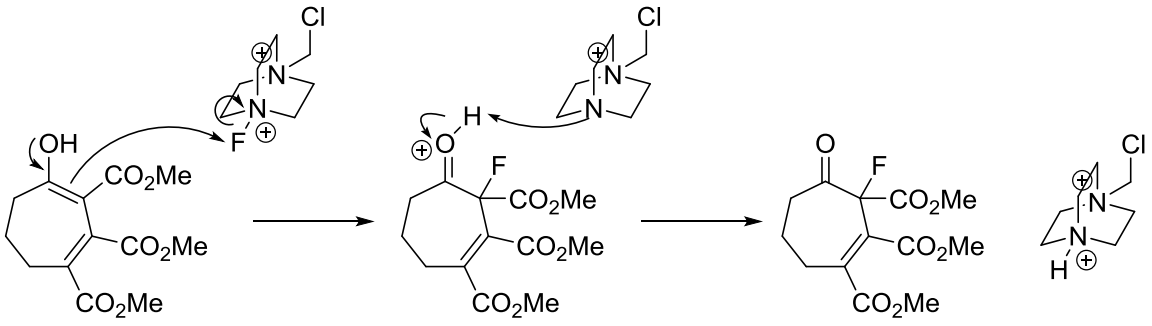


Figure 46. Study of the stability of **209** to base, by ^{1}H NMR in $CDCl_3$ a) **209**, b) **210**, c) **209** + NaH, d) **209** + NaH & DABCO, e) **209** + DABCO, f) **209** + NEt_3, g) **206** + K_2CO_3.

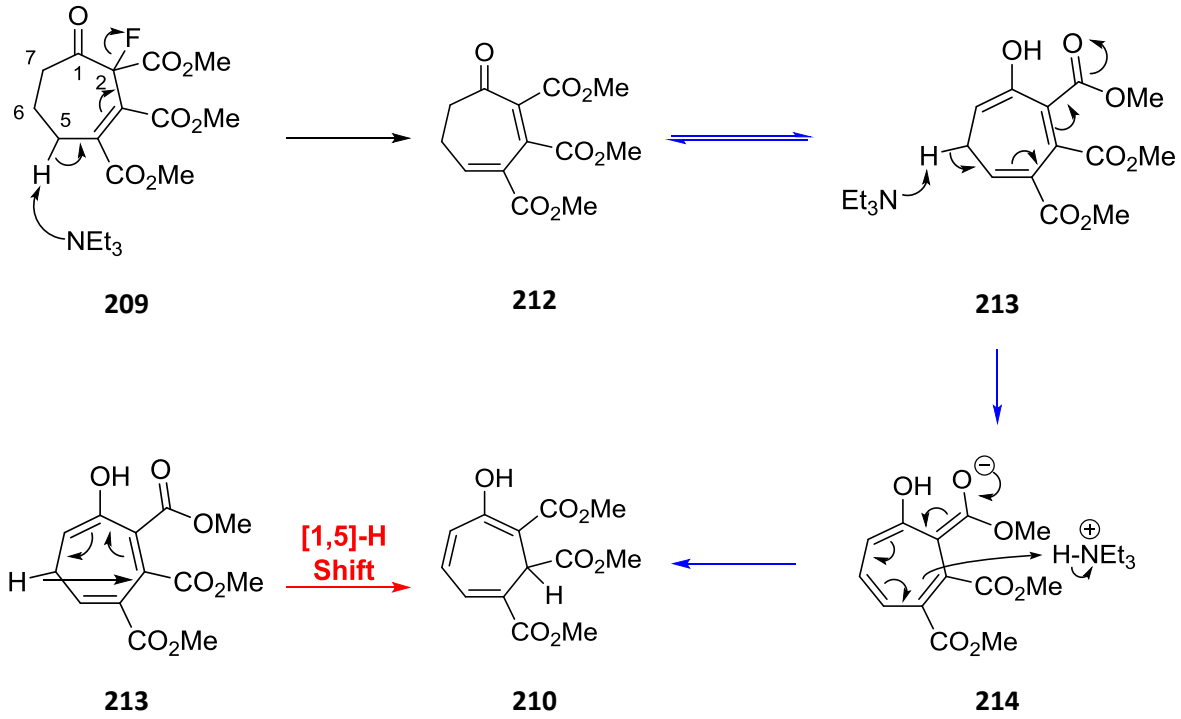
Under neutral conditions, it is proposed that α-fluorination of the enol generates **211**, which is then deprotonated by the DABCO-like base generated from Selectfluor. Therefore there is no remaining base to carry out the conversion of **209** to **210**.



198 **211** **209**

Scheme 100. Mechanism for fluorination of **198** with Selectfluor under neutral conditions.

However, in the presence of additional base, a further reaction can occur. Deprotonation at 5-H (**209**, Scheme **101**), rather than 2-H, facilitates elimination to form diene **212**, which may tautomerise to **213**. However, the coplanarity of the three ester substituents creates a steric strain, which can be relieved by deprotonation at C-6, and reprotonation of the conjugate base **214** to create an sp^3 centre at C-3, between the esters (Scheme **101**, *blue*). An alternative mechanism (Scheme **101**, *red*) invoking a [1,5]-hydride shift to generate **210** directly from **213** was also proposed, however this fails to explain why sodium hydride could not cause this transformation whereas the ionic mechanism can.



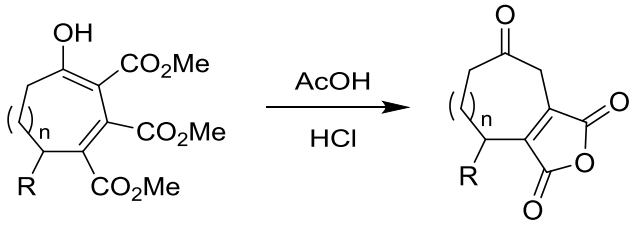
209 **212** **213**

213 **210** **214**

Scheme 101. Proposed ionic (*blue*) and sigmatropic (*red*) mechanism for the formation of **210.**

When treated with sodium hydride, fluoroketone **209** generates the sodium enolate, with the evolution of hydrogen gas. Therefore should elimination to **212** occur, there is no base present to induce formation of **210** and the relief of the steric strain caused by the collinear ester groups. When

the conjugate acid remains in solution, the base can be regenerated by the fluoride anion released, allowing the second deprotonation step to occur. **210** may not be observed because the sodium enolate of **209** is sufficiently stable such that elimination of fluoride is not favourable.



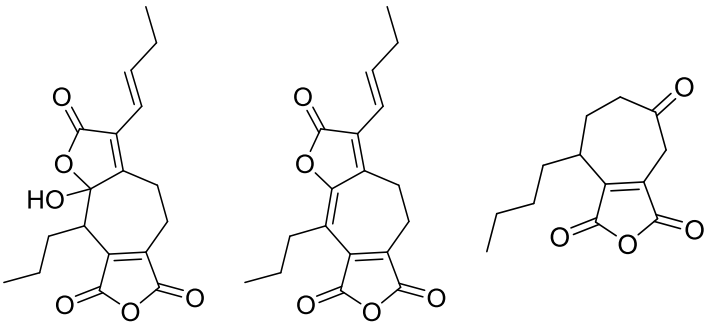
198-**201**

	n	R	Yield / %
215	1	H	**84**
216	1	^{n}Bu	**46**
217	1	^{n}Pen	**38**
218	3	H	**76**

Scheme 102. Formation of maleic anhydrides from triesters **198**-**201**.

Through understanding the mechanism by which **210** forms, it became clear that α-halo-β-ketoesters would not be appropriate substrates for the desired α'-carbonylation. The instability of these α-halo-β-ketoesters to both mild and strongly basic conditions means α'-carbonylation of these compounds (in order to set up a second ring expansion to the 9-membered core of scytalidin) is unlikely to be a viable route. This confirms the degradation experienced when the α'-methoxycarbonylation of **206** was investigated (Scheme **97**) are the result of an unsuitable substrate, and it is highly unlikely that further screening of conditions would give a different result.

As **198**-**201** were not appropriate substrates for the continuation of the total synthesis of scytalidin **12**, they were converted to the anhydride form **215**-**218** by treatment with refluxing acetic acid and hydrochloric acid (Scheme **102**). As these compounds show structural similarity to the maleidrides, in particular the agnestradrides (Figure **47**), it was hoped they would retain some of their biological activity. These substrates were tested for biocidal activity by Syngenta, however none showed appreciable activity in any of their screens.

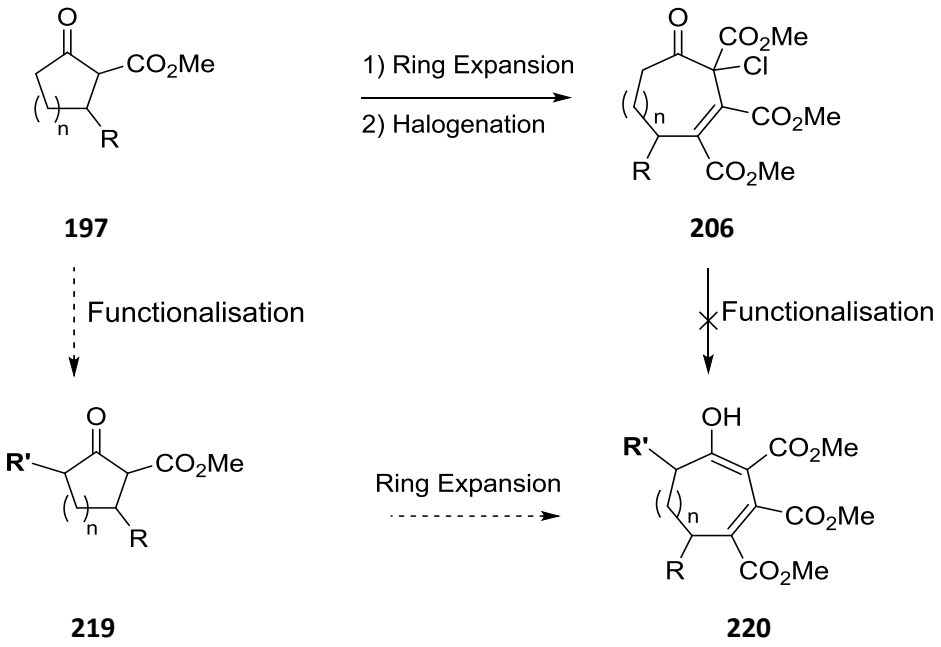


Agnestradride A **16** Agnestradride B **17** **216**

Figure 47. Comparison of the structure of the agnestradrides with heptradridic anhydride **216**.

5.3.3 **Pre-Installation of α'-Functionality**

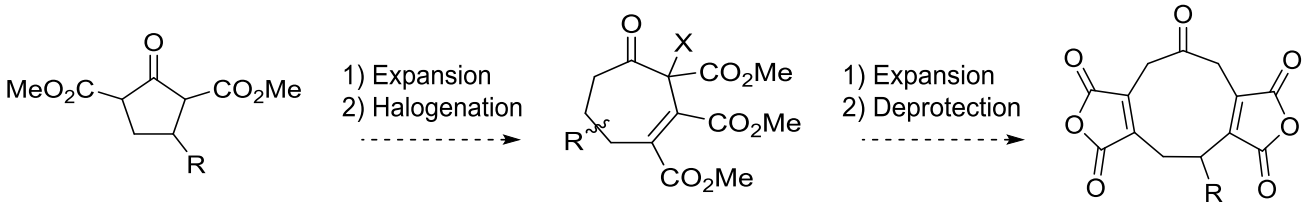
For the continuation of the total synthesis, it was evident that the α'-position of **197** required functionalisation (**219**) prior to ring expansion with NaH/DMAD. The moiety chosen would then be used directly for a second ring expansion or allow β-ketoester functionality for that purpose to be unveiled, to yield **220**.



197 **206** **219** **220**

Scheme 103. α'-Functionalisation of **197** prior to ring expansion may allow the instability problems arising from α-halo-β-ketoester **206** to be circumvented.

The next stage of our investigation was therefore formation of diester **221**. Knowing that "double expansion" on the same side of the ring was not possible (see Scheme **94**), the 1,4-diester appeared an attractive substrate. Whilst the ring expansion may lead to regioisomers **222**, it was hoped that these would converge to the same product **223** after the second ring expansion (see Scheme **104**)

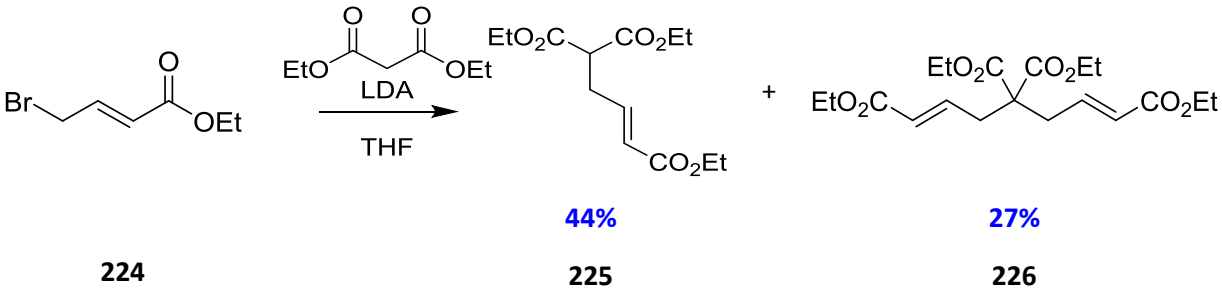


221 **222** **223**

Scheme 104. Proposed route from diester **221** to the nonadride core

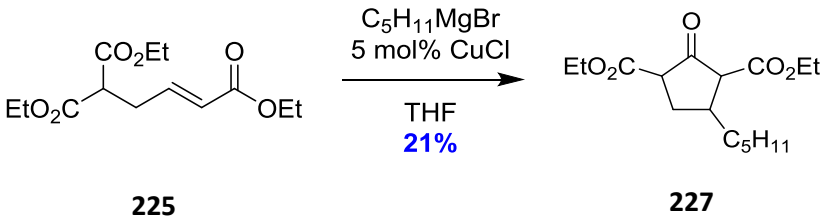
The required diester **221** could be prepared by the conjugate addition - Dieckmann condensation chemistry previously used. Addition of ethyl 4-bromocrotonate **224** to the lithium enolate of diethyl malonate gave the desired triester **225**, however this reaction was complicated by the formation of the double addition product **226** (Scheme **105**). Unfortunately, varying the quantity of base or diethyl

malonate did not improve the yield of triester **225**, with the reaction instead returning unreacted diethyl malonate or the tetra-ester by-product. The 44% yield obtained is in accord with that reported by Wang and coworkers.[198]



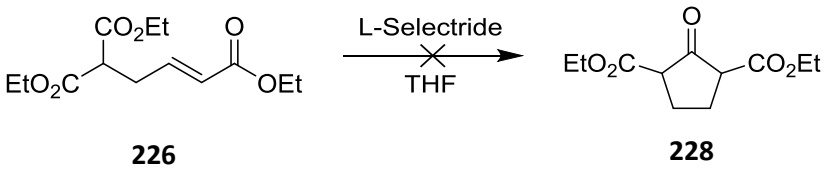
Scheme 105. Alkylation of diethyl malonate with ethyl 4-bromocrotonate **224**.

Triester **225** was then treated with pentylmagnesium bromide/CuCl to give cyclopentanone **227**, shown in Scheme **106**. The formation of three stereocentres in this reaction gave a mixture of diastereomers, isolation and characterisation of which proved complex, although high resolution mass spectrometry (HRMS) did show formation of the desired product mass ($[M+Na]^+$ found 321.1665, expected 321.1665).



Scheme 106. Conjugate addition - Dieckmann cyclisation to give **227**.

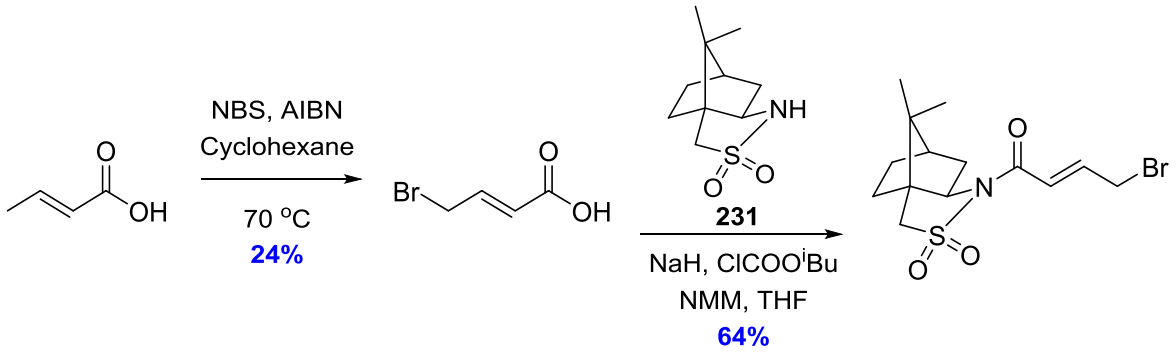
In order to reduce the number of diastereomers formed in this reaction, the conjugate addition with L-Selectride was first explored. The sidechain was not strictly necessary in order to investigate the proposed route, and performing this reduction would form one less asymmetric centre, making analysis of the products formed easier. Unfortunately, this reaction was unsuccessful, returning only starting material.



Scheme 107. Reductive Dieckmann cyclisation.

The use of a chiral auxiliary to control the conjugate addition step was then explored. A sultam-derivative was chosen, as it had previously been shown to give control over conjugate addition reactions, including alkylation of the resultant enolate.[199–201] β-Ketoamides are additionally more

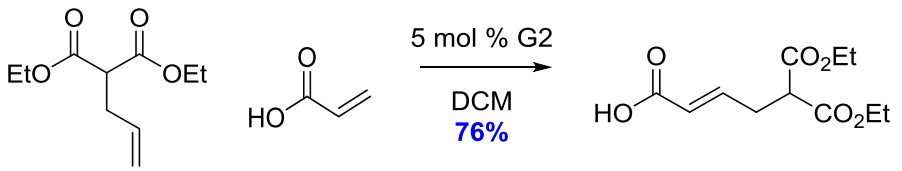
acidic than β-ketoesters, and so potentially regiocontrol in the ring expansion step might be possible.[202] To this end, crotonic acid **229** was brominated under Wohl-Ziegler conditions to yield 4-bromocrotonic acid **230**. Coupling with the Oppolzer sultam auxiliary **231** (synthesised in two steps from (-)-camphorsulfonic acid[203]) using a mixed anhydride approach gave **232** in 64% yield.



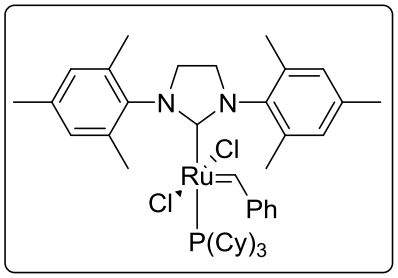
229 **230** **232**

Scheme 108. Synthesis of (1*S*, 2*R*)-*N*-[(4-bromocrotonate]-bornane-10,2-sultam **232**

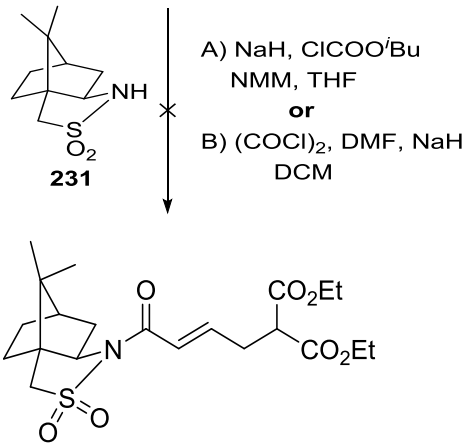
Unfortunately, the bromide displacement using the diethyl malonate anion was unsuccessful. A complex mixture of products were formed, with ^{1}H NMR analysis of the crude products showing no alkene protons present, indicating that instead a conjugate addition had taken place. A variety of bases were investigated (including NaH, LDA, K_2CO_3 and NaOEt), each without success.



233 **234** **235**



Grubbs second generation Catalyst (G2)

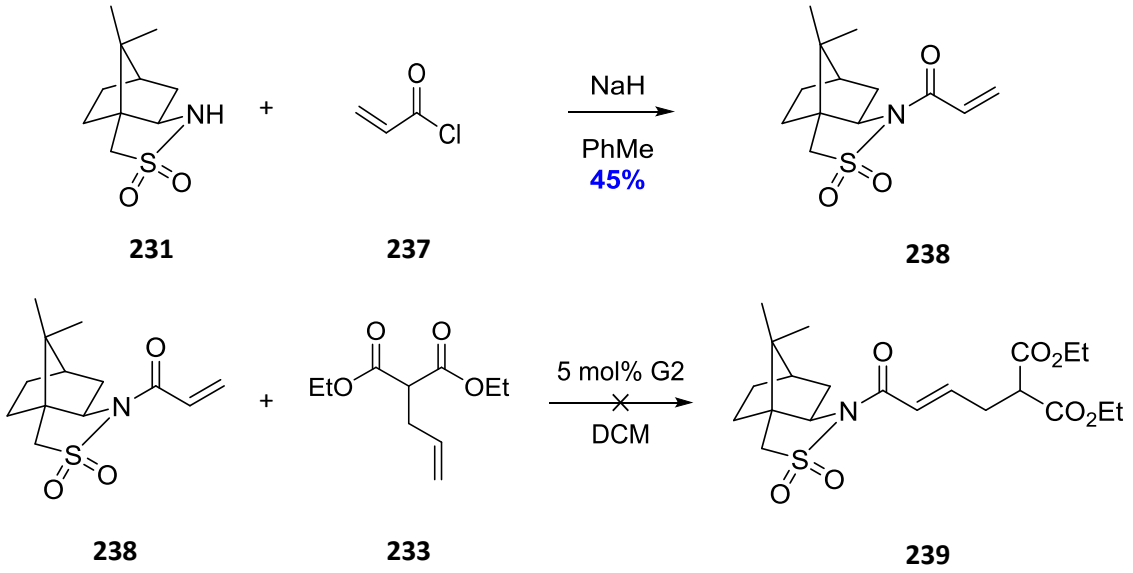


231

236

Scheme 109. Cross Metathesis of diethyl allylmalonate **233** with acrylic acid, then attempting coupling to a sultam auxiliary.

At this point, two alternate routes were explored, both employing metathesis reactions. Firstly, cross metathesis of diethyl allylmalonate **233** and acrylic acid **234** (Scheme **109**) gave carboxylic acid **235** in 76% yield. Coupling of the carboxylic acid to the sultam auxiliary using the same mixed anhydride conditions as before proved unsuccessful. Coupling via the acid chloride appeared to show some (<10%) product formation by ^{1}H NMR analysis, however this was inseparable from the starting sultam **231** by silica chromatography.

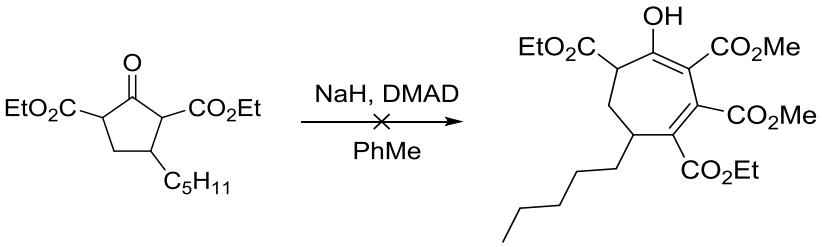


231 **237** **238**

238 **233** **239**

Scheme 110. Synthesis of Oppolzer's sultam acrylamide **238**, and investigation of cross metathesis with allylmalonate **233**.

The second route involved treatment of the sultam **231** with acryloyl chloride **237** to generate the sultam acrylamide **238** (Scheme **110**) with the intention of coupling this to the allylmalonate by cross metathesis. Literature conditions for the cross metathesis of sultam derivatives were followed,[204] however it was found that dimerisation of the allylmalonate **233** occurred exclusively, with only the homodimer and the unreacted acrylamide **238** isolated from the reaction. Addition of the allylmalonate to a refluxing solution of the acrylamide and Grubbs catalyst over 14 hours failed to alleviate this problem. The Grubbs group have reported cross metathesis using oxazolidinone acrylamides, so it is possible that the choice of another chiral auxiliary may allow this problem to be circumvented.[205] At this stage however, we returned to the diester **227** which was prepared as shown in Scheme **106**. To our disappointment, under the standard reaction conditions for the ring expansion (NaH, DMAD, PhMe), no evidence of ring expansion was observed, and only the starting materials returned. Performing the reaction at room temperature, for up to 16 h, or with an additional equivalent of base or DMAD each failed to give the required product **240**.



227 **240**

Scheme 111. Ring expansion of the diester **227** was not successful

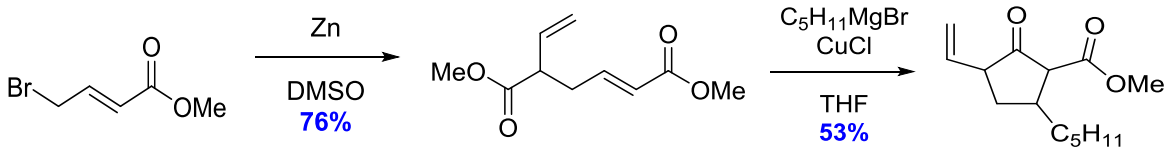
This result was surprising, especially as it was known that α'-functionality did not prevent this reaction occurring (*vide infra*).[4] The presence of a second acidic proton was postulated as the source of the problem. Therefore, reducing the acidity by masking the β-ketoester was investigated.

Use of masked esters for late-stage introduction of β-ketoester functionality

219 **241**

Scheme 112. Ring expansion of substrate **219** would allow a β-ketoester functionality to be unveiled, giving substrate **241** for a second ring expansion.

An alternative moiety from which an ester could be unveiled later in the synthesis was desired. For this purpose a vinyl group was chosen, which could subsequently be oxidatively cleaved.[206,207] A zinc(0)-mediated coupling of methyl 4-bromocrotonate **242** gave vinylhexenedioate **243** in 76% yield.[208]



242 **243** **246**

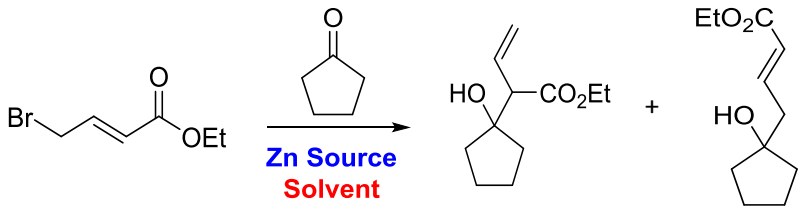
Scheme 113. Bromocrotonate coupling and tandem conjugate addition - Dieckmann cyclisation

As can be seen in Scheme **113**, the γ-bromo compound **242** undergoes α-alkylation in this reaction, as reported by Hudlicky *et al*.[209] The intermediary zinc reagent exhibits delocalised behaviour, and how this nucleophilic π-allyl complex reacts with an electrophile is dependent on the zinc source and

[4] These results are described in the following subsections, however the research was carried out concomitantly.

solvent utilised. The activation of zinc with hydrochloric acid has been shown to dramatically alter the selectivity compared to non-activated zinc, as can be seen in Table **14**.

Highlighted also in Table **14** is the effect of solvent. Whilst Hudlicky *et al.* showed complete selectivity for α-alkylation in diethyl ether, in the dimerisation of bromocrotonates reported by Orsini, DMSO is preferred.[208] In our experience, α-alkylation predominated if the reaction was performed at below 10 °C, and if the reaction was allowed to warm to room temperature or higher then the γ-alkylated product was also observed. This reaction therefore provided a facile route to the substituted hexenedioate **243** desired.

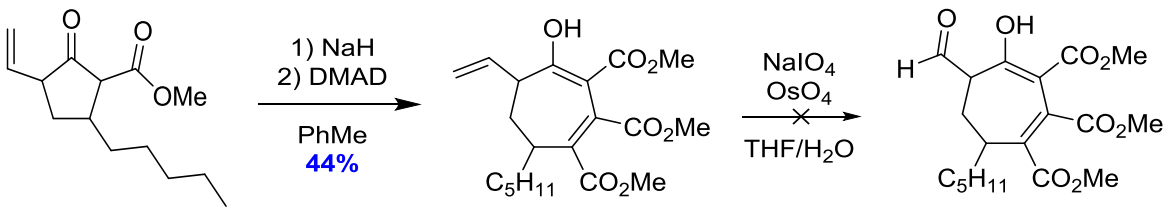


224 **244 (α)** **245 (γ)**

Solvent	Zn source (α/γ ratio)		
	Zn (act. HCl)	**ZnCu (dry)**	**Zn (dry)**
Diethyl Ether	100:0	92:8	94:6
Benzene	87:13	40:60	25:75
Cyclohexane	70:30	22:78	29:71

Table 14. The effect of solvent and zinc source on the α/γ selectivity in a Reformatsky reaction.[209]

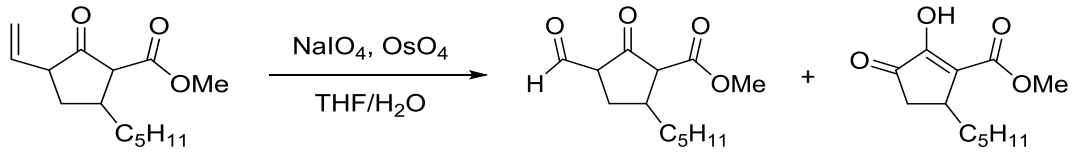
Vinylhexenedioate **243** was used in the tandem conjugate addition – Dieckmann cyclisation as before, giving a complex mixture of diastereomers of **246**. Ring expansion of **246** was achieved in 44% yield, to give **247**. Thus with a vinyl side-chain now successfully installed, the oxidative cleavage with $OsO_4/NaIO_4$ was carried out, however a complex mixture of unidentifiable products was formed, and no **248** was detected.



246 **247** **248**

Scheme 114. Ring expansion of **246**

Oxidative cleavage of the β,γ-vinylketone group under Lemieux-Johnson conditions was also investigated prior to ring expansion, with substrate **246**. This gave a complex mixture of products, most notable of which being the ketone **250**. This suggests that under the reaction conditions, the alkene isomerises into conjugation with the ketone prior to oxidative cleavage. The β-ketoester **246** was also observed to be unstable upon storage, indicating this isomerisation is very facile. Isomerisation of β,γ-unsaturated ketones to α,β-unsaturated ketones has previously been reported under very mild conditions.[210–212]

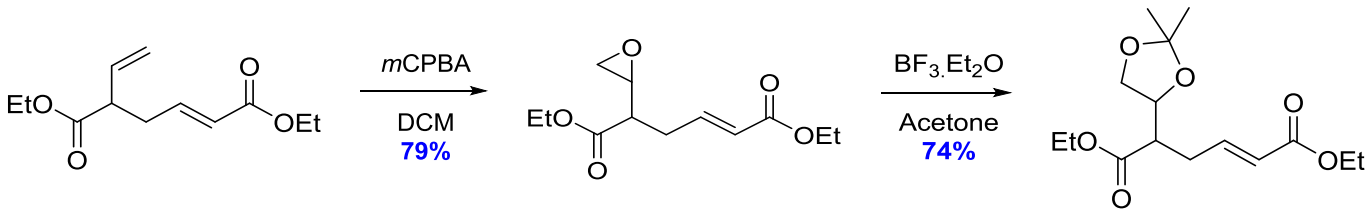


246 **249** **250**

0% **15%**

Scheme 115. Oxidative cleavage of **246**

Therefore, selective oxidation of the vinyl moiety of **246** at an earlier stage in the synthesis was investigated. Vinylhexenedioate **251**[5] proved remarkably stable towards oxidation, with no reaction observed under Upjohn, Prévost-Woodward or Sharpless dihydroxylation conditions.[213] It was found that epoxide **252** could be formed with *m*CBPA over seven days in 79% yield, as a separable mixture of stereoisomers. Each of these epoxides **252** were then converted to the acetonide **253** by the reaction with $BF_3{\cdot}OEt_2$ in acetone, in 74% yield (Scheme **116**).[214–217]

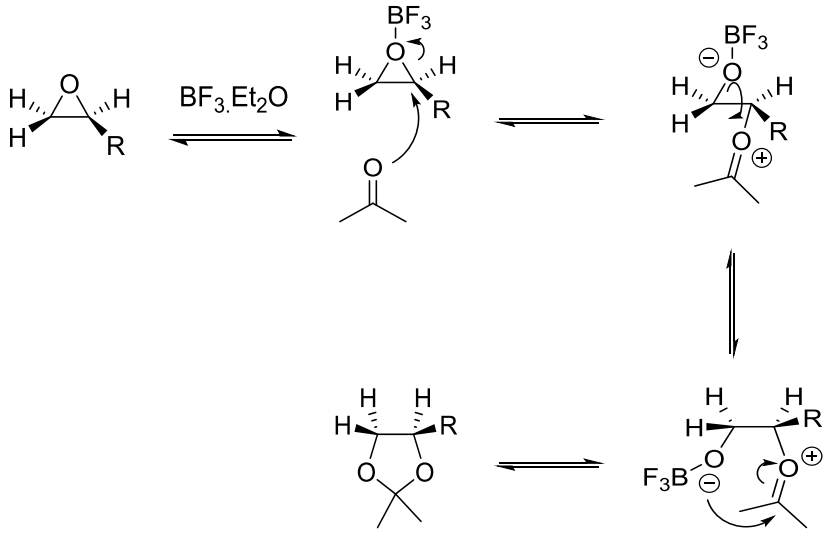


251 **252** **253**

Scheme 116. Epoxidation and acetonide synthesis from vinylhexenedioate **251**.

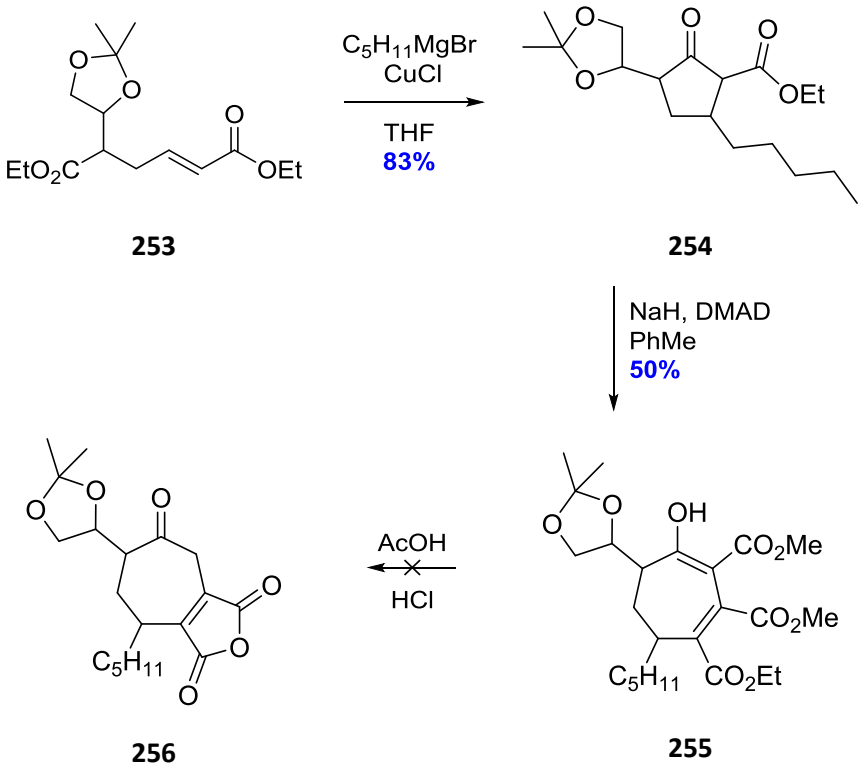
Acetonide formation occurred in a stereospecific manner, in keeping with the mechanism proposed by Wright.[218] When the separated diastereomers of epoxide **252** reacted with $BF_3{\cdot}OEt_2$, each yielded a different, single diastereomer of acetonide **253**. Unfortunately overlapping signals in the ^1H NMR spectrum made positive identification of each diastereomer impossible.

[5] The diethyl vinylhexenedioate **251** was used from this point forwards, on the grounds of cost of starting materials. Preparation as given for dimethyl vinylhexenedioate **243**.



Scheme 117. Mechanism of acetonide formation from terminal epoxides, as reported by Wright.[218]

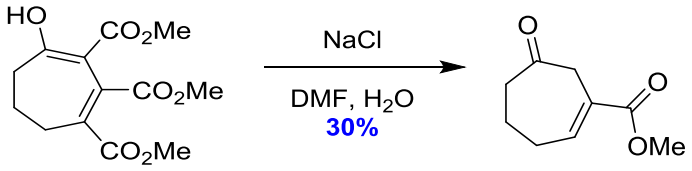
Acetonide **253** was shown to be a substrate for conjugate addition – Dieckmann cyclisation with $C_5H_{11}MgBr$/CuCl, yielding β-ketoester **254** in 83% yield. Ring expansion of **254** with NaH/DMAD successfully formed **255** in 50% yield (Scheme **118**).



Scheme 118. Dieckmann cyclisation, ring expansion of the acetonide-protected substrate **253**.

Hydrolysis of the triester **255** under the strongly acidic conditions previously applied (Scheme **102**) also removed the acetonide, and led to significant degradation. Basic conditions were discounted, as it had previously been shown that the maleic anhydride forms without decarboxylation of the β-ketoester.[184] Alternative deprotection conditions were explored, such as a Krapcho decarboxylation

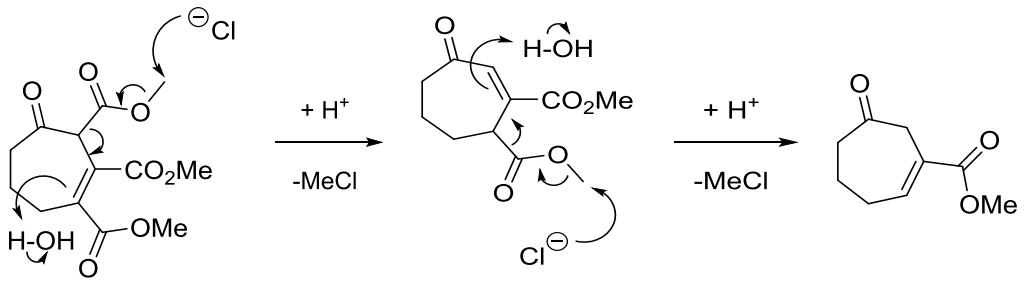
with LiCl/DMSO and DABCO/xylene, but none were successful.[219,220] With NaCl in wet DMF, the monoester **257** was the only product isolated, with the structure confirmed by comparison to literature NMR data.[221]



198 **257**

Scheme 119. Krapcho decarboxylation of **198** also led to removal of a second ester group.

Mechanistically, this can be rationalised as shown in Scheme **120**. Rather than a traditional Krapcho decarboxylation of a β-ketoester, this reaction should proceed via delocalisation into the γ-ester. Protonation then yields diester **258**, in which the γ-ester now has an appropriate relationship with the ketone for a second decarboxylation.



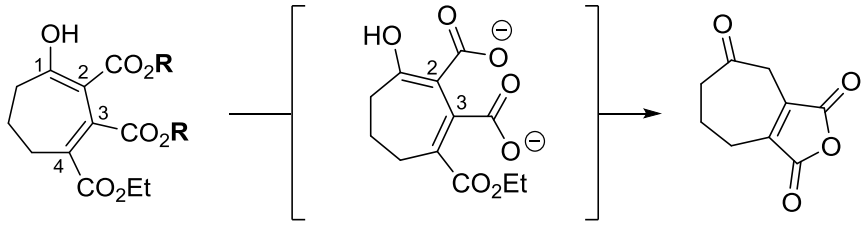
198 **258** **257**

Scheme 120. Plausible mechanism to rationalise the formation of **257**.

Milder conditions were tried, such as Taber's selective dealkoxycarbonylation of β-ketoesters with DMAP in phosphate buffer, and the use of DMSO/H_2O without an inorganic salt.[222,223] In all cases either starting material, the monoester **257** or a complex mixture of degradation products were obtained. Aliquots from reaction mixtures at different timepoints offered no further insight, with no intermediate stages in the degradation identified. With this in mind, it appeared that no S_N2-based deprotection mechanism would offer the selectivity necessary, and that whilst hydrolytic conditions could form the anhydride, as shown in Scheme **102**, it was unlikely that conditions could be found in which the acetonide remained intact.

As a methyl ester could not be successfully removed from **198**, it seemed prudent to change the methyl ester to an alternative ester which could be removed under more mild conditions. This required the synthesis of an alternative acetylene diester with which to perform the ring expansion. It was hoped that once the esters were removed, the C2 carboxylate **259** (Scheme **121**) would

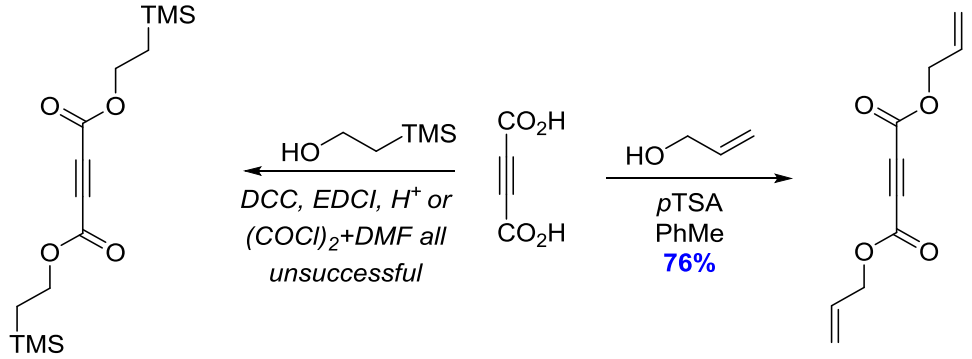
decarboxylate as would be expected for a β-ketoacid, whilst the C3 carboxylate would undergo a nucleophilic addition to the remaining C4 ester, resulting in anhydride formation.



259 **215**

Scheme 121. Proposed deprotection mechanism to yield anhydride **215** by removal of two ester groups

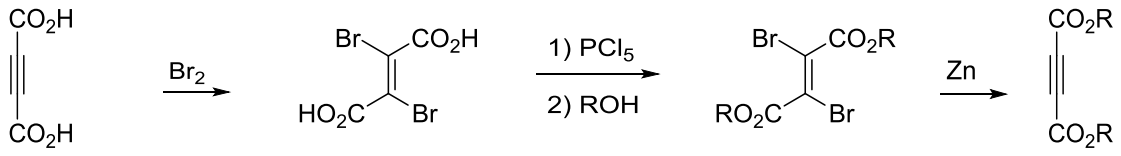
Initially, the use of a TMSE (trimethylsilylethyl) protecting group was investigated, which could then easily be removed with a fluoride source. However starting from the acetylene diacid **260**, the desired diester **261** could not be synthesised with acid catalysis, a DCC or EDCI coupling, or via the acid chloride (Scheme **122**). Transesterification of DMAD with $Ti(^{i}OPr)_4$ was also unsuccessful.



261 **260** **262**

Scheme 122. Conditions investigated for the synthesis of TMSE diester **261** from diacid **260**, and successful synthesis of diallyl diester **262**.

The problems of synthesising acetylenediesters have been previously documented, leading to Charlton and coworkers' proposal of a three-step synthesis. Issues include cyclotrimerisation of the starting material and product, as well as competing 1,4-addition of the alcohol to the reactive alkynoate moiety.[224]

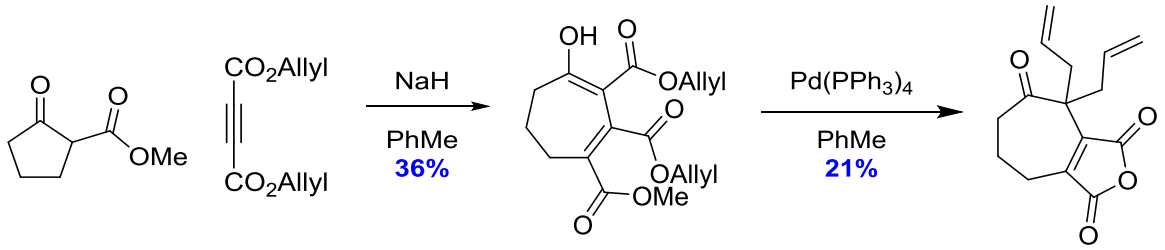


260 **263** **264**

Scheme 123. Charlton's synthesis of acetylene diesters **264** via dibromofumaric acid **263**.

Whilst the Charlton route has been used for the synthesis of a wide range of acetylenic diesters, the reported variable, low yields for the final zinc-promoted dibromination were off-putting, and so other protecting groups were explored first.

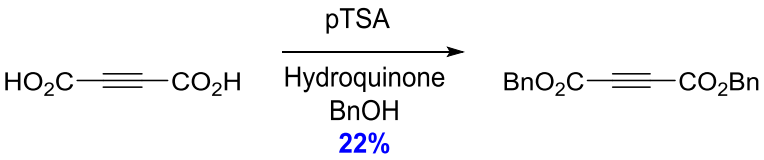
An allyl protecting group was next considered, as it could be easily deprotected with a palladium catalyst.[225] Diallyl ester **262** was synthesised from the diacid **260** with *p*TSA and allyl alcohol in 76% yield (Scheme **122**), and was then used for the ring expansion of **188** with NaH, yielding triester **265** in 36% yield.[184] However, upon treatment with $Pd(PPh_3)_4$ under a variety of conditions, only the α-diallylated product **266** was observed, in a reaction analogous to a Carroll rearrangement.[226–228]



188 **262** **265** **266**

Scheme 124. Ring expansion could be performed with the diallyl ester **262**, but subsequent treatment with Pd(0) gave only α-diallylation via a Carroll rearrangement.

Whilst a benzyl ester is not an ideal protecting group for our purpose, as competing hydrogenation of the alkene moiety during deprotection could be an added complication, a literature synthesis of this diester was encouraging.[229] Treatment of diacid **260** with *p*TSA and benzyl alcohol gave diester **267** in 22% yield. The presence of hydroquinone in the reaction mixture was beneficial, without it only traces of the desired diester **267** were detected. Hydroquinone has been reported to reduce cyclotrimerisation of acetylene diesters.[230] Dibenzyl ether is formed as a by-product in this reaction, which is difficult to remove due to its high boiling point. The reaction was therefore performed with the minimum amount of benzyl alcohol possible (2.1 equivalents) to reduce the quantity of ether formed.

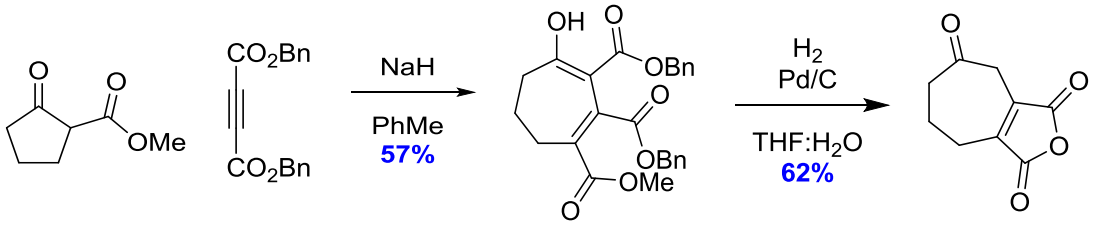


260 **267**

Scheme 125. Synthesis of dibenzyl acetylenedicarboxylate

To test that benzyl diester **267** was an appropriate replacement for DMAD in the ring expansion reaction, and could be removed to form the anhydride through hydrogenolysis, this was first

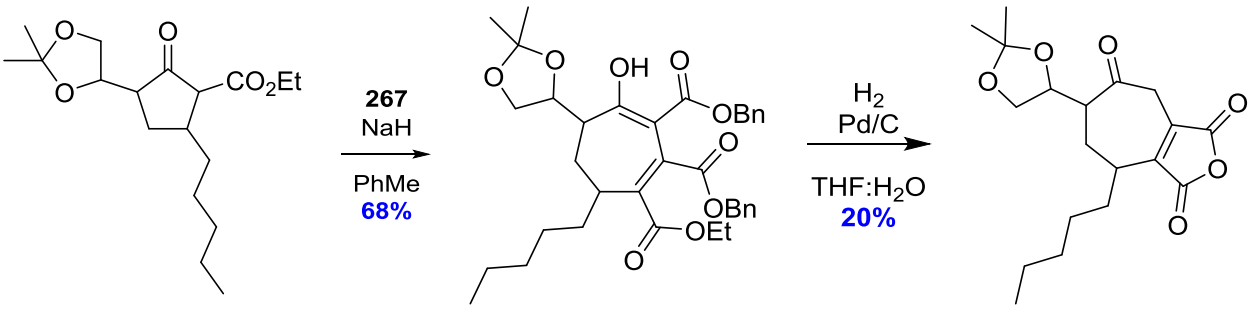
investigated on the simple substrate **188**. Ring expansion of **188** with NaH in toluene, gave triester **268** in 57% yield. Hydrogenolysis of **268** then gave the required anhydride **198** (Scheme **121**). Over-reduction was observed with extended reaction times, but with careful monitoring the maleic anhydride product **198** could be obtained in high purity.



188 **267** **268** **198**

Scheme 126. Ring expansion and subsequent hydrogenolysis to anhydride **198.**

With this chemistry proven, it was then applied to the α'-acetonide-β-ketoester substrate **254**. Ring expansion with dibenzyl ester **267** and NaH in toluene gave triester **269** in 68% yield, with subsequent hydrogenolysis to anhydride **270** in 20% yield.



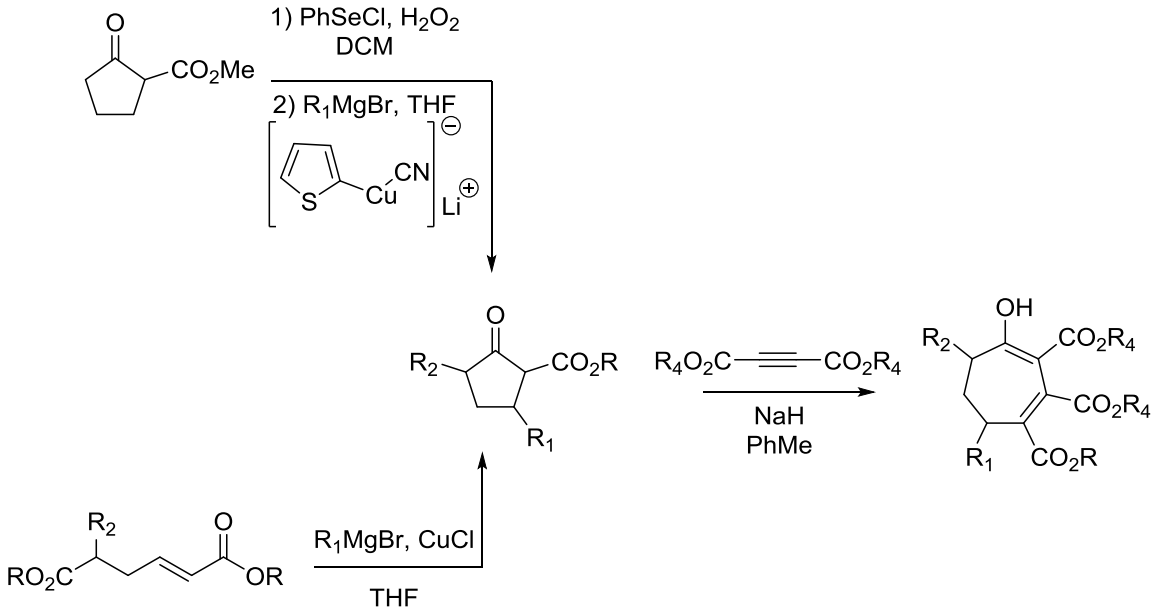
254 **269** **270**

Scheme 127. Ring expansion and subsequent hydrogenolysis with dibenzyl acetylenedicarboxylate

Unfortunately, subsequent oxidative cleavage with HIO_6 was unsuccessful,[231] as was deprotection of the acetonide to the diol. Time and material constraints meant this product could not be explored further.

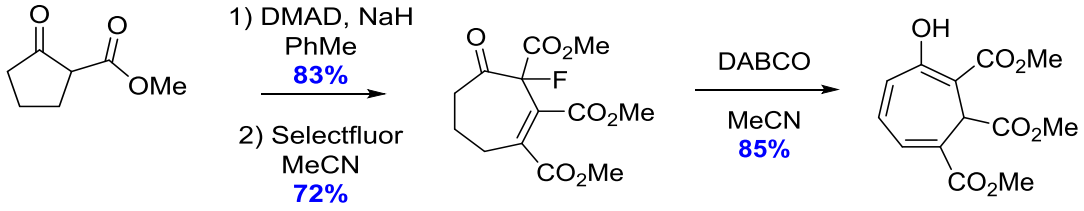
5.4 Conclusions and Future Work

In conclusion, an iterative ring expansion methodology has been explored towards the synthesis of scytalidin. This work has developed two methods for the synthesis of 2-methoxycarbonyl-3-alkylcyclopentanones, and significantly broadened the scope of the Frew-Proctor ring expansion. It has been demonstrated that dimethyl, diallyl and dibenzyl esters can be used in conjunction with 3-, 5- and 3,5-substituted β-ketoesters.



Scheme 128. Synthesis of 2-methoxycarbonyl-2-alkylcyclopentanones by two routes, and ring expansion with several acetylenic diesters has been reported.

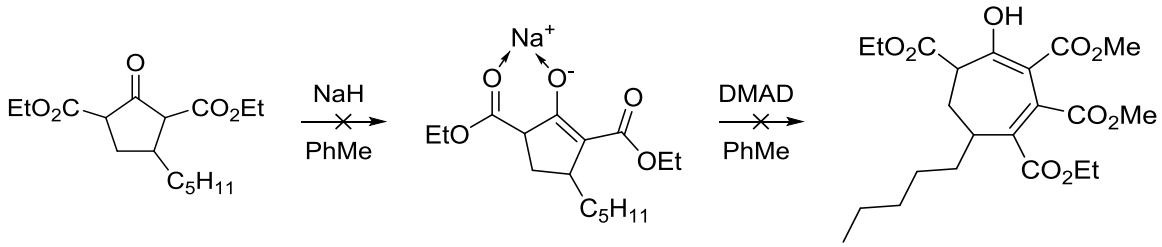
The application of this methodology to the synthesis of scytalidin has been complicated by difficulties arising from the synthesis of an appropriate β-ketoester for a second ring expansion. Methoxycarbonylation after the ring expansion is not possible, due to the instability of the α-halo-β-ketoesters investigated to basic conditions. This led to an interesting triene byproduct **210** but rendered this an end-point for this approach to scytalidin.



197 **209** **210**

Scheme 129. Basic conditions cause α-halo-β-ketoester **209** to form the triene **210**

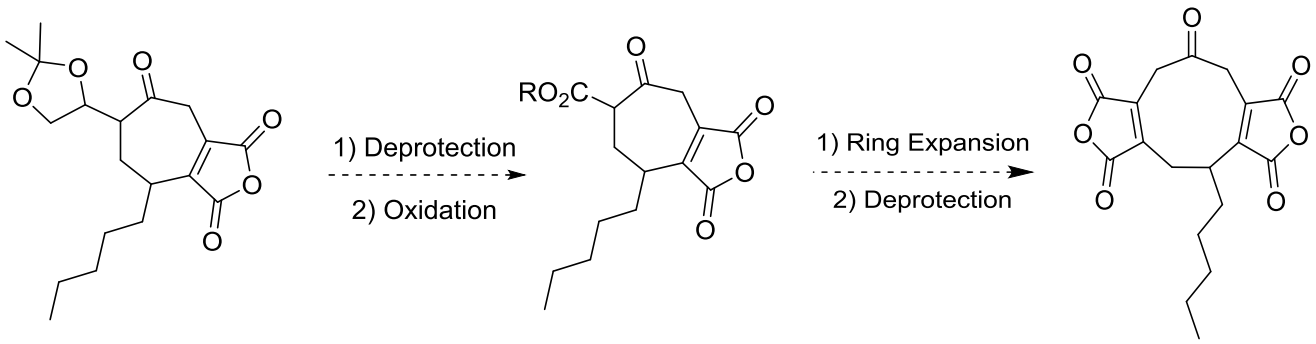
If an ester is pre-installed at the α'-position, the ring expansion reaction is unsuccessful. Presumably due to the presence of an additional acidic proton, and the ability to adopt a highly stabilised conformer. Such a chelate has previously been reported in the alkoxycarbonylation of cyclic ketones with Stiles reagent.[232]



227 **228**

Scheme 130. Proposed stable chelate which prevents further reaction of diester **227**

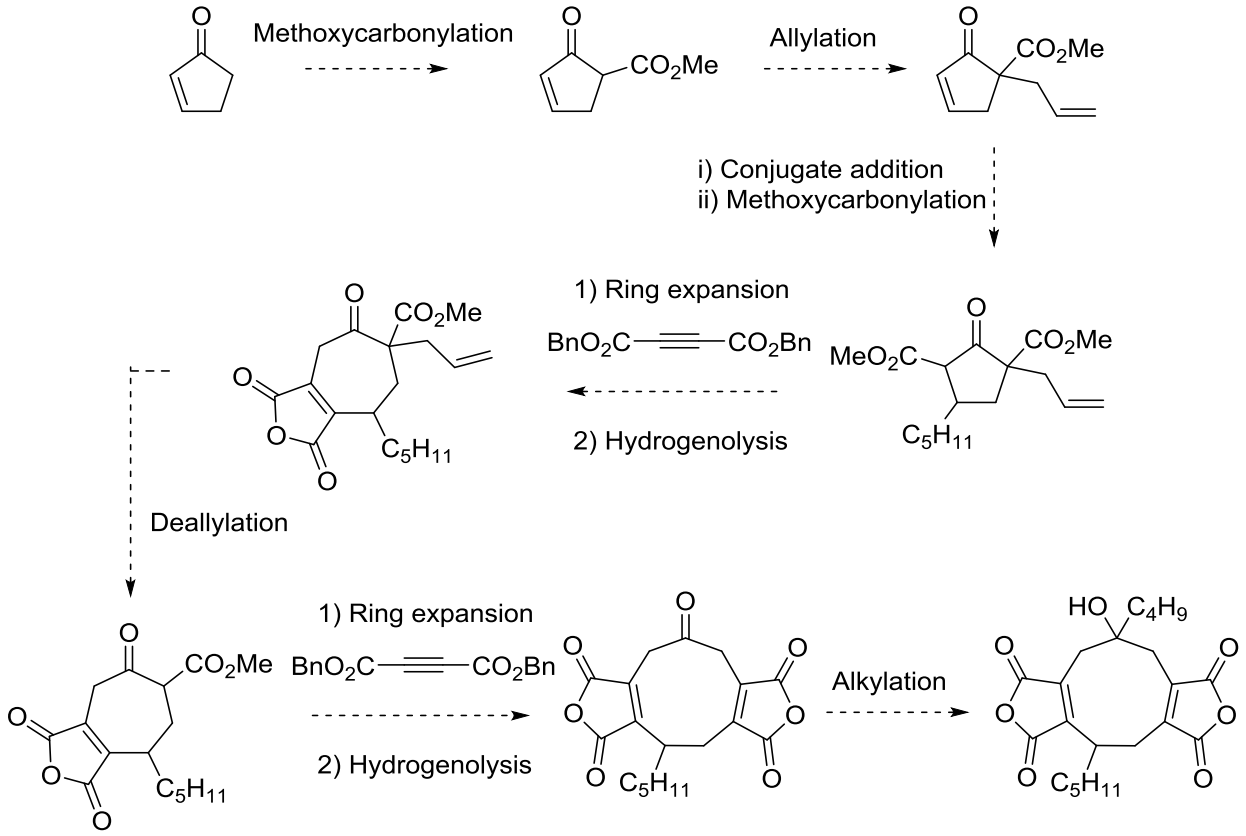
Our final approach was to mask the necessary ester as an alternative moiety, allowed deprotection to the ester when required. The ring expansion substrates thus devised with an α'-acetonide substituent were able to undergo the ring expansion successfully, and a novel use of benzyl protecting groups allowed the maleic anhydride group to be deprotected under much more mild conditions than previously reported, yielding **270**. Further screening of conditions for the transformation of the acetonide to β-ketoester **271** is required, before ring expansion and ester hydrolysis should lead to the formation of the core cyclononadiene structure **272** of scytalidin **12**.



270 **271** **272**

Scheme 131. Proposed endgame for the synthesis of the cyclononadiene core of scytalidin **12**

An alternative synthesis is proposed in Scheme **132**. By protecting an acidic C-H with an easily removed allyl group.[233] It is hoped that the use of dibenzyl acetylenedicarboxylate **267** will allow differentiation of β-ketoesters, thus allowing the synthesis of scytalidin **12** to be completed.



Scheme 132. Proposed synthesis of scytalidin **12**, using an allyl protecting group strategy

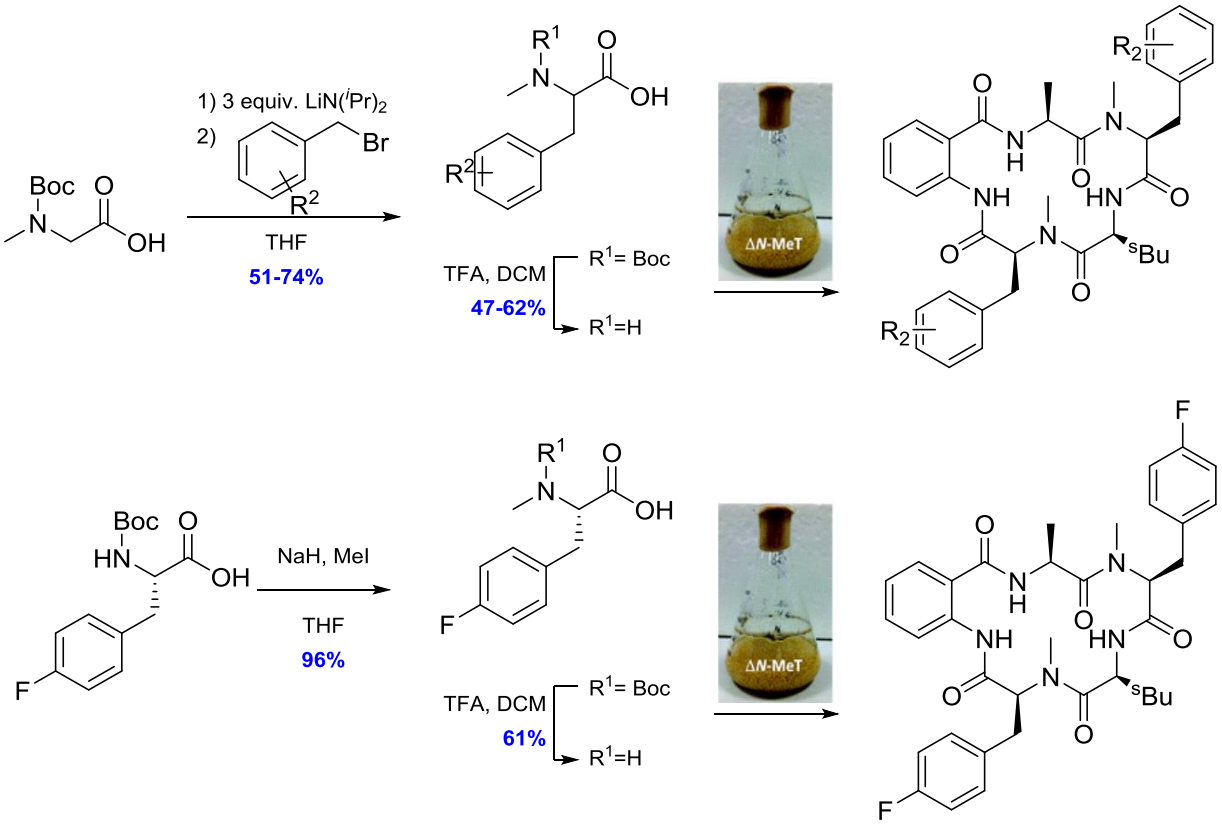
6. Contribution to other projects

The cycloaspeptides: uncovering a new model for methylated nonribosomal peptide biosynthesis

K.M.J. de Mattos-Shipley, C. Greco, D.M. Heard, G. Hough, N.P. Mulholland, J.L. Vincent, J. Micklefield, T.J. Simpson, C.L. Willis, R.J. Cox, A.M. Bailey

Chemical Science, **2018**, 9, 4109-4117, DOI: 10.1039/C8SC00717A

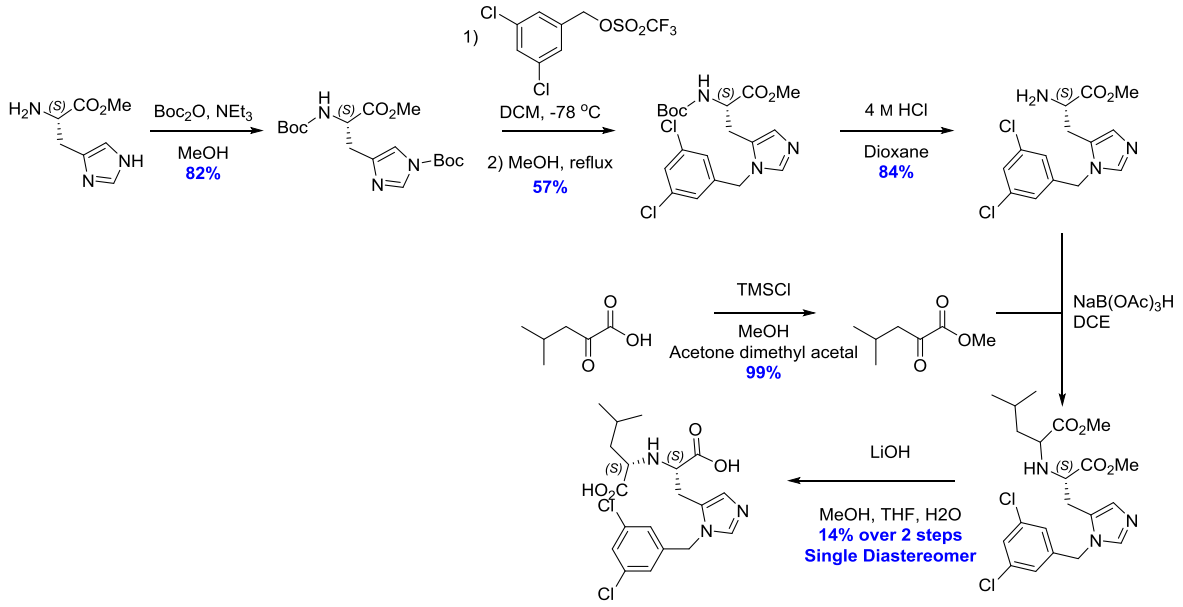
Genome sequencing, bioinformatics, and heterologous expression in *Aspergillus oryzae* was used to study the biosynthesis of the cycloaspeptides, bioactive cyclic pentapeptides produced by several *Penicillium* strains of filamentous fungi. It was shown that a nonribosomal peptide synthetase (NRPS) uses methylated amino acids produced by a trans-acting *N*-methyltransferase to produce the cycloaspeptides. An engineered *A. oryzae* strain with the *N*-methyltransferase removed was produced, and by feeding several natural and unnatural *N*-methyl amino acids to this strain, the metabolite profile could be changed. This was used to produce highly bioactive cycloaspeptides previously isolated in very low titres, and a novel fluorinated cycloaspeptide derivative. My contribution to this project included the synthesis of the required *N*-methyl amino acids.



Scheme 133. Synthesis of *N*-methylated amino acids, and novel cycloaspeptides produced through mutasynthesis. R_2= *p*-Me or *p*-OMe.

Synthesis of an ACE2 Inhibitor for the Study of Alzheimer's in Murine Models

Collaborators at the University of Bristol Institute of Clinical Neurosciences had identified a promising compound which appears to significantly improve the cognition of mice engineered to exhibit symptoms of Alzheimer's pathology. The angiotensin-converting enzyme 2 (ACE2) was anticipated to play a key part in the function of this compound, and therefore to prove this hypothesis, an inhibitor of ACE2 was required. My contribution included the synthesis of a reported ACE2 inhibitor on gram scale, the route employed is shown in Scheme **134**.[234] Results from murine studies will be published in due course.



Scheme 134. The desired ACE2 inhibitor was synthesised as a single diastereomer in five steps.

CHAPTER 7: Experimental

7. Experimental

7.1 General Experimental

Culture Work

S. album strains UAMH 3611 and UAMH 3620 were purchased from the University of Alberta Mold Herbarium and Culture Collection. All glassware, media and miscellaneous items were sterilised using an Astell Autoclave at 121 °C for 15 minutes. Potato Dextrose Agar (PDA): 39 g of Potato Dextrose Agar was dissolved in 1 L distilled water and sterilised prior to use. Potato Dextrose Broth (PDB): 12 g potato dextrose dissolved in 0.5 L distilled water. Malt Extract Broth (MEB): 10 g malt extract dissolved in 0.5 L distilled water.

Synthesis

All reagents were sourced from commercial suppliers and were used without further purification unless stated otherwise. All reactions using anhydrous solvents were performed using standard Schlenk syringe-septa techniques, with flame dried glassware under a positive pressure of nitrogen. Anhydrous THF, Et_2O, hexane, toluene, acetonitrile and CH_2Cl_2 were dried by passing through a modified Grubbs system of alumina columns, manufactured by Anhydrous Engineering and stored over 3 Å molecular sieves. Dry DMF was obtained from Sigma-Aldrich, dry DMSO and acetone from Acros Organics, each were used without further purification or drying. Diisopropylamine and triethylamine were distilled over CaH_2 prior to use in moisture-sensitive reactions. All stated temperatures below ambient are the temperatures of the cooling baths, unless otherwise stated.

Purification

Flash column chromatography was performed according to the procedures used by Still *et. al.*[235] using silica gel 60 (Fisher Scientific or Aldrich) and a suitable eluent. TLC analysis was performed with aluminium backed silica TLC plates (Merck-Kieselgel 60 F_{254}) with a suitable solvent system and was visualised using UV fluorescence (254 & 366 nm) and/or developed with potassium permanganate solution.

HPLC Instrumentation

The Waters 2795HT HPLC system was equipped with the following detectors:

- Waters 998 diode array detector for UV between 200 and 400 nm.
- Electrospray (ES) Waters ZQ mass spectrometry with detection between 150 and 600 m/z units in positive and negative modes.

The Waters 2445SFO HPLC system was equipped the following detectors:

- Waters 2298 diode array detector for UV between 200 and 400 nm.
- Electrospray (ES) Waters ZQ mass spectrometry with detection between 150 and 1200 m/z units.
- Waters Quattro Micro ESI mass spectrometer in positive and negative modes, with detection between 150 and 1200 m/z units.
- Phenomenex Kinetex column (2.6 µ, C18, 100 Å, 4.6 x 100 mm) fitted with a Phenomenex Security Guard precolumn (Luna C5 300 Å).

Spectroscopy & Spectrometry

Infra-red spectra were recorded on a Perkin Elmer Spectrum 100 FTIR with an ATR accessory and frequencies are reported in wavenumbers (cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded using Jeol ECS 400 MHz, Varian 400-MR (400 MHz), Varian VNMRS500a (500 MHz), Varian VNMRS500b (500 MHz), Bruker Advance III HD 500 Cryo (500 MHz), Varian VNMR S600 Cryo (600 MHz), and Bruker Avance III HD 700 (1.7mm micro-cryo) (700 MHz) spectrometers at ambient temperature. Chemical shifts (δ) are quoted in parts per million (ppm) and coupling constants (*J*) are in hertz (Hz), rounded to 0.5 Hz intervals. Two-dimensional NMR techniques (HSQC, COSY, HMBC) were used routinely for structural assignment. Use of NOESY, TOCSY and EXSIDE techniques is indicated as approriate. Residual solvent peaks were used as the internal reference for proton and carbon chemical shifts. HRMS ESI were performed on either a Bruker Daltonics Apex 4, 7 Tesla FTICR or microTOF II. Samples were submitted in MeOH or CH_2Cl_2. Specific rotations ($[\alpha]_D^T$) were measured on a Bellingham and Stanley Ltd. ADP220 polarimeter and are quoted in (° ml)(g dm)$^{-1}$.

Single Crystal XRD Analysis

X-ray diffraction experiments were performed by Dr H.A Sparkes at the University of Bristol, using a Bruker Apex II diffractometer with Mo-K_α radiation (λ = 0.71073 Å). Single crystals were mounted on glass fibre, and data collected with a CCD area detector. Structures were solved using the SIR2004 package within Olex2, using Direct Methods and refinement via the XH refinement package (CGLS Minimisation).

Contributions

All experiments were performed by David Heard, with the following exceptions:

- Isolation of deoxyscytalidin **35** performed by Dr Claudio Greco
- Some steps in the 2nd generation synthesis of tricladic acids A and B, performed by Emyr Tayler under the supervision of David Heard. Denoted with a (*) after the compound name.
- Synthesis and Carroll rearrangement of **266**, performed by Bethan Donnelly under the supervision of David Heard. Denoted with a (†) after the compound name.

7.2 Synthesis of Compounds

7.2.1 Experimental Procedures for Chapter 2

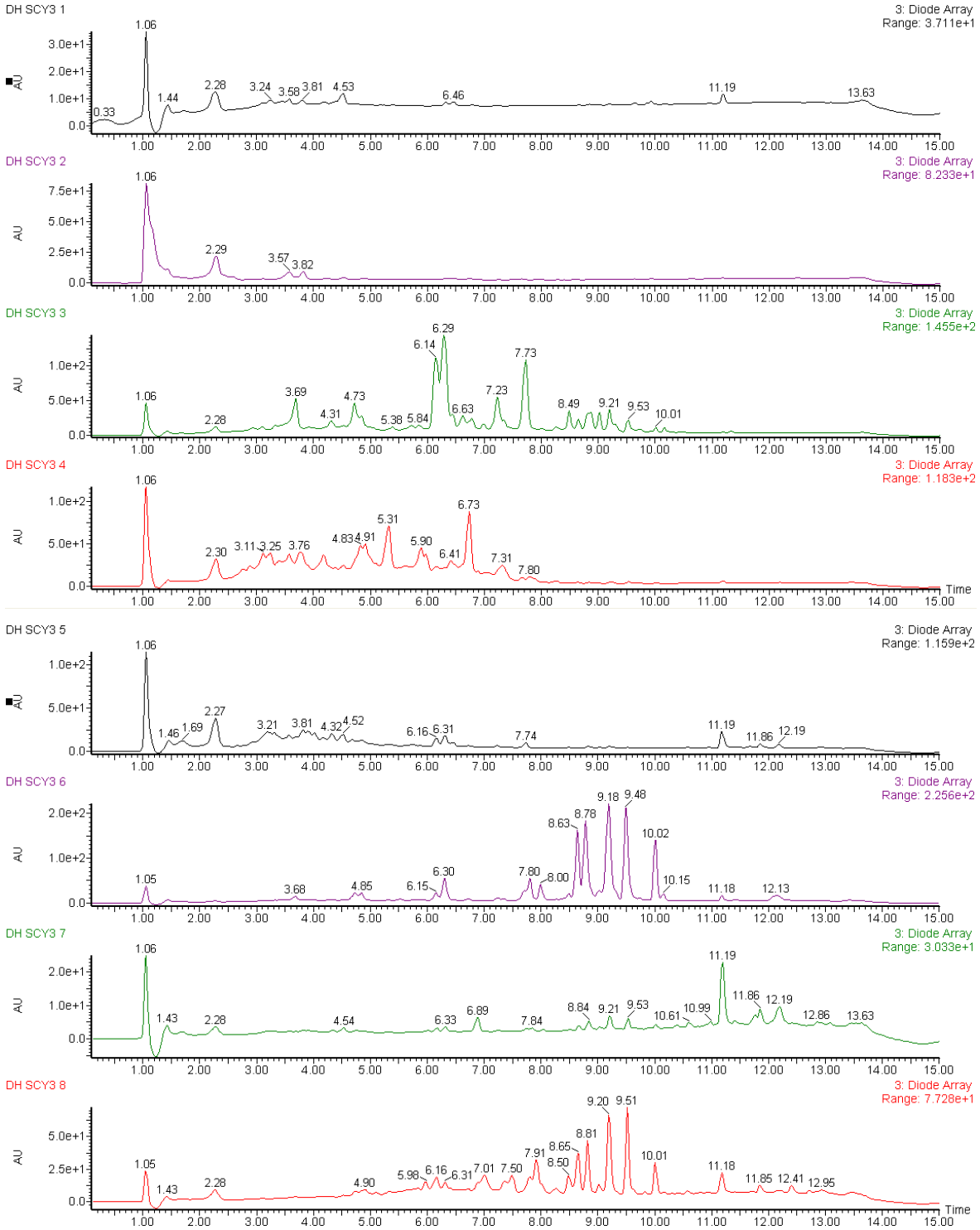
Screening of conditions for scytalidin production from *S. album*

S. album strains UAMH 3620 and 3611 were grown on PDA, the agar plates homogenised with 10 mL of MEB, then inoculated into 500 mL flasks containing 100 mL sterilised media. The flasks were shaken at 200 rpm at 25 °C for 17 days. The mycelia were separated by Buchner filtration, and stirred overnight in acetone, filtered, concentrated *in vacuo*, extracted with ethyl acetate (3 × 100 mL), dried with $MgSO_4$ and concentrated *in vacuo*. The aqueous media were extracted with ethyl acetate (3 × 300 mL), dried with $MgSO_4$ and concentrated *in vacuo*. The mass of each extract is recorded in the table below. Each sample was analysed by analytical HPLC. All crude extracts were prepared to a concentration of 10 mg/mL in HPLC grade acetonitrile and placed in LCMS vials. 20 µL of the extracts were injected and analysed on a Waters 2795HT or Waters 2545SFO HPLC system. Solvents used were: HPLC grade H_2O containing 0.05 % formic acid (A) and HPLC grade acetonitrile containing 0.05 % formic acid (C). The method used was: 0 min, 5% C; 10 min, 95% C; 12 min, 95% C; 13 min, 5% C; 15 min, 5% C. Flow rate 1 mL/min.

Quantity of organic extract produced under each set of conditions was used as a surrogate for quality of growth. Conditions were explored further when a high quantity of organic extract was noted, and the presence of scytalidin was detected by HPLC.

Growth Conditions	***S. Album* strain**	3611 Mycelia	3611 Spore	3620 Mycelia
		Crude extract mass / media volume		
MEB, baffled flasks	Mycelial extract	70.7 mg / 0.4 L (SCY3 7)	10.8 mg/ 0.3 L (SCY3 10)	20.8 mg/ 0.3 L (SCY3 11)
	Media extract	30.5 mg / 0.4 L (SCY3 1)	24.9 mg/ 0.3 L (SCY3 4)	36.7 mg/ 0.3 L (SCY3 5)
PDB, baffled flasks	Mycelial extract	64.3 mg/ 0.2 L (SCY3 12)	21.8 mg/ 0.1 L (SCY3 8)	43.2 mg/ 0.5 L (SCY4-D) 18.7 mg/ 0.1 L (SCY3 9)
	Media extract	189.3 mg/ 0.2 L (SCY3 6)	397.4 mg/ 0.1 L (SCY3 2)	100.8 mg/ 0.5 L (SCY4-B) 37.1 mg/ 0.1 L (SCY3 3)
PDB, non-baffled flasks	Mycelial extract	-	-	140.1 mg/ 0.5 L (SCY4-C)
	Media extract	-	-	121.5 mg/0.5 L (SCY4-A)

Table 15. Crude organic extract mass, and volume from which this was obtained. (Code in brackets is the HPLC trace from Figure **48**, below)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6.29
6.63
7.23
7.73
8.49
9.21
9.53
10.01
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2.30
3.11
3.25
3.76
4.83
4.91
5.31
5.90
6.41
6.73
7.31
7.80
Time
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3.21
3.81
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4.52
6.16
6.31
7.74
11.19
11.86
12.19
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4.85
6.15
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7.80
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8.63
8.78
9.18
9.48
10.02
10.15
11.18
12.13
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1.43
2.28
4.54
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7.84
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9.21
9.53
10.61
10.99
11.19
11.86
12.19
12.86
13.63
DH SCY3 8
3: Diode Array
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2.28
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6.31
7.01
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9.51
10.01
11.18
11.85
12.41
12.95
Time

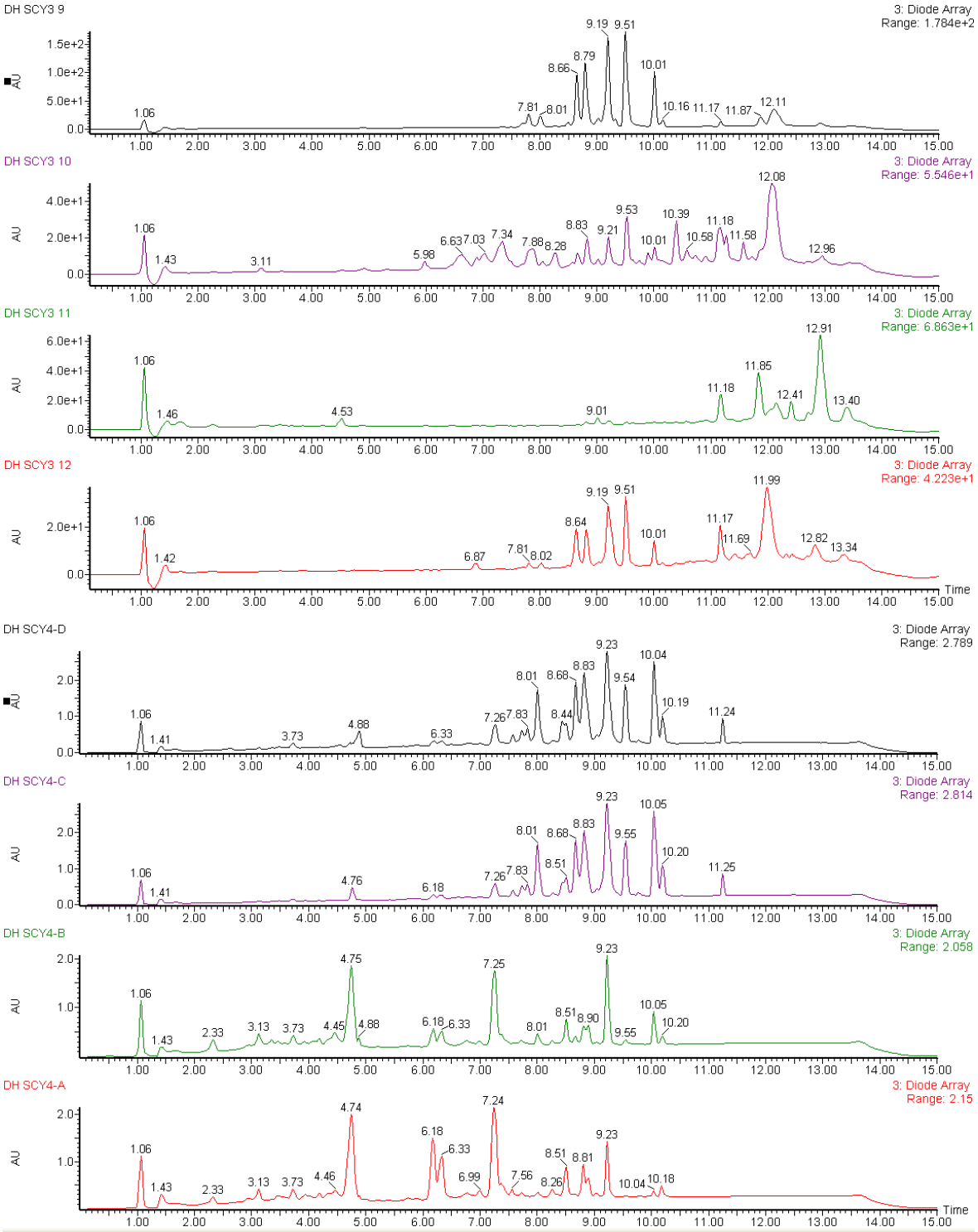
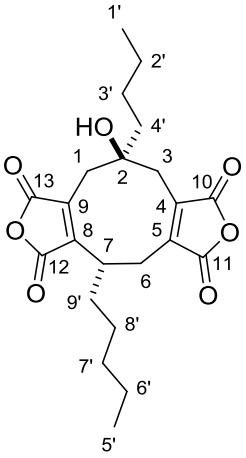


Figure 48. HPLC traces of crude extracts from *S. album* strains UAMH 3620 and UAMH 3611. Conditions tabulated above.

Scytalidin 12

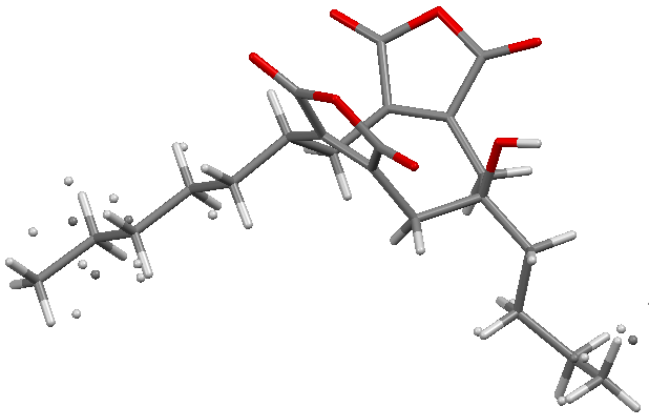


12

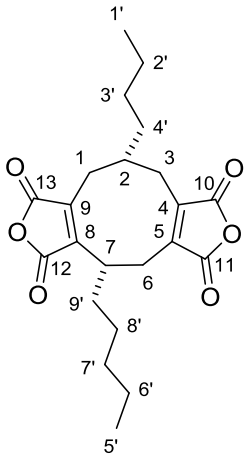
Scytalidium album UAMH 3620 was grown on malt extract agar, and once mature, one agar plate was homogenised with 50 mL malt MEB. 15 mL of this suspension inoculated into non-baffled flasks of malt extract broth (2 × 100 mL). After 4 days, these starter cultures were split evenly between fresh non-baffled flasks of malt extract broth (20 × 100 mL) and grown for 27 days, shaking at 200 rpm at 25 °C. The cultures were combined and the mycelia separated by centrifugation (8000 rpm, 0.4 h). The dry mycelia was blended in acetone (150 mL), filtered, and extracted with an additional (2 × 100 mL) acetone. The combined acetone extracts were concentrated *in vacuo*, suspended in water (300 mL), extracted with ethyl acetate (3 × 500 mL), and the combined organic layers dried with $MgSO_4$, filtered, and concentrated *in vacuo* to yield a brown residue. Scytalidin **12** (20 mg) was isolated from this residue by repeated trituration from diethyl ether (3 × 5 mL) and purified by recrystallisation from diethyl ether, yielding colourless needles. Alternatively, a similar yield of **12** could be obtained via purification by HPLC. $[\alpha]_D^{22}$ -57.8 (*c* 0.47, EtOAc) [lit. $[\alpha]_D^{21}$ −66.6 (*c* 0.4745, EtOAc)][30]; δ_H (500 MHz, CD_2Cl_2) 3.42 (1H, m, 7-H), 3.04-2.71 (5H, m, 1-*H*H, 3-H_2 and 6-H_2) 2.57 (1H, d, *J* 13.0, 1-H*H*), 2.02 (1H, s, OH), 1.85-1.74 (2H, m, 3'-*H*H and 4'-*H*H), 1.72-1.63 (2H, m, 9'-H_2), 1.58-1.47 (2H, m, 3'-H*H* and 4'-H*H*), 1.46-1.37 (3H, m, 2'-H_2 and 8'-*H*H), 1.36-1.27 (5H, m, 6'-H_2, 7'-H_2 and 8'-H*H*), 0.98 (3H, t, *J* 7.5, 1'-H_3), 0.89 (3H, t, *J* 7.0, 5'-H_3); δ_C(125 MHz, CD_2Cl_2) 168.0 (C=O), 166.0 (C=O), 165.9 (C=O), 165.4 (C=O), 145.5 (C=C), 143.5 (C=C), 141.9 (C=C), 76.6 (C-2), 46.2 (C-4'), 35.7, 35.42 and 35.39 (C-1, C-3, C-7 and C-9'), 32.0 (C-6' or C-7'), 28.6 (C-6), 27.3 (C-8'), 26.2 (C-3'), 23.5 (C-2'), 23.0 (C-6' or C-7'), 14.31 (C-1'), 14.28 (C-5'); ν_{max} (film) 3549, 2957, 2933, 2862, 1827, 1766, 1670, 1458, 1277, 1253, 928; HRMS (ESI) calc for $C_{22}H_{28}NaO_7$ $[M+Na]^+$ 427.1727, found 427.1732.

NMR data in accordance with literature.[63] 2 carbons cannot be seen (HSQC indicates around 140-145ppm and 35.5 ppm).

The *cis*-arrangement of alkyl side chains was established through X-ray crystallography.



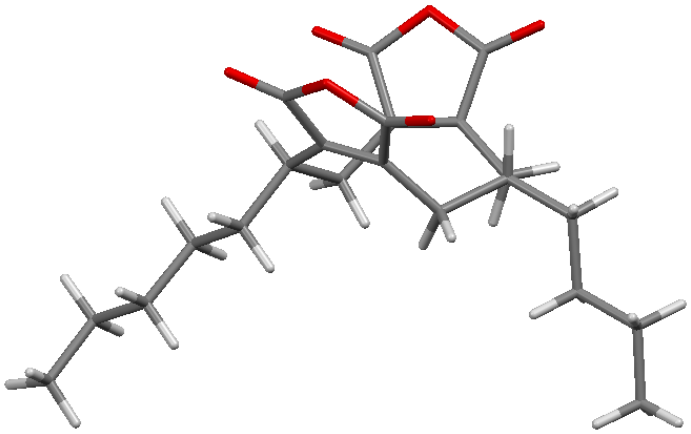
Deoxyscytalidin 35



35

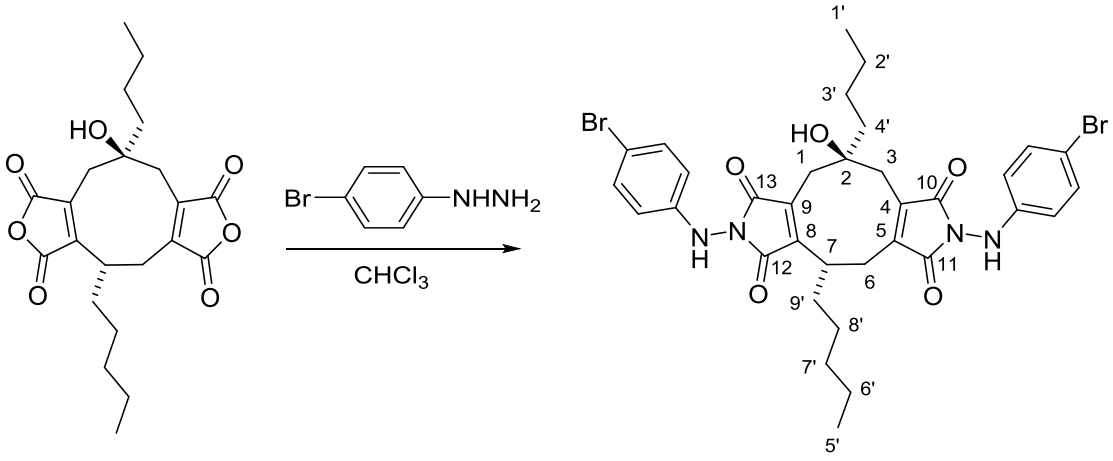
Cultures of *S. album* UAMH 3620 were grown as previously described, and deoxyscytalidin **35** (20 mg) isolated by preparative HPLC as a crystalline solid. $[\alpha]_D^{22}$ −52.5 (*c* 0.13, $CHCl_3$) [lit. $[\alpha]_D$ -82.2 (*c* 0.135, $CHCl_3$)][55]; δ_H(500 MHz, CD_2Cl_2) 3.36 (1H, m, 7-H), 2.97-2.81 (2H, m, 6-H_2), 2.74-2.62 (2H, m, 1-H_2 or 3-H_2), 2.40-2.28 (2H, m, 1-H_2 or 3-H_2), 1.93 (1H, m, 2-H), 1.77-1.23 (14H, m, 2'-H_2 to 4'-H_2 and 6'-H_2 to 9'-H_2), 0.97 (3H, t, *J* 7.0, 1'-H_3), 0.90 (3H, t, *J* 6.0, 5'-H_3); δ_C(125 MHz, CD_2Cl_2) 166.2 (C=O), 166.0 (C=O), 165.9 (C=O), 165.4 (C=O), 145.1 (C=C), 144.8 (C=C), 144.2 (C=C), 143.8 (C=C), 38.8 (C-2), 37.5 (CH_2), 35.5 (C-7), 34.8 (CH_2), 32.0 (CH_2), 31.1, 30.3 (C-1 and C-3), 29.6 (CH_2), 28.6 (C-6), 27.5 (CH_2), 23.1 (CH_2), 23.0 (CH_2), 14.29 (CH_3), 14.28 (CH_3); ν_{max} (film) 2959, 2931, 2860, 1825, 1770, 1669, 1458, 1254, 927; HRMS (ESI) calc for $C_{22}H_{28}NaO_6$ $[M+Na]^+$ 411.1778, found 411.1779.

NMR data in accordance with literature.[55]



The *cis*-arrangement of alkyl side chains was established through X-ray crystallography.

Scytalidin bis(4-bromophenylhydrazide) maleimide derivative 51

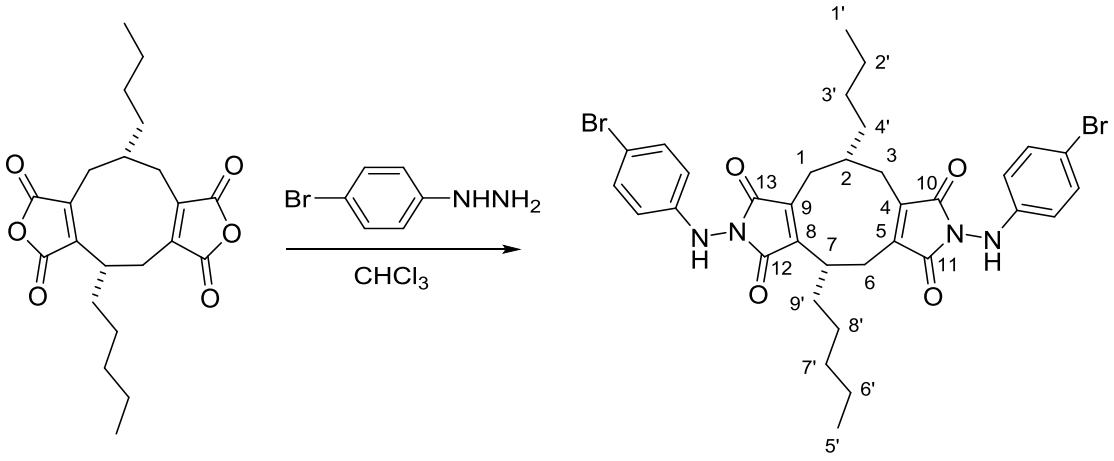


12 **51**

p-Bromophenylhydrazine hydrochloride (110 mg, 0.50 mmol, 4 equiv.) was dissolved in water (5 mL) and sodium hydroxide (1 M, 0.8 mL). After 0.25 h, the reaction mixture was extracted with diethyl ether (3 × 15 mL), and the combined organic layers dried over $MgSO_4$ and concentrated *in vacuo*. The residue was redissolved in chloroform (5 mL) and scytalidin **12** (50 mg, 0.12 mmol) added. The resulting orange solution was stirred at room temperature for 4 h, then the solvent removed *in vacuo*. The crude material was dissolved in ethyl actetate (5 mL), filtered, then washed with water (5 mL). The organic layer was dried over $MgSO_4$, filtered, and then concentrated *in vacuo*. The crude material was purified by column chromatography using methanol/dichloromethane (0-2% methanol) to give bis(maleimide) **51** as an orange oil (12 mg, 13%). $[\alpha]_D^{22}$ −72.0 (*c* 0.25, $CHCl_3$); δ_H (500 MHz, CD_2Cl_2) 7.30 (4H, 2 × d, *J* 9.0, Ar-H), 6.55 (4H, 2 × d, *J* 9.0, Ar-H), 6.01 (1H, s, NH), 5.99 (1H, s, NH), 3.43 (1H, m, 7-H), 3.03-2.71 (5H, m, 1-*H*H, 3-H_2, 6-H_2). 2.57 (1H, d, *J* 13.0, 1-H*H*), 2.27 (1H, s, OH), 1.83-1.70 (2H, m, 3'-H_2 and 4'-H_2), 1.59-1.25 (12H, m, 3'-H*H*, 4'-H*H*, 9'-H_2, 8'-H_2, 7'-H_2, 6'-H_2, 2'-H_2), 0.96 (3H, t, *J* 7.5, 1'-H_3), 0.88 (3H, t, *J* 7.0, 5'-H_3); δ_C (125 MHz, CD_2Cl_2) 172.1 (C=O), 169.8 (C=O), 169.6 (C=O), 169.0 (C=O), 145.7 (Ar), 145.6 (Ar), 141.7 (C=C), 140.1 (C=C), 138.9 (C=C), 138.0 (C=C),

132.7 (Ar), 132.6 (Ar), 115.7 (Ar), 115.6 (Ar), 114.6 (Ar), 114.4 (Ar), 75.9 (C-2), 46.3 (C-4'), 36.5 (C-1), 35.3 (C-3 or C-6), 35.0 (C-7), 31.2 (CH_2), 30.3 (CH_2), 29.2 (C-3 or C-6), 27.8 (CH_2), 26.3 (CH_2), 23.6 (CH_2), 23.0 (CH_2), 14.4 (C-1'), 14.3 (C-5'); ν_{max} (film) 3505, 3323, 2955, 2929, 2858, 1720, 1595, 1488; HRMS (ESI) calc for $C_{34}H_{39}{}^{79}Br_2N_4O_5$ $[M+H]^+$ 741.1287, found 741.1281.

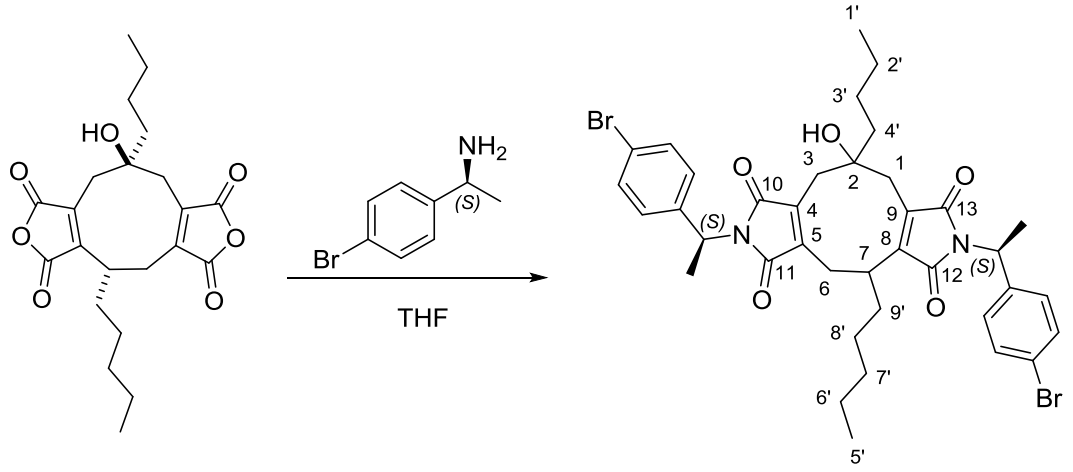
Deoxyscytalidin bis(4-bromophenylhydrazide) maleimide derivative 52



35 **52**

p-Bromophenylhydrazine hydrochloride (138 mg, 0.62 mmol, 4 equiv.) was dissolved in water (10 mL) and sodium hydroxide (1 M, 1 mL). After 0.25 h, the reaction mixture was extracted with diethyl ether (3 × 15 mL), and the combined organic layers dried over $MgSO_4$, then concentrated *in vacuo*. The residue was redissolved in chloroform (5 mL) and deoxyscytalidin **35** (60 mg, 0.16 mmol) added. The resulting orange solution was stirred at room temperature for 4 h, then the solvent removed *in vacuo*. The crude material was dissolved in ethyl actetate (5 mL), filtered, then washed with water (5 mL). The organic layer was dried over $MgSO_4$, filtered, and then concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (5-25% ethyl acetate) to give bis(maleimide) **52** as an orange oil (90 mg, 80%). $[\alpha]_D^{22}$ −60.0 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 7.31-7.25 (4H, m, Ar-H), 6.53-6.49 (4H, m, Ar-H), 6.11 (1H, s, NH), 6.06 (1H, s, NH), 3.43 (1H, br s, 7-H), 2.93-2.81 (2H, m, 6-H_2), 2.77-2.60 (2H, m, 1-*H*H and 3-*H*H), 2.38-2.22 (2H, m, 1-H*H* and 3-H*H*), 1.89 (1H, m, 2-H), 1.81-1.71 and 1.60-1.27 (14H, m, 2'-H_2, 3'-H_2, 4'-H_2, 6'-H_2, 7'-H_2, 8'- H_2, 9'-H_2), 0.96-0.87 (6H, m, 1'-H_3 and 5'-H_3); δ_C (125 MHz, $CDCl_3$) 169.5 (C=O), 169.3 (C=O), 169.2 (C=O), 168.7 (C=O), 145.0 (Ar), 144.9 (Ar), 140.5 (C=C), 139.8 (C=C), 139.7 (C=C), 139.7 (C=C), 132.3 (Ar), 132.2 (Ar), 115.6 (Ar), 115.4 (Ar), 114.5 (Ar), 114.5 (Ar), 40.9 (CH_2), 37.3 (CH_2), 34.6 (C-7), 33.9 (C-2), 31.6 (CH_2), 30.7 (CH_2), 29.2 (CH_2), 28.6 (CH_2), 28.5 (CH_2), 27.6 (CH_2), 22.7 (CH_2), 22.6 (CH_2), 14.1, 14.1 (C-1' and C-5'); ν_{max} (film) 3323, 2928, 1721, 1489, 1241; HRMS (ESI) calc for $C_{34}H_{38}{}^{79}Br_2NaN_4O_4$ $[M+Na]^+$ 747.1152, found 747.1142.

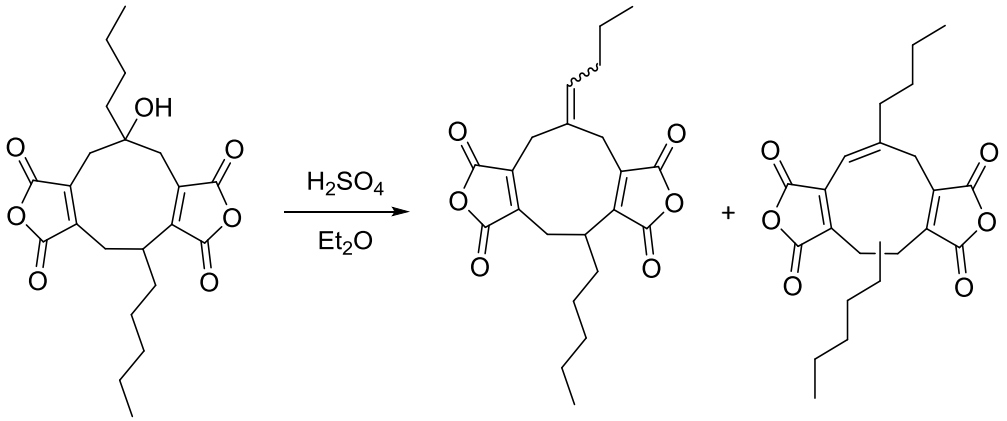
Scytalidin bis(4-bromo-α-methylbenzylamidyl) maleimide derivative 53



12 **53**

Scytalidin **12** (34 mg, 0.08 mmol) was dissolved in dry THF (1 mL) and (*S*)-(−)-4-bromo-α-methylbenzylamine (360 µL, 505 mg, 2.53 mmol, 30 equiv.) added. The resulting solution was heated at reflux for 16 h. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate (5 mL) and 1 M HCl (2 mL) added. The layers were separated, and the aqueous layer further extracted with ethyl acetate (2 × 10 mL). The combined organic layers were dried over $MgSO_4$, filtered, and the concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/hexane (5-20% ethyl acetate) to give bis(maleimide) **53** as a colourless oil (20 mg, 40%). $[\alpha]_D^{22}$ -108.0 (*c* 0.50, $CHCl_3$); δ_H (500 MHz, CD_2Cl_2) 7.38 (4H, 2 × d, *J* 8.5, Ar-H), 7.16 (4H, 2 × d, *J* 8.5, Ar-H), 5.19 (2H, m, 2 × ArC<u>H</u>CH_3), 3.32 (1H, m, 7-H), 2.86-2.57 (5H, m, 1-*H*H, 3-H_2, 6-H_2), 2.43-2.33 (2H, m, 1-H*H*, OH), 1.68 (3H, d, *J* 7.5, C<u>H</u>$_3$CH), 1.66 (3H, d, *J* 7.5, C<u>H</u>$_3$CH), 1.61-1.23 (12H, m, 3'-H*H*, 4'-H*H*, 9'-H_2, 8'-H_2, 7'-H_2, 6'-H_2, 2'-H_2), 0.95 (3H, t, *J* 7.0, 1'-H_3), 0.86 (3H, t, *J* 7.0, 5'-H_3); δ_C (125 MHz, CD_2Cl_2) 174.4 (C=O), 171.6 (C=O), 171.5 (C=O), 171.1 (C=O), 142.7 (C=C), 140.9 (C=C), 140.5 (Ar), 140.3 (Ar), 139.5 (C=C), 138.5 (C=C), 132.1 (Ar), 132.0 (Ar), 129.4 (Ar), 129.1 (Ar), 121.8 (Ar), 121.8 (Ar), 75.9 (C-2), 50.1 (Ar<u>C</u>HCH$_3$), 49.6 (Ar<u>C</u>HCH$_3$), 45.9 (C-4'), 35.9 (C-3 or C-6), 34.9 (C-7), 34.5 (C-1), 32.1 (CH_2), 30.3 (CH_2), 28.7 (C-3 or C-6), 27.7 (CH_2), 26.3 (CH_2), 23.6 (CH_2), 23.0 (CH_2), 18.5 (ArCH<u>C</u>H$_3$), 18.23 (ArCH<u>C</u>H$_3$), 14.4 (C-1'), 14.3 (C-5'); ν_{max} (film) 3525, 2929, 2857, 1700, 1592, 1390, 1358, 1009; HRMS (ESI) calc for $C_{38}H_{45}{}^{79}Br_2N_2O_5$ $[M+H]^+$ 767.1695, found 767.1689.

Elimination of Scytalidin



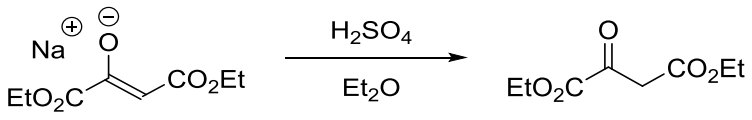
12 **55** **56**

To a solution of scytalidin **12** (100 mg, 0.25 mmol) in chloroform (3 mL) was added concentrated sulfuric acid (3 drops), and the reaction mixture was heated to 60 °C for 1 h in a sealed tube. After cooling to room temperature, the reaction mixture was treated with saturated aqueous $NaHCO_3$ (5 mL), dried over $MgSO_4$, then filtered. The crude material was concentrated *in vacuo*, then purified by column chromatography using dichloromethane/hexane (50-100% dichloromethane) to give a mixture of four alkenes as a colourless oil (35 mg, 39%). A 7:1 ratio of *exocyclic*-**55:** *endocylic*-**56** was observed, with a 2:1 ratio of the *exo*-stereoisomers, and a 1.2:1 ratio of the *endo*-stereoisomers. $[\alpha]_D^{22}$ −11.0 (*c* 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 6.29 – 6.25 (0.08H, s, **56** C=C-H), 6.13 (0.06H, s, **56** C=C-H), 5.89 (0.34H, t, *J* 7.5, **55** C=C-H), 5.83 (0.68H, t, *J* 7.5, **55** C=C-H), 3.46 (0.37H, d, *J* 14.0), 3.41 – 3.21 (3.9H, m), 3.18 – 2.98 (2.7H, m), 2.87 (0.37H, dd, *J* 14.0, 4.5), 2.77 (0.68H, dd, *J* 15.0, 4.0), 2.36 – 2.12 (2.6H, m), 1.81 – 1.66 (2.6H, m), 1.49 – 1.21 (11H, m), 0.96-0.92 (3H, m, CH_3), 0.89-0.86 (3H, m); δ_C (125 MHz, $CDCl_3$) 165.5 (C=O), 165.4 (C=O), 165.3 (C=O), 165.2 (C=O), 165.0 (C=O), 164.9 (C=O), 164.9 (C=O), 164.8 (C=O), 145.6 (C=C), 144.2 (C=C), 143.3 (C=C), 143.3 (C=C), 142.6 (C=C), 142.1 (C=C), 141.8 (C=C), 141.1 (C=C), 137.9 (C=C-H), 137.5 (C=C-H), 125.7 (C=C), 125.2 (C=C), 35.6 (CH), 35.4 (CH), 34.3 (CH_2), 33.4 (CH_2), 32.9 (CH_2), 32.8 (CH_2), 31.5 (CH_2), 31.5 (CH_2), 30.7 (CH_2), 30.6 (CH_2), 27.7 (CH_2), 27.6 (CH_2), 27.5 (CH_2), 27.1 (CH_2), 26.3 (CH_2), 25.8 (CH_2), 22.5 (CH_2), 22.5 (CH_2), 22.4 (CH_2), 22.4 (CH_2), 14.1 (CH_3), 14.0 (CH_3), 13.9 (CH_3), 13.9 (CH_3); ν_{max} (film) 3566, 2957, 2931, 2861, 1828, 1764, 1662, 1253; HRMS (ESI) calc for $C_{22}H_{26}NaO_6$ $[M+Na]^+$ 409.1621, found 409.1623.

NB: ^{13}C NMR data given for **55** only.

7.2.2 Experimental Procedures for Chapter 3

Diethyl oxaloacetate 63

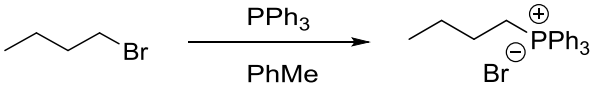


64 **63**

To a solution of diethyloxalacetate sodium salt **64** (10.5 g, 43.7 mmol) in water (100 mL) was added concentrated sulfuric acid (5 mL, 18.7 M). The mixture was stirred for 1 h, then extracted with diethyl ether (3 × 100 mL). The combined organic layers were dried with $MgSO_4$, filtered, and concentrated *in vacuo* to give diethyl oxaloacetate **63** (8.17g, 99 %) as an orange oil. δ_H (400 MHz, $CDCl_3$, NMR shows 1:1 keto:enol forms) 11.59 (0.5 H, br s, OH), 5.97 (0.5H, s, C=C-H), 4.34-4.11 (4H, m, OCH_2), 3.77 (1H, s, CH_2), 1.36-1.19 (6H, m, CH_3); δ_C(100 MHz, $CDCl_3$) 186.7 (C=O), 171.9 (C=O), 166.4 (C=O), 161.8 (C=O), 160.0 (C=O), 159.3 (C=O), 97.0 (C=C-H), 63.0 (OCH_2), 62.4 (OCH_2), 61.8 (OCH_2), 61.3 (OCH_2), 45.3 (CH_2), 14.1 (CH_3), 14.0 (CH_3).

NMR data in accordance with literature.[236]

Butyltriphenylphosphonium bromide S1

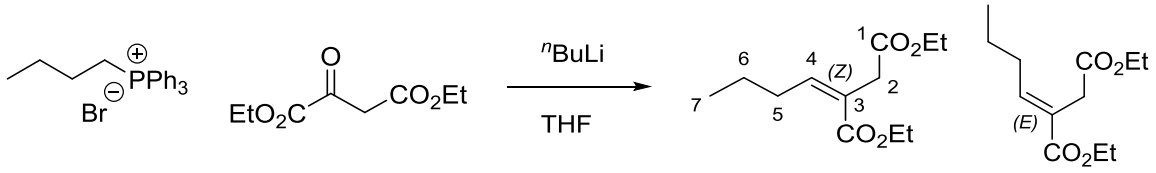


S1

Triphenylphosphine (19.1 g, 72.9 mmol, 1.0 equiv.) and 1-bromobutane (10.0 g, 7.85 mL, 72.9 mmol, 1.0 equiv.) were dissolved in toluene (50 mL) and heated to reflux for 16 h. The reaction mixture was then allowed to cool to room temperature, and the precipitate isolated by filtration, washed with diethyl ether (2 × 50 mL), then concentrated *in vacuo* to give **S1** as a white solid (18.1 g, 62%). δ_H (400 MHz, $CDCl_3$) 7.83-7.73 (9H, m, Ar-H), 7.70-7.64 (6H, m, Ar-H), 3.73-3.65 (2H, m, CH_2), 1.69-1.50 (4H, m, CH_2), 0.86 (3H, t, *J* 7.0, CH_3); δ_C (100 MHz, $CDCl_3$) 135.1 (Ar), 133.7 (d, *J* 10.0, Ar), 130.6 (d, *J* 12.5, Ar), 118.3 (d, *J* 86.0, Ar), 24.7 (d, *J* 4.5, CH_2), 23.8 (d, *J* 16.5, CH_2), 22.7 (d, *J* 49.5, CH_2), 13.8 (CH_3).

NMR data in accordance with literature.[237]

Ethyl (*E*/*Z*)-3-ethoxycarbonylhept-3-enoate 66A

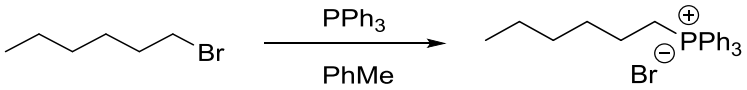


S1 **63** **66A**

Butyltriphenylphosphonium bromide **S1** (18.02 g, 45.1 mmol, 1.1 equiv.) was slurried in dry THF (150 mL) and cooled to 0 °C. *n*-Butyllithium (27.6 mL, 1.47 M, 40.6 mmol, 1.0 equiv.) was added slowly, resulting in a red solution. The reaction mixture was allowed to warm to room temperature over 0.5 h, then cooled to -78 °C. Diethyl oxaloacetate **63** (7.63 g. 40.6 mmol, 1.0 equiv.) in dry THF (10 mL) was added over 0.5 h, resulting in an orange solution, which was allowed to warm to room temperature and stirred for 16 h. The reaction mixture was poured with rapid stirring into hexane (100 mL). This mixture was filtered through Celite®, rinsing with 1:1 hexane: diethyl ether (2 × 25 mL). The filtrate was concentrated *in vacuo*, then the crude material was purified by column chromatography using ethyl acetate/petrol (5-10% ethyl acetate) to give **66A** (1.17 g, 11%) as an inseparable 1:1:0.4 ratio of (*E*-keto:*Z*-keto:*Z*-enol) isomers. δ_H (400 MHz, $CDCl_3$) 11.63 (0.4H, s, *Z*-OH (enol form)), 6.93 (1H, td, *J* 7.5, 4.0, *E*-4-H), 6.05-5.97 (1.4H, m, *Z*-4-H), 5.27(0.4H, m, *Z*-2-H (enol form)), 4.34-4.29 (0.8H, m, *Z*-enol-OCH_2), 4.21-4.28 (0.8H, m, *Z*-enol-OCH_2), 4.20-4.07 (8H, m, OCH_2), 3.31 (2H, s, *E*-2-H_2), 3.22 (2H, s, *Z*-2-H_2), 2.55-2.47 (2H, m, *Z*-5-H_2), 2.18-2.10 (2H, m, *E*-5-H_2), 1.51-1.40 (4H, m, *E*-6-H_2 and *Z*-6-H_2), 1.35-1.17 (15.2H, m, *Z*-enol-6-H_2, $CH_2C\underline{H}_3$), 0.94 (7.2H, m, 7-H_3); δ_C (100 MHz, $CDCl_3$) 172.0, 171.6, 171.0, 167.1, 166.7, 159.4, 147.4, 147.3, 145.4, 125.9, 125.3, 97.0, 96.9, 62.4, 61.4, 60.8, 60.4, 40.5, 32.5, 31.6, 31.0, 22.5, 21.9, 14.2, 13.9, 13.9; HRMS (ESI) calc for $C_{12}H_{20}NaO_4$ $[M+Na]^+$ 251.1254, found 251.1264.

NMR data in accordance with literature.[89]

Hexyltriphenylphosphonium bromide S2

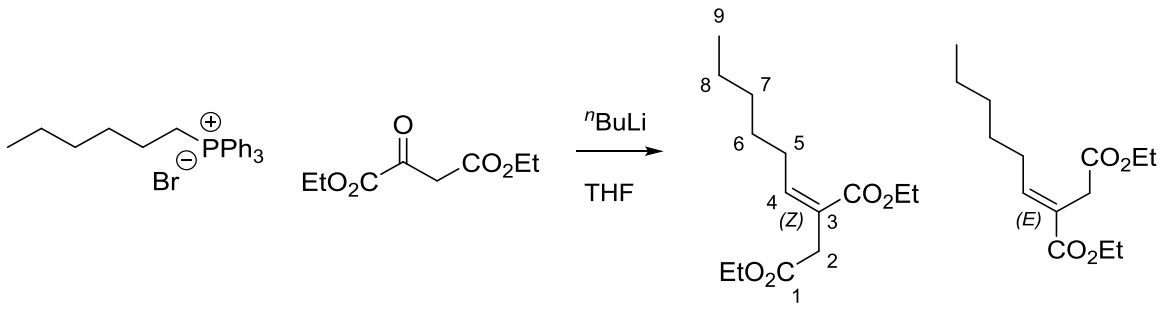


S2

Triphenylphosphine (3.18 g, 12.1 mmol, 1 equiv.) and 1-bromohexane (2.00 g, 1.70 mL, 12.1 mmol, 1 equiv.) were dissolved in toluene (12 mL) and heated to reflux for 16 h. The reaction mixture was then allowed to cool to room temperature, and the precipitate isolated by filtration, washed with diethyl ether (2 × 25 mL) and concentrated *in vacuo* to give **S2** as a white solid (3.52 g, 68%). δ_H (400 MHz, $CDCl_3$) 7.88 – 7.75 (9H, m, Ar-H), 7.72-7.66 (6H, m, Ar-H), 3.80-3.72 (2H, m, CH_2), 1.67-1.55 (4H, m, 2 × CH_2), 1.27-1.16 (4H, m, 2 × CH_2), 0.80 (3H, t, *J* 7.0, CH_3); δ_C(100 MHz, $CDCl_3$) 135.0 (Ar), 133.7 (d, *J* 10.0, Ar), 130.5 (d, *J* 12.5, Ar), 118.4 (d, *J* 85.5, Ar), 31.3 (CH_2), 30.1 (d, *J* 15.5, CH_2), 22.6 (d, *J* 4.5, CH_2), 22.2 (CH_2), 13.9 (CH_3).

NMR data in accordance with literature.[238,239]

Diethyl (*E*/*Z*)-2-hexylidenesuccinate 66B

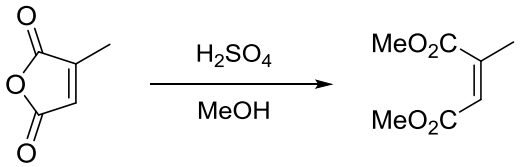


S2 **63** **66B**

Hexyltriphenylphosphonium bromide (3.45 g, 8.07 mmol, 1.1 equiv.) was slurried in dry THF (50 mL) and cooled to 0 °C. *n*-Butyllithium (5.0 mL, 1.47 M, 7.37 mmol, 1.0 equiv.) was added slowly, resulting in a red solution. The reaction mixture was allowed to warm to room temperature over 0.5 h, then cooled to -78 °C. Diethyl oxaloacetate **63** (1.39 g. 7.37 mmol, 1.0 equiv.) in dry THF (5 mL) was added over 0.5 h, resulting in an orange solution, which was allowed to warm to room temperature and stirred for 16 h. The reaction mixture was poured with rapid stirring into hexane (100 mL). This mixture was filtered through Celite®, rinsing with 1:1 hexane: diethyl ether (2 × 25 mL). The filtrate was concentrated *in vacuo*, then the crude material was purified by column chromatography using ethyl acetate/petrol (5-10% ethyl acetate) to give **66B** (112 mg, 5%) as an inseparable 3.55:1 ratio of (*Z:E*) isomers. δ_H (400 MHz, $CDCl_3$) 6.94 (1H, t, *J* 7.5, *E*-4-H), 6.03 (3.55H, t, *J* 7.5, *Z*-4-H), 4.21 – 4.08 (18.2H, m, OCH_2), 3.31 (2H, s, *E*-2-H_2), 3.23 (7.1H, s, *Z*-2-H_2), 2.53 (7.1H, q, *J* 7.5, *Z*-5-H_2), 2.16 (2H, q, *J* 7.5, *E*-5-H_2), 1.47-1.37 (9.1H, m, *E*-6-H_2 and *Z*-6-H_2), 1.33 – 1.18 (45.5H, m, 2 × *E*-$OCH_2C\underline{H}_3$, 2 × *Z*-$OCH_2C\underline{H}_3$, *E*-7-H_2, *Z*-7-H_2, *E*-8-H_2, *Z*-8-H_2), 0.91 – 0.83 (14.7H, m, *E*-9-H_3, *Z*-9-H_3); δ_C (100 MHz, $CDCl_3$) 171.6 (C=O), 170.9 (C=O), 167.0 (C=O), 166.6 (C=O), 147.6 (*Z*-C-4), 145.6 (*E*-C-4), 125.6 (*E*-C-3), 125.0 (*Z*-C-3), 60.7, 60.7, 60.6, 60.3 (4 × OCH_2), 40.4 (*Z*-C-2), 32.4 (*E*-C-2), 31.5, 29.5, 28.9, 28.2, 23.9, 22.5 (6 × CH_2), 14.2, 14.1, 14.1, 14.0, 14.0, 13.9 (6 × CH_3); HRMS (ESI) calc for $C_{14}H_{24}NaO_4$ $[M+Na]^+$ 279.1567, found 279.1577.

NMR data in accordance with literature.[240]

Dimethyl citraconate 71



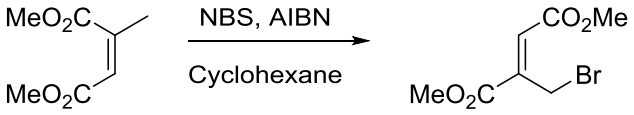
70 **71**

Citraconic anhydride **70** (3.60 mL, 4.50 g, 40.0 mmol) was dissolved in distilled methanol (40 mL) and concentrated sulfuric acid added (1 mL). The colourless solution was heated to 75 °C for 16 h, then allowed to cool to room temperature and concentrated *in vacuo*. The resulting oil was dissolved in

ethyl acetate (50 mL) and washed with water (20 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give diester **71** as a colourless oil (6.14 g, 97%). δ_H (400 MHz, $CDCl_3$) 2.05 (3H, s, CH_3), 3.71 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 5.85 (1H, s, CH); δ_C (100 MHz, $CDCl_3$) 20.6 (CH_3), 52.0 (OCH_3), 52.5 (OCH_3), 120.8 (CH), 145.8 (C), 165.5 (C=O), 169.5 (C=O).

NMR data in accordance with literature.[241]

Dimethyl (*E*)-2-(bromomethyl)maleate 68

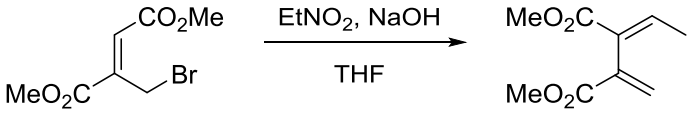


71 **68**

To diester **71** (3.58 g, 22.7 mmol) in acetonitrile (100 mL) was added *N*-bromosuccinimide (5.30 g, 29.8 mmol, 1.3 equiv.) and AIBN (145 mg, 0.88 mmol, 0.03 equiv.). The resulting suspension was heated to reflux at 81 °C for 36 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*, resuspended in toluene (100 mL), filtered, washed with water (2 × 25 mL), dried over $MgSO_4$, filtered, and then concentrated *in vacuo* to an orange oil. The crude material was purified by column chromatography using ethyl acetate/petrol (1-6% ethyl acetate) to give **68** as a colourless oil (3.81 g, 71%). δ_H (400 MHz, $CDCl_3$) 6.77 (1H, s, CH), 4.67 (2H, s, CH_2), 3.83 (3H, s, OCH_3), 3.78 (3H, s, OCH_3); δ_C (100 MHz, $CDCl_3$) 165.2 (C=O), 165.0 (C=O), 142.8 (C=C), 128.3 (C=C), 53.1 (OCH_3), 52.3 (OCH_3), 22.5 (CH_2).

NMR data in accordance with literature.[242]

Dimethyl (*E*)-2-ethylidene-3-methylenesuccinate 72



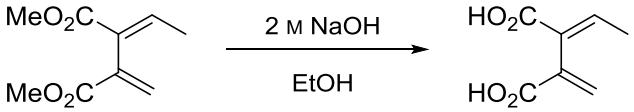
68 **72**

Nitroethane (0.30 mL, 316 mg, 4.22 mmol, 2.0 equiv) and sodium hydroxide (10 mL, 0.6 M) were stirred at room temperature for 2 h, then bromide **68** (495 mg, 2.1 mmol, 1.0 equiv) in THF (5 mL) was added. The resulting solution was stirred at room temperature for 2.5 h, then diluted with water (20 mL) and extracted with diethyl ether (3 × 40 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to a colourless oil. The crude material was purified by column chromatography using ethyl acetate/petrol (5-10% ethyl acetate) to give diester **72** as a colourless oil (62 mg, 16%). δ_H (400 MHz, $CDCl_3$) 7.06 (1H, q, *J* 7.0, C*H*CH_3), 6.48 (1H, s, C*H*H), 5.56 (1H, s, CH*H*), 3.70 (3H, s, OCH_3), 3.68 (3H, s, OCH_3), 1.78 (3H, d, *J* 7.0, CH_3); δ_C (100 MHz, $CDCl_3$) 166.6

(C=O), 166.4 (C=O), 141.3 (<u>C</u>HCH$_3$), 134.9 (C-2), 130.9 (C-3), 129.7 (CH$_2$), 52.2 (OCH$_3$), 52.0 (OCH$_3$), 15.4 (CH$_3$); ν_{max} (film) 2953, 1714, 1649, 1624, 1436, 1247.

NMR data in accordance with literature.[90]

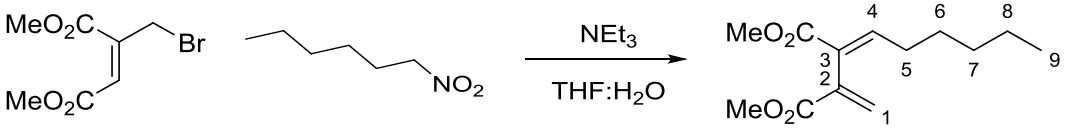
(*E*)-2-Ethylidene-3-methylenesuccinic acid 73



72 **73**

Diester **72** (62 mg, 0.33 mmol) was dissolved in ethanol (5 mL) and sodium hydroxide (5 mL, 2 M) added. The reaction mixture was stirred at room temperature for 16 h, then aqueous sodium sulfate (4%, 25 mL) added and the reaction mixture acidified to pH 1 with hydrochloric acid (6 M). The aqueous mixture was extracted with diethyl ether (4 × 20 mL), and the combined organic layers dried over MgSO$_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol/acetic acid (50% ethyl acetate, 1% acetic acid) to give diacid **73** as a white solid (35 mg, 70%). δ_H (400 MHz, MeOD) 7.07 (1H, q, *J* 7.0, CH), 6.48 (1H, s, C*H*H), 5.64 (1H, s, CH*H*), 1.82 (3H, d, *J* 7.0, CH$_3$); δ_C (100 MHz, MeOD) 169.6 (C-1), 169.3 (C-4), 142.0 (CH), 137.5 (C-2), 133.2 (C-3), 130.0 (CH$_2$), 15.6 (CH$_3$); ν_{max} (film) 2876, 1672, 1619, 1437, 1289; HRMS (ESI) calc for $C_7H_7O_4$ $[M\text{-}H]^-$ 155.0350, found 155.0344.

Dimethyl (*E*)-2-hexylidene-3-methylenesuccinate 153 (Optimisation study)



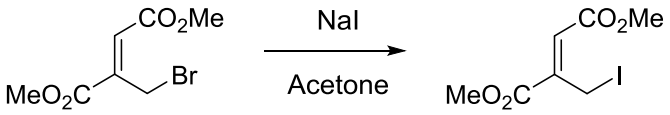
68 **S3** **153**

1-Nitrohexane **S3** (180 mg, 1.37 mmol) and triethylamine (440 mg, 4.3 mmol) were dissolved in a mixture of H$_2$O (7 ml) and THF (7 ml). The solution was stirred for 2 h at room temperature. Allyl bromide **68** (249 mg, 1.05 mmol) dissolved in THF (7 ml) was added over 2 hours at 0 °C. The resultant orange solution was stirred for 16 h at room temperature then diluted with water (10 ml). The aqueous layer was extracted with diethyl ether (3 × 75 ml). The organic extracts were combined and concentrated *in vacuo*. The residue was purified by column chromatography using ethyl acetate/petroleum ether (1-4% ethyl acetate) to give **153** as a colourless oil (152 mg, 60%). δ_H (400 MHz, CDCl$_3$) 6.99 (1H, *t*, J 7.0, 4-H), 6.49 (1H, d, *J* 1.5, 1-*H*H), 5.58 (1H, d, *J* 1.5, 1-H*H*), 3.74 (3H, s, OCH$_3$), 3.72 (3H, s, OCH$_3$), 2.16 (2H, app. q, *J* 7.5, 5-H$_2$), 1.48- 1.38 (2H, m, 6-H$_2$), 1.33-1.23 (4H, m, 7-H$_2$ and 8-H$_2$), 0.87 (3H, m, 9-H$_3$); δ_C (100 MHz, CDCl$_3$) 166.9 (3-C=O), 166.6 (2-C=O), 146.7 (C-4), 135.4, 129.9 (C-2 and C-3), 129.5 (C-1), 52.3 (OCH$_3$), 52.1 (OCH$_3$), 31.6 (C-7), 29.6 (C-5), 28.5 (C-6), 22.5 (C-

8), 14.1 (9-H_3); ν_{max} (film) 2953, 2928, 1719, 1435, 1246, 1199, 1065; HRMS (ESI) calc for $C_{13}H_{21}O_4$ [M+H] 241.1434, found 241.1436.

Reaction optimisation detailed in Table **5**, ^{1}H NMR yields used for optimisation, using ethylene carbonate as the standard.

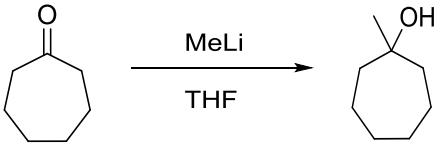
Dimethyl (*E*)-2-(iodomethyl)maleate S4



68 **S4**

To a solution of bromide **68** (497 mg, 2.1 mmol) in acetone (20 mL) was added sodium iodide (470 mg, 3.13 mmol, 1.5 equiv.) in one portion at room temperature. The resulting orange suspension was stirred for 7 h, then concentrated *in vacuo* and dissolved in chloroform (50 mL) and water (50 mL). The organic layer was washed with saturated aqueous $Na_2S_2O_3$ (50 mL) and water (50 mL), then dried over $MgSO_4$, filtered, and concentrated *in vacuo*, to give iodide **S4** (425 mg, 71%) as an orange oil that did not require further purification. δ_H (400 MHz, $CDCl_3$) 6.72 (1H, s, CH), 4.69 (2H, s, CH_2), 3.85 (3H, s, OCH_3), 3.80 (3H, s, OCH_3); δ_C (100 MHz, $CDCl_3$) 165.5 (C=O), 165.3 (C=O), 145.0 (C-2), 126.2 (C-3), 53.1 (OCH_3), 52.3 (OCH_3), 5.4 (CH_2); ν_{max} (film) 2953, 1719, 1636, 1434, 1272, 1214, 1146, 1014, 783; HRMS (ESI) calc for $C_7H_9INaO_4$ $[M+Na]^+$ 306.9438, found 306.9435.

1-Methylcycloheptan-1-ol 81

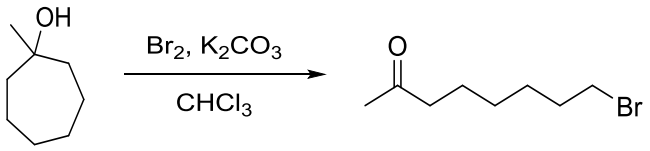


80 **81**

Methyllithium (33 mL, 22 mmol, 1.5 M in hexanes, 2 equiv.) was added to dry THF (75 mL) at -78 °C, followed by cycloheptanone **80** (1.3 mL, 1.24 g, 11.0 mmol). After 2 h, the reaction was quenched by the addition of a concentrated NH_4OH/saturated NH_4Cl solution (20 mL, 90:10). The reaction mixture was extracted with diethyl ether (3 × 50 mL), and the combined organic layers dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (5% ethyl acetate) to give alcohol **81** as a colourless oil (987 mg, 70%). δ_H (400 MHz, $CDCl_3$) 1.70-1.46 (10H, m, 2-H_2, 3-H_2, 4-*H*H), 1.41-1.31 (3H, m, 4-H*H* and OH), 1.20 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 74.4 (C-1), 43.5 (C-2), 31.6 (CH_3), 30.2 (C-3), 23.1 (C-4).

NMR data in accordance with literature.[243]

8-Bromooctan-2-one 82

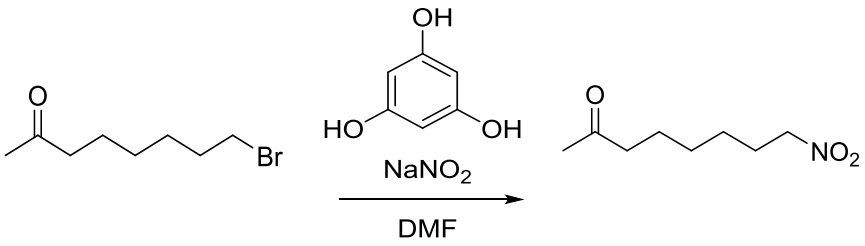


81 **82**

To a solution of alcohol **81** (1.42 g, 11.1 mmol, 1 equiv.) in chloroform (50 mL) at 0 °C was added potassium carbonate (9.19 g, 66.5 mmol, 6 equiv.) in one portion. After 10 min, bromine (2.9 mL, 9.05 g, 56.6 mmol, 5 equiv.) was added over 5 min, and the dark red solution stirred at 0 °C for 5 h. Half-saturated aqueous $Na_2S_2O_3$ (25 mL) was added, and the layers separated. The organic phase was washed with saturated aqueous $Na_2S_2O_3$ (40 mL) and water (40 mL), then dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (5% ethyl acetate) to give ketone **82** as a brown oil (2.00 g, 87%). δ_H (400 MHz, $CDCl_3$) 3.41-3.34 (2H, m, 8-H_2), 2.41 (2H, t, *J* 7.5, 3-H_2), 2.11 (1 H, d, *J* 4.0, 1-H_3), 1.87-1.79 (2H, m, 7-H_2), 1.62-1.51 (2H, m, 4-H_2), 1.47-1.37 (2H, m, 6-H_2), 1.34–1.23 (2H, m, 5-H_2); δ_C (100 MHz, $CDCl_3$) 209.0 (C-2), 43.6 (C-3), 33.9 (C-8), 32.6 (C-7), 30.0 (C-1), 28.3 (C-5), 28.0 (C-6), 23.6 (C-4).

NMR data in accordance with literature.[101]

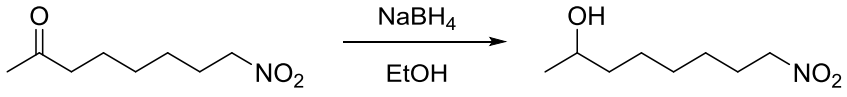
8-Nitrooctan-2-one 83



82 **83**

To a solution of sodium nitrite (682 mg, 9.9 mmol, 2.0 equiv.) and phloroglucinol (671 mg, 5.3 mmol, 1.1 equiv.) in DMF (30 mL) at 0 °C was added bromide **82** (1.002 g, 4.8 mmol) in DMF (5mL) dropwise. After 0.5 h, the reaction was allowed to warm to room temperature and stirred for 16 h. Water was then added (30 mL), and then the reaction mixture extracted with diethyl ether (100 mL) and washed with water (2 × 100 mL). The ethereal layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (10-50% ethyl acetate) to give **83** (321 mg, 38%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 4.32 (2H, t, *J* 7.0, 8-H_2), 2.37 (2H, t, *J* 7.5, 3-H_2), 2.06 (3H, s, 1-H_3), 1.98 – 1.88 (2H, m, 7-H_2), 1.51 (2H, p, *J* 7.5, 4-H_2), 1.37 – 1.22 (4H, m, 5-H_2 and 6-H_2); δ_C (100 MHz, $CDCl_3$) 208.8 (C-2), 75.5 (C-8), 43.3 (C-3), 29.9 (C-1), 28.2 (C-5), 27.1 (C-7), 26.0 (C-6), 23.2 (C-4); ν_{max} (film) 2935, 2862, 1711, 1550, 1371, 910, 731; HRMS (ESI) calc for $C_8H_{15}NNaO_3$ $[M+Na]^+$ 196.0944, found 196.0953.

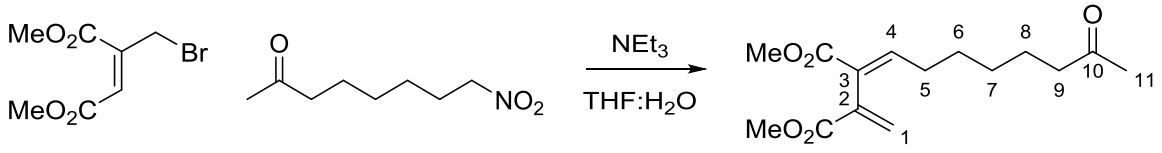
8-Nitrooctan-2-ol 77



83 **77**

To a solution of ketone **83** (100 mg, 0.58 mmol) in ethanol (5 mL) at 0 °C was added sodium borohydride (11 mg, 0.29 mmol, 0.5 equiv.) in a single portion. After 2 h at 0 °C, the reaction was quenched by the addition of saturated aqueous NH_4Cl solution (5 mL), then concentrated *in vacuo*. Water (25 mL) was added to the resulting solid, which was then extracted with ethyl acetate (3 × 25 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (15-30% ethyl acetate) to give alcohol **77** (98 mg, 97%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 4.37 (2H, t, *J* 7.0, 8-H_2), 3.77 (1H, m, 2-H), 1.99 (2H, q, *J* 7.0, 7-H_2), 1.55 (1H, m, OH), 1.46-1.30 (8H, m, 3-H_2, 4-H_2, 5-H_2, 6-H_2), 1.17 (3H, dd, *J* 6.0, 2.5, 1-H_3); δ_C (100 MHz, $CDCl_3$) 75.8 (C-8), 68.1 (C-2), 27.4 (C-7), 39.1, 28.9, 26.3, 25.5 (C-3, C-4, C-5 and C-6), 23.7 (C-1); ν_{max} (film) 3406, 2932, 2860, 2254, 1551, 1381, 907, 731; HRMS (ESI) calc for $C_8H_{17}NNaO_3$ $[M+Na]^+$ 198.1101, found 198.1096.

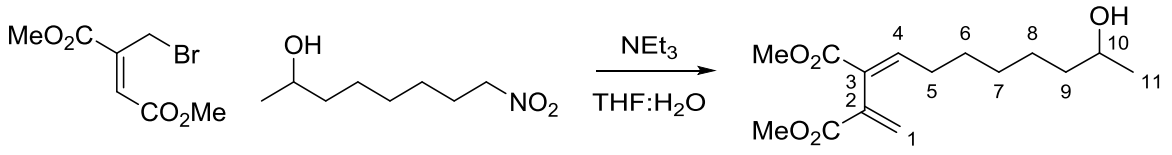
Dimethyl (E)-2-methylene-3-(7-oxooctylidene)succinate 84



68 **83** **84**

Nitroalkane **83** (100 mg, 0.55 mmol, 1.3 equiv.) and triethylamine (0.15 mL, 0.11 g, 1.1 mmol, 2.5 equiv.) were dissolved in THF (3.5 mL) and water (3.5 mL) and stirred for 2 h. Bromide **68** (101 mg, 0.42 mmol) in THF (2 mL) was added at 0 °C, and the reaction mixture stirred for an additional 16 h at room temperature. The orange solution was diluted with water (20 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (5-25% ethyl acetate) to give diester **84** (65 mg, 54%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 6.96 (1H, t, *J* 7.5, 4-H), 6.48 (1H, d, *J* 1.5, 1-*H*H), 5.57 (1H, d, *J* 1.5, 1-H*H*), 3.73 (3H, s, OCH_3), 3.71 (3H, s, OCH_3), 2.40 (2H, t, *J* 7.5, 9-H_2), 2.16 (2 H, q, *J* 7.5, 5-H_2), 2.11 (3H, s, 11-H_3), 1.54 (2H, p, *J* 7.5, 6-H_2, 7-H_2 or 8-H_2), 1.44 (2H, p, *J* 7.5, 6-H_2, 7-H_2 or 8-H_2), 1.33 – 1.23 (2H, m, 6-H_2, 7-H_2 or 8-H_2); δ_C (100 MHz, $CDCl_3$) 209.3 (C-10), 166.8 (3-C=O), 166.5 (2-C=O), 146.3 (C-4), 135.3 (C-2), 130.0 (C-3), 129.7 (C-1), 52.3 (OCH_3), 52.2 (OCH_3), 43.6 (C-9), 30.0 (C-11), 29.4 (C-5), 28.9, 28.5, 23.6 (C-6, C-7 and C-8); ν_{max} (film) 2950, 2858, 1713, 1436, 1249, 732; HRMS (ESI) calc for $C_{15}H_{22}NaO_5$ $[M+Na]^+$ 305.1359, found 305.1353.

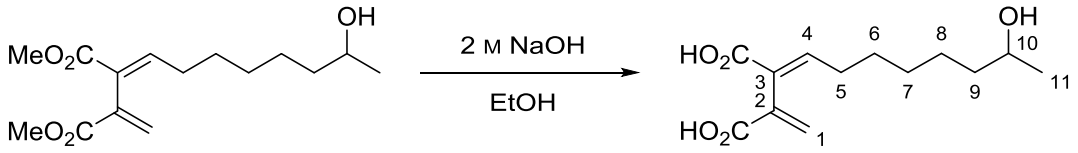
Dimethyl (*E*)-2-(7-hydroxyoctylidene)-3-methylenesuccinate 85



68 **77** **85**

Nitroalkane **77** (92 mg, 0.54 mmol, 1.3 equiv.) and triethylamine (0.20 mL, 0.145 g, 1.43 mmol, 3.4 equiv.) were dissolved in THF (5 mL) and water (5 mL) and stirred for 2 h. Bromide **68** (102 mg, 0.42 mmol) in THF (2 mL) was added at 0 °C, and the reaction mixture stirred for an additional 16 h at room temperature. The orange solution was diluted with water (20 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (10-30% ethyl acetate) to give diester **85** (63 mg, 53%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 6.97 (1H, t, *J* 7.5, 4-H), 6.47 (1H, d, *J* 1.5, 1-*H*H), 5.57 (1H, d, *J* 1.5, 1-H*H*), 3.81-3.62 (7H, m, 2 × OCH_3, 10-H), 2.16 (2H, q, *J* 7.5, 5-H_2), 1.96 (1H, m, OH), 1.49-1.22 (6H, m, 6-H_2, 7-H_2, 8-H_2 and 9-H_2), 1.15 (3H, d, *J* 6.0, 11-H_3); δ_C (100 MHz, $CDCl_3$) 166.8 (3-C=O), 166.6 (2-C=O), 146.6 (C-4), 135.3 (C-2), 129.9 (C-3), 129.6 (C-1), 68.1 (C-10), 52.3 (OCH_3), 52.1 (OCH_3), 39.2, 29.5, 29.3, 28.7, 25.6 (C-5, C-6, C-7, C-8 and C-9), 23.6 (C-11); ν_{max} (film) 3419, 2930, 2858, 1717, 1436, 1256, 1057; HRMS (ESI) calc for $C_{15}H_{24}NaO_5$ $[M+Na]^+$ 307.1516, found 307.1527.

(*E*)-2-(6-Hydroxyheptylidene)-3-methylenesuccinic acid 36 (Tricladic Acid A)

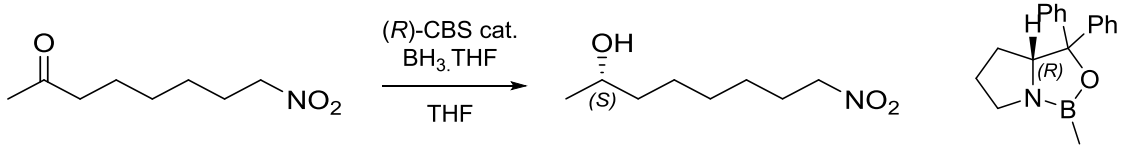


85 **36**

Diester **85** (50 mg, 0.18 mmol) was dissolved in ethanol (10 mL) and added to aqueous sodium hydroxide (10 mL, 2 M) at 0 °C over 20 minutes. The solution was stirred at room temperature for 16 h. Sodium sulfate solution (4%, 25 mL) was added, then the reaction mixture was acidified to pH 1 with aqueous HCl (6 M). The solution was extracted with diethyl ether (4 × 25 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by flash column chromatography using petroleum ether/ethyl acetate/acetic acid (75% ethyl acetate, 1% acetic acid) to give **36** as a viscous oil (25 mg, 55%). δ_H (400 MHz, CH_3OD) 6.96 (1H, t, *J* 7.5, 4- H), 6.43 (1H, d, *J* 1.5, 1-H*H*), 5.59 (1H, d, *J* 1.5, 1-H*H*), 3.70 (1H, m, 10-H), 2.21 (2H, q, *J* 7.5, 5-H_2), 1.50-1.25 (8H, m, 6-H_2, 7-H_2, 8-H_2, 9-H_2), 1.14 (3H, d, *J* 6.0, 11-H_3); δ_C (100 MHz, CH_3OD) 168.6 (3-C=O), 168.3 (2-C=O), 145.3 (C-4), 136.9 (C-2), 131.2 (C-3), 128.0 (C- 1), 67.1 (C-10), 38.6 (C-9), 29.1 (C-5), 29.0 (C-7), 28.3 (C-6), 25.2 (C-8), 22.1 (C-11).

NMR data accordance with literature.[53]

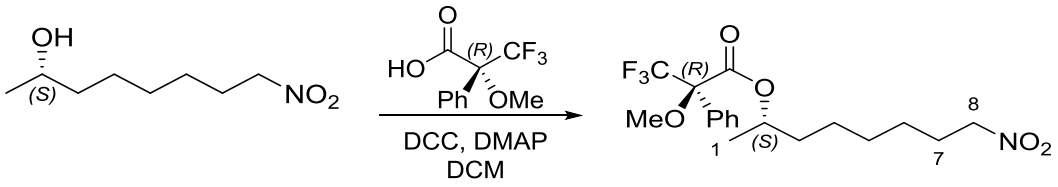
(*S*)-8-Nitrooctan-2-ol 86



83 **86** **(*R*)-CBS oxazaborolidine**

Ketone **83** (100 mg, 0.58 mmol) was dried under vacuum, then dry THF (5 mL) added. The reaction mixture was cooled to 0 °C, and *R*-CBS oxazaborolidine (58 µL, 1 M in toluene, 0.06 mmol, 0.1 equiv.) added, followed by the dropwise addition of BH_3·THF (350 µL, 1 M in THF, 0.035 mmol, 0.6 equiv.). After 0.25 h, the reaction was complete by TLC and quenched by the addition of hydrochloric acid (5 mL, 0.5 M). After stirring for 0.25 h, the reaction mixture was extracted with diethyl ether (3 × 15 mL), and the combined organic layers dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by flash column chromatography using hexane/ethyl acetate (20-30% ethyl acetate) to give **86** as a colourless oil (80 mg, 80%, e.e. 68%). $[\alpha]_D^{22}$ +9.0 (*c* 1.0, $CHCl_3$) Spectroscopic data as previously recorded. Enantiomeric excess determined via Mosher's method (below).[109]

(*S*)-8-Nitrooctan-2-yl (*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate 87



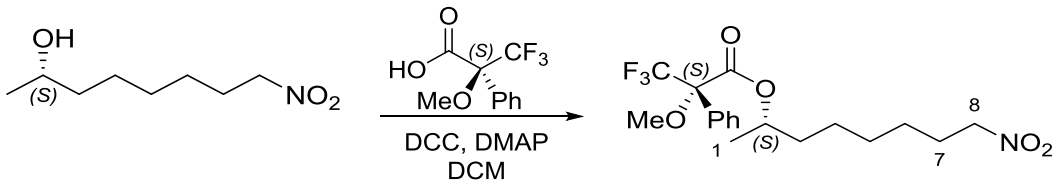
86 **87**

To **86** (9.8 mg, 0.056 mmol) in dry DCM (1 mL) at room temperature was added *R*-MTPA-OH (43 mg, 30 µL, 0.18 mmol, 3.1 equiv.) and *N*,*N*'-dicyclohexylcarbodiimide (42 mg, 0.20 mmol, 3.5 equiv.) and DMAP (22 mg, 0.18 mmol, 3.1 equiv.). The reaction mixture was stirred at room temperature for 16 h, then filtered through a cotton wool plug and concentrated *in vacuo*. The crude material was purified by flash column chromatography using hexane/ethyl acetate (5-15% ethyl acetate) to give **87** as a colourless oil (11 mg, 48%). $[\alpha]_D^{22}$ = +40.4 (*c.* 0.39, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.62-7.49 (2H, m, Ar-H), 7.42-7.38 (3H, m, Ar-H), 5.18-5.09 (1H, m, 2-H), 4.38-4.32 (2H, m, 8-H_2), 3.57 (2.5H, s, OCH_3), 3.54 (0.5H, s, OCH_3), 2.00-1.89 (2H, m, 7-H_2), 1.61 (1H, m, 3-*H*H), 1.52 (1H, m, 3-H*H*), 1.37-1.16 (9H, m, 1-H_3, 4-H_2, 5-H_2, 6-H_2); δ_C (100 MHz, $CDCl_3$)[6] 166.3 (C=O), 132.7 (Ar), 129.7 (Ar), 128.5 (*R*,*R*-Ar),

[6] (*R*,*R*-C…) indicates peaks arising from the minor diastereomer.

128.5 (Ar), 127.5 (*R*,*R*-Ar), 127.3 (Ar), 123.5 (app. d, *J* 288.5, CF_3), 75.7 (C-8), 73.9 (C-2), 55.6 (OCH_3), 35.5 (*R*,*R*-C-3), 35.5 (C-3), 28.7 (*R*,*R*-CH_2), 28.6 (CH_2), 27.4 (*R*,*R*-C-7), 27.3 (C-7), 26.2 (*R*,*R*-CH_2), 26.2 (CH_2), 25.1 (*R*,*R*-CH_2), 24.8 (CH_2), 20.0 (C-1), 19.6 (*R*,*R*-C-1); ν_{max} (film) 2937, 2860, 1742, 1552, 1452, 1382, 1267, 1168; HRMS (ESI) calc for $C_{18}H_{24}F_3NNaO_5$ $[M+Na]^+$ 414.1499, found 414.1503.

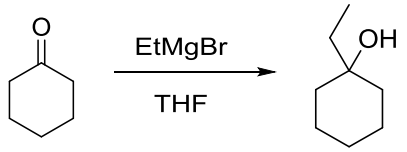
(*S*)-8-Nitrooctan-2-yl (*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate 88



86 **88**

To **86** (10.0 mg, 0.057 mmol) in dry DCM (1 mL) at room temperature was added *S*-MTPA-OH (43 mg, 30 µL, 0.18 mmol, 3.1 equiv.) and *N*,*N*'-dicyclohexylcarbodiimide (42 mg, 0.20 mmol, 3.5 equiv.) and DMAP (22 mg, 0.18 mmol, 3.1 equiv.). The reaction mixture was stirred at room temperature for 16 h, then filtered through a cotton wool plug, and concentrated *in vacuo*. The crude material was purified by flash column chromatography using hexane/ethyl acetate (5% ethyl acetate) to give **88** as a colourless oil (15 mg, 66%). $[\alpha]_D^{22}$ = -38.4 (*c.* 0.47, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.55-7.49 (2H, m, Ar-H), 7.43-7.37 (3H, m, Ar-H), 5.17-5.09 (1H, m, 2-H), 4.39-4.32 (2H, m, 8-H_2), 3.57 (0.5H, s, OCH_3), 3.54 (2.5H, s, OCH_3), 2.02-1.89 (2H, m, 7-H_2), 1.68 (1H, m, 3-*H*H), 1.56 (1H, m, 3-H*H*), 1.37-1.16 (9H, m, 1-H_3, 4-H_2, 5-H_2, 6-H_2); δ_C (100 MHz, $CDCl_3$) 166.3 (C=O), 132.4 (Ar), 129.7 (Ar), 129.7 (*R*,*S*-Ar), 128.5 (Ar), 128.5 (*R*,*S*-Ar), 127.5 (Ar), 127.5 (*R*,*S*-Ar), 123.5 (d, *J* 288.5, CF_3), 75.7 (C-8), 74.0 (C-2), 73.9 (*R*,*S*-C-2), 55.5 (OCH_3), 35.5 (C-3), 35.5 (*R*,*S*-C-3), 28.7 (CH_2), 28.5 (*R*,*S*-CH_2), 27.4 (C-7), 27.3 (*R*,*S*-C-7), 26.2 (CH_2), 26.2 (*R*,*S*-CH_2), 25.1 (CH_2), 24.8 (*R*,*S*-CH_2), 20.0 (*R*,*S*-C-1), 19.6 (C-1); ν_{max} (film) 2935, 2860, 1741, 1552, 1451, 1382, 1267, 1168; HRMS (ESI) calc for $C_{18}H_{24}F_3NNaO_5$ $[M+Na]^+$ 414.1499, found 414.1499.

1-Ethylcyclohexan-1-ol 90



89 **90**

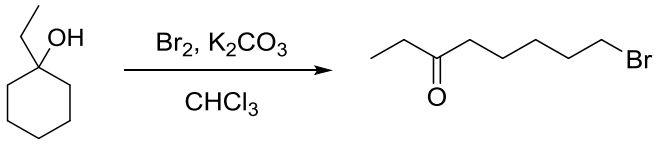
To a solution of cyclohexanone **89** (3.20 mL, 3.03 g, 30.6 mmol) in dry THF (50 mL) at -15 °C was added ethylmagnesium bromide (18 mL, 2 M in diethyl ether, 36.0 mmol, 1.2 equiv.) over 0.25 h. The pale yellow solution was allowed to warm to room temperature over 3 h, then quenched with saturated NH_4Cl solution (10 mL). The reaction mixture was concentrated *in vacuo* then redissolved in ethyl acetate (100 mL) and water (50 mL). The layers were separated and the aqueous layer

additionally extracted with ethyl acetate (2 × 70 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (5-15% ethyl acetate) to give alcohol **90** as a colourless oil (1.26 g, 32%). δ_H (400 MHz, $CDCl_3$) 1.62-1.34 (10H, m, CH_2's), 1.29-1.18 (2H, m, CH_2's), 0.88 (3H, t, *J* 7.5, CH_3); δ_C (100 MHz, $CDCl_3$) 71.6 (C-1), 37.0, 34.9, 26.0, 22.4 (4 × CH_2), 7.3 (CH_3). MP (petrol) 34-35 °C (lit. 34.5-35 °C)[244]

NMR data in accordance with literature.[245]

Cerium chloride heptahydrate (11.5 g, 30.9 mmol, 1.5 equiv.) was ground to a fine powder in a mortar, then dried under vacuum at 145 °C for 2 h. The powder was then allowed to cool to room temperature under N_2, then THF (100 mL) added. The resulting suspension was stirred at room temperature overnight, then cooled to 0 °C and ethylmagnesium bromide (10.2 mL, 3.0 M in diethyl ether, 30.6 mmol, 1.5 equiv.) added. After 1.5 h, cyclohexanone **89** (2.1 mL, 1.99 g, 20.4 mmol, 1.0 equiv.) was added slowly. The reaction mixture was allowed to warm to room temperature over 2 h, then quenched at 0 °C with hydrochloric acid (2 M, 50 mL) and extracted with diethyl ether (3 × 100 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$, then dried over $MgSO_4$, filtered, then concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (5-20% ethyl acetate) to give alcohol **90** as a colourless oil (2.222 g, 85%). Spectroscopic data as previously described.

8-Bromooctan-3-one 91

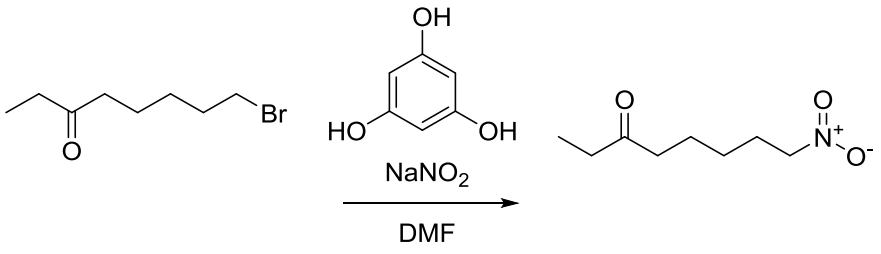


90 **91**

To a solution of alcohol **90** (2.00 mg, 15.6 mmol) in chloroform (75 mL) at 0 °C was added potassium carbonate (13.0 g, 93.8 mmol, 6 equiv.) in one portion. After 10 min, bromine (4.0 mL, 12.5 g, 78.0 mmol, 5 equiv.) was added over 15 min, and the dark red solution stirred at 0 °C for 5 h. Half-saturated aqueous sodium thiosulfate solution (30 mL) was added, and the layers separated. The aqueous layer was extracted with chloroform (2 × 100 mL). The combined organic layers were washed with saturated aqueous $Na_2S_2O_3$ (50 mL) and water (60 mL), then dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (5% ethyl acetate) to give ketone **91** as a colourless oil (2.31 g, 72%). δ_H (400 MHz, $CDCl_3$) 3.39-3.31 (2H, m, 8-H_2), 2-44-2.32 (4H, m, 2-H_2 and 4-H_2), 1.88-1.76 (2H, m, 7-H_2), 1.62-1.49 (2H, m, 5-H_2), 1.46-1.33 (2H, m, 6-H_2), 1.05-0.96 (3H, m, 1-H_3); δ_C (100 MHz, $CDCl_3$) 211.3 (C-3), 42.1 (C-4), 36.0 (C-2), 33.7 (C-8), 32.6 (C-7), 27.8 (C-6), 23.0 (C-5), 7.9 (C-1).

NMR data in accordance with literature.[101]

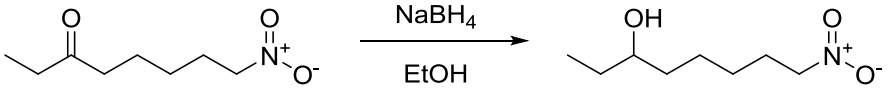
8-Nitrooctan-3-one 92



91 **92**

To a solution of sodium nitrite (2.04 g, 29.6 mmol, 2.0 equiv.) and phloroglucinol (2.02 g, 15.9 mmol, 1.1 equiv.) in DMF (60 mL) and DMSO (60 mL) at 0 °C was added bromide **91** (3.00 g, 14.5 mmol) in DMF (10 mL) dropwise. After 0.5 h, the reaction was allowed to warm to room temperature and stirred for 16 h. Water was then added (100 mL), and then the reaction mixture extracted with diethyl ether (100 mL) and washed with water (2 × 100 mL). The ethereal layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (5-20% ethyl acetate) to give **92** (851 mg, 34%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 4.32 (2H, t, *J* 7.0, 8-H_2), 2.42-2.32 (4H, m, 2-H_2 and 4-H_2), 1.94 (2H, p, *J* 7.0, 7-H_2), 1.55 (2H, p, *J* 7.5, 5-H_2), 1.36-1.26 (2H, m, 6-H_2), 0.97 (3H, t, *J* 7.5, 1-H_3); δ_C (100 MHz, $CDCl_3$) 211.1 (C-3), 75.4 (C-8), 41.6 (C-4), 35.9 (C-2), 27.2 (C-7), 25.8 (C-6), 22.8 (C-5), 7.8 (C-1); ν_{max} (film) 2939, 1710, 1549, 1378, 912, 731; HRMS (ESI) calc for $C_8H_{15}NNaO_3$ $[M+Na]^+$ 196.0944, found 196.0949.

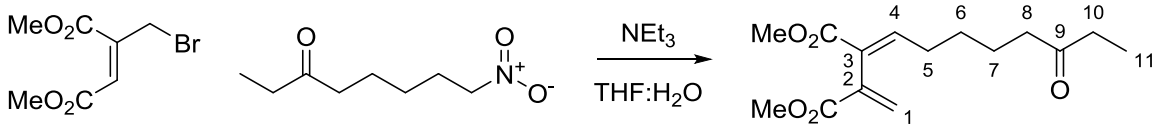
8-Nitrooctan-3-ol 96



92 **96**

To a solution of ketone **92** (250 mg, 1.44 mmol) in ethanol (15 mL) at 0 °C was added sodium borohydride (22 mg, 0.58 mmol, 0.4 equiv.) in a single portion. After 6 h at 0 °C, the reaction was quenched by the addition of saturated aqueous NH_4Cl (10 mL), then concentrated *in vacuo*. Water (25 mL) was added to the resulting solid, which was then extracted with ethyl acetate (3 × 25 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (10% ethyl acetate) to give alcohol **96** (163 mg, 65%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 4.38 (2H, t, *J* 7.0, 8-H_2), 3.57-3.44 (1H, m, 3-H), 2.02 (2H, p, *J* 7.0, 7-H_2), 1.57-1.31 (9H, m, 2-H_2, 4-H_2, 5-H_2, 6-H_2, OH), 0.93 (3H, t, *J* 7.5, 1-H_3); δ_C (100 MHz, $CDCl_3$) 76.1 (C-8), 73.4 (C-3), 27.8 (C-7), 36.9, 30.7, 26.7, 25.4 (C-2, C-4, C-5, C-6), 10.3 (C-1); ν_{max} (film) 3369, 2930, 2861, 1549, 1382, 968; HRMS (ESI) calc for $C_8H_{17}NNaO_3$ $[M+Na]^+$ 198.1101, found 198.1101.

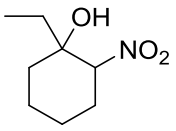
Dimethyl (E)-2-methylene-3-(6-oxooctylidene)succinate 95



68 **92** **95**

Nitroalkane **92** (256 mg, 1.44 mmol, 1.3 equiv.) and triethylamine (0.50 mL, 0.35 g, 3.4 mmol, 2.6 equiv.) were dissolved in THF (10 mL) and water (10 mL) and stirred for 2 h. Bromide **68** (262 mg, 1.10 mmol, 1 equiv.) in THF (4 mL) was added at 0 °C, and the reaction mixture stirred for an additional 16 h at room temperature. The orange solution was diluted with water (20 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (10% ethyl acetate) to give diester **95** (116 mg, 37%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 6.96 (1H, t, *J* 7.5, 4-H), 6.48 (1H, d, *J* 1.5, 1-*H*H), 5.57 (1H, d, *J* 1.5, 1-H*H*), 3.73 (3H, s, OCH_3), 3.71 (3H, s, OCH_3), 2.43 – 2.35 (4H, m, 8-H_2 and 10-H_2), 2.17 (2H, q, *J* 7.5, 5-H_2), 1.61-1.52 (2H, m, 7-H_2), 1.47 – 1.37 (2H, m, 6-H_2), 1.03 (3H, t, *J* 7.5, 11-H_3); δ_C (100 MHz, $CDCl_3$) 211.3 (C-9), 166.7 (3-C=O), 166.5 (2-C=O), 145.9 (C-4), 135.3 (C-2), 130.2 (C-3), 129.7 (C-1), 52.3 (OCH_3), 52.2 (OCH_3), 42.0 (C-8), 36.1 (C-10), 29.4 (C-5), 28.3 (C-6), 23.6 (C-7), 7.9 (C-11); ν_{max} (film) 2952, 1713, 1437, 1255, 907, 729; HRMS (ESI) calc for $C_{15}H_{23}O_5$ $[M+H]^+$ 283.1540, found 283.1548.

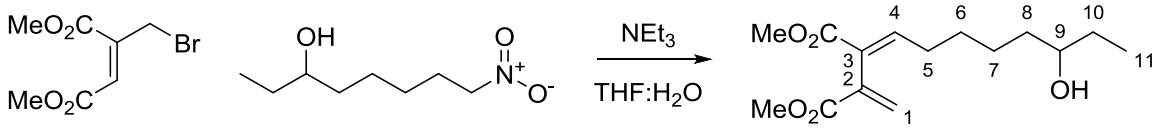
Also isolated were two cyclic nitroalkanols **94**:



Nitroalkanol A - δ_H (400 MHz, $CDCl_3$) 4.48 (1H, dd, *J* 12.5, 4.0, $CHNO_2$), 2.90 (1H, d, *J* 2.5, OH), 2.34 (1H, qd, *J* 12.5, 4.0, C*H*H), 2.02 (1H, dd, *J* 12.5, 3.5, C*H*H), 1.86 (2H, dd, *J* 12.5, 3.5, C*H*H), 1.79-1.68 (1H, m, C*H*H), 1.59-1.45 (3H, m 3 × C*H*H), 1.36-1.19 (2H, m, 2 × C*H*H), 0.94 (3H, t, *J* 7.5, CH_3); δ_C (100 MHz, $CDCl_3$) 91.3 ($CHNO_2$), 71.9, 33.7, 32.8, 27.8, 24.3, 20.0 (6 × CH_2), 7.7 (CH_3); ν_{max} (film) 3557, 2945, 1544, 905, 729; HRMS (ESI) calc for $C_8H_{15}NNaO_3$ $[M+Na]^+$ 196.0944, found 196.0944

Nitroalkanol B - δ_H (400 MHz, $CDCl_3$) 4.47 (1H, dd, *J* 11.0, 4.5, $CHNO_2$), 2.87 (1H, s, OH), 2.22 – 2.06 (2H, m, 2 × C*H*H), 2.02-1.83 (2H, m, 2 × C*H*H), 1.79-1.56 (2H, m, 2 × C*H*H), 1.44-1.18 (4H, m, 4 × C*H*H), 0.93 (3H, t, *J* 7.5, CH_3); δ_C (100 MHz, $CDCl_3$) 92.9 ($CHNO_2$), 73.8, 34.2, 27.0, 24.9, 23.6, 21.8 (6 × CH_2), 6.5 (CH_3); ν_{max} (film) 3524, 2939, 2867, 1542, 1371; HRMS (ESI) calc for $C_8H_{15}NNaO_3$ $[M+Na]^+$ 196.0944, found 196.0939

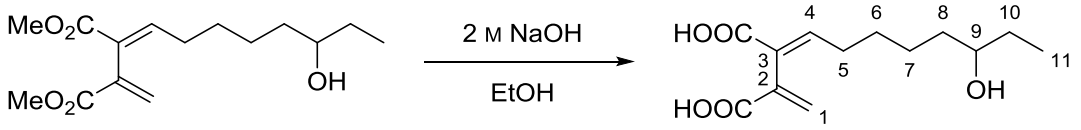
Dimethyl (*E*)-2-(6-hydroxyoctylidene)-3-methylenesuccinate 97



68 **96** **97**

Nitroalkane **96** (152 mg, 0.87 mmol, 1.3 equiv.) and triethylamine (0.33 mL, 0.24 g, 3.40 mmol, 3.5 equiv.) were dissolved in THF (7.5 mL) and water (7.5 mL) and stirred for 2 h. Bromide **68** (161 mg, 0.67 mmol, 1 equiv.) in THF (2 mL) was added at 0 °C, and the reaction mixture stirred for an additional 16 h at room temperature. The orange solution was diluted with water (20 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (10-30% ethyl acetate) to give diester **97** (112 mg, 59%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 6.99 (1H, t, *J* 7.5, 4-H), 6.49 (1H, d, *J* 1.5, 1-*H*H), 5.59 (1H, d, *J* 1.5, 1-H*H*), 3.74 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 3.50 (1H, m, 9-H), 2.19 (2H, q, *J* 7.0, 5-H_2), 1.55-1.30 (9H, m, 6-H_2, 7-H_2, 8-H_2, 10-H_2 and OH), 0.92 (3H, t, *J* 7.5, 11-H_3); δ_C (100 MHz, $CDCl_3$) 166.8 (C=O), 166.6 (C=O), 146.4 (C-4), 135.3 (C-2), 130.0 (C-3), 129.6 (C-1), 73.2 (C-9), 52.3 (OCH_3), 52.2 (OCH_3), 36.7 (C-8), 30.4 (C-10), 29.6 (C-5), 28.8 (C-6), 25.5 (C-7), 10.0 (C-11); ν_{max} (film) 3427, 2932, 2858, 1715, 1436, 1248; HRMS (ESI) calc for $C_{15}H_{25}O_5$ $[M+H]^+$ 285.1697, found 285.1704.

(±)-(*E*)-2-(6-Hydroxyoctylidene)-3-methylenesuccinic acid 37 (Tricladic acid B)

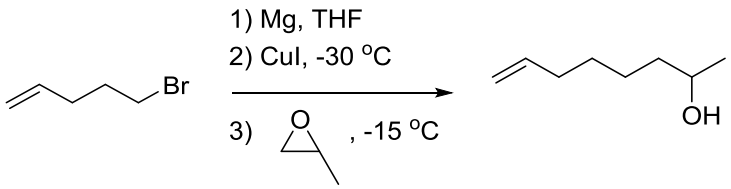


97 **37**

Diester **97** (66 mg, 0.23 mmol) was dissolved in ethanol (3 mL) and sodium hydroxide (5 mL, 2 M) added. The reaction mixture was stirred at room temperature for 16 h, then aqueous sodium sulfate (4%, 25 mL) added and the reaction mixture acidified to pH 1 with hydrochloric acid (6 M). The aqueous mixture was extracted with diethyl ether (4 × 20 mL), and the combined organic layers dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol/acetic acid (75% ethyl acetate, 1% acetic acid) to give diacid **37** (32 mg, 71%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 6.97 (1H, t, *J* 7.5, 4-H), 6.45 (1H, d, *J* 2.0, 1-*H*H), 5.62 (1H, d, *J* 2.0, 1-H*H*), 3.54 (1H, m, 9-H), 2.22 (2H, q, *J* 7.0, 5-H_2), 1.56-1.33 (8H, m, 6-H_2, 7-H_2, 8-H_2, 10-H_2), 0.93 (3H, t, *J* 7.5, 11-H_3); δ_C (100 MHz, $CDCl_3$) 169.7 (3-C=O), 169.4 (2-C=O), 146.9 (C-4), 137.9 (C-2), 132.3 (C-3), 129.7 (C-1), 73.7 (C-9), 37.6 (C-8), 31.0 (C-10), 30.5 (C-5), 29.8 (C-6), 26.5 (C-7), 10.4 (C-11); ν_{max} (film) 3381, 2932, 2483, 2076, 1688, 1455, 1117, 970; HRMS (ESI) calc for $C_{13}H_{20}NaO_5$ $[M+Na]^+$ 279.1203, found 279.1206.

NMR data in accordance with literature.[53]

(±)-2-Hydroxyoct-7-ene 99(*)

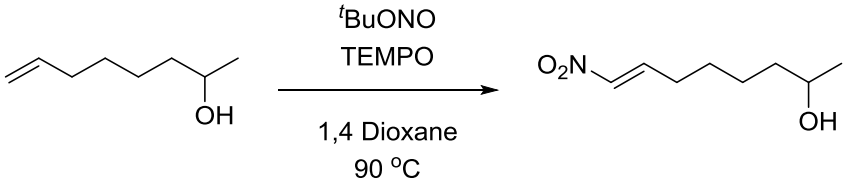


98 **99**

To a suspension of magnesium (0.20 g, 8.20 mmol, 1.25 equiv.) in dry THF (10 mL) under a nitrogen atmosphere, 5-bromo-pent-1-ene (0.16 mL, 0.20 g, 1.3 mmol) was added. The solution was warmed to initiate the reaction. Once initiated, the remaining 5-bromo-pent-1-ene (0.64 mL, 0.80 g, 5.4 mmol) in dry THF (20 mL) was added dropwise over 0.4 h. The reaction mixture was stirred for 1 h. The reaction mixture was cooled to −30 ˚C and CuI (1.30 g, 6.7 mmol) was added. The resultant purple solution was stirred for 5 minutes at −30 ˚C. (±)-Propylene oxide (0.30 mL, 0.25 g, 4.30 mmol) was added. The reaction was warmed to -15 ˚C and stirred for a further 2 h. The reaction mixture was quenched with saturated aqueous NH_4Cl (10 mL). After warming to room temperature, diethyl ether was added (10 mL) and the layers were separated. The organic layer was washed sequentially with a saturated solution of aqueous $NaHCO_3$ (10 mL) and then brine (10 mL). The combined aqueous layers were extracted with diethyl ether (3 × 50 mL). The organic extracts were combined, dried over MgSO4, filtered, then concentrated *in vacuo*. The crude product was purified by column chromatography using petroleum ether/diethyl ether (15% diethyl ether) to give **99** as a colourless oil (303 mg, 55%). δ_H (400 MHz, $CDCl_3$) 5.87 (1H, ddt, *J* 17.0, 10.0, 6.5, 7-H), 4.92 (1H, dq, *J* 17.0, 1.5, 8-H*H*), 4.87 (1H, dtd, *J* 10.0, 2.5, 1.5, 8-H*H*), 3.73 (1H, m, 2-H), 2.00 (2H, q, *J* 6.5, 6-H) 1.50-1.20 (6H, m, 3-H_2, 4-H_2, 5-H_2) 1.12 (3H, d, *J* 6.0, 1-H_3); δ_C (100 MHz, $CDCl_3$) 138.9 (C-7), 114.4 (C-8), 68.1 (C-2), 39.2 (C-3), 33.7 (C-6), 28.9, 25.2 (C-4 and C- 5), 23.5 (C-1).

NMR data in accordance with literature.[132,246]

(±)-(*E*)-2-Hydroxy-8-nitrooct-7-ene 100 (*)

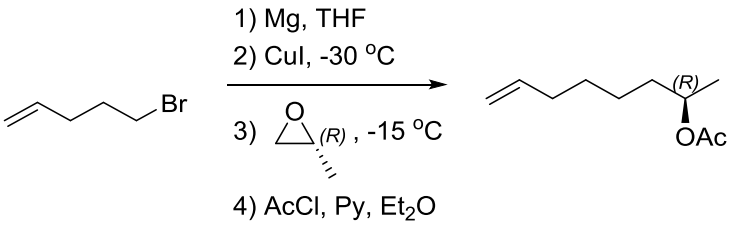


99 **100**

To a screw capped test tube charged with a magnetic stirrer bar, TEMPO, (102 mg, 0.65 mmol), *tert*-butyl nitrite (0.38 mL, 0.33 g, 3.20 mmol) and alcohol **99** (202 mg, 1.57 mmol) were dissolved in 1,4-dioxane (3 mL). The solution was heated for 8 h at 90 °C. The resultant orange solution was diluted

with diethyl ether (5 mL), washed with saturated aqueous $Na_2S_2O_3$ (3 × 5 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography using petroleum ether/ethyl acetate (15 % ethyl acetate) to give **100** (30 mg, 11%); δ_H (400 MHz, $CDCl_3$) 7.27 (1H, dt, *J* 13.5, 7.5, 7-H), 6.98 (1H, dt, *J* 13.5, 1.5, 8-H), 3.80 (1H, m, 2-H), 2.29 (2H, qd, *J* 7.5, 1.5, 6-H_2), 1.60-1.35 (6H, m, 3-H_2, 4-H_2, 5-H_2, 6-H_2), 1.20 (3H, d, *J* 6.5, CH_3). δ_C (100 MHz, $CDCl_3$) 142.4 (C-7), 139.7 (C-8), 67.8 (C-2), 38.8 (C-3), 28.4 (C-6), 27.8, 25.3 (C-4 and C-5), 23.7 (C-1); ν_{max} (film) 3366, 2930, 1645, 1521, 1351; HRMS (ESI) calc for $C_8H_{15}NO_3Na$ $[M+Na]^+$ 196.0949, found 196.0943.

(*R*)-2-Acetoxyoct-7-ene 101 (*)

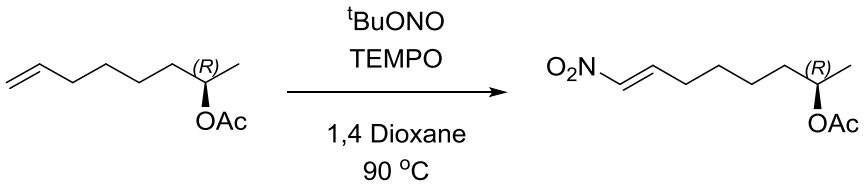


98 **101**

5-Bromo-1-pentene **98** (4.6 mL, 5.80 g, 39 mmol) was dissolved in dry THF (30 mL). A small portion of which was added to a suspension of magnesium turnings (1.80 g, 75 mmol) in THF (45 mL) under a nitrogen atmosphere. The solution was sonicated for 5 minutes to initiate the Grignard reaction. Once initiated, the remaining 5-bromo-1-pentene was added dropwise over 20 minutes. The reaction mixture was stirred for 1 h. The reaction mixture was cooled to −30 ˚C before CuI (150 mg, 0.78 mmol) was added. (*R*)-Propylene oxide (1.05 mL, 0.87 g, 15.0 mmol) was added and the reaction was warmed to −15 ˚C. The reaction was stirred for a further 2 h and then quenched with saturated aqueous NH_4Cl (10 mL). After warming to room temperature, diethyl ether was added (30 mL) and the layers were separated. The organic layer was washed sequentially with water (30 mL) and brine (30 mL). The combined aqueous layers were extracted with diethyl ether (3 × 75 mL). The organic extracts were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The crude residue and acetyl chloride (2.10 ml, 2.30 g, 30.0 mmol) were dissolved in diethyl ether (45 mL). Pyridine (2.4 mL, 2.4 g, 30 mmol) was added to the reaction mixture resulting in the formation of a white suspension. The solution was heated to reflux at 35 °C until TLC analysis (10% ethyl acetate, hexane) indicated that the starting material was consumed (1 h). After cooling the reaction mixture to room temperature, aqueous HCl (1 M, 40 mL) was added. The aqueous layer was separated and extracted with diethyl ether (3 × 75 mL). The organic extracts were combined, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography using petroleum ether/ethyl acetate (3% ethyl acetate) to give **101** as a colourless oil (2.10 g, 82%); $[\alpha]_D^{22}$ = +3.0 (*c* 1.00, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 5.73 (1H, ddt, *J* 17.0, 10.0, 6.5, 7-H), 4.93 (1H, dq, *J* 17.0, 2.0, 8-H*H*), 4.87 (1H,

ddt, *J* 10.0, 2.0, 1.5, 8-H*H*), 4.62 (1H, sext, *J* 6.0, 2-H), 1.98 (2H, m, 6-H_2), 1.96 (3H, s, 10-H_3), 1.54-1.21 (6H, m, 3-H_2, 4-H_2, 5-H_2), 1.13 (3H, d, *J* 6.0, 1-H_3); δ_C (100 MHz, $CDCl_3$) 170.8 (C-9), 138.7 (C-7), 114.4 (C-8), 71.0 (C-2), 35.7 (C-3), 33.6 (C-6), 28.7 (C-5), 24.8 (C-4), 21.4 (C-10), 20.0 (C-1); ν_{max} (film) 3074, 2977, 2932, 1735, 1640, 1240; HRMS (ESI) calc for $C_{10}H_{18}O_2Na$ $[M+Na]^+$ 193.1199, found 193.1194.

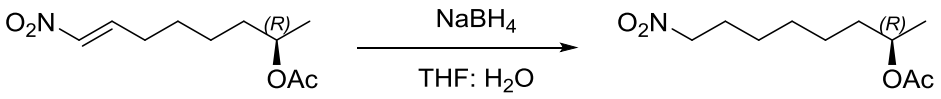
(*R,E*)-2-Acetoxy-8-nitrooct-7-ene 102 (*)



101 **102**

Acetylated alcohol **101** (1.20 g, 7.00 mmol), *tert*-butyl nitrite (1.80 mL, 1.60 g, 15.0 mmol) and TEMPO (268 mg, 1.72 mmol) were dissolved in 1,4-dioxane (18 mL) and heated to reflux in air at 90 °C for 16 h. The orange solution was washed through a Celite® pad, concentrated *in vacuo* and purified by column chromatography using petroleum ether/diethyl ether (14-25% diethyl ether) to give nitroalkene **102** as an orange oil (1.16 g, 77%). $[\alpha]_D^{22}$ = +14 (*c.* 1.00, $CHCl_3$); δ_H (400 MHz, CD_2Cl_2) 7.18 (1H, dt, *J* 13.0, 7.0, 7-H), 6.91 (1H, dt, *J* 13.0, 1.5, 8-H), 4.78 (1H, sext, *J* 6.5, 2-H), 2.19 (2H, qd, *J* 7.0, 1.5, 6-H_2), 1.91 (3H, s, 10-H_3), 1.55-1.25 (6H, m, 3-H_2, 4-H_2, 5-H_2), 1.10 (3H, d, *J* 6.0, 1-H_3); δ_C (100 MHz, CD_2Cl_2) 170.4 (C-9), 142.6 (C-7), 139.6 (C-8), 70.4 (C-2), 35.4 (C-3), 28.3 (C-6), 27.5 (C-5), 24.8 (C-4), 21.1 (C-10), 19.7 (C-1); ν_{max} (film) 2977, 2936, 1729, 1648, 1522, 1351, 1242; HRMS (ESI) calc for $C_{10}H_{17}NO_4Na$ $[M+Na]^+$ 238.1055, found 238.1056.

(*R*)-2-Acetoxy-8-nitrooctane 103 (*)

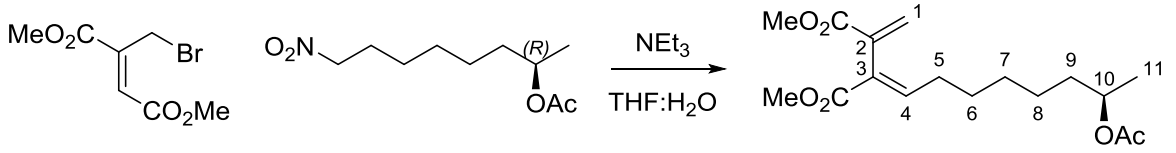


102 **103**

To a solution of nitroalkene, **102** (400 mg, 1.86 mmol) in THF:methanol (10:1, 18 mL) at 0 °C under a nitrogen atmosphere, $NaBH_4$ (140 mg, 3.72 mmol) was added in five portions. The reaction was stirred for 1 h at 0 °C. the resultant orange solution was quenched with saturated aqueous $NaHCO_3$ (5 mL), diluted with water (50 mL) and extracted with ethyl acetate (4 × 50 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography using hexane/ethyl acetate (12% ethyl acetate) to give nitroalkane **103** as a colourless oil (284 mg, 70%). $[\alpha]_D^{22}$ = +21 (*c* 0.66, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 4.88 (1H, sext, *J* 6.5, 2-H), 4.37 (2H, t, *J* 7.5, 8-H_2), 2.02 (3H, s, 10-H_3), 2.00 (2H, quin, *J*

7.5, 7-H_2), 1.60-1.27 (8H, m, 3-H_2, 4-H_2, 5-H_2, 6-H_2), 1.20 (3H, d, *J* 6.5, 1-H_3); δ_C (100 MHz, $CDCl_3$) 170.8 (C-9), 75.6 (C-8), 70.8 (C-2), 35.7 (C-3), 28.6 (C-5), 27.3 (C-7), 26.1 (C-6), 25.0 (C-4), 21.4 (C-10), 20.0 (C-1); ν_{max} (film) 2975, 2860, 2933, 1731, 1552, 1372, 1244; HRMS (ESI) calc for $C_{10}H_{19}NO_4Na$ $[M+Na]^+$ 240.1212, found 240.1209.

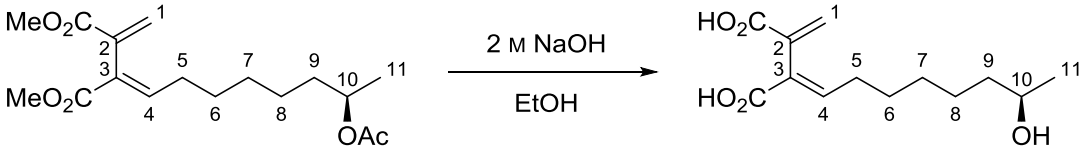
Dimethyl (*R*,*E*)-2-(6-acetoxyheptylidene)-3-methylenesuccinate 104 (*)



68 **103** **104**

Nitroalkane **103** (200 mg, 0.92 mmol) and triethylamine (0.58 mL, 0.42 g, 4.10 mmol) were dissolved in THF (8 mL) and water (8 mL). The solution was stirred for 2 h at room temperature. Allyl bromide **68** (175 mg, 0.74 mmol) in THF (2 mL) was added over 2 h at 0 °C with a syringe pump and stirred for an additional 16 h at room temperature. The yellow solution was diluted with water (30 mL) and extracted with diethyl ether (4 × 75 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude material was purified by column chromatography using petroleum ether/ethyl acetate (9-17% ethyl acetate) to give triester **104** as a colourless oil (150 mg, 65%). $[\alpha]_D^{22}$ = +16 (*c* 0.66, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 6.97 (1H, t, *J* 7.5, 4-H), 6.49 (1H, d, *J* 1.5, 1-H*H*), 5.57 (1H, d, *J* 1.5, 1-H*H*), 4.85 (1H, sext, *J* 6.5, 10-H), 3.74 (3H, s, OCH3), 3.71 (3H, s, OCH_3), 2.16 (2H, q, *J* 7.5, 5-H_2), 2.01 (3H, s, 13-H_3), 1.59-1.38 (4H, m, 7-H_2, 9-H_2), 1.34-1.25 (4H, m, 6-H_2, 8-H_2), 1.18 (3H, d, *J* 6.5, 11-H_3); δ_C (100 MHz, $CDCl_3$) 170.7 (C-12), 166.7 (3-C=O), 166.4 (2-C=O), 146.2 (C-4), 135.2 (C-2), 129.9 (C-3), 129.4 (C-1), 70.9 (C-10), 52.2 (2-OCH_3), 52.0 (3-OCH_3), 35.8 (C-9), 29.4 (C-5), 29.1 (C-6), 28.5 (C-7), 25.1 (C-8), 21.4 (C-13), 19.9 (C-11); ν_{max} (film) 2934, 2859, 1721, 1623, 1242, 1199; HRMS (ESI) calc for $C_{17}H_{26}O_6Na$ $[M+Na]^+$ 349.1627, found 349.1625.

(*R*,*E*)-2-(6-Hydroxyheptylidene)-3-methylenesuccinic acid (*R*)-36 ((*R*)-Tricladic acid A) (*)



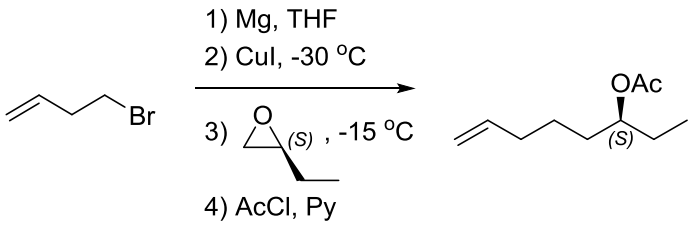
104 **(*R*)-36**

Triester **104** (140 mg, 0.45 mmol) was dissolved in ethanol (5 mL) and added to aqueous sodium hydroxide (10 mL, 2 M) at 0 °C over 20 minutes. The solution was stirred at room temperature for 16 h. The reaction mixture was acidified to pH 1 with aqueous HCl (6 M). The solution was extracted with diethyl ether (4 × 50 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude material was purified by flash column chromatography using petroleum ether/ethyl acetate/acetic acid (70% ethyl acetate, 0.8% acetic

acid) to give **(*R*)-36** as a viscous oil (115 mg, 56%). $[\alpha]_D^{22}$ = −5 (*c.* 0.91, MeOH) [lit. $[\alpha]_D^{20}$ −0.4 (*c* 0.35, MeOH)][53]; δ_H (400 MHz, CH_3OD) 6.96 (1H, t, *J* 7.5, 4- H), 6.43 (1H, d, *J* 1.5, 1-H*H*), 5.59 (1H, d, *J* 1.5, 1-H*H*), 3.70 (1H, m, 10-H), 2.21 (2H, q, *J* 7.5, 5-H_2), 1.50-1.25 (8H, m, 6-H_2, 7-H_2, 8-H_2, 9-H_2), 1.14 (3H, d, *J* 6.0, 11-H_3); δ_C (100 MHz, CH_3OD) 168.6 (3-C=O), 168.3 (2-C=O), 145.3 (C-4), 136.9 (C-2), 131.2 (C-3), 128.0 (C-1), 67.1 (C-10), 38.6 (C-9), 29.1 (C-5), 29.0 (C-7), 28.3 (C-6), 25.2 (C-8), 22.1 (C-11); ν_{max} (film) 3385, 2931, 2858, 1693, 1621, 1279, 1130; HRMS (ESI) calc for $C_{13}H_{20}O_5Na$ $[M+Na]^+$ 279.1208, found 279.1208.

^1H and ^{13}C spectra obtained after further purification by preparative HPLC, and is in accordance with literature.[53]

(*S*)-3-Acetoxyoct-7-ene 106 (*)



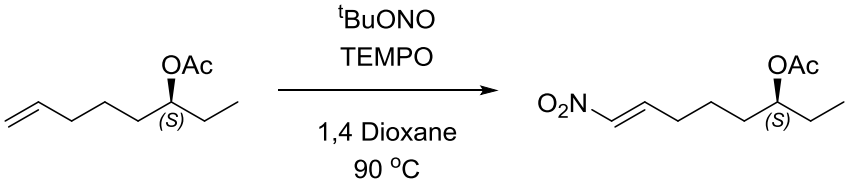
105 **106**

4-Bromo-1-butene **105** (2.64 ml, 3.51 g, 26.0 mmol) was dissolved in dry THF (20 mL). A small portion of which was added to a suspension of magnesium (1.21 g, 50.2 mmol) in THF (40 mL). The solution was sonicated for 5 minutes to initiate the reaction. Once initiated, the remaining 4-bromo-1-butene was added dropwise over 20 minutes. The reaction mixture was stirred for 1 h and then cooled to −30 ˚C before CuI (150 mg, 0.78 mmol) was added. (*S*)-1,2-Epoxybutane (1.30 mL, 1.10 g, 15.0 mmol) was added resulting in a purple solution. The reaction was warmed to -15 ˚C. The reaction was stirred for a further 2 h and then quenched with saturated aqueous NH_4Cl (10 mL). After warming to room temperature, diethyl ether was added (30 mL) and the layers were separated. The organic layer was washed sequentially with H_2O (30 mL) and brine (30 mL). The combined aqueous layers were extracted with diethyl ether (3 × 75 mL). The organic extracts were combined, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue and acetyl chloride (2.30 ml, 2.32 g, 30 mmol) were dissolved in diethyl ether (45 mL). Pyridine (2.40 mL, 2.42 g, 30 mmol) was added to the solution. On addition, a white suspension was formed. The solution was heated to reflux at 35 °C until TLC analysis (10% ethyl acetate, hexane) indicated that the starting material was consumed (1 h). After cooling the reaction mixture to room temperature, aqueous HCl (40 mL, 1 M) was added. The organic layer was separated, washed with water (30 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography using petroleum ether/ethyl acetate (3% ethyl acetate) to give **106** as a colourless oil (2.33 g, 91%). $[\alpha]_D^{22}$ = −2 (*c* 1.00,

$CHCl_3$); δ_H (400 MHz, $CDCl_3$) 5.78 (1H, ddt, *J* 17.0, 10.0, 6.5, 7-H), 5.00 (1H, dq, *J* 17.0, 1.5, 8-H*H*), 4.94 (1H, ddt, *J* 10.0, 2.0, 1.5, 8-H*H*), 4.81 (1H, quin, *J* 6.5, 3-H), 2.05 (2H, m, 6-H_2), 2.04 (3H, s, 10-H_3), 1.60-1.48 (4H, m, 2-H_2, 4-H_2), 1.46-1.32 (2H, m, 5-H_2), 0.87 (3H, t, *J* 7.5, 1-H_3); δ_C (100 MHz, $CDCl_3$) 170.9 (C-9), 138.5 (C-7), 114.7 (C-8), 75.3 (C-3), 33.5 (C-6), 33.0 (C-4), 27.0 (C-2), 24.6 (C-5), 21.2 (C-10), 9.6 (C-1).

Data were consistent with that reported in literature.[247]

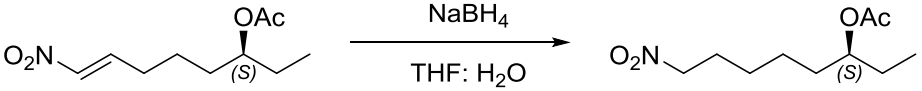
(*S*,*E*)-3-Acetoxy-8-nitrooct-7-ene 107 (*)



106 **107**

Alkene **106** (1.35 g, 7.93 mmol), *tert*-butyl nitrite (2.0 mL, 1.2 g, 16.0 mmol) and TEMPO (513 mg, 3.28 mmol) were dissolved in 1,4-dioxane (20 mL) and heated to reflux in air at 90 °C for 16 h. The orange solution was washed through a Celite® pad, concentrated *in vacuo*, and purified by column chromatography using petroleum ether/diethyl ether (14-25% diethyl ether) to give nitroalkene **107** as an orange oil (1.18 g, 69%). $[\alpha]_D^{22}$ = −11 (*c.* 1.00, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.17 (1H, dt, *J* 13.5, 7.5, 7-H), 9.91 (1H, dt, *J* 13.5, 1.5, 8-H), 2.20 (2H, m, 6-H_2), 1.94 (3H, s, 10-H_3), 1.53-1.39 (6H, m, 2-H_2, 4-H_2, 5-H_2), 0.80 (3H, t, *J* 7.5, 1-H_3) δ_C (100 MHz, $CDCl_3$) 170.6 (C-9), 142.3 (C-7), 139.8 (C-8), 74.5 (C-3), 33.0 (C-4), 28.2 (C-6), 27.0 (C-2), 23.6 (C-5), 20.7 (C-10), 9.3 (C-1); ν_{max} (film) 2939, 2878, 1728, 1649, 1522, 1351, 1241; HRMS (ESI) calc for $C_{10}H_{17}NO_4Na$ $[M+Na]^+$ 238.1055, found 238.1057.

(*S*,*E*)-3-Acetoxy-8-nitrooctane 108 (*)

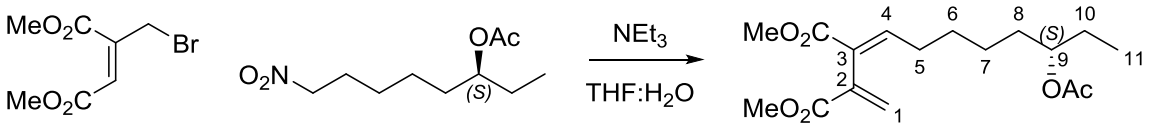


107 **108**

To a solution of nitroalkene **107** (1.00 g, 4.60 mmol) in THF:methanol (10:1, 45 mL) at 0 °C under a nitrogen atmosphere, $NaBH_4$ (263 mg, 6.97 mmol) was added in five portions. The reaction was stirred for 1 h at 0 °C. the resultant red solution was quenched with saturate aqueous $NaHCO_3$ (10 mL), diluted with water (100 mL) and extracted with ethyl acetate (4 × 75 mL). The combined organic layers were washed with brine (30 mL), dried $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography using hexane/ethyl acetate (10-12% ethyl acetate) to give nitroalkane **108** as a colourless oil (706 mg, 70%). $[\alpha]_D^{22}$ = −11 (*c* 1.00, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 4.73 (1H, quin, *J* 6.0, 3-H), 4.31 (2H, t, *J* 7.0, 8-H_2), 1.98 (3H, s, 10-H_3), 1.94 (2H, quin, *J* 7.5, 7-H_2),

1.55-1.42 (4H, m, 2-H_2, 4-H_2), 1.38-1.22 (4H, m, 5-H_2, 6-H_2), 0.81 (3H, t, *J* 7.5, 1-H_3); δ_C (100 MHz, $CDCl_3$) 170.9 (C-9), 75.5 (C-8), 75.0 (C-3), 33.3 (C-4), 27.3 (C-7), 27.0 (C-2), 26.1 (C-6), 24.6 (C-5), 21.2 (C-10), 9.6 (C-1); ν_{max} (film) 2938, 2864, 1727, 1550, 1372, 1241; HRMS (ESI) calc for $C_{10}H_{19}NO_4Na$ $[M+Na]^+$ 240.1212, found 240.1208.

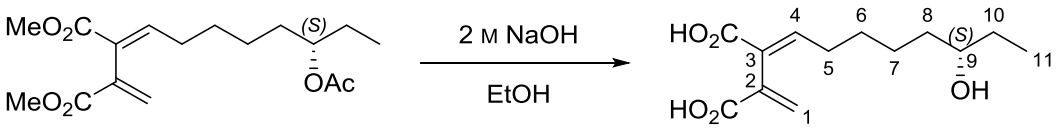
Dimethyl (*S*, *E*)-2-(5-acetoxyheptylidene)-3-methylenesuccinate 109 (*)



68 **108** **109**

Nitroalkane **108** (300 mg, 1.38 mmol, 1.1 equiv.) and triethylamine (0.87 mL, 0.63 mg, 6.20 mmol, 5 equiv.) were dissolved in THF (12 mL) and water (12 mL). Following 2 h of stirring a red solution was observed. Bromide **68** (285 mg, 1.20 mmol) in THF (3 mL) was added over 2 h at 0 °C with a syringe pump and stirred for an additional 14 h at room temperature. The yellow solution was diluted with water (40 mL) and extracted with diethyl ether (4 × 75 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using petroleum ether/ethyl acetate (9-17% ethyl acetate) to give triester **109** as a colourless oil (266 mg, 71%). $[\alpha]_D^{22}$ = −8 (*c* 1.00, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 6.97 (1H, t, *J* 7.5, 4-H), 6.49 (1H, d, *J* 1.5, 1-H*H*), 5.56 (1H, d, *J* 1.5, 1-H*H*), 4.78 (1H, quin, *J* 6.0, 9-H), 3.74 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 1.56-1.40 (6H, m, 6-H_2, 8-H_2, 10-H_2), 1.36-1.23 (2H, m, 7-H_2), 0.86 (3H, t, *J* 7.5, 11-H_3); δ_C (100 MHz, $CDCl_3$) 170.9 (C-12), 166.7 (C=O), 166.4 (C=O), 146.0 (C-4), 135.2 (C-2), 130.0 (C-3), 129.5 (C-1), 75.2 (C-9), 52.2, 52.0 (2-OCH_3 and 3-OCH_3), 33.3 (C-8), 29.4 (C-5), 28.5 (C-6), 27.0 (C-10), 25.1 (C-7), 21.2 (C-13), 9.6 (C-11); ν_{max} (film) 2948, 2860, 1721, 1623, 1240; HRMS (ESI) calc for $C_{17}H_{26}O_6Na$ $[M+Na]^+$ 349.1627, found 349.1632.

(*S*,*E*)-2-(6-Hydroxyoctylidene)-3-methylenesuccinic acid (*S*)-37 ((*S*)-Tricladic acid B) (*)



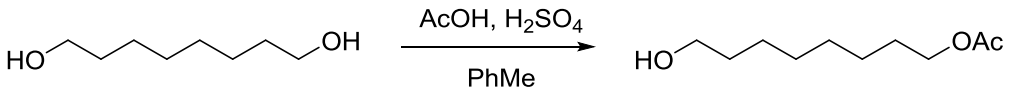
109 **(*S*)-37**

Triester **109** (210 mg, 0.67 mmol) was dissolved in ethanol (7 mL) and added to aqueous sodium hydroxide (14 mL, 2 M) at 0 °C over 0.3 h. The solution was stirred at room temperature for 16 h. The reaction mixture was acidified to pH 1 with aqueous hydrochloric acid (6 M). The solution was extracted with ethyl acetate (4 × 75 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by flash column chromatography using petroleum ether/ethyl acetate/acetic acid (70% ethyl acetate, 0.8% acetic acid) to give **(*S*)-37** as a viscous oil (124 mg, 72%). $[\alpha]_D^{22}$ = +4 (*c.* 1.70, MeOH) [Lit $[\alpha]_D^{22}$ +2.0 (*c* 0.34,

MeOH)][53]; δ_H (400 MHz, $CDCl_3$) 6.96 (1H, t, *J* 7.5, 4-H), 6.44 (1H, d, *J* 1.5, 1-H*H*), 5.61 (1H, d, *J* 1.5, 1-H*H*), 3.43 (1H, m, 9-H), 2.21 (2H, q, *J* 7.5, 5-H_2), 1.50-1.25 (8H, m, 6-H_2, 7-H_2, 8-H_2, 10-H_2), 1.14 (3H, t, *J* 7.5, 11- H_3); δ_C (100 MHz, $CDCl_3$) 168.4 (3-C=O), 168.1 (2-C=O), 145.4 (C-4), 136.7 (C-2), 131.0 (C-3), 128.2 (C-1), 72.3 (C-9), 36.2 (C-8), 29.7 (C-10), 29.1 (C-5), 28.4 (C-6), 25.1 (C-7), 9.0 (C-11); ν_{max} (film) 3371, 2933, 2860, 1691, 1622, 1266, 1129; HRMS (ESI) calc for $C_{13}H_{20}O_5Na$ $[M+Na]^+$ 279.1208, found 279.1205.

^1H and ^{13}C spectra obtained after further purification by preparative HPLC, and is in accordance with literature.[53]

8-Hydroxyoctyl acetate 111



110 **111**

1,8-octanediol **110** (13.4 g, 91.6 mmol, 1.1 equiv.), acetic acid (4.80 mL, 5.0 g, 83.3 mmol, 1 equiv.) and sulfuric acid (0.2 mL) were heated to reflux in toluene (200 mL) for 16 h. The mixture was then concentrated *in vacuo*, and the residue poured into water (100 mL), neutralised with saturated $NaHCO_3$ solution (100 mL) then extracted with ethyl acetate (3 × 150 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (10-50% ethyl acetate) to give monoacetate **111** as a colourless oil (7.87 g, 50%) and the diacetylated alcohol **S5** (4.37 g).

8-hydroxyoctyl acetate **111**:

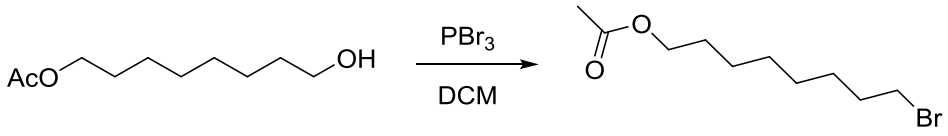
δ_H (400 MHz, $CDCl_3$) 4.00 (2H, t, *J* 7.0, 1-H_2), 3.58 (2H, t, *J* 6.5, 8-H_2), 2.00 (3H, s, CH_3), 1.81 (1H, m, OH), 1.66 – 1.43 (4H, m, 2-H_2 and 7-H_2), 1.36 – 1.20 (8H, m, 3-H_2, 4-H_2, 5-H_2 and 6-H_2); δ_C (100 MHz, $CDCl_3$) 171.4 (C=O), 64.7 (C-1), 62.9 (C-8), 32.8 (C-2 or C-7), 29.4 (CH_2), 29.3 (CH_2), 28.6 (C-2 or C-7), 25.9 (CH_2), 25.7 (CH_2), 21.1 (CH_3).

Octane-1,8-diyl diacetate **S5**:

δ_H (400 MHz, $CDCl_3$) 4.02 (4H, t, *J* 7.0, 1-H_2 and 8-H_2), 2.02 (6H, s, 2 × CH_3), 1.63-1.54 (4H, m, 2-H_2 and 7-H_2), 1.36-1.26 (8H, m, 3-H_2, 4-H_2, 5-H_2 and 6-H_2); δ_C (100 MHz, $CDCl_3$) 171.3 (C=O), 64.7 (C-1 and C-8), 28.7 (C-2 and C-7), 29.2 and 25.9 (C-3, C-4, C-5 and C-6), 21.1 (CH_3).

NMR data in accordance with literature.[248]

8-Bromooctyl acetate 112

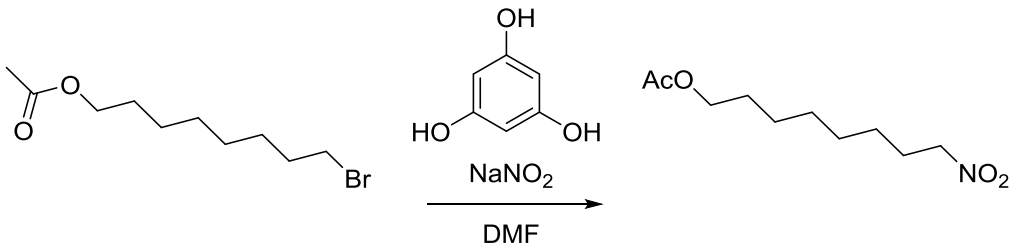


111 **112**

To a solution of alcohol **111** (3.00 g, 15.9 mmol, 1 equiv.) in dry DCM (85 mL) at 0 °C was added phosphorus tribromide (1.80 mL, 5.18 g, 19.2 mmol, 1.2 equiv.) and the reaction mixture was allowed to warm to room temperature and stirred for 16 h. Water (5 mL) was added slowly, then the organic layer was washed with water (2 × 50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (5% ethyl acetate) to give bromide **112** (1.94 g, 49%). δ_H (400 MHz, $CDCl_3$) 4.03 (2H, t, *J* 6.5, 1-H_2), 3.39 (2H, t, *J* 7.0, 8-H_2), 2.03 (3H, s, CH_3), 1.83 (2H, p, *J* 7.0, 2-H_2), 1.60 (2H, p, *J* 7.0, 7-H_2), 1.40 (2H, q, *J* 7.0) and 1.35-1.23 (6H, m, 3-H_2, 4-H_2, 5-H_2 and 6-H_2); δ_C (100 MHz, $CDCl_3$) 171.3 (C=O), 64.6 (C-1), 34.0 (C-8), 32.9 (C-2), 29.1 (CH_2), 28.7 (CH_2), 28.7 (CH_2), 28.2 (CH_2), 25.9 (CH_2), 21.1 (CH_3).

NMR data in accordance with literature.[249]

8-Nitrooctyl acetate 113

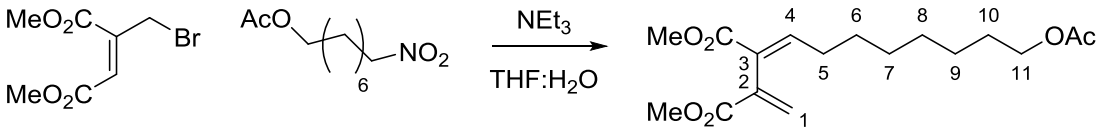


112 **113**

Bromide **112** (1.01 g, 4.04 mmol) in DMF (5 mL) was added dropwise to a solution of sodium nitrite (518 mg, 7.5 mmol, 1.9 equiv.) and phloroglucinol (504 mg, 4.0 mmol, 1.0 equiv.) in DMF (30 mL) at 0 °C. After 0.5 h, the reaction mixture was allowed to warm to room temperature and stirred for 16 h. Water (60 mL) and hexane (100 mL) were added, the layers separated, and the organic layer further extracted with water (2 × 100 mL), then dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (0-10% ethyl acetate) to give nitroalkane **113** as a colourless oil (522 mg, 60%). δ_H (400 MHz, $CDCl_3$) 4.33 (2H, t, *J* 7.0, 8-H_2), 3.99 (2H, t, *J* 6.5, 1-H_2), 2.05-1.90 (5H, m, CH_3 and 7-H_2), 1.56 (2H, q, *J* 6.5, 2-H_2), 1.36-1.25 (8H, m, 3-H_2, 4-H_2, 5-H_2 and 6-H_2); δ_C (100 MHz, $CDCl_3$) 171.2, (C=O), 75.7 (C-8), 64.4 (C-1), 28.9 (CH_2), 28.7 (CH_2), 28.5 (C-2), 27.3 (C-7), 26.1 (CH_2), 25.7 (CH_2), 21.0 (CH_3); ν_{max} (film) 2932, 2859, 2256, 1731, 1551, 1366, 1242, 909, 730; HRMS (ESI) calc for $C_{10}H_{20}NO_4$ $[M+H]^+$ 218.1387, found 218.1381.

Nitrite ester **S6** also isolated (249 mg, 29%): δ_H (400 MHz, $CDCl_3$) 4.62 (2H, br t, *J* 7.0, 8-H_2), 3.98 (2H, t, *J* 6.5, 1-H_2), 1.97 (3H, s, CH_3), 1.66 (2H, p, *J* 7.0, CH_2), 1.55 (2H, p, *J* 7.0, CH_2), 1.35-1.24 (8H, m, 4 × CH_2); δ_C (100 MHz, $CDCl_3$) 171.4 C(=O), 64.7 (CH_2), 63.0 (CH_2), 32.8 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 28.7 (CH_2), 25.9 (CH_2), 25.7 (CH_2), 21.1 (CH_3); ν_{max} (film) 2930, 2857, 1737, 1641, 1366, 1238, 1036, 792; HRMS (ESI) calc for $C_{10}H_{19}NNaO_4$ $[M+Na]^+$ 240.1206, found 240.1197. Sample degrades on standing.

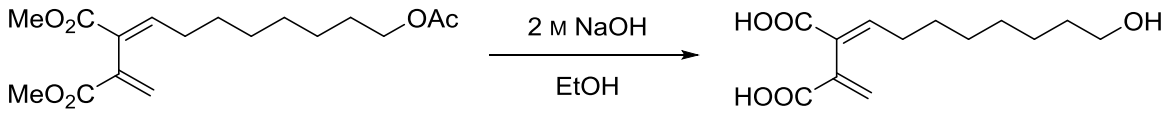
Dimethyl (*E*)-2-(8-acetoxyoctylidene)-3-methylenesuccinate 114



68 **113** **114**

Nitroalkane **113** (379 mg, 1.76 mmol, 1.3 equiv.) and triethylamine (0.50 mL, 0.35 g, 3.40 mmol, 2.6 equiv.) were dissolved in THF (15 mL) and water (15 mL) and stirred for 2 h. Bromide **68** (318 mg, 1.35 mmol, 1 equiv.) in THF (2 mL) was added at 0 °C, and the reaction mixture stirred for an additional 16 h at room temperature. The orange solution was diluted with water (20 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (2-10% ethyl acetate) to give diester **114** (133 mg, 30%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 6.97 (1H, t, *J* 7.5, 4-H), 6.47 (1H, s, 1-*H*H), 5.56 (1H, s, 1-H*H*), 4.02 (2H, t, *J* 7.0, 11-H_2), 3.72 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 2.15 (2H, q, *J* 8.0, 5-H_2), 2.02 (3H, s, CH_3), 1.58 (2H, p, *J* 7.0, CH_2), 1.42 (2H, p, *J* 7.0, CH_2), 1.33-1.23 (6H, m, 3 × CH_2); δ_C (100 MHz, $CDCl_3$) 171.4 (CH_3C=O), 166.9 (3-C=O), 166.6 (2-C=O), 146.6 (C-4), 135.3 (C-2), 129.9 (C-3), 129.6 (C-1), 64.7 (C-11), 52.3 (OCH_3), 52.2 (OCH_3), 29.6 (C-5), 29.3, 29.1, 28.7, 28.7, 25.9 (C-6, C-7, C-8, C-9 and C-10), 21.2 (CH_3); ν_{max} (film) 2930, 2856, 1722, 1436, 1365, 1240, 1053; HRMS (ESI) calc for $C_{17}H_{27}O_6$ $[M+H]^+$ 327.1802, found 327.1796.

(*E*)-2-(8-Hydroxyoctylidene)-3-methylenesuccinic acid 38 (Tricladic Acid C)



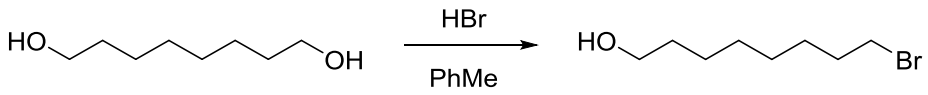
114 **38**

Diester **114** (98.0 mg, 0.30 mmol) was dissolved in ethanol (8 mL) and sodium hydroxide (9 mL, 2 M) added. The reaction mixture was stirred at room temperature for 16 h, then aqueous sodium sulfate (4%, 25 mL) added and the reaction mixture acidified to pH 1 with hydrochloric acid (6 M). The aqueous mixture was extracted with diethyl ether (4 × 20 mL), and the combined organic layers dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol/acetic acid (75% ethyl acetate, 1% acetic acid) to give

diacid **38** (72 mg, 91%) as a colourless oil. δ_H (400 MHz, MeOD) 6.92 (1H, t, *J* 7.5, 4-H), 6.42 (1H, d, *J* 1.5, 1-*H*H), 5.57 (1H, d, *J* 1.5, 1-H*H*), 3.50 (2H, t, *J* 6.5, 11-H_2), 2.16 (2H, q, *J* 7.5, 5-H_2), 1.52-1.39 (4H, m, 6-H_2 and 10-H_2), 1.38-1.22 (6H, m, 7-H_2, 8-H_2 and 9-H_2); δ_C (100 MHz, MeOD) 169.6 (3-C=O), 169.3 (2-C=O), 147.1 (C-4), 137.7 (C-2), 132.1 (C-3), 129.8 (C-1), 62.9 (C-11), 33.5 (C-10), 30.4 (C-5), 30.3, 30.2 (C-7 and C-8), 29.6 (C-6), 26.7 (C-9); ν_{max} (film) 2931, 2858, 2489, 2230, 2072, 1686, 1621, 1329, 1117, 1056, 971; HRMS (ESI) calc for $C_{13}H_{19}O_5$ $[M-H]^-$ 255.1238, found 255.1235.

^1H and ^{13}C spectra in accordance with literature.[53]

8-Bromooctan-1-ol 115

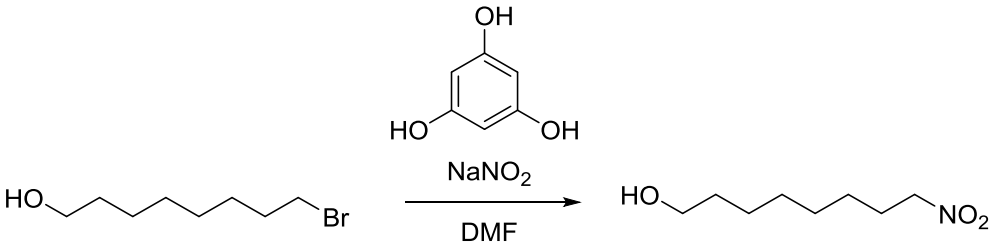


110 **115**

1,8-octanediol **110** (5.01 g, 34.2 mmol) was dissolved in toluene (75 mL), and then HBr (48% aq, 4.75 mL, 42.8 mmol) added. The mixture was heated to 112 °C for 16 h, then cooled to room temperature and water (50 mL) added. The aqueous layer was extracted with diethyl ether (3 × 20 mL) and the combined organic layers washed with HCl (1 M, 70 mL), then dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (50% ethyl acetate) to give alcohol **115** (6.546 g, 92%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 3.64-3.58 (2H, m, 1-H_2), 3.41-3.36 (2H, m, 8-H_2), 1.88-1.79 (2H, m, CH_2), 1.65-1.47 (3H, m, CH_2 and OH), 1.46-1.27 (8H, m, 4 × CH_2); δ_C (100 MHz, $CDCl_3$) 63.0 (C-1), 34.1 (C-8), 32.9, 32.8, 29.3, 28.8, 28.2, 25.8 (C-2, C-3, C-4, C-5, C-6 and C-7).

NMR data in accordance with literature.[250]

8-Nitrooctan-1-ol 116



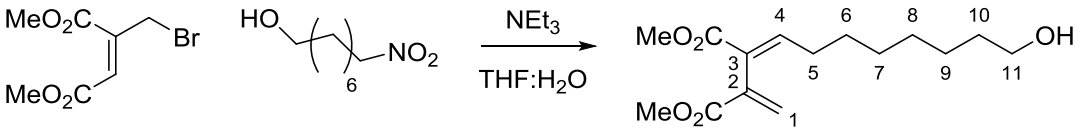
115 **116**

To a solution of sodium nitrite (2.030 g, 29.4 mmol, 2.0 equiv.) and phloroglucinol (1.92 g, 15.2 mmol, 1.1 equiv.) in DMF (100 mL) and DMSO (100 mL) at 0 °C was added bromide **115** (3.004 g, 14.4 mmol) in DMF (5mL) dropwise. After 0.5 h, the reaction was allowed to warm to room temperature and stirred for 16 h. Water was then added (300 mL), and then the reaction mixture extracted with diethyl ether (200 mL) and washed with water (2 × 100 mL). The ethereal layer was dried over $MgSO_4$,

filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (10-50% ethyl acetate) to give nitroalkanol **116** (1.10 g, 44%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 4.35 (2H, t, *J* 7.0, 8-H_2), 3.60 (2H, t, *J* 6.5, 1-H_2), 1.98 (2H, p, *J* 7.0, CH_2, 7-H_2), 1.59-1.49 (3H, m, 2-H_2 and OH), 1.39-1.28 (8H, m, 4 × CH_2). δ_C (100 MHz, $CDCl_3$) 75.8 (C-8), 63.0 (C-1), 32.7 (C-2), 29.1, 28.9 (2 × CH_2), 27.5 (C-7), 26.2, 25.7 (2 × CH_2); ν_{max} (film) 3335, 2929, 2857, 1550, 1463, 1435, 1383, 1055; HRMS (ESI) calc for $C_8H_{17}NNaO_3$ $[M+Na]^+$ 198.1101, found 198.1092.

^{1}H NMR matches previously reported.[251]

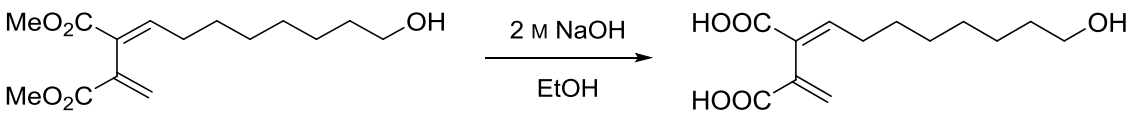
Dimethyl (*E*)-2-(8-hydroxyoctylidene)-3-methylenesuccinate 117



68 **116** **117**

Nitroalkanol **116** (251 mg, 1.43 mmol, 1.3 equiv.) and triethylamine (0.54 mL, 0.39 g, 3.8 mmol, 3.5 equiv.) were dissolved in THF (7.5 mL) and water (7.5 mL) and stirred for 2 h. Bromide **68** (263 mg, 1.10 mmol, 1 equiv.) in THF (3 mL) was added at 0 °C, and the reaction mixture stirred for an additional 16 h at room temperature. The orange solution was diluted with water (20 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (25-50% ethyl acetate) to yield diester **117** (167 mg, 54%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 6.91 (1H, t, *J* 7.5, 4-H), 6.41 (1H, d, *J* 1.5, 1-*H*H), 5.51 (1H, d, *J* 1.5, 1-H*H*), 3.66 (3H, s, OCH_3), 3.64 (3H, s, OCH_3), 3.51 (2H, t, *J* 6.5, 11-H_2), 2.26 (1H, s, OH), 2.09 (2H, q, *J* 7.5, 5-H_2), 1.46 (2H, m, 10-H_2), 1.36 (2H, p, *J* 7.0, 6-H_2), 1.29-1.19 (6H, m, 7-H_2, 8-H_2, 9-H_2); δ_C (100 MHz, $CDCl_3$) 166.7 (3-C=O), 166.4 (2-C=O), 146.5 (C-4), 135.1 (C-2), 129.7 (C-3), 129.5 (C-1), 62.6 (C-11), 52.1 (OCH_3), 51.9 (OCH_3), 32.6 (C-10), 29.4 (C-5), 29.2, 29.1, 28.5, 22.6 (C-6, C-7, C-8 and C-9); ν_{max} (film) 3515, 2931, 2857, 1714, 1437, 1255, 908, 730; HRMS (ESI) calc for $C_{15}H_{24}NaO_5$ $[M+Na]^+$ 307.1516, found 307.1514.

(*E*)-2-(8-Hydroxyoctylidene)-3-methylenesuccinic acid 38 (Tricladic Acid C)



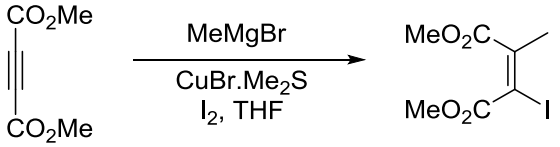
117 **38**

Diester **117** (150 mg, 0.53 mmol) was dissolved in ethanol (15 mL) and sodium hydroxide (15 mL, 2 M) added. The reaction mixture was stirred at room temperature for 16 h, then aqueous sodium

sulfate (4%, 40 mL) added and the reaction mixture acidified to pH 1 with hydrochloric acid (6 M). The aqueous mixture was extracted with diethyl ether (4 × 25 mL), and the combined organic layers dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol/acetic acid (75% ethyl acetate, 1% acetic acid) to give diacid **38** (120 mg, 90%) as a colourless oil. Characterisation data as previously reported (page 166).

7.2.3 Experimental Procedures for Chapter 4

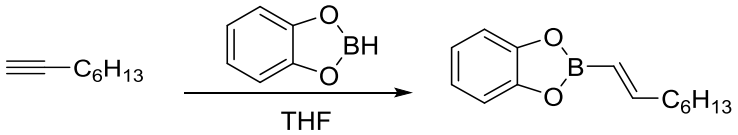
Dimethyl 2-iodo-3-methylmaleate 141



140 **141**

A 3-necked, 250 mL round-bottomed flask was equipped with a low temperature thermometer, nitrogen inlet and septum. THF (30 mL) was cooled to −40 °C and $CuBr.Me_2S$ (1.12 g, 5.51 mmol, 1.1 equiv.) and methylmagnesium bromide (1.82 mL, 3 M in diethyl ether, 5.5 mmol, 1.1 equiv.) added. After 1 h, the resulting yellow solution was cooled to −75 °C and dimethyl acetylenedicarboxylate **140** (0.61 mL, 700 mg, 4.9 mmol, 1 equiv.) in THF (7 mL) was added over 0.5 h via syringe pump, maintaining an internal temperature of −75 °C. After 1 h, iodine (1.43 g, 5.65 mmol, 1.2 equiv.) in THF (12 mL) was added over 0.75 h via syringe pump, maintaining an internal temperature of −70 °C. The resulting orange solution was stirred for 1.5 h at -75 °C, then quenched by addition of 25% saturated aqueous NH_4Cl (25 mL) and allowed to warm to room temperature. The mixture was poured into water (20 mL) and extracted with diethyl ether (3 × 30 mL). The combined organic layers were washed with sat. aq. $Na_2S_2O_3$ (2 × 25 mL), water (25 mL) and brine (25 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a yellow oil (1.11 g, 53%) containing a 1:1 mixture of the desired product **141** and starting material **140** (determined by ^1H NMR). This oil was used without further purification.

(*E*)-2-(Oct-1-en-1-yl)benzo[d][1,3,2]dioxaborole 143

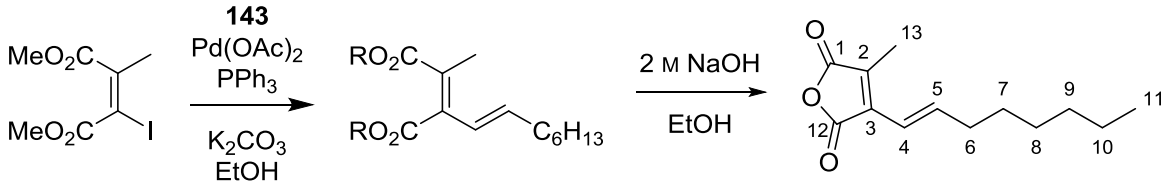


142 **143**

1-Octyne **142** (3.3 mL, 2.50 g, 22.7 mmol, 2 equiv.) and catecholborane (11.5 mL, 11.5 mmol, 1 M in THF, 1 equiv.) were heated to reflux at 75 °C for 2 h. After cooling to room temperature and concentration *in vacuo*, the residue was dissolved in DCM (20 mL), washed with water (20 mL), dried

over $MgSO_4$, filtered, and concentrated *in vacuo* to a yellow oil. The crude material was passed through a plug of silica to remove unreacted 1-octyne, then used without further purification.

(*E*)-3-Methyl-4-(oct-1-en-1-yl)furan-2,5-dione 145 (Tricladolide D)



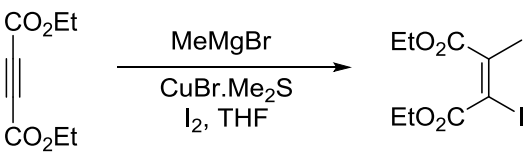
141 **144** **145**

Potassium carbonate (978 mg, 7.0 mmol, 1.8 equiv.), triphenylphosphine (67 mg, 0.24 mmol, 0.08 equiv.) and palladium(II) acetate (31 mg, 0.13 mmol, 0.03 equiv.) were dissolved in absolute ethanol (25 mL). Solutions of unpurified vinyl iodide **141** (1.11 g) and boronic ester **143** (1.10 g) each in ethanol (8 mL) were added sequentially, and the resulting dark brown suspension was heated to reflux at 80 °C for 16 h. The dark brown mixture was then cooled to room temperature, poured into water (30 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic layers were washed with water (2 × 25 mL) and brine (30 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using diethyl ether/petrol (5-20% diethyl ether) as an eluent to give a mixture of Me/Me, Me/Et, Et/Me and Et/Et esters **144**.

Diester mixture **144** (190 mg, 0.67 mmol) was dissolved in ethanol (15 mL) and sodium hydroxide (2 M, 15 mL) added. The resulting solution was stirred at room temperature for 16 h, then sodium sulfate (4%, 15 mL) was added and the reaction mixture acidified to pH 1 with hydrochloric acid (6 M). The aqueous mixture was extracted with diethyl ether (3 × 25 mL), and the combined organic layers were washed with water (30 mL), dried over $MgSO_4$, filtered, then concentrated *in vacuo* to give the crude maleic anhydride **145** (147 mg, 98% from **140**). δ_H (400 MHz, $CDCl_3$) 7.11 (1H, dt, *J* 15.0, 7.0, 5-H), 6.23 (1H, d, *J* 15.0, 4-H), 2.28 (2H, q, *J* 7.0, 6-H), 2.10 (3H, s, 13-H_3), 1.53-1.39 (2H, m, 7-H_2), 1.38-1.18 (6H, m, 8-H_2, 9-H_2 and 10-H_2), 0.88 (3H, t, *J* 7.0, 11-H_3); δ_C (100 MHz, $CDCl_3$) 166.5 (C-1), 164.7 (C-12), 148.2 (C-5), 137.4 (C-3), 135.0 (C-2), 117.2 (C-4), 34.6 (C-6), 31.7 (C-9), 29.0 (C-8), 28.5 (C-7), 22.7 (C-10), 14.2 (C-11), 9.4 (C-13).

NMR data in accordance with literature.[50,53]

Diethyl 2-iodo-3-methylmaleate 148

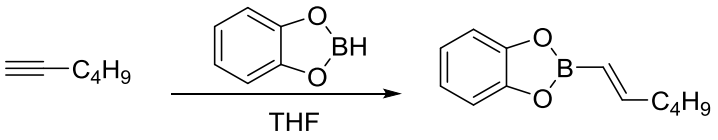


147 **148**

A three-necked 500 mL round-bottomed flask was equipped with a low temperature thermometer, nitrogen inlet and septum. THF (70 mL) was cooled to -40 °C and $CuBr.Me_2S$ (2.186 g, 10.4 mmol, 1.1 equiv.) and methylmagnesium bromide (3.5 mL, 3 M in diethyl ether, 10.4 mmol, 1.1 equiv.) added. After 1 h, the resulting yellow solution was cooled to −75 °C and diethyl acetylenedicarboxylate **147** (1.5 mL, 1.60 g, 9.4 mmol, 1 equiv.) in THF (10 mL) was added over 0.75 h via syringe pump, maintaining an internal temperature of −75 °C. After 1 h, iodine (2.88 g, 11.3 mmol, 1.2 equiv.) in THF (20 mL) was added over 0.75 h via syringe pump, maintaining an internal temperature of −75 °C. The resulting orange solution was stirred for 1.5 h at -75 °C, then quenched by dropwise addition of 25% saturated aqueous NH_4Cl (25 mL) and allowed to warm to room temperature. The mixture was poured into water (20 mL) and extracted with diethyl ether (3 × 30 mL). The combined organic layers were washed with saturated aqueous $Na_2S_2O_3$ (2 × 25 mL), water (25 mL) and brine (25 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield a yellow oil **148** (2.78 g, 95%). δ_H (400 MHz, $CDCl_3$) 4.25 (2H, q, *J* 7.0, OCH_2), 4.22 (2H, q, *J* 7.0, OCH_2), 2.20 (3H, s, CH_3), 1.32 (3H, t, *J* 7.0, $OCH_2C\underline{H}_3$), 1.29 (3H, t, *J* 7.0, $OCH_2C\underline{H}_3$); δ_C (100 MHz, $CDCl_3$) 165.8 (C=O), 164.5 (C=O), 141.7 (C-3), 100.8 (C-2), 62.6 (OCH_2), 62.1 (OCH_2), 24.8 (CH_3), 14.1 ($OCH_2C\underline{H}_3$), 13.9 ($OCH_2C\underline{H}_3$).

NMR data in accordance with literature.[149]

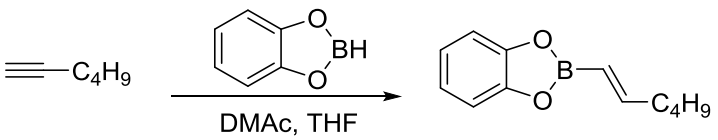
(*E*)-2-(Hex-1-en-1-yl)benzo[d][1,3,2]dioxaborole 150



149 **150**

1-Hexyne (3.5 mL, 2.50 g, 30.5 mmol, 2 equiv.) and catecholborane (15.5 mL, 15.5 mmol, 1 M in THF, 1 equiv.) were heated to reflux at 75 °C for 2 h. After cooling to room temperature and concentrating *in vacuo*, the residue was dissolved in DCM (20 mL), washed with water (20 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to a pale yellow oil. The crude material was passed through a plug of silica to remove unreacted 1-hexyne, then used without further purification.

Alternate preparation[154]:

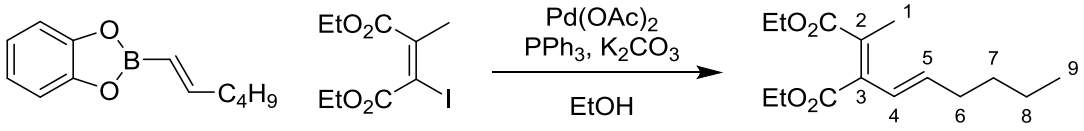


149 **150**

1-Hexyne (3.5 mL, 2.50 g, 30.5 mmol) was dissolved in THF (5 mL) and *N*,*N*-dimethylacetamide (0.2 mL) added. Catecholborane (3.9 mL, 3.67 g, 30.5 mmol, 1 equiv.) was added dropwise, and then the reaction heated to reflux for 3 h. The reaction mixture was cooled to room temperature, concentrated *in vacuo*, then purified by column chromatography using ethyl acetate/petrol (10-50% ethyl acetate) to give catecholboronic ester **150** as a yellow oil (4.22 g, 69%). δ_H (400 MHz, $CDCl_3$) 7.24-7.20 (2H, m, Ar-H), 7.10-7.05 (2H, m, Ar-H), 6.86 (1H, m, 2-H), 5.80 (1H, dd, *J* 18.0, 1.5, 1-H), 2.30 (2H, tdd, *J* 8.0, 6.0, 1.5, 3-H_2) 1.54-1.45 (2H, m, 4-H_2), 1.44-1.35 (2H, m, 5-H_2), 0.94 (3H, t, *J* 7.5, 6-H_3); δ_C (100 MHz, $CDCl_3$) 158.2 (C-2), 148.4 (Ar), 122.6 (Ar), 121.4 (C-1), 115.6 (C-1), 112.4 (Ar), 35.9 (C-3), 30.4 (C-4), 22.4 (C-5), 14.1 (C-6).

NMR data in accordance with literature.[252]

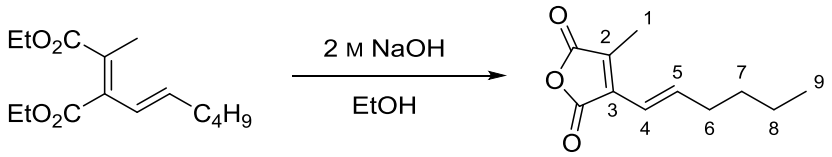
Diethyl 2-((*E*)-hex-1-en-1-yl)-3-methylmaleate 151



150 **148** **151**

Potassium carbonate (1.60 g, 11.5 mmol, 1.8 equiv.), triphenylphosphine (110 mg, 0.42 mmol, 0.08 equiv.) and palladium(II) acetate (47 mg, 0.20 mmol, 0.03 equiv.) were dissolved in absolute ethanol (40 mL). Solutions of unpurified vinyl iodide **148** (2.84 g) and boronic ester **150** (1.566 g) each in ethanol (10 mL) were added sequentially, and the resulting dark brown suspension was heated to reflux at 80 °C for 16 h. The dark mixture was then cooled to room temperature, poured into water (30 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic layers were washed with water (2 × 25 mL) and brine (30 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using diethyl ether/petrol (5-20% diethyl ether) to give diester **151** (1.012 g, 40% over two steps). δ_H (400 MHz, $CDCl_3$) 6.31 (1H, dt, *J* 16.0, 1.5 Hz, 4-H), 5.94 (1H, dt, *J* 16.0, 7.0 Hz, 5-H), 4.29 (2H, q, *J* 7.0 Hz, OCH_2), 4.17 (2H, q, *J* 7.0 Hz, OCH_2), 2.18 (2H, q, *J* 7.0, 6-H_2), 1.97 (3H, s, 1-H_3), 1.44-1.35 (2H, m, 7-H_2), 1.34-1.23 (8H, m, 8-H2, 2 × $OCH_2C\underline{H}_3$), 0.88 (3H, t, *J* 7.0, 9-H_3); δ_C (100 MHz, $CDCl_3$) 169.1 (C=O), 167.2 (C=O), 141.5 (C-2), 141.1 (C-4), 124.2 (C-3), 124.0 (C-5), 61.2 (OCH_2), 61.1 (OCH_2), 33.4 (C-6), 30.9 (C-7), 22.3 (C-8), 14.2 ($OCH_2\underline{C}H_3$), 14.2 ($OCH_2\underline{C}H_3$), 13.9 (C-9), 13.6 (C-1); ν_{max} (film) 2981, 2932, 1721, 1368, 1259, 1181, 1108, 1033; ; HRMS (ESI) calc for $C_{15}H_{24}NaO_4$ $[M+Na]^+$ 291.1567, found 291.1571.

(*E*)-3-Methyl-4-(hex-1-en-1-yl)furan-2,5-dione 146

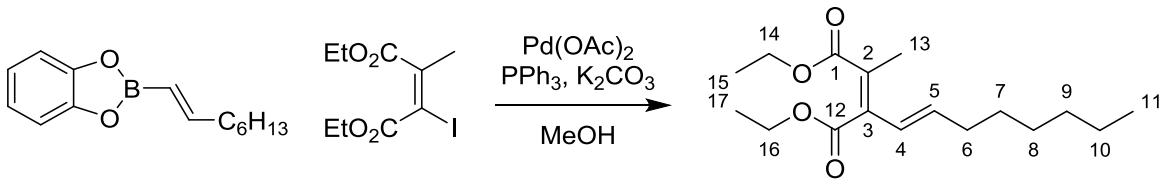


151 **146**

Diester **151** (750 mg, 2.8 mmol) was dissolved in ethanol (35 mL) and sodium hydroxide (2 M, 45 mL) added. The resulting solution was stirred at room temperature for 16 h, then sodium sulfate (4%, 50 mL) added and the reaction mixture acidified to pH 1 with hydrochloric acid (6 M). The aqueous mixture was extracted with diethyl ether (4 × 50 mL), and the combined organic layers washed with water (50 mL), then dried over $MgSO_4$, filtered, and then concentrated *in vacuo* to give maleic anhydride **146** (1.49 g, 91%). δ_H (400 MHz, $CDCl_3$) 7.12 (1H, dt, *J* 16.0, 7.0, 5-H), 6.23 (1H, dt, *J* 16.0, 1.5, 4-H), 2.28 (2H, qd, *J* 7.0, 1.5, 6-H_2), 2.11 (3H, s, 1-H_3), 1.53-1.43 (2H, m, 7-H_2), 1.41-1.31 (2H, m, 8-H_2), 0.92 (3H, t, *J* 7.5, 9-H_3); δ_C(100 MHz, $CDCl_3$) 166.5 (C=O), 164.7 (C=O), 148.2 (C-5), 137.4 (C-3), 135.0 (C-2), 117.3 (C-4), 34.3 (C-6), 30.7 (C-7), 22.4 (C-8), 14.0 (C-9), 9.4 (C-1); HRMS (ESI) calc for $C_{11}H_{14}NaO_3$ $[M+Na]^+$ 217.0835, found 217.0840.

NMR data in accordance with literature.[52]

Diethyl 2-((*E*)-oct-1-en-1-yl)-3-methylmaleate S7



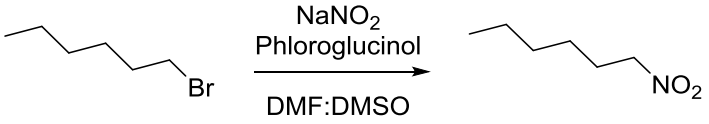
143 **148** **S7**

Potassium carbonate (1.04 g, 5.81 mmol, 1.8 equiv.), triphenylphosphine (73 mg, 0.30 mmol, 0.1 equiv.) and palladium(II) acetate (31 mg, 0.15 mmol, 0.03 equiv.) were dissolved in absolute ethanol (20 mL). Solutions of unpurified vinyl iodide **148** (1.3 g) and boronic ester **143** (1.3 g) each in ethanol (5 mL) were added sequentially, and the resulting dark brown suspension was heated to reflux at 80 °C for 16 h. The dark mixture was then cooled to room temperature, poured into water (30 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic layers were washed with water (2 × 25 mL) and brine (30 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using diethyl ether/petrol (5-30% diethyl ether) as an eluent to give diester **S7** (347 mg, 25% over two steps). δ_H (400 MHz, $CDCl_3$) 6.32 (1H, dt, *J* 15.5, 1.5, 4-H), 5.95 (1 H, dt, *J* 15.5, 7.0, 5-H), 4.31 (2H, q, *J* 7.0, 16-H_2), 4.18 (2H, q, *J* 7.0, 14-H_2), 2.19 (2H, q, *J* 7.0, 6-H_2), 1.98 (3H, s, 13-H_3), 1.45-1.37 (2H, m, 7-H_2), 1.35-1.20 (12H, m, 8-H_2, 9-H_2, 10-H_2, 15-H_3 and 17-H_3), 0.94-0.80 (3H, m, 11-H_3). δ_C (100 MHz, $CDCl_3$) 169.1 (C-12), 167.1 (C-1), 141.5 (C-2), 141.2

(C-4), 124.1 (C-3), 123.9 (C-5), 61.2 (OCH_2), 61.1 (OCH_2), 33.7 (C-6), 31.7 and 28.9 (C-8 and C-9), 28.7 (C-7), 22.6 (C-10), 14.2, 14.1 and 14.1 (C-11, C-15 and C-17), 13.6 (C-13).

NMR data in accordance with literature.[50]

1-Nitrohexane S3

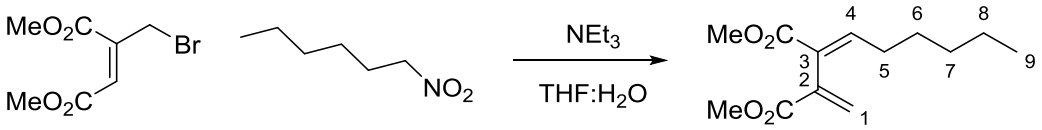


S8 **S3**

1-Bromohexane **S8** (1.7 mL, 2.0 g, 12.1 mmol) was added dropwise to a stirred solution of sodium nitrite (1.029 g, 14.9 mmol, 1.2 equiv.) and phloroglucinol (0.595 g, 4.83 mmol, 0.4 equiv.) in DMSO (20 mL) and DMF (20 mL) at 0 °C. After 2 h, water (60 mL) and hexane (100 mL) were added, the layers separated, and the organic layer further extracted with water (2 × 100 mL), then dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield 1-nitrohexane **S3** as a colourless oil (831 mg, 52% yield – 96% pure with 4% hexyl nitrite). δ_H (400 MHz, $CDCl_3$) 4.37 (2H, t, *J* 7.0, 1-H_2), 2.00 (2H, p, *J* 7.0, 2-H_2), 1.42-1.26 (6H, m, 3-H_2, 4-H_2 and 5-H_2), 0.89 (3H, t, *J* 7.0, 6-H_3); δ_C (100 MHz, $CDCl_3$) 75.9 (C-1), 31.1 (C-2), 27.5, 26.0, 22.4 (C-3, C-4 and C-5), 14.0 (C-6)

NMR data in accordance with literature.[103]

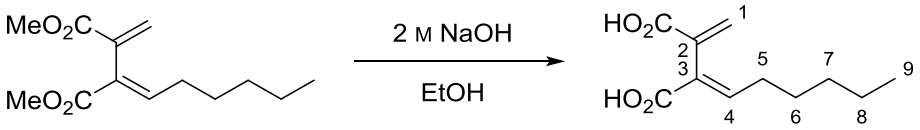
Dimethyl (*E*)-2-hexylidene-3-methylenesuccinate 153



68 **S3** **153**

1-Nitrohexane **S3** (900 mg, 6.9 mmol, 1.3 equiv.) and triethylamine (3.0 mL, 2.1 g, 21.1 mmol, 4 equiv.) were dissolved in THF (15 mL) and water (15 mL) and stirred for 2 h. Bromide **68** (1.245 g, 5.25 mmol) in THF (7 mL) was added, and the reaction mixture stirred for an additional 16 h. The orange solution was diluted with water (20 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (1-4% ethyl acetate) to give diester **153** as a colourless oil (298 mg, 24%). δ_H (400 MHz, $CDCl_3$) 6.99 (1H, t, *J* 7.0, 4-H), 6.49 (1H, d, *J* 1.5, 1-*H*H), 5.58 (1H, d, *J* 1.5, 1-H*H*), 3.74 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 2.16 (2H, app. q, *J* 7.5, 5-H_2), 1.48-1.38 (2H, m, 6-H_2), 1.33-1.23 (4H, m, 7-H_2 and 8-H_2), 0.87 (3H, t, *J* 7.0, 9-H_3); δ_C (100 MHz, $CDCl_3$) 166.9 (C=O), 166.6 (C=O), 146.7 (C-4), 135.4, 129.9 (C-2 and C-3), 129.5 (C-1), 52.3 (OCH_3), 52.1 (OCH_3), 31.6 (C-7), 29.6 (C-5), 28.5 (C-6), 22.5 (C-8), 14.1 (9-H_3); ν_{max} (film) 2953, 2928, 1719, 1435, 1246, 1199, 1065; HRMS (ESI) calc for $C_{13}H_{21}O_4$ $[M+H]^+$ 241.1434, found 241.1436.

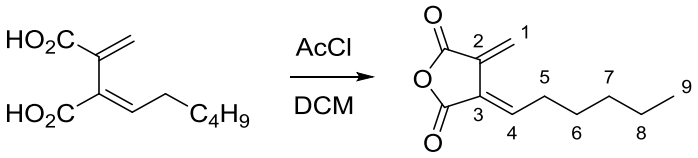
(*E*)-2-Hexylidene-3-methylenesuccinic acid 152



153 **152**

Diester **153** (295 mg, 1.20 mmol) was dissolved in ethanol (12 mL) and sodium hydroxide (18 mL, 2 M) added. The reaction mixture was stirred at room temperature for 16 h, then aqueous sodium sulfate (4%, 25 mL) added and the reaction mixture acidified to pH 1 with hydrochloric acid (6 M). The aqueous mixture was extracted with diethyl ether (4 × 20 mL), and the combined organic layers dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol/acetic acid (25-40% ethyl acetate, 1% acetic acid) to yield diacid **152** as a white solid (158 mg, 60%). δ_H (400 MHz, MeOD) 6.95 (1H, t, *J* 7.5, 4-H), 6.44 (1H, s, 1-*H*H), 5.60 (1H, s, 1-H*H*), 2.18 (2H, app. q, *J* 7.5, 5-H_2), 1.46 (2H, m, 6-H_2), 1.38-1.25 (4H, m, 7-H_2 and 8-H_2), 0.90 (3H, t, *J* 6.5, 9-H_3); δ_C (100 MHz, MeOD) 168.2 (C=O), 167.9 (C=O), 145.6 (C-4), 136.4 (C-3), 130.8 (C-2), 128.3 (C-1), 31.2 (C-7), 29.0 (C-5), 28.0 (C-6), 22.0 (C-8), 12.9 (C-9); ν_{max} (film) 2956, 2928, 2859, 2650, 1683, 1620, 1426, 1281; HRMS (ESI) calc for $C_{11}H_{16}NaO_4$ $[M+Na]^+$ 235.0941, found 235.0930.

(E)-3-Hexylidene-4-methylenedihydrofuran-2,5-dione 154

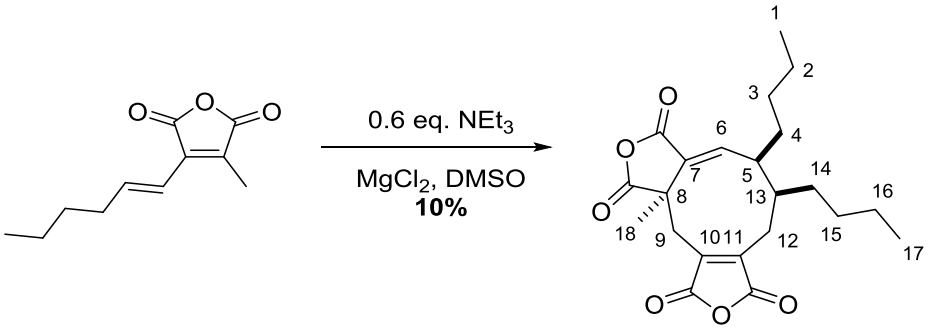


152 **154**

Diacid **152** (55 mg, 0.25 mmol) in dry DCM (2 mL) was cooled to −30 °C, then acetyl chloride (0.1 mL, 6 equiv.) added dropwise. The temperature was maintained at −30 °C for 96 h, after which the solvent was removed under first a stream of N_2 then *in vacuo*, maintaining an internal temperature of −30 °C. Pre-cooled carbon tetrachloride (2 mL) was added, with an aliquot (0.8 mL) then removed after 0.25 h for NMR analysis. The remaining solution was concentrated *in vacuo* at −30 °C, and used immediately without further purification. δ_H (500 MHz, CCl_4) 7.10 (1H, br. s, 4-H), 6.50 (1H, br. s, 1-*H*H), 6.04 (1H, br. s, 1-H*H*), 2.70-1.90 (2H, m, 5-H_2), 1.75-1.08 (6H, m, 6-H_2, 7-H_2, 8-H_2), 1.00-0.76 (3H, m, 9-H_3); δ_C (125 MHz, CCl_4) 162.3 (C=O), 162.2 (C=O), 147.1 (C-4), 130.7 (C-3), 123.4 (C-1), 122.4 (C-2), 31.4 (C-6, C-7 or C-8), 29.6 (C-5), 27.7 (C-6, C-7 or C-8), 22.3 (C-6, C-7 or C-8), 13.8 (C-9); δ_H (400 MHz, $CDCl_3$) 7.21 (1H, t, *J* 7.0, 4-H), 6.56 (1H, s, 1-*H*H), 6.12 (1H, s, 1-H*H*), 2.52 (2H, q, *J* 7.0, 5-H_2), 1.66-1.57 (2H, m, CH_2), 1.40-1.20 (4H, m, 2 × CH_2), 0.93-0.85 (3H, m, 9-H_3)

Compound rapidly decomposes to multiple products, including **146**. IR and MS could not be collected, nor would MS be instructive.

***Iso*-glaucanic acid analogue - (5*S*,6*S*,10a*R*,*E*)-5,6-dibutyl-10a-methyl-5,6,10a,11-tetrahydro-1H-cyclonona[1,2-c:4,5-c']difuran-1,3,8,10(4H)-tetraone 155**

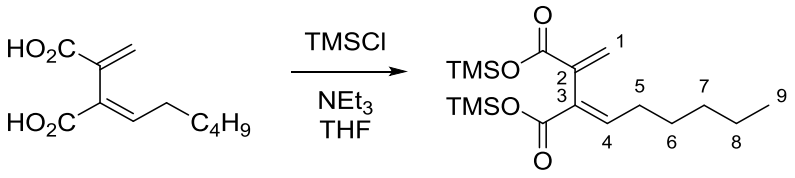


146 **155**

To $MgCl_2$ (13 mg, 0.14 mmol, 0.5 equiv.) in dry DMSO (3.6 mL) was added anhydride **146** (50 mg, 0.26 mmol) and triethylamine (23 µL, 17 mg, 0.17 mmol, 0.65 equiv.). The purple solution was stirred at room temperature for 16 h, then quenched by the addition of hydrochloric acid (5 mL, 3 M). The orange solution was extracted with diethyl ether (3 × 20 mL), then the combined organic layers were weashed with water (20 mL) and brine (20 mL). After drying over $MgSO_4$, filtration, and concentration *in vacuo*, the crude material was purified by column chromatography using diethyl ether/petrol/formic acid (10-30% ethyl acetate, 2% formic acid) to give **155** as a yellow oil (5 mg, 10%). δ_H (500 MHz, $CDCl_3$) 6.93 (1H, d, *J* 11.5, 6-H), 3.26 (1H, d, *J* 13.5, 9-*H*H), 2.77 (1H, dd, *J* 13.5, 3.5, 12-*H*H), 2.63 (1H, d, *J* 13.5, 9-H*H*), 2.35 (1H, dd, *J* 13.5, 5.0, 12-H*H*), 1.99-1.86 (2H, m, 5-H and 13-H), 1.75-1.60 (2H, m, 4-*H*H and 14-*H*H), 1.53 (3H, s, 18-H_3), 1.38-1.12 (10H, m, 4-H*H*, 14-H*H*, 2-H_2, 3-H_2, 15-H_2, 16-H_2), 0.97 (3H, t, *J* 7.5, 1-H_3), 0.86 (3H, t, *J* 7.5, 17-H_3); δ_C (125 MHz, $CDCl_3$) 173.8 (C=O), 165.6 (C=O), 165.3 (C=O), 164.2 (C=O), 152.3 (C-6), 147.3 (C-10), 141.4 (C-11), 129.9 (C-7), 48.8 (C-8), 43.1 (C-5), 32.9 (C-9), 31.7 (C-4), 30.3 (C-15), 30.2 (C-3), 29.6 (C-14), 23.2 (C-16), 22.9 (C-2), 20.3 (C-18), 14.2 (C-17), 14.0 (C-1).

NMR data in accordance with literature.[52]

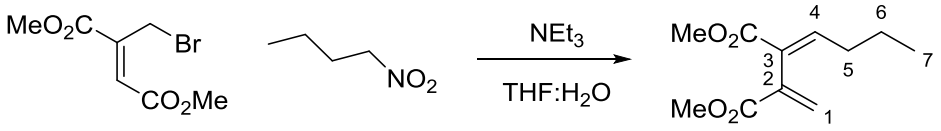
Bis(trimethylsilyl) (*E*)-2-hexylidene-3-methylenesuccinate 158



152 **158**

To a solution of diacid **152** (100 mg, 0.47 mmol) in dry THF (5 mL) was added triethylamine (0.15 mL, 110 mg, 1.04 mmol, 2.2 equiv.), then the reaction mixture was cooled to 0 °C. Chlorotrimethylsilane (0.13 mL, 113 mg, 1.04 mmol, 2.2 equiv.) was added, and the reaction mixture allowed to warm to room temperature over 1 h. The resulting mixture was filtered through Celite® under a nitrogen atmosphere, and the filtrate washed with dry THF (2 × 5 mL). The residue was concentrated *in vacuo* and used without further purification (122 mg ,73%). δ_H (400 MHz, $CDCl_3$) 6.94 (1H, t, *J* 8.0, 4-H), 6.45 (1H, d, *J* 2.0, 1-*H*H), 5.56 (1H, d, *J* 2.0, 1-H*H*), 2.16 (2H, q, *J* 8.0, 5-H_2), 1.52 – 1.22 (6H, m, 6-H_2, 7-H_2, 8-H_2), 0.96 – 0.83 (3H, m, 9-H_3), 0.29 (18H, m, 2 × OSi(C$\underline{H}_3$)$_3$); δ_C (125 MHz, $CDCl_3$) 170.6 (C=O), 170.1 (C=O), 144.9 (C-4), 138.2 (C-3), 132.6 (C-2), 127.8 (C-1), 31.4 (C-5), 29.2, 28.6, 22.4 (C-6, C-7 and C-8), 13.9 (C-9), 0.96 (OSi(C$\underline{H}_3$)$_3$).

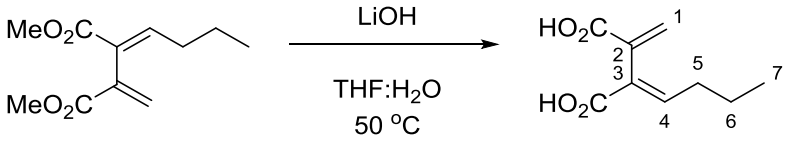
Dimethyl (*E*)-2-butylidene-3-methylenesuccinate 166



68 **S9** **166**

1-nitrobutane **S9** (425 mg, 4.11 mmol, 1.3 equiv.) and triethylamine (2.20 mL, 1.60 g, 15.8 mmol, 5 equiv.) were dissolved in THF (20 mL) and water (20 mL) and stirred for 2 h. The reaction mixture was cooled to 0 °C, bromide **68** (750 mg, 3.21 mmol) in THF (7 mL) was added over 8 h, and then the reaction mixture stirred for an additional 16 h. The orange solution was diluted with water (20 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (1-4% ethyl acetate) to give diester **166** as a colourless oil (314 mg, 47%). δ_H (400 MHz, $CDCl_3$) 6.97 (1H, t, *J* 7.5, 4-H), 6.46 (1H, d, *J* 1.5, 1-*H*H), 5.55 (1H, d, *J* 1.5, 1-H*H*), 3.71 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 2.12 (2H, q, *J* 7.5, 5-H_2), 1.44 (2H, q, *J* 7.5, 6-H_2), 0.89 (3H, t, *J* 7.5, 7-H_3); δ_C (100 MHz, $CDCl_3$) 166.8 (C=O), 166.5 (C=O), 146.4 (C-4), 135.3 (C-3), 130.0 (C-2), 129.5 (C-1), 52.2 (OCH_3), 52.0 (OCH_3), 31.6 (C-5), 22.0 (C-6), 13.9 (C-7); ν_{max} (film) 3416, 2959, 1935, 1719, 1459, 1145; HRMS (ESI) calc for $C_{11}H_{16}NaO_4$ $[M+Na]^+$ 235.0941, found 235.0945.

(*E*)-2-Butylidene-3-methylenesuccinic acid 167

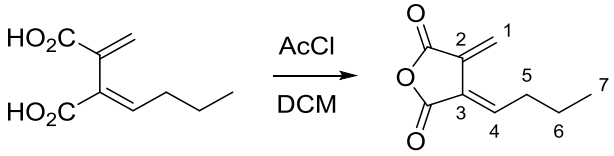


166 **167**

To a solution of diester **166** (500 mg, 2.4 mmol) in water (40 mL) and THF (40 mL) was added lithium hydroxide (1.13 g, 47 mmol, 20 equiv.). The reaction mixture was heated to 50 °C for 16 h, then cooled to room temperature. Saturated aqueous $NaHCO_3$ solution (15 mL) was added, then the reaction mixture was extracted with diethyl ether (3 × 20 mL). The aqueous layer was acidified to pH 4 with solid citric acid, then extracted with diethyl ether (4 × 20 mL). The combined organic layers were then extracted with water (5 × 20 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give diacid **167** as an amorphous white solid (388 mg, 89%). δ_H (400 MHz, $CDCl_3$) 7.14 (1H, t, *J* 7.5, 4-H), 6.62 (1H, d, *J* 1.5, 1-*H*H), 5.71 (1H, d, *J* 1.5, 1-H*H*), 2.20 (2H, q, *J* 7.5, 5-H_2), 1.49 (2H, q, *J* 7.5, 6-H_2), 0.93 (3H, t, *J* 7.5, 7-H_3); δ_C (125 MHz, $CDCl_3$) 171.9 (C=O), 171.7 (C=O), 149.0 (C-4), 134.8 (C-3), 131.5 (C-3), 129.5 (C-1), 31.8 (C-5), 22.0 (C-6), 14.0 (C-7); ν_{max} (film) 2963, 2660, 2570, 1675, 1624, 1436, 1295, 1145; HRMS (ESI) calc for $C_9H_{12}O_4$ $[M\text{-}H]^-$ 183.0663, found 188.0659.

NMR on addition of 0.5 eq. of DABCO: δ_H (500 MHz, $CDCl_3$) 6.96 (1H, t, *J* 7.5, 4-H), 6.43 (1H, d, *J* 1.5, 1-*H*H), 5.56 (1H, d, *J* 1.5, 1-H*H*), 2.19 (2H, q, *J* 7.5, 5-H_2), 1.46 (2H, q, *J* 7.5, 6-H_2), 0.92 (3H, t, *J* 7.5, 7-H_3); δ_C (125 MHz, $CDCl_3$) 171.6 (C=O), 171.2 (C=O), 146.7 (C-4), 137.1 (C-3), 131.5 (C-2), 129.2 (C-1), 31.7 (C-5), 22.2 (C-6), 14.0 (C-7).

(*E*)-3-Butylidene-4-methylenedihydrofuran-2,5-dione 139



167 **139**

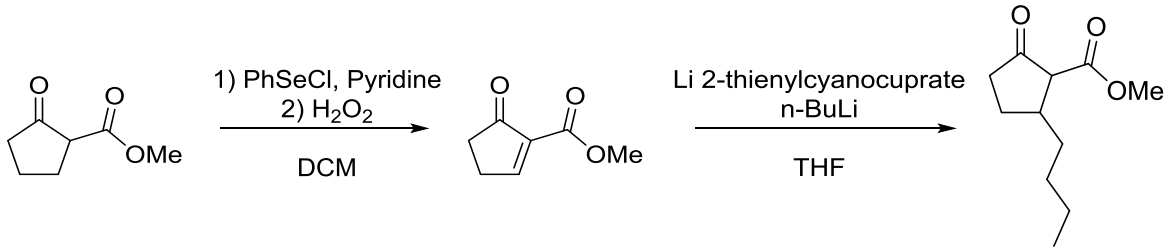
Diacid **167** (75 mg, 0.41 mmol) in dry DCM (2 mL) was cooled to −20 °C, then acetyl chloride (0.1 mL, 6 equiv.) added dropwise. The temperature was maintained at −20 °C for 96 h, after which the solvent was removed under first a stream of N_2 then *in vacuo*, maintaining an internal temperature of −30 °C. Pre-cooled carbon tetrachloride (2 mL) was added, with an aliquot (0.8 mL) then removed after 0.25 h for NMR analysis. δ_H (500 MHz, $CDCl_3$) 7.24 (1H, t, *J* 7.5, 4-H), 6.59 (1H, s, 1-*H*H), 6.13 (1H, s, 1-H*H*), 2.52 (2H, q, *J* 7.5, 5-H_2), 1.68 (2H, q, *J* 7.5, 6-H_2), 1.04 (3H, t, *J* 7.5, 7-H_3); δ_C (125 MHz,

$CDCl_3$) 163.9 (C=O), 163.8 (C=O), 149.3 (C-4), 130.4 (C-3), 125.3 (C-1), 122.3 (C-2), 32.0 (C-5), 21.6 (C-6), 14.0 (C-7).

Compound rapidly decomposes to multiple products. IR and MS could not be collected, nor would MS be instructive.

7.2.4 Experimental Procedures for Chapter 5

Methyl 2-butyl-5-oxocyclopentane-1-carboxylate 193



188 **189** **193**

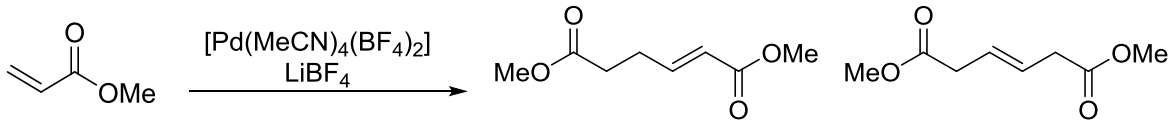
Phenylselenyl chloride (376 mg, 1.84 mmol, 1.05 equiv.) in dry DCM (12 mL) was cooled to 0 °C, and pyridine (0.16 mL, 1.94 mmol, 1.1 equiv.) added. After 0.25 h, 2-methoxycarbonylcyclopentanone **188** (0.218 mL, 1.76 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was washed with hydrochloric acid (1 M, 2 × 10 mL) and the organic layer treated with hydrogen peroxide (30%, 0.1 mL) at 0 °C. After 0.25 h, an additional portion of hydrogen peroxide (0.3 mL) was added, and the reaction mixture stirred for 1 h. Water (5 mL) was added, and the organic layer separated and washed with saturated aqueous $NaHCO_3$ (5 mL). After drying over $MgSO_4$, filtration and concentration *in vacuo* gave methyl 5-oxocyclopent-1-ene-1-carboxylate **189** (165 mg) as a yellow oil which was used without further purification.

A solution of lithium 2-thienylcyanocuprate (4.7 mL, 0.25 M in THF, 1.1 mmol, 1.1 equiv.) was cooled to −78 °C, and *n*-BuLi (0.77 mL, 1.54 M in hexanes, 1 equiv.) added dropwise. The resulting green solution was stirred for 1 h at −78 °C, then a solution of methyl 5-oxocyclopent-1-ene-1-carboxylate **189** (165 mg, 1.00 mmol) in THF (2 mL) was added dropwise. After 1.5 h, the now yellow solution was deemed complete by TLC and quenched with saturated aqueous NH_4Cl solution, and stirred for 0.5 h at room temperature. The layers were separated, and the organic phase washed with saturated aqueous NH_4Cl (3 × 15 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using EtOAc/petrol (1-20% EtOAc) to give methyl 2-butyl-5-oxocyclopentane-1-carboxylate **193** (114 mg, 38% over 2 steps) as a colourless oil; δ_H (400 MHz, $CDCl_3$) 3.74 (3H, s, OCH_3), 2.82 (1H, dd, *J* 11.0, 1.1, 1-H), 2.58 (1H, m, 2-H), 2.48-2.14 (3H, m, 4-H_2 and 3-H), 1.60-1.50 (1H, m, 6-H), 1.44 (2H, m, 3-H and 6-H), 1.37-1.22 (4H, m,

7-H_2 and 8-H_2), 0.95-0.83 (3H, m, CH_3); δ_C (100 MHz, $CDCl_3$) 170.2 (C=O), 62.0 (C-1), 52.5 (OCH_3), 41.5 (C-2), 38.6 (C-4), 34.8 (C-6), 29.4 (CH_2), 27.5 (C-3), 22.8 (CH_2), 14.1 (CH_3); HRMS (ESI) calc for $C_{11}H_{18}NaO_3$ $[M+Na]^+$ 221.1148, found 221.1152

Spectroscopic data in accordance with literature.[189]

Dimethyl (*E*)-hex-2-enedioate 195



194 **195** **S10**

A mixture of methyl acrylate (10.5 mL, 122 mmol), lithium tetrafluoroborate (1.86 g, 19.8 mmol, 0.17 equiv.) and $[Pd(MeCN)_4(BF_4)_2]$ (259 mg, 0.58 mmol, 0.005 equiv.) was heated at 40 °C for 27 h. The reaction mixture was cooled to room temperature, then quenched with saturated aqueous $NaHCO_3$ solution (10 mL) and extracted with diethyl ether (3 × 30 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Distillation under reduced pressure (146 °C, 40 Torr) gave dimethyl hex-2-enedioate **195** and dimethyl hex-3-enedioate **S10** (1.149 g, 11%) as a colourless oil.

Dimethyl hex-2-enedioate **195**:

δ_H (400 MHz, $CDCl_3$): 6.92 (1H, dt, *J* 15.5, 6.5, 3-H), 5.84 (1H, dt, *J* 15.5, 1.5, 2-H), 3.70 (3H, s, OCH_3), 3.66 (3H, s, OCH_3) 2.56-2.42 (4H, m, 4-H_2 and 5-H_2); δ_C (101 MHz, $CDCl_3$): 172.7 (C=O), 166.8 (C=O), 146.9 (C-3), 122.0 (C-2), 51.9 (OCH_3), 51.6 (OCH_3), 32.3 (C-5), 27.3 (C-4).

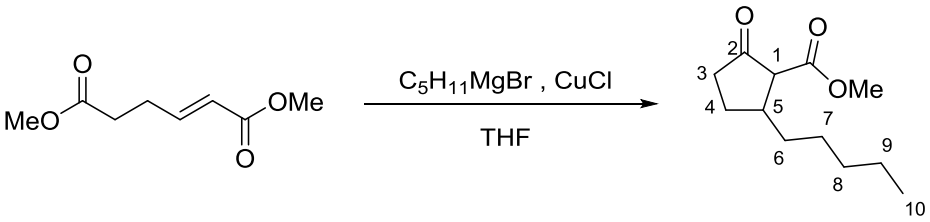
Spectroscopic data in accordance with literature.[253]

Dimethyl hex-3-enedioate **S10**:

δ_H (400 MHz, $CDCl_3$): 5.69-5.66 (2H, m, 3-H and 4-H), 3.65 (3H, s, OCH_3) 3.09-3.06 (4H, m, 2-H_2 and 5-H_2); δ_C(101 MHz, $CDCl_3$): 172.0 (C=O), 126.0 (C-3 and C-4), 52.0 (OCH_3), 37.8 (C-2 and C-5).

Spectroscopic data in accordance with literature.[192]

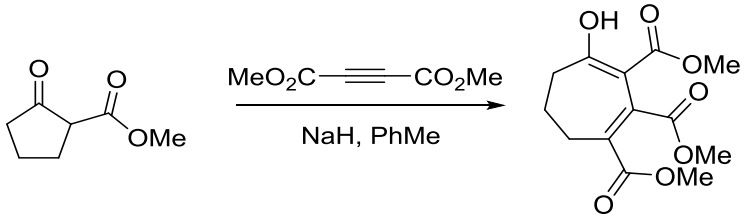
Methyl 2-oxo-5-pentylcyclopentane-1-carboxylate 196



195 **196**

To a solution of pentylmagnesium bromide (4.65 mL, 2 M in THF, 9.3 mmol, 2 equiv.) in THF (5 mL) under an N_2 atmosphere was added copper (I) chloride (23 mg, 0.23 mmol, 0.05 equiv.) and the reaction mixture cooled to 0 °C. Dimethyl (*E*)-hex-2-enedioate **195** (800 mg, 4.65 mmol) in THF (10 mL) was added dropwise, and the resulting solution stirred at 0 °C for 50 mins, then at room temperature for 20 mins. The reaction was quenched with sat. NH_4Cl solution (5 mL) and extracted with chloroform (3 × 20 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (5-15% ethyl acetate) to give a diastereomeric mixture of ketoester **196** (647 mg, 66%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 3.74 (3H, s, OCH_3), 2.82 (1H, dd, *J* 11.0, 1.0, 1-H), 2.58 (1H, m, 5-H), 2.47-2.27 (2H, m, 3-H_2), 2.23 (1H, m, 4-H), 1.65-1.22 (9H, m, 4-H, 6-H_2, 7-H_2, 8-H_2 and 9-H_2), 0.88 (3H, t, *J* 7.0, CH_3); δ_C (100 MHz, $CDCl_3$) 170.2 (C=O), 62.1 (C-1), 52.5 (OCH_3), 41.6 (C-5), 38.6 (C-3), 35.1 (CH_2), 31.9 (CH_2), 27.5 (C-4), 27.0 (CH_2), 22.7 (CH_2), 14.1 (C-10); ν_{max} (film) 2955, 2926, 2857, 1757, 1727, 1458, 1435, 1259, 1122; HRMS (ESI) calc for $C_{12}H_{20}NaO_3$ $[M+Na]^+$ 235.1310, found 235.1265.

Trimethyl 4-hydroxycyclohepta-1,3-diene-1,2,3-tricarboxylate 198



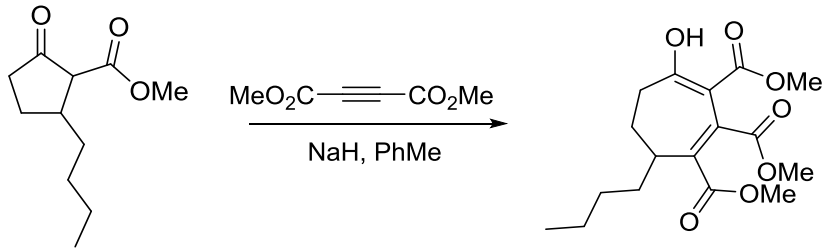
188 **198**

To sodium hydride (60% dispersion in mineral oil, 1.83 g, 45.7 mmol, 1.3 equiv.) in dry toluene (45 mL) was added β-ketoester **188** (4.30 mL, 4.92 g, 34.6 mmol) with stirring at 0 °C under an N_2 atmosphere. After 1 h, dimethylacetylenedicarboxylate (4.8 mL, 5.49 g, 38.6 mmol, 1.1 equiv.) was added over 10 mins. After 4 h, the reaction was cooled to 0 °C before adding glacial acetic acid (10 mL) dropwise. Hydrochloric acid (2 M, 100 mL) was added, and the aqueous layer separated and washed with toluene (3 × 50 mL). The combined organic phases were washed with water (50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a red oil. The crude material was purified by column chromatography using ethyl acetate/petrol (10-50% ethyl acetate) to give trimethyl 4-hydroxycyclohepta-1,3-diene-1,2,3-tricarboxylate **198** as needles (8.31 g, 83%), m.p.

(methanol) 101-103 °C (lit. 106 °C)[184]; δ_H (400 MHz, $CDCl_3$): 13.04 (1H, s, OH), 3.78 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 3.73 (3H, s, OCH_3), 2.49-2.37 (4H, m, 2 × CH_2), 2.30 (2H, m, CH_2); δ_C (100 MHz, $CDCl_3$) 182.3 (C-4), 170.9 (C=O), 169.0 (C=O), 167.4 (C=O), 138.6 and 134.7 (C-1 and C-2), 98.9 (C-3), 52.5 (OCH_3), 52.4 (OCH_3), 52.1 (OCH_3), 33.8, 31.7 and 28.4 (CH_2).

Spectroscopic data in accordance with literature.[184]

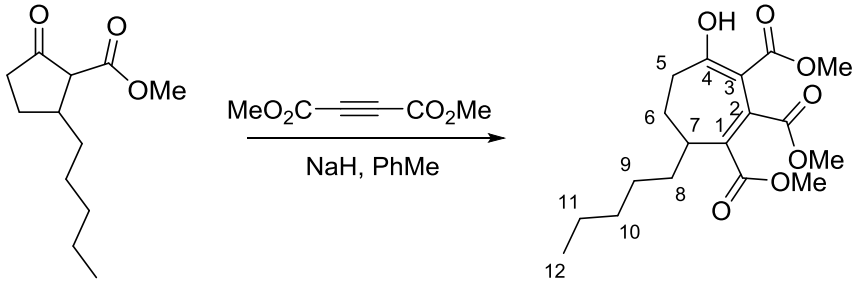
Trimethyl 7-butyl-4-hydroxycyclohepta-1,3-diene-1,2,3-tricarboxylate 199



193 **199**

To sodium hydride (60% dispersion in mineral oil, 32 mg, 0.81 mmol, 1.6 equiv.) in dry toluene (3 mL) was added a solution of methyl 2-butyl-5-oxocyclopentane-1-carboxylate **193** (104 mg, 0.51 mmol) in toluene (5 mL) with stirring at 0 °C. After 1 h, dimethylacetylenedicarboxylate (68 µL, 79 mg, 0.56 mmol, 1.1 equiv.) was added. After 3 h, the reaction was cooled to 0 °C before adding glacial acetic acid (0.30 mL). Hydrochloric acid (1 M, 5 mL) was added, and the aqueous layer separated and washed with toluene (3 × 20 mL). The combined organic phases were washed with water (5 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a red oil. The crude material was purified by column chromatography using ethyl acetate/petrol (5-15% ethyl acetate) to give triester **199** as a colourless oil (78 mg, 46 %); δ_H (400 MHz, $CDCl_3$): 12.65 (1H, s, OH), 3.79 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 3.67 (3H, s, OCH_3), 2.46 (3H, m, 7-H, 5-H and 6-H), 2.26 (1H, m, 5-H), 1.96 (1H, m, 6-H), 1.62 (1H, m, 8-H), 1.24 (5H, m, 8-H, 9-H_2 and 10-H_2), 0.84 (3H, t, *J* 6.7, CH_3); δ_C (100 MHz, $CDCl_3$) 179.9 (C-4), 170.9 (C=O), 169.0 (C=O), 166.1 (C=O), 147.9 (C-1 or C-2), 127.9 (C-1 or C-2), 97.7 (C-3), 52.1 (OCH_3), 52.0 (OCH_3), 51.7 (OCH_3), 39.7 (C-7), 38.9 (C-6), 31.4 (C-8), 30.9 (C-5), 29.9, 22.7 (C-9 and C-10), 13.9 (C-11); ν_{max} (film) 2953, 2928, 2860, 1724, 1651, 1600, 1441, 1331, 1305, 1213; HRMS (ESI) calc for $C_{17}H_{24}NaO_7$ $[M+Na]^+$ 363.1414, found 363.1415

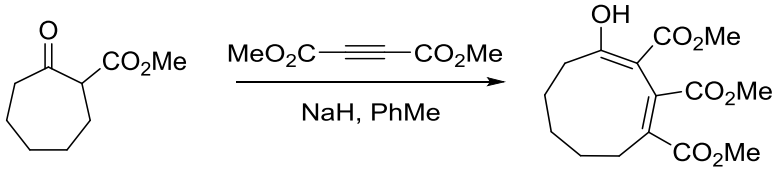
Trimethyl 4-hydroxy-7-pentylcyclohepta-1,3-diene-1,2,3-tricarboxylate 200



196 **200**

To sodium hydride (60% dispersion in mineral oil, 93 mg, 2.43 mmol, 1.5 equiv.) in dry toluene (16 mL) was added a solution of ketoester **196** (344 mg, 1.64 mmol) in toluene (4 mL) with stirring at 0 °C. After 1 h, dimethylacetylenedicarboxylate (230 µL, 258 mg, 1.82 mmol, 1.1 equiv.) was added. After 4 h, the reaction was cooled to 0 °C before adding glacial acetic acid (1 mL). Hydrochloric acid (1 M, 20 mL) was added, and the aqueous layer separated and washed with toluene (3 × 20 mL). The combined organic phases were washed with water (10 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a red oil. The crude material was purified by column chromatography using ethyl acetate/petrol (5-10% ethyl acetate) to give triester **200** as a colourless oil (357 mg, 61%). δ_H (400 MHz, $CDCl_3$) 12.67 (1H, s, OH), 3.81 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 2.62-2.38 (3H, m, 7-H, 5-H and 6-H), 2.31 (1H, m, 5-H or 6-H), 1.98 (1H, m, 5-H or 6-H), 1.63 (1H, m, 8-H), 1.37-1.17 (7H, m, 8-H, 9-H_2, 10-H_2 and 11-H_2), 0.86 (3H, t, *J* 6.9, 12-CH_3); δ_C (100 MHz, $CDCl_3$) 179.9 (C-4), 170.9 (C=O), 169.0 (C=O), 166.1 (C=O), 148.0 (C-1 or C-2), 144.1 (C-1 or C-2), 97.7 (C-3), 52.2 (OCH_3), 52.1 (OCH_3), 51.7 (OCH_3), 39.8 (C-7), 38.9 (C-5 or C-6), 31.9 (C-9, C-10 or C-11), 31.7 (C-8), 30.9 (C-5 or C-6), 27.5 (C-9, C-10 or C-11), 22.5(C-9, C-10 or C-11), 14.0 (C-12); HRMS (ESI) calc for $C_{18}H_{26}NaO_7$ $[M+Na]^+$ 377.1571, found 377.1583

Trimethyl 4-hydroxycyclonona-1,3-diene-1,2,3-tricarboxylate 201



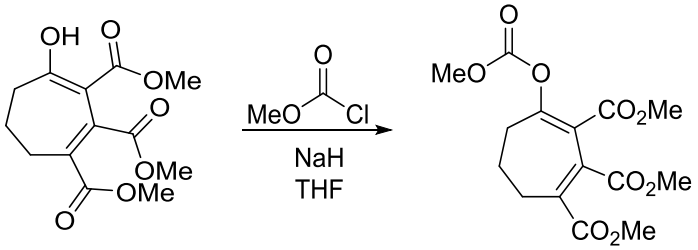
S11 **201**

Trimethyl 4-hydroxycyclonona-1,3-diene-1,2,3-tricarboxylate **201** was prepared from β-ketoester **S11** (500 mg, 2.94 mmol) as above, to give **201** (625 mg, 68%) as needles, m.p. (methanol) 111-112 °C (lit. 113 °C)[184]. δ_H (400 MHz, $CDCl_3$) 12.71 (1H, s, OH), 3.82 (3H, s, OCH_3), 3.72-3.71 (6H, 2 × s, 2 × OCH_3), 2.62 (1H, ddd, 14.0, 6.5, 3.5, C<u>H</u>H), 2.48-2.24 (3H, m, CH_2 and C<u>H</u>H), 1.83-1.53 (5H, m, 2 × CH_2, C<u>H</u>H), 1.44 (1H, m, C<u>H</u>H); δ_C (100 MHz, $CDCl_3$) 179.3 (C-4), 171.6 (C=O), 170.0 (C=O), 166.8 (C=O), 148.1 (C-1), 128.9 (C-2), 98.4 (C-3), 52.5 (OCH_3), 52.4 (OCH_3), 52.1 (OCH_3), 33.6 (CH_2), 33.5

(CH_2), 29.3 (CH_2), 25.4 (CH_2), 24.7 (CH_2). HRMS (ESI) calc for $C_{15}H_{20}NaO_7$ [M+Na] 335.1101, found 335.1104.

Spectroscopic data in accordance with literature.[184]

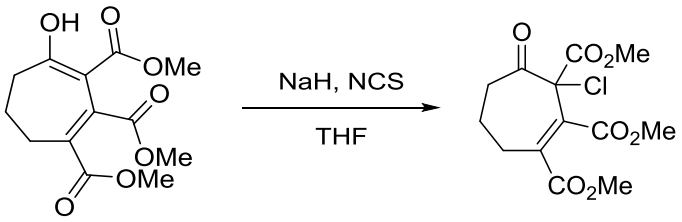
Trimethyl 4-((methoxycarbonyl)oxy)cyclohepta-1,3-diene-1,2,3-tricarboxylate 204



198 **204**

Sodium hydride (42 mg, 1.06 mmol, 1 equiv.) in THF (10 mL) was cooled to 0 °C under an N_2 atmosphere, and triester **198** (300 mg, 1.06 mmol) added in a single portion. After 1 h, methyl chloroformate (0.1 mL, 1.27 mmol, 1.2 equiv.) was added, and the reaction mixture allowed to warm to room temperature. After 4 h, the reaction was quenched by addition of saturated aqueous NH_4Cl (15 mL), and extracted with diethyl ether (2 × 30 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and the solvent removed *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (25-50% ethyl acetate) to give **204** as a colourless oil (350 mg, 97%). δ_H (400 MHz, $CDCl_3$) 3.89 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 3.71 (3H, s, OCH_3), 3.67 (3H, s, OCH_3), 2.50-2.43 (2H, m, CH_2), 2.43-2.35 (4H, m, CH_2); δ_C (100 MHz, $CDCl_3$) 169.4 (C=O), 165.2 (C=O), 163.3 (C=O), 162.7 (C=O), 152.50 ($(RO)_2C$=O), 146.2 (C=C), 130.9 (C=C), 117.8 (C=C), 55.9 (OCH_3), 52.7 (OCH_3), 52.5 (OCH_3), 52.1 (OCH_3), 35.8 (CH_2), 31.4 (CH_2), 29.1 (CH_2); ν_{max} (film) 2955, 1766, 1728, 1436, 1260, 1206; HRMS (ESI) calc for $C_{15}H_{18}NaO_9$ $[M+Na]^+$ 365.0843, found 365.0853.

Trimethyl 3-chloro-4-oxocyclohept-1-ene-1,2,3-tricarboxylate 206

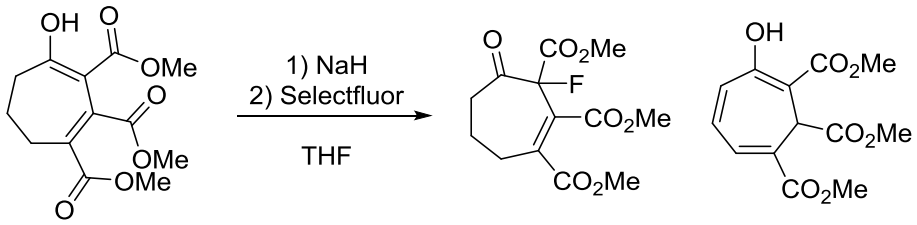


198 **206**

Sodium hydride (51 mg, 1.27 mmol, 1.2 equiv.) in THF (10 mL) was cooled to 0 °C under an N_2 atmosphere, and triester **198** (300 mg, 1.06 mmol) added in a single portion. After 0.5 h, *N*-chlorosuccinimide (155 mg, 1.16 mmol, 1.1 equiv.) was added in a single portion to the yellow solution, and the resulting suspension allowed to warm to room temperature and stirred for 3 h. THF was removed *in vacuo*, and the residue suspended in diethyl ether (20 mL) and extracted with water

(20 mL). The layers were separated, and the aqueous layer extracted with diethyl ether (2 × 20 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and the solvent removed *in vacuo* to give a yellow oil. The crude material was purified by column chromatography using ethyl acetate/petrol (25-35% ethyl acetate) to give **206** as a colourless oil (251 mg, 75%). δ_H (400 MHz, $CDCl_3$): 3.83 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.75 (3H, s, OCH_3), 3.07 (1H, m, C*H*H), 2.85-2.72 (1H, m, CH*H*), 2.60-2.42 (2H, m, CH_2), 2.28-2.08 (2H, m, CH_2); δ_C (100 MHz, $CDCl_3$) 198.1 (C-4), 168.2 (C=O), 164.9 (d, C=O), 141.0 (C-1 or C-2), 133.8 (C-1 or C-2), 76.0 (C-3), 54.5 (OCH_3), 52.9 (OCH_3), 35.5 (CH_2), 26.9 (CH_2), 24.2 (CH_2); ν_{max} (film) 3694, 3026, 2955, 1773, 1727, 1435, 1270, 756; HRMS (ESI) calc for $C_{13}H_{15}{}^{35}ClNaO_7$ $[M+Na]^+$ 341.0399, found 341.0397.

Trimethyl 3-fluoro-4-oxocyclohept-1-ene-1,2,3-tricarboxylate 209



198 **209** **210**

Sodium hydride (344 mg, 8.58 mmol, 1.2 equiv.) in THF (17 mL) was cooled to 0 °C under an N_2 atmosphere, and triester **198** (1.99 g, 7.04 mmol) added portionwise. After 0.5 h, Selectfluor (2.74 g, 7.74 mmol, 1.1 equiv.) was added in a single portion, and the resulting suspension allowed to warm to room temperature and stirred for 16 h. THF was removed *in vacuo*, and the residue suspended in ethyl acetate and filtered. The filtrate was concentrated *in vacuo*, and the crude material purified by column chromatography using ethyl acetate/petrol (20-50% ethyl acetate) to yield the fluorinated product **209** (697 mg, 34%) as a colourless oil, and by-product **210** (196 mg, 9%) as yellow plates, m.p. (methanol) 112-114 °C.

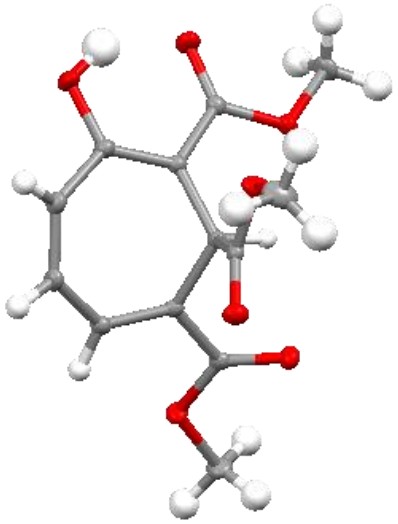
Trimethyl 3-fluoro-4-oxocyclohept-1-ene-1,2,3-tricarboxylate **209**:

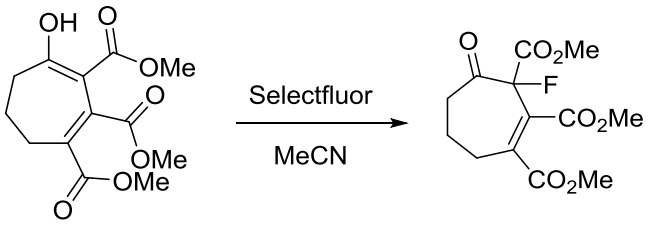
δ_H (400 MHz, $CDCl_3$) 3.83 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 3.74 (3H, s, OCH_3) 2.88 (2H, t, *J* 7.5, 5-H_2), 2.60-2.44 (2H, m, 7-H_2), 2.19-2.05 (2H, m, 6-H_2); δ_C (101 MHz, $CDCl_3$) 199.0 (d, $^2J_{CF}$ 22.0, C-4), 168.3 (C1-C=O), 165.3 (d, $^2J_{CF}$ 26.5, C3-C=O), 164.5 (d, $^3J_{CF}$ 1.5, C2-C=O), 147.1 (d, $^3J_{CF}$ 7.0, C-1), 129.7 (d, $^2J_{CF}$ 20.0, C-2), 96.6 (d, $^1J_{CF}$ 202.0, C-3), 36.7 (d, $^3J_{CF}$ 1.5 ,C-5), 27.9 (C-7), 21.9 (C-6); δ_F(282 MHz, $CDCl_3$): −150.8; ν_{max} (film) 2956, 1776, 1729, 1644, 1436, 1258; HRMS (ESI) calc for $C_{13}H_{15}FNaO_7$ $[M+Na]^+$ 325.0694, found 325.0699.

Trimethyl 4-hydroxycyclohepta-3,5,7-triene-1,2,3-tricarboxylate **210**:

δ_H (400 MHz, $CDCl_3$): 12.22 (1H, s, OH), 7.30 (1H, d, *J* 6.0, 7-H), 6.73 (1H, dd, *J* 12.0 and 6.0, 6-H), 6.63 (1H, d, *J* 12.0, 5-H), 5.51 (1H, s, 2-H), 3.86 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.53 (3H, s, OCH_3); δ_C (100 MHz, $CDCl_3$): 171.5 (C=O), 171.1 (C=O), 166.9 (C-4), 166.1 (C-1-C=O), 133.8 (C-6), 132.7 (C-7), 131.4 and 131.2 (C-5 and C-1), 99.6 (C-3), 37.9(C-2); ν_{max} (film) 2954, 1738, 1713, 1652, 1607, 1566, 1442, 1282, 1233; HRMS (ESI) calc for $C_{13}H_{14}NaO_7$ $[M+Na]^+$ 305.0632, found 305.0632.

Crystal structure obtained, space group C2/c:

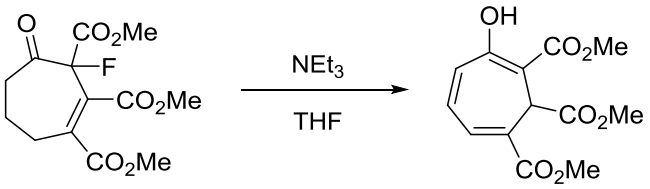




198 **209**

To a solution of triester **198** (1.00 g, 3.52 mmol) in acetonitrile (20 mL) was added Selectfluor (1.41 g, 3.98 mmol, 1.1 equiv.) in one portion, and the resulting white suspension stirred at room temperature for 48 h. DCM (20 mL) and water (20 mL) were added to the now pink solution, and the layers separated. The organic layer was washed with water (20 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to a colourless oil. This was purified by column chromatography using ethyl acetate/petrol (10-35% ethyl acetate) to yield the fluorinated compound **209** (801 mg, 72%). Spectroscopic data as previously described.

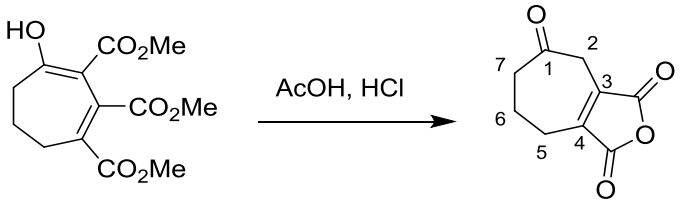
Trimethyl 4-hydroxycyclohepta-3,5,7-triene-1,2,3-tricarboxylate 210



209 **210**

To a solution of fluoro-ketoester **209** (1.51 g, 5.0 mmol) in dry THF (12 mL) was added triethylamine (0.85 mL, 6.0 mmol, 1.2 equiv.), and the resulting yellow solution stirred for 1.5 h at room temperature. Half saturated NH_4Cl (20 mL) was added, and the reaction mixture extracted with DCM (3 × 40 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to a waxy yellow solid. Recrystallisation from methanol gave triene **210** (1.20 g, 85%) as yellow plates. Spectroscopic data as previously described.

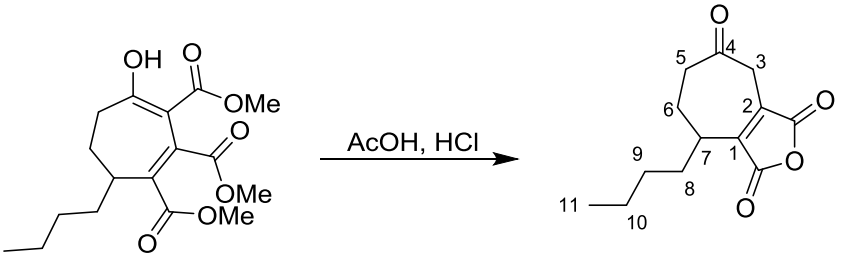
1-Oxo-cyclohept-3-ene-3,4-dicarboxylic acid anhydride 215



198 **215**

To triester **198** (201 mg, 0.71 mmol) was added glacial acetic acid (2 mL) and concentrated hydrochloric acid (37%, 2 mL). The reaction mixture was heated at 70 °C for 72 h, then concentrated *in vacuo* to give anhydride **215** (107 mg, 84%) as a brown oil; δ_H (400 MHz, $CDCl_3$): 3.65 (2H, t, *J* 2.5, 2-H_2), 2.77-2.69 (4H, m, 5-H_2 and 7-H_2), 2.16-2.09 (2H, m, 6-H_2); δ_C (100 MHz, $CDCl_3$) 203.8 (C-1), 164.9 (C=O), 164.7 (C=O), 144.7, 135.6 (C-3 and C-4), 43.5 (C-7), 38.8 (C-2), 25.9 (C-5), 19.5 (C-6); ν_{max} (film) 1835, 1762, 1711, 1601, 1422, 1268; HRMS (ESI) calc for $C_9H_8NaO_4$ $[M+Na]^+$ 203.0315, found 203.0208.

1-Oxo-5-butylcyclohept-3-ene-3,4-dicarboxylic acid anhydride 216

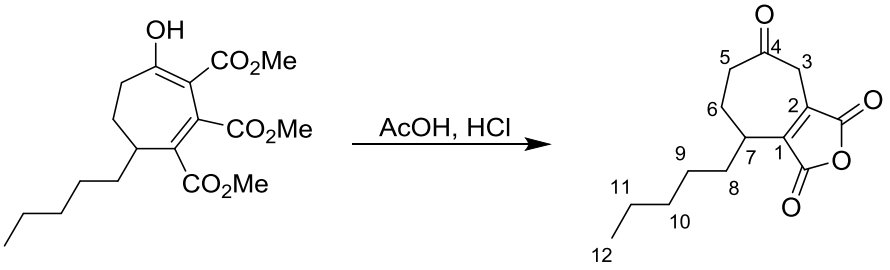


199 **216**

To triester **199** (43 mg, 0.13 mmol) was added glacial acetic acid (3 mL) and concentrated hydrochloric acid (37%, 3 mL). The reaction mixture was heated at 90 °C for 24 h, then concentrated *in vacuo*, adding toluene (3 × 10 mL) to remove residual acetic acid as its azeotrope. The crude material was purified by column chromatography using ethyl acetate/petrol (10-25% ethyl acetate) to give anhydride **216** as a yellow oil (16 mg, 53%). δ_H (400 MHz, $CDCl_3$) 3.65 (2H, s, 3-H_2), 2.95 (1H, m, 7-H), 2.79 (1H, ddd, *J* 17.5, 10.0, 3.0, 5-*H*H), 2.62 (1H, ddd, *J* 17.5, 8.0, 3.0, 5-H*H*), 2.20-2.03 (2H, m, 6-H_2), 1.81 (1H, m, 8-*H*H), 1.52 (1H, m, 8-H*H*), 1.42-1.29 (4H, m, 9-H_2 and 10-H_2), 0.92 (3H, t, *J* 6.0, 11-H_3); δ_C (100 MHz, $CDCl_3$) 203.8 (C-4), 164.7 (C=O), 164.6 (C=O), 148.3 (C-1), 135.2 (C-2), 40.2 (C-5), 38.6 (C-3), 36.2 (C-7), 31.4 (C-8), 29.0 (C-9 or C-10), 23.9 (C-6), 22.6 (C-9 or C-10), 14.0 (C-11); ν_{max} (film) 2924, 2863, 1830, 1767, 1718, 1599, 1261; HRMS (ESI) calc for $C_{13}H_{16}NaO_4$ $[M+Na]^+$ 259.0946, found 259.0929.

NB: Atom labelling does not correspond to compound name.

1-Oxo-5-pentylcyclohept-3-ene-3,4-dicarboxylic acid anhydride 217

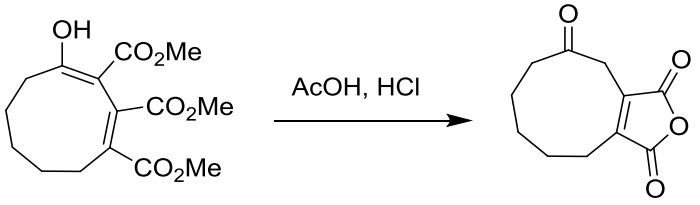


200 **217**

To triester **200** (276 mg, 0.78 mmol) was added glacial acetic acid (3 mL) and concentrated hydrochloric acid (37%, 3 mL). The reaction mixture was heated at 90 °C for 24 h, then concentrated *in vacuo*, adding toluene (3 × 10 mL) to remove residual acetic acid as its azeotrope. The crude material was purified by column chromatography using ethyl acetate/petrol (10-25% ethyl acetate) to give anhydride **217** as a yellow oil (76 mg, 38%). δ_H (400 MHz, $CDCl_3$) 3.63 (2H, s, 3-H_2), 2.93 (1H, m, 7-H), 2.77 (1H, ddd, *J* 17.5, 10.0, 3.0, 5-*H*H), 2.60 (1H, ddd, *J* 17.5, 8.0, 3.0, 5-H*H*), 2.20-2.01 (2H, m, 6-H_2), 1.84-1.73 (1H, m, 8-*H*H), 1.54-1.43 (1H, m, 8-H*H*), 1.43-1.18 (6H, m, 9-H_2, 10-H_2 and 11-H_2), 0.87 (3H, t, *J* 6.5, 12-H_3); δ_C (100 MHz, $CDCl_3$) 203.9 (C-4), 164.7, 164.6 (C=O), 148.2, 135.2 (C-1 and C-2), 40.1 (C-5), 38.5 (C-3), 36.2 (C-7), 31.6 (C-8), 31.5 (C-9, C-10 or C-11), 26.5 (C-9, C-10 or C-11), 23.8 (C-6), 22.5 (C-9, C-10 or C-11), 14.1 (C-12); ν_{max} (film) 2929, 2859, 1831, 1764, 1717, 1598, 1256; HRMS (ESI) calc for $C_{14}H_{18}NaO_4$ $[M+Na]^+$ 273.1103, found 273.1098.

NB: Atom labelling does not correspond to compound name.

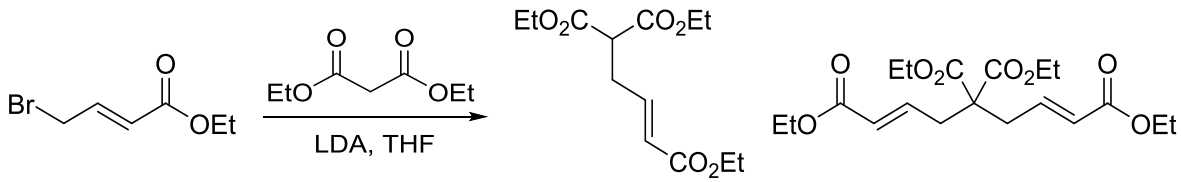
1-Oxo-cyclonona-3-ene-3,4-dicarboxylic acid anhydride 218



201 **218**

To triester **201** (376 mg, 1.20 mmol) was added glacial acetic acid (5 mL) and concentrated hydrochloric acid (37%, 5 mL). The reaction mixture was heated at 90 °C for 24 h, then concentrated *in vacuo*, adding toluene (3 × 10 mL) to remove residual acetic acid as its azeotrope. The crude material was purified by column chromatography using ethyl acetate/petrol (25% ethyl acetate) to give anhydride **218** (190 mg, 76%) as needles, m.p. (methanol) 114-116 °C. δ_H (400 MHz, $CDCl_3$) 3.60 (2H, s, 2-CH_2), 2.67-2.62 (2H, m, 5-H_2), 2.56-2.51 (2H, m, 9-H_2), 1.85-1.79 (2H, m, 8-H_2), 1.79-1.73 (2H, m, 6-H_2), 1.43-1.36 (2H, m, 7-H_2); δ_C (100 MHz, $CDCl_3$) 206.7 (C-1), 165.1 (C=O), 165.1 (C=O), 145.2 (C-4), 137.5 (C-3), 44.9 (C-9), 38.9 (C-2), 25.4 (C-7), 25.1 (C-6), 22.8 (C-8), 22.4 (C-5); ν_{max} (film) 2953, 2869, 1842, 1771, 1698, 1668, 1442, 1253; HRMS (ESI) calc for $C_{11}H_{12}NaO_4$ [M+Na] 231.0633, found 231.0642.

Triethyl (*E*)-but-3-ene-1,1,4-tricarboxylate 225



224 **225** **226**

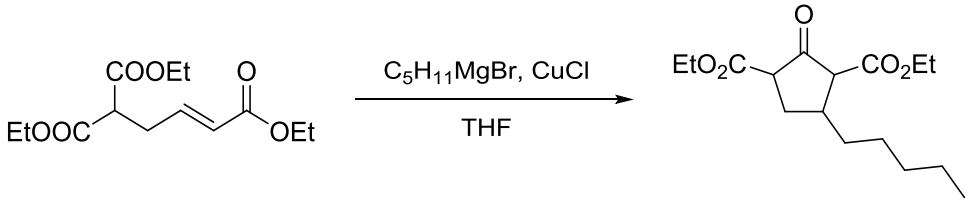
To lithium diisopropylamide (4.4 mmol, 1.1 equiv.) in dry THF (35 mL) at −78 °C was added diethyl malonate (0.62 mL, 650 mg, 4.06 mmol) dropwise. After 1 h, ethyl 4-bromocrotonate **224** (861 mg, 4.46 mmol, 1.1 equiv.) was added at −78 °C, and after stirred at −78 °C for 2 h the reaction mixture was raised to room temperature and stirred overnight. The reaction was quenched with sat. NH_4Cl solution (15 mL) and extracted with dichloromethane (3 × 30 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to an orange oil. The crude material was purified by column chromatography using ethyl acetate/petrol (5-10% ethyl acetate) to give unsaturated ester **225** (493 mg, 44%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 6.87 (1H, dt, *J* 15.5, 7.0, 4-H), 5.89 (1H, ddd, *J* 15.5, 2.0, 1.0, 3-H), 4.29-4.10 (6H, m, OCH_2), 3.48 (1H, t, *J* 7.5, 1-H), 2.78 (2H, td, *J* 7.0, 1.5, 2-H_2), 1.26-1.30 (9H, m, CH_3), δ_C (100 MHz, $CDCl_3$) 168.3 (C=O), 166.0 (C=O), 143.7 (C-4), 123.8 (C-3), 61.7 (OCH_2), 60.4 (OCH_2), 50.7 (C-1), 31.1 (C-2), 14.2 (CH_3), 14.0 (CH_3); HRMS (ESI) calc for $C_{13}H_{20}NaO_6$ $[M+Na]^+$ 295.1158, found 295.1157

Spectroscopic data in accordance with literature.[198]

By-product, diethyl (2*E*,7*E*)-5,5-diethoxycarbonylnona-2,7-dienedioate, **226** isolated in yields of 9-27%. δ_H (400 MHz, $CDCl_3$) 6.75 (2H, dt, *J* 15.5, 7.5, 3-H and 7-H), 5.90 (2H, dt, *J* 15.5, 1.0, 2-H and 8-H), 4.27-4.06 (8H, m, OCH_2), 2.78 (4H, dt, *J* 7.5, 1.0, 4-H_2 and 6-H_2), 1.30-1.19 (12H, m, CH_3), δ_C (100 MHz, $CDCl_3$): 169.8 (C=O), 165.9 (C-1 and C-9), 141.9 (C-3 and C-7), 125.7 (C-2 and C-8), 62.1 (OCH_2), 60.6 (OCH_2), 56.9 (C-5), 35.8 (C-4 and C-6), 14.4 (CH_3), 14.2 (CH_3).

Spectroscopic data in accordance with literature.[254]

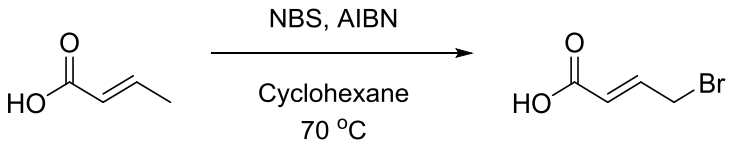
Diethyl 2-oxo-4-pentylcyclopentane-1,3-dicarboxylate 227



225 **227**

To a solution of pentylmagnesium bromide (0.73 mL, 2 M in THF, 1.1 mmol, 2 equiv.) in THF (2 mL) under an N_2 atmosphere, was added copper (I) chloride (4 mg, 0.04 mmol, 0.05 equiv.) and the reaction mixture cooled to 0 °C. Triethyl (*E*)-but-3-ene-1,1,4-tricarboxylate **225** (198 mg, 0.73 mmol) in THF (3 mL) was added dropwise, and the resulting solution stirred at 0 °C for 0.5 h, then at room temperature for 4 h. The reaction was quenched with saturated aqueous NH_4Cl (5 mL) and extracted with chloroform (3 × 50 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (5-10% ethyl acetate) to give a pale green oil, tentatively assigned as a mixture of diastereomers of diethyl 2-oxo-4-pentylcyclopentane-1,3-dicarboxylate **227** (45 mg, 21%). ν_{max} (film) 2982, 1724, 1658, 1369, 1153; HRMS (ESI) calc for $C_{16}H_{26}NaO_5$ $[M+Na]^+$ 321.1672, found 321.1665. ^1H and ^{13}C NMR spectra can be found in the spectral appendix.

4-Bromocrotonic acid 230



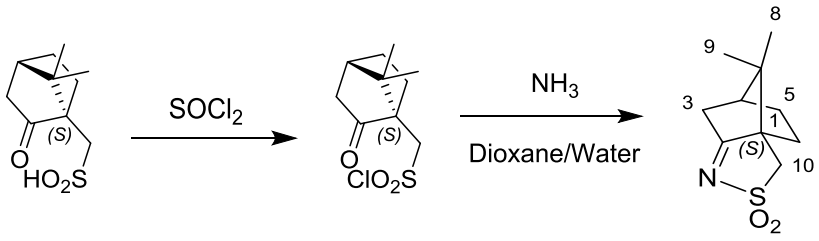
229 **230**

Crotonic acid **229** (6.01 g, 69.7 mmol) and *N*-bromosuccinimide (13.69 g, 76.8 mmol, 1.1 equiv.) were dissolved in cyclohexane (100 mL) and AIBN (239 mg, 1.4 mmol, 0.02 equiv.) added. The reaction mixture was heated to 70 °C for 2 h, then cooled to room temperature. Further AIBN (237 mg, 1.4 mmol, 0.02 equiv.) was added, and the reaction heated to 70 °C for 3 h. The cooled reaction mixture

was filtered, and the filtrate washed with sat. aq. $NaHCO_3$ (3 × 20 mL). The combined aqueous layers were acidified to pH 2 with 2 M HCl, then extracted with ethyl acetate (3 × 30 mL), and the combined organic layers dried over $MgSO_4$ and filtered. The crude material was purified by column chromatography using ethyl acetate/petrol/acetic acid (10% ethyl acetate, 1% acetic acid) to give 4-bromocrotonic acid **230** as a deliquescent white solid (2.708 g, 24%). δ_H(400 MHz, $CDCl_3$) 11.32 (br s, COOH) 7.12 (1H, dt, *J* 15.0, 7.5, 3-H), 6.05 (1H, dt, *J* 15.0, 1.5, 2-H), 4.03 (2H, dd, *J* 7.5, 1.5, 4-H_2); MP: 69-72 °C (lit. 74.7-75.3 °C)[255].

^{1}H NMR in accordance with literature.[255]

(1*S*)-(−)-10,2-Camphorsulfonylimine S14

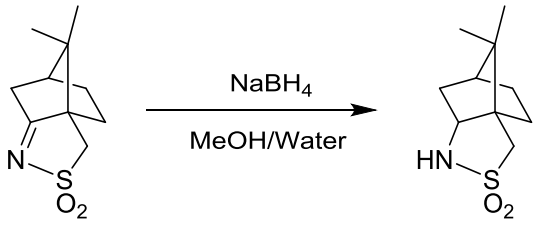


S12 **S13** **S14**

Thionyl chloride (15 mL, 173.1 mmol, 2 equiv.) was added dropwise to (1*S*)-(+)-camphorsulfonic acid **S12** (20.1 g, 86.5 mmol) and the resulting solution heated to reflux at 90 °C for 3 h. DMF (two drops) was then added and the reaction mixture heated at 90 °C for an additional 1 h. Excess thionyl chloride was then removed by evaporation, toluene (2 × 10 mL) was used to remove traces of thionyl chloride by co-distillation. The resulting orange oil **S13** was dissolved in dioxane (25 mL), cooled on ice and concentrated NH_4OH (190 mL) added dropwise. The reaction mixture was stirred overnight at room temperature, and the resulting white precipitate isolated by filtration, and washed with water (2 × 25 mL) to give the camphorsulfonylimine **S14** as a white solid (14.5 g, 79%). $[\alpha]_D^{22}$ -41 (*c* 1.0, $CHCl_3$) [lit. $[\alpha]_D^{14}$ -39 (*c* 1.0, $CHCl_3$)][256]; δ_H (400 MHz, $CDCl_3$) 3.17 (1H, d, *J* 13.0, 10-*H*H), 2.97 (1H, d, J 13.0, 10-H*H*), 2.76 (1H, ddd, *J* 19.0, 4.5, 2.0, 3-*H*H), 2.37 (1H, d, *J* 19.0, 3-H*H*), 2.25 (1H, m, 4-H), 2.01-2.11 (2H, m, 6-H_2), 1.84-1.73 (1H, m, 5-*H*H), 1.53-1.42 (1H, m, 5-H*H*), 1.08 (3H, s, 8-H_3 or 9-H_3), 0.86 (3H, s, 8-H_3 or 9 H_3); δ_C (100 MHz, $CDCl_3$) 195.4 (C), 64.5 (C), 49.4 (CH_2), 47.9 (C), 44.6 (CH), 35.9 (CH_2), 28.4 (CH_2), 26.6 (CH_2), 19.4 (CH_3), 18.9 (CH_3); HRMS (ESI) calc for $C_{10}H_{15}NNaO_2S$ $[M+Na]^+$ 236.0721, found 236.0720.

Spectroscopic data in accordance with literature.[257]

(1*S*,2*R*)-(−)-bornane-10,2-sultam 231

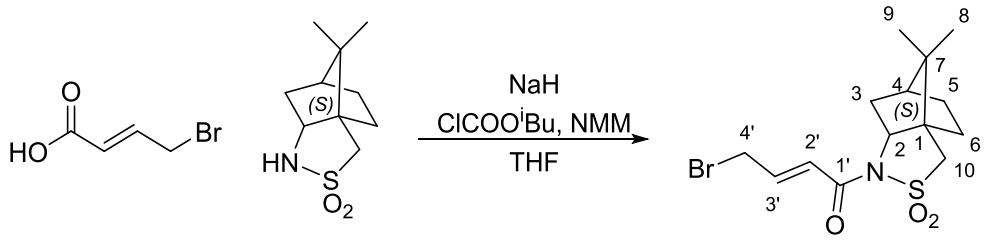


S14 **231**

Sodium borohydride (5.14 g, 135.9 mmol, 2 equiv.) was added portionwise to a solution of camphorsulfonylimine **S14** (14.5 g, 68.0 mmol) in methanol (150 mL) and water (30 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. The methanol was removed *in vacuo* and DCM (100 mL) added. The solution was acidified using sulfuric acid (540 mL, 2 N). The layers were separated, and the aqueous layer extracted with DCM (2 × 150 mL). The combined extracts were washed with brine and dried over $MgSO_4$. After filtration, the dichloromethane was removed *in vacuo*, followed by recrystallisation from ethanol to give (1*S*,2*R*)-bornane-10,2-sultam **231** (10.1 g, 66%) as colourless crystals. $[\alpha]_D^{22}$ -38 (*c* 1.0, $CHCl_3$)) [lit. $[\alpha]_D^{25}$ -33 (*c* 5, $CHCl_3$)][258]; δ_H (400 MHz, $CDCl_3$) 4.11 (1H, br, s, NH), 3.42 (1H, m, 2-H), 3.13 (1H, d, *J* 14.0, 10-*H*H), 3.08 (1H, d, *J* 14.0, 10-H*H*), 2.02-1.80 (5H, m, 3-H_2, 5-*H*H, 6-*H*H and 4-H), 1.44 (1H, m, 6-H*H*), 1.29 (1H, m, 5-H*H*), 1.12 (3H, s, 8-H_3 or 9-H_3), 0.93 (3H, s, 8-H_3 or 9-H_3); δ_C (100 MHz, $CDCl_3$) 63.0 (C-2), 55.3 (C-1), 50.5 (C-10), 47.6 (C-7), 44.9 (C-4), 36.2 (C-3), 32.0 (C-6), 27.0 (C-5), 20.7 and 20.6 (C-8 and C-9); HRMS (ESI) calc for $C_{10}H_{17}NNaO_2S$ $[M+Na]^+$ 238.0878, found 238.0877.

Spectroscopic data in accordance with literature.[257]

(1*S*,2*R*)-*N*-[(4-bromocrotonate]-bornane-10,2-sultam 232



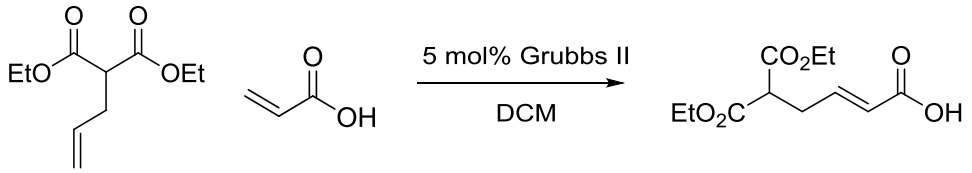
230 **231** **232**

A solution of 10,2-camphorsultam **231** (1.96 g, 9.1 mmol) in dimethoxyethane (15 mL) was added dropwise to a suspension of sodium hydride (60% suspension in mineral oil, 407 mg, 10.0 mmol, 1.1 equiv.) in dimethoxyethane (15 mL). The solution was stirred for 1 h. In parallel, isobutyl chloroformate (1.3 mL, 10.0 mmol, 1.1 equiv.) and *N*-methylmorpholine (1.1 mL, 10 mmol, 1.1 equiv.) were added at −15 °C to a solution of 4-bromocrotonic acid **230** (1.502 g, 9.1 mmol, 1 equiv.) in dimethoxyethane (30 mL). After 0.25 h at −15 °C, this preformed mixed anhydride was filtered under nitrogen through a Celite® pad, directly into the sultam solution. The reaction mixture was

stirred at −15 °C for 1 h, then was allowed to warm to room temperature and stirred for 1 h. The reaction was quenched with water (50 mL) and concentrated *in vacuo*. Ethyl acetate (50 mL) was added, and the organic layer washed with saturated aqueous NH_4Cl, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified via column chromatography using EtOAc/Petrol (5-10% EtOAc) to give camphorsultam derivative **232** as a white solid (2.09 g, 64%). $[\alpha]_D^{22}$ -108 (*c* 1, $CHCl_3$); δ_H(400 MHz, $CDCl_3$) 7.09 (1H, dt, *J* 15.0, 7.5, 3'-H), 6.75 (1H, dt, *J* 15.0, 1.0, 2'-H), 4.06 – 4.02 (2H, m, 4'-H_2), 3.94 (1H, m, 2-H), 3.47 (2H, AB, 10-H_2), 2.22-2.06 (2H, m, 3-H_2), 1.97-1.82 (3H, m, 6-*H*H, 5-*H*H, 4-H), 1.55-1.31 (2H, m, 5-H*H*, 6-H*H*), 1.18 and 0.98 (2 × 3H, 2 × s, 8-H_3 and 9-H_3); δ_C (100 MHz, $CDCl_3$) 163.0 (C-1'), 142.8 (C-2'), 123.7 (C-3'), 65.3 (C-2), 53.3 (C-10), 48.8 (C-7), 48.0 (C-1), 44.8 (C-4), 38.5 (C-3), 33.0 (C-5), 29.3 (C-4'), 26.6 (C-6), 21.0 and 20.0 (C-8 and C-9); MP 108-112 °C (lit. 115 °C)[259]; HRMS (ESI) calc for $C_{14}H_{20}{}^{79}BrNNaO_3S$ $[M+Na]^+$ 384.0239, found 384.0239.

^1H NMR in accordance with literature.[259]

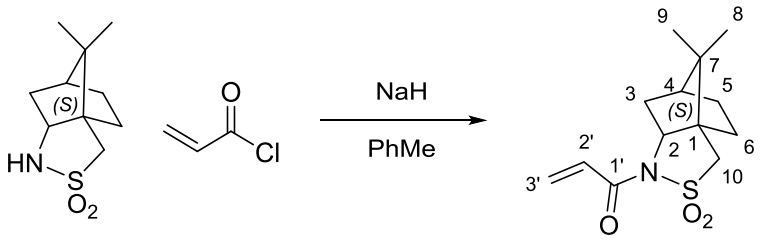
5,5-Di(ethoxycarbonyl)-pent-2-enoic acid 235



233 **234** **235**

To a solution of diethyl allylmalonate **233** (0.2 mL, 1 mmol, 1 equiv.) and acrylic acid **234** (0.1 mL, 1.30 mmol, 1.3 equiv.) in dry DCM (9 mL), was added a solution of G2 (42 mg, 5 mol%) in DCM (1 mL). The resulting red solution was heated at 40 °C for 16 h. Once cooled, the orange solution was diluted with DCM (20 mL), and washed with saturated aqueous $NaHCO_3$ (30 mL). The aqueous layer was further extracted with DCM (20 mL), and then acidified with HCl (1 M, 20 mL). The aqueous layer was extracted with DCM (3 × 50 mL), and the combined organic extracts dried with $MgSO_4$, filtered, and concentrated *in vacuo* to a brown oil. The crude material was purified by column chromatography using EtOAc/petrol (20% EtOAc) to give 5,5-di(ethoxycarbonyl)-pent-2-enoic acid **235** as a yellow oil (186 mg, 76%). δ_H (400 MHz, $CDCl_3$) 7.00 (1H, dt, *J* 15.5, 7.0, 3-H), 5.90 (1H, dt, *J* 15.5, 1.5, 2-H), 4.25-4.17 (4H, 2 × q, *J* 7.0, OCH_2), 3.50 (1H, t, *J* 7.5, 5-H), 2.82 (2H, td, *J* 7.5, 1.5, 4-H_2), 1.27 (6H, t, *J* 7.0, CH_3); δ_C (100 MHz, $CDCl_3$) 171.2 (C-1), 168.4 (C=O), 147.0 (C-3), 123.2 (C-2), 62.0 (OCH_2), 50.6 (C-5), 31.3 (C-4), 14.2 (CH_3); ν_{max} (film) 2982, 1728, 1699, 1655, 1278, 1228, 1156, 1033; HRMS (ESI) calc for $C_{11}H_{15}O_6$ $[M-H]^-$ 243.0869, found 243.0865

(1*S*,2*R*)-*N*-(Acrylamide)-bornane-10,2-sultam 238

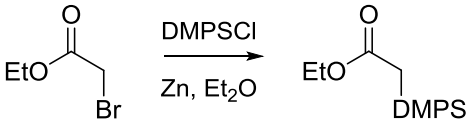


231 **237** **238**

Sultam **231** (0.63 g, 2.97 mmol) was added to a suspension of sodium hydride (185 mg, 4.5 mmol, 1.5 equiv.) in toluene (8 mL) at 0 °C under an N_2 atmosphere. After 1 h, acryloyl chloride **237** (0.30 mL, 3.5 mmol, 1.2 equiv.) was added dropwise, and the reaction mixture allowed to warm to room temperature. After 3 h, the reaction mixture was cooled to 0 °C, and quenched by the addition of water (5 mL). The resulting white solution was extracted with ethyl acetate (3 × 30 mL), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using EtOAc/petrol (10-20% EtOAc) to give Oppolzer's sultam acrylamide **238** (358 mg, 45%) as a white solid. $[\alpha]_D^{22}$ −110 (*c* 1.0, $CHCl_3$) [lit. $[\alpha]_D^{20}$ −99 (*c* 1, CH_2Cl_2)][260]; δ_H (400 MHz, $CDCl_3$) 6.87 (1H, dd, *J* 16.5, 10.5, 2'-H), 6.51 (1H, dd, *J* 16.5, 1.5, 3'-H), 5.86 (1H, dd, *J* 10.5, 1.5, 3'-H), 3.95 (1H, dd, *J* 7.5, 5.0, 2-H), 3.49 (2H, ABq, *J* 13.8, 10-H_2), 2.24-2.05 (2H, m, 3-H_2), 2.02-1.82 (3H, m, 4-H and 6-H_2), 1.50-1.30 (2H, m, 5-H_2), 1.18 and 0.98 (2 × 3H, 2 × s, 8-H_3 and 9-H_3); δ_C (100 MHz, $CDCl_3$) 164.0 (C-1'), 131.5 (C-3'), 127.9 (C-2'), 65.3 (C-2), 53.3 (C-10), 48.7 (C-1), 48.0 (C-7), 44.8 (C-4), 38.6 (C-3), 33.0 (C-6), 26.6 (C-5), 21.0 and 20.0 (C-8 and C-9); HRMS (ESI) calc for $C_{13}H_{19}NNaO_3S$ $[M+Na]^+$ 292.0978, found 292.0978.

NMR data in accordance with literature.[261]

Ethyl 2-(dimethyl(phenyl)silyl)acetate S16



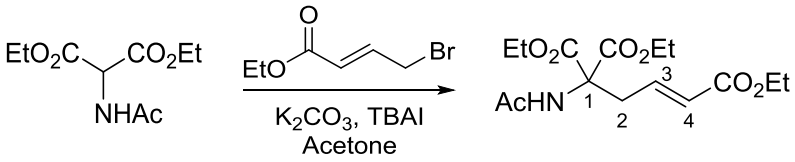
S15 **S16**

Zinc dust (0.28 g, 4.3 mmol) was stirred with 2% HCl (2 × 25 mL), water (3 × 25 mL), acetone (2 × 25 mL) and diethyl ether (1 × 50 mL), filtering off each wash solution, before being dried *in vacuo*. To the dry, activated zinc dust was added dry diethyl ether (8 mL), and the resulting grey suspension heated to 34 °C for 0.25 h. Chloro(dimethyl)phenylchlorosilane (0.60 mL, 605 mg, 3.52 mmol) and ethyl 2-bromoacetate (0.49 mL, 734 mg, 4.40 mmol, 1.25 equiv.) were then added simultaneously at 34 °C. The reaction mixture was maintained at 34 °C for 3 h, then the resulting colourless solution cooled to room temperature and quenched by the addition of saturated aqueous NH_4Cl (20 mL). The layers were separated and the aqueous layer extracted with diethyl ether (3 × 30 mL). The combined

organic layers were then dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using Et_2O/petrol (1-5& Et_2O) to give the silylated compound **S16** as a colourless oil (542 mg, 69%). δ_H (400 MHz, $CDCl_3$) 7.57-7.53 (2H, m, Ar-H), 7.41-7.36 (3H, m, Ar-H), 4.05 (2H, q, *J* 7.0, OCH_2), 2.13 (2H, s, CH_2), 1.17 (3H, t, J 7.0, $OCH_2C\underline{H}_3$), 0.42 (6H, s, $Si(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 172.7 (C=O), 137.1 (Ar), 133.6 (Ar), 129.6 (Ar), 128.0 (Ar), 60.0 (OCH_2), 26.4 (CH_2), 14.4 ($OCH_2\underline{C}H_3$), -2.7 ($Si(CH_3)_2$).

NMR data in accordance with literature.[262]

Triethyl (*E*)-1-acetamidobut-3-ene-1,1,4-tricarboxylate S18

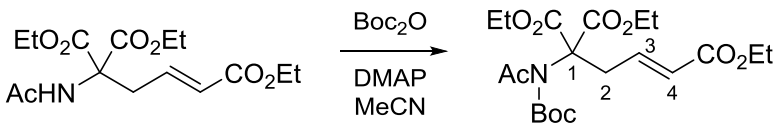


S17 **S18**

To diethyl acetamidomalonate **S17** (3.00 g, 13.8 mmol) in acetone (50 mL) was added TBAI (285 mg, 0.77 mmol, 0.06 equiv.), potassium carbonate (2.87 g, 20.8 mmol, 1.5 equiv.) and ethyl 4-bromocrotonate (2.7 mL, 85% purity, 3.22 g, 16.7 mmol, 1.2 equiv.). The resulting suspension was heated to 56 ° C for 16 h, then allowed to cool to room temperature, filtered, and concentrated *in vacuo*. The residue was dissolved in ethyl acetate (50 mL), and washed with water (2 × 25 mL) and brine (25 mL), then dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified via column chromatography using 25-50% EtOAc/hexane as the eluent to yield **S18** as a white solid (2.85 g, 63%). MP (MeOH) 58-62 °C (lit. 62-68 °C)[263]; δ_H (400 MHz, $CDCl_3$) 6.78 (1H, s, NH), 6.65 (1H, dt, *J* 15.5, 7.5, 3-H), 5.85 (1H, app. dd, *J* 15.5, 1.5, 4-H), 4.25 (4H, q, *J* 7.0, OCH_2), 4.15 (2H, q, *J* 7.0, OCH_2), 3.24 (2H, dd, *J* 7.5, 1.5, 2-H_2), 2.03 (3H, s, CH_3), 1.29-1.22 (9H, m, $OCH_2C\underline{H}_3$); δ_C (100 MHz, $CDCl_3$) 169.4 (C=O), 167.3 (C=O), 165.9 (C=O), 141.2 (C-3), 125.8 (C-4), 65.8 (C-1), 63.1 (OCH_2), 60.6 (OCH_2), 35.4 (C-2), 23.1 (CH_3), 14.3 ($OCH_2\underline{C}H_3$), 14.1 ($OCH_2\underline{C}H_3$); ν_{max} (film) 1739, 1719, 1661, 1504, 1298, 1201; HRMS (ESI) calc for $C_{15}H_{23}NaNO_7$ [M+Na] 352.1367, found 352.1381.

NMR data in accordance with literature.[263]

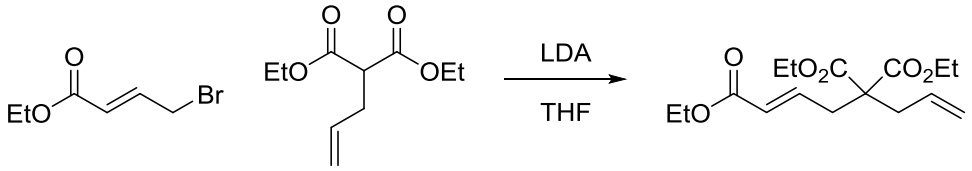
Triethyl (*E*)-1-(*N*-(tert-butoxycarbonyl)acetamido)but-3-ene-1,1,4-tricarboxylate S19



S18 **S19**

To a solution of **S18** (400 mg, 1.22 mmol) in dry acetonitrile (20 mL) was added DMAP (30 mg, 0.24 mmol, 0.2 equiv.) and di-*tert*-butyl dicarbonate (700 mg, 3.21 mmol, 2.6 equiv.). The reaction mixture was heated to 40 °C for 96 h, then cooled to room temperature and concentrated *in vacuo*. The residue was redissolved in diethyl ether (25 mL), washed with sat. aq. $KHSO_4$, $NaHCO_3$, brine, and then dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified via column chromatography using 25-50% EtOAc/hexane as the eluent to yield **S19** as a pale yellow oil (284 mg, 54%, 76% b.r.s.m) and unreacted starting material **S18** (116 mg). δ_H (400 MHz, $CDCl_3$) 7.00 (1H, m, 3-H), 5.76 (1H, m, 4-H), 4.29-4.09 (6H, m, 3 × OCH_2), 3.26 (2H, dd, *J* 8.0, 1.5 , 2-H_2), 2.38 (3H, s, CH_3), 1.48 (9H, s, C($\underline{C}H_3$)$_3$), 1.31-1.14 (9H, m, 3 × $OCH_2\underline{C}H_3$); δ_C (100 MHz, $CDCl_3$) 172.6 (C=O), 166.3 (C=O), 166.2 (C=O), 151.9 (C=O), 144.1 (C-3), 124.4 (C-4), 85.6 ($\underline{C}(CH_3)_3$), 71.3 (C-1), 62.7 (OCH_2), 60.3 (OCH_2), 36.3 (C-2), 27.8 (C($\underline{C}H_3$)$_3$), 26.7 (CH_3), 14.4 ($OCH_2\underline{C}H_3$), 14.0 ($OCH_2\underline{C}H_3$); ν_{max} (film) 1746, 1720, 1701, 1369, 1244, 1148; HRMS (ESI) calc for $C_{20}H_{31}NaNO_9$ $[M+Na]^+$ 452.1891, found 452.1896.

Ethyl 5,5-bis(ethoxycarbonyl)-2,7-octadienoate S20

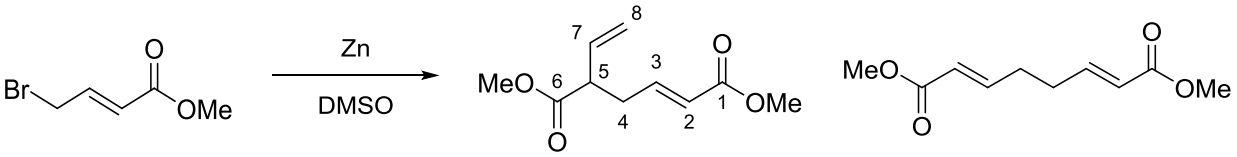


224 **233** **S20**

To a solution of diisopropylamine (0.9 mL, 6.0 mmol, 1.2 equiv.) in THF (30 mL) at −15 °C was added *n*-BuLi (3.5 mL, 1.6 M in hexane, 1.1 equiv.). After 1 h, the reaction was cooled to −78 °C and diethyl allylmalonate **233** (1.0 mL, 5.0 mmol, 1 equiv.) in THF (5 mL) was added. After a further 1 h, ethyl 4-bromocrotonate **224** (1.0 mL, 6.1 mmol, 1.3 equiv.) in THF (5 mL) was added dropwise. The resulting bright yellow solution was allowed to warm to room temperature and stirred for 16 h, then quenched with half saturated aqueous NH_4Cl (30 mL) and extracted with DCM (3 × 50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to an orange oil. The crude material was purified by column chromatography using ethyl acetate/petrol (10% ethyl acetate) to yield triester **S20** (1.30 g, 83%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 6.78 (1H, dt, *J* 15.5, 7.5, 3-H), 5.85 (1H, dt, *J* 15.5, 1.5, 2-H), 5.63 (1H, ddt, *J* 19.0, 9.5, 7.5, 7-H), 5.17-5.08 (2H, m, 8-H_2), 4.25-4.12 (6H, m, 3 × OCH_2), 2.75 (2H, dd, *J* 7.5, 1.5, 4-H_2), 2.64 (2H, d, *J* 7.5, 6-H_2), 1.30-1.20 (9H, m, 3 × CH_3); δ_C (100 MHz, $CDCl_3$) 170.3 (C=O), 166.0 (C=O), 142.6 (C-3), 131.9 (C-7), 125.3 (C-2), 119.8 (C-8), 61.7 (OCH_2), 60.5 (OCH_2), 57.1 (C-5), 37.3 (C-6), 35.4 (C-4), 14.4 (CH_3), 14.2 (CH_3); HRMS (ESI) calc for $C_{16}H_{24}NaO_6$ $[M+Na]^+$ 335.1465, found 335.1451.

NMR data in accordance with literature.[264]

Dimethyl (*E*)-5-vinylhex-2-enedioate 243



243 **243** **S21**

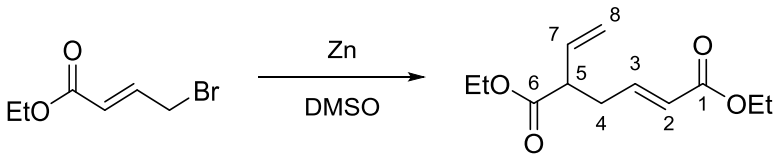
Zinc dust (4.68 g, 70 mmol) was stirred with 2% HCl (2 × 50 mL), water (3 × 50 mL), acetone (2 × 50 mL) and diethyl ether (1 × 50 mL), filtering off each wash solution, before being dried under vacuum. To the dry zinc dust in dry DMSO (20 mL) at 0 °C, was added methyl 4-bromocrotonate (8 mL, 68 mmol) portionwise, then the reaction mixture allowed to warm to room temperature. After 4 h, diethyl ether (100 mL) was added, then the reaction mixture washed with HCl (0.1 M, 100 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic layers were washed with water (100 mL), then dried with $MgSO_4$, filtered, and the solvent removed *in vacuo* to give a green oil **243** (5.16 g, 76%) which was used without further purification. δ_H (400 MHz, $CDCl_3$) 6.84 (1H, dt, *J* 15.5 and 7.0, 3-H), 5.89-5.73 (2H, m, 2-H and 7-H), 5.22-5.12 (2H, m, 8-H_2), 3.71 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 3.17 (1H, q, *J* 7.5, 5-H), 2.66 (1H, m, 4-*H*H), 2.45 (1H, m, 4-H*H*); δ_C (100 MHz, $CDCl_3$) 173.2 (C-6), 166.7 (C-1), 145.2 (C-3), 134.6 (C-7), 123.2 (C-2), 118.4 (C-8), 52.2 (OCH_3), 51.6 (OCH_3), 48.8 (C-5), 34.4 (C-4).

NMR data in accordance with literature.[265]

Additionally, if the reaction was allowed to warm significantly, dimethyl (2*E*,6*E*)-octa-2,6-dienedioate **S21** could be isolated in up to 10% yield. δ_H (400 MHz, $CDCl_3$) 6.93 (2H, dt, *J* 16.0 and 6.5, 3-H and 6-H), 5.85 (2H, d, *J* 16.0, 2-H and 7-H), 3.72 (6H, s, 2 × OCH_3), 2.36 (4H, m, 4-H_2 and 5-H_2); δ_C (100 MHz, $CDCl_3$) 166.9 (C-1 and C-8), 147.3 (C-3 and C-6), 122.1 (C-2 and C-7), 51.6 (OCH_3), 30.6 (C-4 and C-5); ν_{max} (film) 3425, 2952, 1720, 1657, 1435, 1271, 1201, 1154.

NMR data in accordance with literature.[266]

Diethyl (*E*)-5-vinylhex-2-enedioate 251



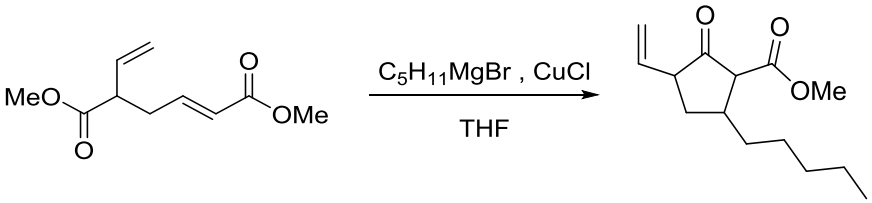
224 **251**

Zinc dust (0.68 g, 10.4 mmol) was stirred with 2% HCl (2 × 50 mL), water (3 × 50 mL), acetone (2 × 50 mL) and diethyl ether (1 × 50 mL), filtering off each wash solution, before being dried under vacuum. To the dry zinc dust in dry DMSO (4 mL) at 0 °C, was added ethyl 4-bromocrotonate **224** (1.9 mL, 10 mmol) portionwise, then the reaction mixture allowed to warm to room temperature.

After 4 h, diethyl ether (50 mL) was added, then the reaction mixture washed with HCl (0.1 M, 50 mL) and extracted with diethyl ether (3 × 20 mL). The combined organic layers were washed with water (50 mL), then dried with $MgSO_4$, filtered, and the solvent removed *in vacuo* to give a green oil. The crude material was purified by column chromatography using ethyl acetate/petrol (5-15% ethyl acetate) to give **251** (0.92 g, 79%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 6.79 (1H, dt, *J* 16.0 and 7.0, 3-H), 5.84-5.72 (2H, m, 2-H and 7-H), 5.16-5.09 (2H, m, 8-H_2), 4.15-4.05 (4H, m, 2 × OCH_2), 3.10 (1H, q, *J* 7.5, 5-H), 2.60 (1H, m, 4-*H*H), 2.41 (1H, m, 4-H*H*), 1.27-1.15 (6H, m, 2 × CH_3); δ_C (100 MHz, $CDCl_3$) 172.6 (C-6), 166.2 (C-1), 144.9 (C-3), 134.7 (C-7), 123.5 (C-2), 118.1 (C-8), 60.9 (OCH_2), 60.3 (OCH_2), 48.9 (C-5), 34.4 (C-4), 14.3 (CH_3), 14.2 (CH_3).

NMR data in accordance with literature.[208,267]

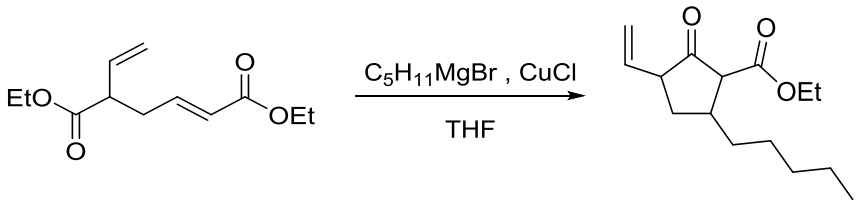
Methyl 2-oxo-5-pentyl-3-vinylcyclopentane-1-carboxylate 246



243 **246**

To a solution of pentylmagnesium bromide (20.5 mL, 51 mmol, 2 equiv., 2 M in THF) in dry THF (20 mL) at 0 °C, was added Cu(I)Cl (130 mg, 1.3 mmol, 5 mol%). After 0.5 h, diester **243** (5.05 g, 25.3 mmol) in THF (15 mL) was added dropwise. After 0.5 h at 0 °C, the black reaction mixture was raised to room temperature and stirred for an additional 3 h. The reaction was quenched with saturated aqueous NH_4Cl (80 mL), and extracted with chloroform (3 × 200 mL). The combined organic layers were washed with water (200 mL), then dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a green oil. The crude material was purified by column chromatography using ethyl acetate/petrol (5-10% ethyl acetate) to give ester **246** as a green oil as a mixture of isomers (3.44 g, 57%). ν_{max} (film) 2952, 2859, 1726, 1650, 1592, 1440, 1287, 1216; HRMS (ESI) calc for $C_{14}H_{22}NaO_3$ $[M+Na]^+$ 261.1461, found 261.1433. ^1H and ^{13}C NMR spectra can be found in the spectral appendix.

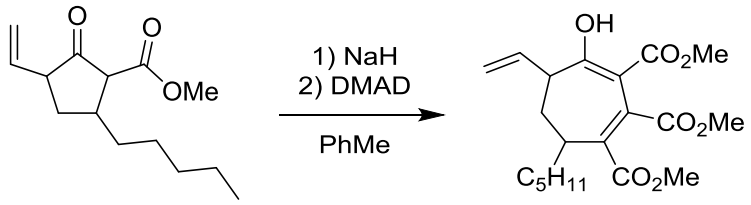
Ethyl 2-oxo-5-pentyl-3-vinylcyclopentane-1-carboxylate S22



251 **S22**

To a solution of pentylmagnesium bromide (3.55 mL, 7.10 mmol, 2 equiv., 2 M in THF) in dry THF (5 mL) at 0 °C, was added Cu(I)Cl (18 mg, 5 mol%). After 0.5 h, diester **251** (732 mg, 3.3 mmol) in THF (10 mL) was added dropwise. After 0.5 h at 0 °C, the black reaction mixture was raised to room temperature and stirred for an additional 3 h. The reaction was quenched with saturated aqueous NH_4Cl (20 mL), and extracted with chloroform (3 × 30 mL). The combined organic layers were washed with water (100 mL), then dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a green oil. The crude material was purified by column chromatography using ethyl acetate/petrol (5-10% ethyl acetate) to give ester **S22** as a green oil as a mixture of isomers (472 mg, 53%). HRMS (ESI) calc for $C_{15}H_{24}NaO_3$ $[M+Na]^+$ 275.1618, found 275.1610. ^1H and ^{13}C NMR spectra can be found in the spectral appendix.

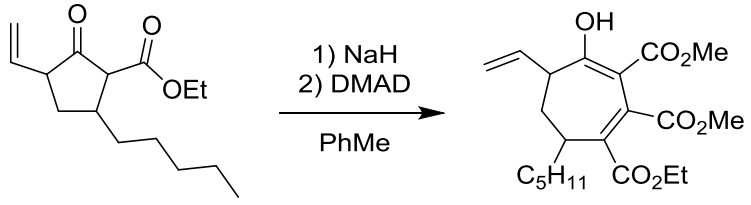
Trimethyl 4-hydroxy-7-pentyl-5-vinylcyclohepta-1,3-diene-1,2,3-tricarboxylate 247



246 **247**

To sodium hydride (60% dispersion in mineral oil, 484 mg, 11.9 mmol, 1.5 equiv.) in dry toluene (20 mL) under an N_2 atmosphere was added a solution of ketoester **246** (1.997 g, 7.93 mmol) in dry toluene (8 mL) with stirring at 0 °C over 0.5 h. After 1 h, DMAD (1.1 mL, 1.24 g, 8.7 mmol, 1.1 equiv.) was added. After 4 h, the reaction was was cooled to 0 °C before adding glacial acetic acid (5 mL). Hydrochloric acid (1 M, 20 mL) was added, and the aqueous layer separated and washed with toluene (3 × 50 mL). The combined organic phases were washed with water (50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a red oil. The crude material was purified by column chromatography using ethyl acetate/petrol (10-18% ethyl acetate) to give triester **247** (1.35 g, 44%) as a colourless oil as a mixture of isomers. HRMS (ESI) calc for $C_{20}H_{28}NaO_7$ $[M+Na]^+$ 403.1727, found 403.1664. ^1H and ^{13}C NMR spectra can be found in the spectral appendix.

1-Ethyl-2,3-dimethyl 4-hydroxy-7-pentyl-5-vinylcyclohepta-1,3-diene-1,2,3-tricarboxylate S23

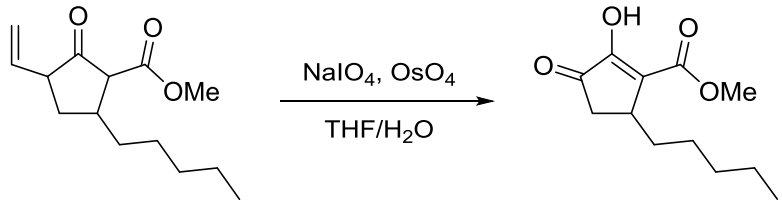


S22 **S23**

To sodium hydride (60% dispersion in mineral oil, 530 mg, 13.1 mmol, 1.5 equiv.) in dry toluene (18 mL) under an N_2 atmosphere was added a solution of ketoester **S22** (2.20 g, 8.72 mmol) in dry

toluene (12 mL) with stirring at 0 °C over 0.5 h. After 1 h, dimethylacetylenedicarboxylate (1.2 mL, 1.36 g, 9.59 mmol, 1.1 equiv.) was added. After 4 h, the reaction was cooled to 0 °C before adding glacial acetic acid (3 mL). Hydrochloric acid (1 M, 20 mL) was added, and the aqueous layer separated and washed with toluene (3 × 40 mL). The combined organic phases were washed with water (30 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to a red oil. The crude material was purified by column chromatography using ethyl acetate/petrol (10-20% ethyl acetate) to give triester **S23** (1.02 g, 31%) as a colourless oil as a mixture of isomers. ν_{max} (film) 2955, 2931, 1721, 1649, 1593, 1442, 1216, 907, 727; HRMS (ESI) calc for $C_{21}H_{30}NaO_7$ $[M+Na]^+$ 417.1884, found 417.1883. ^1H and ^{13}C NMR spectra can be found in the spectral appendix.

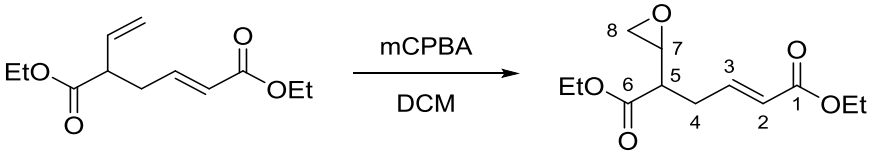
Methyl 2-hydroxy-3-oxo-5-pentylcyclopent-1-ene-1-carboxylate 249



246 **249**

To ester **246** (498 mg, 2.1 mmol) in a 1:1 THF:water solution (40 mL) was added sodium periodate (2.12 g, 9.91 mmol, 5 equiv.) and osmium tetroxide (1 crystal), and the reaction mixture stirred at room temperature for 3 h. Water (50 mL) was added, and the mixture was extracted with ethyl acetate (3 × 50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude brown oil was purified by column chromatography using ethyl acetate/petrol (25% ethyl acetate) to give side-product **249** (73 mg, 15%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 9.12 (1H, br s, OH), 3.89 and 3.73 (3H, 2 × br s, OCH_3), 3.02-2.91 (1H, m, 5-H), 2.59 (1H, dd, *J* 19.5 and 6.5, 4-*H*H), 2.16 (1H, d, *J* 19.5, 4-H*H*), 1.93-1.84 (1H, m, 6-*H*H) 1.40-1.00 (7H, m, 6-H*H*, 7-H_2, 8-H_2 and 9-H_2), 0.86 (3H, t, *J* 6.5, 10-H_3); δ_C (100 MHz, $CDCl_3$) 201.8 (C-3), 168.9 and 160.9 (C=O and C-2), 129.0 (C-1), 52.4 (OCH_3), 39.5 (C-4), 34.4 (C-6), 33.5 (C-5), 31.8, 26.7 and 22.6 (C-7, C-8 and C-9), 14.1 (C-10); ν_{max} (film) 3359, 2955, 2926, 2858, 1722, 1677, 1446, 1218, 1145, 1100; HRMS (ESI) calc for $C_{12}H_{18}NaO_4$ $[M+Na]^+$ 249.1097, found 249.1078.

Diethyl (*E*)-5-(oxiran-2-yl)hex-2-enedioate 252



251 **252**

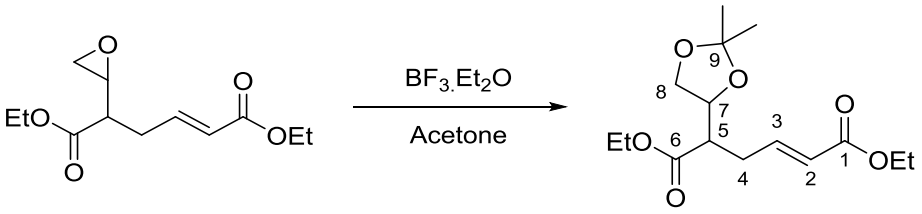
To vinylhexenedioate **251** (1.520 g, 6.7 mmol) in DCM (75 mL) was added *m*CPBA (2.378 g, 13.8 mmol, 2 equiv.) and the resulting solution stirred at room temperature for 7 days. The reaction

mixture was washed with saturated aqueous $NaHCO_3$ (3 × 30 mL), water (30 mL), and then the organic layer dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (10% ethyl acetate) to give epoxide **252** as a 2:1 mixture of separable diastereomers (1.293 g, 79%).

Major diastereomer: δ_H (400 MHz, $CDCl_3$) 6.92 (1H, dt, *J* 16.0 and 7.0, 3-H), 5.90 (1H, dt, *J* 16.0, 1.5, 2-H), 4.17 (4H, 2 × q, *J* 7.0, 2 × OCH_2), 3.09 (1H, ddd, *J* 7.5, 4.0, 2.5, 7-H), 2.83 (1H, dd, *J* 5.0, 4.0, 8-*H*H), 2.75-2.59 (3H, m, 4-H_2 and 8-H*H*), 2.32 (1H, td, J 7.5, 5.5, 5-H), 1.26 (6H, 2 × t, *J* 7.0, 2 × CH_3); δ_C (100 MHz, $CDCl_3$) 171.3 (C-6), 166.1 (C-1), 144.2 (C-3), 123.7 (C-2), 61.1 (OCH_2), 60.3 (OCH_2), 52.1 (C-7), 47.9 (C-5), 46.7 (C-8), 32.2 (C-4), 14.2 (CH_3), 14.2 (CH_3); ν_{max} (film) 2983, 1721, 1656, 1369, 1265, 1182; HRMS (ESI) calc for $C_{12}H_{18}O_5Na$ $[M+Na]^+$ 265.1046, found 265.1051.

Minor diastereomer: δ_H (400 MHz, $CDCl_3$) 6.86 (1H, dt, *J* 15.5, 7.5, 3-H), 5.88 (1H, d, *J* 15.5, 2-H), 4.25 – 4.14 (4H, m, 2 × OCH_2), 3.17 (1H, ddd, *J* 7.5, 4.0, 2.5, 7-H), 2.82 (1H, dd, *J* 4.5, 4.0, 8-*H*H), 2.63 (1H, m, 4-*H*H), 2.57 (1H, dd, J 4.5, 2.5, 8-H*H*), 2.49 (1H, dddd, *J* 14.5, 7.5, 6.5, 1.5, 4-H*H*), 2.39 (1H, m, 5-H), 1.27 (6H, 2 × t, *J* 7.0, 2 × CH_3); δ_C (100 MHz, $CDCl_3$) 172.1 (C-6), 166.1 (C-1), 144.1 (C-3), 124.0 (C-2), 61.4 (OCH_2), 60.6 (OCH_2), 52.1 (C-7), 47.5 (C-5), 46.2 (C-8), 31.3 (C-4), 14.3 (CH_3), 14.3 (CH_3); ν_{max} (film) 2983, 1721, 1656, 1369, 1182; HRMS (MALDI) calc for $C_{12}H_{18}O_5Na$ $[M+Na]^+$ 265.1046, found 265.1052.

Diethyl (*E*)-5-(2,2-dimethyl-1,3-dioxolan-4-yl)hex-2-enedioate 253



252 **253**

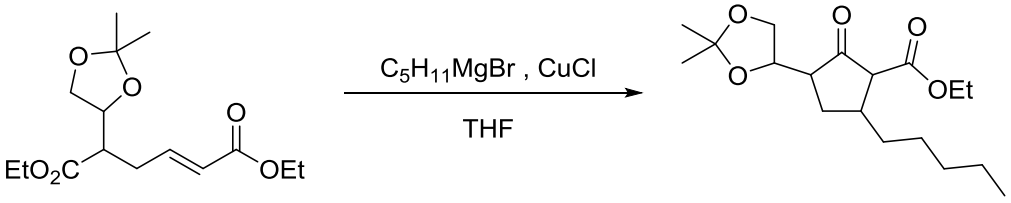
To a solution of a single diastereomer of epoxide **252** (2.22 g, 9.17 mmol) in dry acetone (50 mL) was added boron trifluoride diethyl etherate (100 µL, 0.74 mmol, 0.08 equiv.) at 0 °C. After 1 h, the reaction was allowed to warm to room temperature and stirred for 16 h. The reaction was quenched with saturated aqueous $NaHCO_3$ (20 mL), then extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (5-10% ethyl acetate) to give a single diastereomer of acetonide **253** as a colourless oil (2.03 g, 74%).

First diastereomer: δ_H (400 MHz, $CDCl_3$) 6.90 (1H, dt, *J* 15.5, 7.0, 3-H), 5.87 (1H, dt, *J* 15.5, 1.5, 2-H), 4.24-4.11 (5H, m, 2 × OCH_2, 7-H), 4.08 (1H, dd, *J* 8.5, 6.0, 8-*H*H), 3.74 (1H, dd, *J* 8.5, 6.0, 8-H*H*), 2.71-2.55 (3H, m, 4-H_2, 5-H), 1.40 (3H, s, CH_3), 1.34 (3H, s, CH_3), 1.27 (3H, t, *J* 7.0, CH_3), 1.24 (3H, t, *J*

7.0, CH_3); δ_C (100 MHz, $CDCl_3$) 172.0 (C-6), 166.2 (C-1), 144.8 (C-3), 123.5 (C-2), 109.4 (C-9), 75.3 (C-7), 68.1 (C-8), 60.9 (OCH_2), 60.3 (OCH_2), 48.7 (C-5), 31.5 (C-4), 26.8 (R_3C-$\underline{C}H_3$), 25.3 (R_3C-$\underline{C}H_3$), 14.2 ($OCH_2C\underline{H_3}$), 14.2 ($OCH_2C\underline{H_3}$); ν_{max} (film) 2986, 1718, 1656, 1371, 1192, 1051; HRMS (MALDI) calc for $C_{15}H_{24}O_6Na$ $[M+Na]^+$ 323.1465, found 323.1478.

Second diastereomer: δ_H (400 MHz, $CDCl_3$) 6.86 (1H, dddd, J=15.5, 7.7, 6.8, 1.1 Hz), 5.86 (1H, dt, *J* 15.5, 1.5, 2-H), 4.30 (1H, m, 7-H), 4.18 (4H, 2 × q, *J* 7.0, 2 × OCH_2), 4.04 (1H, ddd, *J* 8.5, 6.0, 1.0, 8-*H*H), 3.79 (1H, ddd, *J* 8.5, 6.0, 1.0, 8-H*H*), 2.72 (1H, dddd, J=10.0, 7.0, 4.5, 1.0, 5-H), 2.53 (1H, dddt, *J* 15.0, 10.0, 7.5, 1.0, 4-*H*H), 2.36 (1H, m, 4-H*H*), 1.41 (3H, s, CH_3), 1.33 (3H, s, CH_3), 1.26 (6H, 2 × t, *J* 7.0, 2 × CH_3); δ_C (100 MHz, $CDCl_3$) 172.3 (C-6), 166.2 (C-1), 144.6 (C-3), 123.7 (C-2), 109.6 (C-9), 76.1 (C-7), 66.9 (C-8), 61.0 (OCH_2), 60.5 (OCH_2), 48.3 (C-5), 30.9 (C-4), 26.7 (R_3C-$\underline{C}H_3$), 25.4 (R_3C-$\underline{C}H_3$), 14.4 ($OCH_2C\underline{H_3}$), 14.4 ($OCH_2C\underline{H_3}$); ν_{max} (film) 2984, 1720, 1656, 1370, 1264, 1209, 1182, 1157, 1048; HRMS (MALDI) calc for $C_{15}H_{24}O_6Na$ $[M+Na]^+$ 323.1465, found 323.1472.

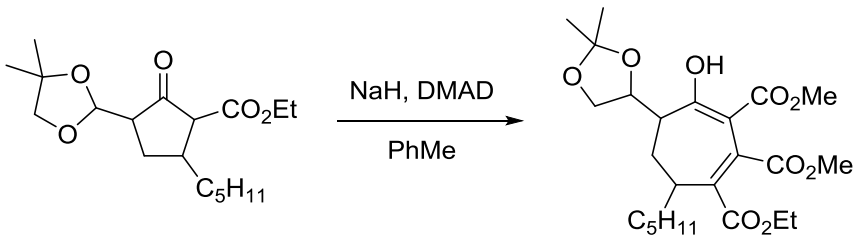
Ethyl 3-(2,2-dimethyl-1,3-dioxolan-4-yl)-2-oxo-5-pentylcyclopentane-1-carboxylate 254



253 **254**

To dry THF (9 mL) at 0 °C was added CuCl (9 mg, 0.09 mmol, 0.05 equiv.) and pentylmagnesium bromide (1.82 mL, 3.64 mmol, 2 equiv, 2 M solution in diethyl ether). After 0.5 h, acetonide **253** (538 mg, 1.79 mmol) in THF (3 mL) was added dropwise. After 1.5 h, the reaction mixture was warmed to room temperature, and then stirred for an additional 2 h. Saturated aqueous NH_4Cl (20 mL) was added, and then the reaction mixture extracted with chloroform (3 × 50 mL). The combined organic layers were washed with water (50 mL), and then dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (5% ethyl acetate) to give ketoester **254** (484 mg, 83%) as a mixture of diastereomers. ν_{max} (film) 2929, 2859, 1752, 1723, 1457, 1371, 1056, 910, 733; HRMS (ESI) calc for $C_{18}H_{30}NaO_5$ $[M+Na]^+$ 349.1985, found 349.1998.

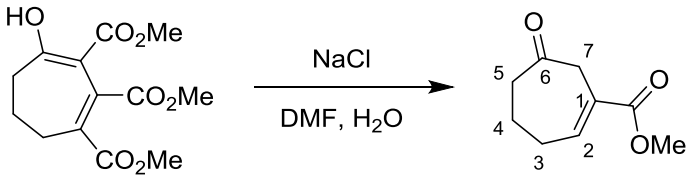
1-Ethyl 2,3-dimethyl 5-(2,2-dimethyl-1,3-dioxolan-4-yl)-4-hydroxy-7-pentylcyclohepta-1,3-diene-1,2,3-tricarboxylate 255



254 **255**

To sodium hydride (60% dispersion in mineral oil, 140 mg, 3.5 mmol, 1.5 equiv., washed with 2 × 8 mL hexane) in dry toluene (10 mL) under an N_2 atmosphere was added a solution of ketoester **254** (760 mg, 2.3 mmol) in toluene (8 mL) with stirring at 0 °C over 0.5 h. After 1 h, dimethylacetylenedicarboxylate (0.32 mL, 0.37 g, 2.6 mmol, 1.1 equiv.) was added. After 4 h, the reaction was was cooled to 0 °C before adding glacial acetic acid (2 mL). Hydrochloric acid (1 M, 20 mL) was added, and the aqueous layer separated and washed with toluene (3 × 40 mL). The combined organic phases were washed with water (50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a red oil. The crude material was purified by column chromatography using ethyl acetate/petrol (10-25% ethyl acetate) to give triester **255** (540 mg, 50%) as a colourless oil as a mixture of isomers. ν_{max} (film) 2954, 2929, 2861, 1723, 1650, 1598, 1443, 1370, 1214, 1052, 910, 732; HRMS (MALDI) calc for $C_{24}H_{39}NaO_9$ $[M+Na]^+$ 491.2252, found 491.2257. ^1H and ^{13}C NMR spectra can be found in the spectral appendix.

Methyl 6-oxo-cyclohept-1-enecarboxylate 257



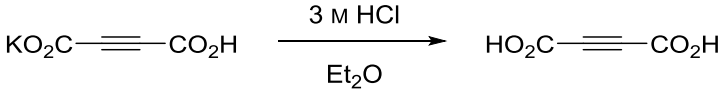
198 **257**

A stirred solution of triester **198** (500 mg, 1.76 mmol) and sodium chloride (617 mg, 10.6 mmol, 6 equiv.) in DMF (5 mL) and water (1 mL) was heated to reflux at 150 °C for 3 h. The resulting orange solution was cooled to room temperature and ethyl acetate (20 mL) and water (20 mL) added. The aqueous layer was separated and washed with further ethyl acetate (2 × 20 mL). The combined organic phases were washed with water (50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (5-15% ethyl acetate) to give ester **257** (88 mg, 30%) as a colourless oil. δ_H(400 MHz, $CDCl_3$) 7.06 (1H, tt, *J*

5.5, 1.5, 2-H), 3.67 (3H, s, OMe), 3.53 (2H, q, *J* 1.5, 7-H_2), 2.54 – 2.49 (2H, m, 4-H_2), 2.46 – 2.38 (2H, m, 3-H_2), 2.02 – 1.89 (2H, m, 5-H_2); δ_C (101 MHz, $CDCl_3$) 208.4 (C-6), 167.1 (C=O), 142.5 (C-2), 125.2 (C-1), 52.2 (OMe), 43.1 (C-4), 41.1 (C-7), 29.1 (C-3), 21.3 (C-5).

Spectroscopic data in accordance with literature.[268]

Acetylenedicarboxylic acid 260

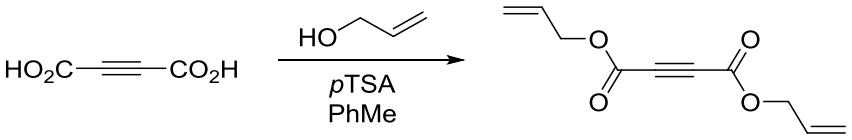


S22 **260**

Acetylenedicarboxylic acid monopotassium salt **S22** was dissolved in diethyl ether (100 mL) and hydrochloric acid (3 M, 100 mL) and stirred for 2 h. The organic layer was separated, diethyl ether (100 mL) added to the aqueous layer and stirred an additional 2 h. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield diacid **260** as a beige solid in quantitative yield. δ_C(100 MHz, D_2O) 155.9 (C=O), 75.5 (C≡C); ν_{max} (ATR) 3512, 3390, 1663, 1423, 1259.

NMR data in accordance with literature.[269]

Diallyl acetylenedicarboxylate 262 (†)

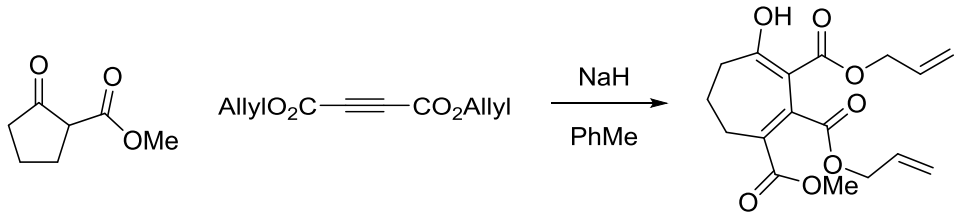


260 **262**

Diacid **260** (4.96 g, 43.5 mmol) was added to a solution of *p*-toluenesulfonic acid monohydrate (0.53 g, 2.80 mmol) and allylic alcohol (5.53 g, 95.2 mmol, 6.47 mL) in toluene (40 mL). The reaction mixture was heated to reflux under Dean-Stark conditions for 20 h. After cooling to room temperature the solvent was removed *in vacuo*, and the resulting orange oil dissolved in ethyl acetate (30 mL). The solution was washed with saturated aqueous $NaHCO_3$ (2 × 25 mL) and the organic layer dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give diester **262** as an orange oil (6.41 g, 76 %). δ_H (400 MHz, $CDCl_3$) 5.90 (2H, ddt, *J* 17.0, 10.5, 6.0, -CH=), 5.37 (2H, dq, *J* 17.0, 1.5, =CH_2), 5.30 (2H, dq, *J* 10.5, 1.0, =CH_2), 4.70 (4H, ddd, *J* 6.0, 1.5, 1.0, -CH_2); δ_C (100 MHz, $CDCl_3$) 151.5 (C=O), 130.4 (-CH=), 120.2 (=CH_2), 74.8 (C≡C), 67.4 (CH_2); ν_{max} (film) 1719, 1231, 1053, 991, 934, 746; m/z (ESI+) 217 $[M+Na]^+$.

NMR data in accordance with literature.[270]

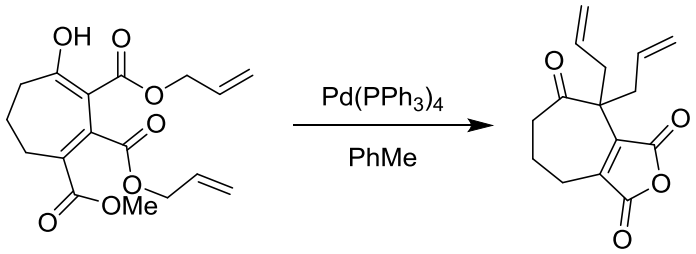
2,3-Diallyl 1-methyl 4-hydroxycyclohepta-1,3-diene-1,2,3-tricarboxylate 265 (†)



188 **262** **265**

Ketoester **188** (2.13 g, 15.0 mmol, 1.86 mL) was added to a solution of sodium hydride (60% dispersion, 1.36 g, 34.0 mmol) in dry toluene (30 mL) at 0 °C under an N_2 atmosphere with stirring over 0.5 h. The resulting white paste was stirred at room temperature for 1 h and then cooled to 0 °C. Diallyl ester **262** (3.20 g, 16.5 mmol) was added with stirring over 0.75 h, maintaining the reaction temperature below 10 °C. After 4 h, the reaction mixture was cooled to 0 °C then glacial acetic acid (6 mL) was added dropwise. Hydrochloric acid (2 M, 10 mL) was added and the aqueous layer was washed with toluene (3 × 10 mL). The organic phase was washed with water (10 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield a dark red oil, which was purified twice by column chromatography (10% EtOAc : Petrol, then 1% MeOH : DCM) to yield the product **265** as a pale yellow oil (1.822 g, 36%). δ_H (400 MHz, $CDCl_3$) 13.04 (1H, s, enol-H), 5.90 (1H, ddt, *J* 17.5, 10.5, 6.0, -CH=CH_2), 5.81 (1H, ddt, *J* 17.0, 10.5, 6.0, -CH=CH_2), 5.31 (1H, dq, *J* 17.0, 1.5, -CH=CH_2), 5.28 (1H, dq, *J* 17.5, 1.5, -CH=CH_2), 5.23 (1H, dq, *J* 10.5, 1.5, -CH=CH_2), 5.21 (1H, dq, *J* 10.5, 1.5, -CH=CH_2), 4.60 (1H, dt, *J* 6.0, 1.5, OCH_2), 4.57 (1H, dt, *J* 6.0, 1.5, OCH_2), 3.74 (3H, s, OCH_3), 2.47-2.23 (6H, m, CH_2); δ_C(100 MHz, $CDCl_3$) 182.6 (enol-C), 170.2 (C=O), 169.1 (C=O), 166.6 (C=O), 138.6 (C=C), 134.7 (C=C), 131.9 (-CH=), 131.4 (-CH=), 119.0 (=CH_2), 118.7 (=CH_2), 98.9 (C), 66.2 (OCH_2), 65.8 (OCH_2), 52.5 (OCH_3), 33.7 (CH_2), 31.7 (CH_2), 28.5 (CH_2); ν_{max} (film) 2953, 1721, 1644, 1585, 1300, 1247, 1195; HRMS (ESI) calc for $C_{17}H_{20}NaO_7$ $[M+Na]^+$ 359.1107, found 359.1111.

1-Oxo-2,2-diallyl-cyclohept-3-ene-3,4-dicarboxylic acid anhydride 266 (†)

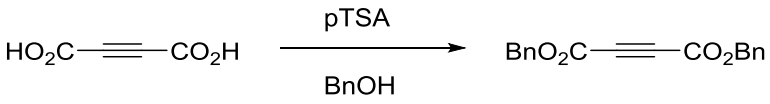


265 **266**

Triester **265** (100 mg, 0.30 mmol) was added to a solution of $Pd(PPh_3)_4$ (17mg, 0.015 mmol, 0.05 equiv.) in dry toluene (5 mL). After 1 h at room temperature, the reaction mixture was concentrated *in vacuo*. The crude product was purified by column chromatography using EtOAc/petrol (10%

EtOAc) to give anhydride **266** as a colourless oil (16 mg, 21%). δ_H (400 MHz, $CDCl_3$) 5.49 (2H, dddd, *J* 16.5, 10.5, 8.5, 7.0, -CH=), 5.09-4.95 (4H, m, $=CH_2$), 2.88 (2H, ddt, *J* 14.0, 7.0, 1.0, CH_2), 2.76 (2H, ddt, *J* 14.0, 8.5, 1.0, CH_2), 2.69 (2H, t, *J* 7.0, CH_2), 2.66-2.56 (2H, m, CH_2) 2.04-1.89 (2H, m, CH_2); δ_C (100 MHz, $CDCl_3$) 208.0 (C=O), 165.0 (C=O), 164.1 (C=O), 145.5 (C=C), 142.1 (C=C), 132.4 (CH=), 119.9 (=CH2), 59.2 (C), 41.6 (CH_2), 40.8 (CH_2), 24.9 (CH_2), 19.9 (CH_2); ν_{max} (film) 2921, 1831, 1762, 1708, 1640, 1448, 1247, 923; MS (EI) 260.4 (M^+)

Dibenzyl acetylenedicarboxylate 267

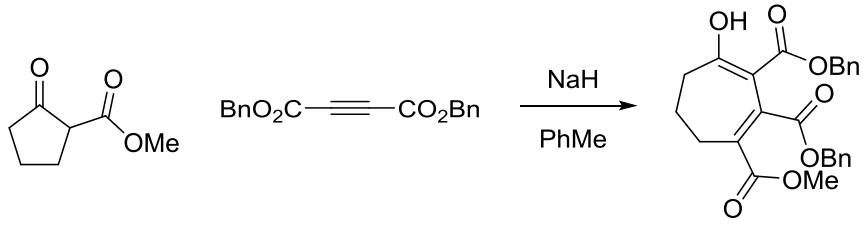


260 **267**

A mixture of diacid **260** (4.00 g, 35.1 mmol), benzyl alcohol (25 mL, 244 mmol, 7 equiv.), *p*-toluenesulfonic acid monohydrate (402 mg, 2.1 mmol, 0.06 equiv.) and hydroquinone (58 mg, 0.53 mmol. 0.015 equiv.) was heated to 100 °C for 16 h. The resulting red solution was distilled under reduced pressure (140 °C, 40 mmHg) to remove benzyl alcohol, then cooled to room temperature and diethyl ether (100 mL) and saturated aqueous $NaHCO_3$ (100 mL) added. The layers were separated, and the organic phase further extracted with saturated aqueous $NaHCO_3$ (2 × 100 mL). The organic layer was then dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using diethyl ether/petrol (5-10% diethyl ether) to give dibenzyl ether (3.93 g) and diester **267** (2.29 g, 22%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 7.41-7.34 (10H, m, Ar-H), 5.25 (4H, s, $OC\underline{H_2}Ph$); δ_C (100 MHz, $CDCl_3$) 151.7 (C=O), 134.1 (Ar-C), 129.1 (Ar-H), 128.9 (Ar-H), 128.8 (Ar-H), 75.0 (C≡C), 68.7 ($O\underline{C}H_2Ph$).

NMR data in accordance with literature.[229]

2,3-Dibenzyl 1-methyl 4-hydroxycyclohepta-1,3-diene-1,2,3-tricarboxylate 268

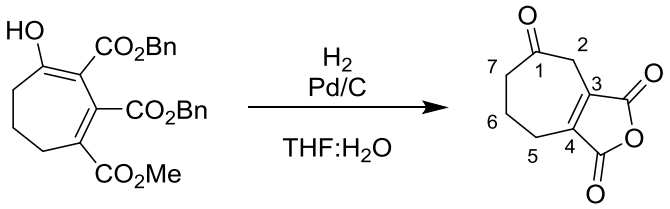


188 **267** **268**

To sodium hydride (60% dispersion in mineral oil, 217 mg, 5.3 mmol, 1.3 equiv.) in dry toluene (25 mL) under an N_2 atmosphere was added ketoester **188** (430 µL, 493 mg, 3.5 mmol) dropwise with stirring at 0 °C. After 1 h, diester **267** (1.20 g, 4.1 mmol, 1.2 equiv.) in toluene (10 mL) was added over 0.5 h. After 4 h, the reaction was cooled to 0 °C before adding glacial acetic acid (2 mL). Hydrochloric acid (1 M, 20 mL) was added, and the aqueous layer separated and washed with toluene

(3 × 40 mL). The combined organic phases were washed with water (50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a red oil. The crude material was purified by column chromatography using ethyl acetate/petrol (5-15% ethyl acetate) to give triester **268** (1.02 g, 57%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 13.14 (1H, s, OH), 7.32-7.27 (10H, m, Ar-H), 7.19-7.14 (2H, m, Ar-H), 5.07 (2H, s, O$\underline{C}H_2$Ph), 4.80 (2H, s, O$\underline{C}H_2$Ph), 3.51 (3H, s, OMe), 2.42 (4H, app. q, *J* 7.0, 5-H_2 and 7-H_2), 2.28 (2H, q, *J* 7.0, 6-H_2); δ_C (100 MHz, $CDCl_3$) 182.9 (C-4), 170.3 ($\underline{C}O_2$Bn), 169.0 ($\underline{C}O_2$Me), 166.8 ($\underline{C}O_2$Bn), 138.3 (C-1), 135.5 (Ar), 135.1 (Ar), 135.0 (C-2), 128.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.5 (Ar), 128.3 (Ar), 99.0 (C-3), 67.0 (O$\underline{C}H_2$Ph), 66.9 (O$\underline{C}H_2$Ph), 52.3 (OMe), 33.9 (C-6), 31.8 (C-5), 28.5 (C-7); ν_{max} (film) 2950, 1718, 1646, 1583, 1302, 1248, 1196, 909, 731; HRMS (ESI) calc for $C_{25}H_{24}NaO_7$ $[M+Na]^+$ 459.1414, found 459.1412.

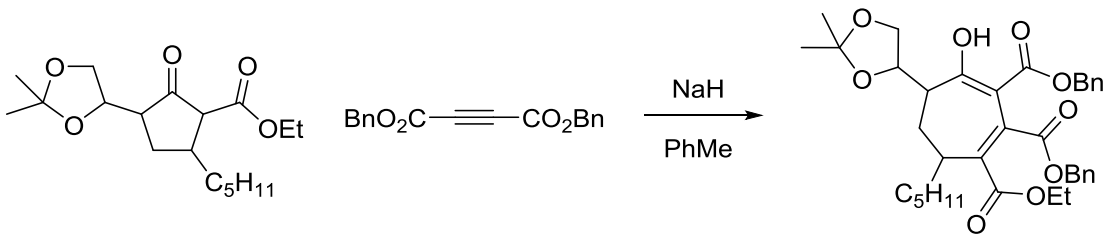
1-Oxo-cyclohept-3-ene-3,4-dicarboxylic acid anhydride 198



268 **198**

To triester **268** (200 mg, 0.46 mmol) in THF (6 mL) and water (0.5 mL) was added palladium (10 wt% on carbon, 49 mg, 0.046 mmol, 0.1 equiv.). A balloon of hydrogen was fixed to the vessel and the reaction mixture purged with hydrogen. The empty balloon was removed, and replaced with a fresh balloon of hydrogen, and the suspension was stirred at room temperature. After 1.25 h, the hydrogen atmosphere was removed and replace with nitrogen. The reaction mixture was then filtered through a bed of Celite® and $MgSO_4$, then concentrated *in vacuo.* The crude material was purified by column chromatography using ethyl acetate/petrol/acetic acid (25% ethyl acetate, 1% acetic acid) to give anhydride **198** as a yellow oil (51 mg, 62%). NMR data as reported above.

2,3-Dibenzyl 1-ethyl 5-(2,2-dimethyl-1,3-dioxolan-4-yl)-4-hydroxy-7-pentylcyclohepta-1,3-diene-1,2,3-tricarboxylate 269

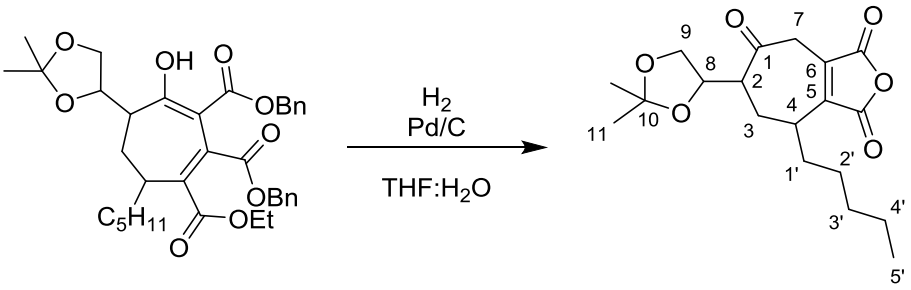


254 **267** **269**

To sodium hydride (60% dispersion in mineral oil, 295 mg, 7.2 mmol, 1.3 equiv.) in dry toluene (45 mL) was added a solution of ketoester **254** (1.796 g, 5.5 mmol) in dry toluene (15 mL) dropwise

with stirring at 0 °C over 0.5 h. After 1 h, diester **267** (2.12 g, 7.2 mmol, 1.3 equiv.) in dry toluene (12 mL) was added over 0.5 h. After 4 h, the reaction was cooled to 0 °C before glacial acetic acid (2 mL) was added. Hydrochloric acid (1 M, 20 mL) was added, and the aqueous layer separated and washed with toluene (3 × 40 mL). The combined organic phases were washed with water (50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to a red oil. The crude material was purified by column chromatography using ethyl acetate/petrol (2-7% ethyl acetate) to give a mixture of diastereomers of triester **269** (2.34 g, 68%) as an orange oil. ν_{max} (film) 2930, 2859, 1721, 1644, 1596, 1455, 1213; HRMS (ESI) calc for $C_{36}H_{44}NaO_9$ $[M+Na]^+$ 643.2877, found 643.2874. ^1H and ^{13}C NMR spectra can be found in the spectral appendix.

1-Oxo-2-(2,2-dimethyl-1,3-dioxolan-4-yl)-4-pentyl-cyclohept-5-ene-5,6-dicarboxylic acid anhydride 270



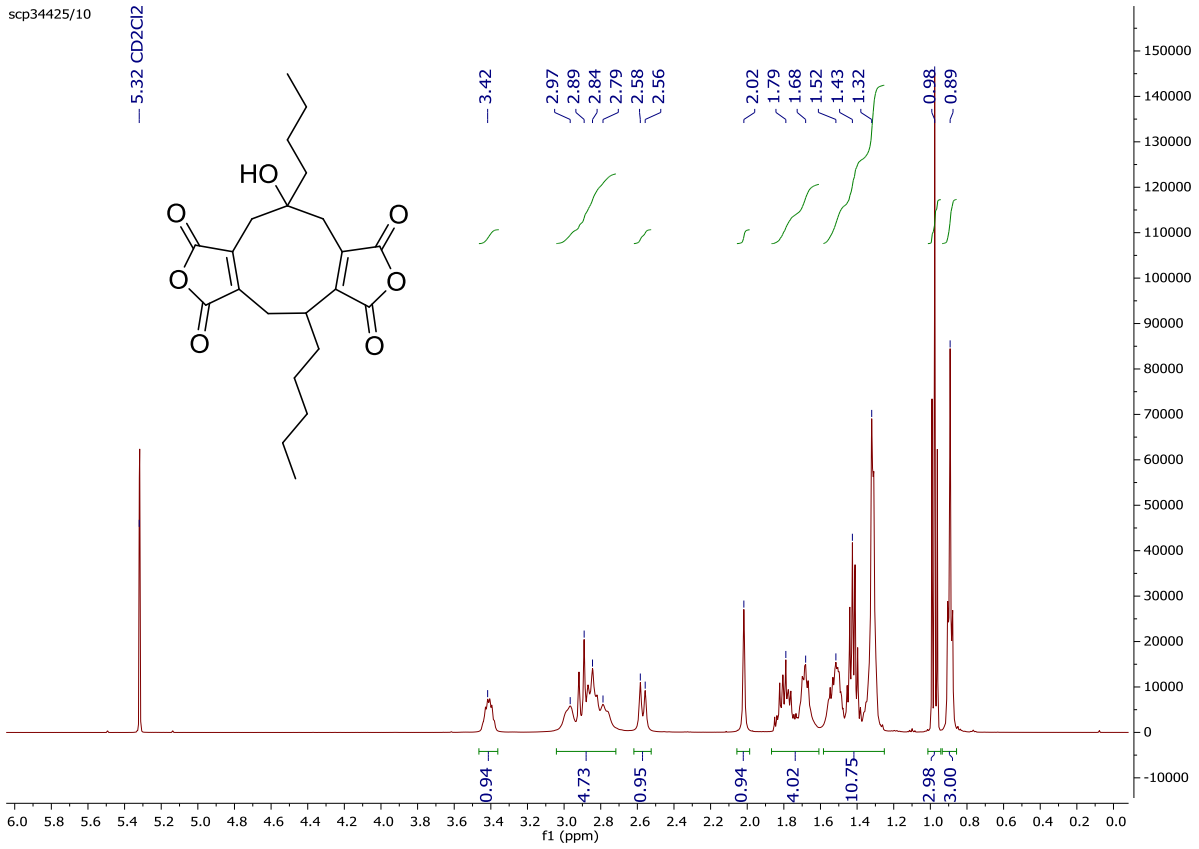
269 **270**

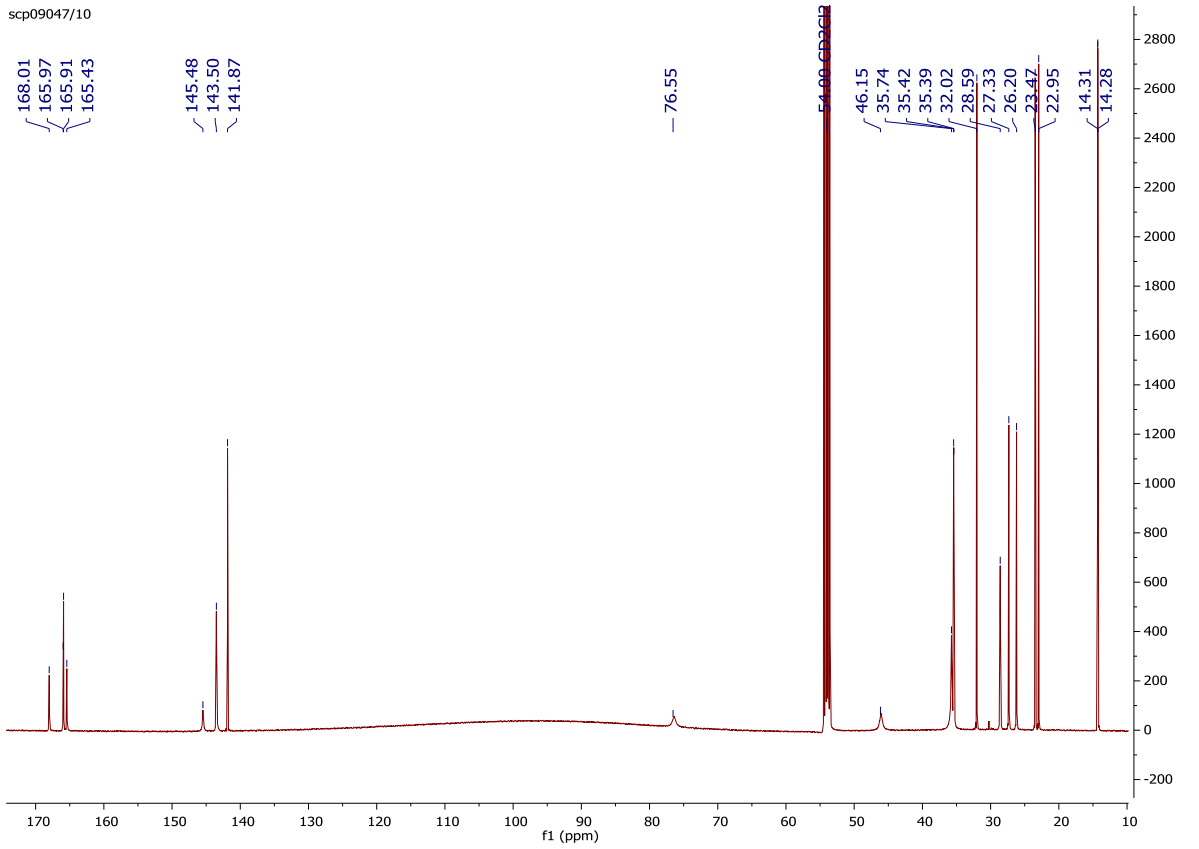
To triester **269** (150 mg, 0.24 mmol) in THF (3 mL) and water (0.2 mL) was added palladium (10 wt% on carbon, 26 mg, 0.024 mmol, 0.1 equiv.). A balloon of hydrogen was fixed to the vessel and the reaction mixture purged with hydrogen. The empty balloon was removed, and replaced with a fresh balloon of hydrogen, and the suspension was stirred at room temperature. After 1 h, the hydrogen atmosphere was removed and replace with nitrogen. The reaction mixture was then filtered through a bed of Celite® and $MgSO_4$, then concentrated *in vacuo.* The crude material was purified by column chromatography using ethyl acetate/petrol/acetic acid (10-25% ethyl acetate, 1% acetic acid) to give anhydride **270** as a yellow oil (17 mg, 20%). δ_H (400 MHz, $CDCl_3$) 4.38 (1H, m, 8-H), 4.14 (1H, dd, *J* 9.0, 6.5, 9-*H*H), 3.69-3.70 (2H, m, 9-H*H* and 7-*H*H), 3.57 (1H, d, *J* 18.0, 7-H*H*), 3.07-2.95 (2H, m, 2-H and 4-H), 2.18 (1H, dt, *J* 15.0, 3.5, 3-*H*H), 2.02 (1H, ddd, *J* 15.0, 12.0, 3.5, 3-H*H*), 1.70 (1H, m, 1'-*H*H), 1.51 (1H, m, 2'-*H*H), 1.47 – 1.25 (12H, m 2 × 11-H_3, 1'-H*H*, 2'-H*H*, 3'-H_2, 4'-H_2), 0.89 (3H, t, *J* 6.5, 5'-H_3); δ_C (100 MHz, $CDCl_3$) 204.0 (C-1), 164.7 (C=O), 164.6 (C=O), 148.2 (C-5), 135.3 (C-6), 109.2 (C-10), 75.6 (C-8), 66.4 (C-9), 50.7 (C-2), 40.1 (C-7), 34.1 (C-4), 31.5 (C-3'), 30.6 (C-1'), 27.0 (C-2'), 26.3 (C-11), 26.1 (C-3), 24.8 (C-11), 22.6 (C-4'), 14.1 (C-5'); ν_{max} (film) 2931, 2862, 1765, 1713, 1456, 1380, 1259, 1044, 921; HRMS (ESI) calc for $C_{19}H_{26}NaO_6$ $[M+Na]^+$ 373.1622, found 373.1620.

CHAPTER 8: Selected Spectra

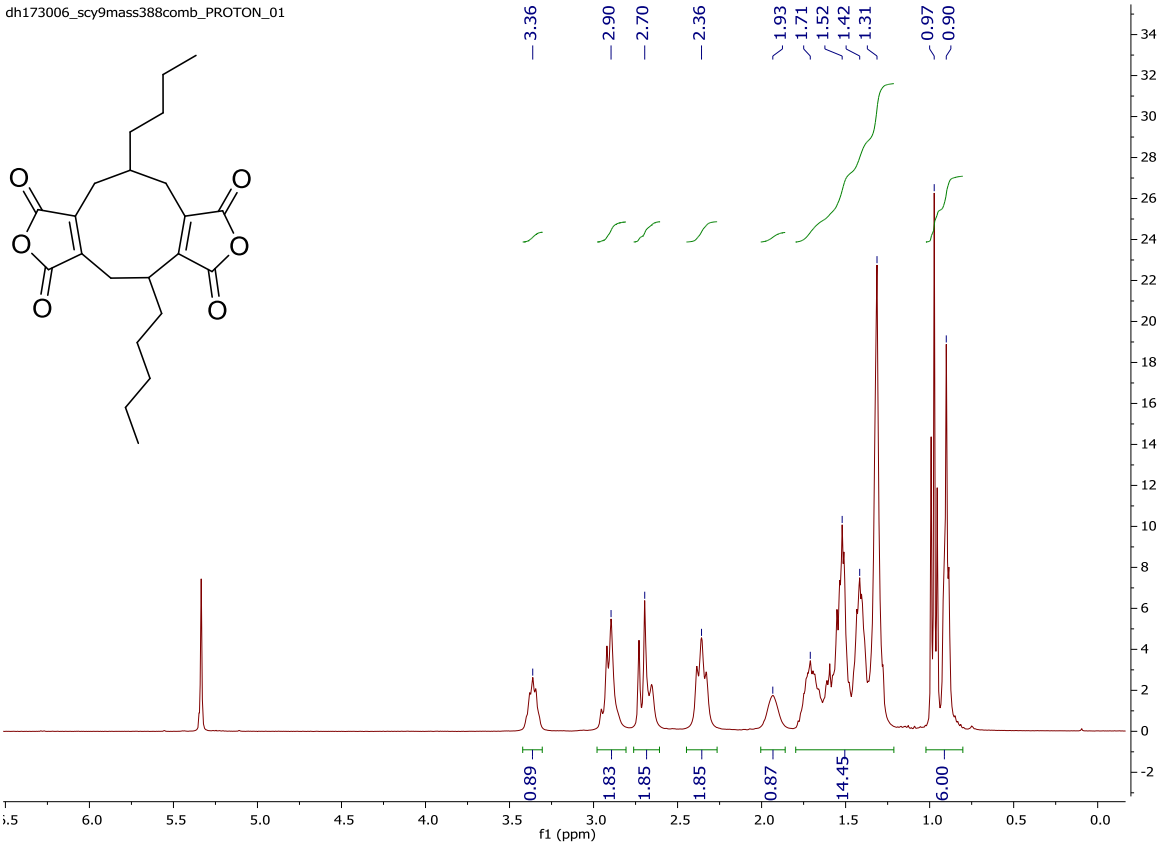
8. Selected NMR Spectra

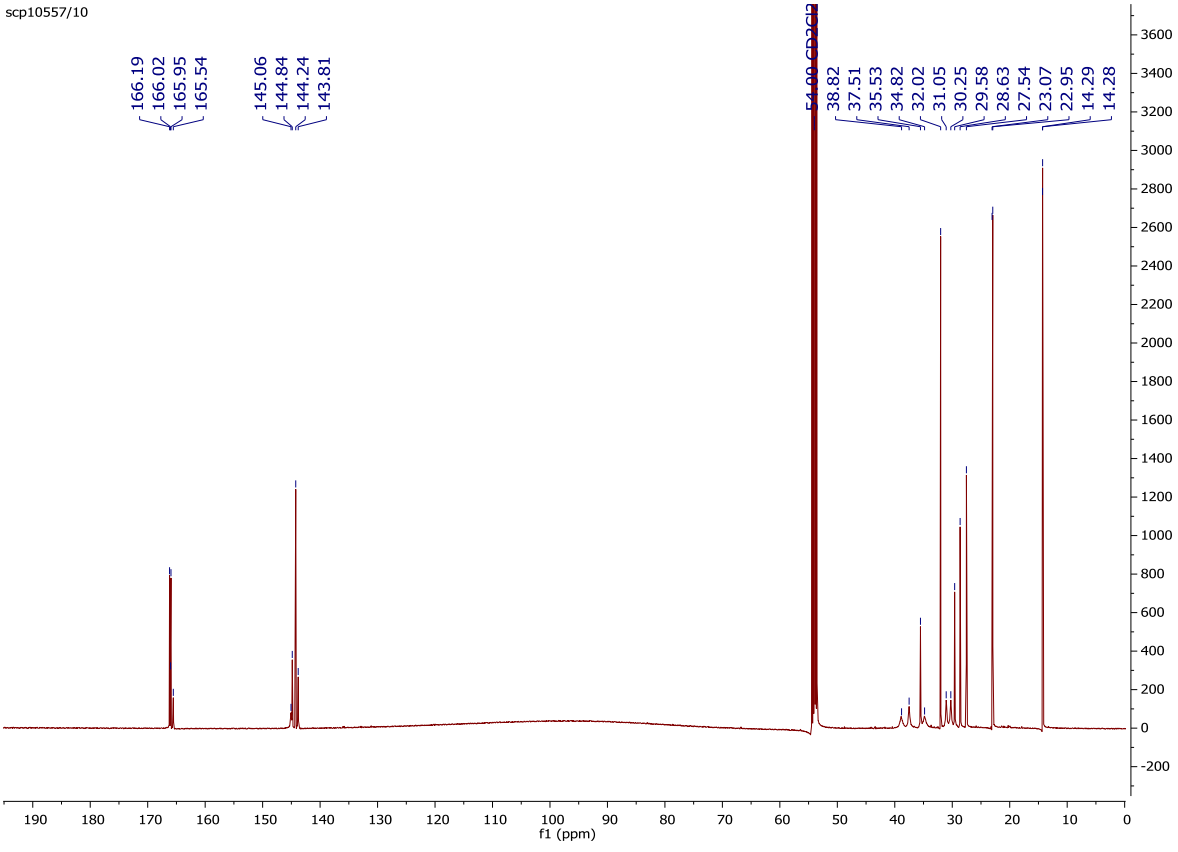
Scytalidin 12



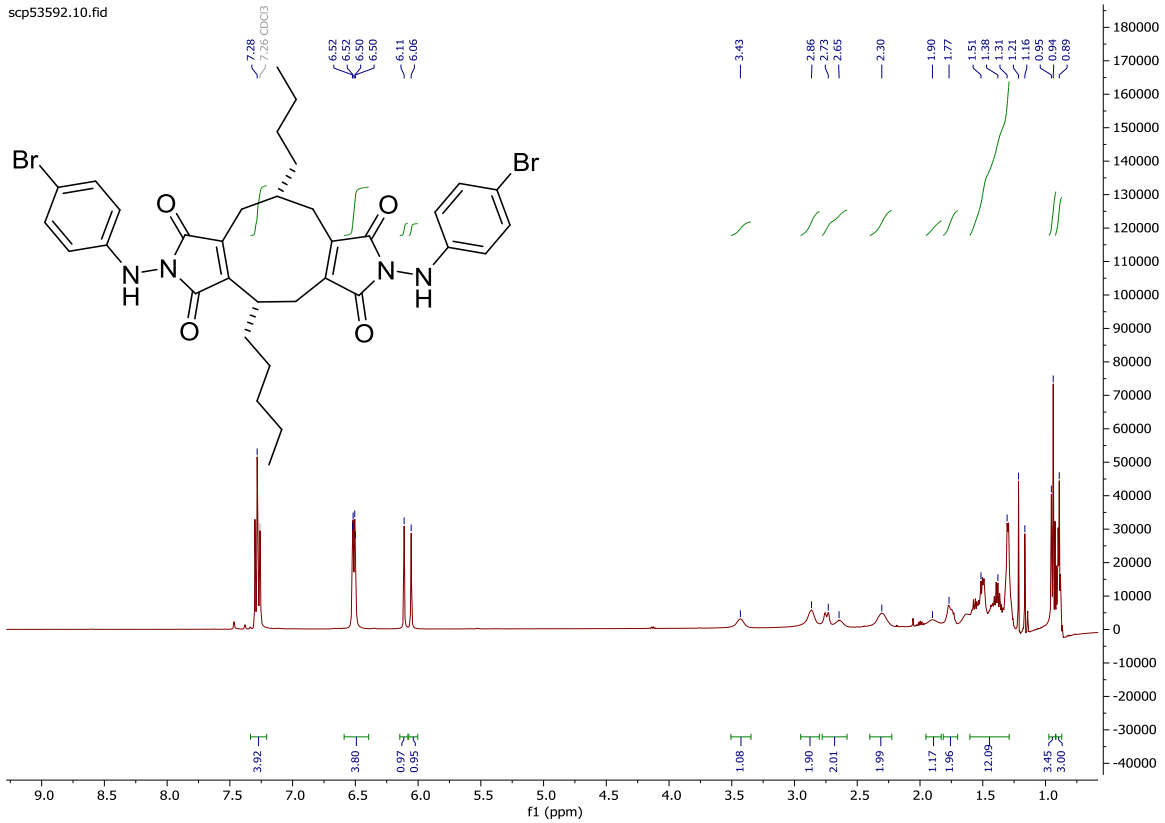


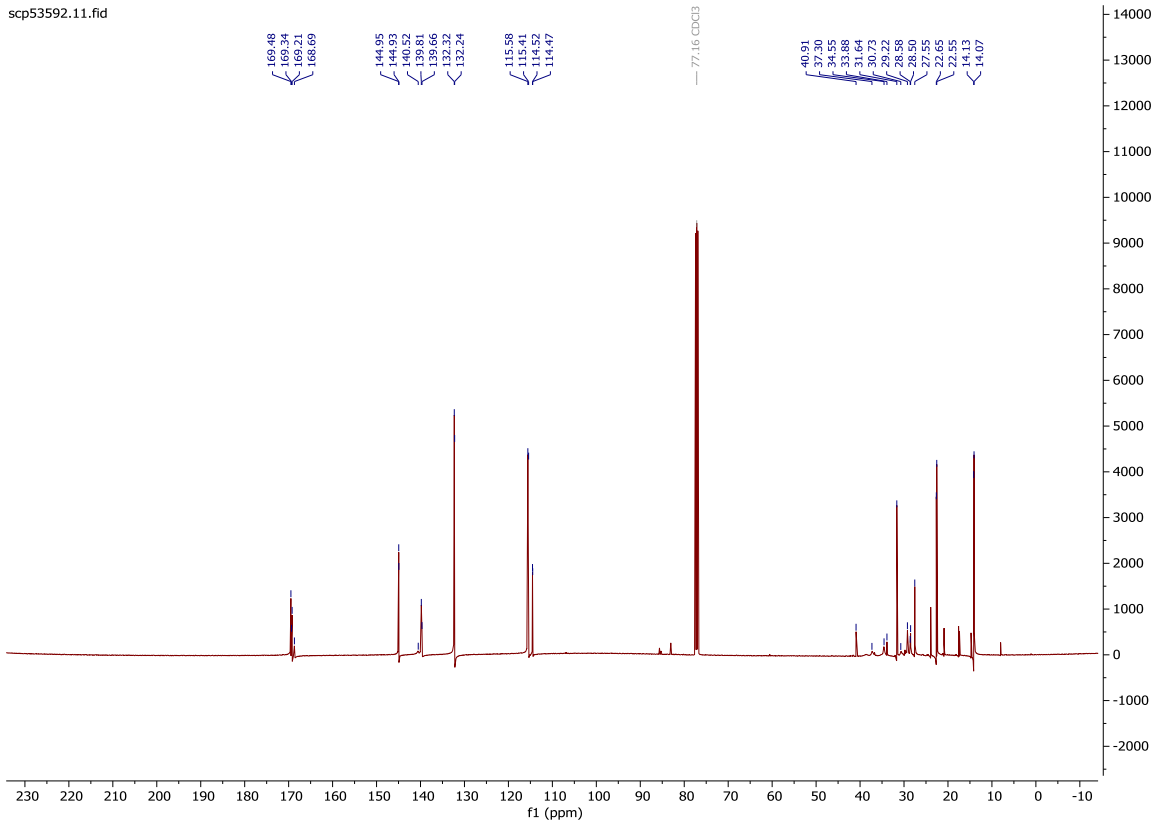
Deoxyscytalidin 35



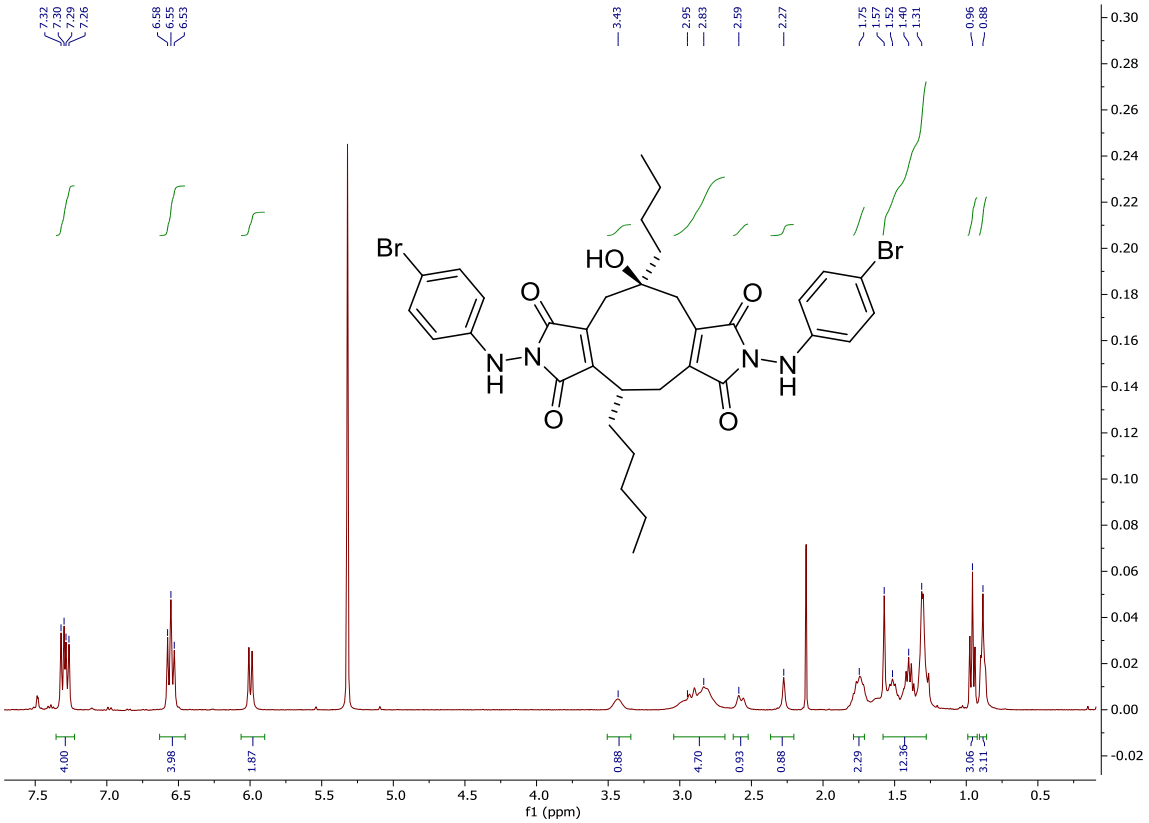


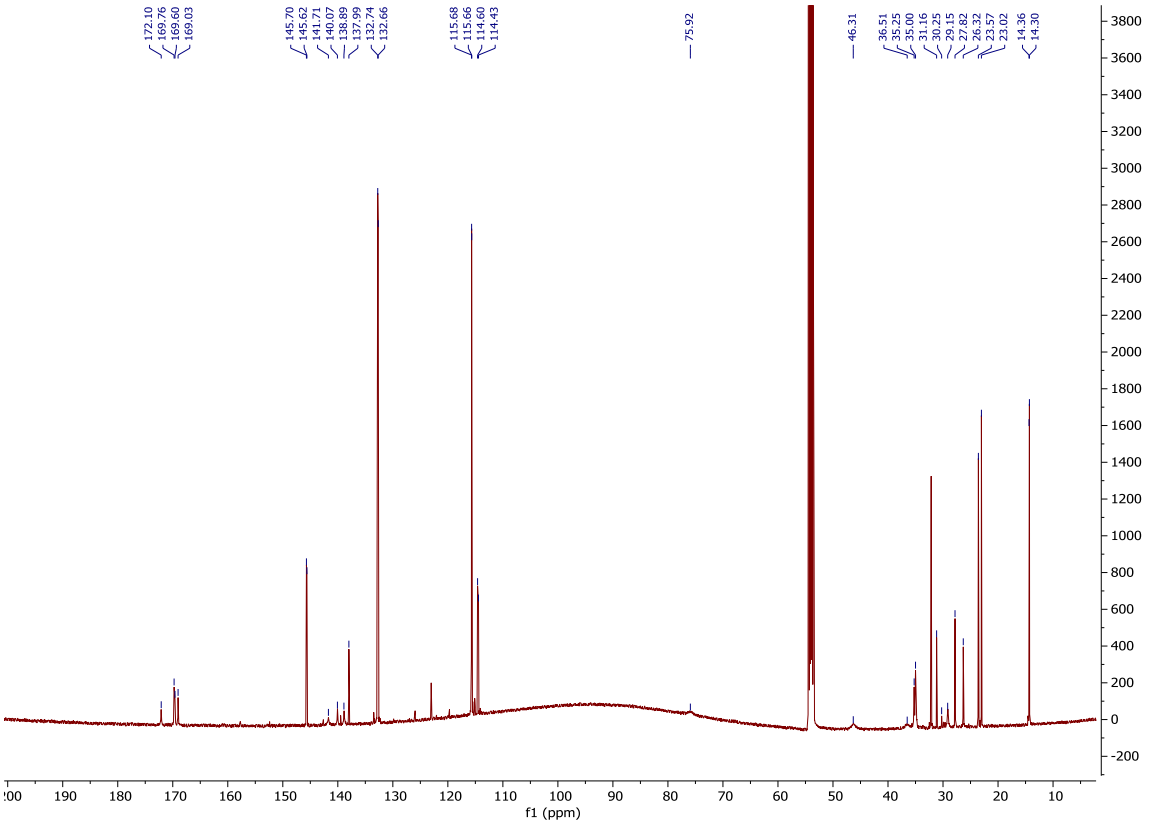
Deoxyscytalidin bis(4-bromophenylhydrazide) maleimide derivative 52



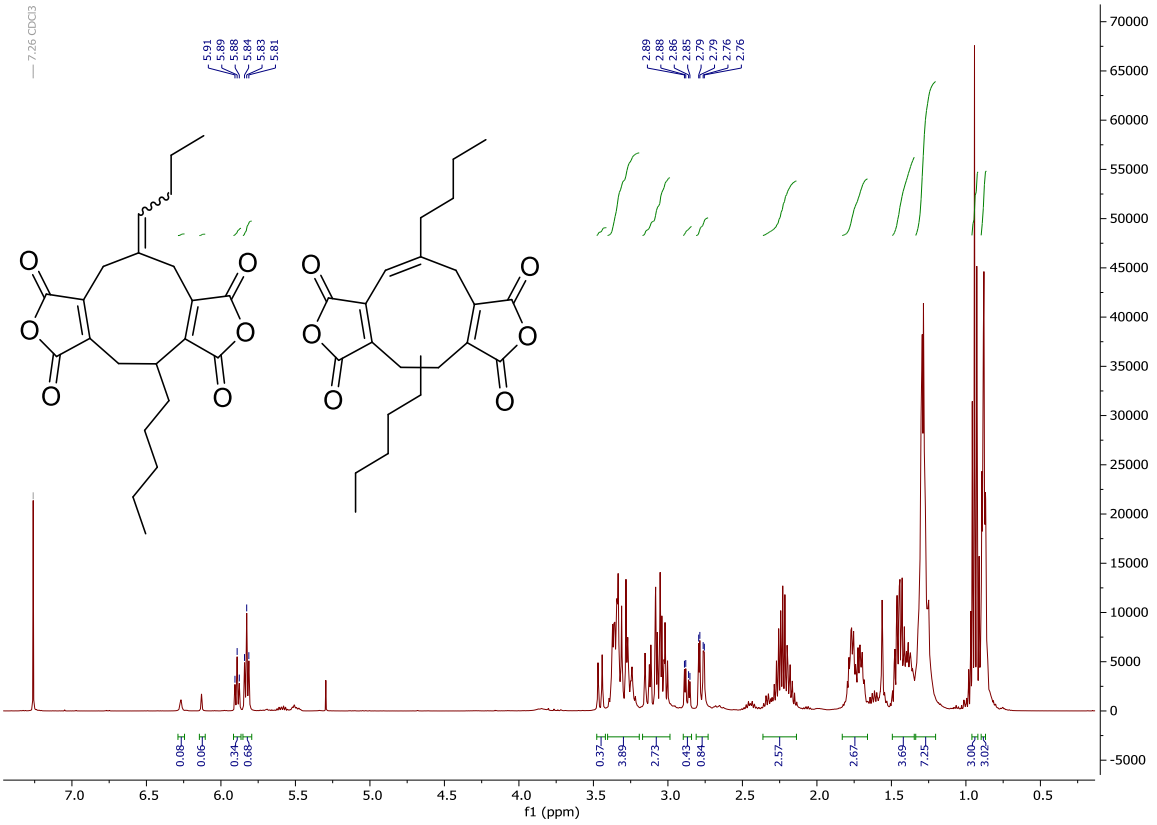


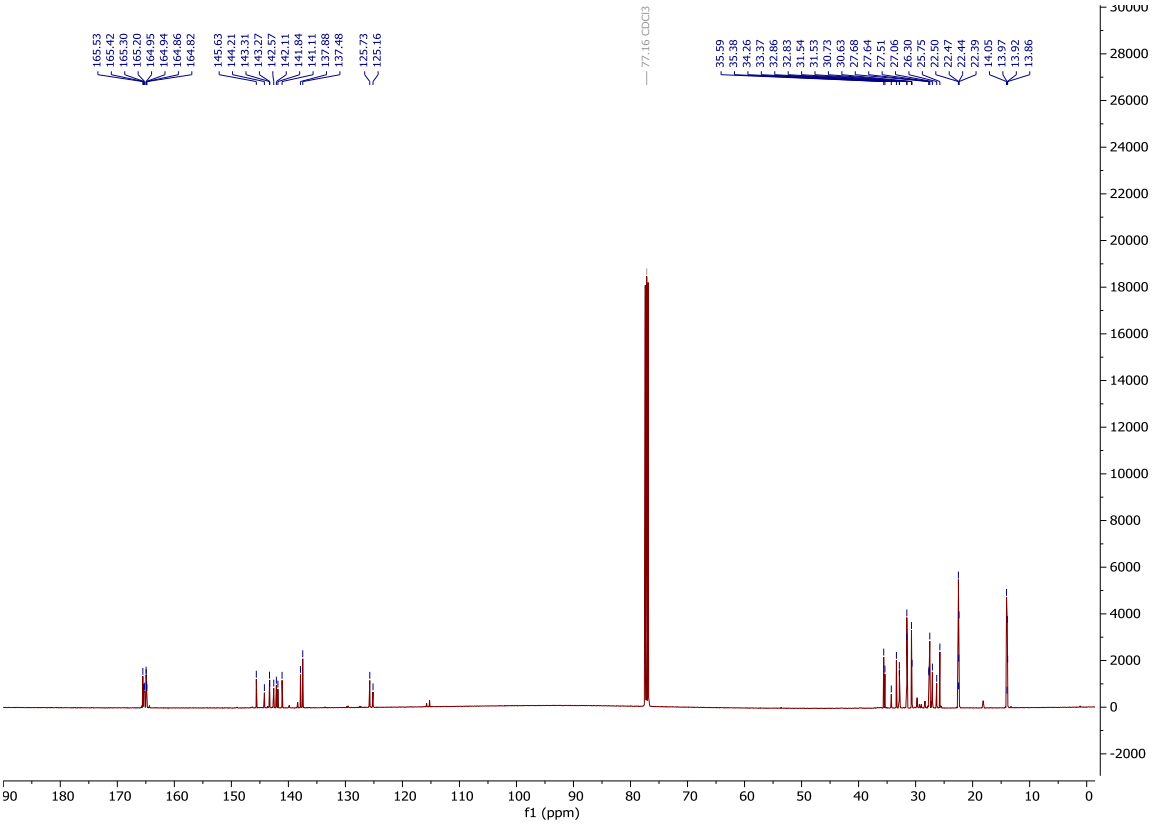
Scytalidin bis(4-bromophenylhydrazide) maleimide derivative 51



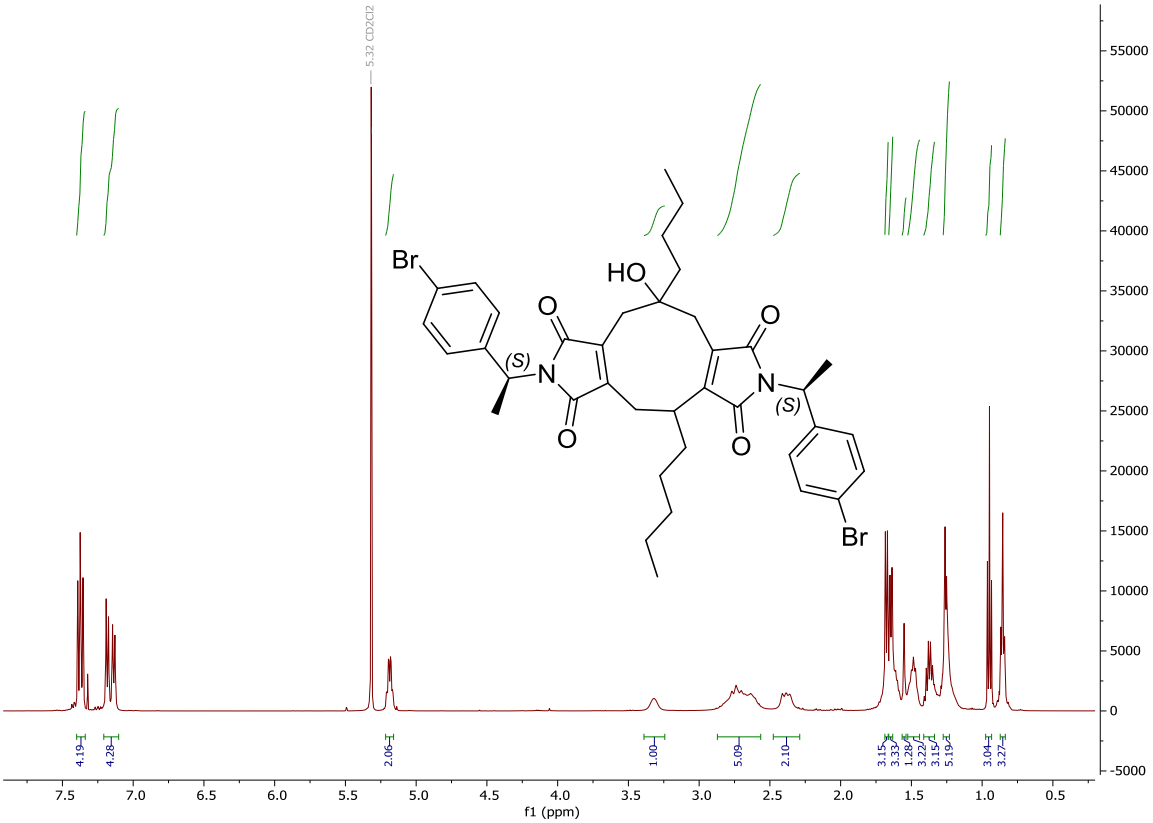


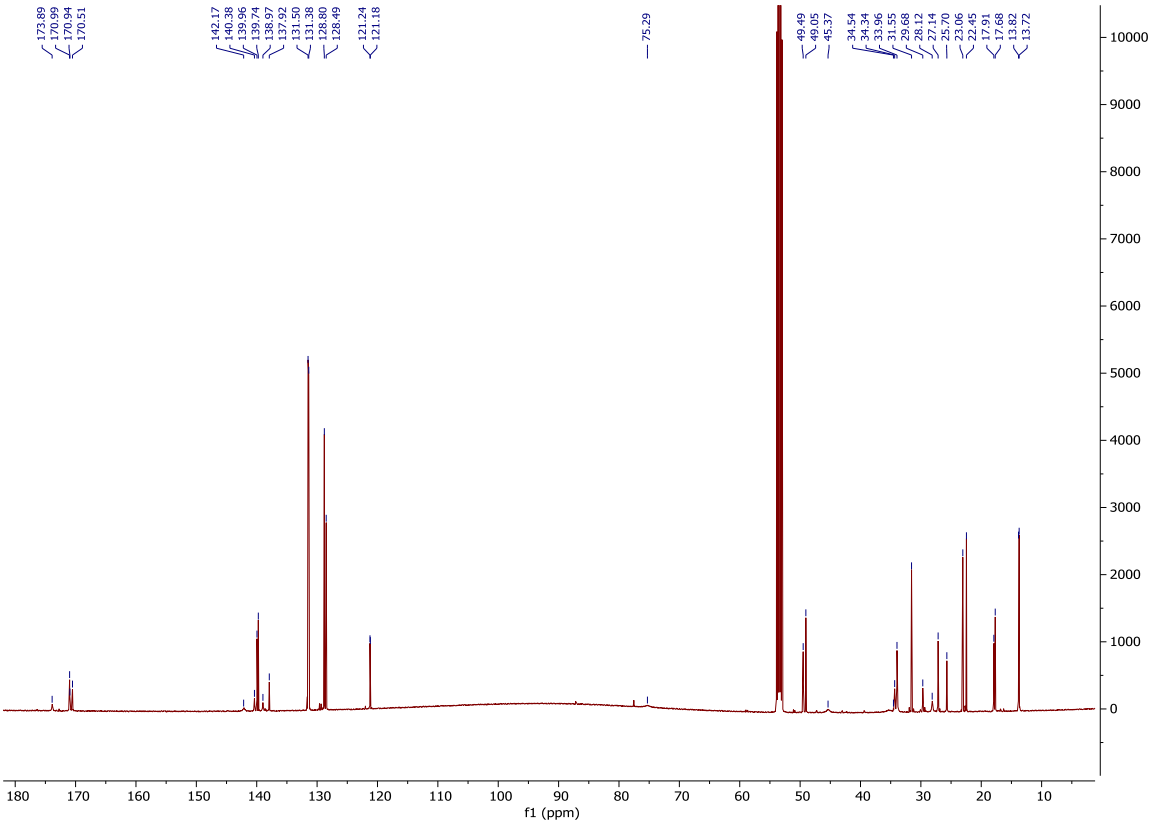
Elimination of Scytalidin 55 + 56



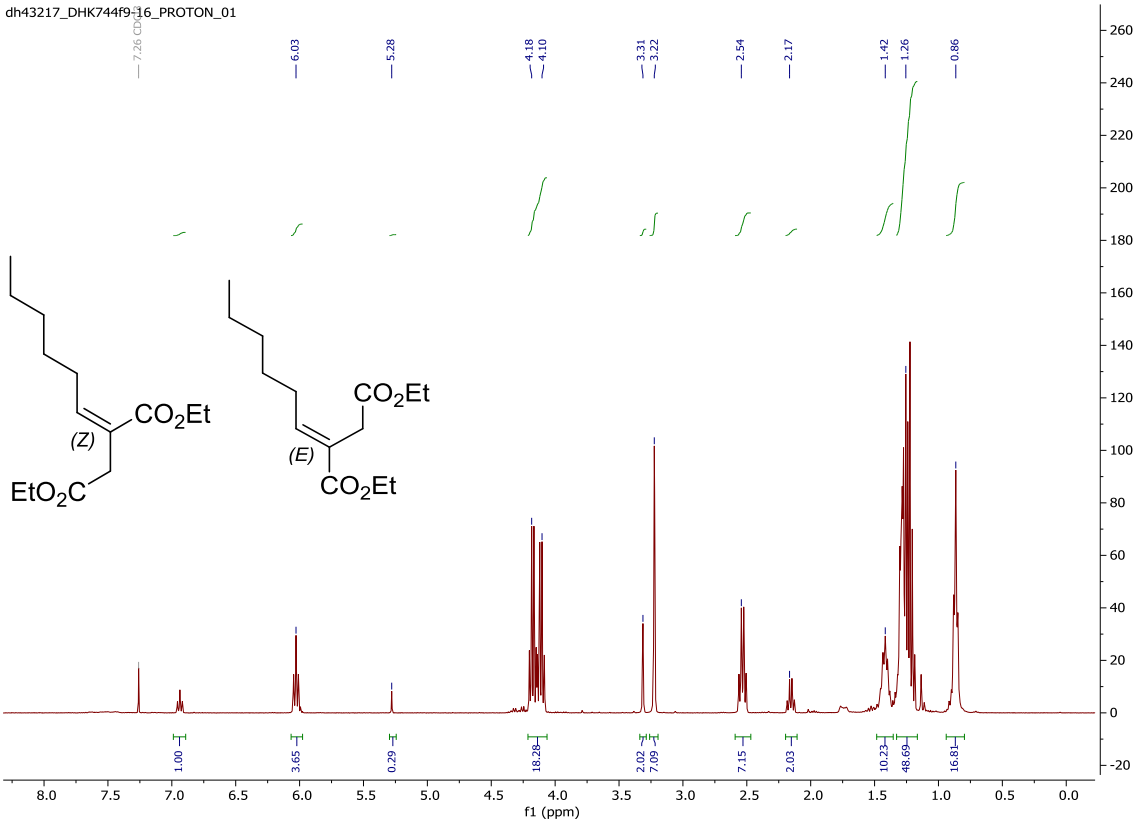


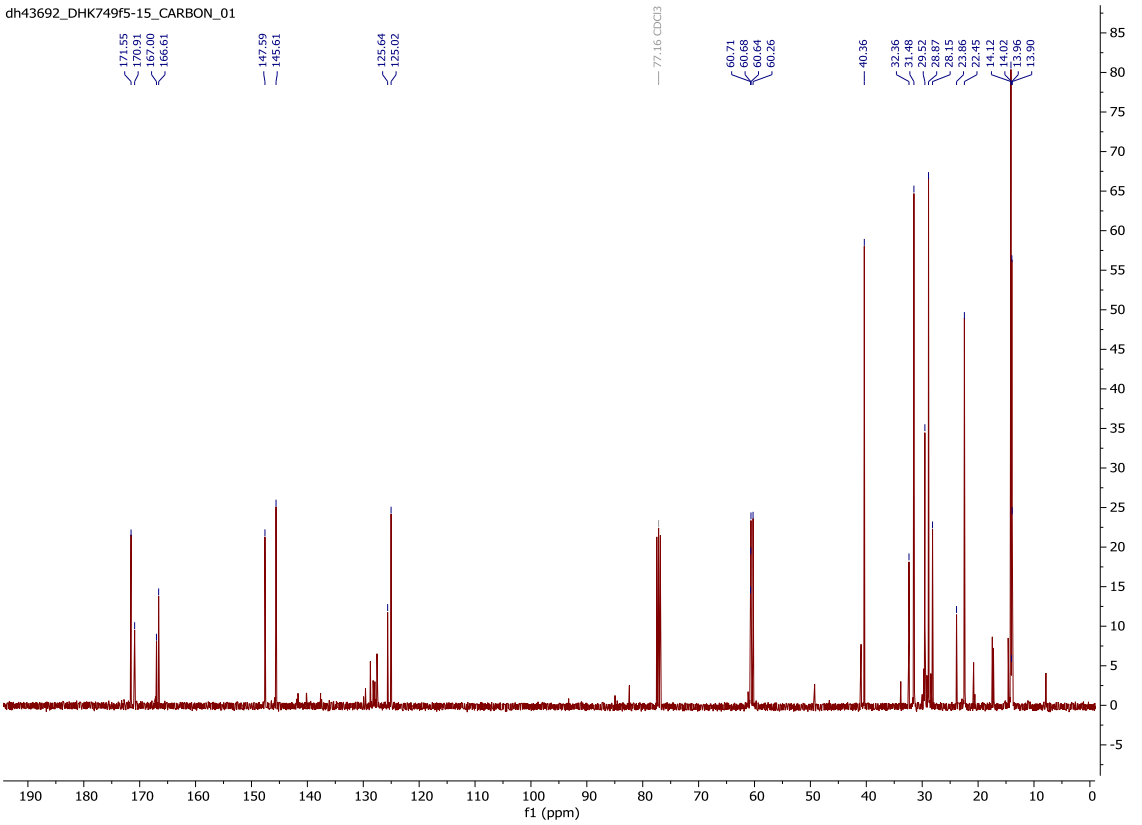
Scytalidin bis(4-bromo-α-methylbenzylamidyl) maleimide derivative 53



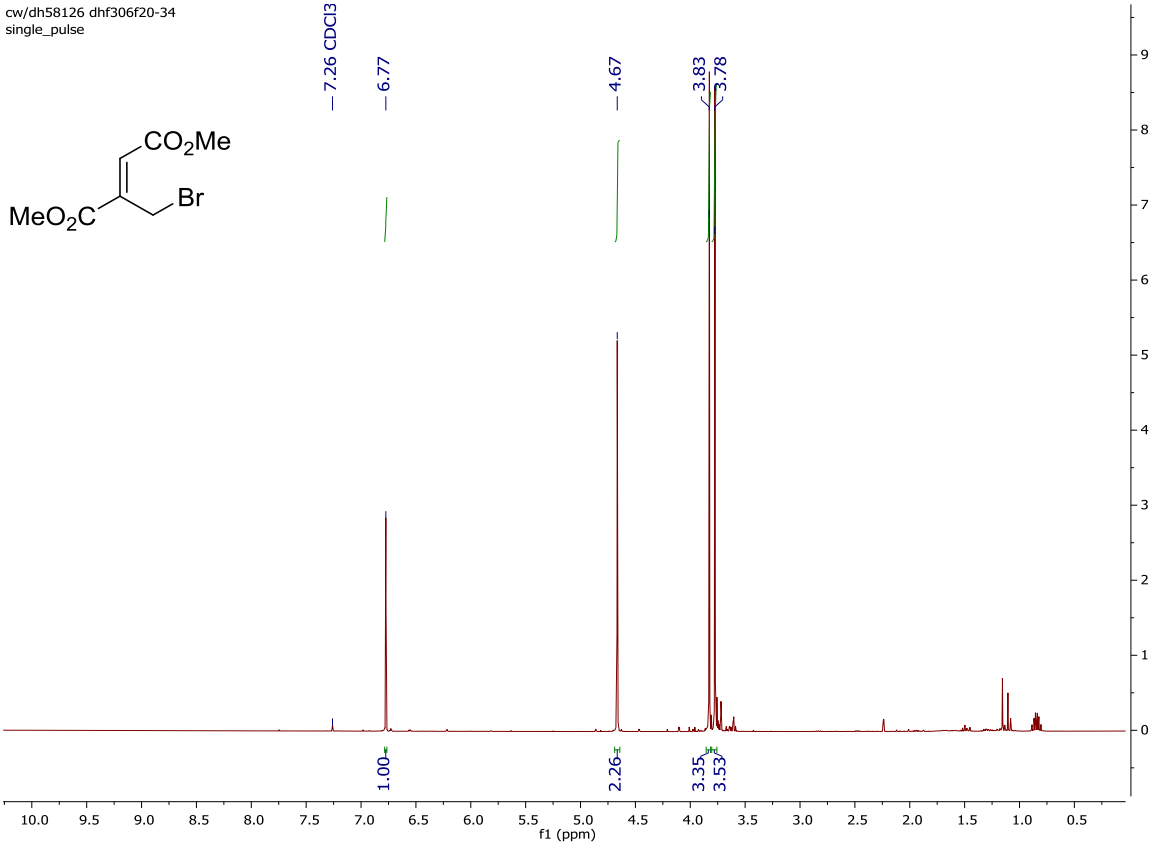


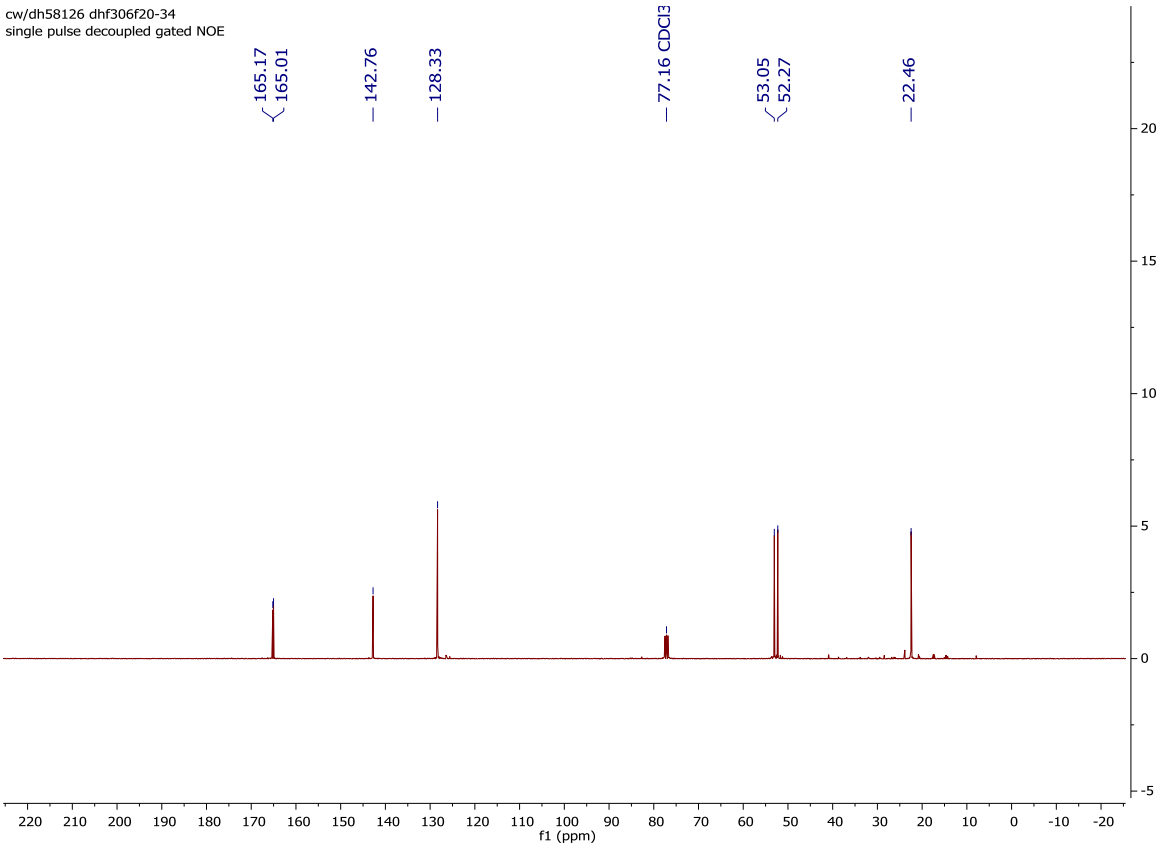
Diethyl (*E*/*Z*)-2-hexylidenesuccinate 66B



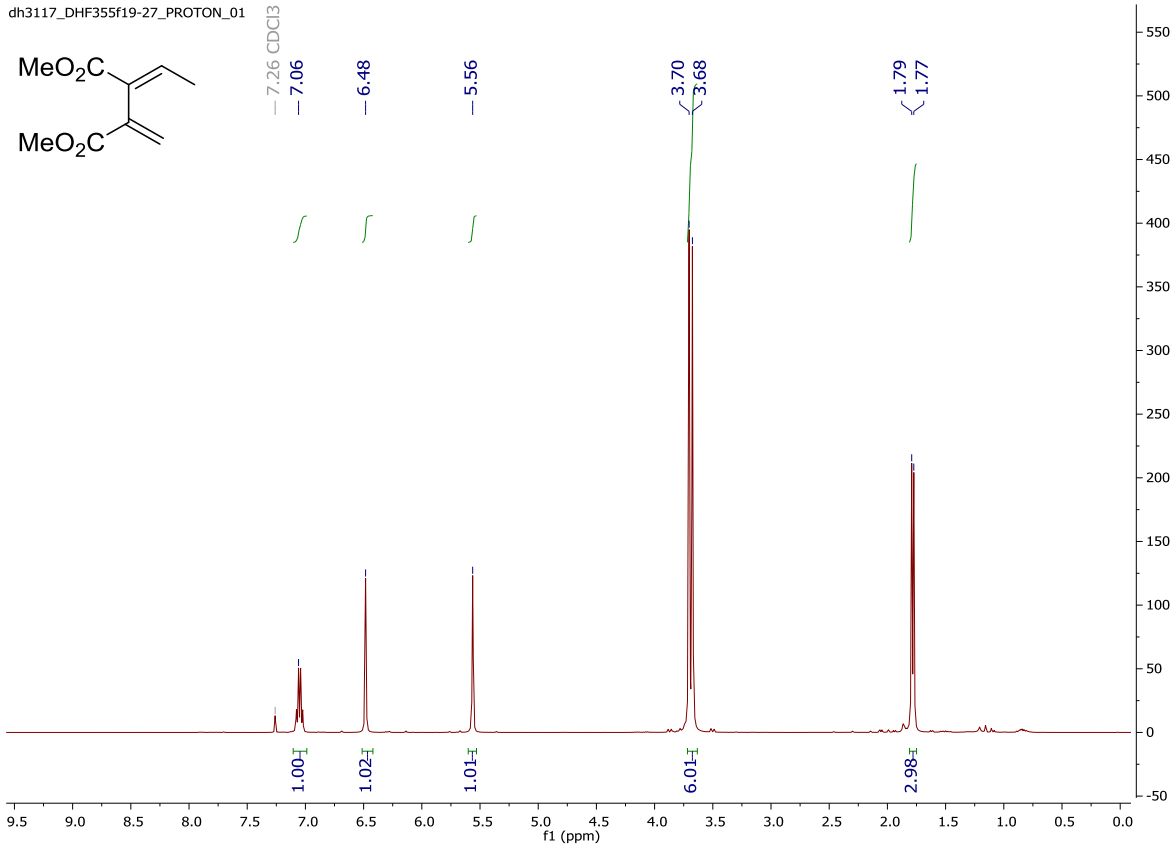


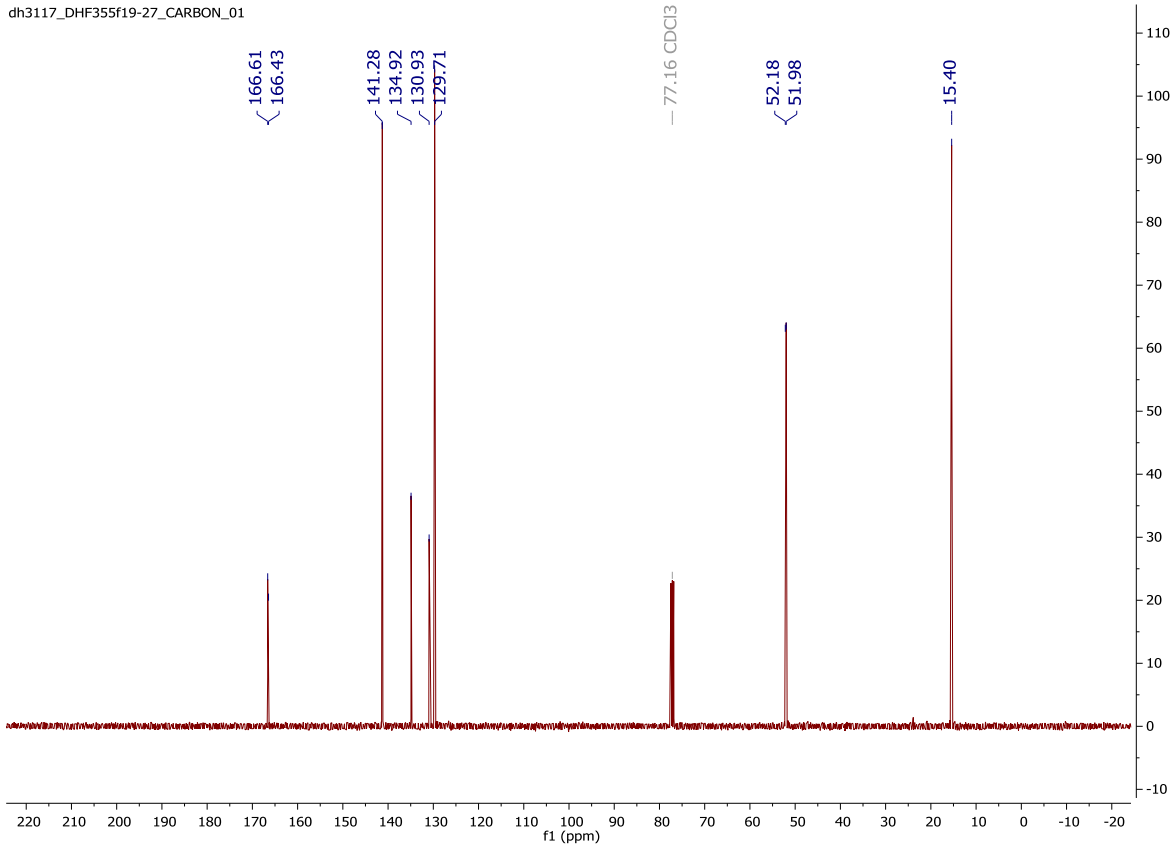
Dimethyl 2-(bromomethyl)maleate 68



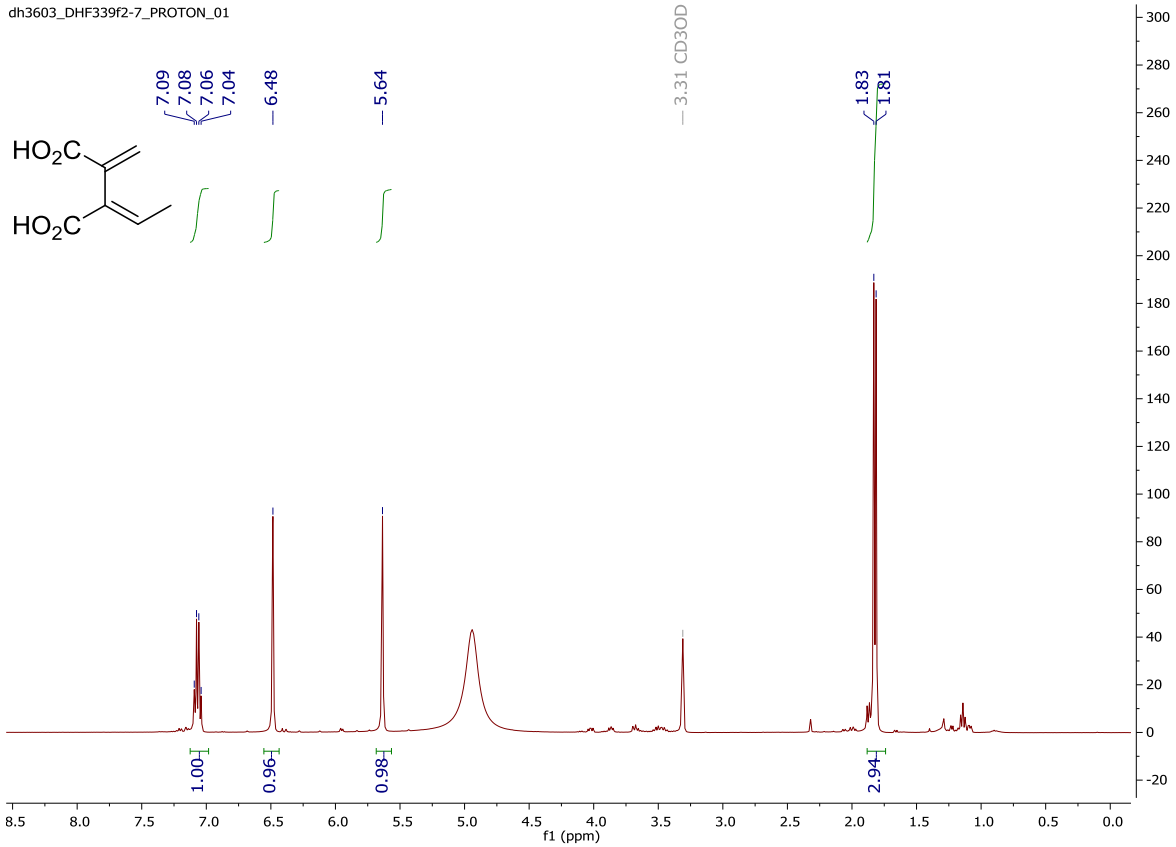


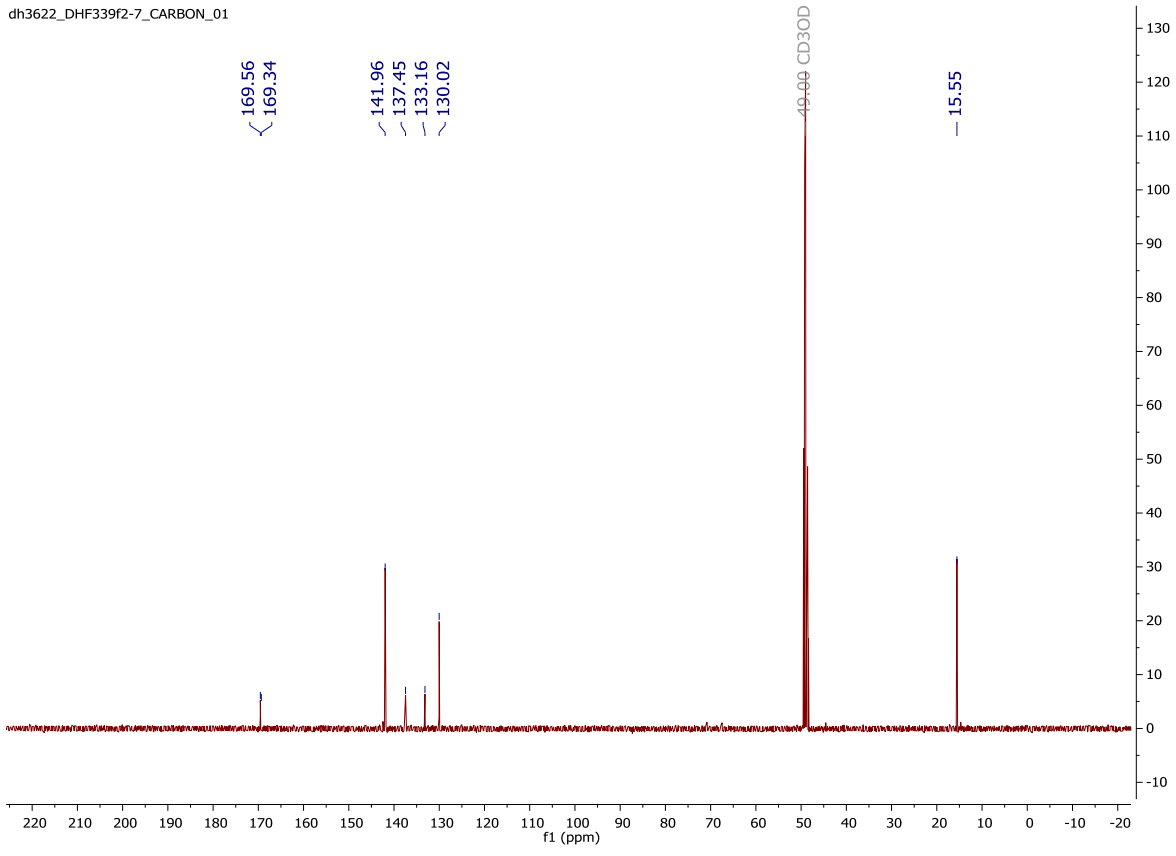
Dimethyl (*E*)-2-ethylidene-3-methylenesuccinate 72



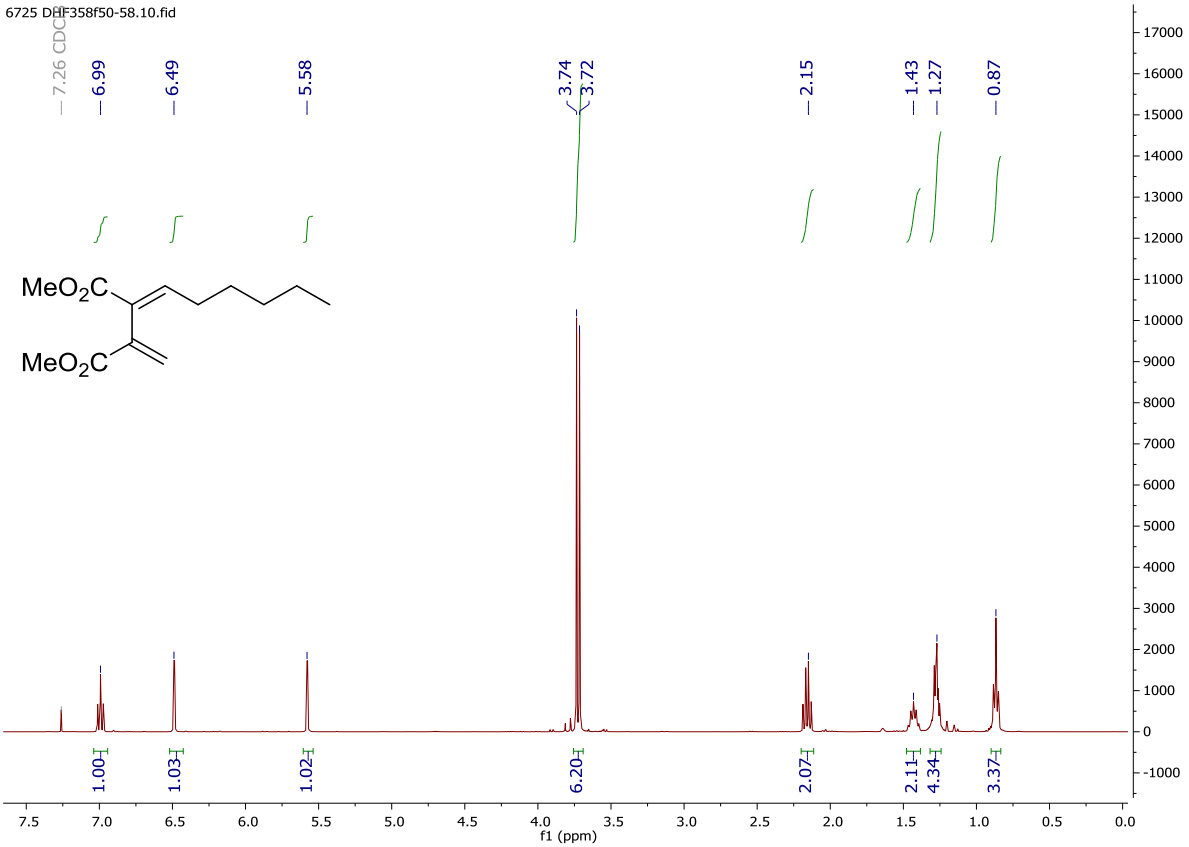


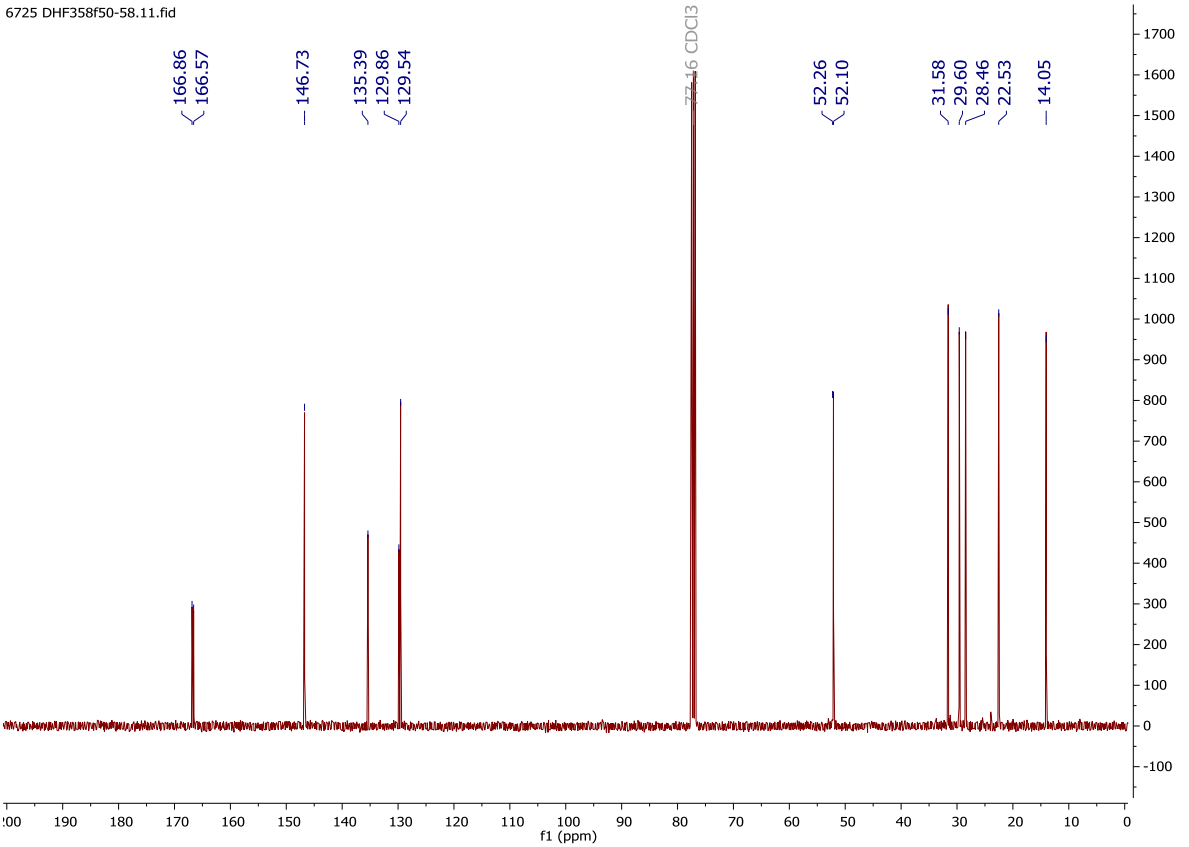
(*E*)-2-Ethylidene-3-methylenesuccinic acid 73



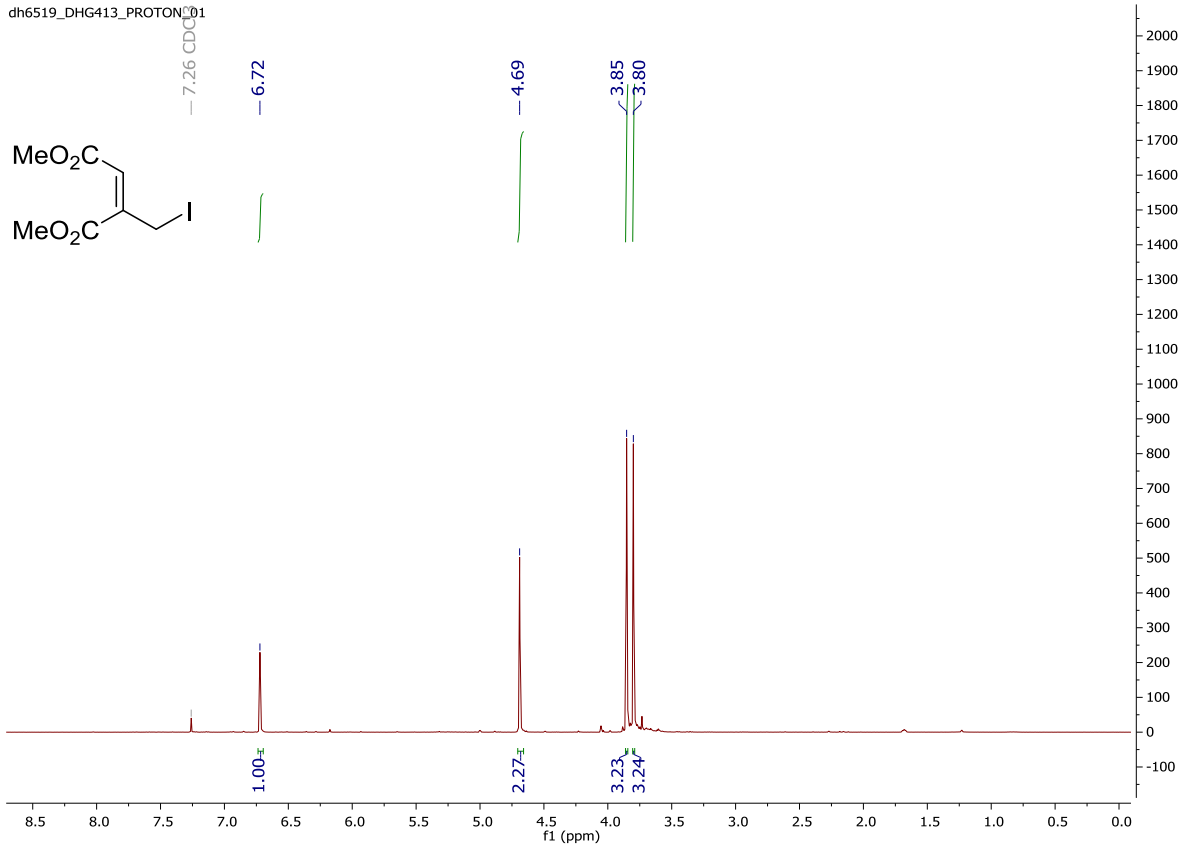


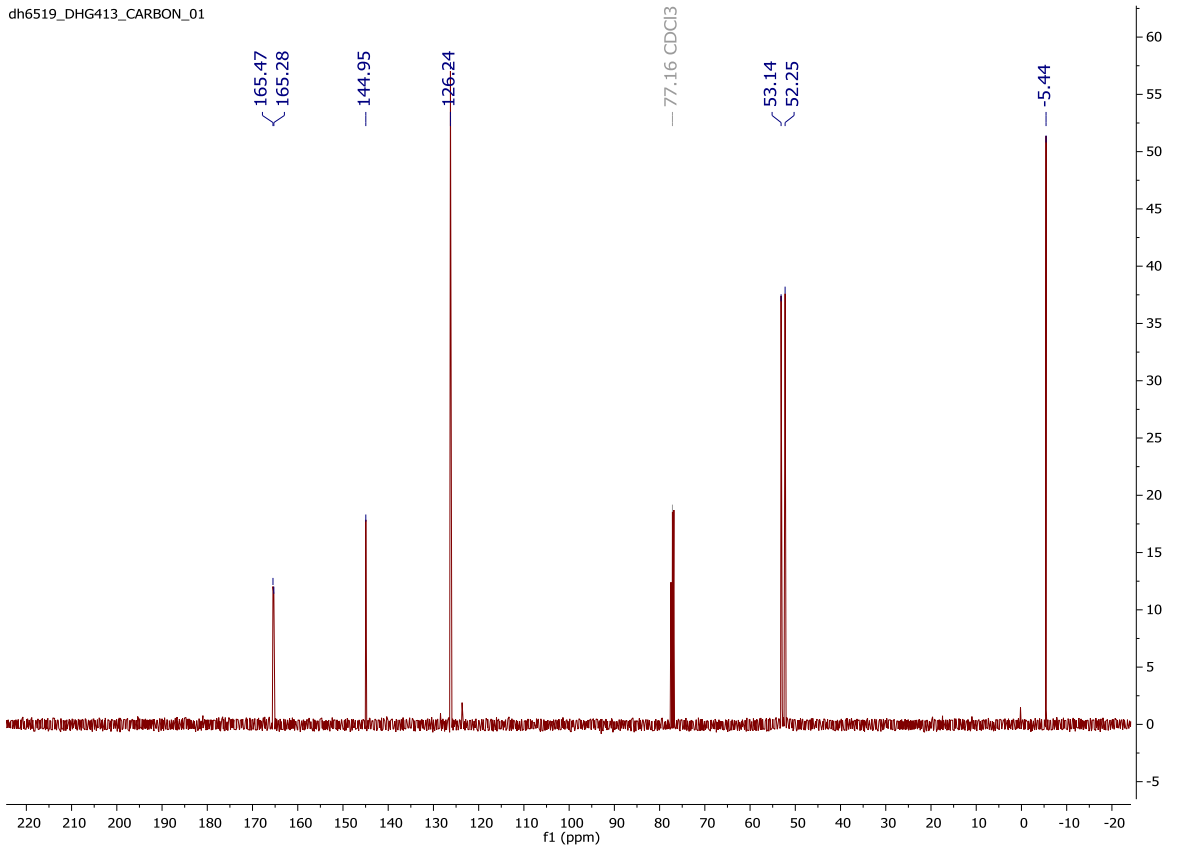
Dimethyl (*E*)-2-hexylidene-3-methylenesuccinate 153



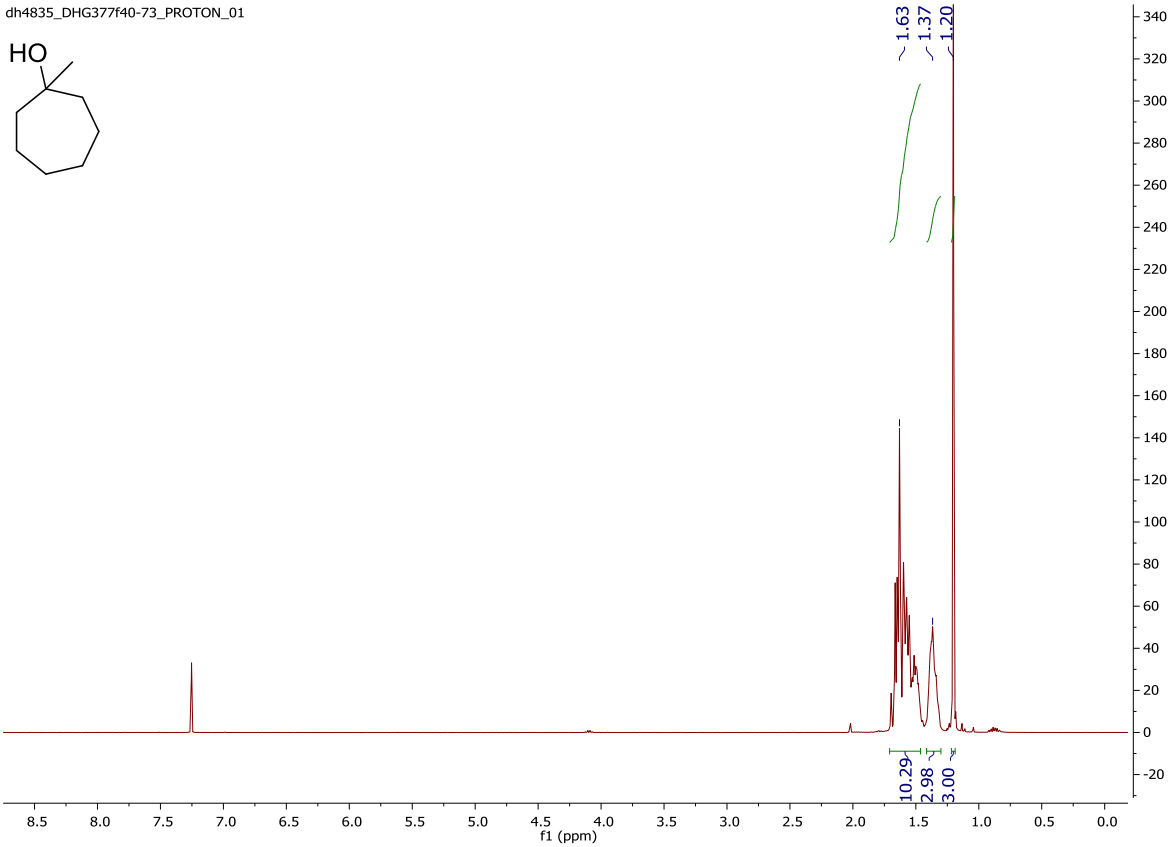


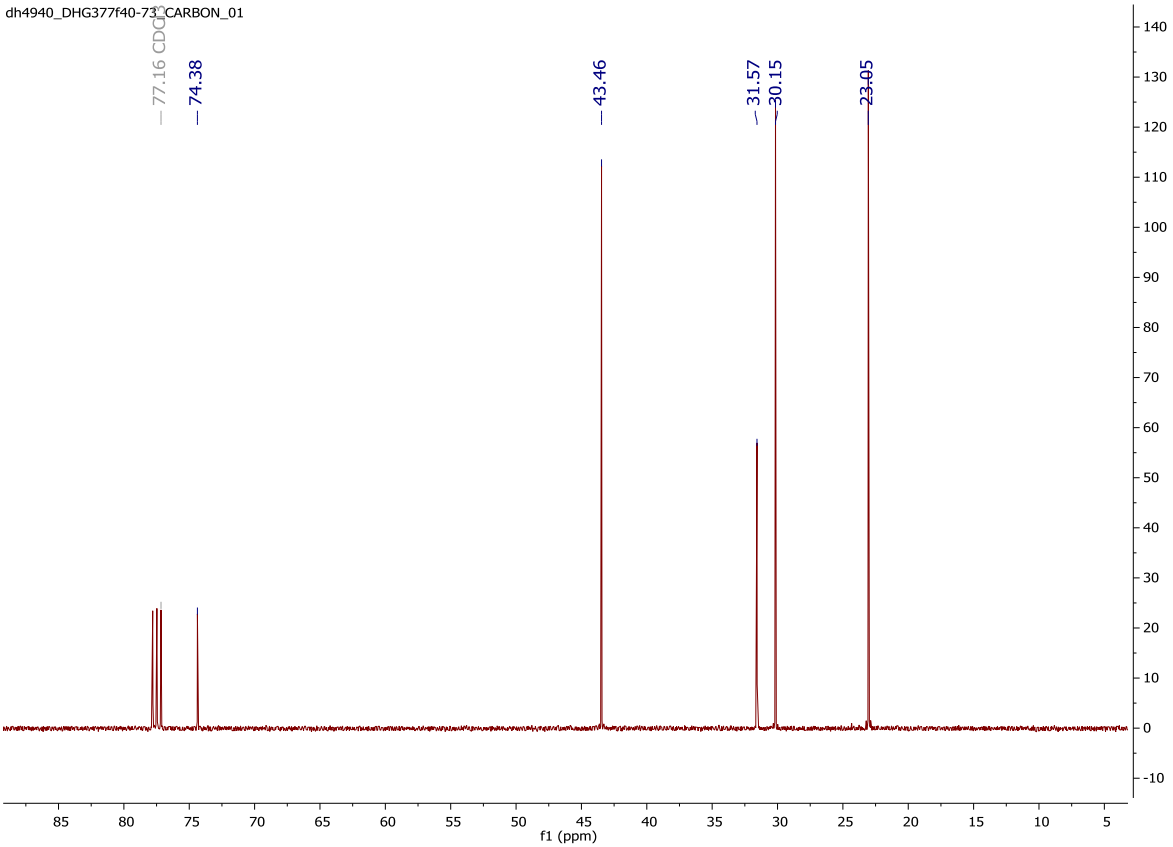
Dimethyl (2-iodomethyl)maleate S4



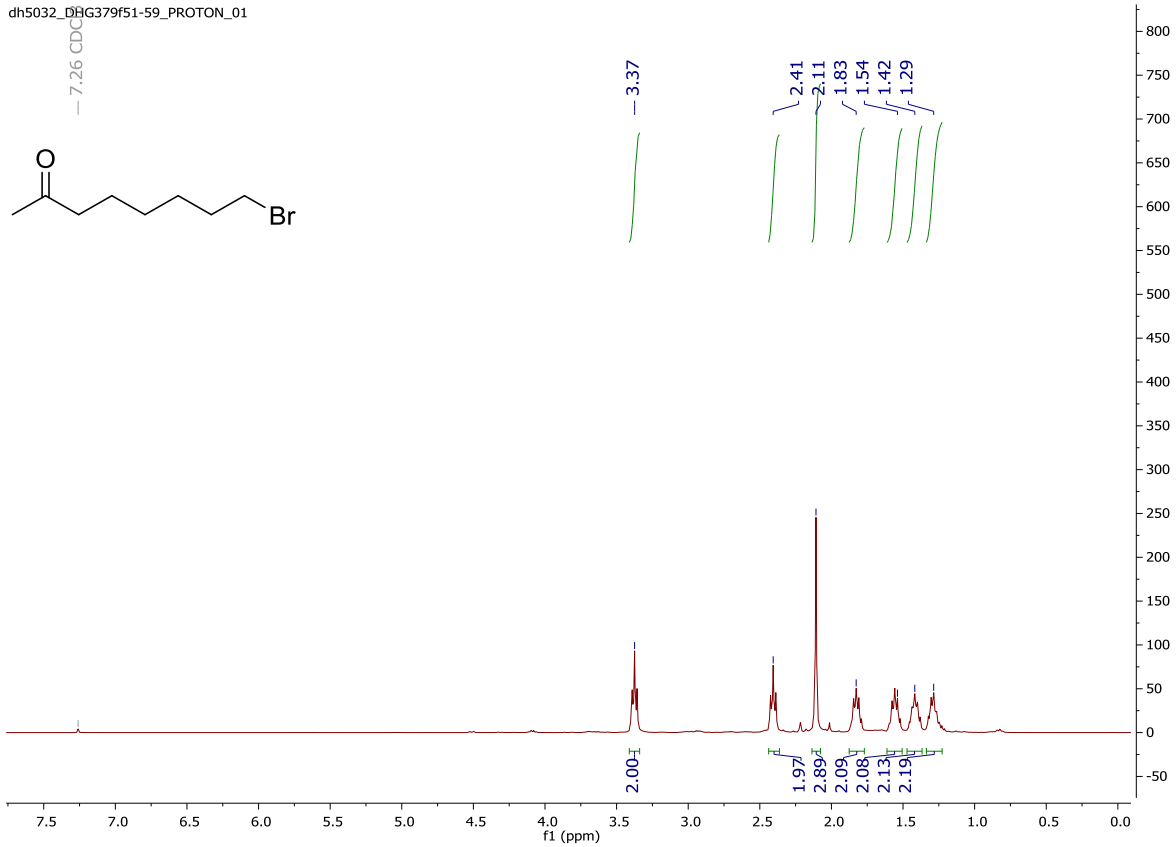


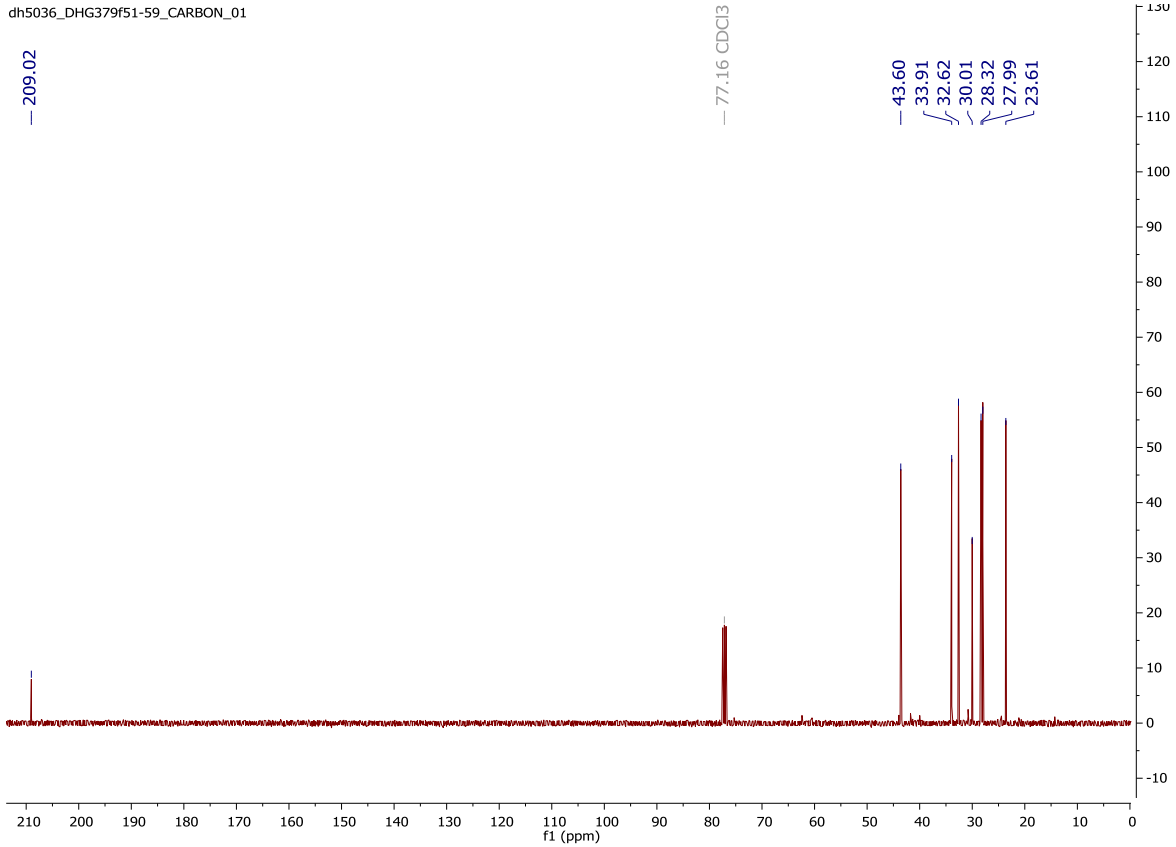
1-Methylcycloheptan-1-ol 81



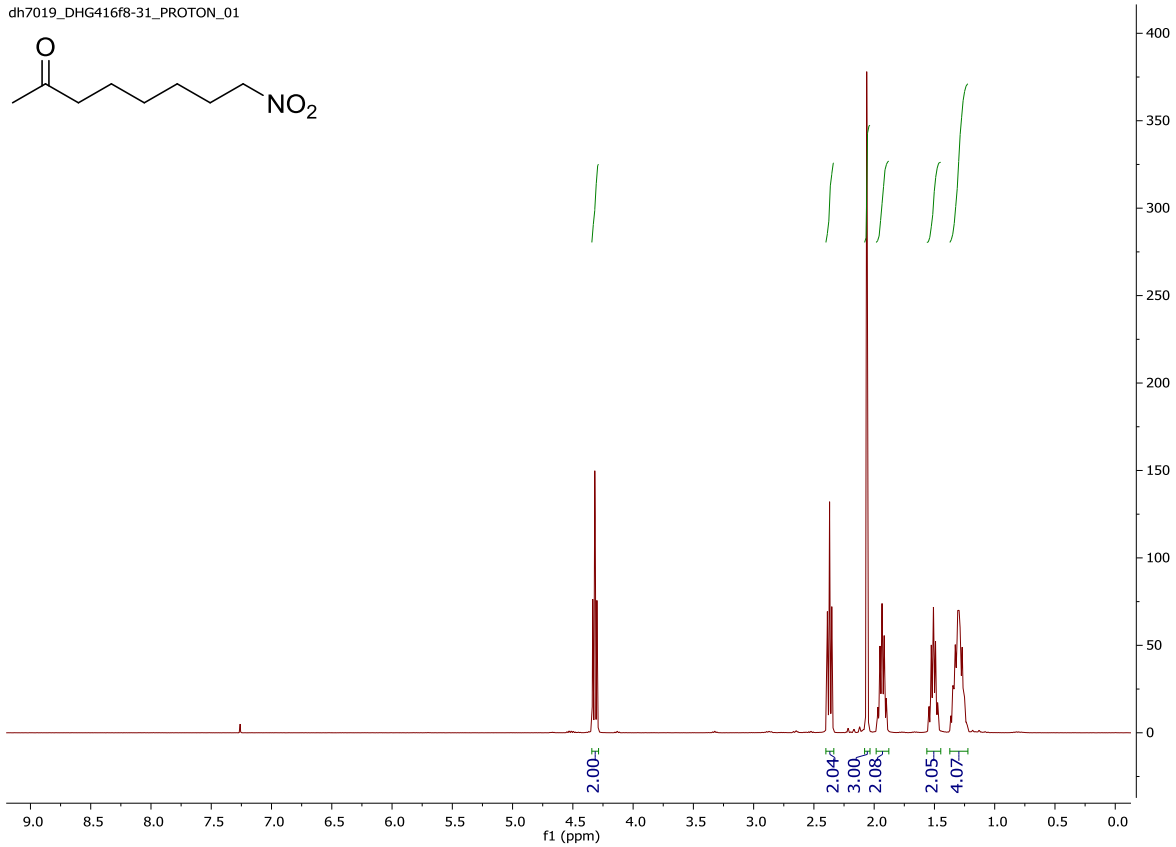


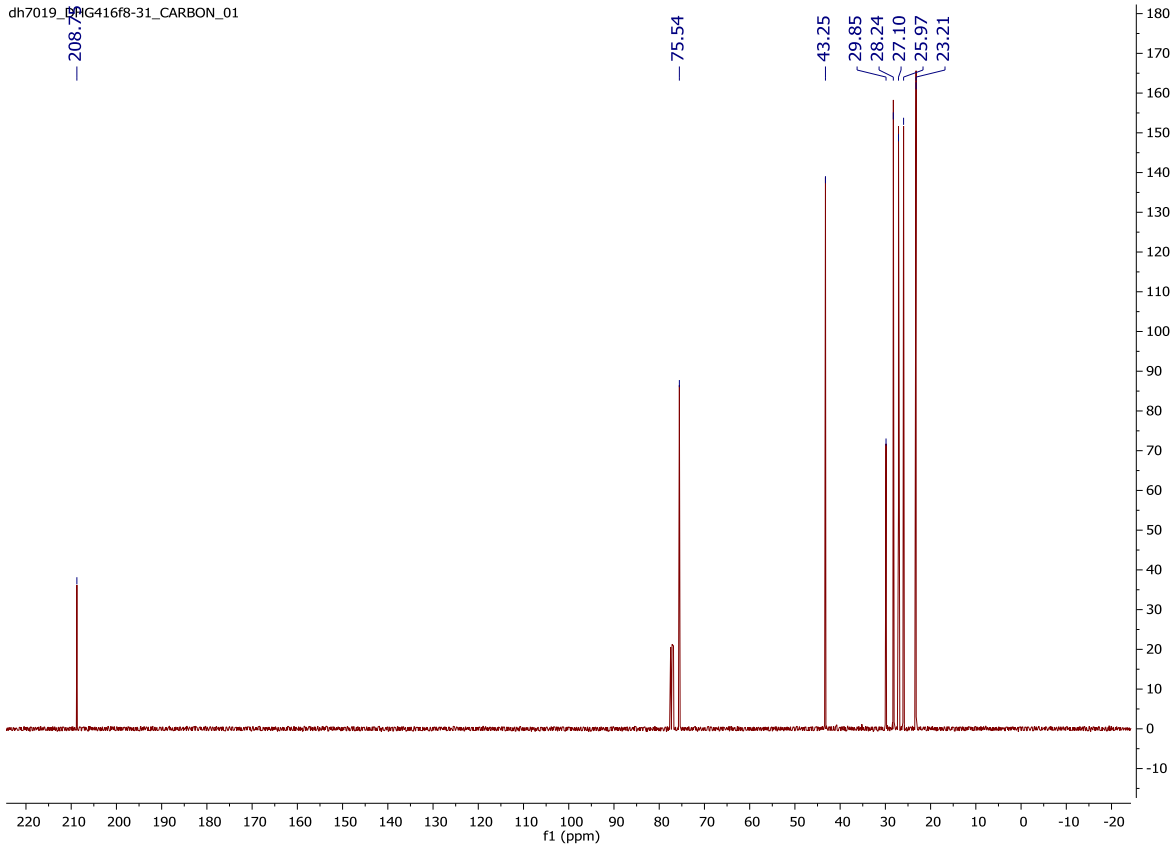
8-Bromooctan-2-one 82



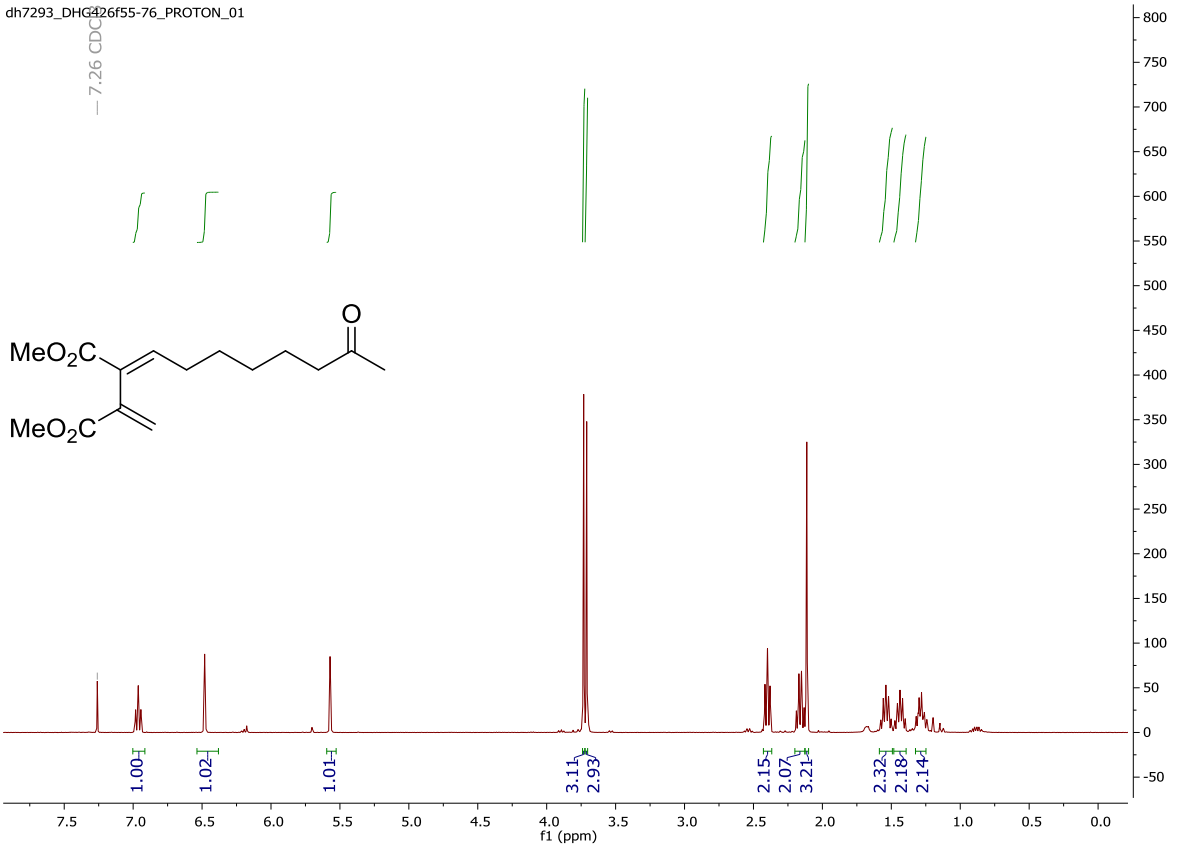


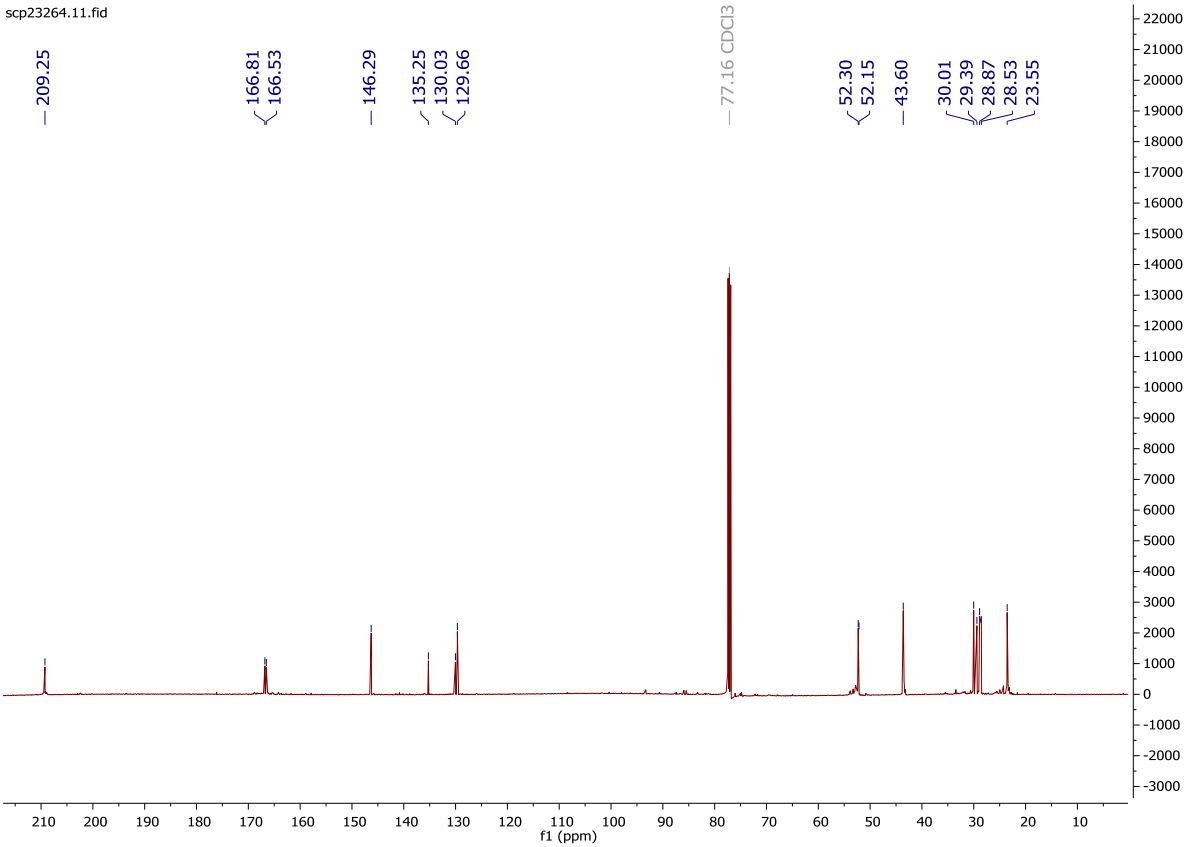
8-Nitrooctan-2-one 83



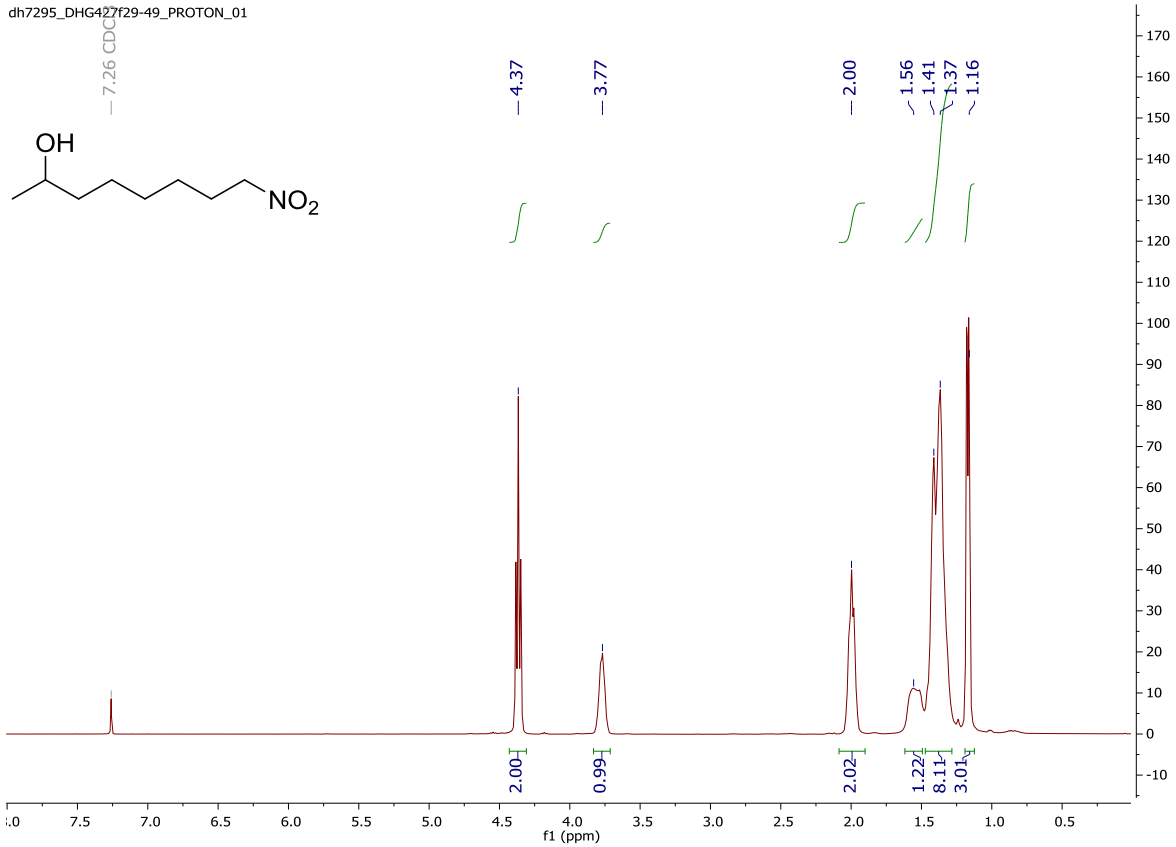


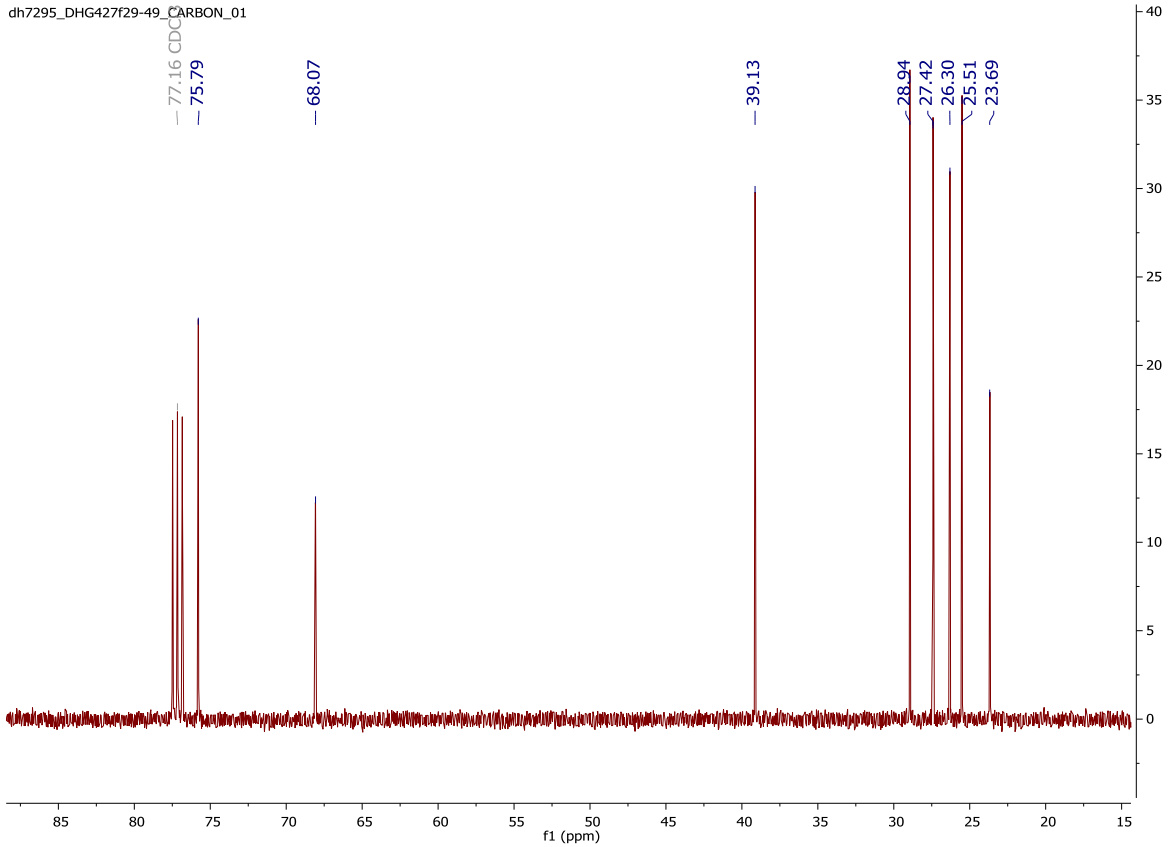
Dimethyl (*E*)-2-methylene-3-(7-oxooctylidene)succinate 84



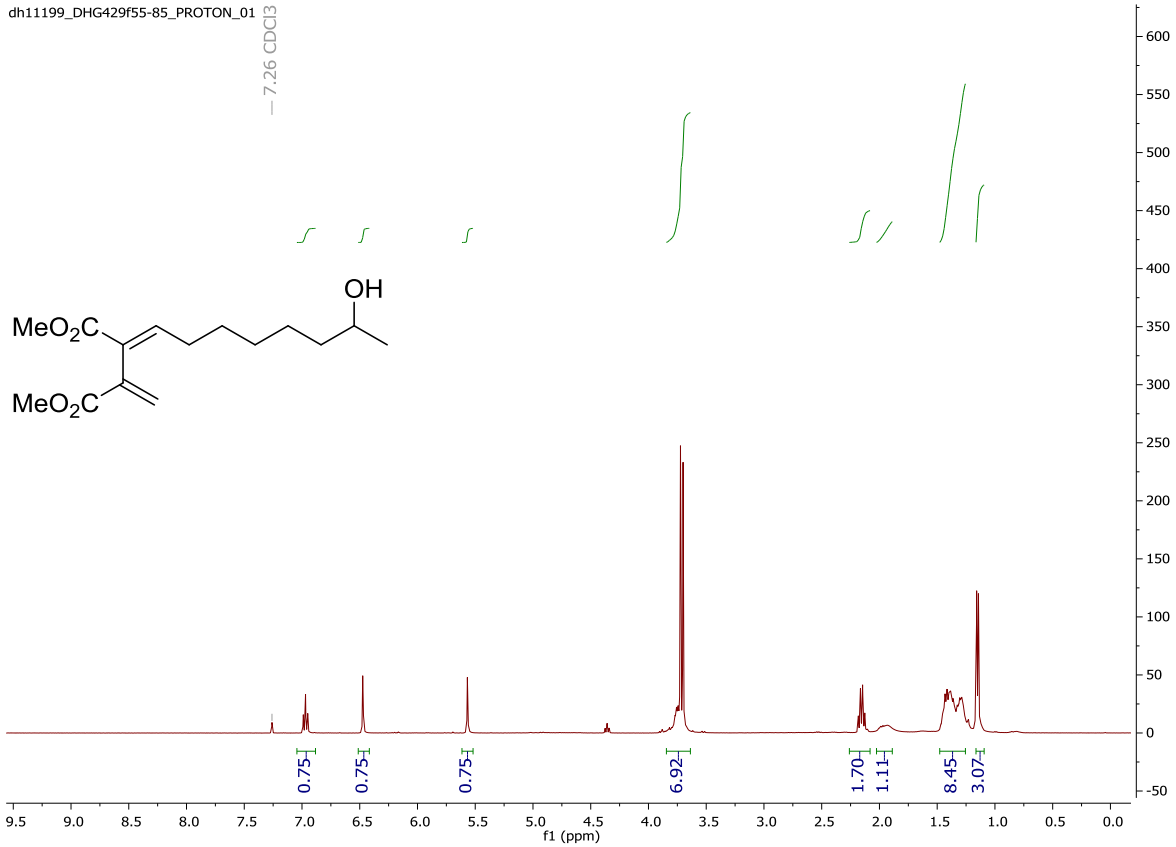


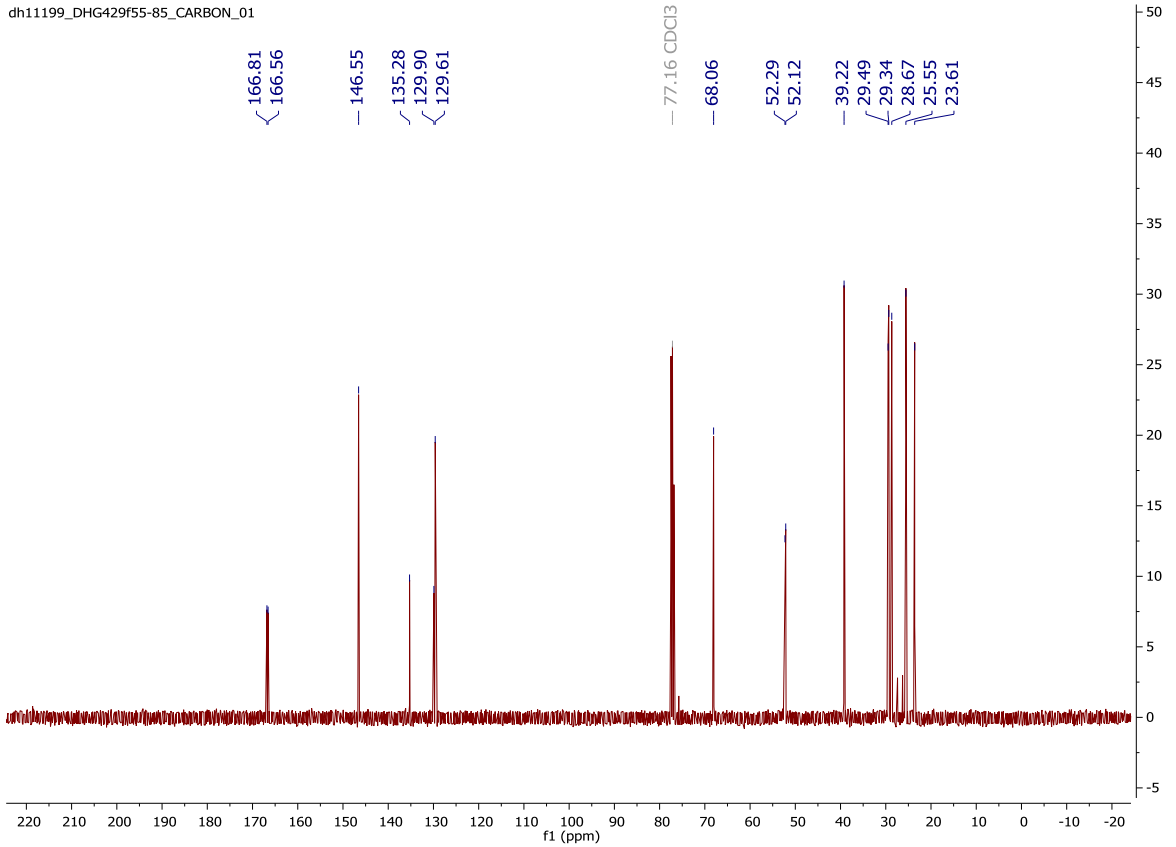
8-Nitrooctan-2-ol 77



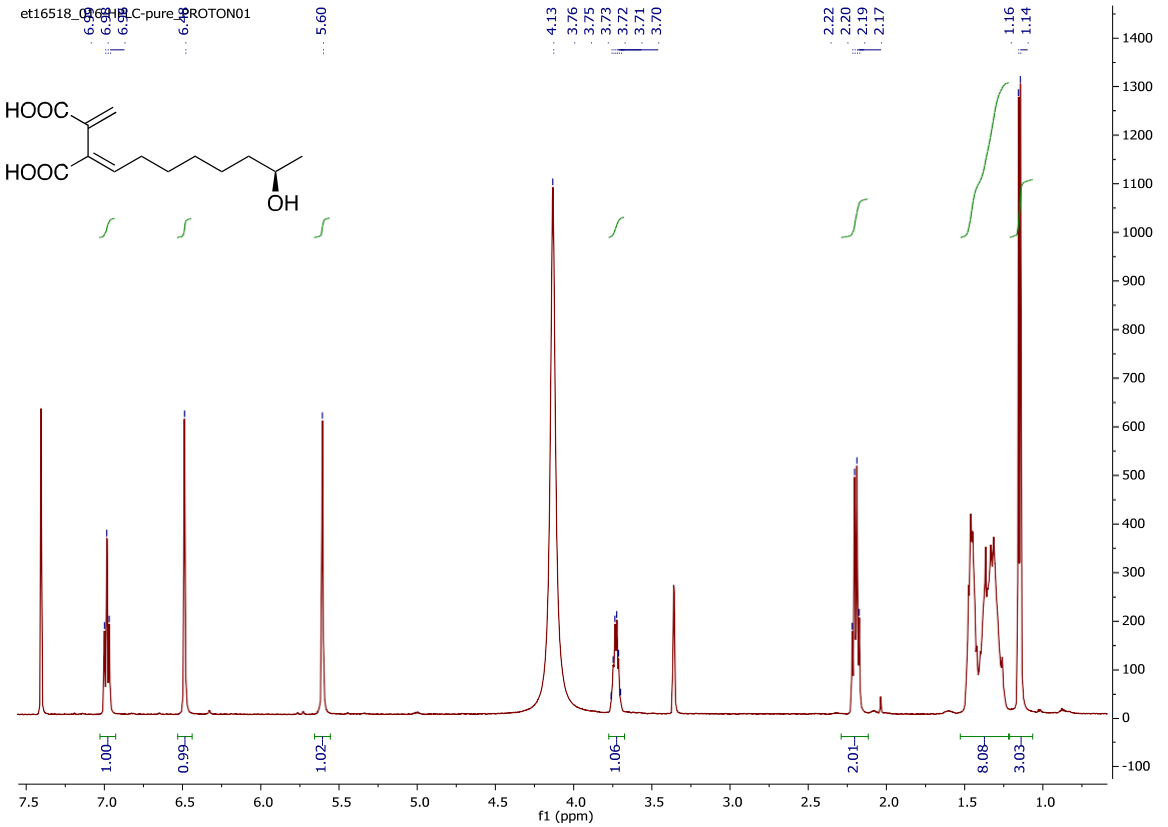


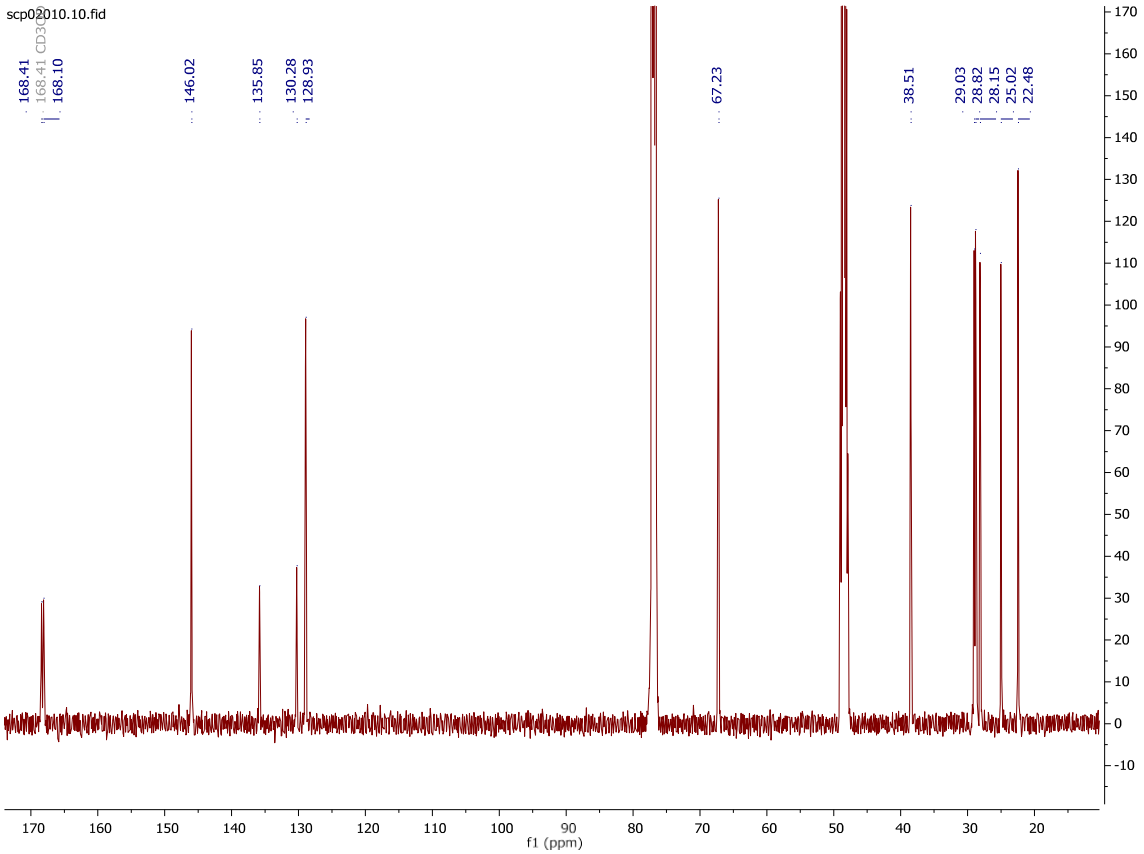
Dimethyl (*E*)-2-(7-hydroxyoctylidene)-3-methylenesuccinate 85



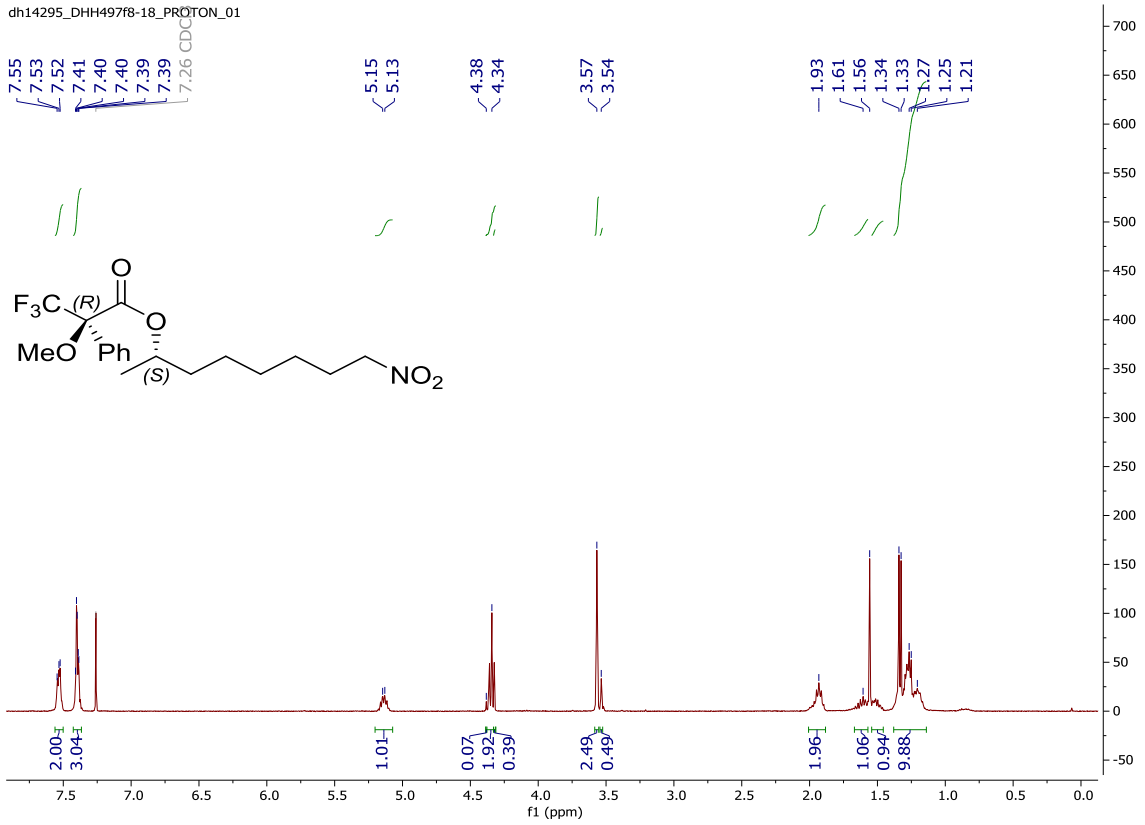


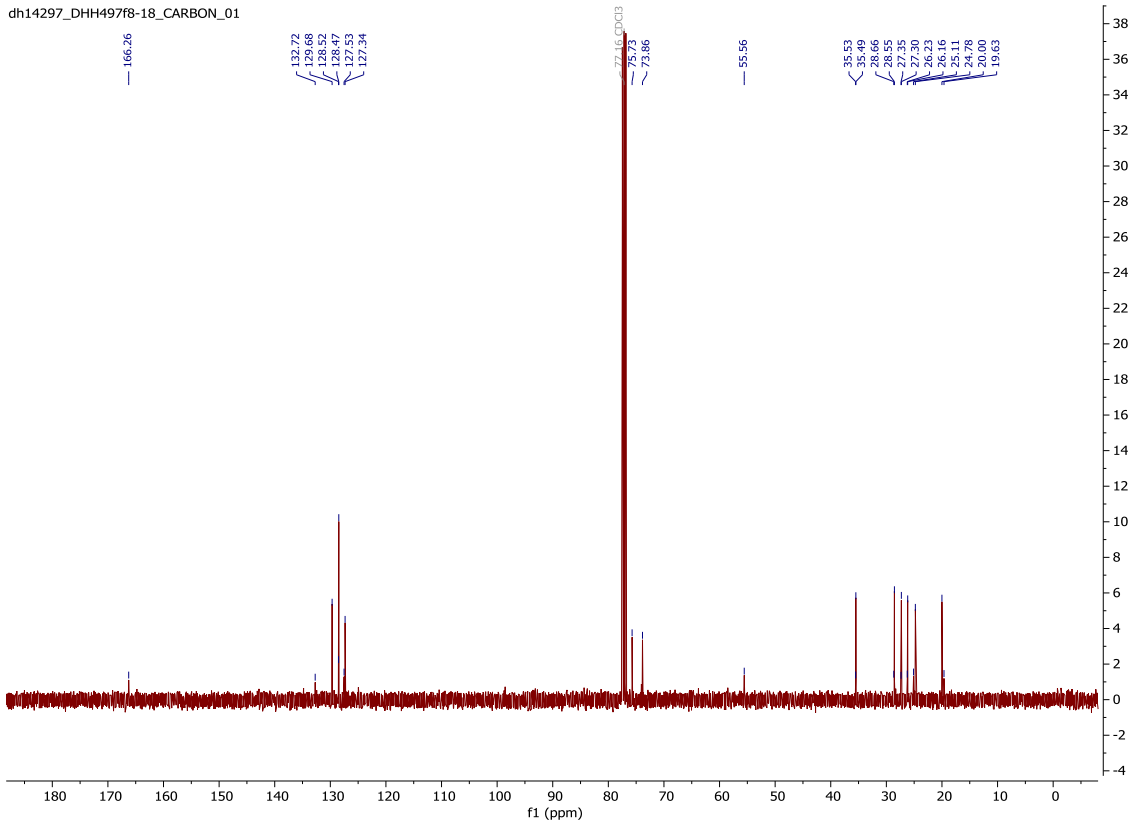
(*E*)-2-(6-Hydroxyheptylidene)-3-methylenesuccinic acid 36 (Tricladic Acid A)



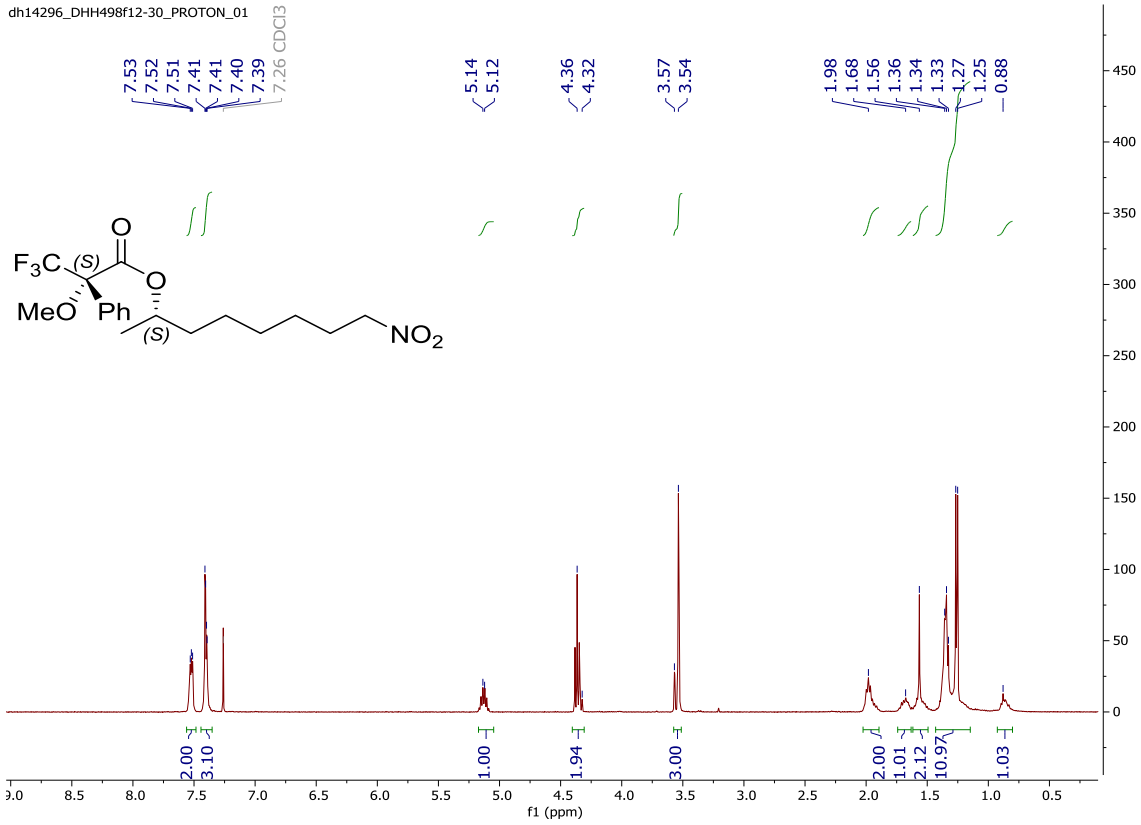


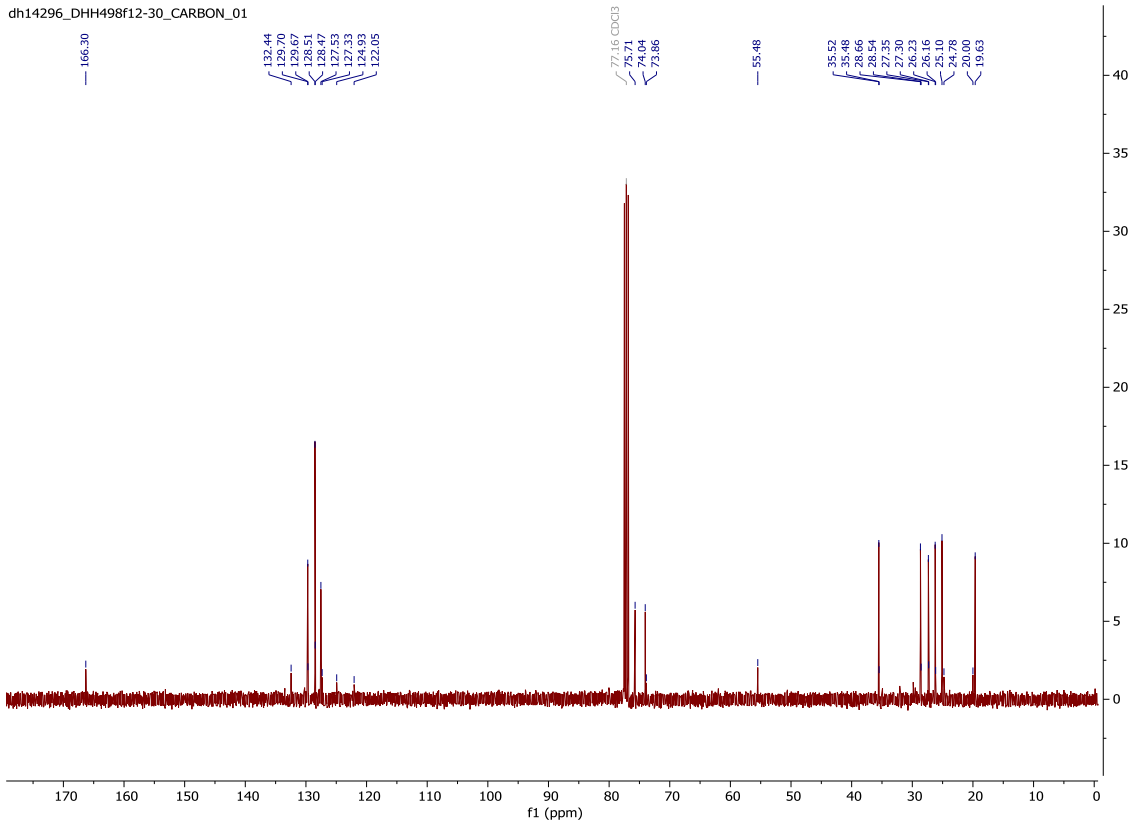
(*S*)-8-Nitrooctan-2-yl (*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate 87



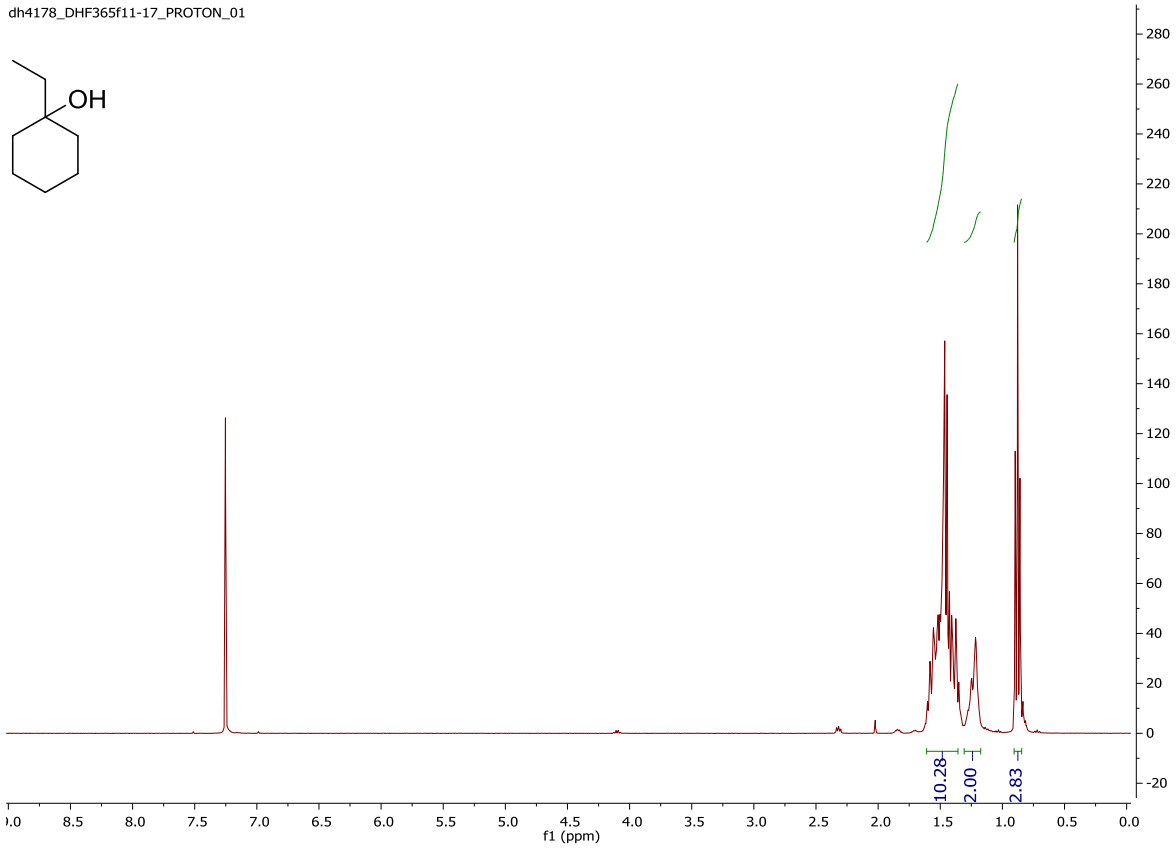


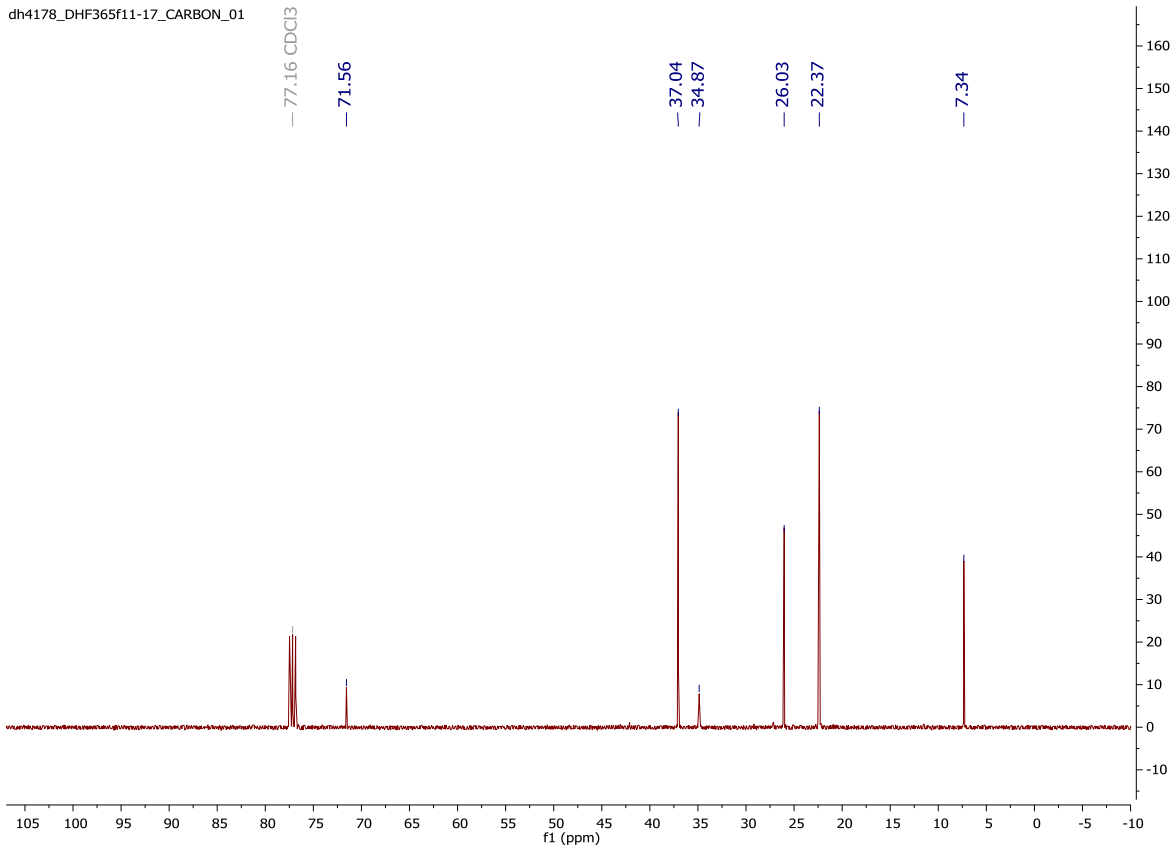
(*S*)-8-Nitrooctan-2-yl (*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate 88



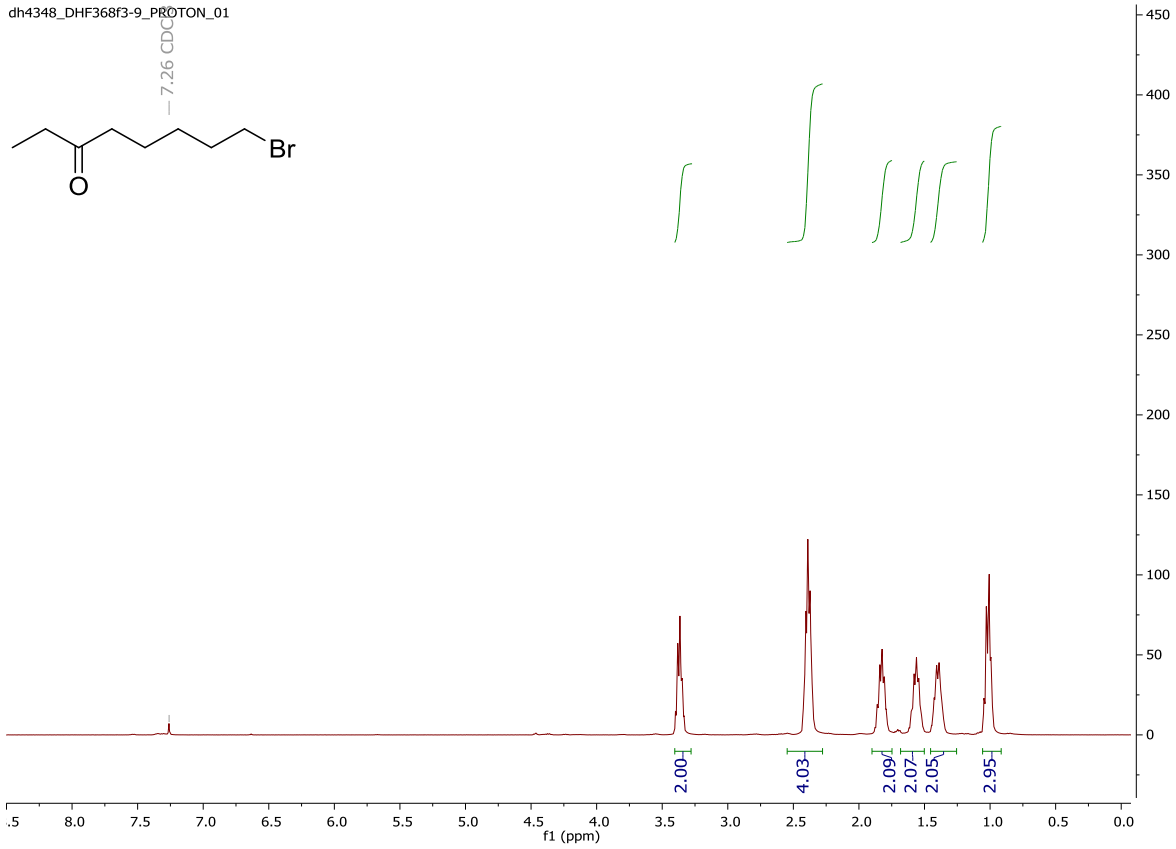


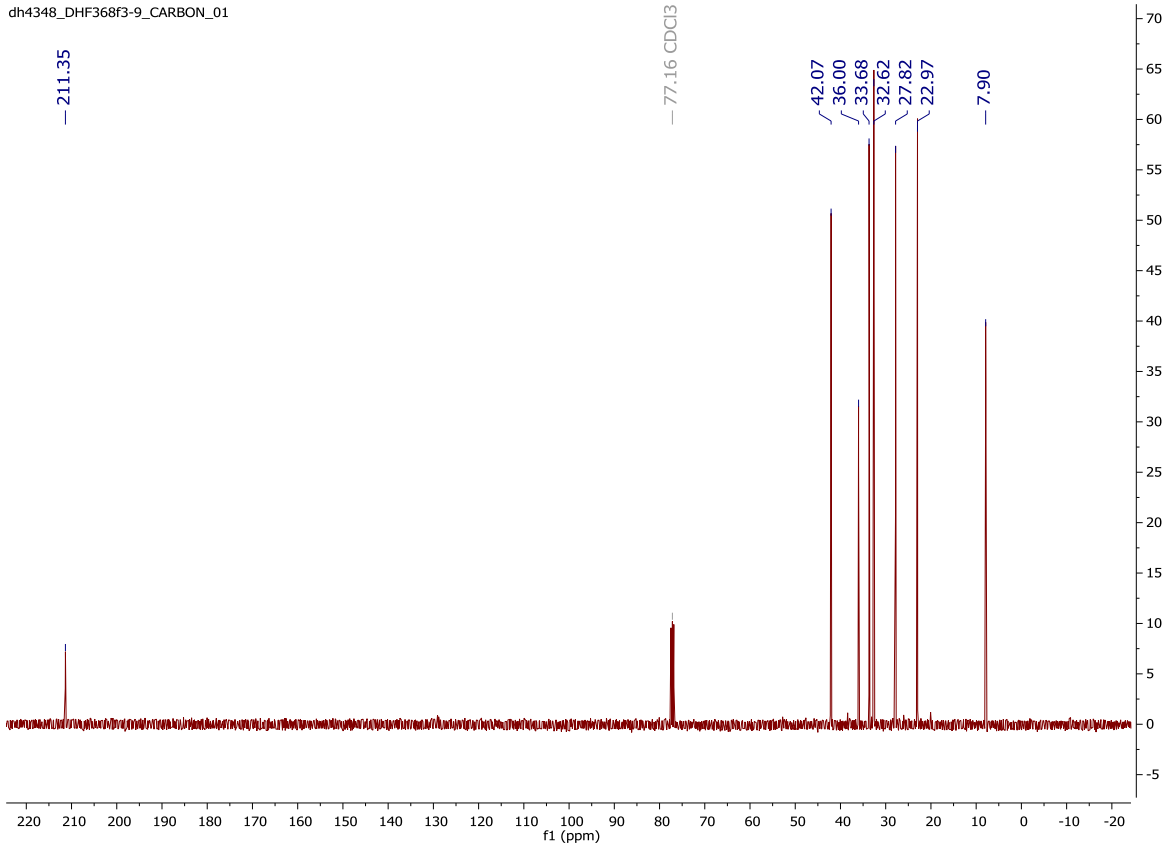
1-Ethylcyclohexan-1-ol 90



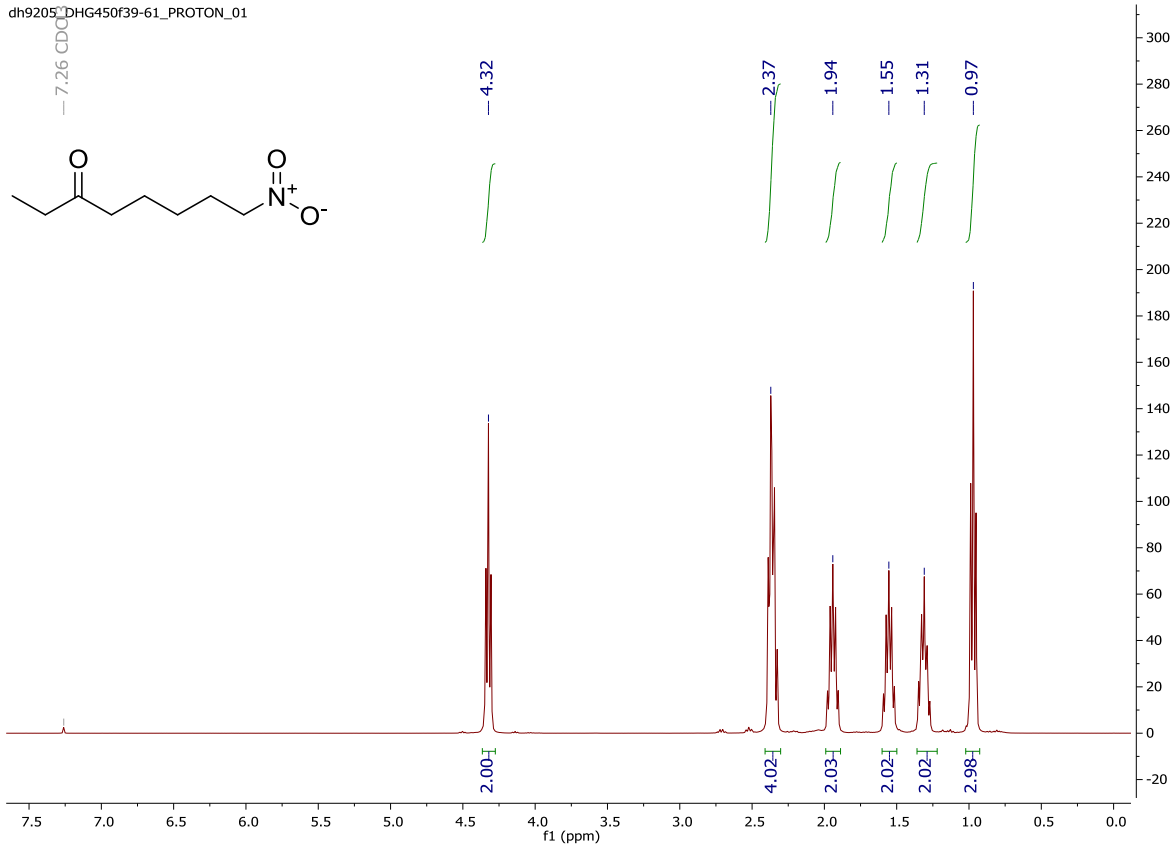


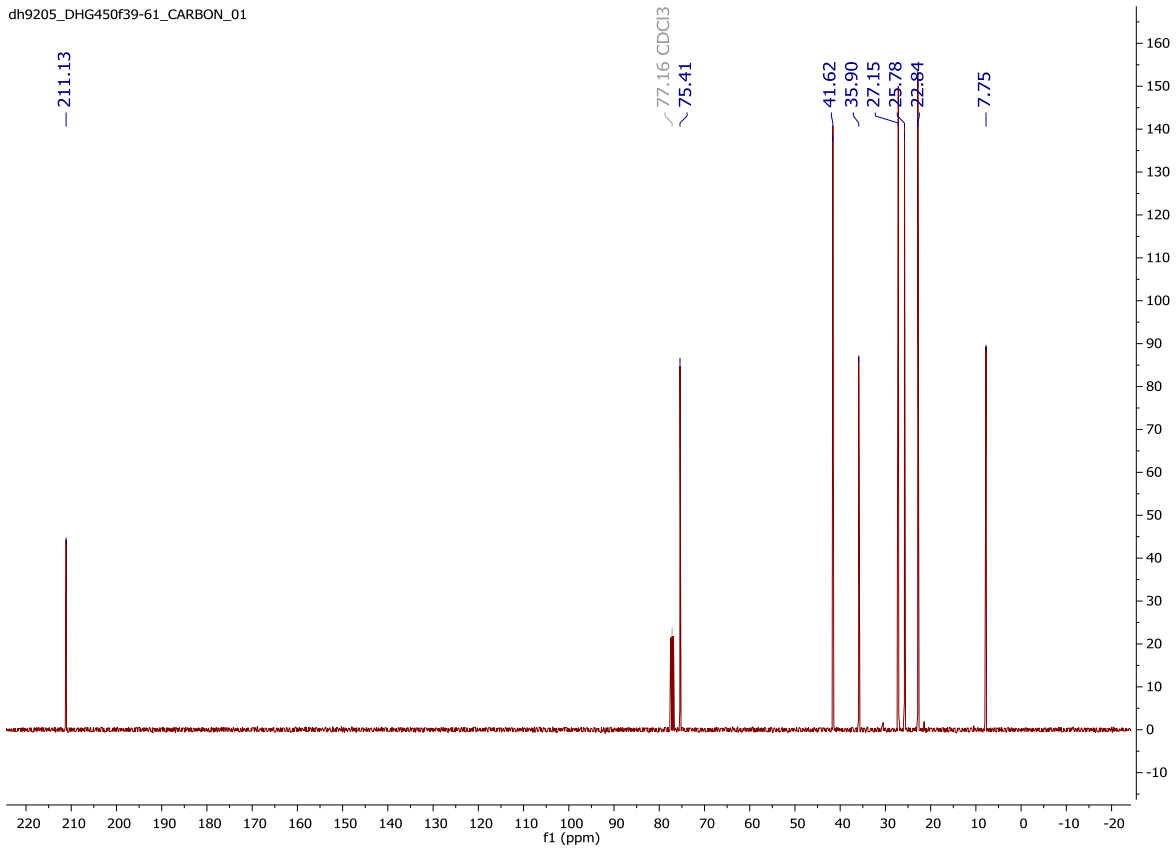
8-Bromooctan-3-one 91



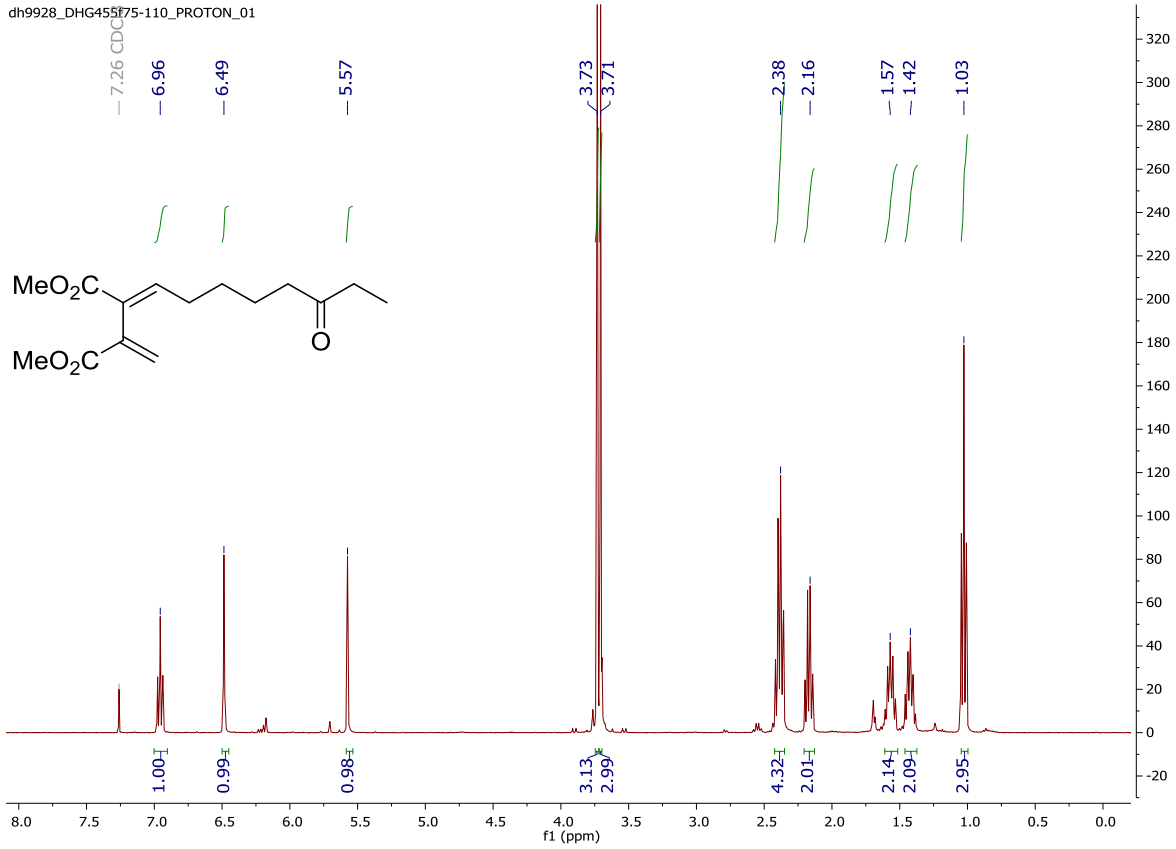


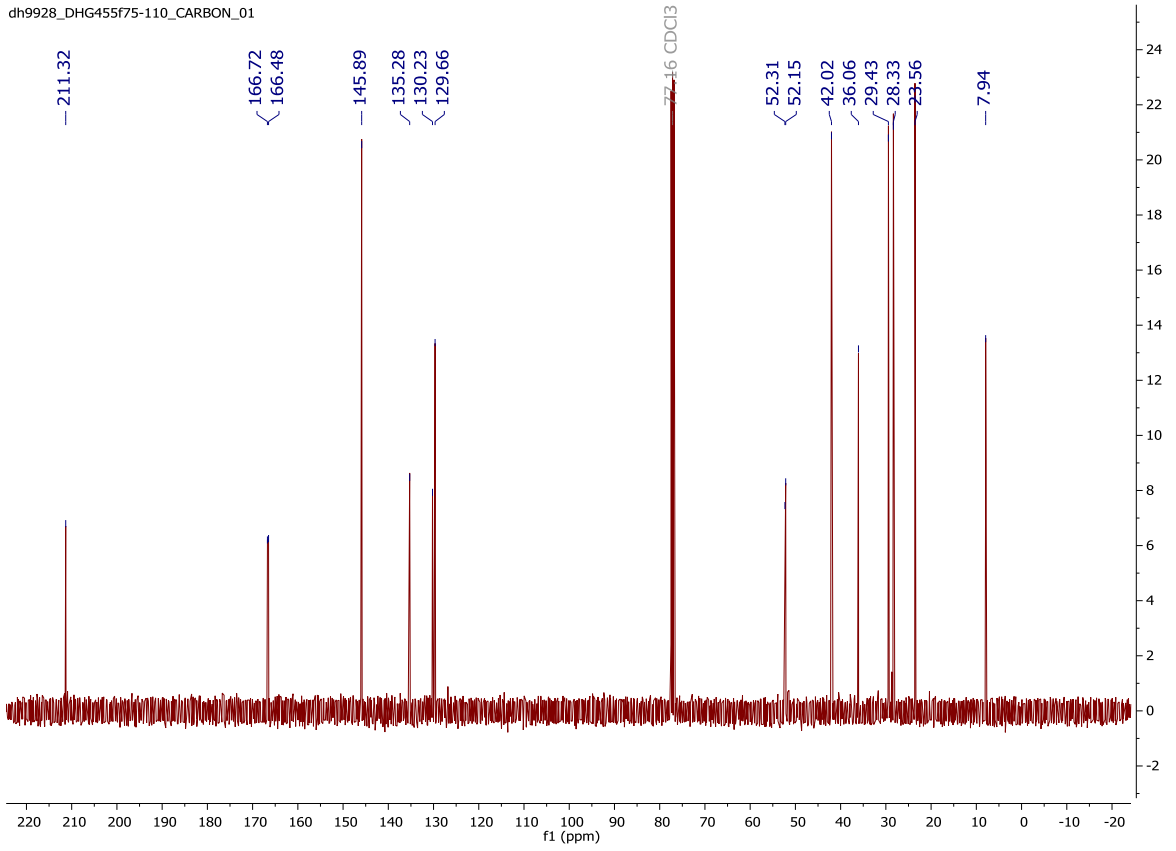
8-Nitrooctan-3-one 92



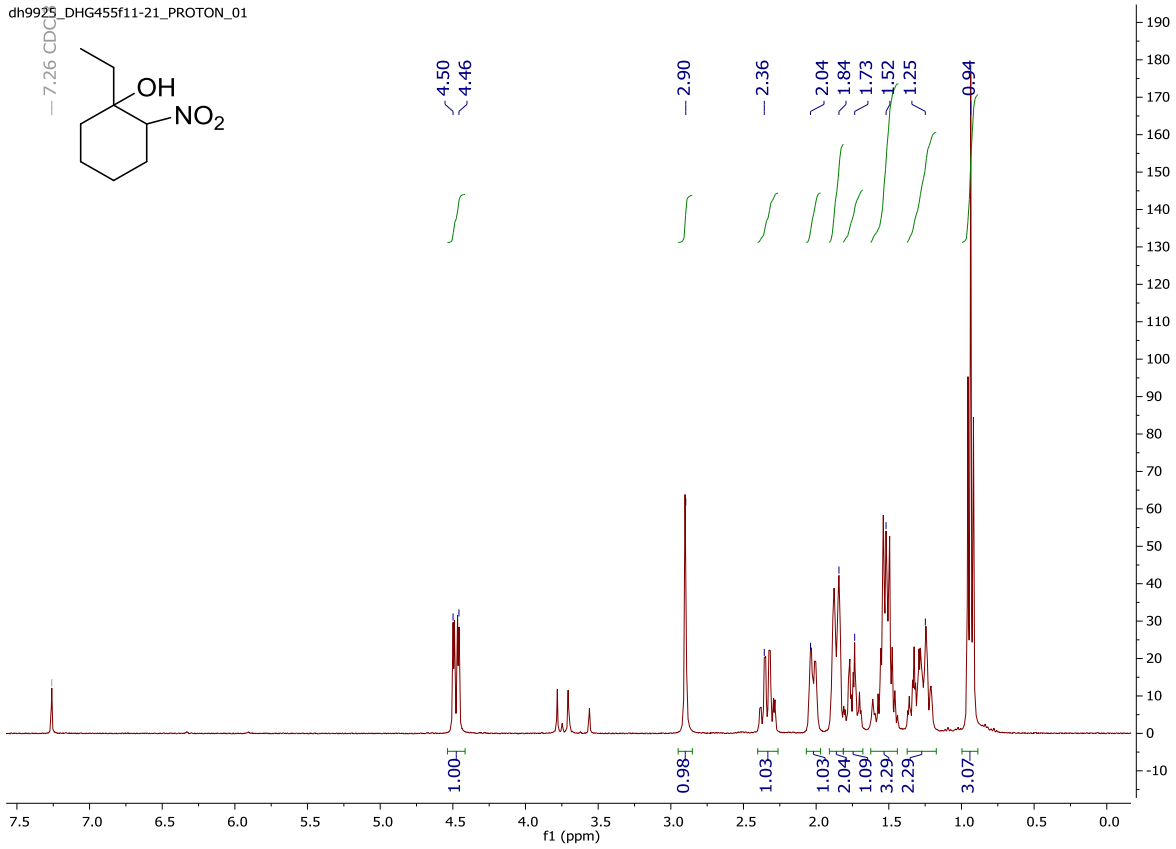


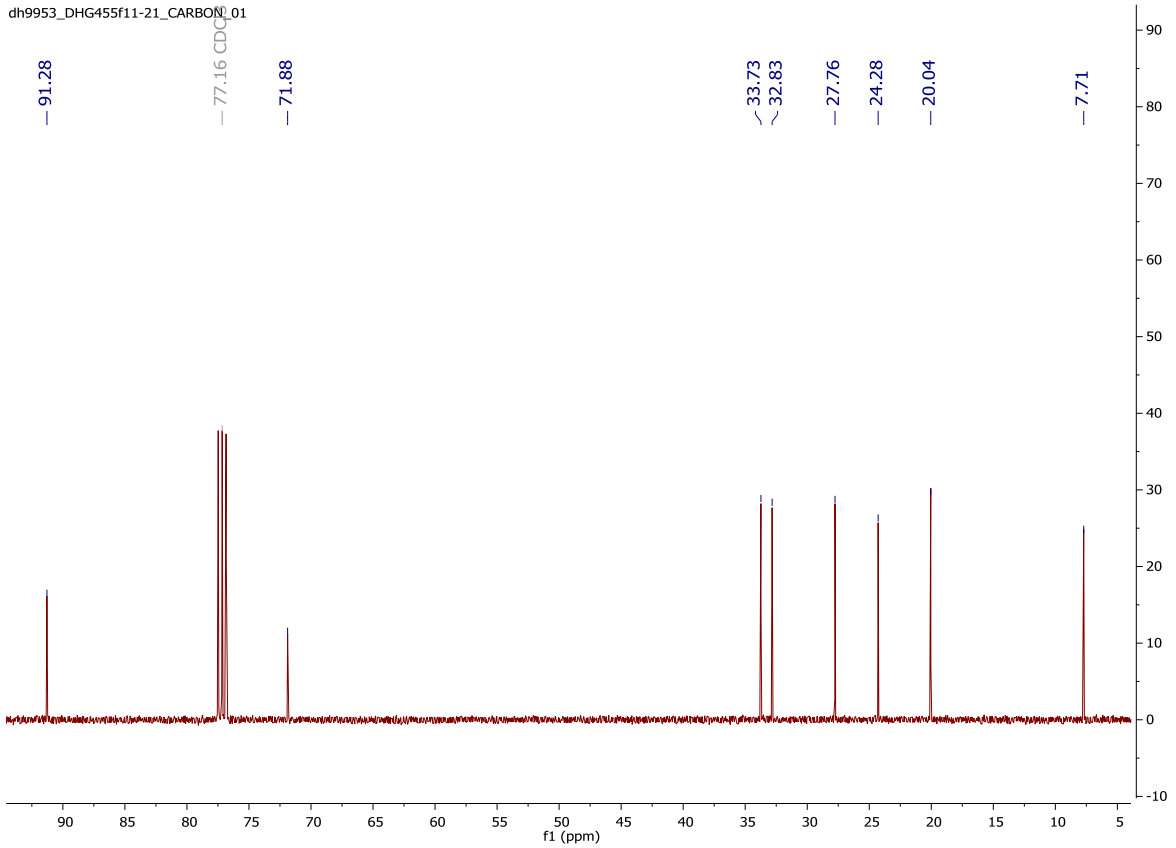
Dimethyl (*E*)-2-methylene-3-(6-oxooctylidene)succinate 95



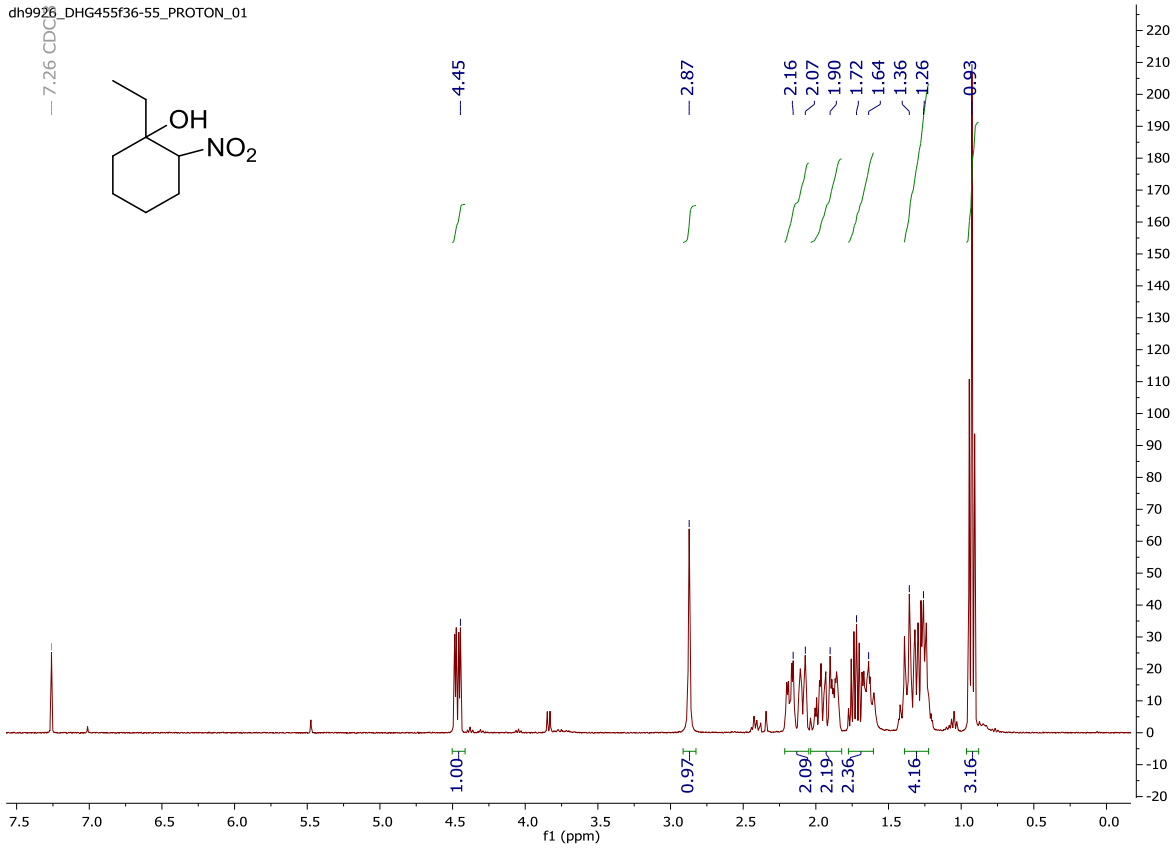


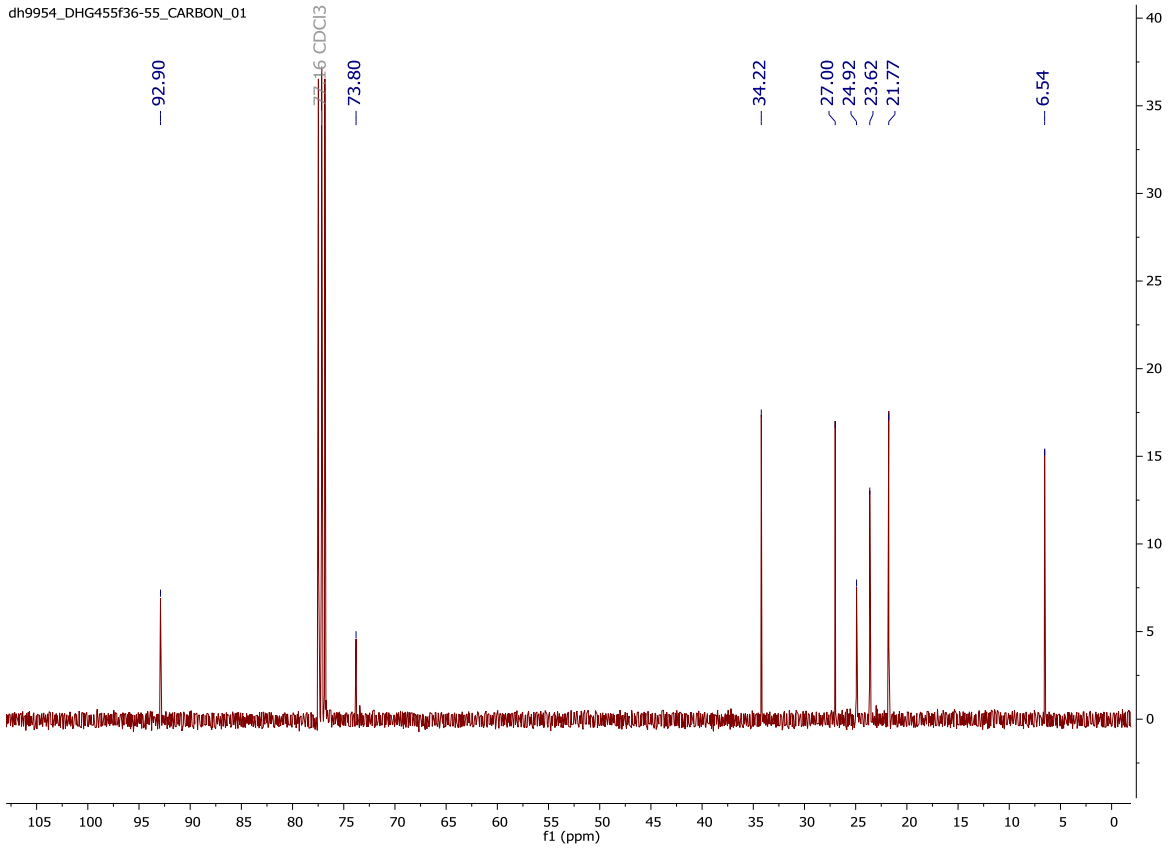
1-Ethyl-2-nitrocyclohexan-1-ol 94 (Diastereomer A)



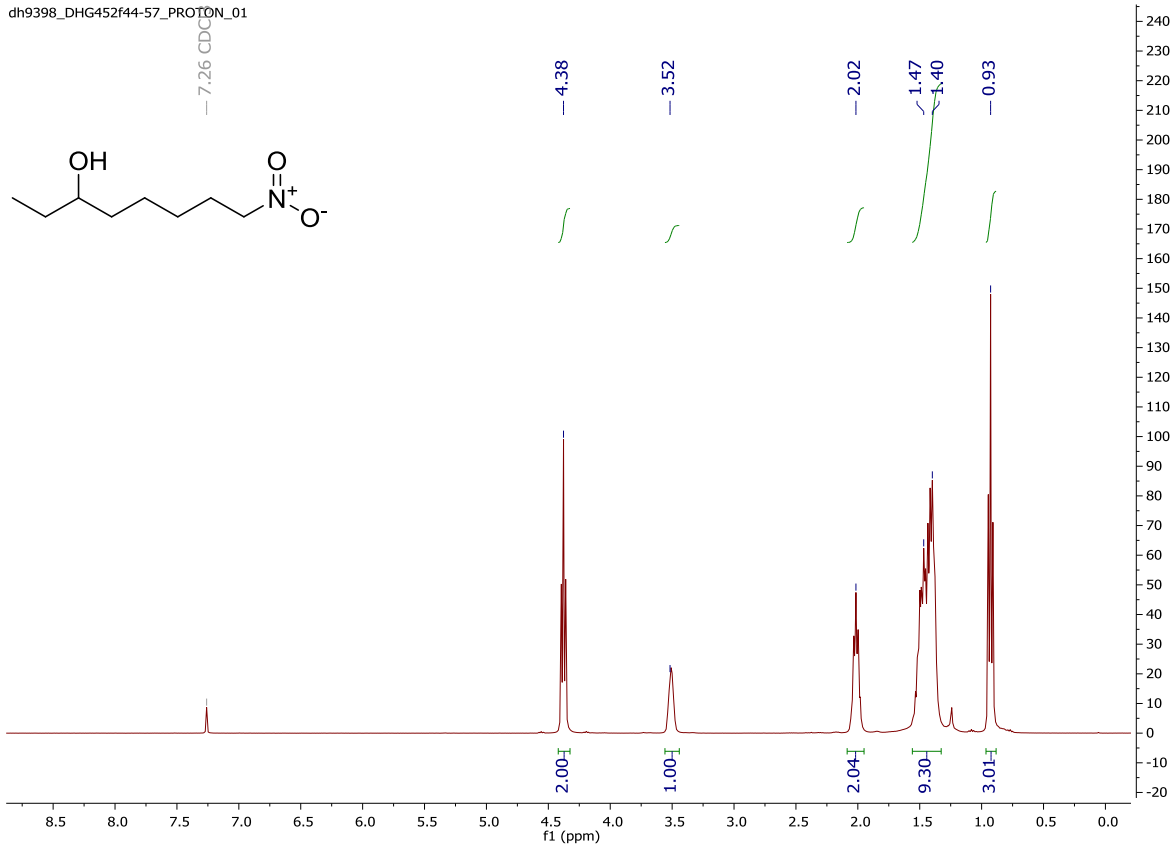


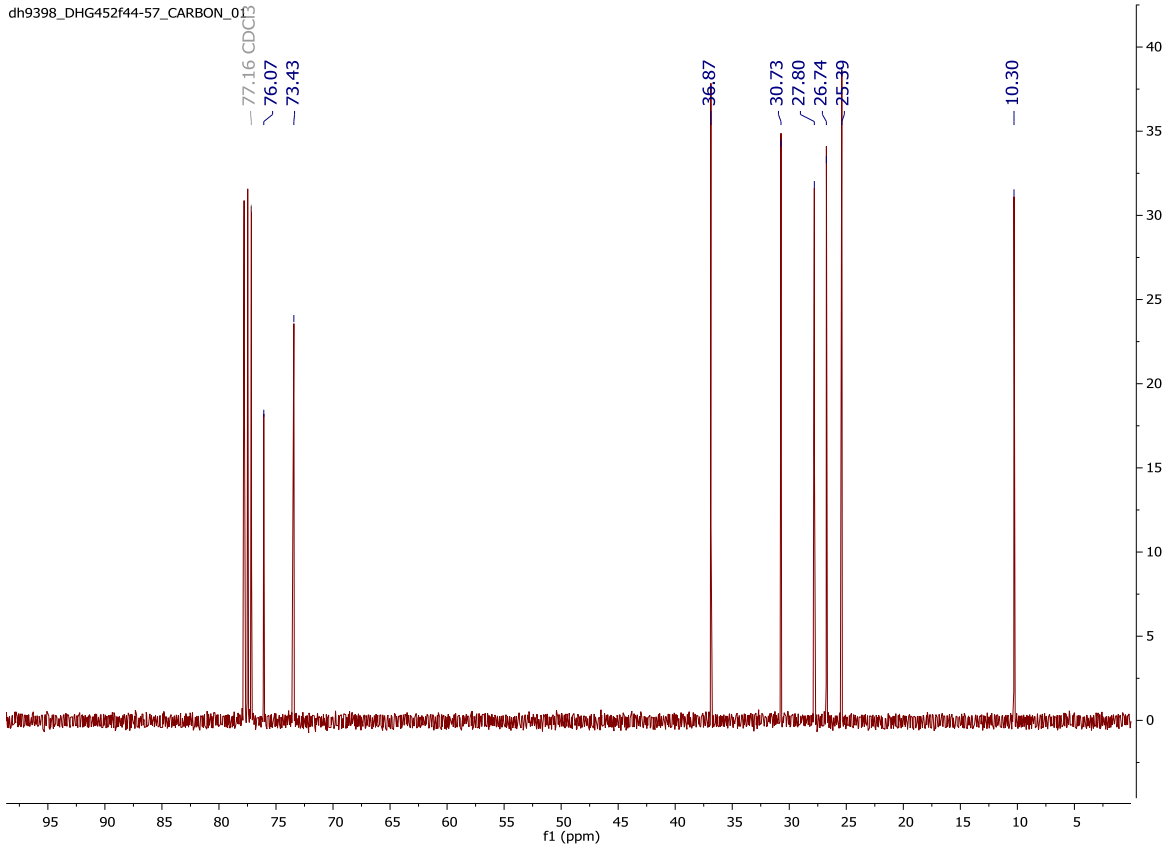
1-Ethyl-2-nitrocyclohexan-1-ol 94 (Diastereomer B)



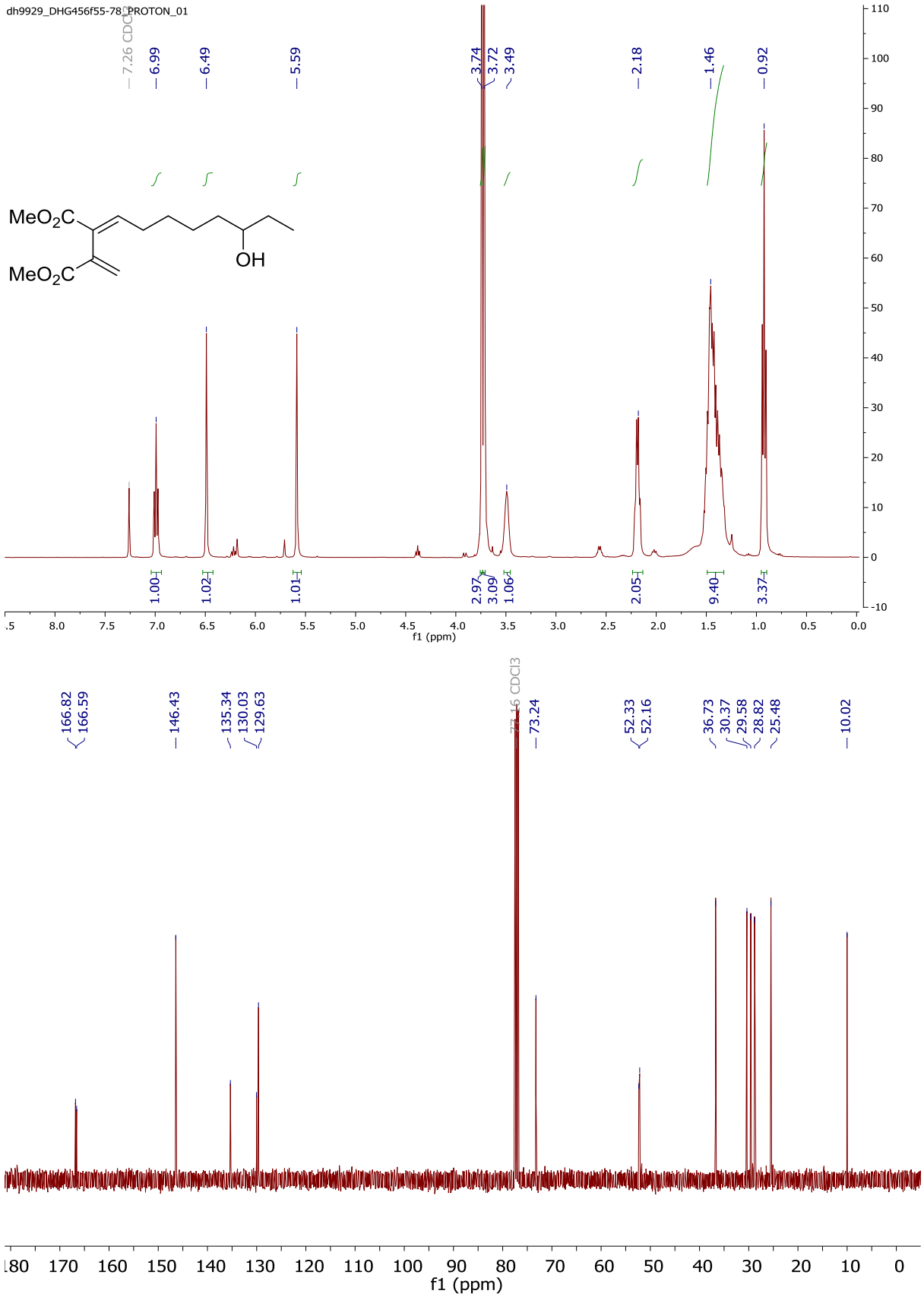


8-Nitrooctan-3-ol 96

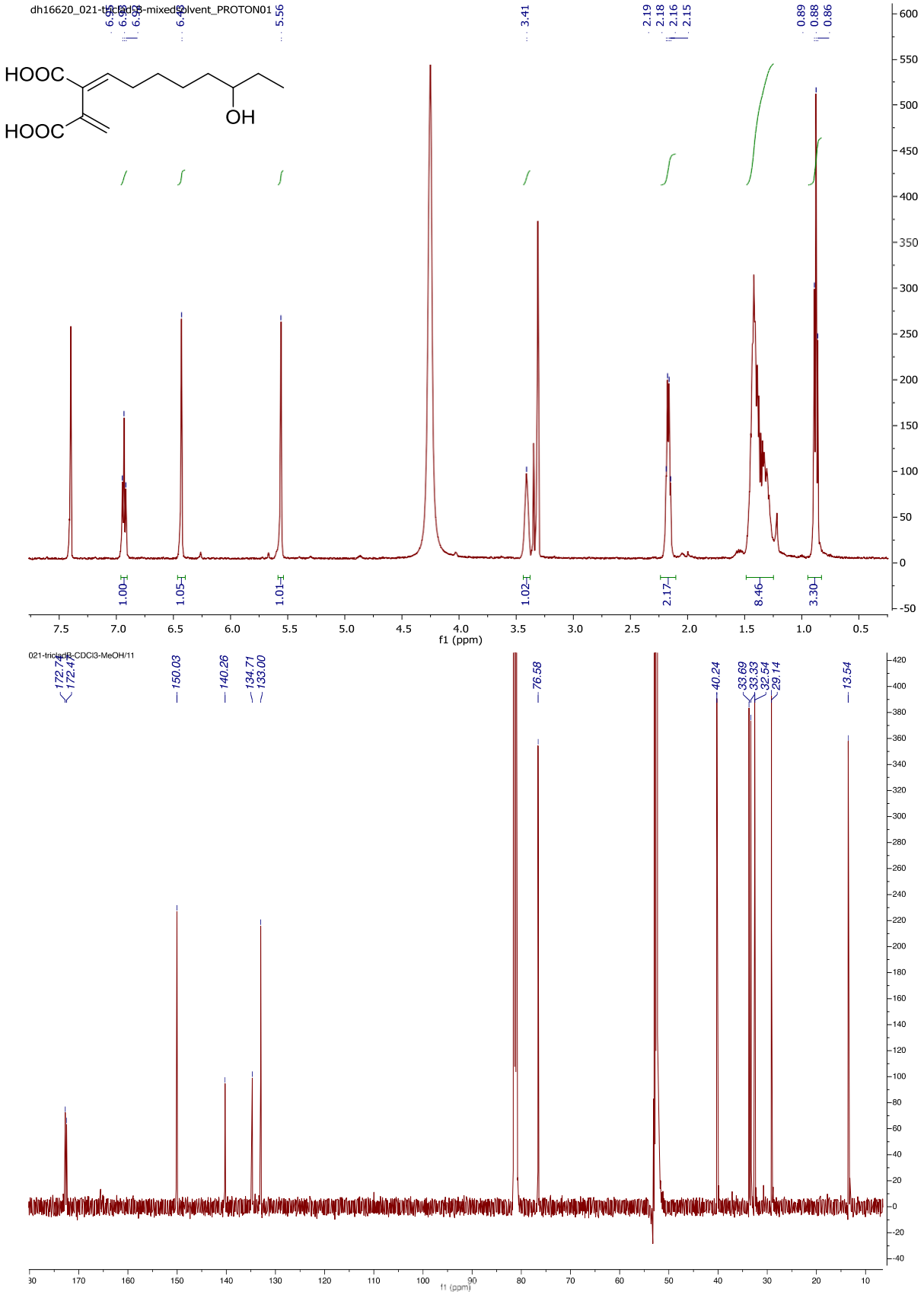




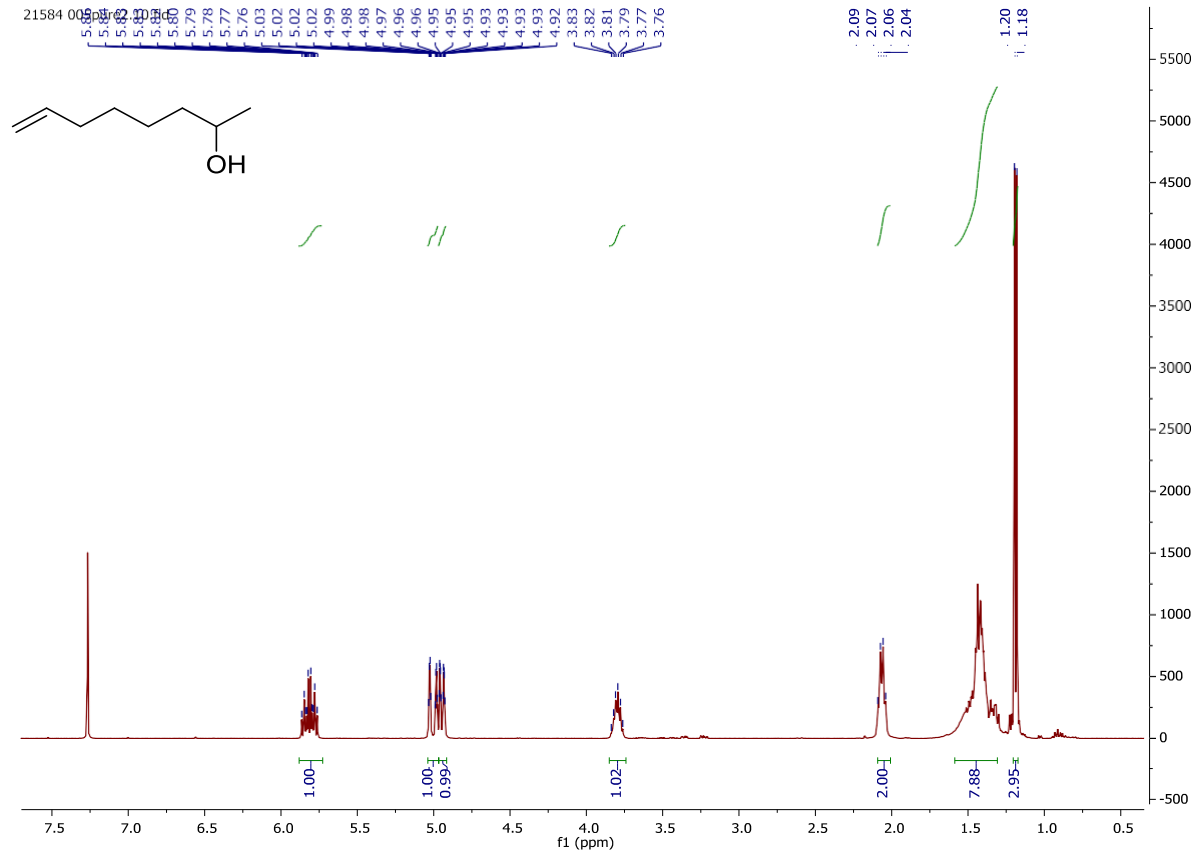
Dimethyl (*E*)-2-(6-hydroxyoctylidene)-3-methylenesuccinate 97

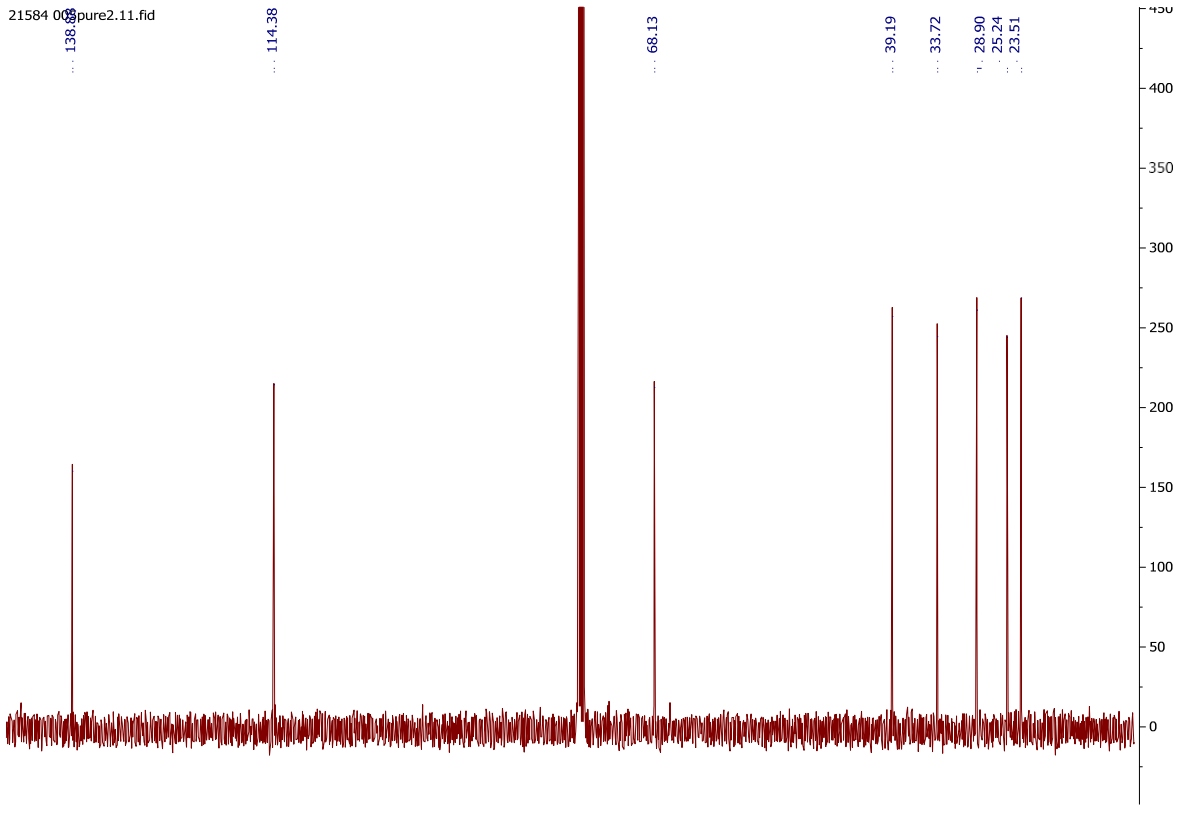


(*E*)-2-(6-Hydroxyoctylidene)-3-methylenesuccinic acid 37 (Tricladic acid B)

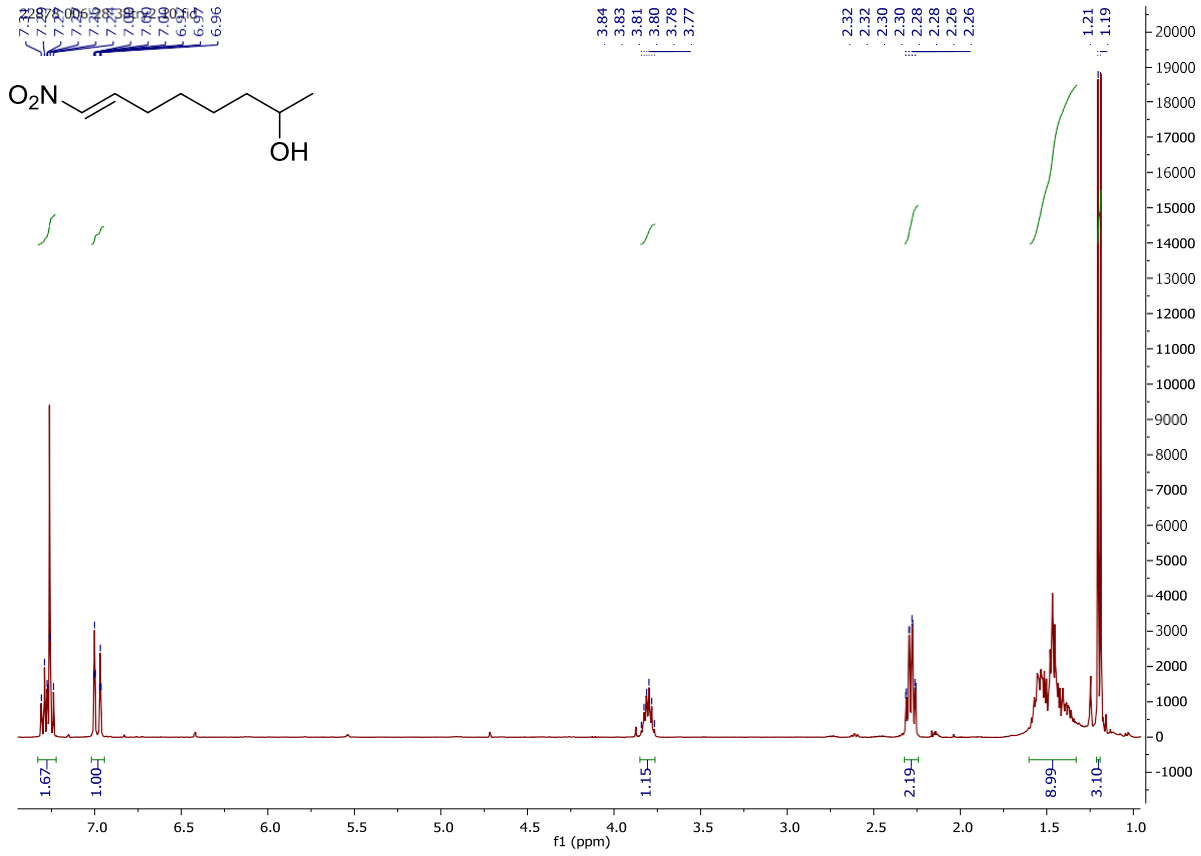


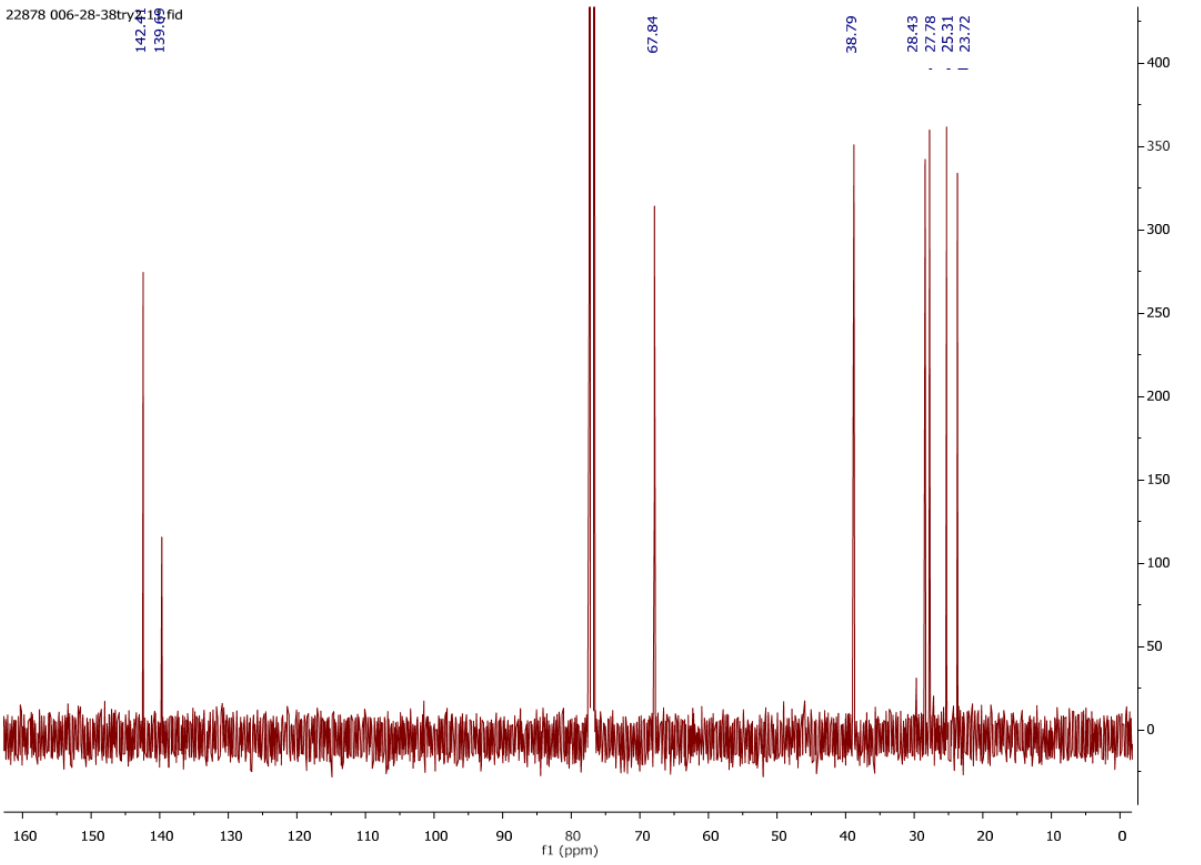
(±)-2-Hydroxyoct-7-ene 99 (*)



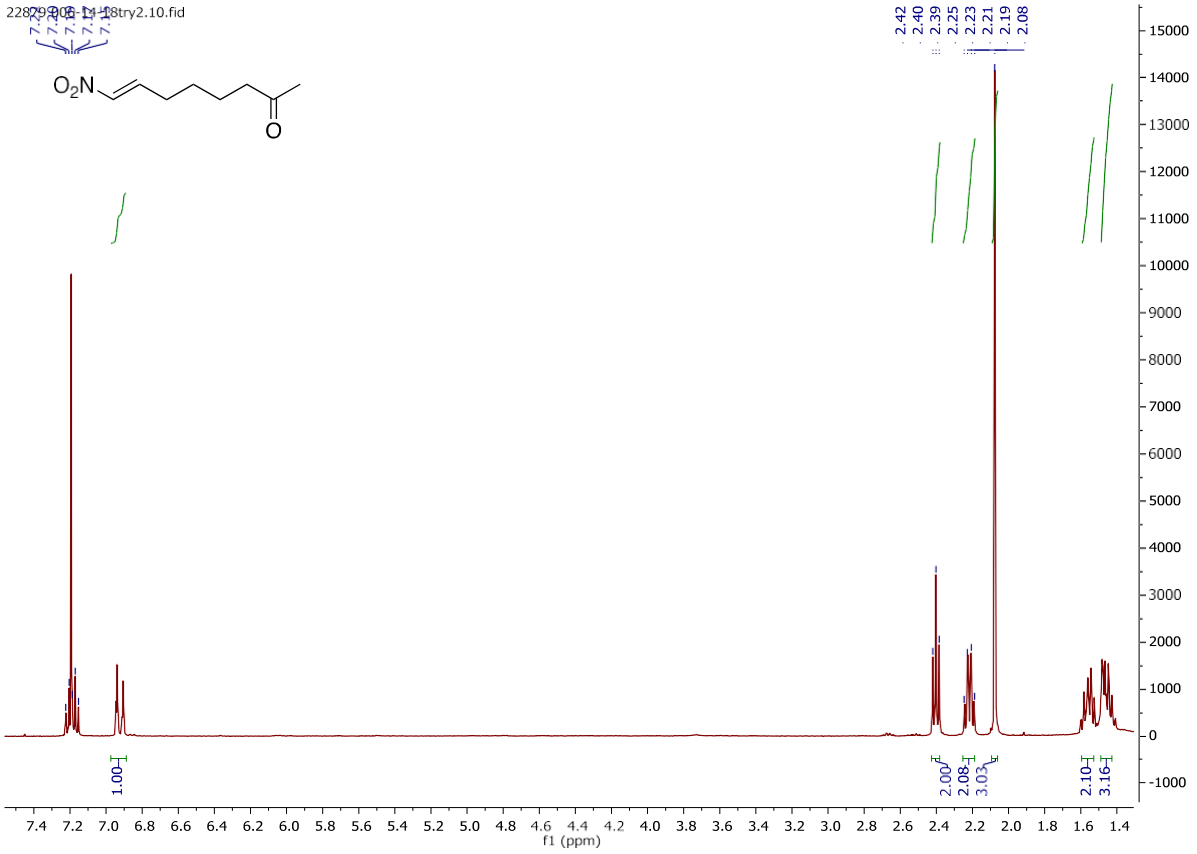


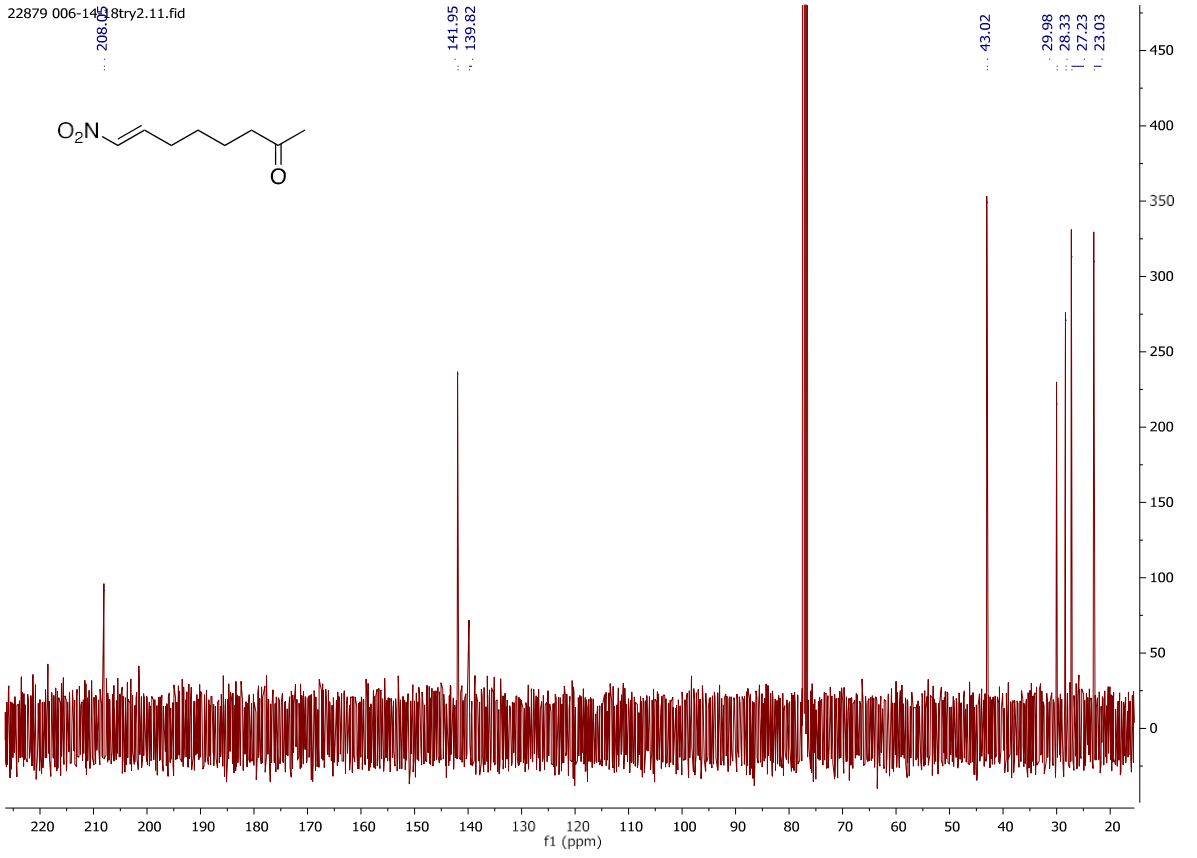
(±)-(*E*)-2-Hydroxy-8-nitrooct-7-ene 100 (*)



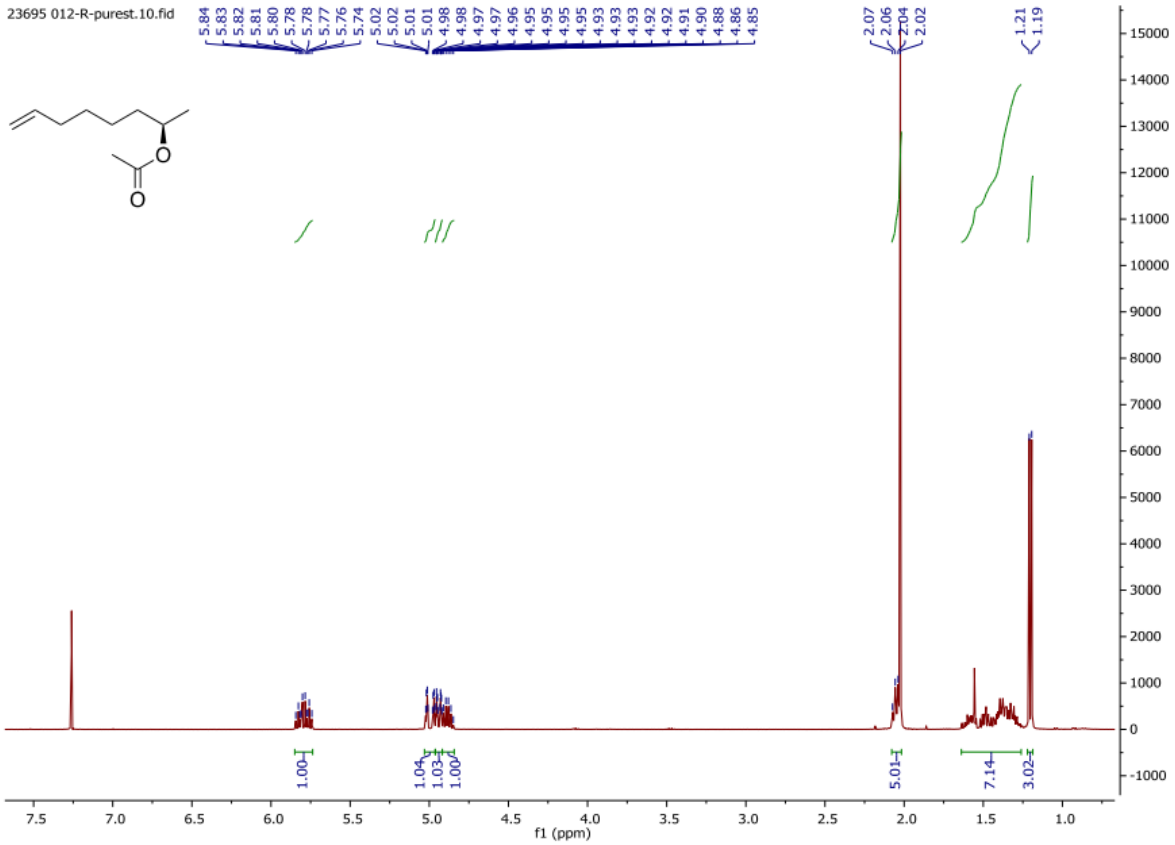


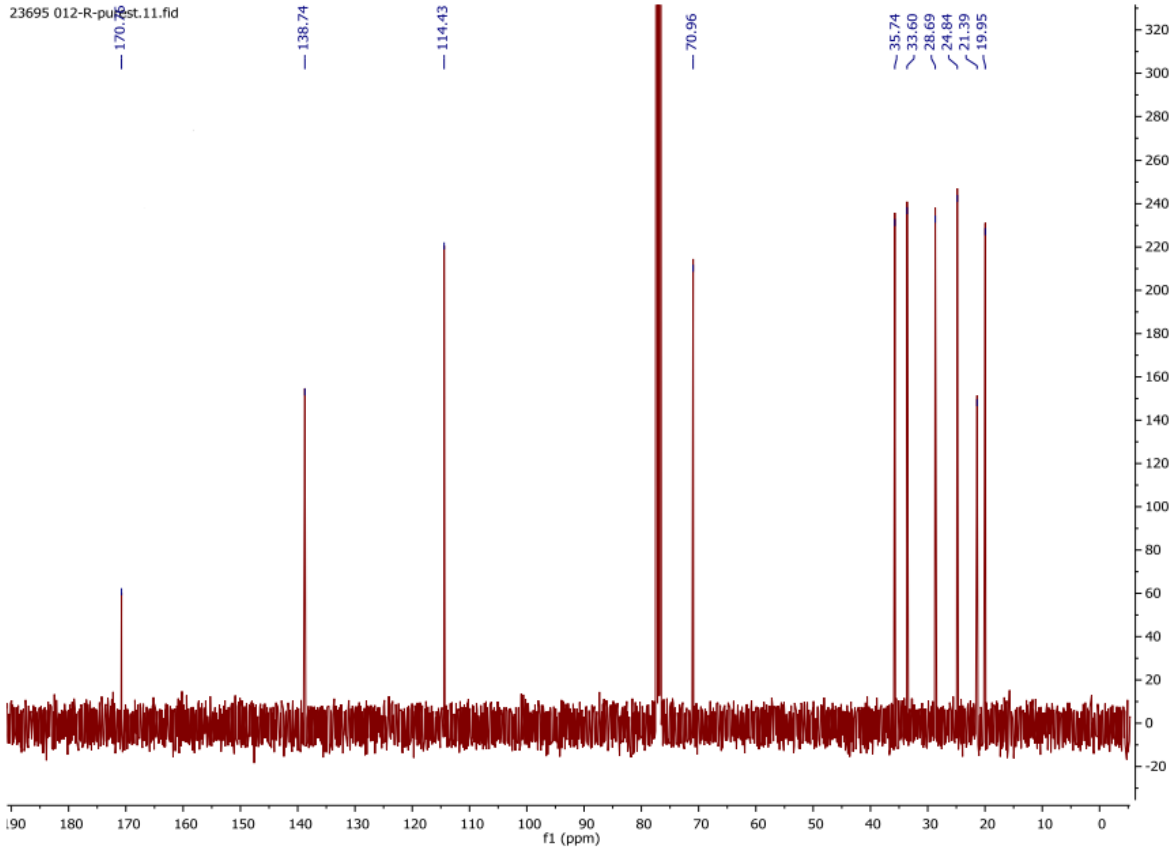
(*E*)-8-Nitrooct-7-en-2-one S23



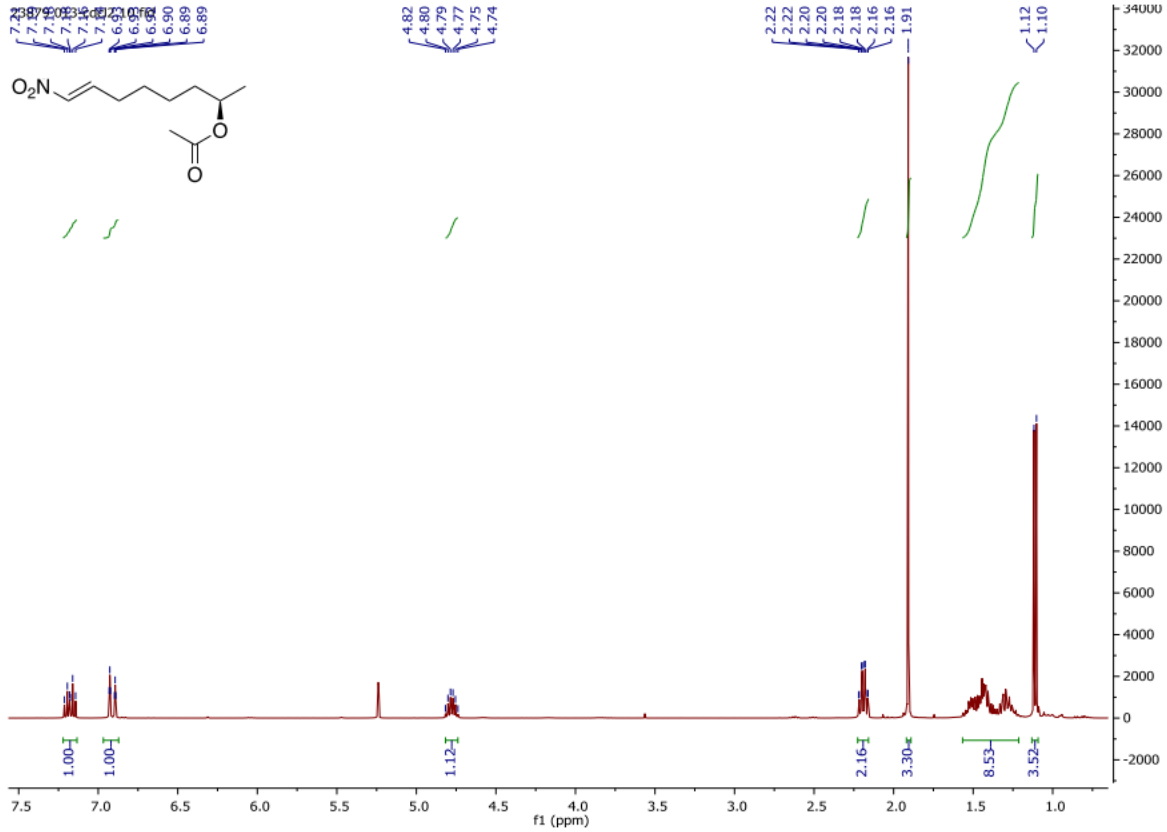


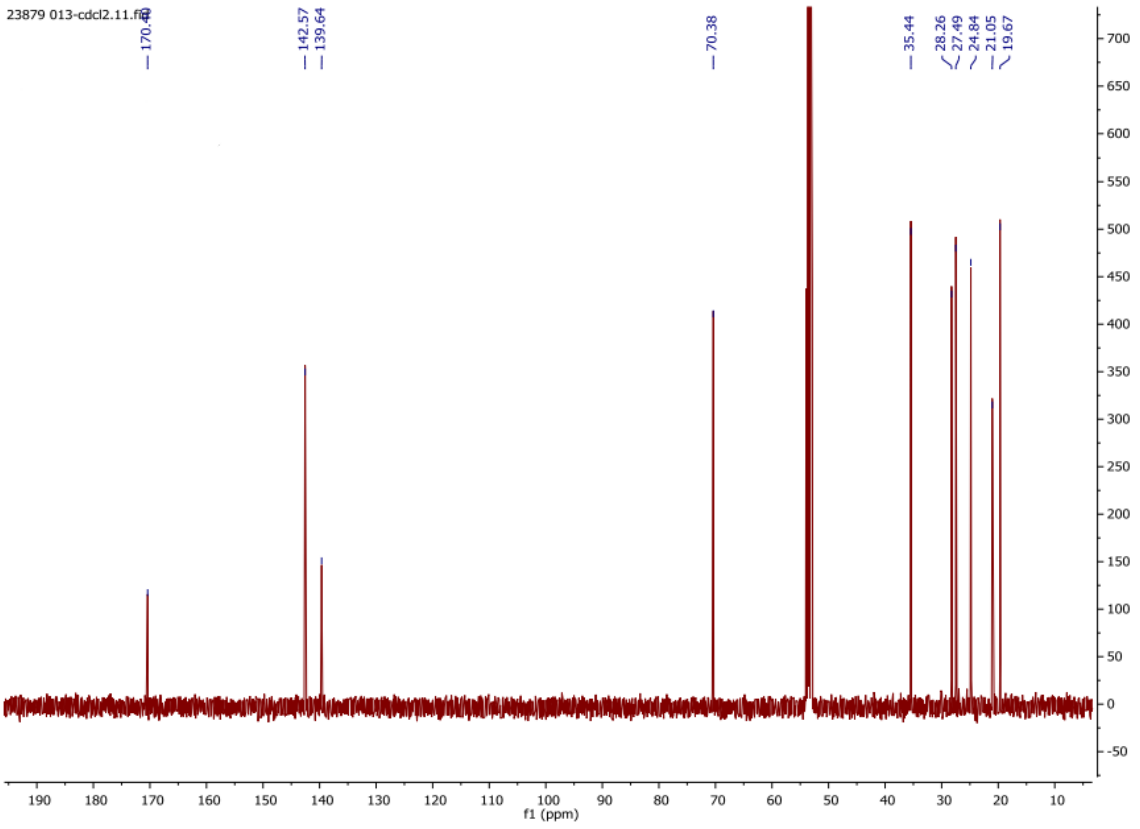
(*R*)-2-Acetoxyoct-7-ene 101 (*)



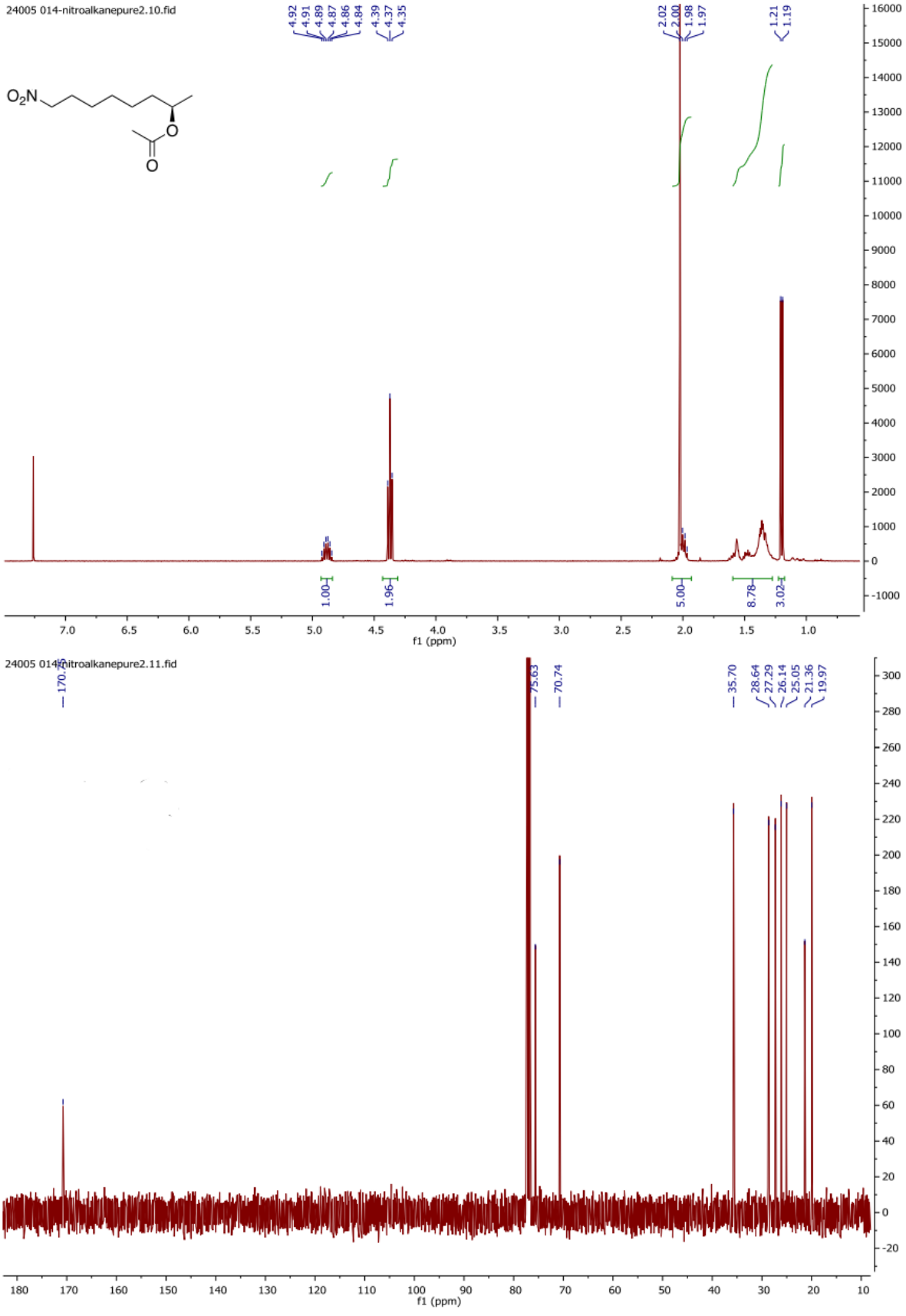


(*R,E*)-2-Acetoxy-8-nitrooct-7-ene 102 (*)

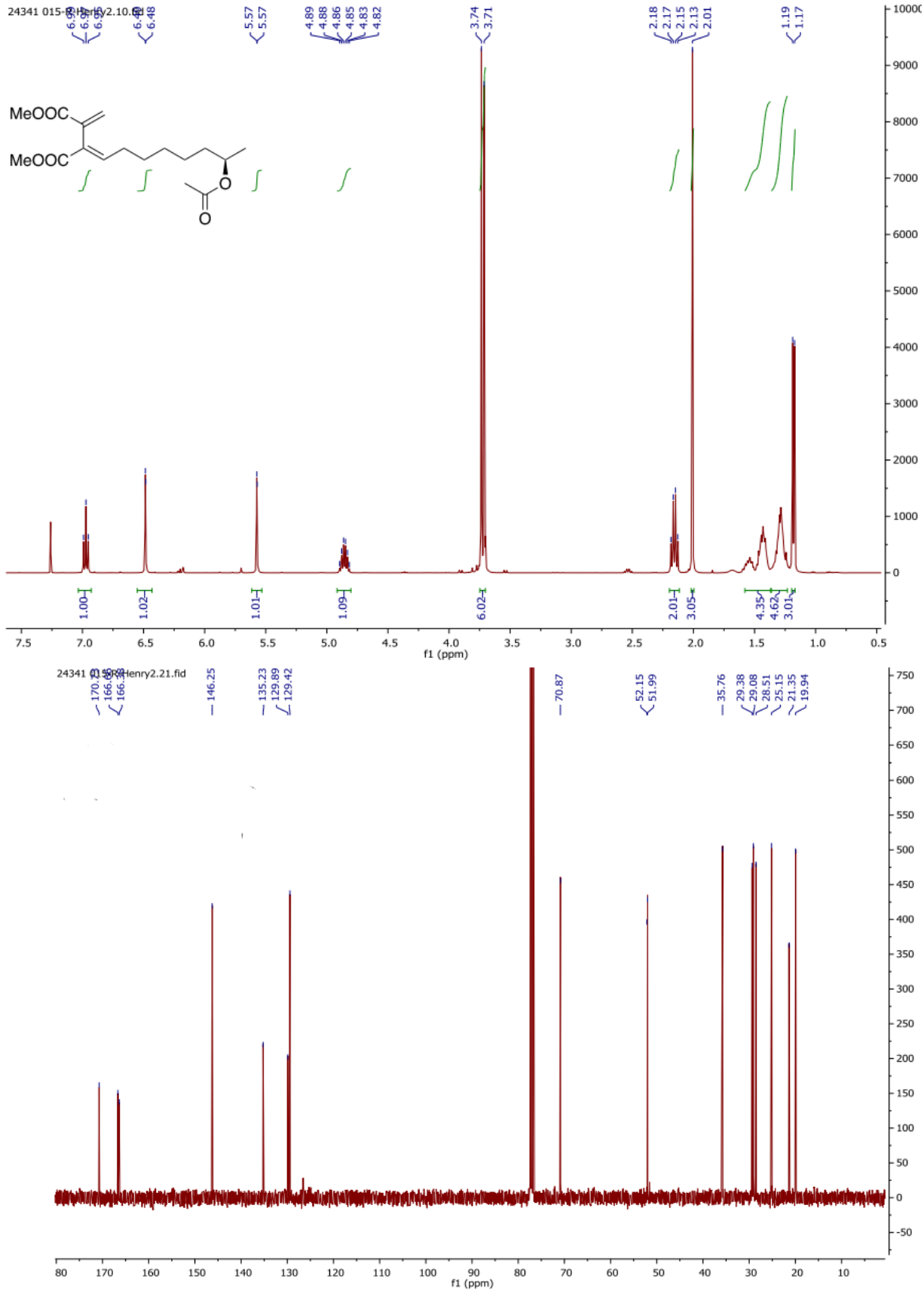




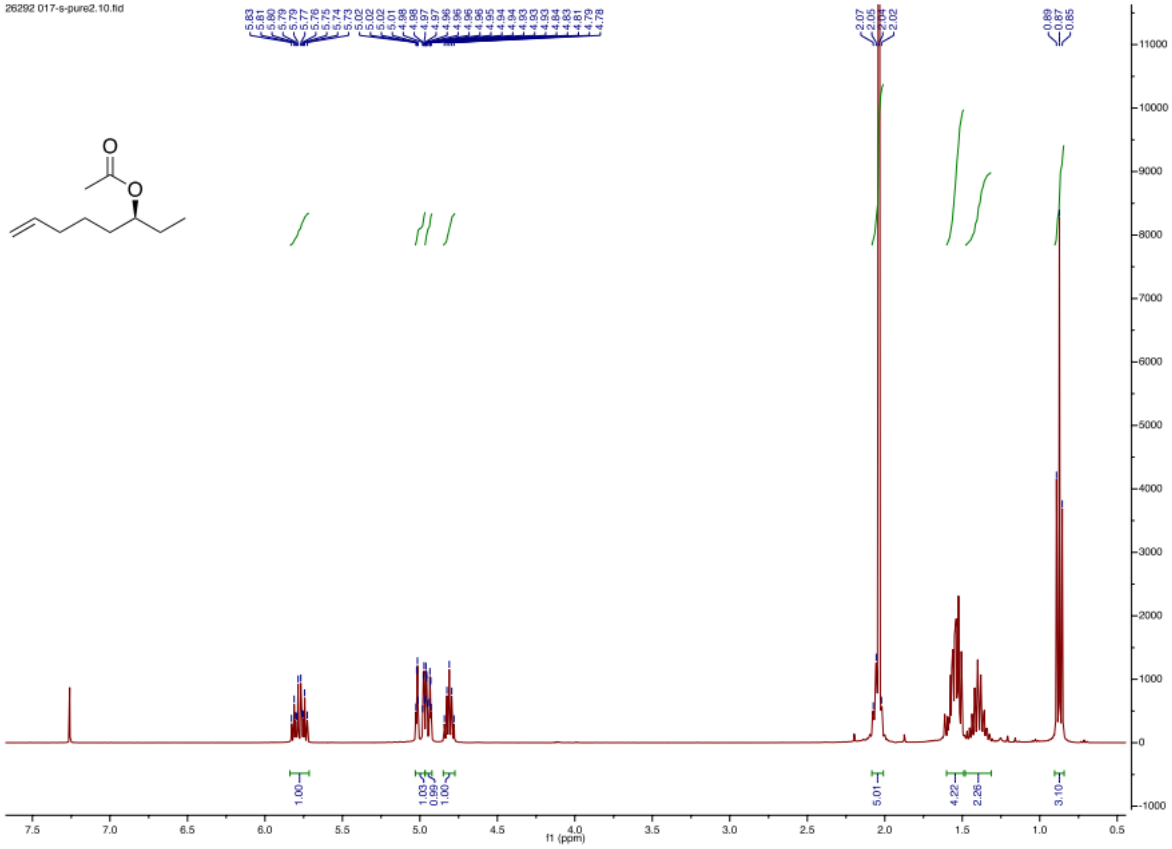
(*R*)-2-Acetoxy-8-nitrooctane 103 (*)

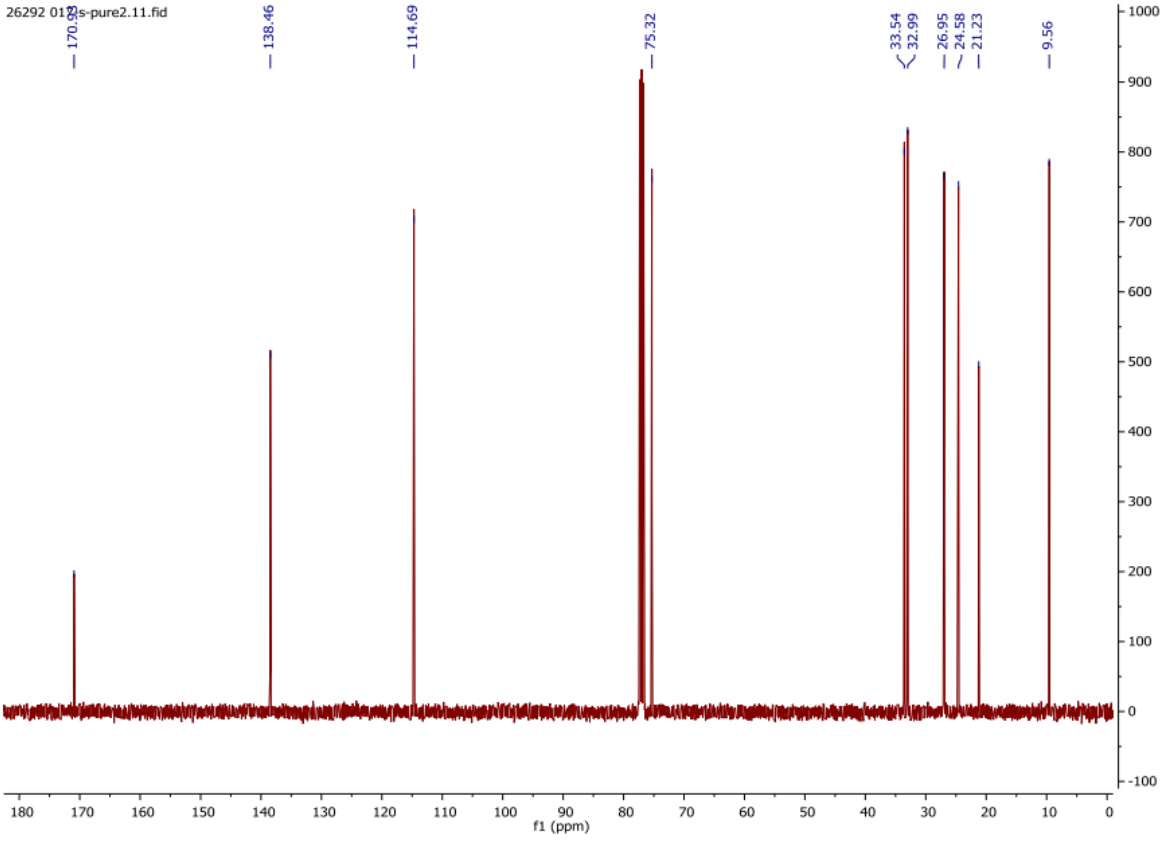


Dimethyl (*R*,*E*)-2-(6-acetoxyheptylidene)-3-methylenesuccinate 104 (*)

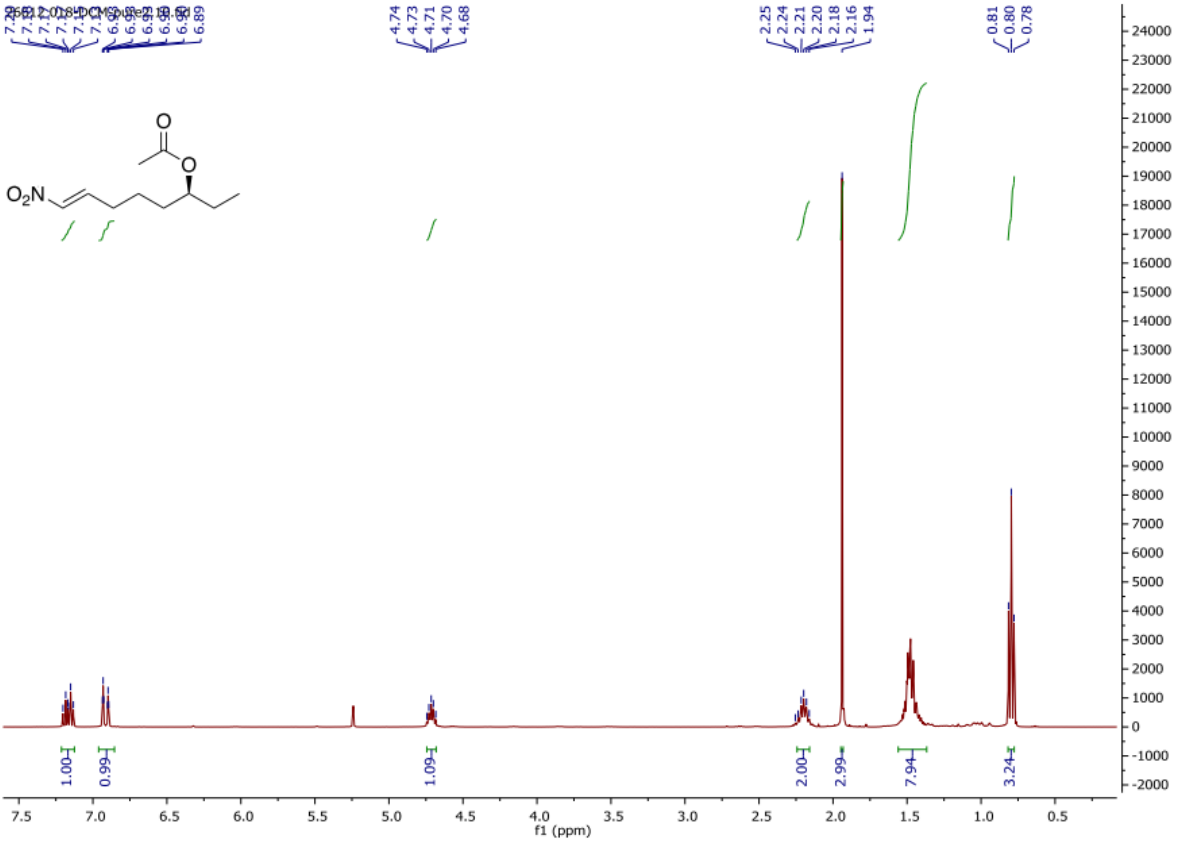


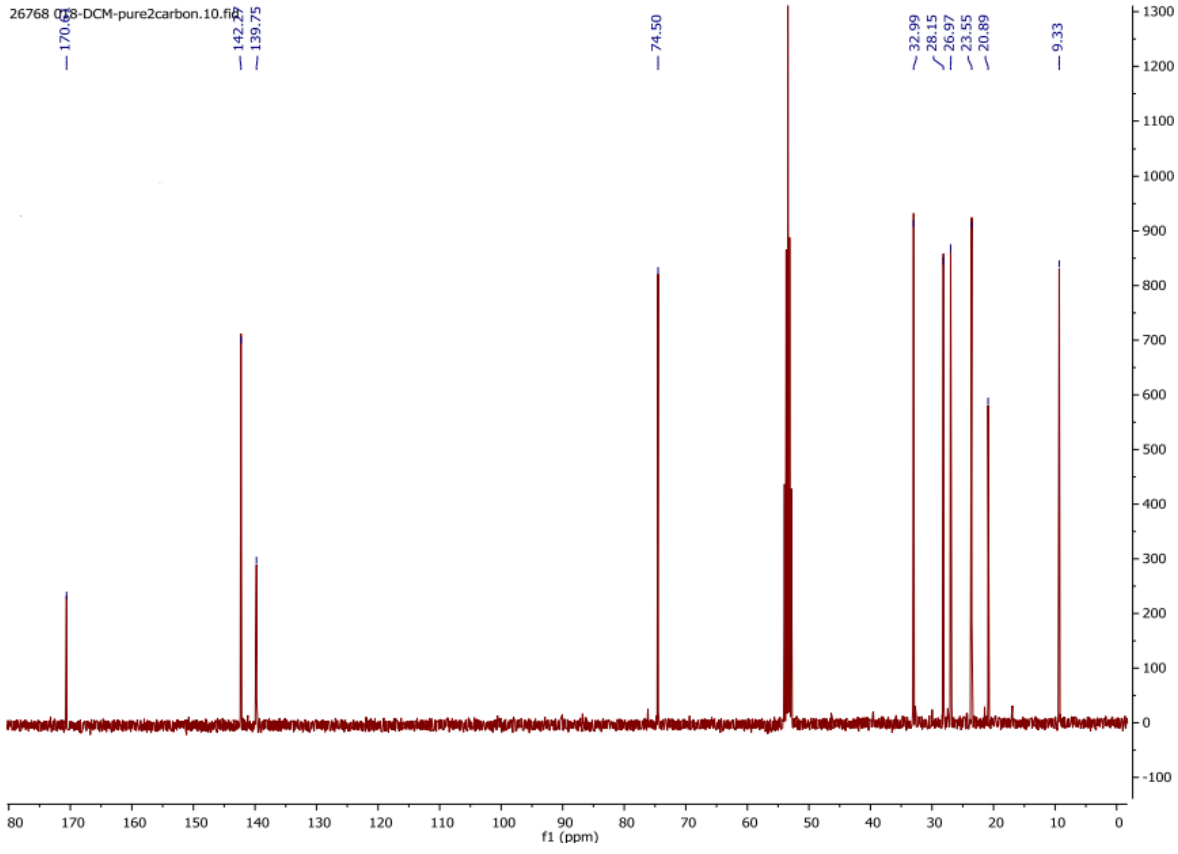
(*S*)-3-Acetoxyoct-7-ene 106 (*)



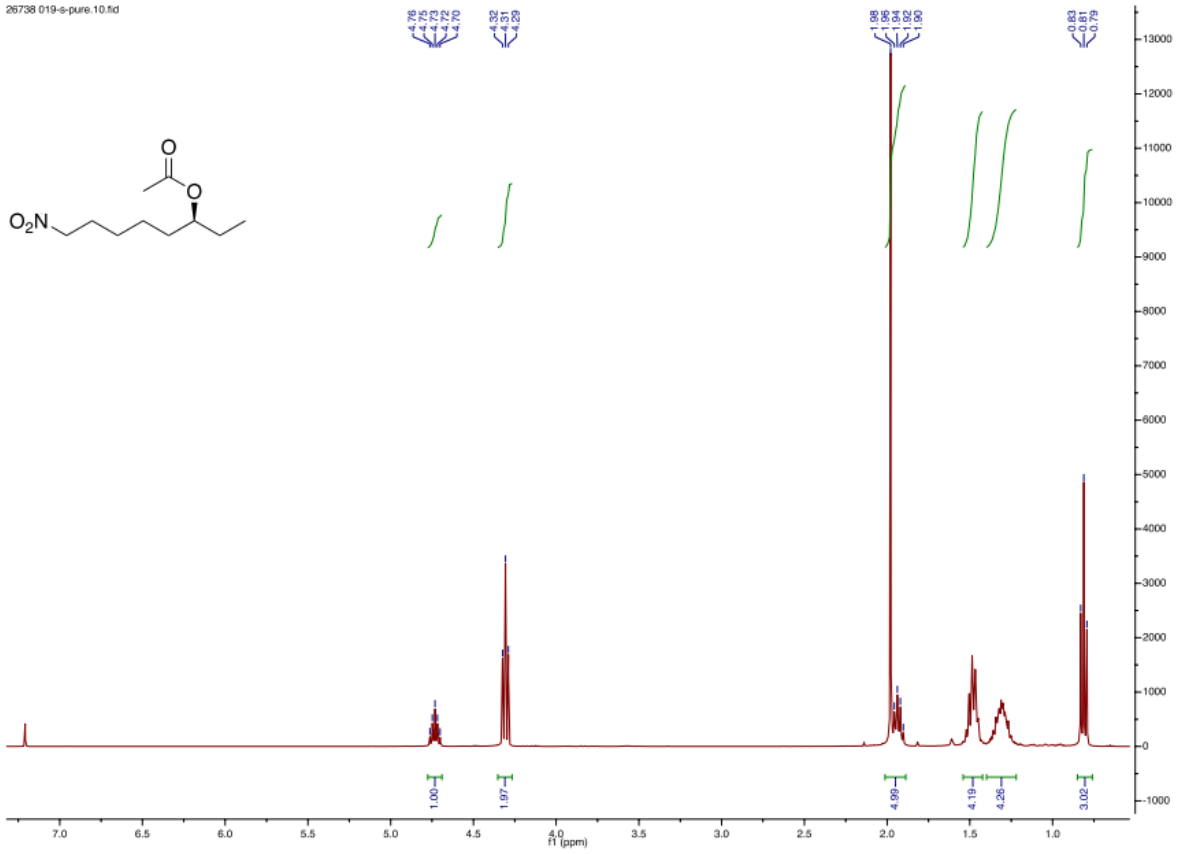


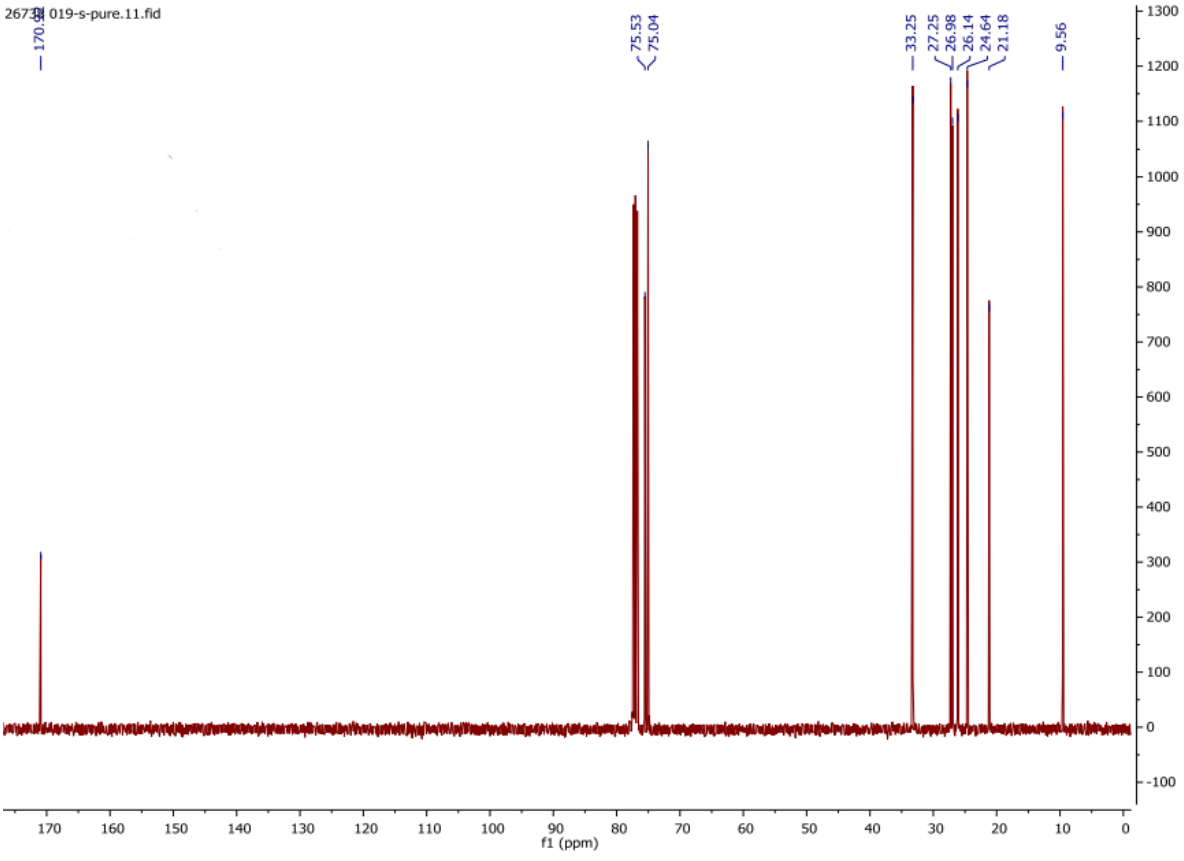
(*S*,*E*)-3-Acetoxy-8-nitrooct-7-ene 107 (*)



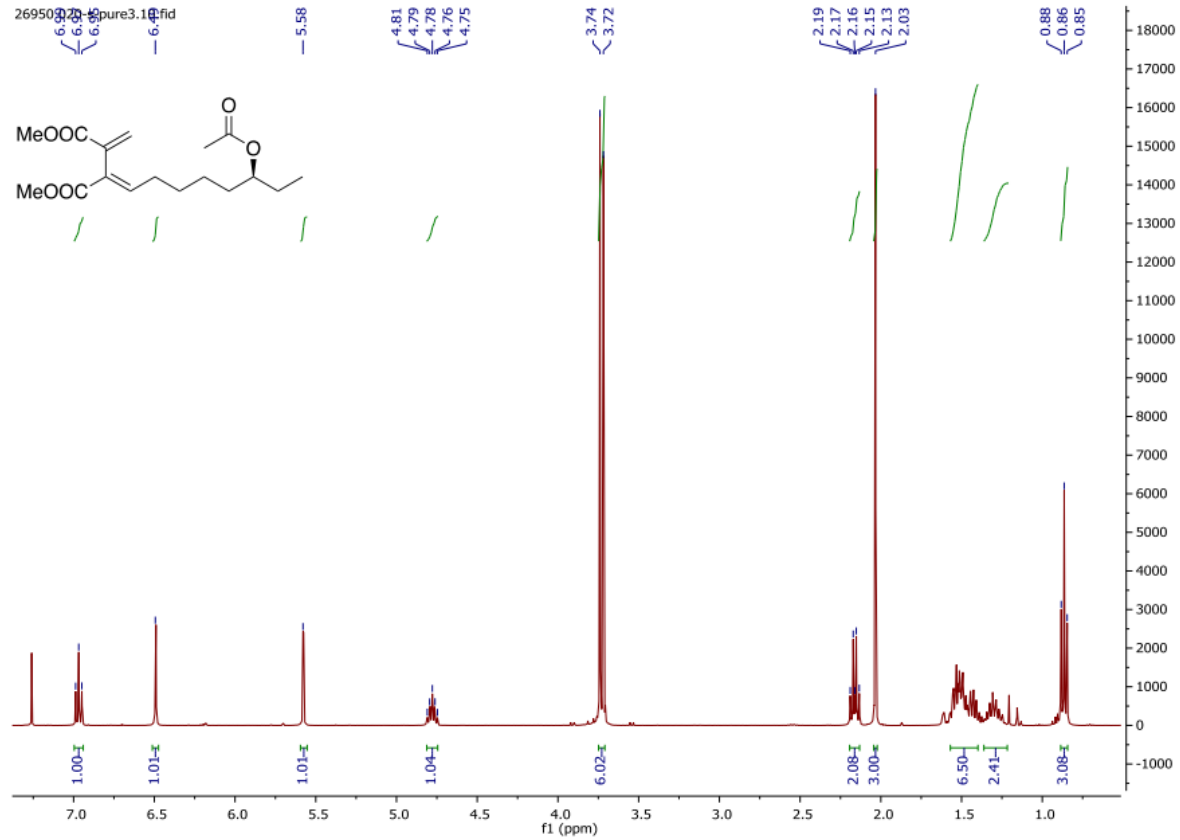


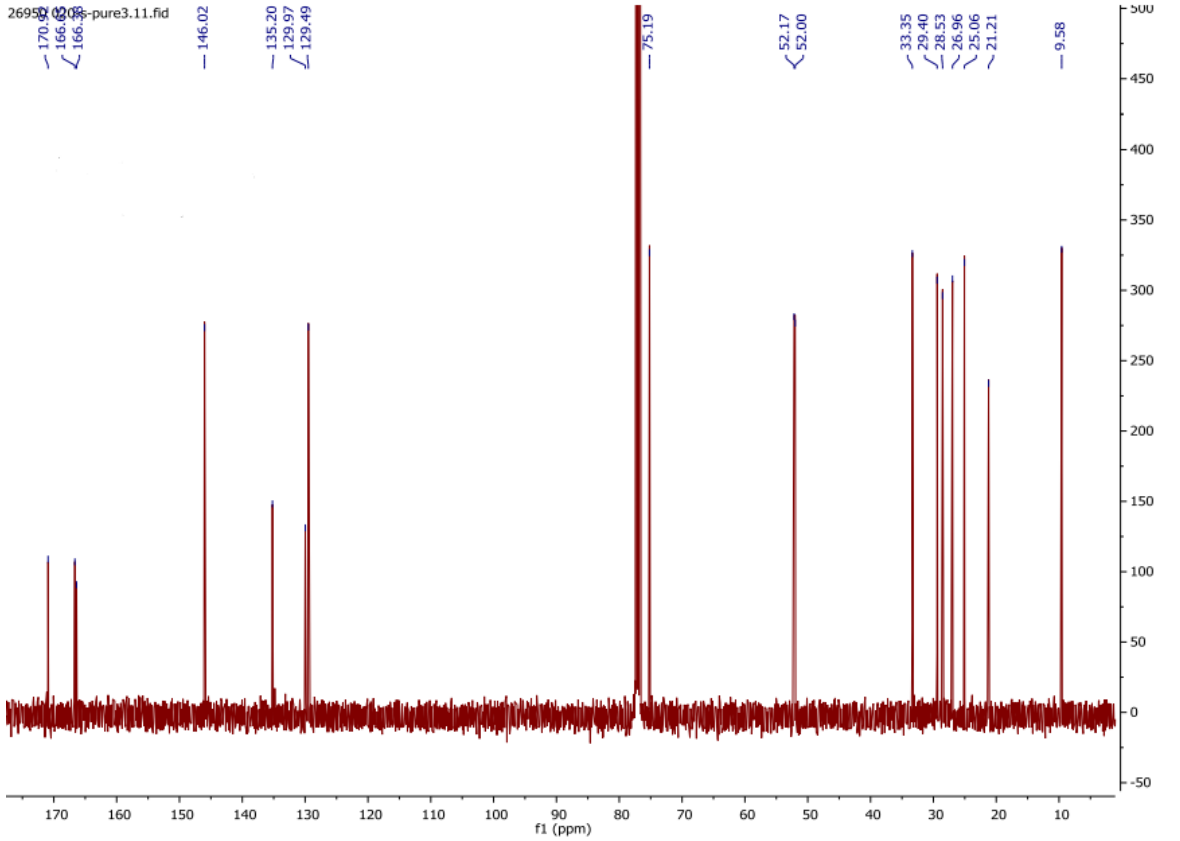
(*S*,*E*)-3-Acetoxy-8-nitrooctane 108 (*)



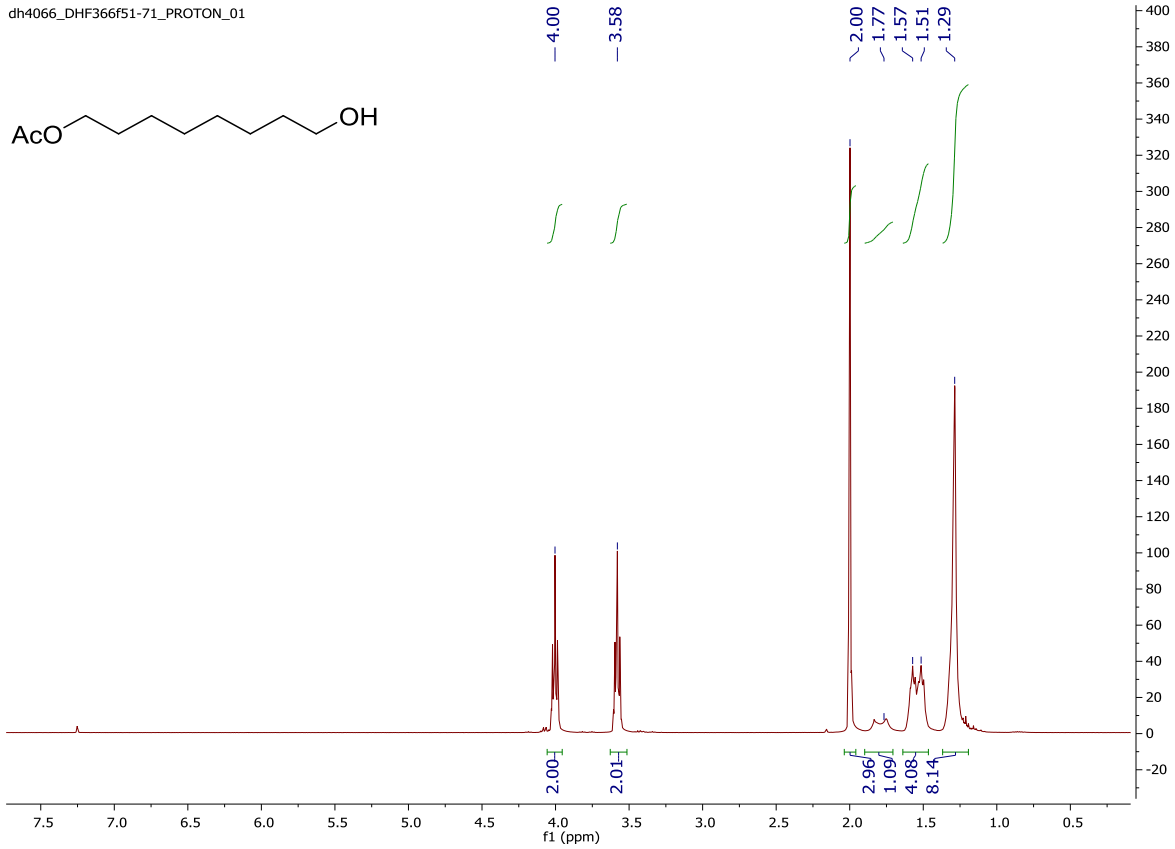


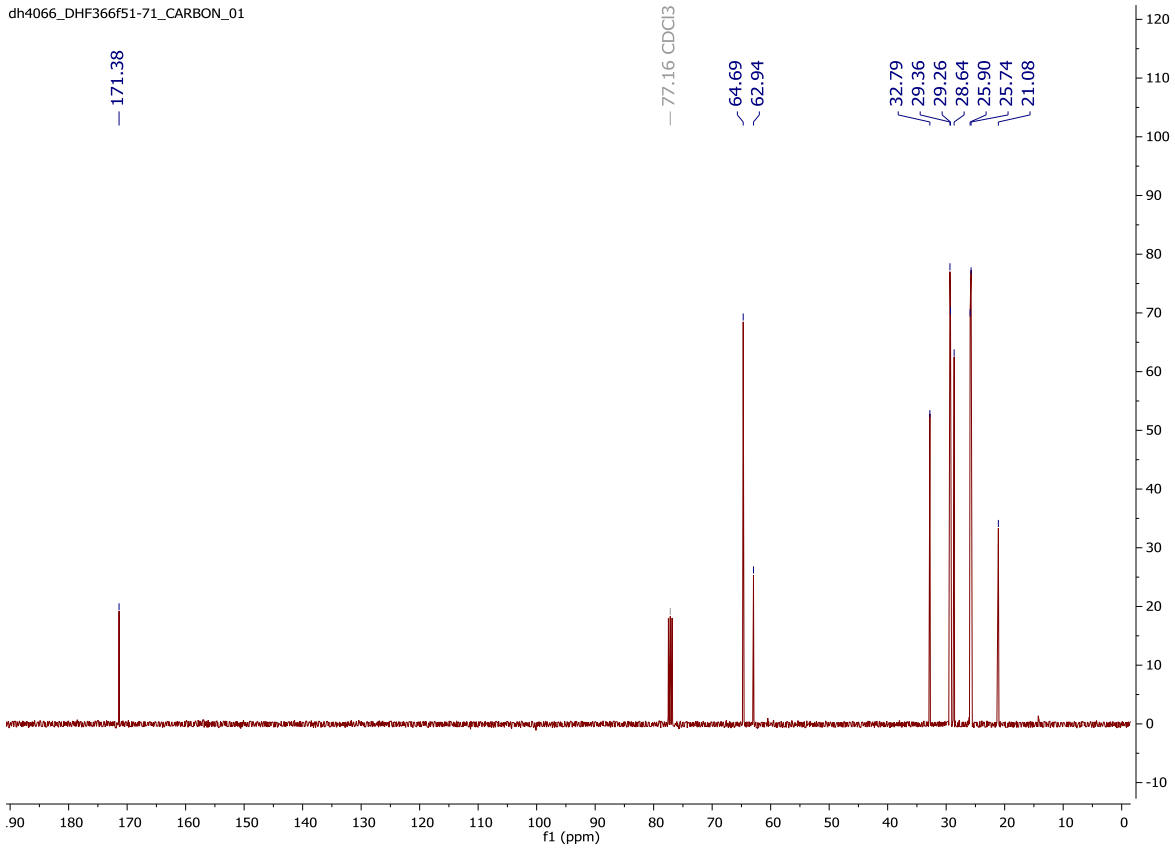
Dimethyl (*S*,*E*)-2-(5-acetoxyheptylidene)-3-methylenesuccinate 109 (*)



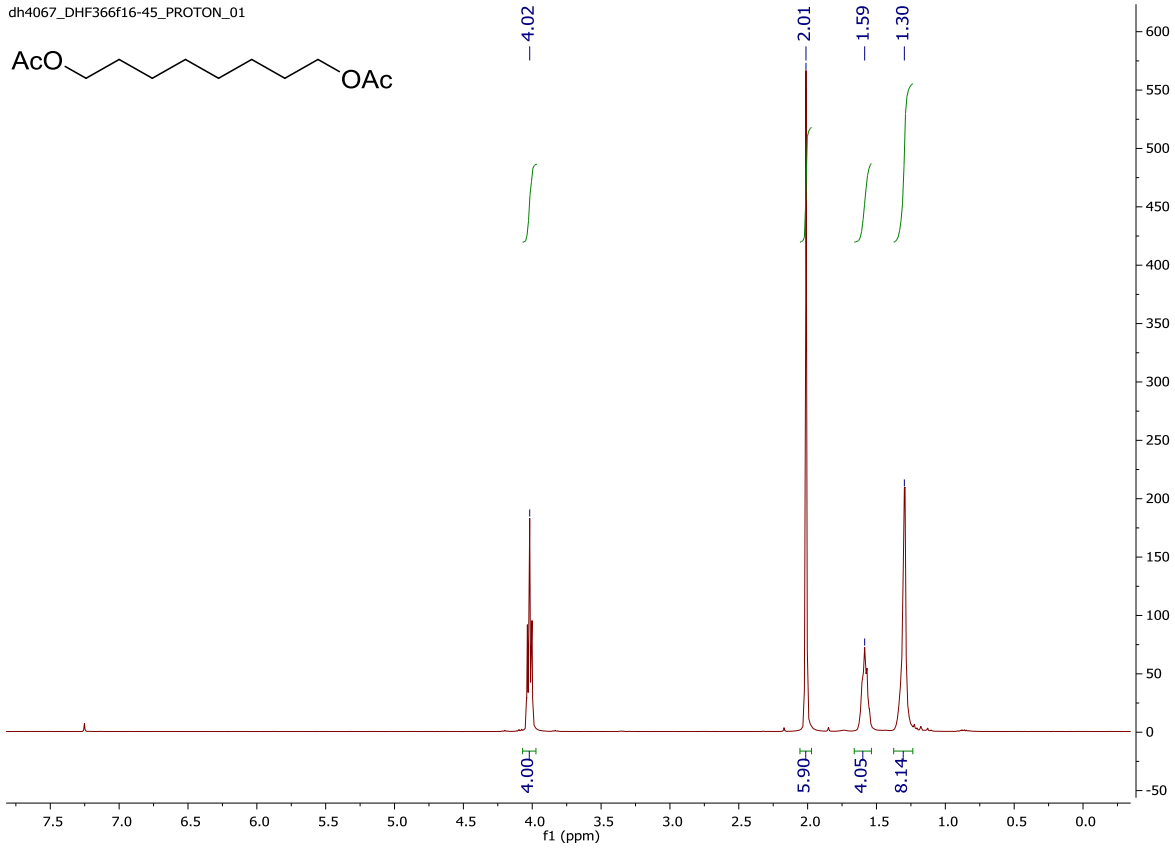


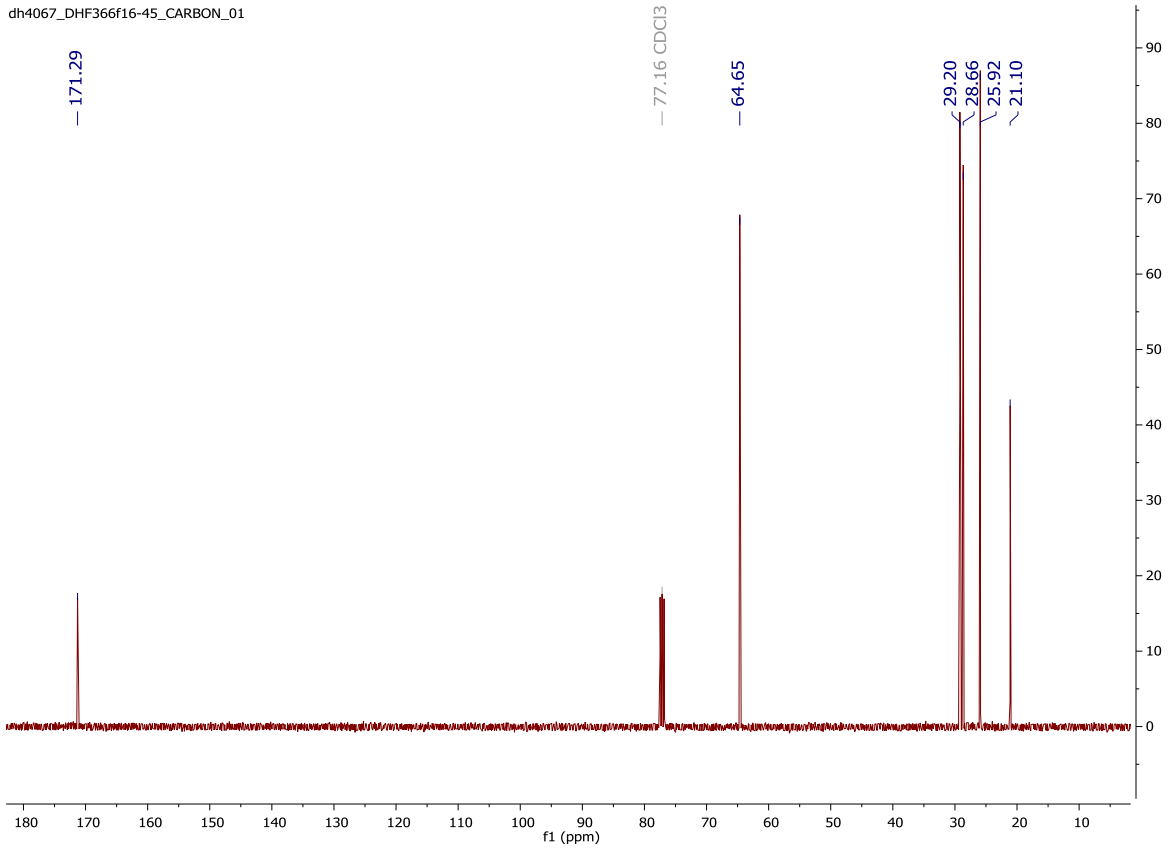
8-Hydroxyoctyl acetate 111



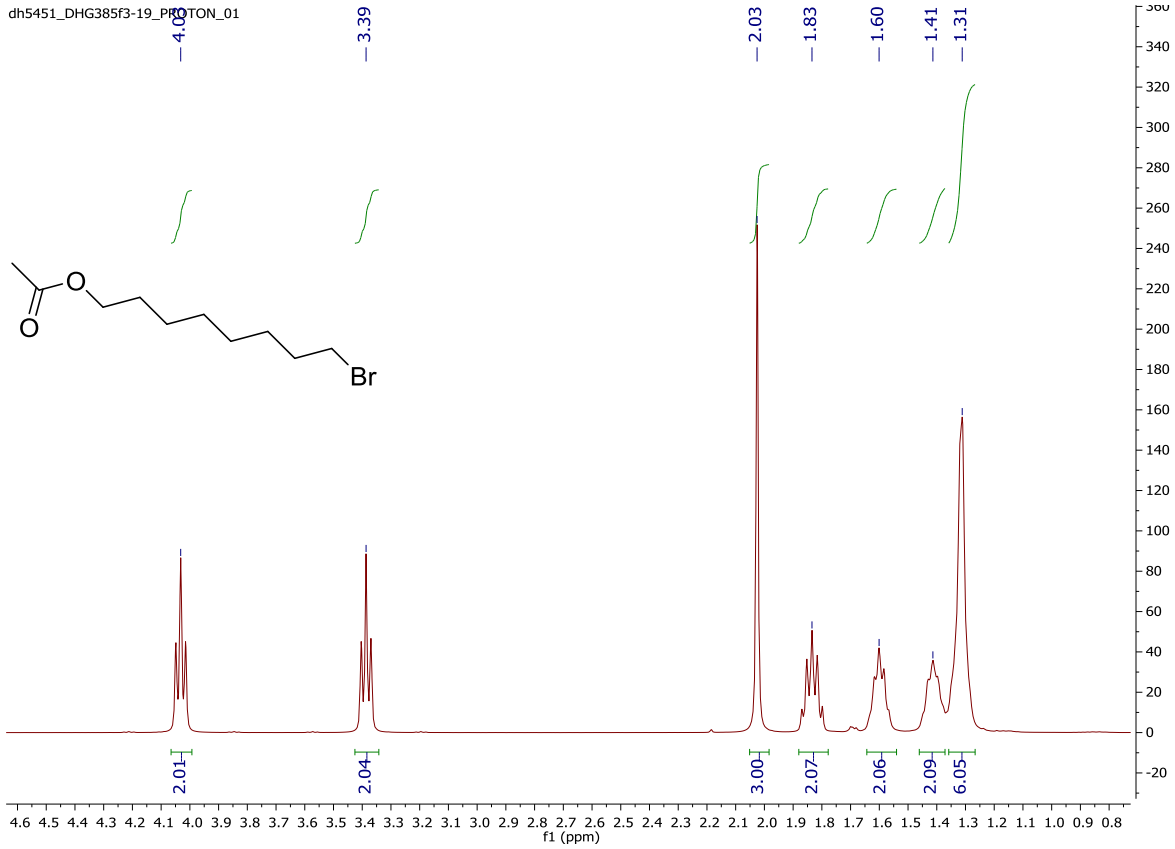


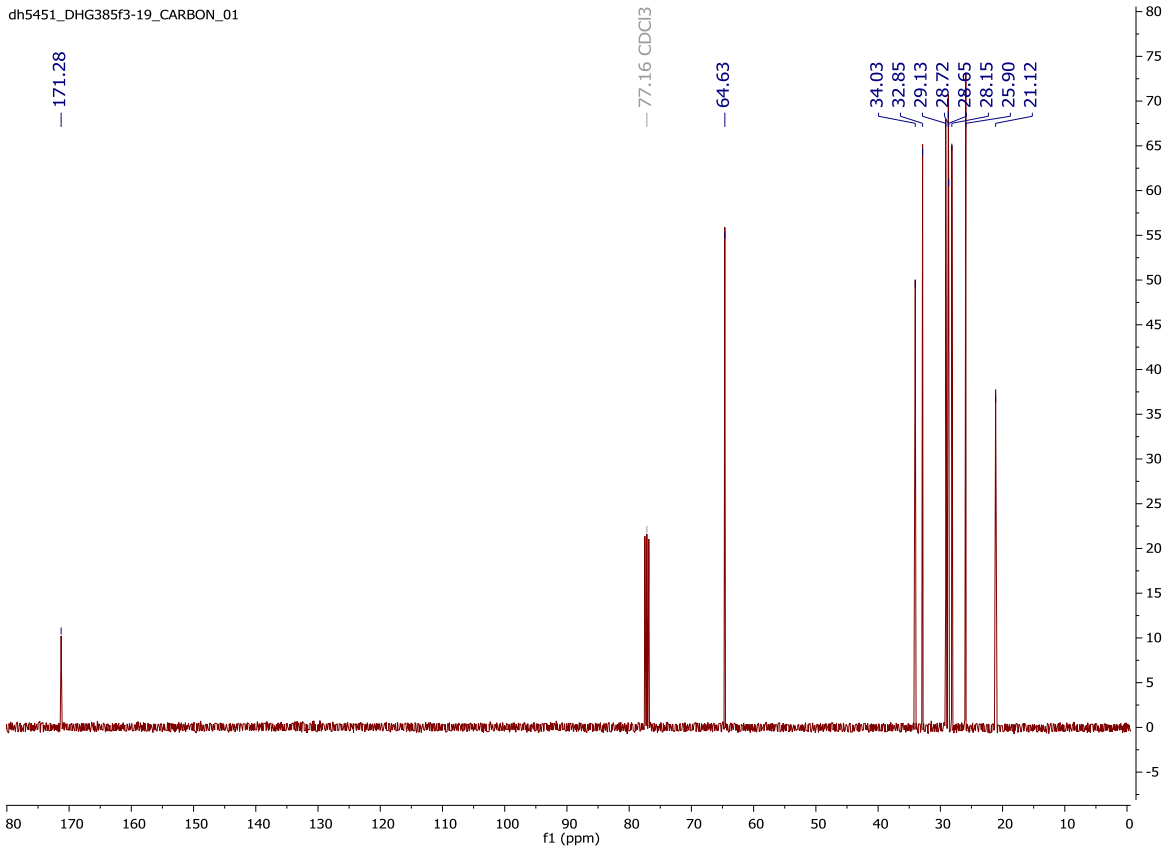
Octane-1,8-diol diacetate S5



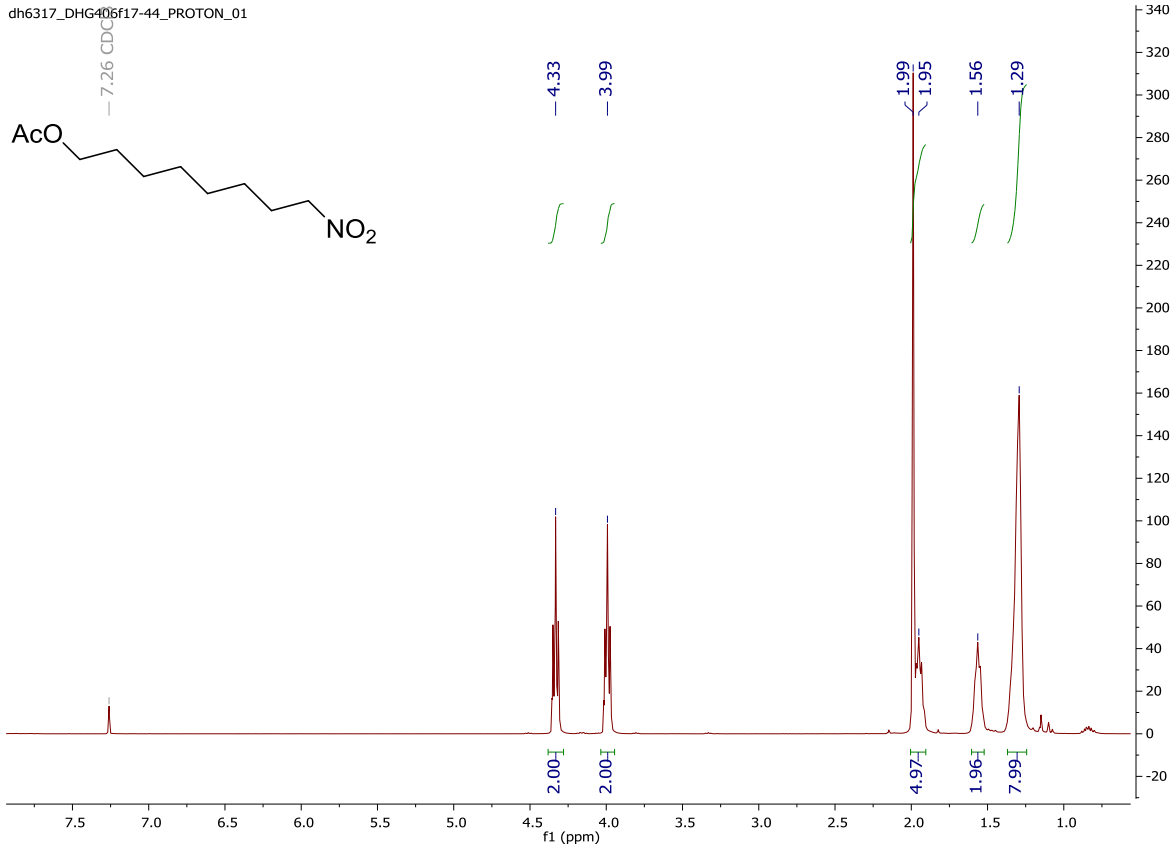


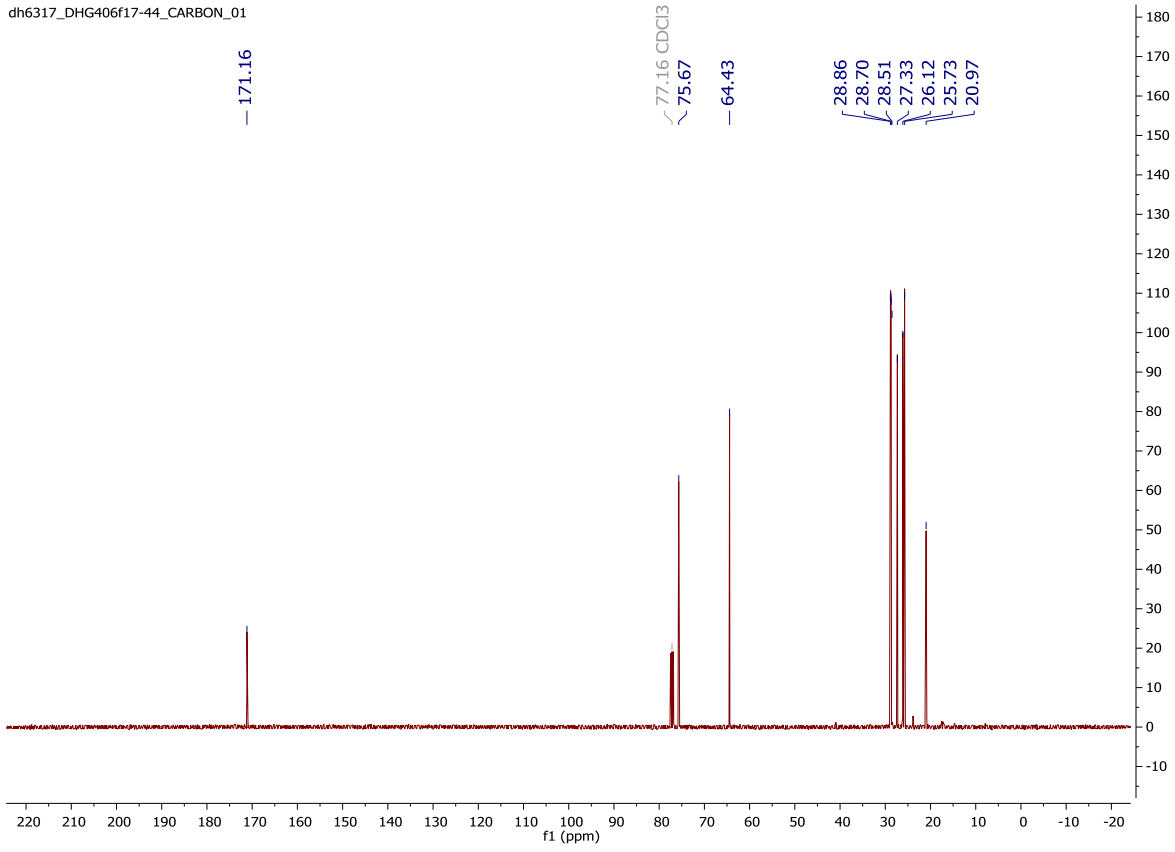
8-Bromooctyl acetate 112



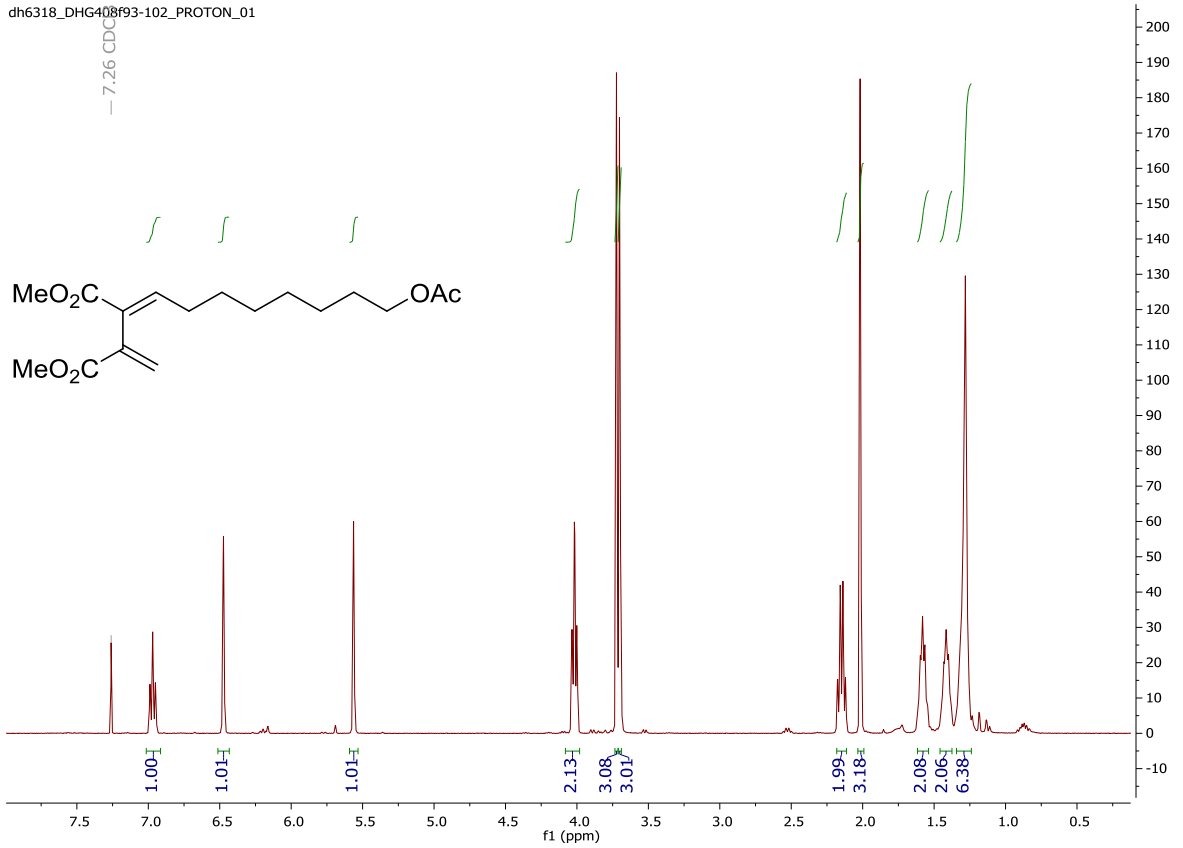


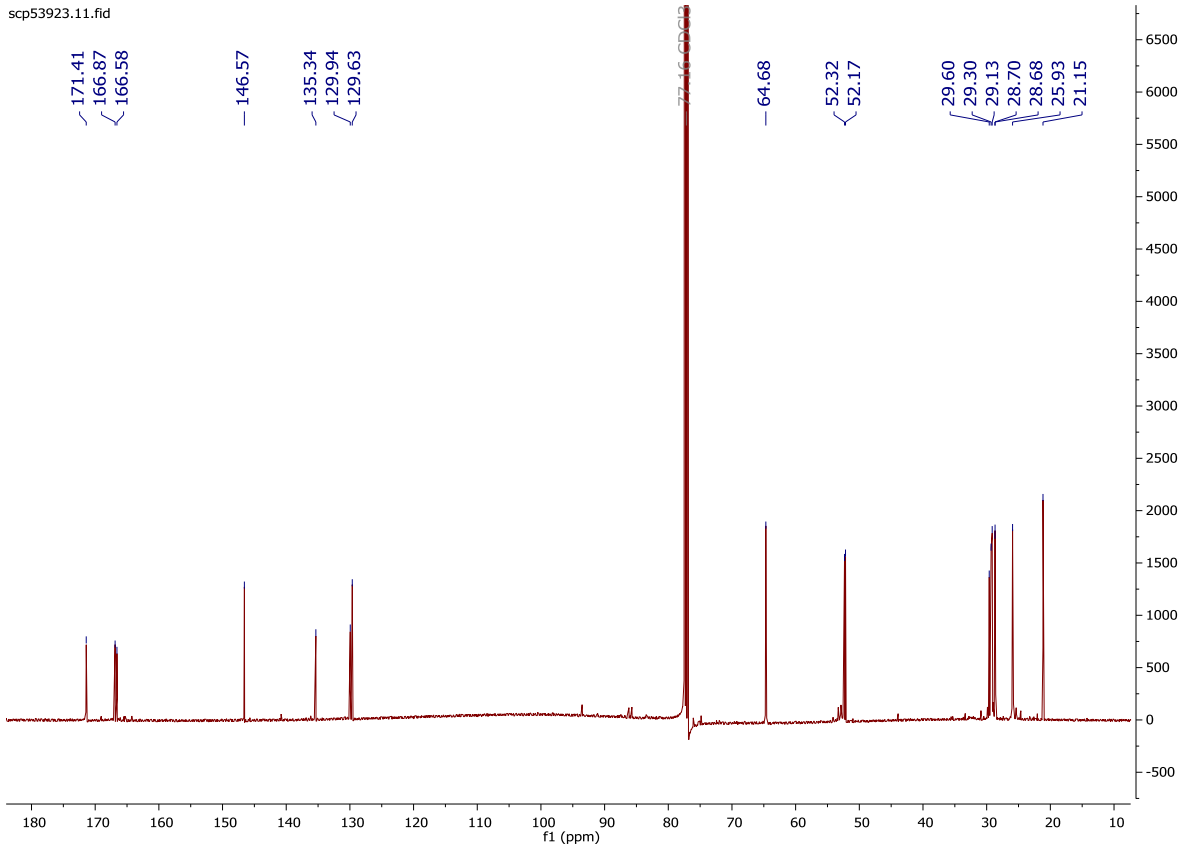
8-nitrooctyl acetate 113



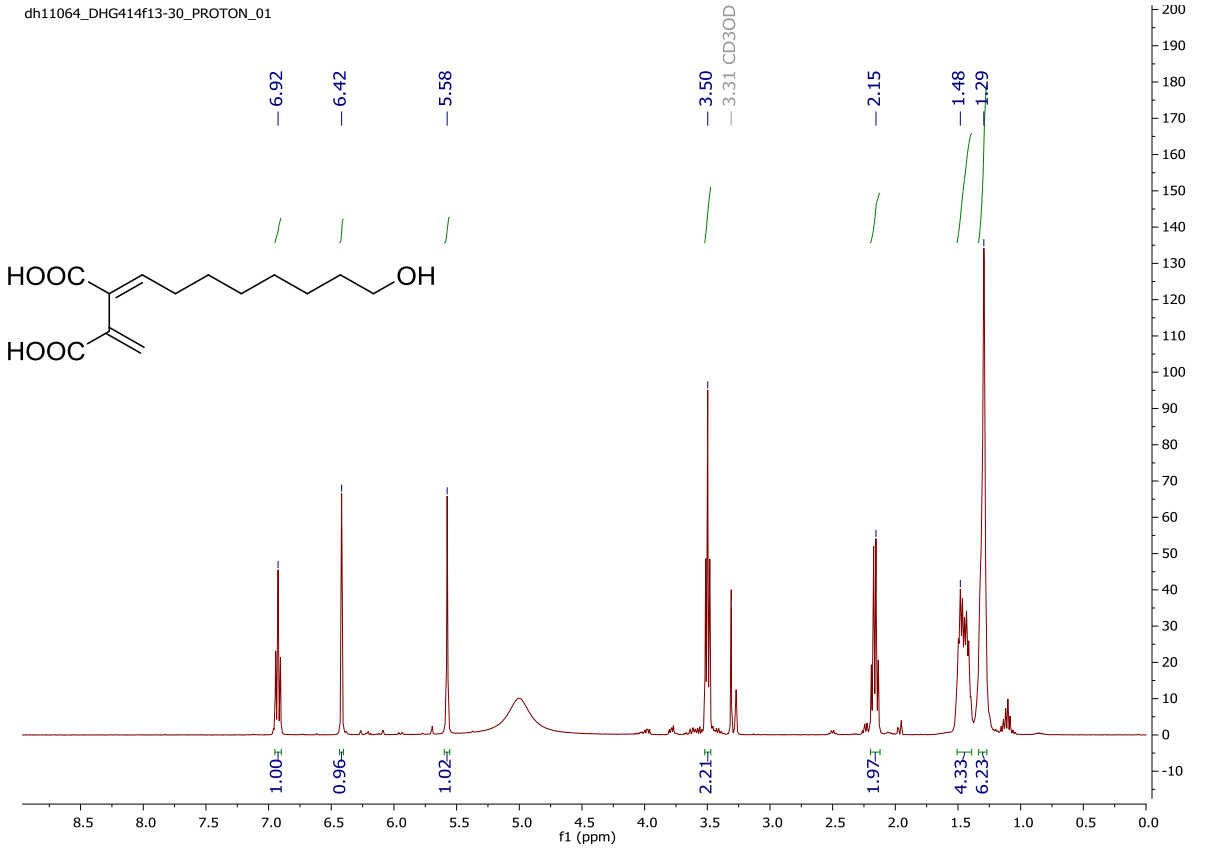


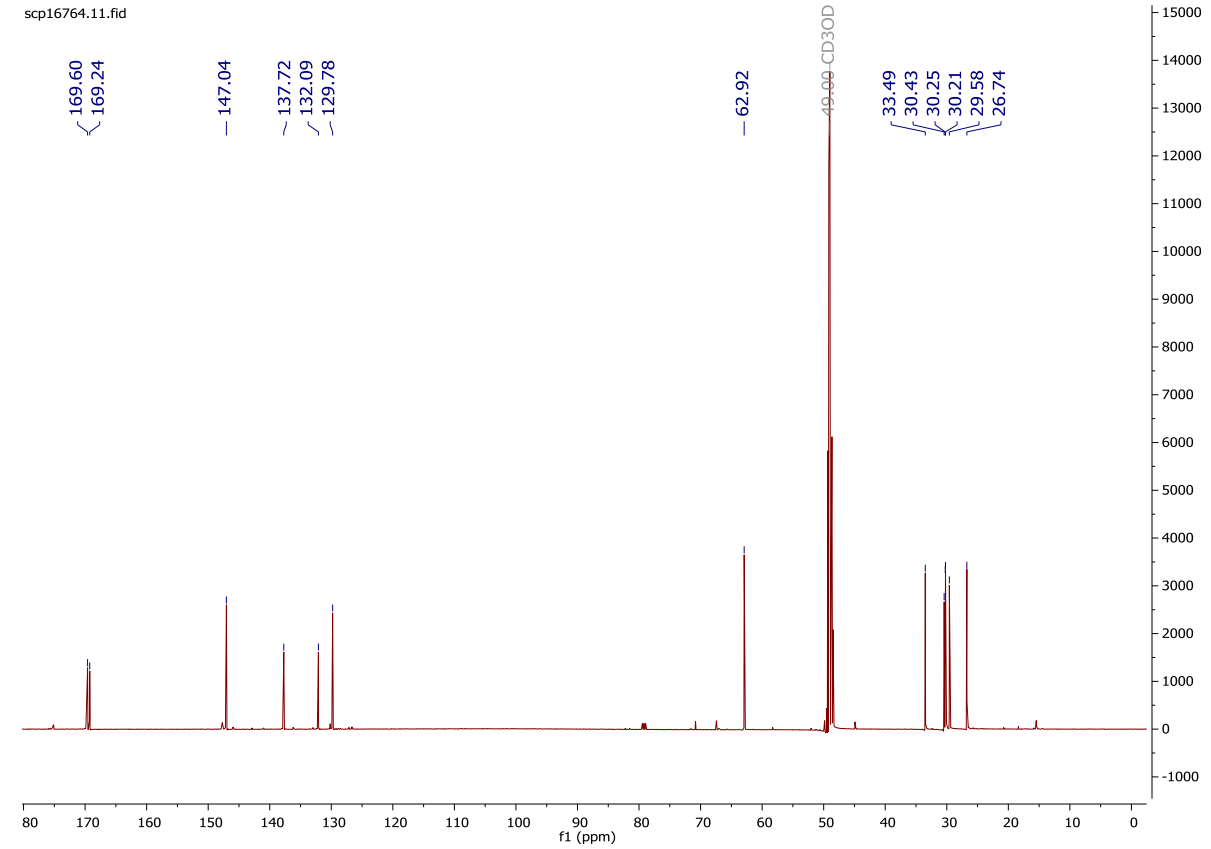
Dimethyl (*E*)-2-(8-acetoxyoctylidene)-3-methylenesuccinate 114



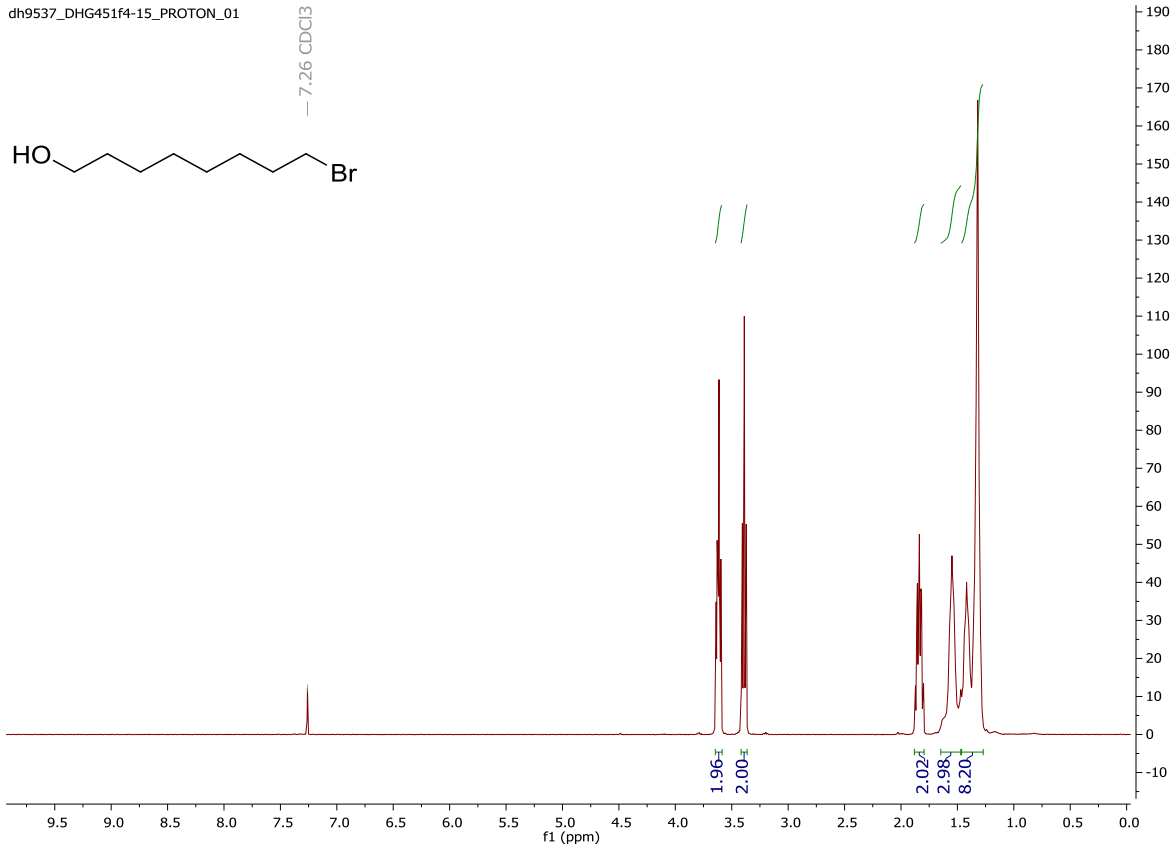


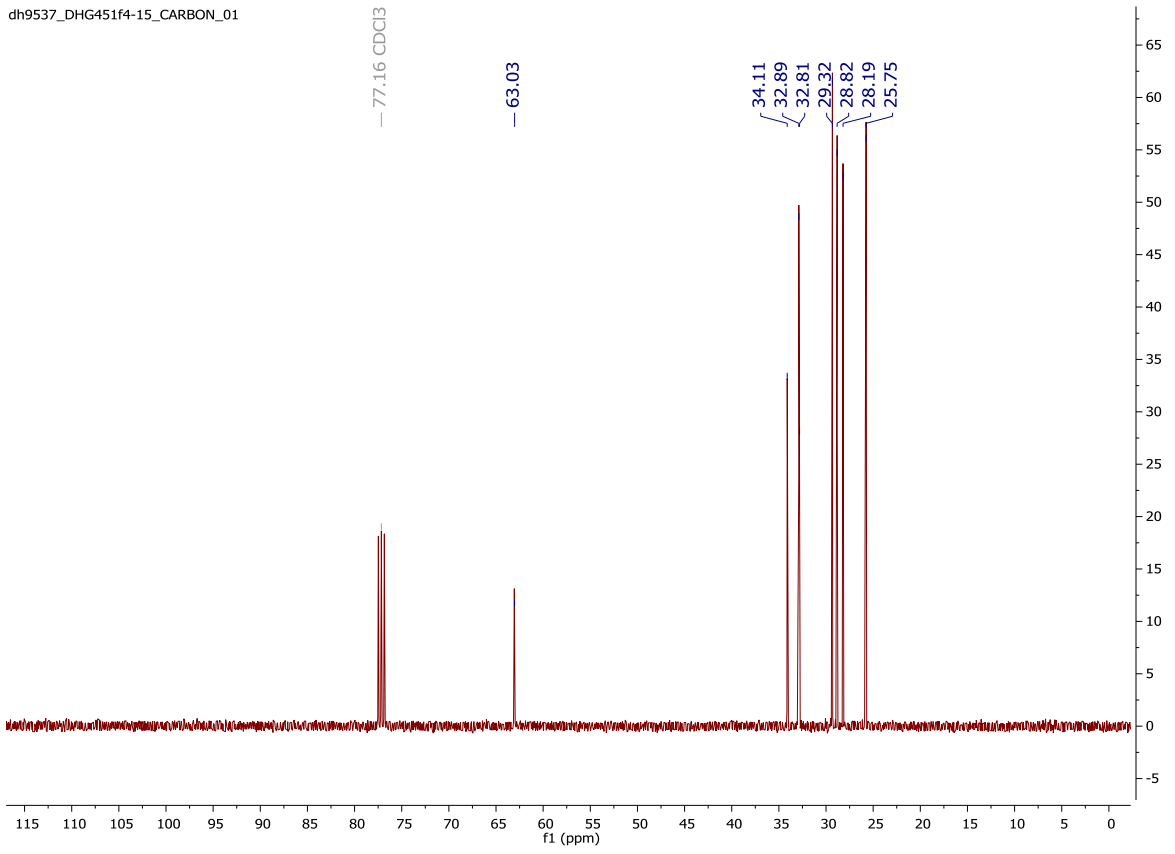
(*E*)-2-(8-Hydroxyoctylidene)-3-methylenesuccinic acid 38 (Tricladic Acid C)



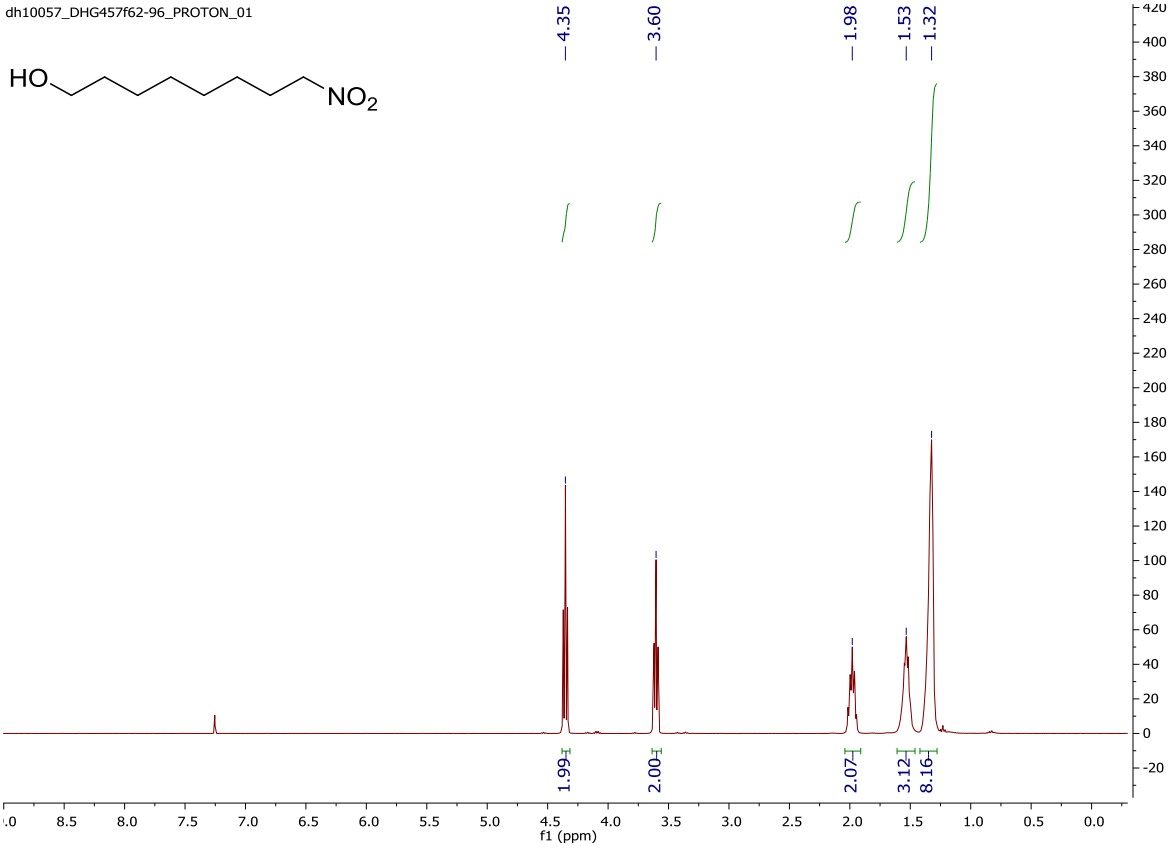


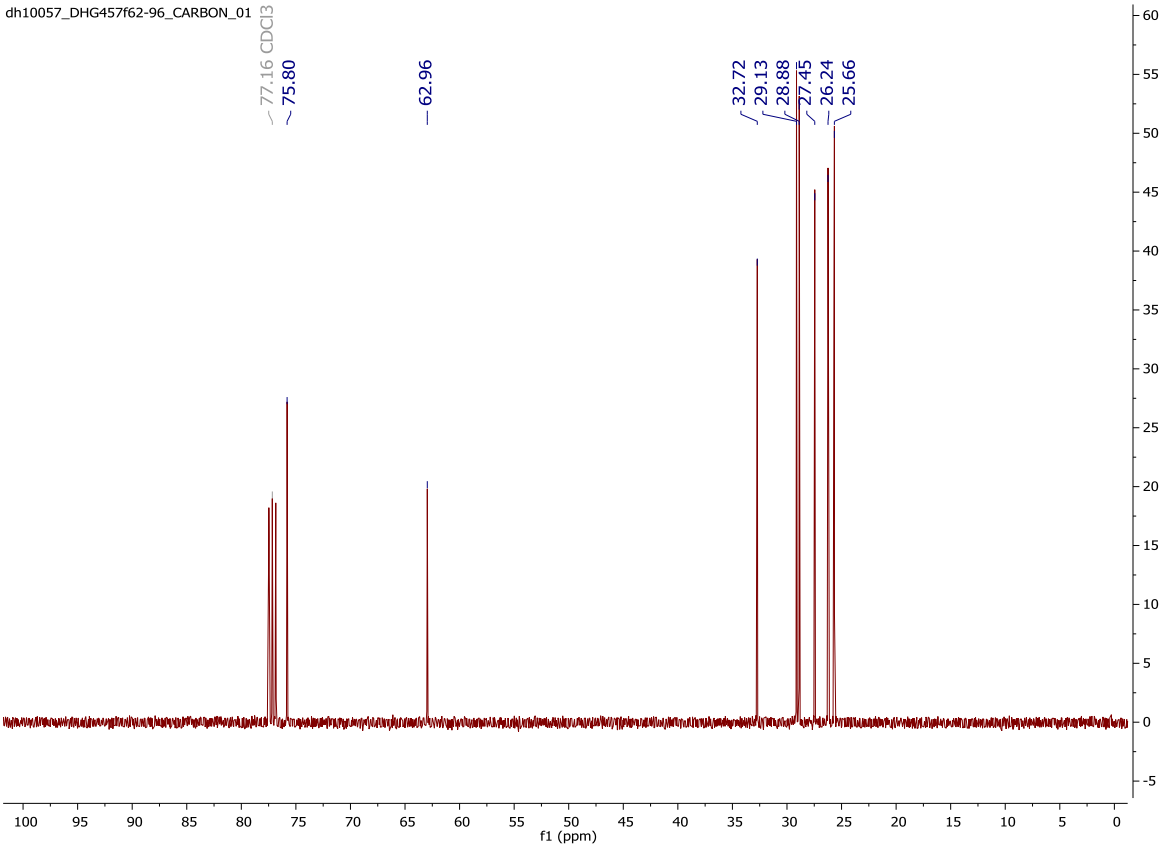
8-Bromooctan-1-ol 115



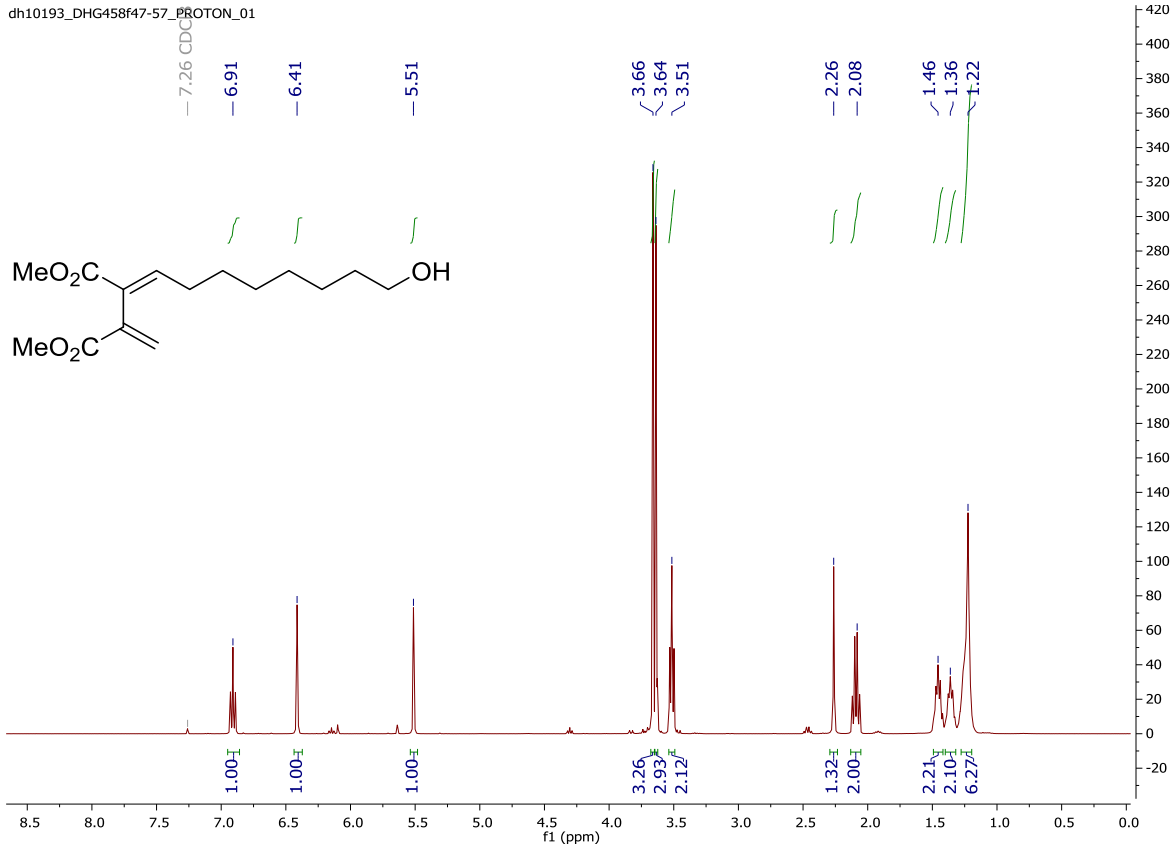


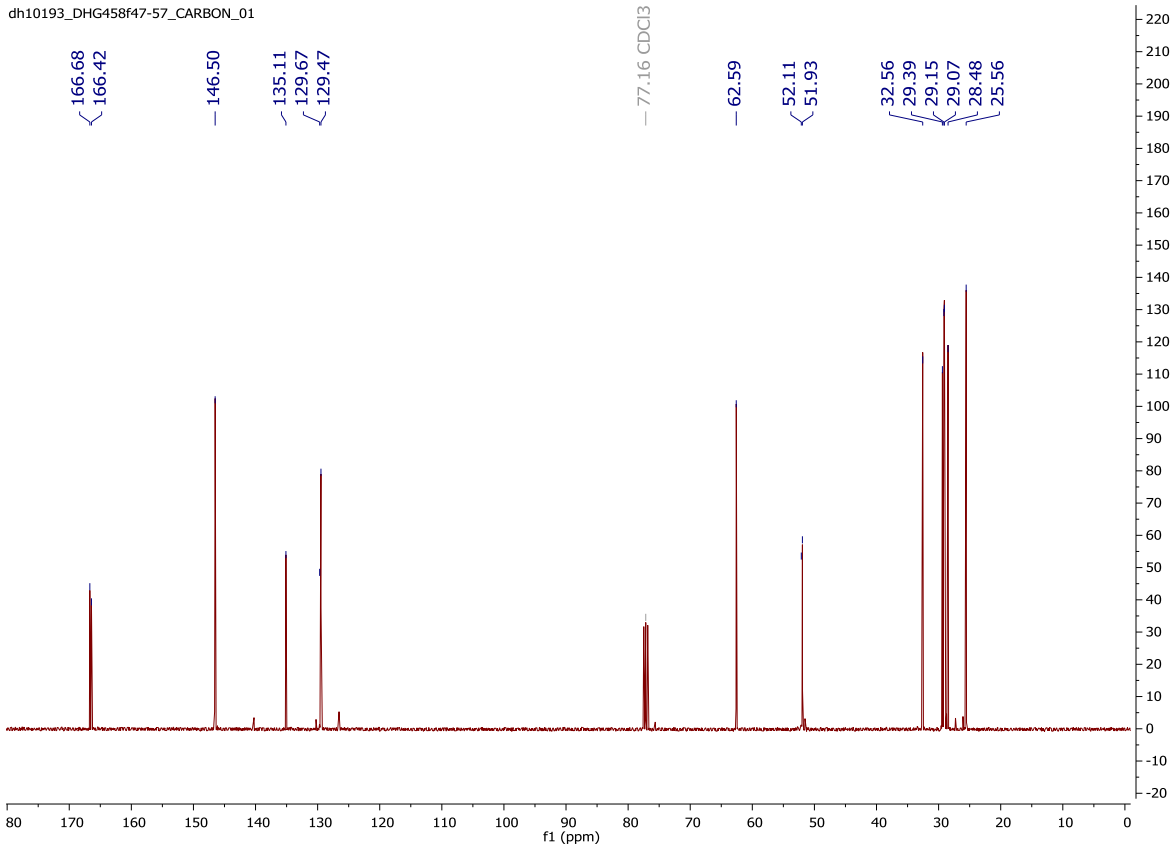
8-Nitrooctan-1-ol 116



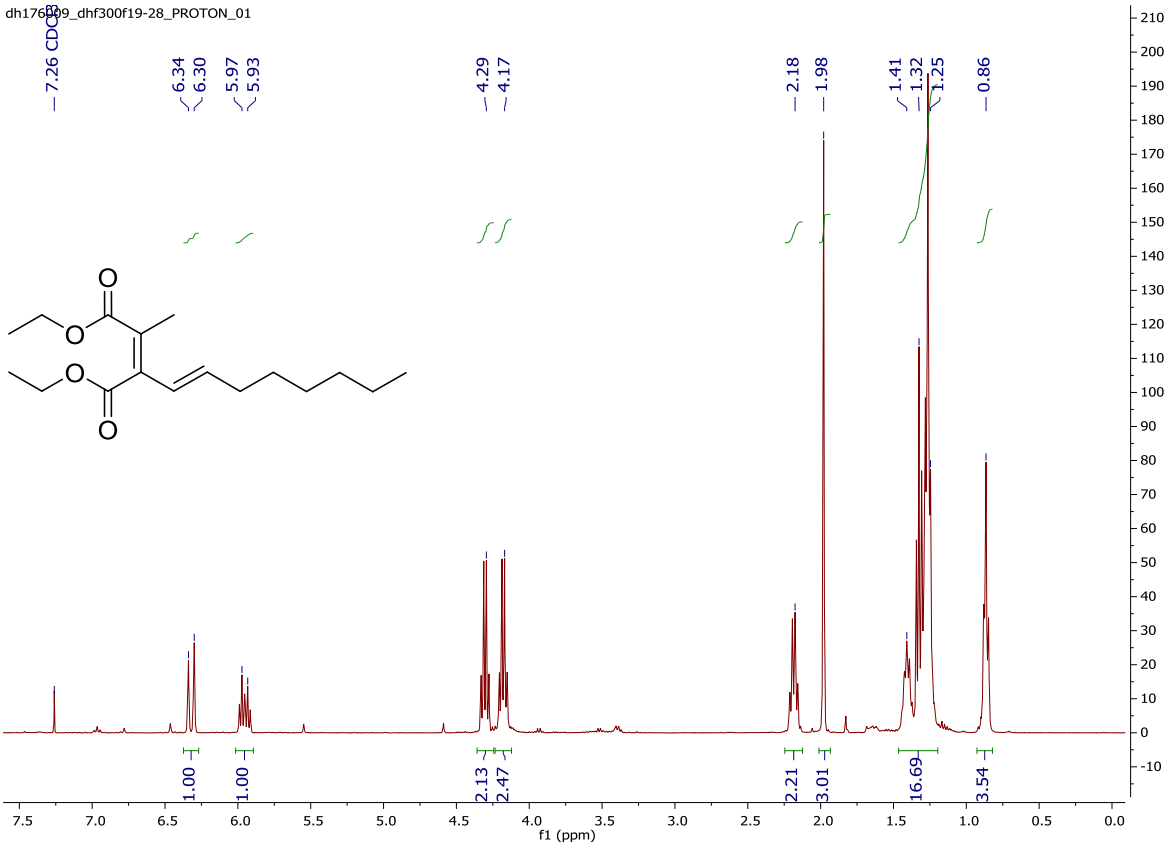


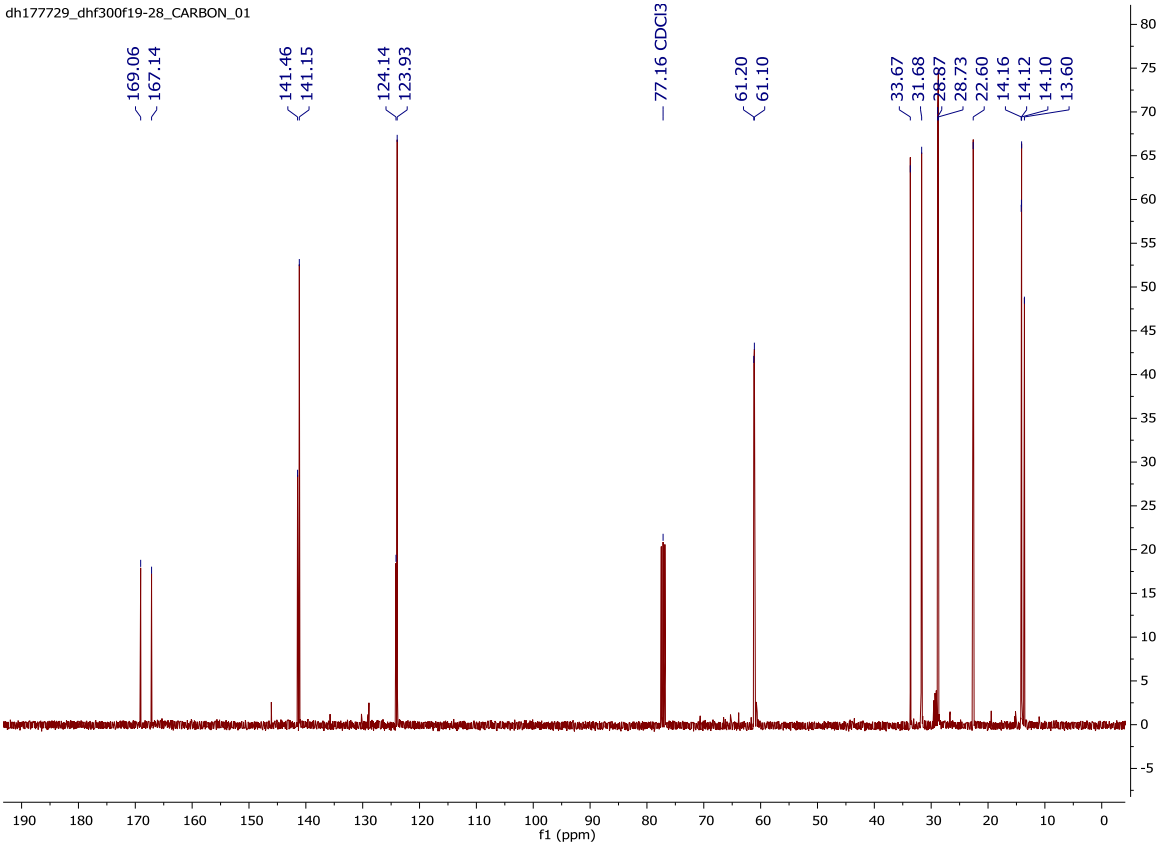
Dimethyl (*E*)-2-(8-hydroxyoctylidene)-3-methylenesuccinate 117



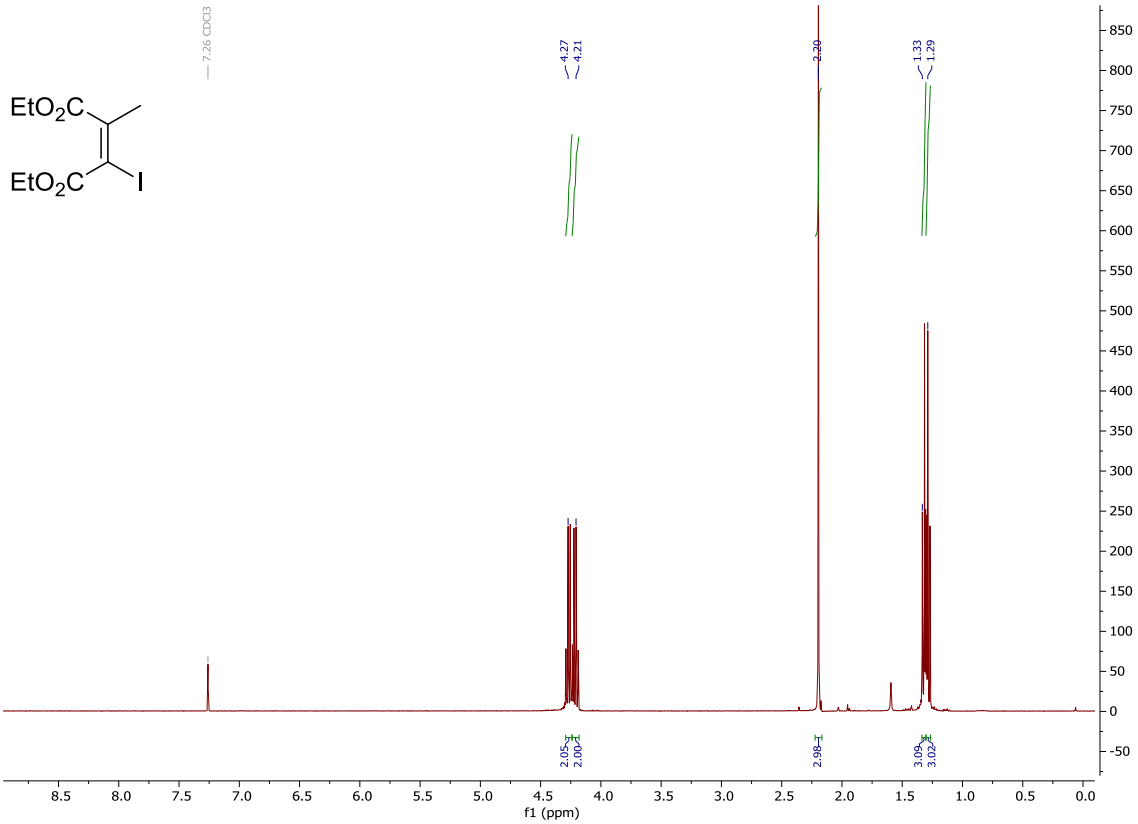


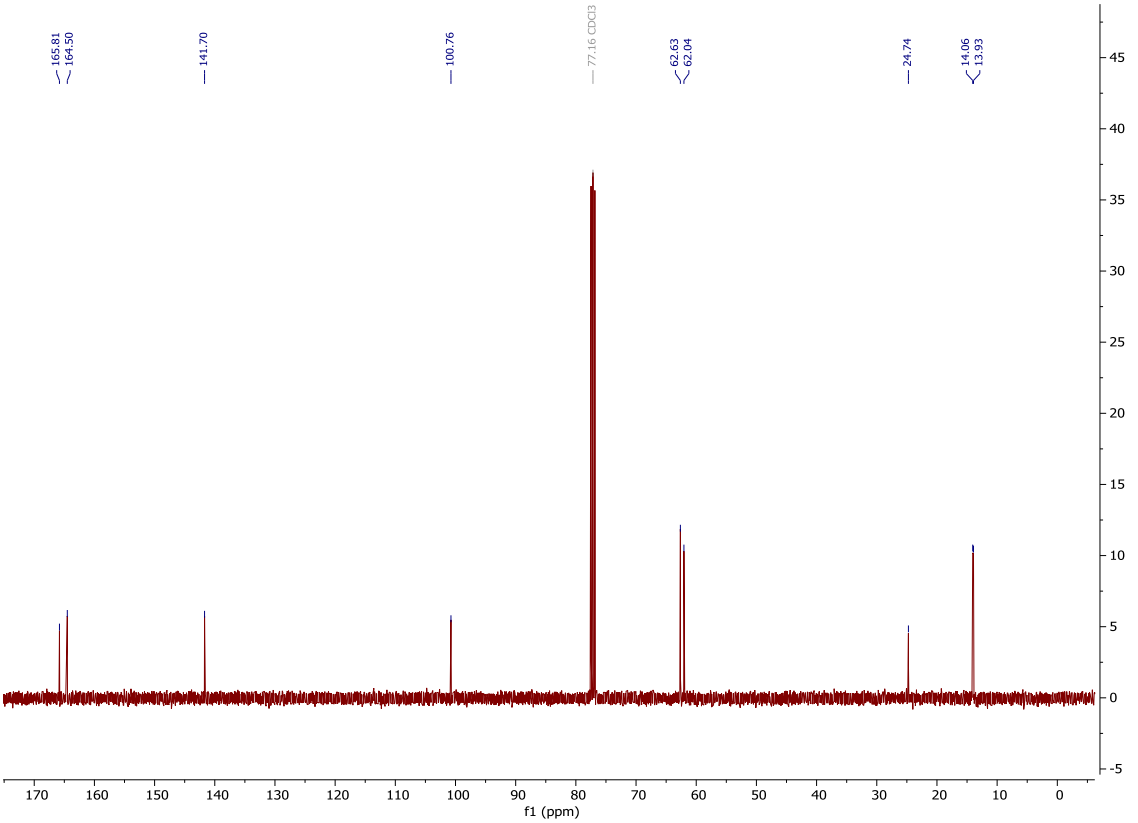
Diethyl 2-((*E*)-oct-1-en-1-yl)-3-methylmaleate S7



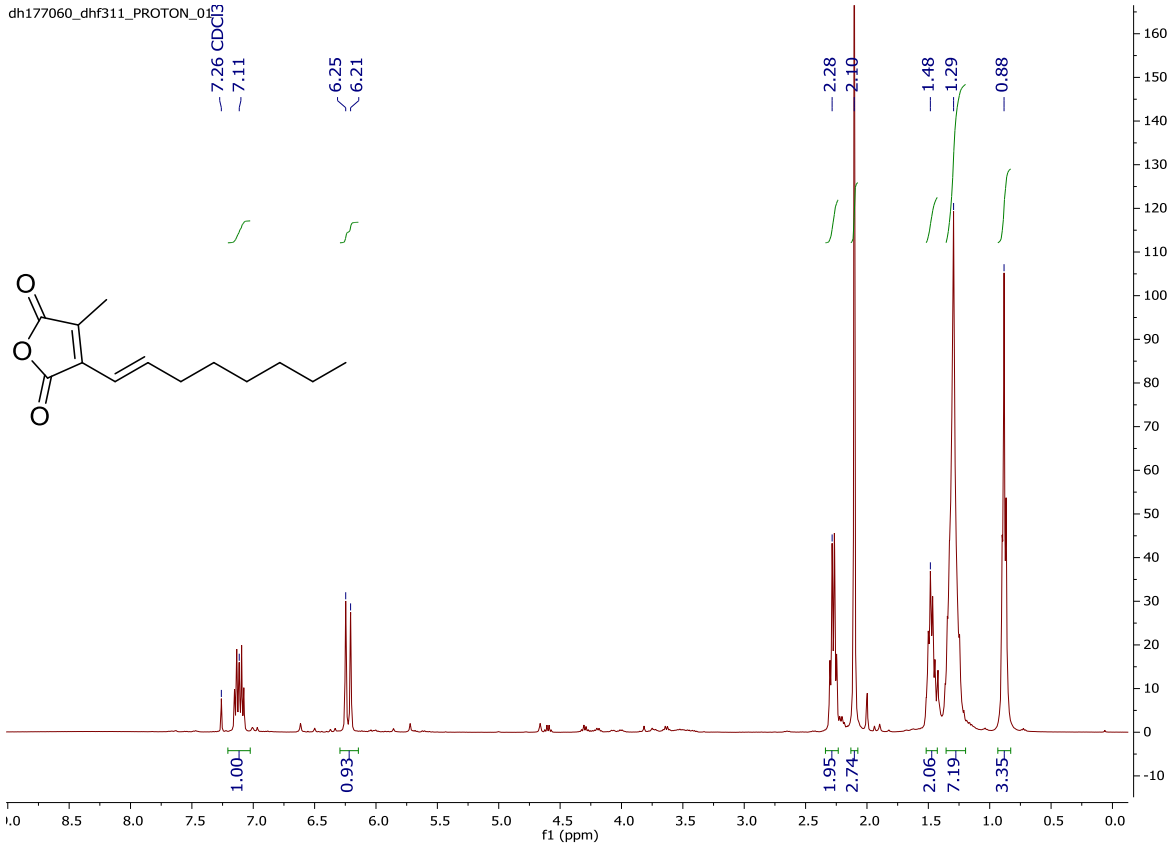


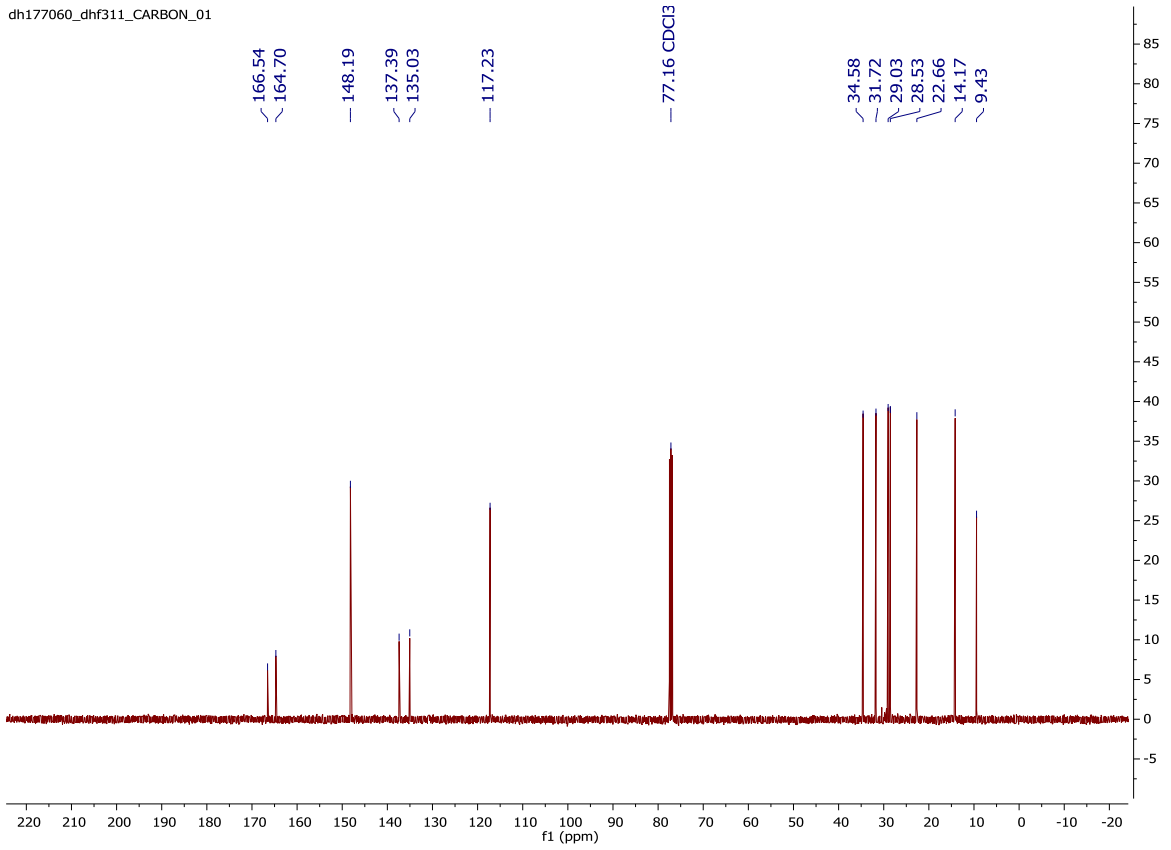
Diethyl 2-iodo-3-methylmaleate 148



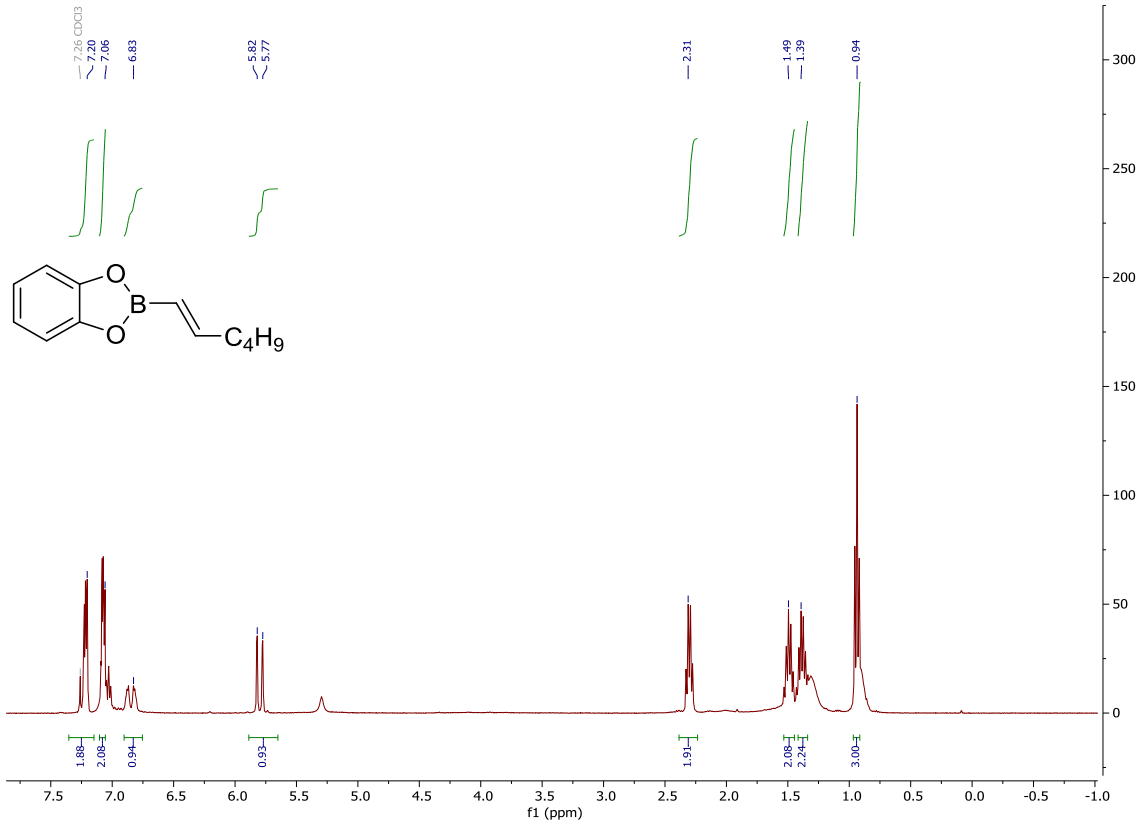


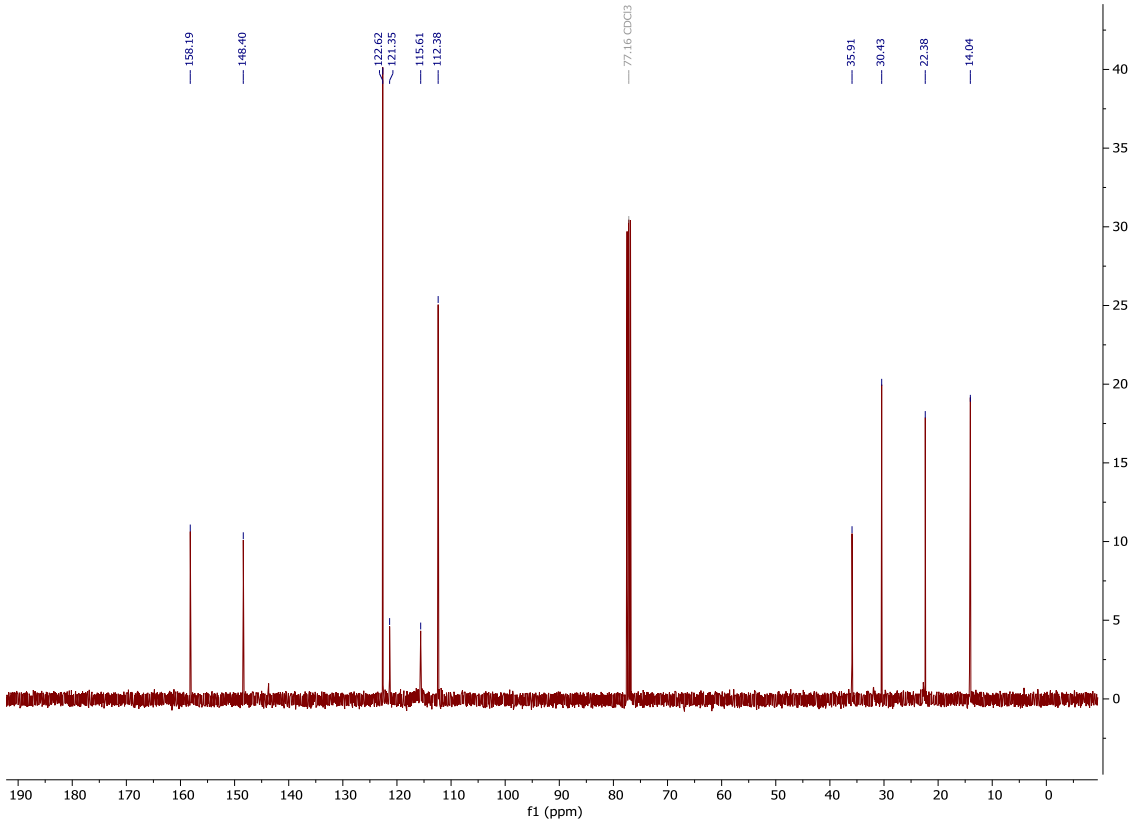
(*E*)-3-Methyl-4-(oct-1-en-1-yl)furan-2,5-dione 145 (Tricladolide D)



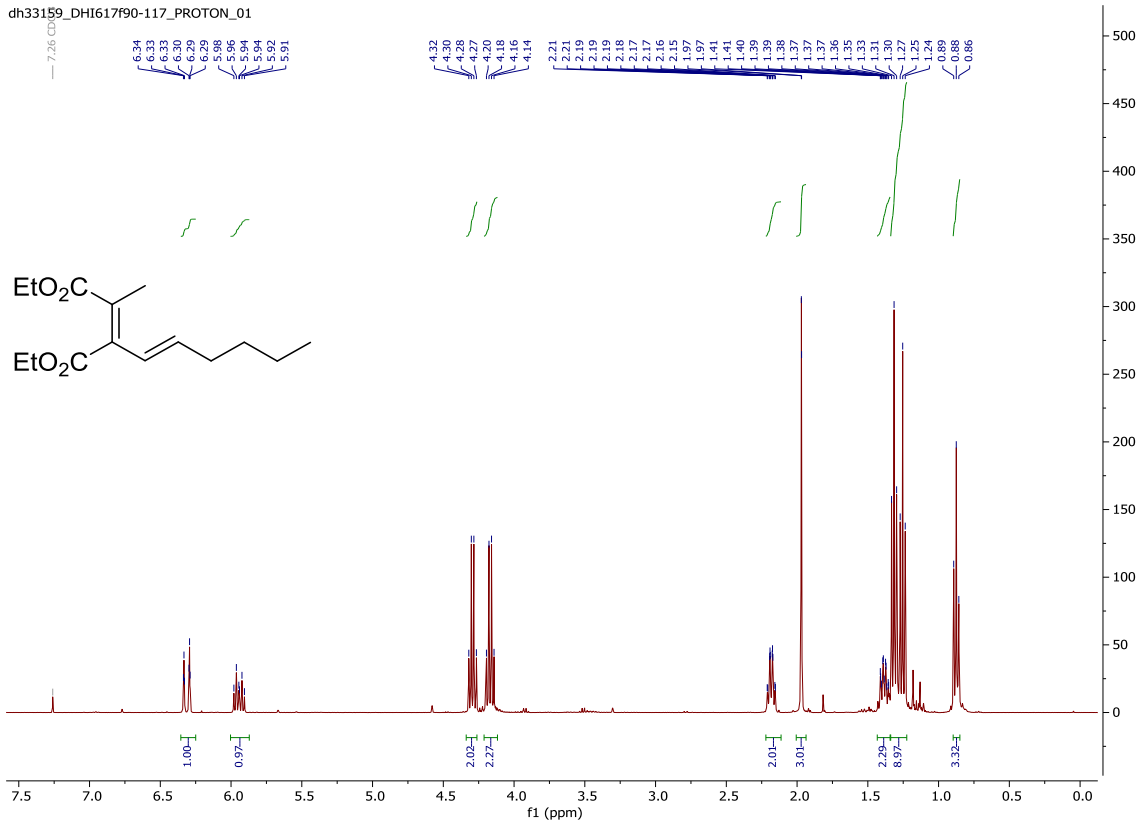


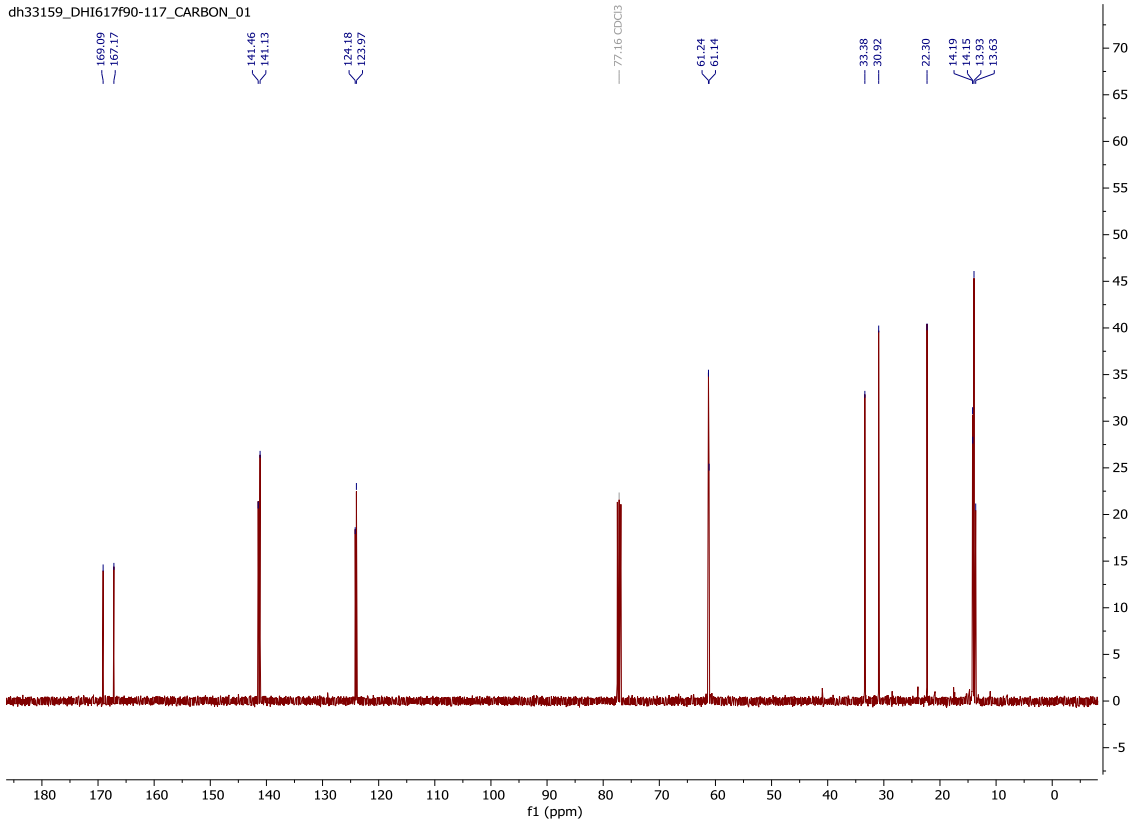
(*E*)-2-(Hex-1-en-1-yl)benzo[d][1,3,2]dioxaborole 150



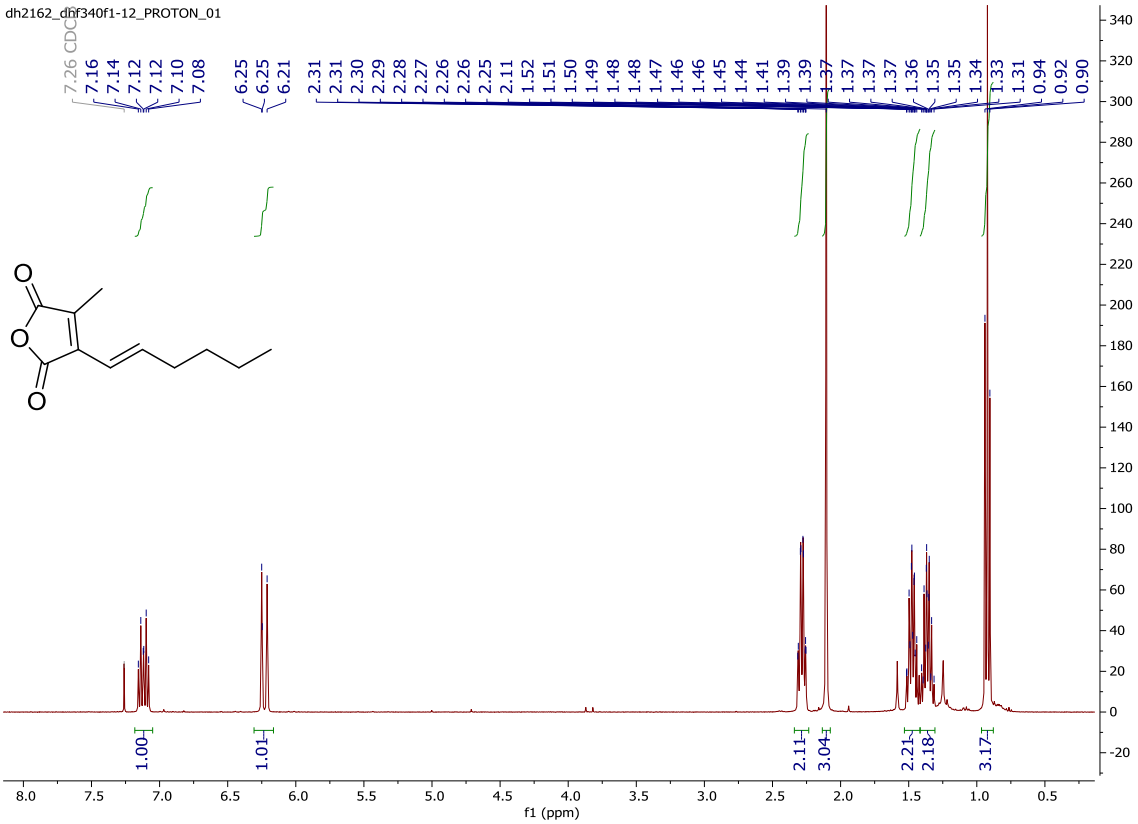


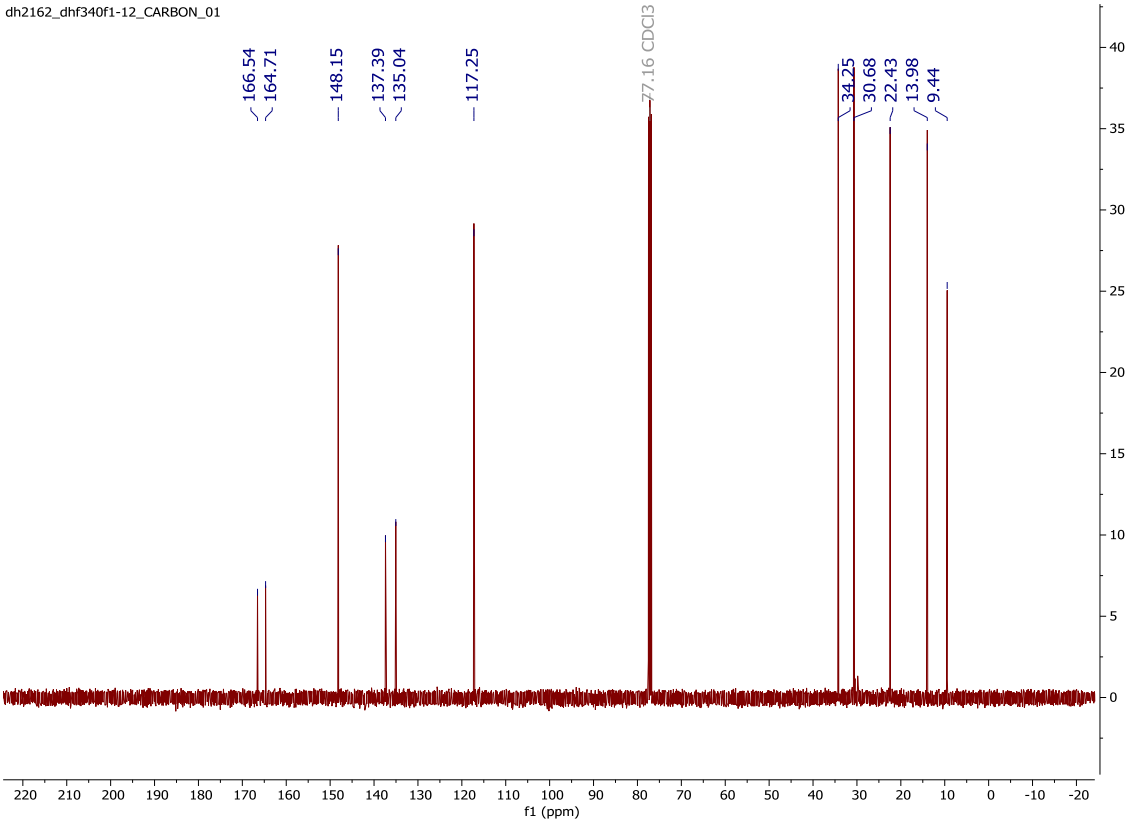
Diethyl 2-((*E*)-hex-1-en-1-yl)-3-methylmaleate 151



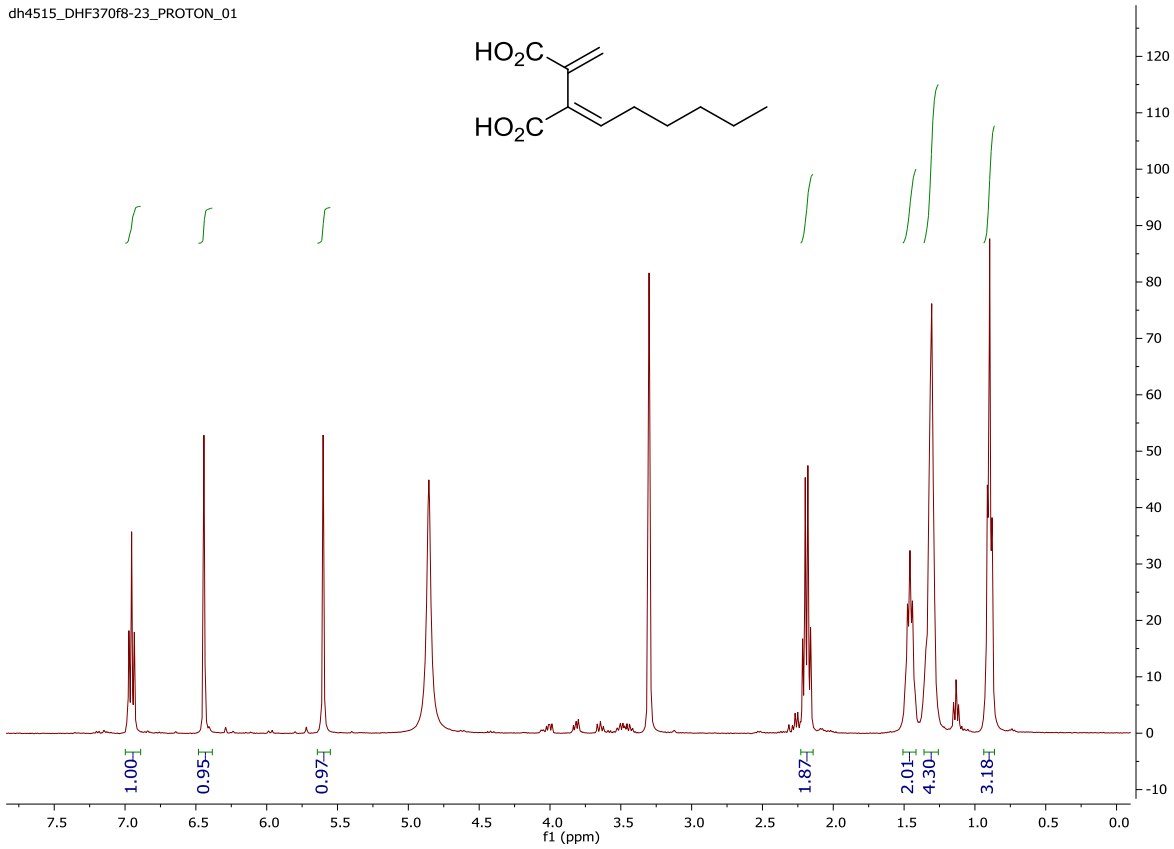


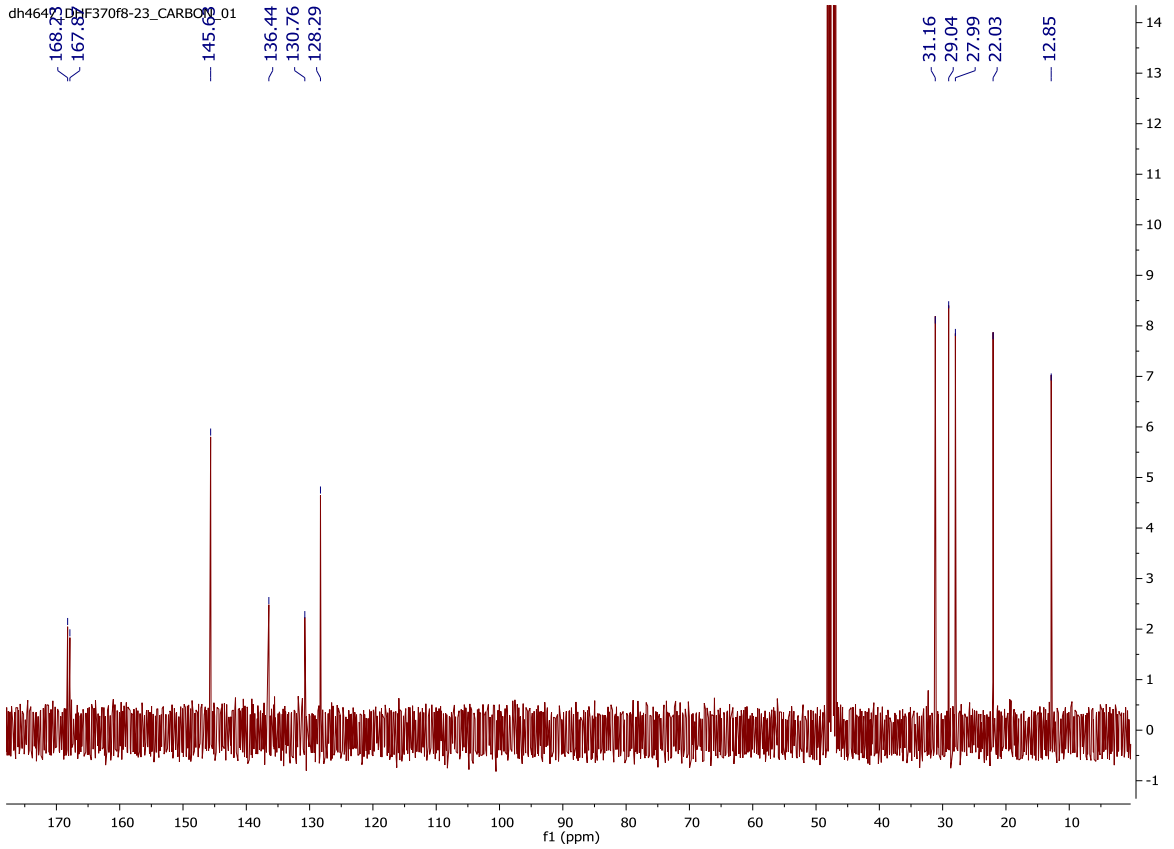
(*E*)-3-Methyl-4-(hex-1-en-1-yl)furan-2,5-dione 146



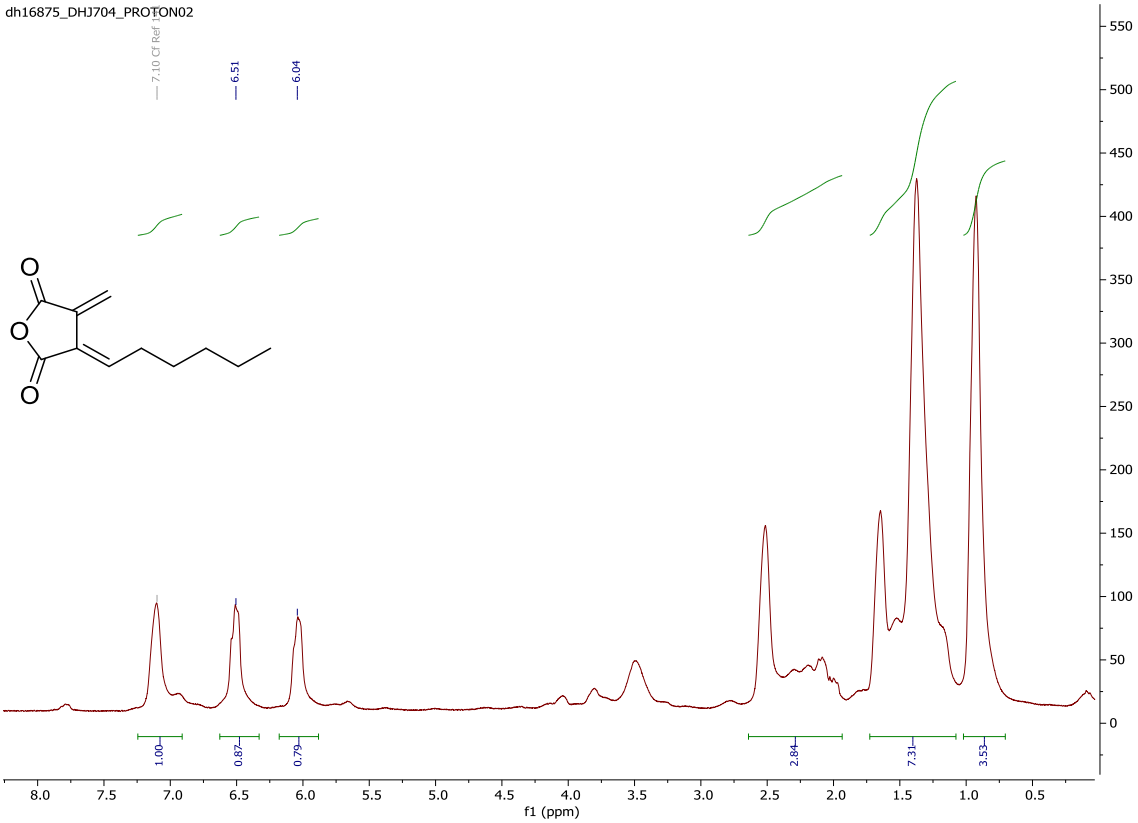


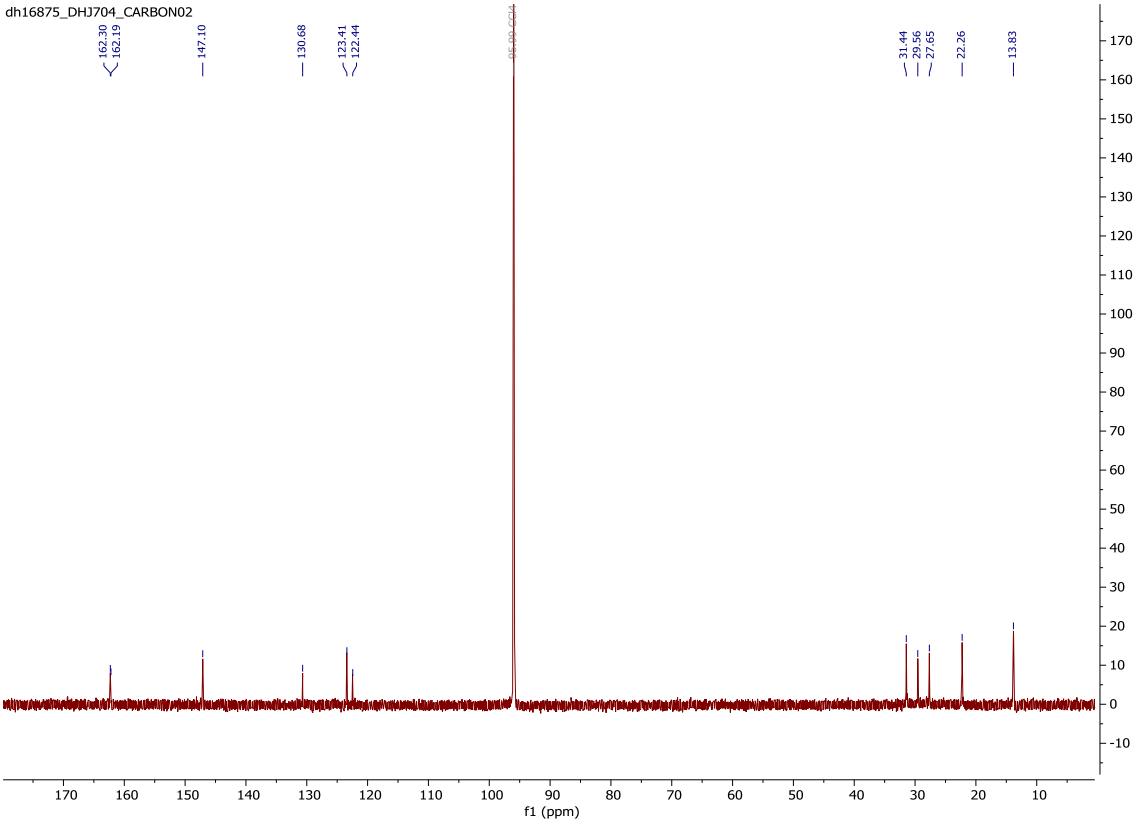
(*E*)-2-hexylidene-3-methylenesuccinic acid 152



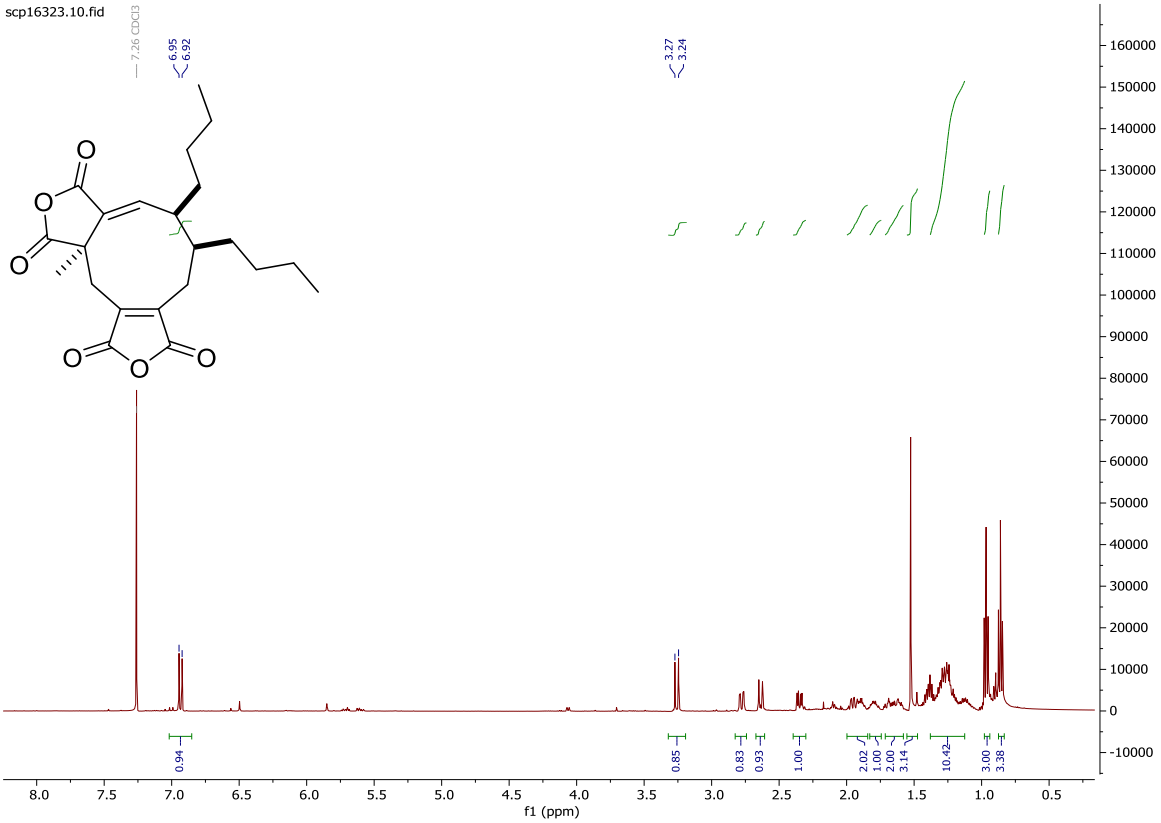


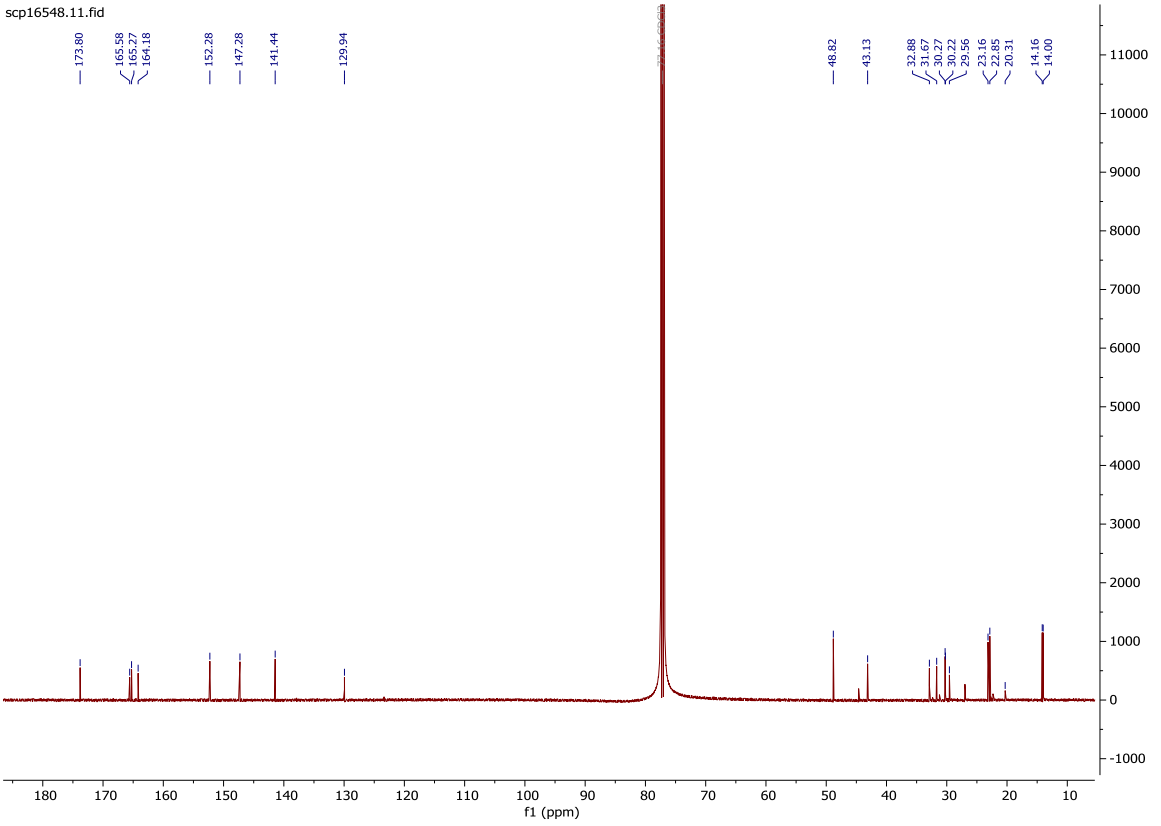
(*E*)-3-Hexylidene-4-methylenedihydrofuran-2,5-dione 154



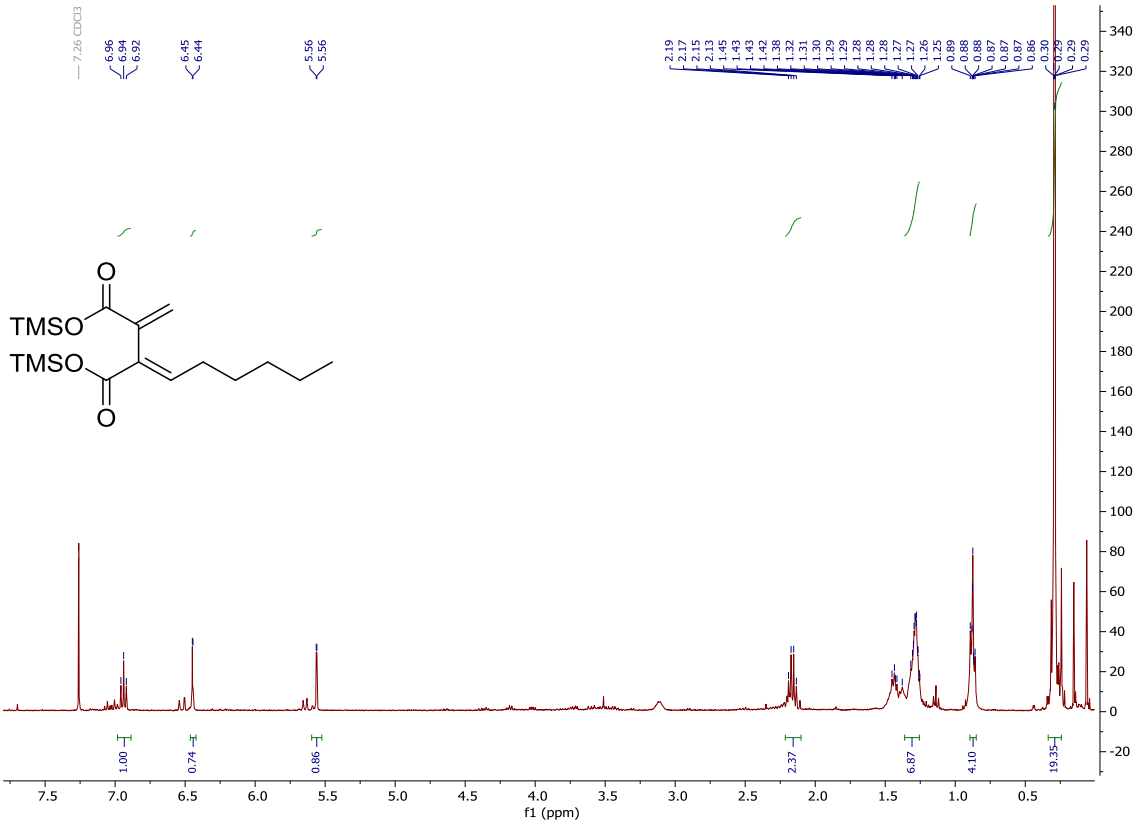


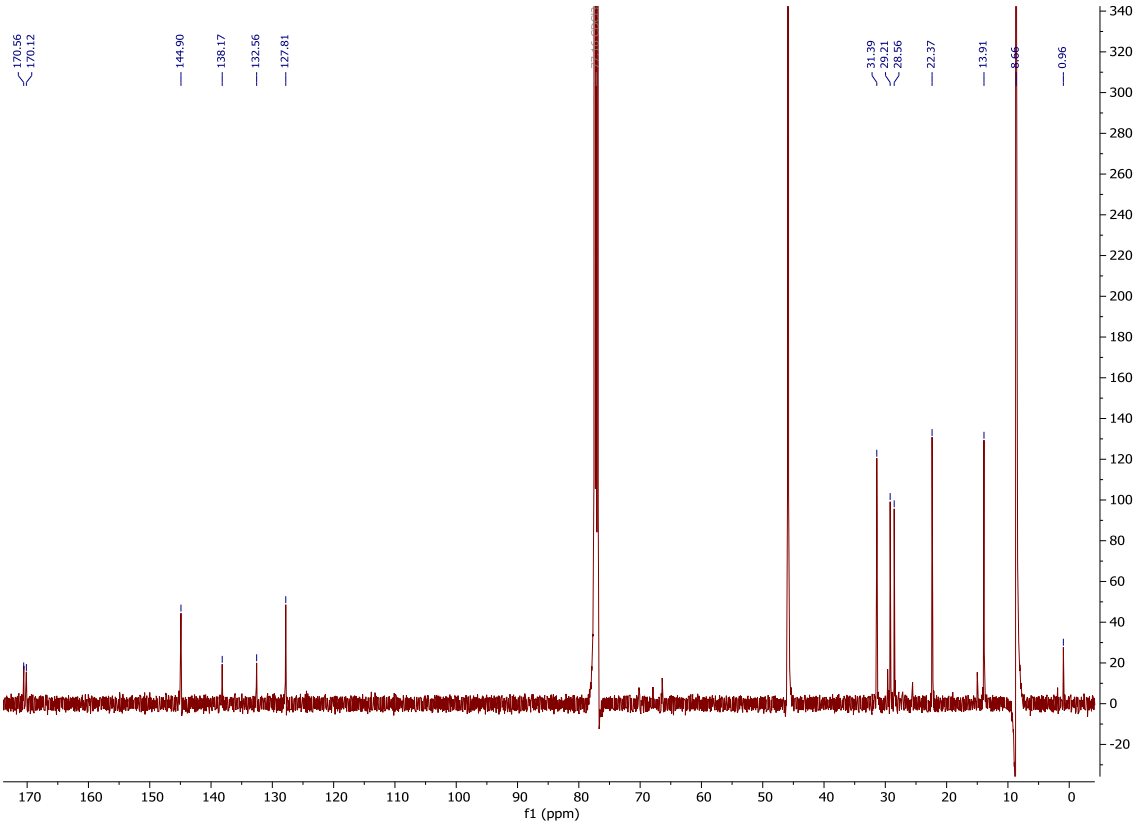
***Iso*-glaucanic acid analogue - (5*S*,6*S*,10a*R*,*E*)-5,6-dibutyl-10a-methyl-5,6,10a,11-tetrahydro-1H-cyclonona[1,2-c:4,5-c']difuran-1,3,8,10(4H)-tetraone 155**



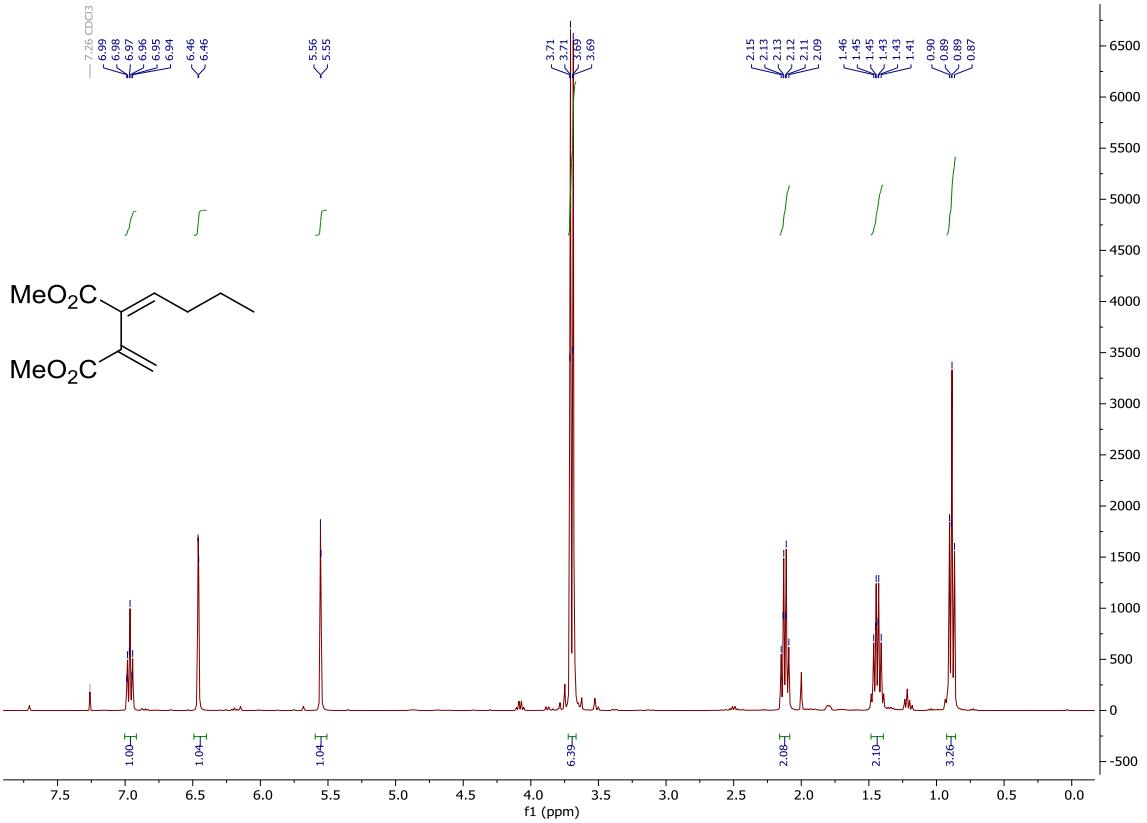


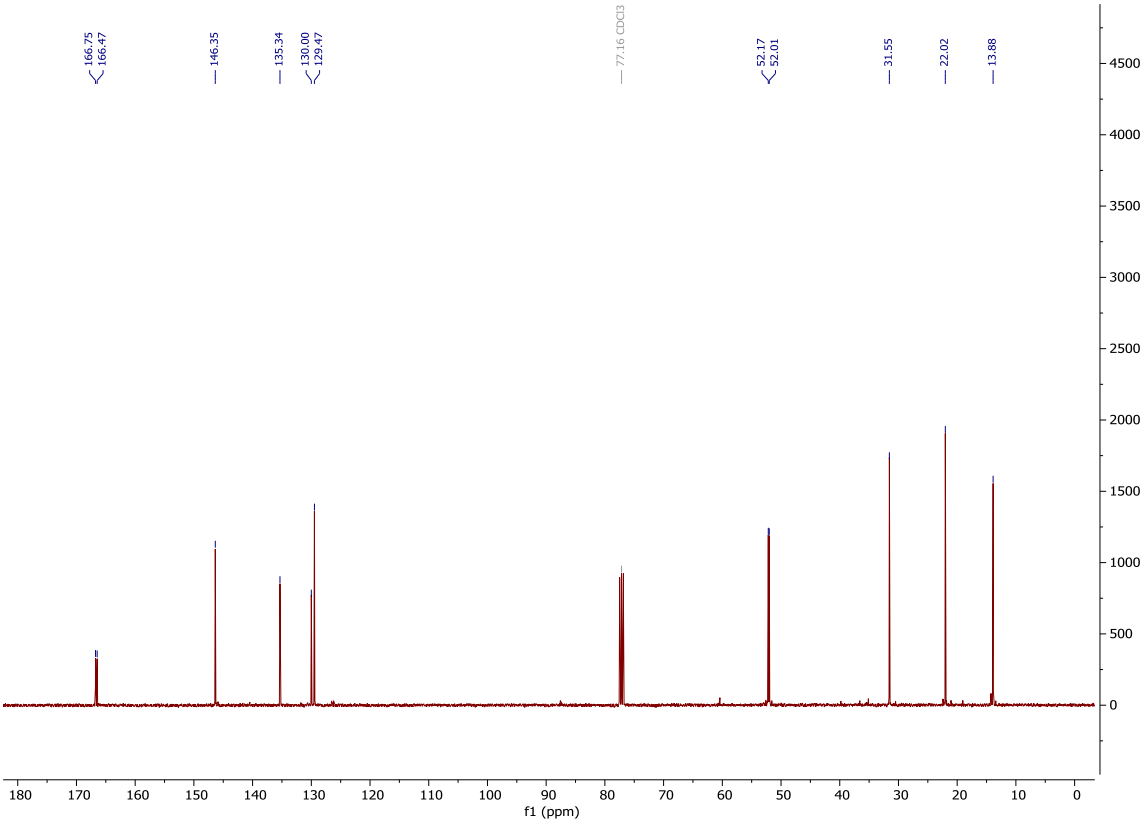
Bis(trimethylsilyl) (*E*)-2-hexylidene-3-methylenesuccinate 158



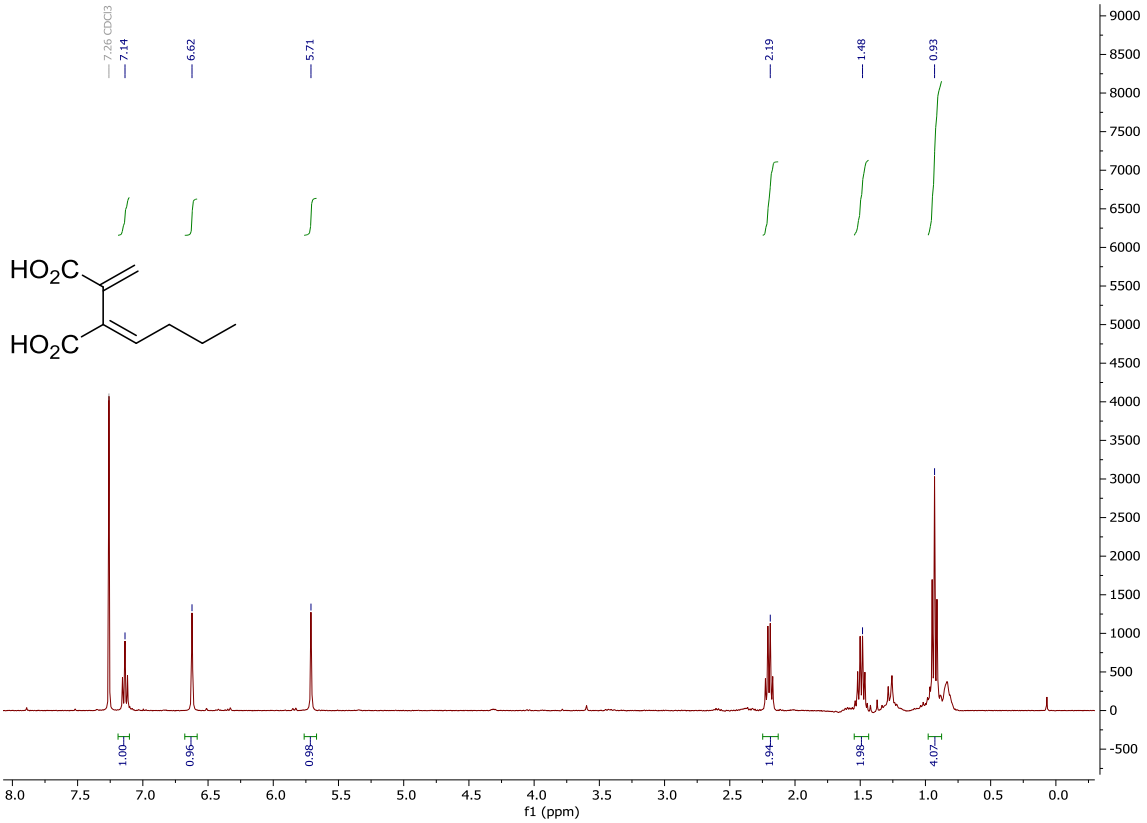


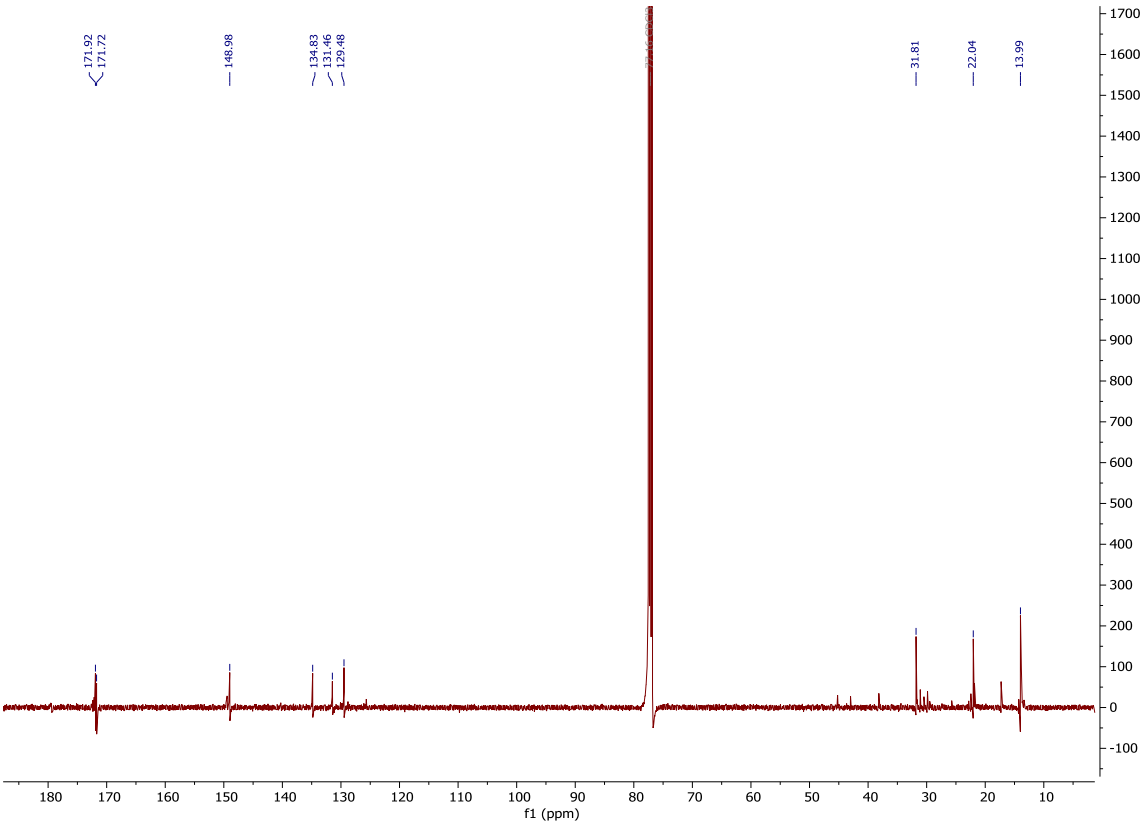
Dimethyl (*E*)-2-butylidene-3-methylenesuccinate 166



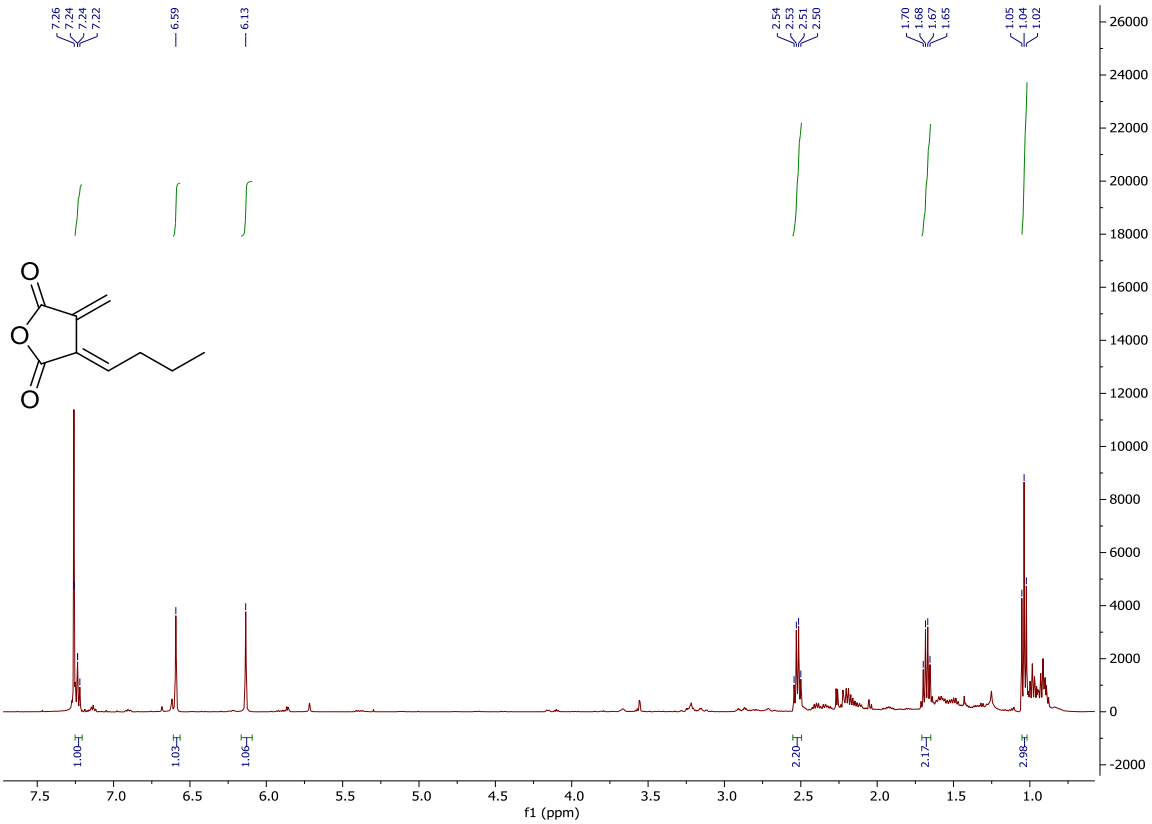


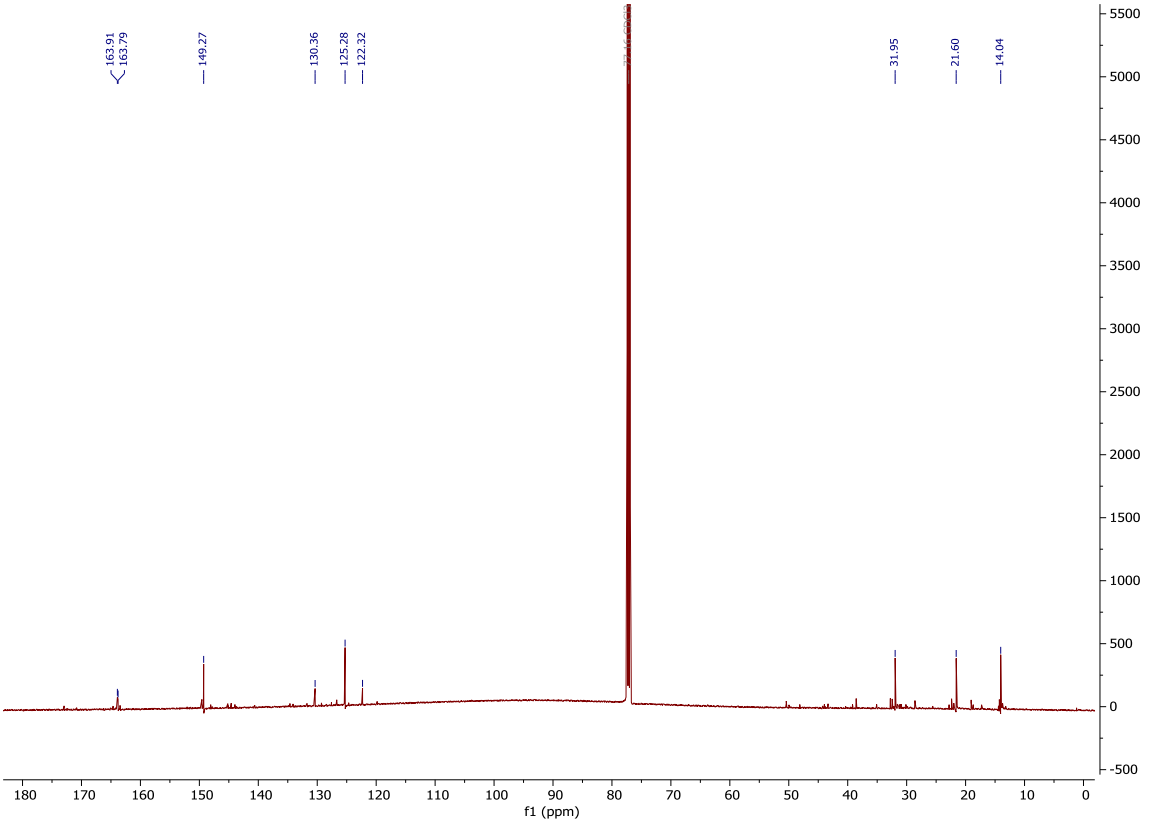
(*E*)-2-Butylidene-3-methylenesuccinic acid 167



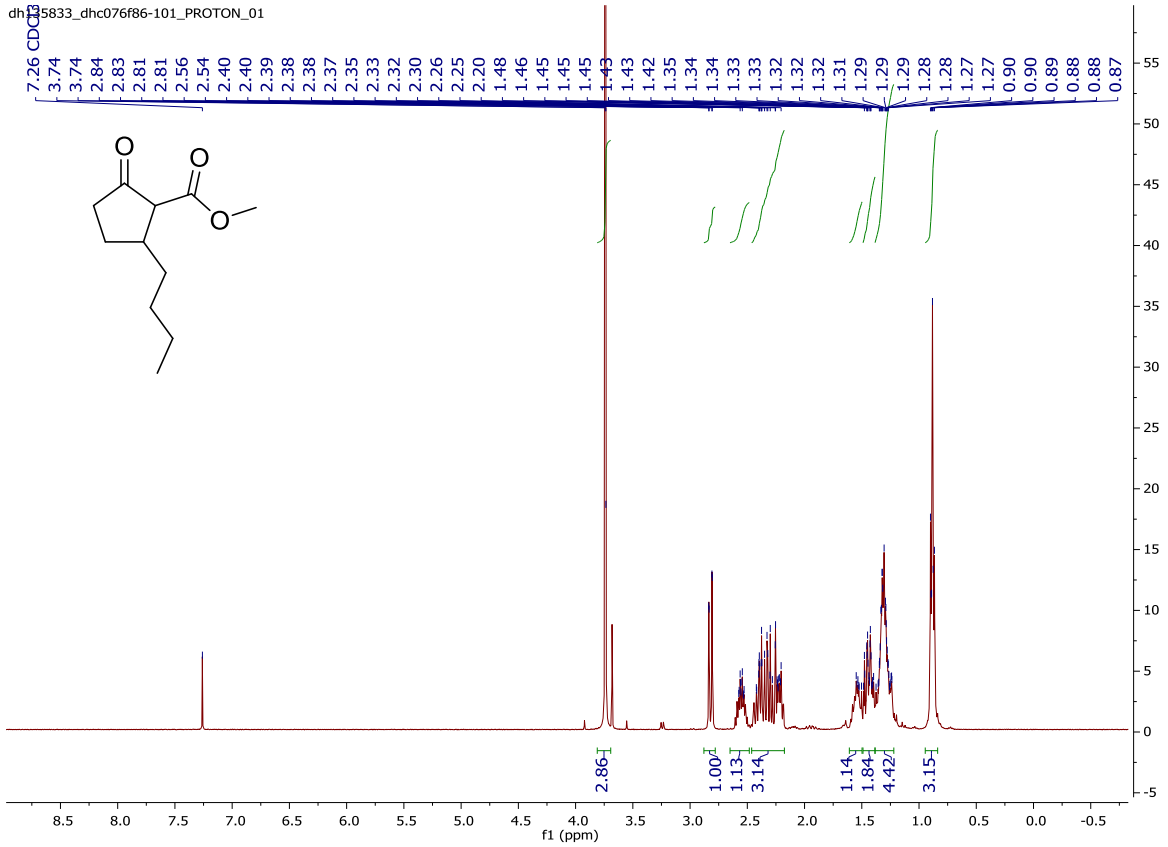


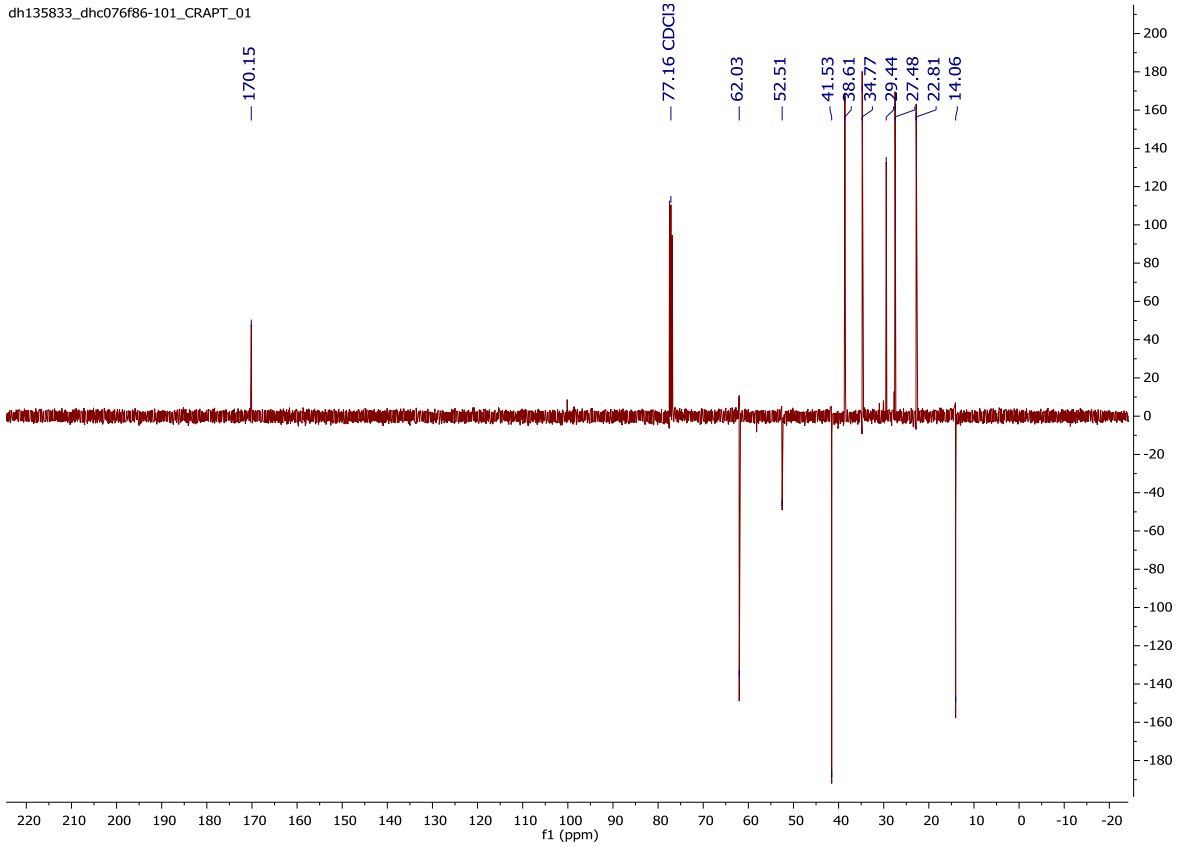
(*E*)-3-Butylidene-4-methylenedihydrofuran-2,5-dione 139



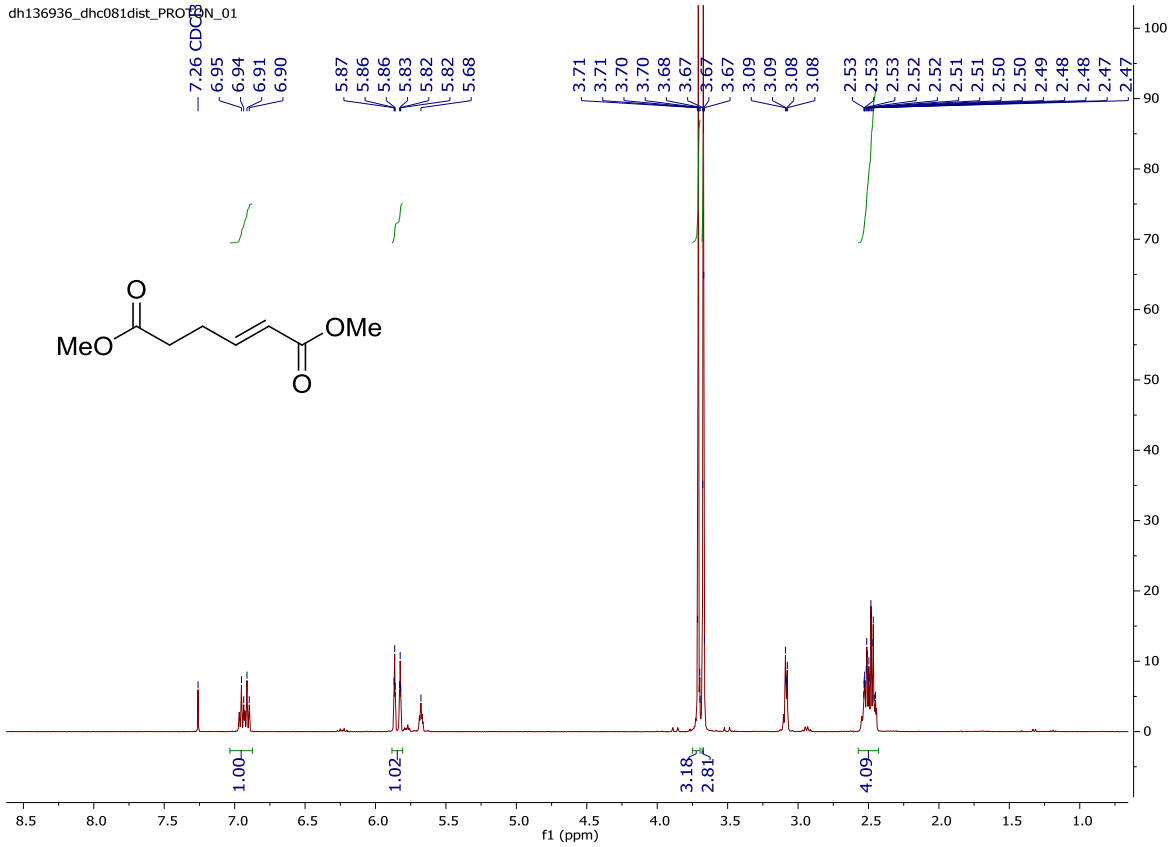


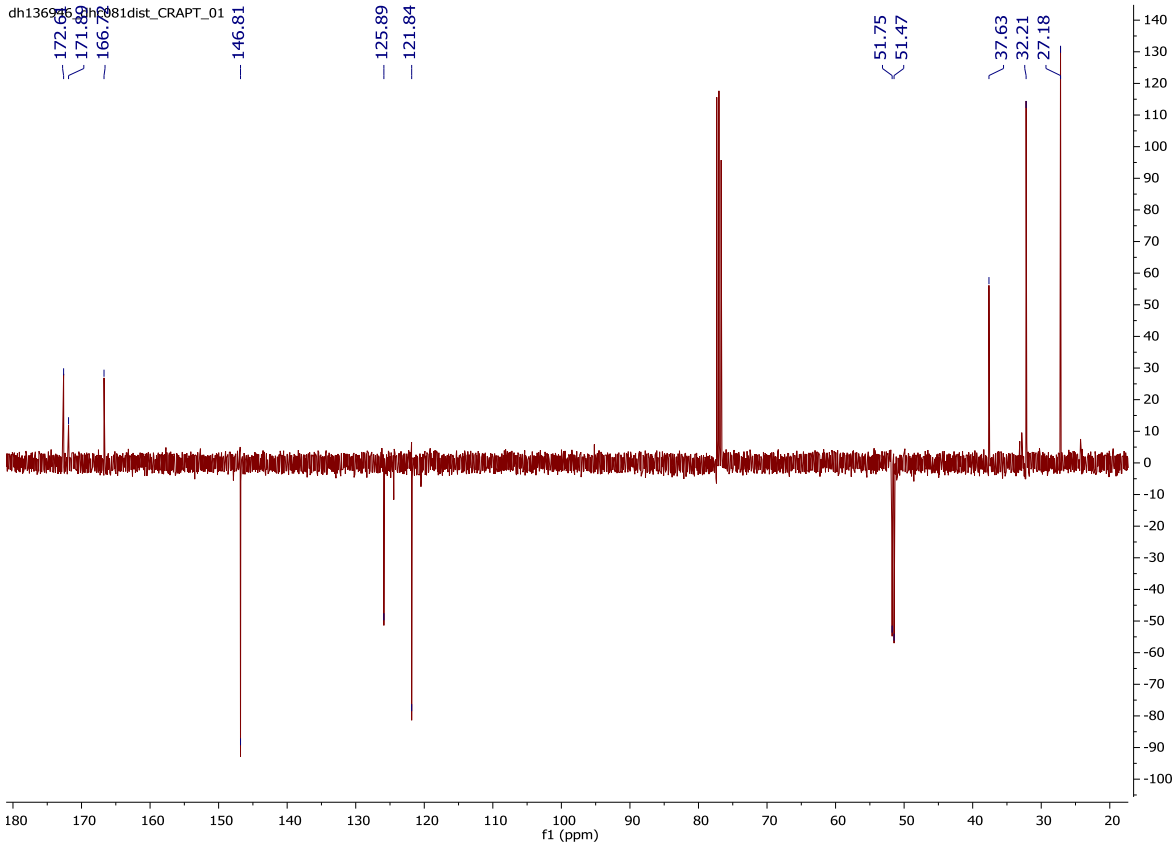
Methyl 2-butyl-5-oxocyclopentane-1-carboxylate 193





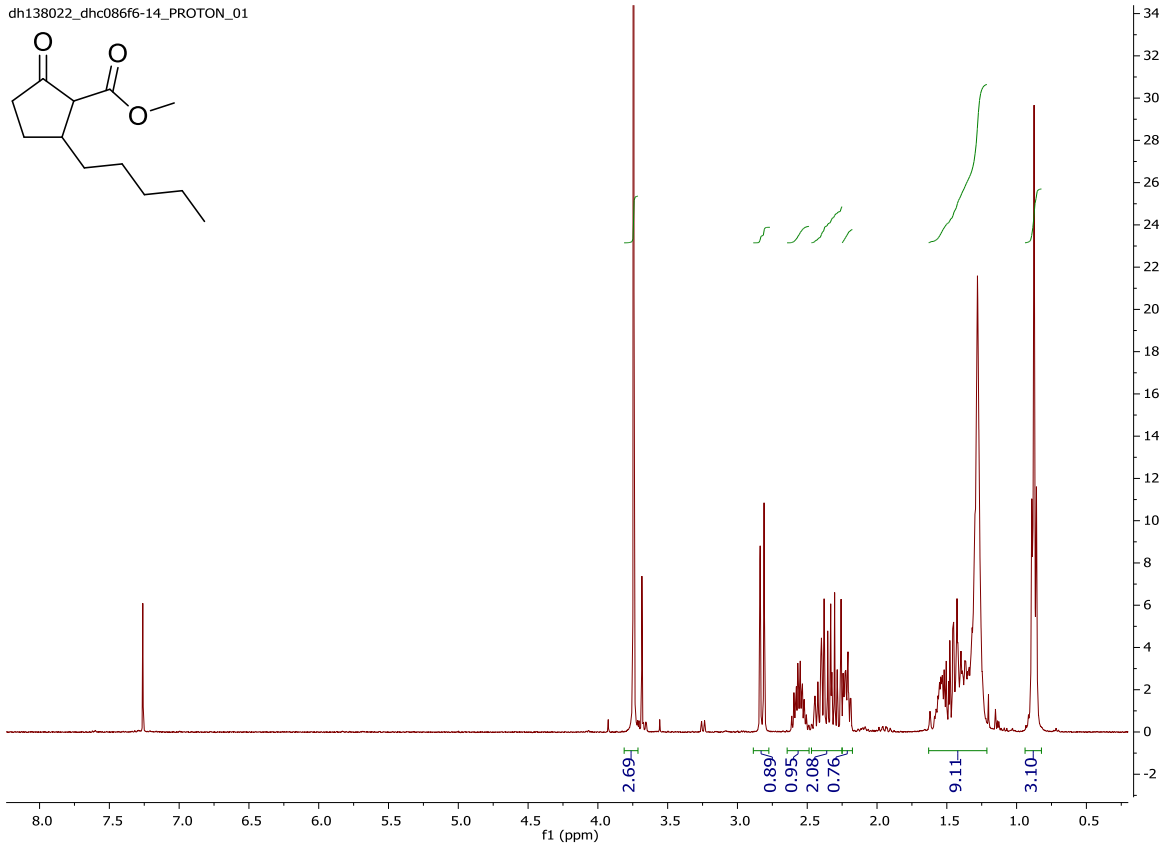
Dimethyl (*E*)-hex-2-enedioate 195

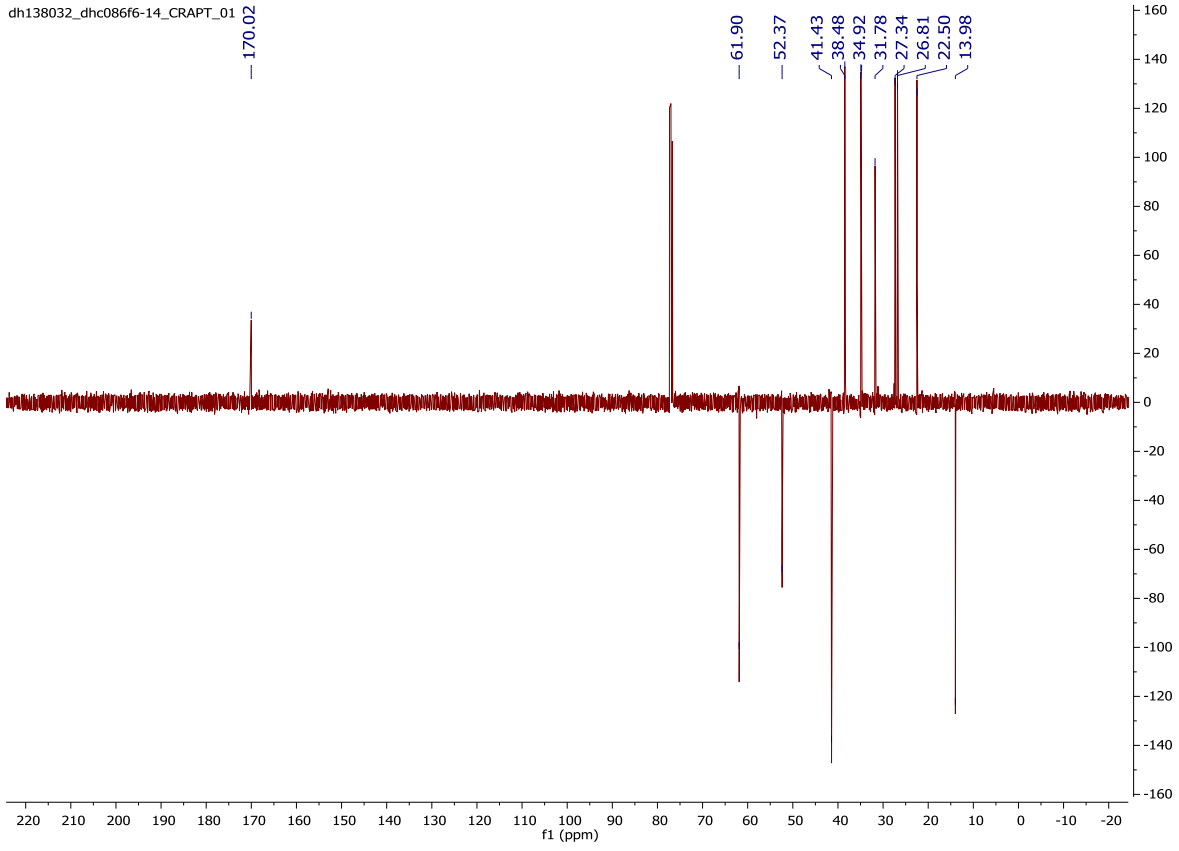




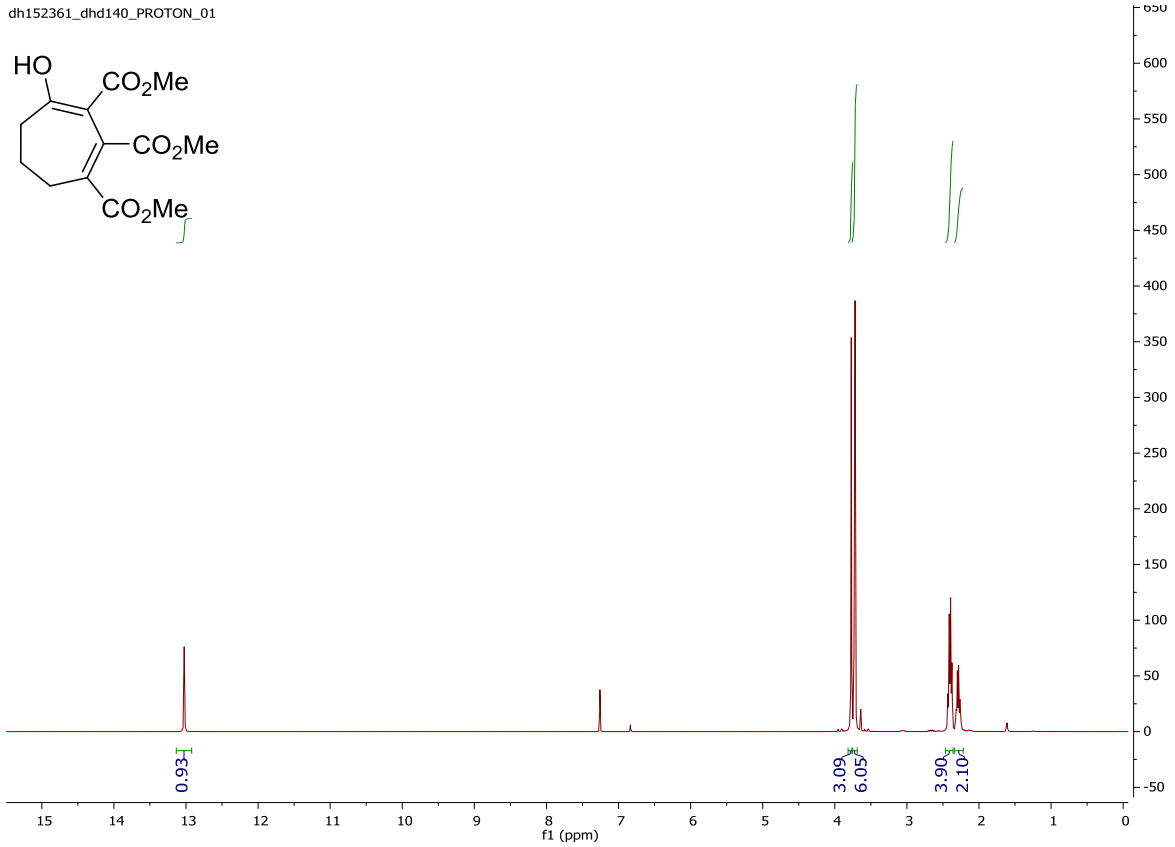
(^{13}C Peaks are identified for both compounds present)

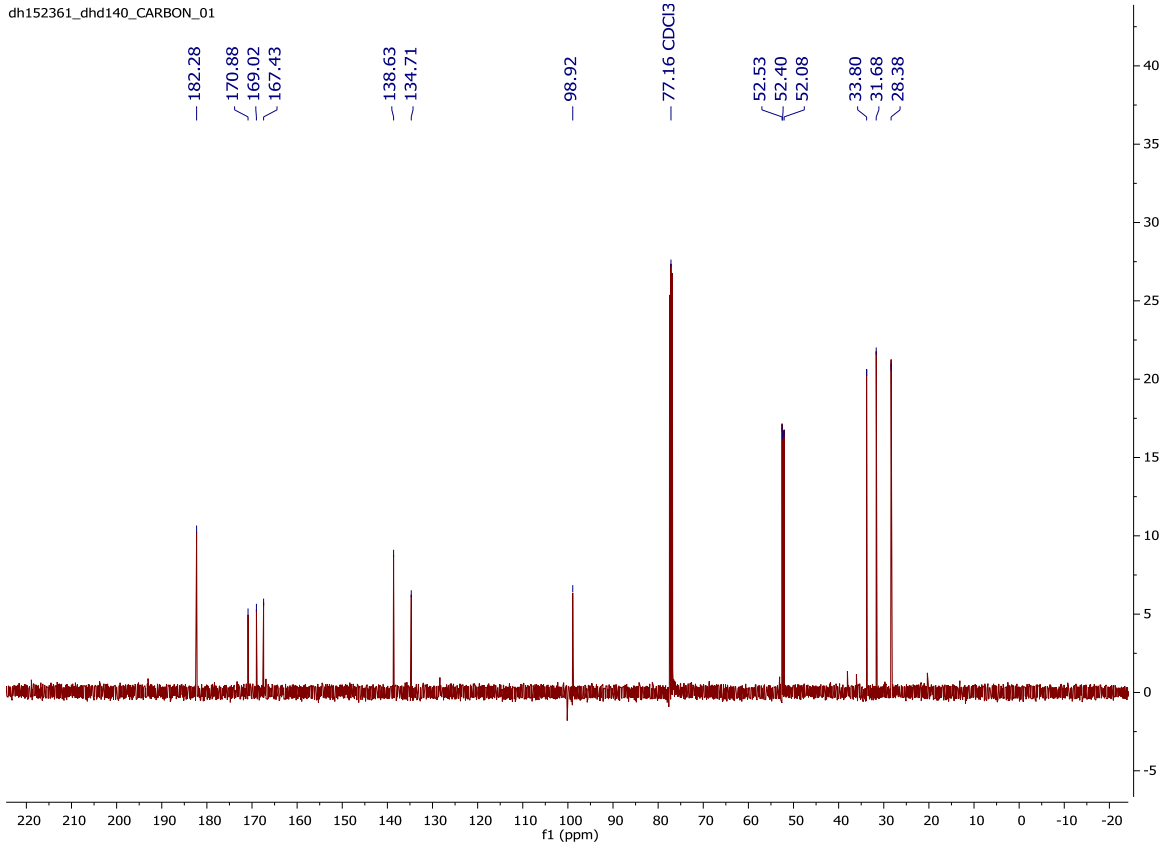
Methyl 2-oxo-5-pentylcyclopentane-1-carboxylate 196



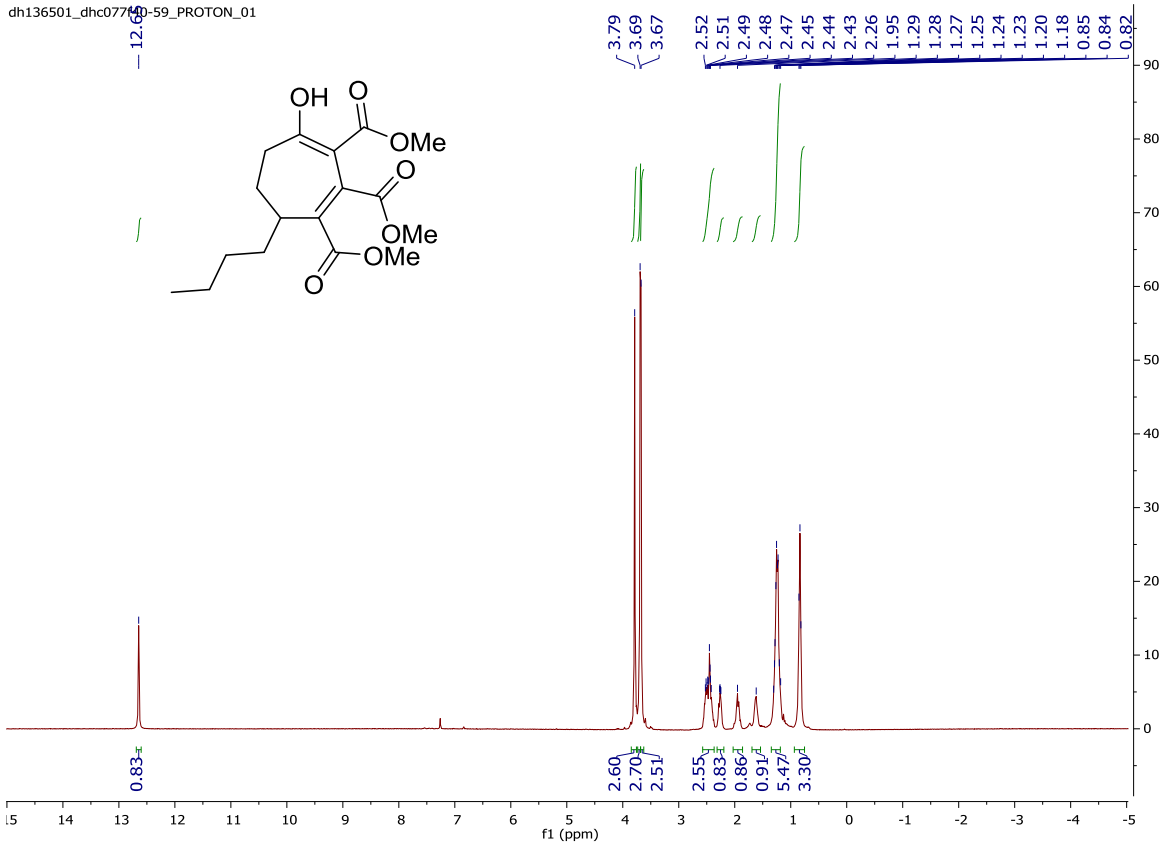


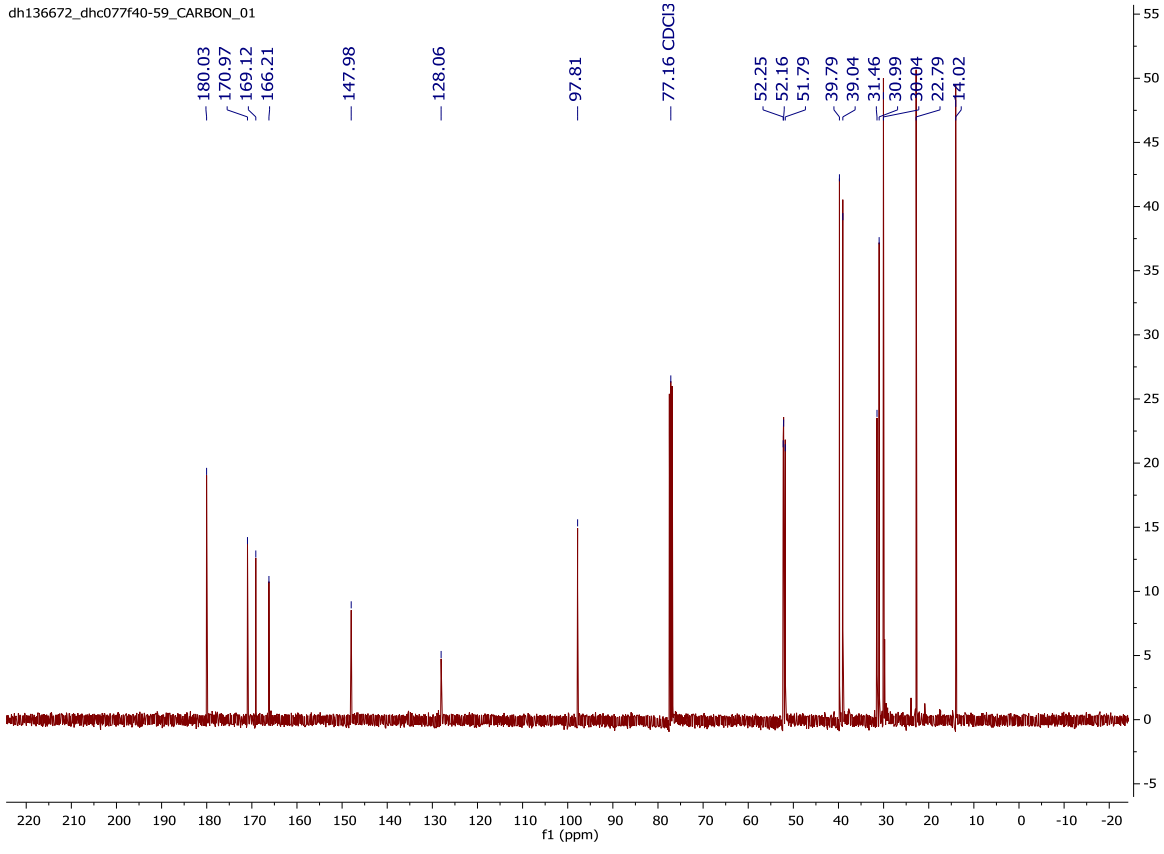
Trimethyl 4-hydroxycyclohepta-1,3-diene-1,2,3-tricarboxylate 198



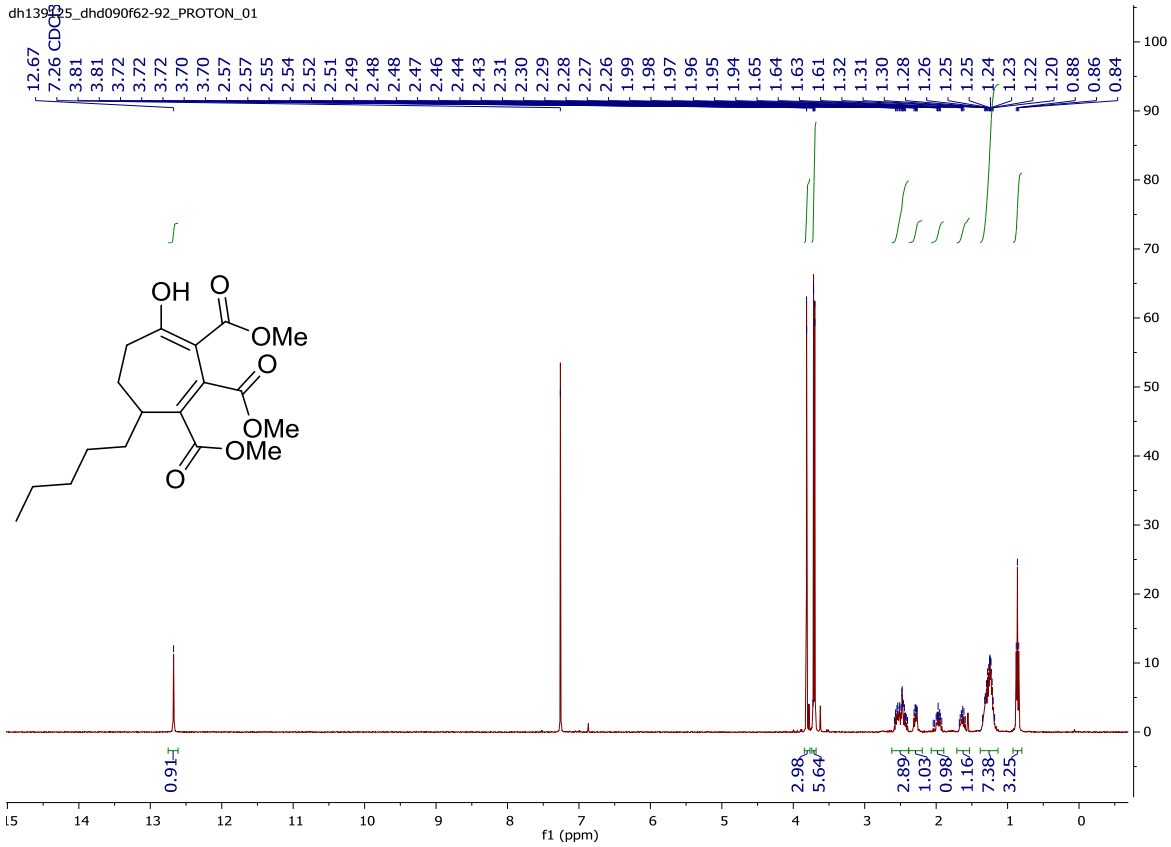


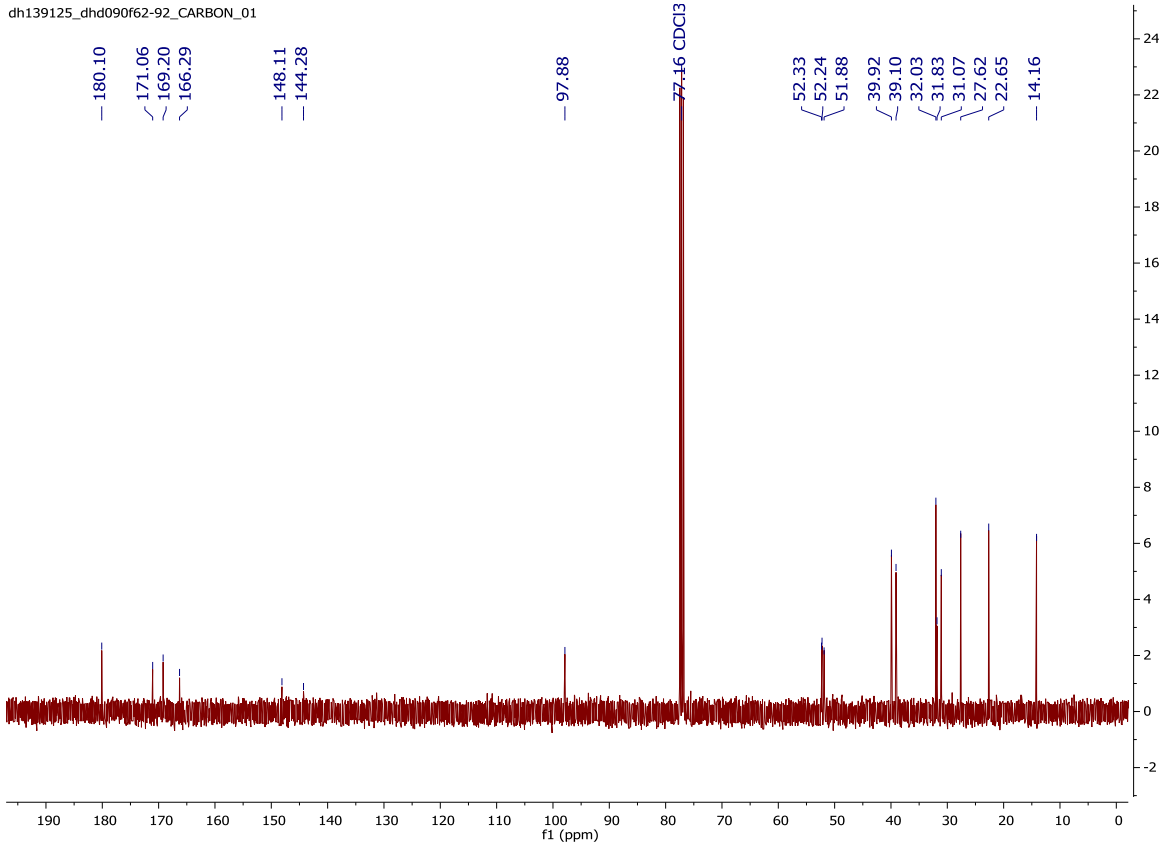
Trimethyl 7-butyl-4-hydroxycyclohepta-1,3-diene-1,2,3-tricarboxylate 199



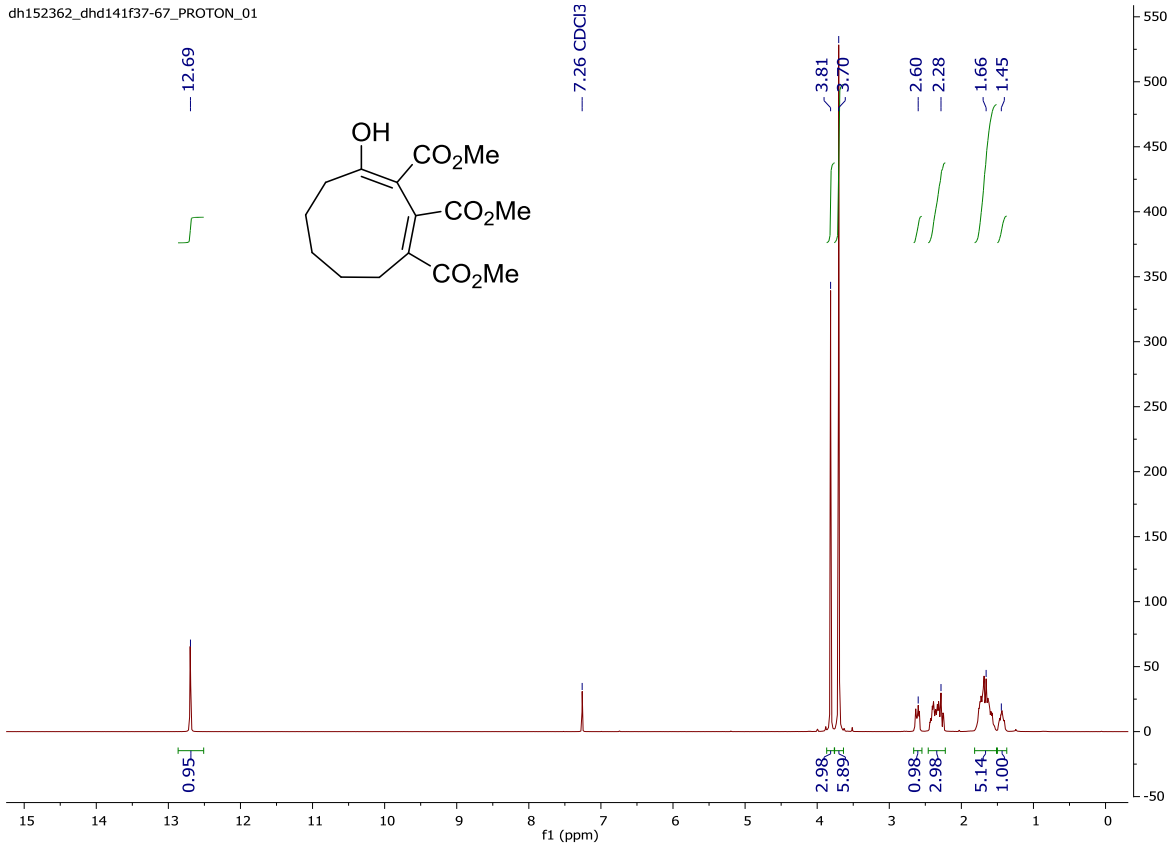


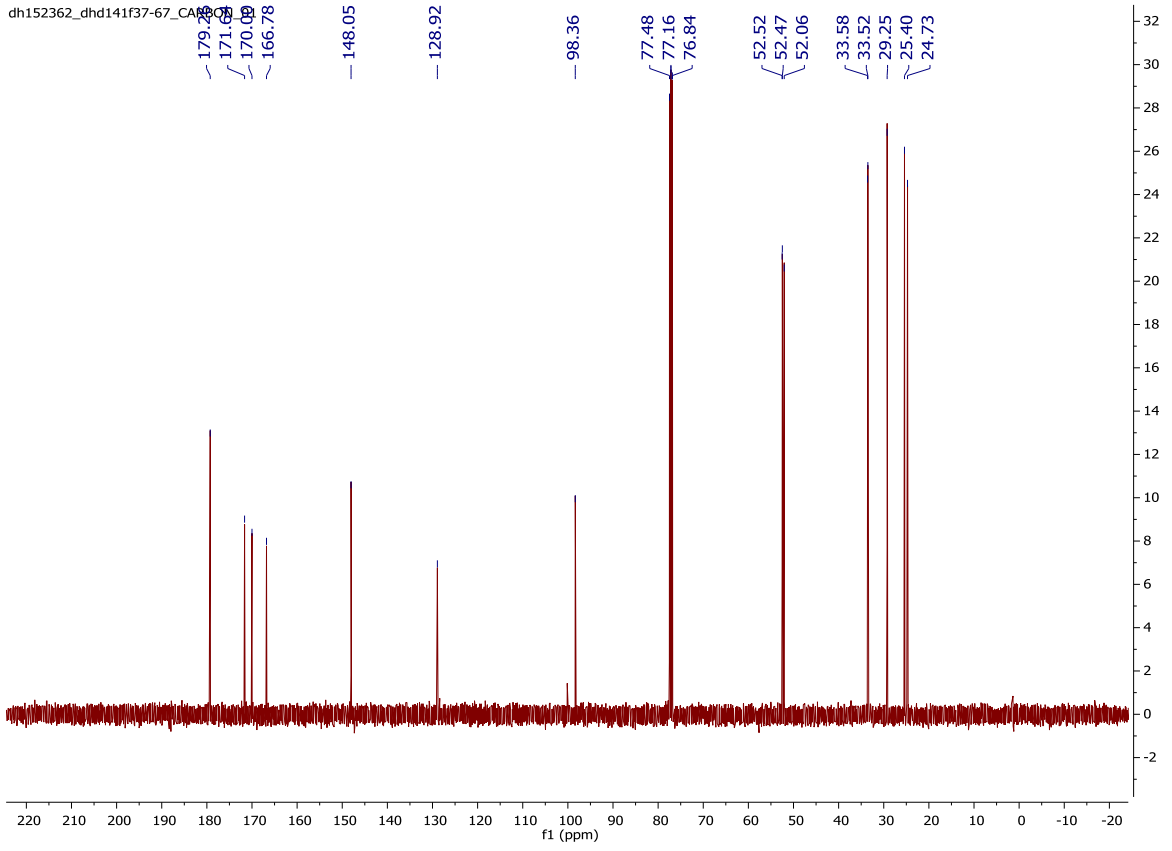
Trimethyl 4-hydroxy-7-pentylcyclohepta-1,3-diene-1,2,3-tricarboxylate 200



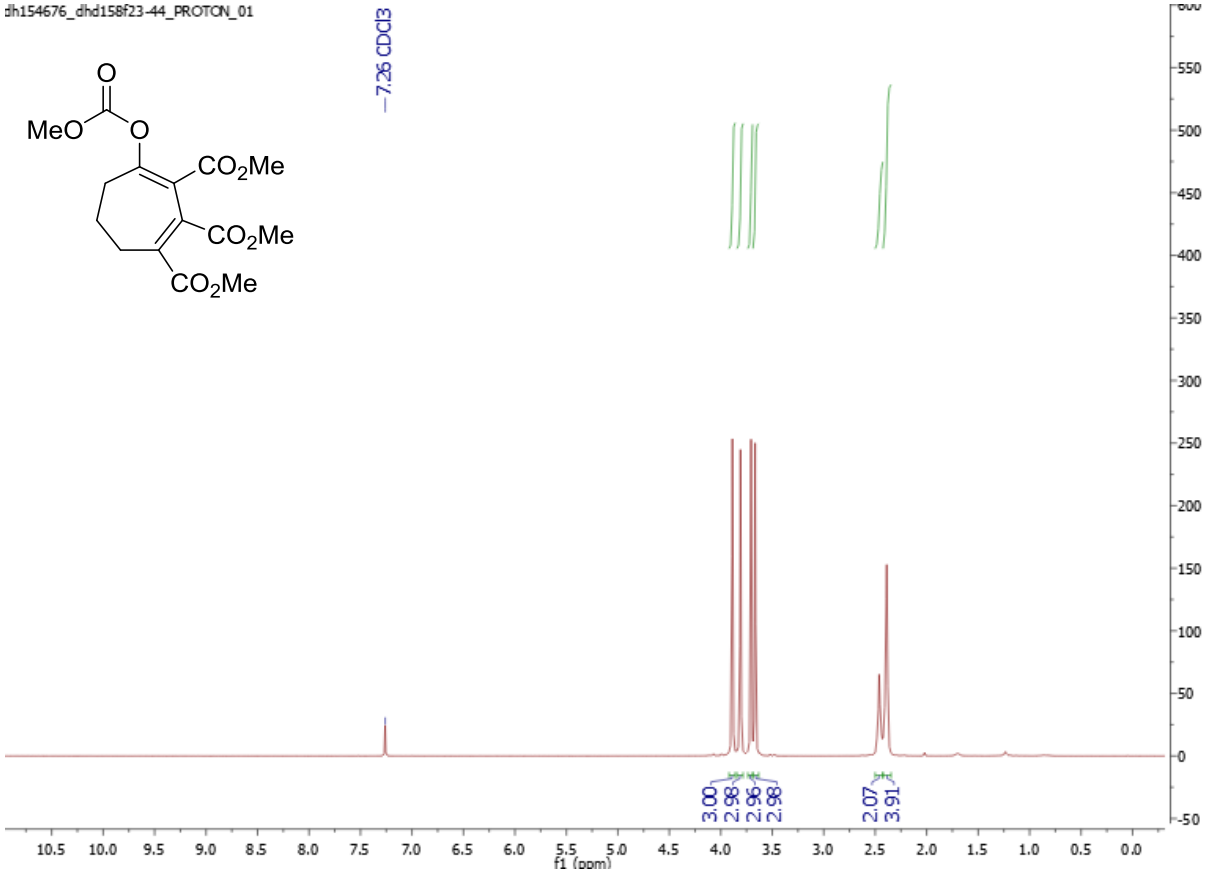


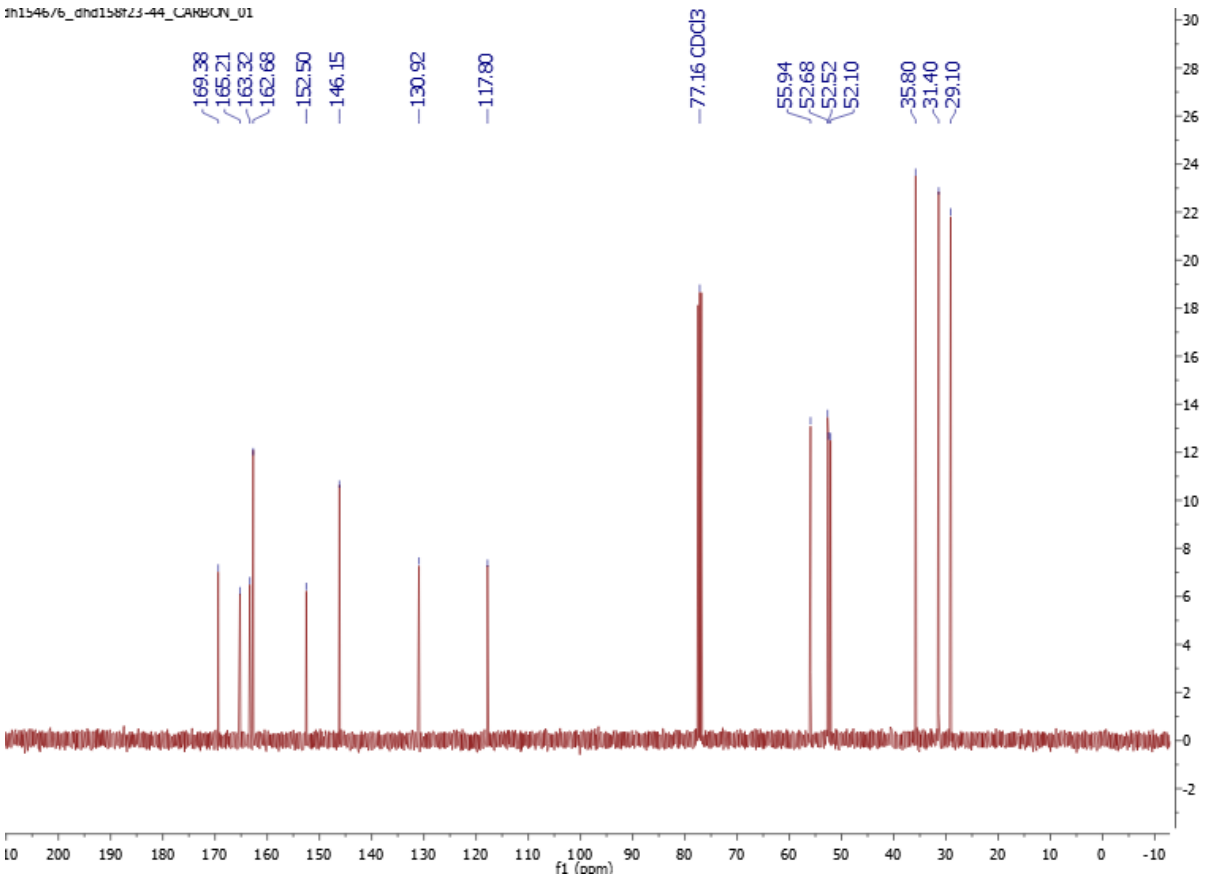
Trimethyl 4-hydroxycyclonona-1,3-diene-1,2,3-tricarboxylate 201



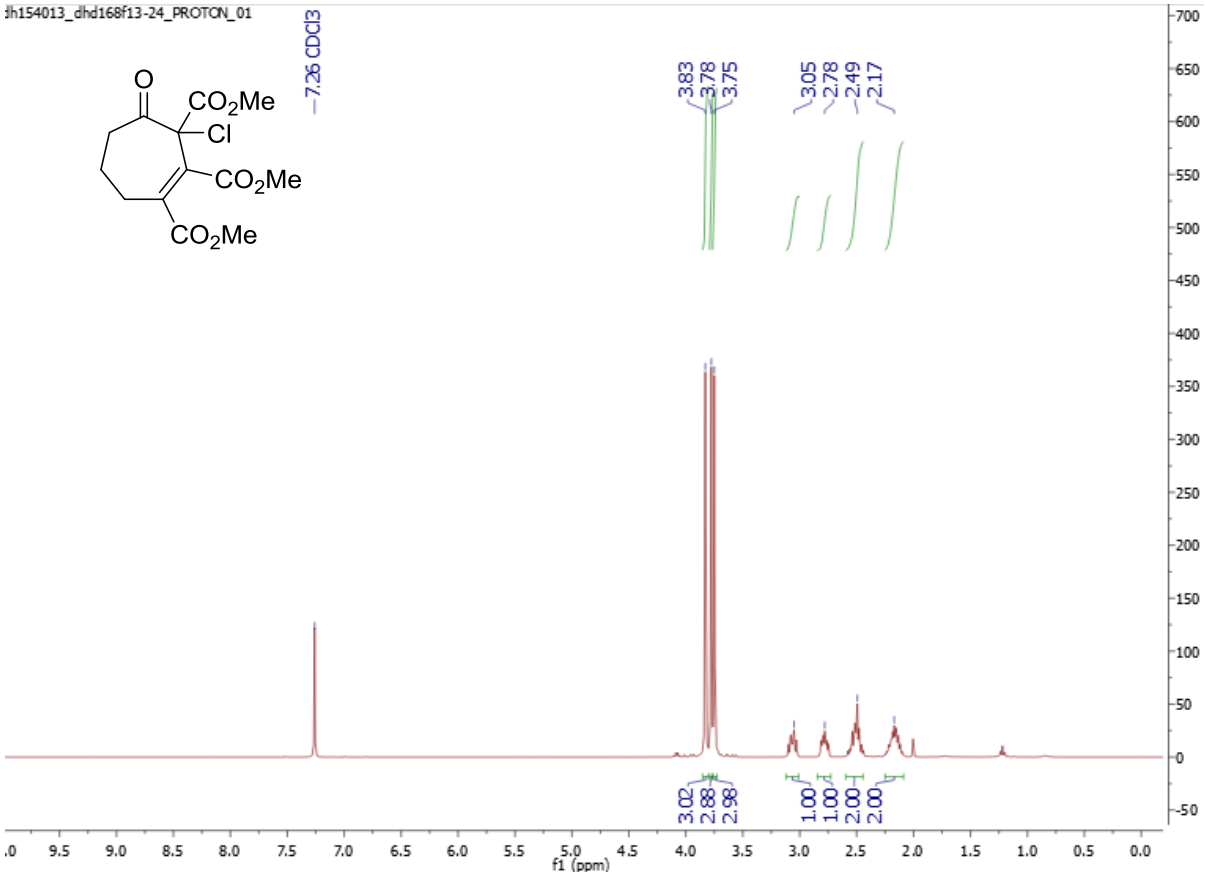


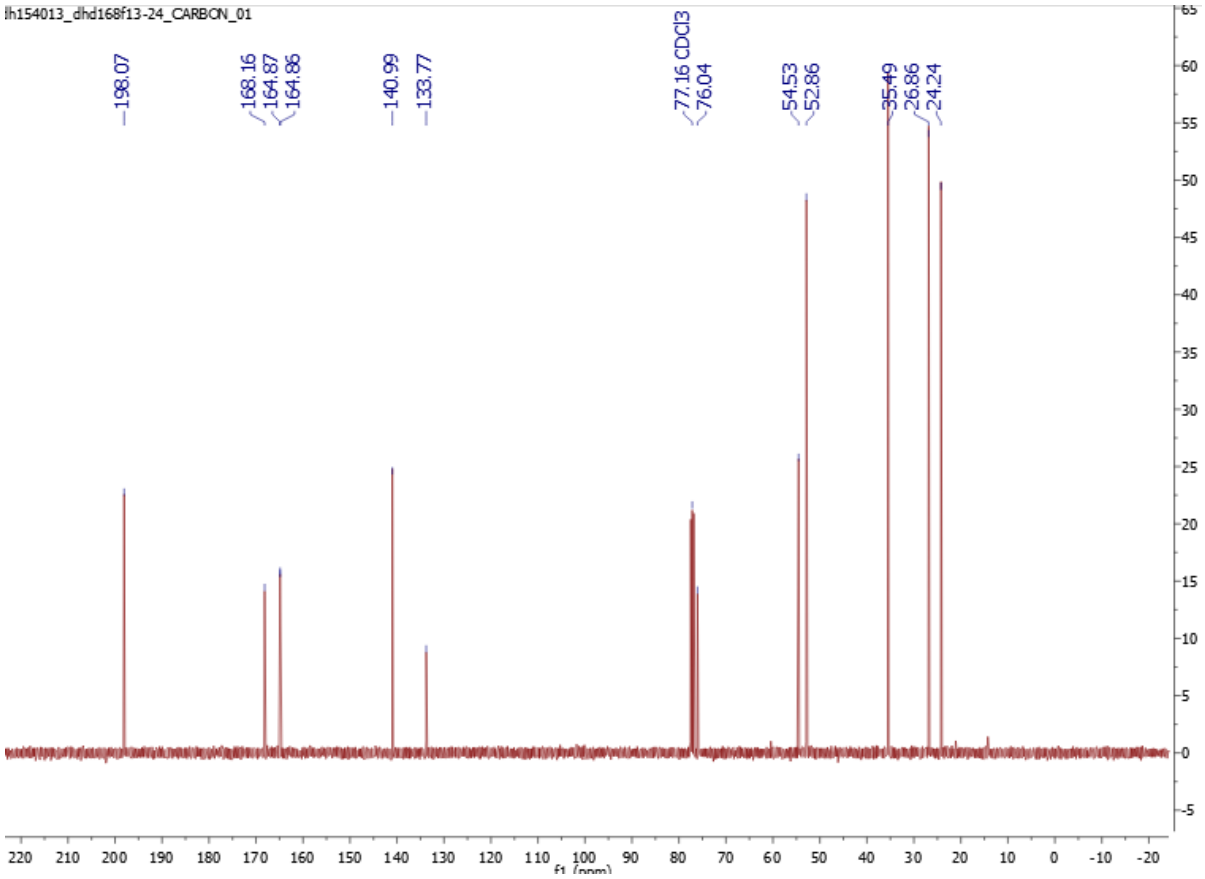
Trimethyl 4-((methoxycarbonyl)oxy)cyclohepta-1,3-diene-1,2,3-tricarboxylate 204



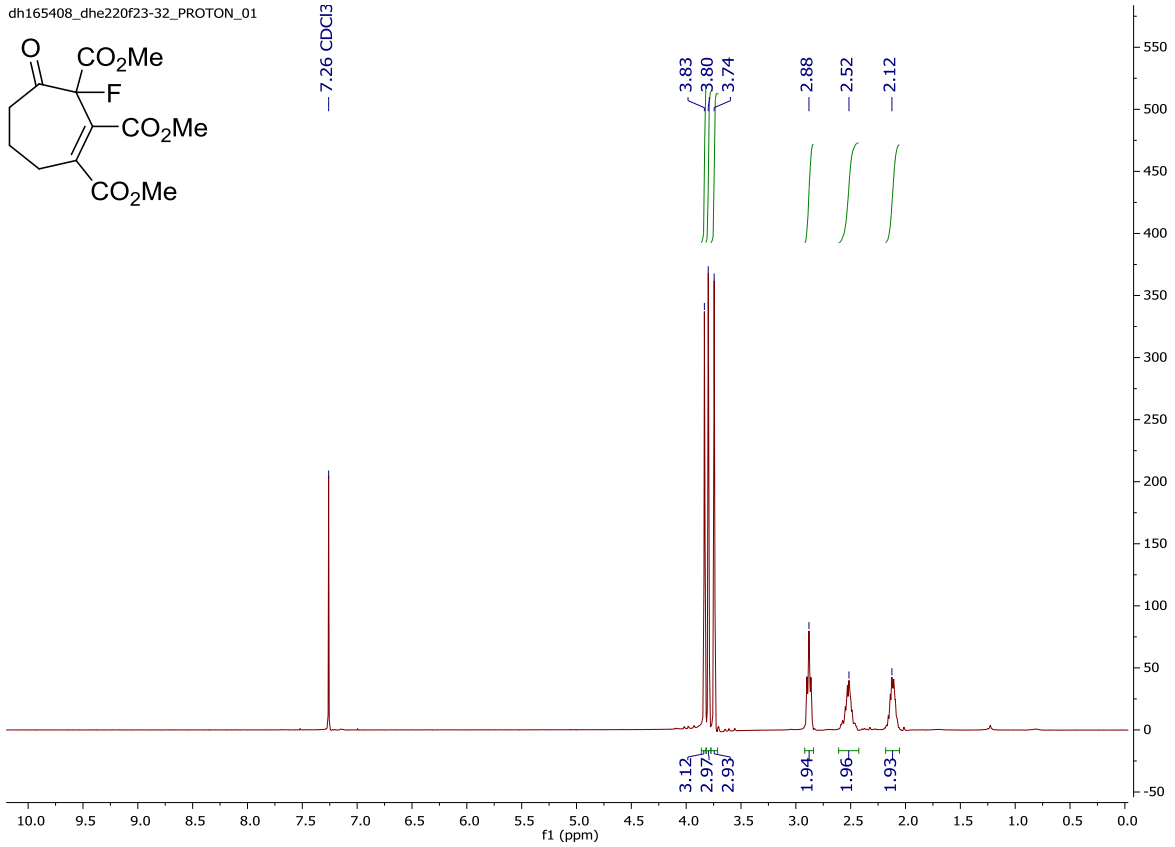


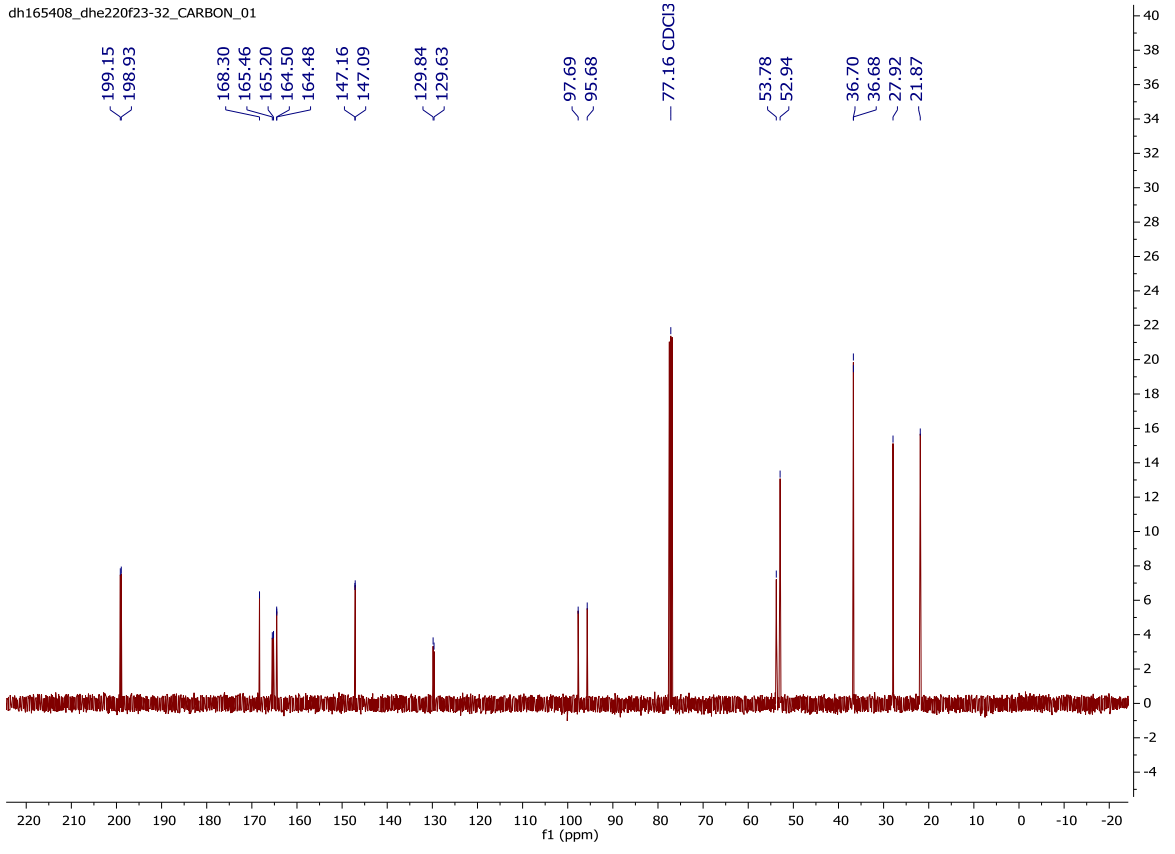
Trimethyl 3-chloro-4-oxocyclohept-1-ene-1,2,3-tricarboxylate 206



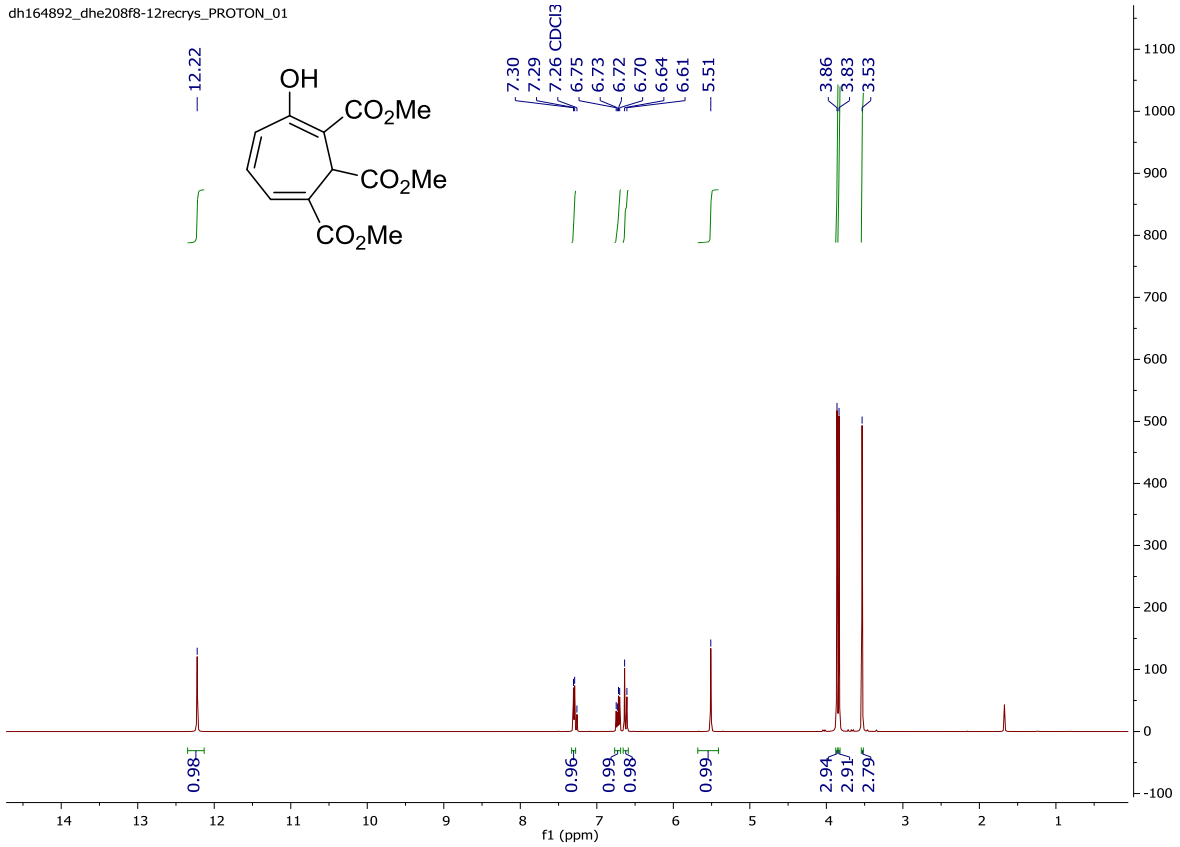


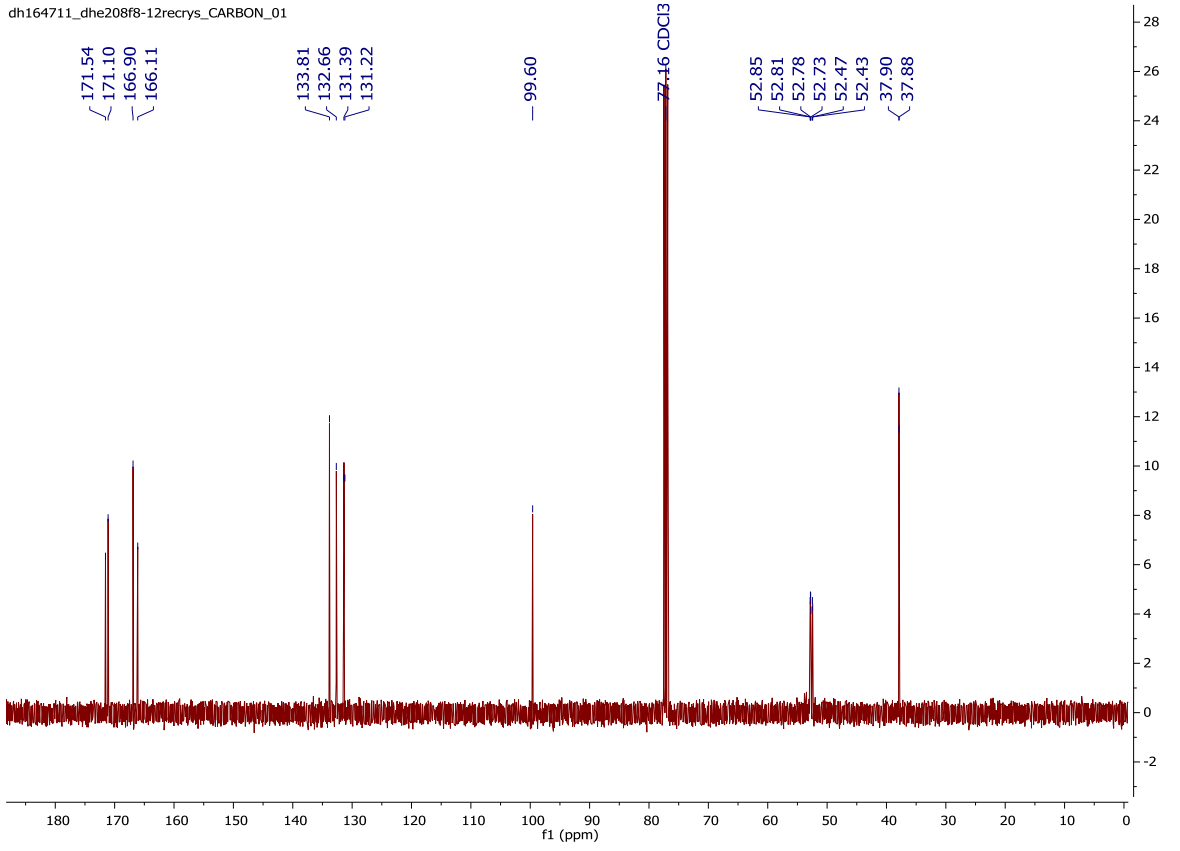
Trimethyl 3-fluoro-4-oxocyclohept-1-ene-1,2,3-tricarboxylate 209



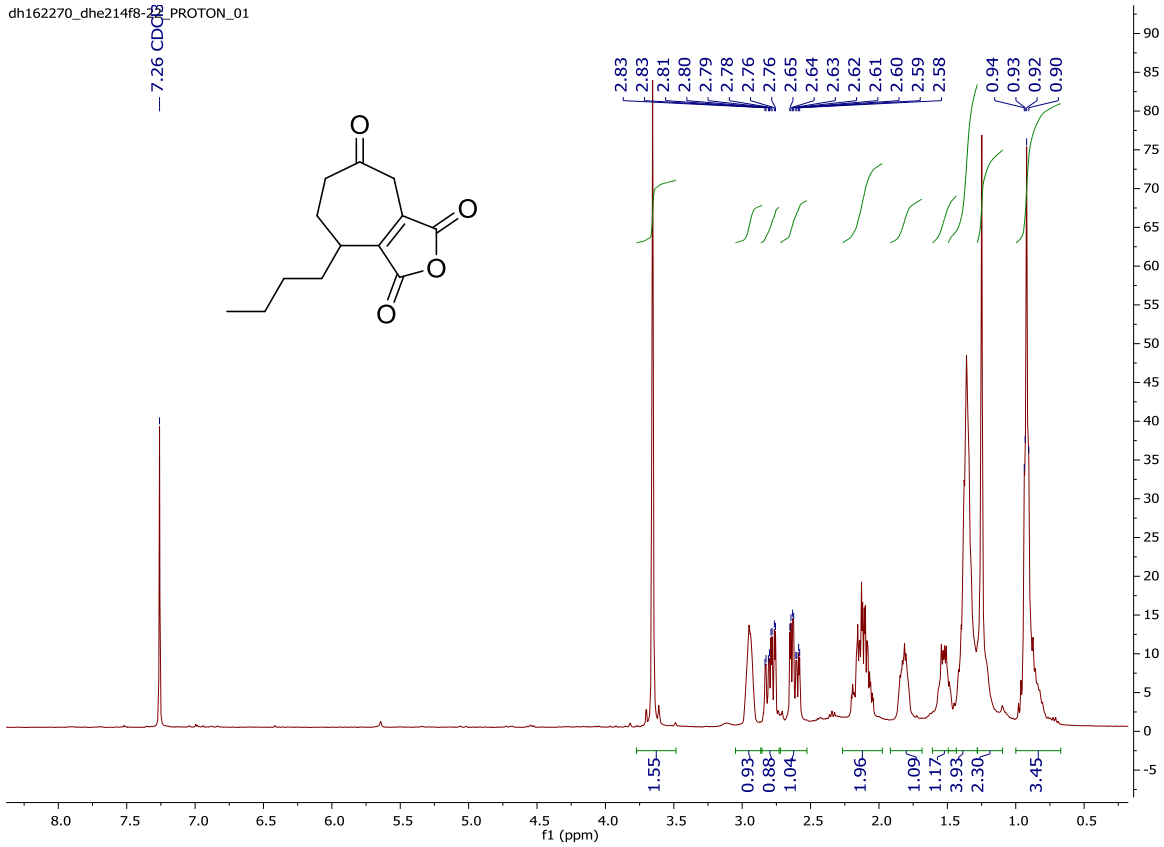


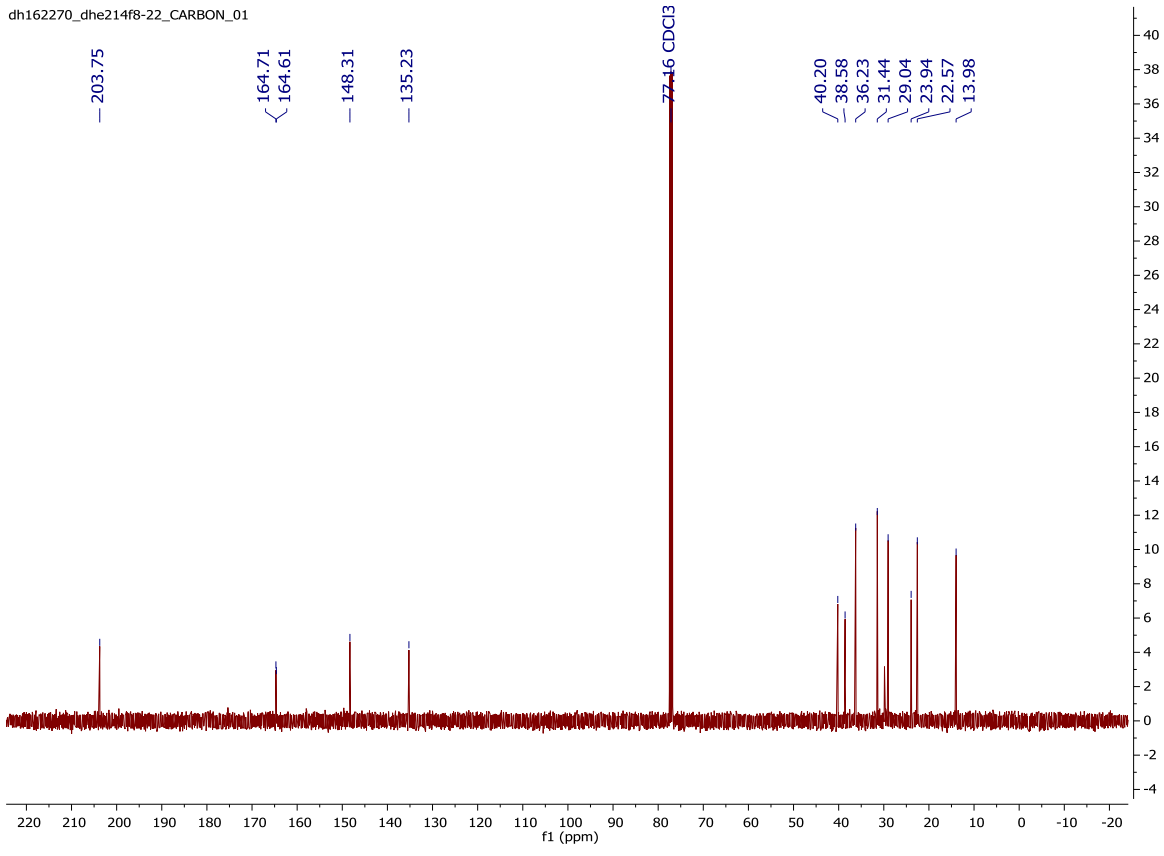
Trimethyl 4-hydroxycyclohepta-3,5,7-triene-1,2,3-tricarboxylate 210



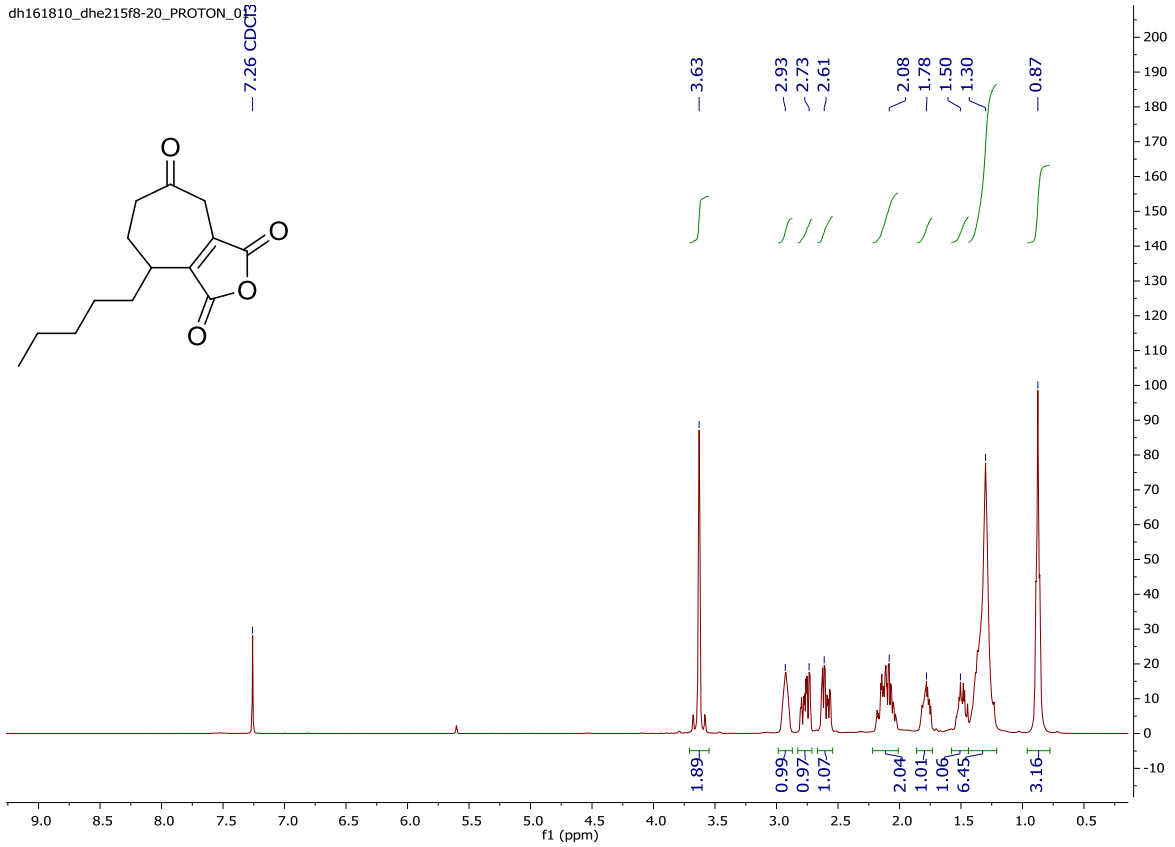


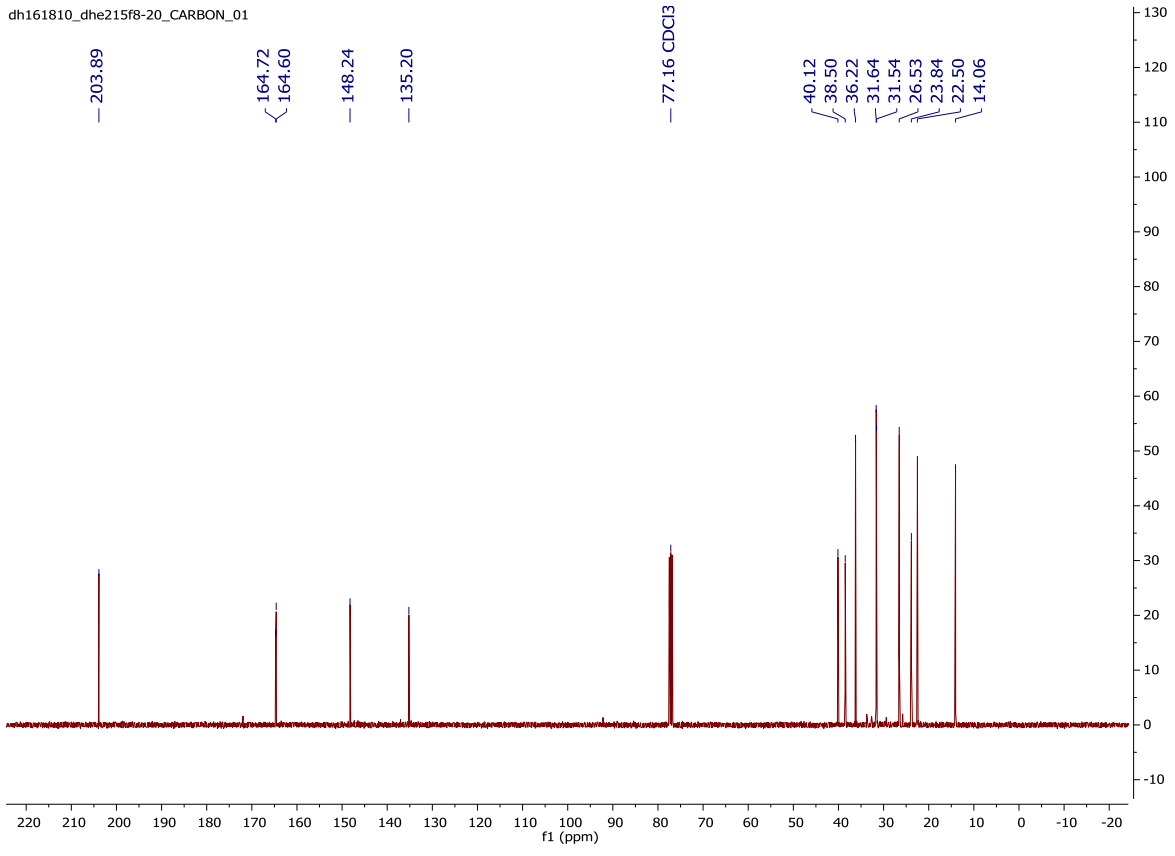
1-Oxo-5-butylcyclohept-3-ene-3,4-dicarboxylic acid anhydride 216



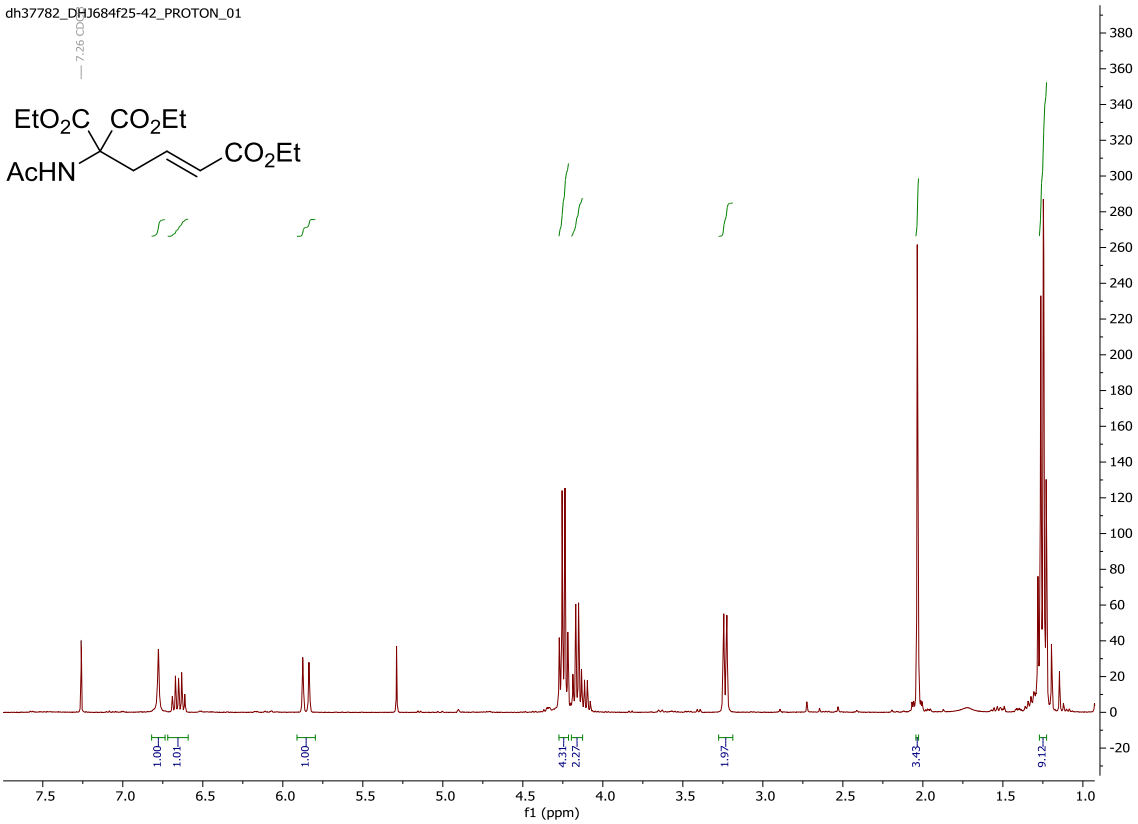


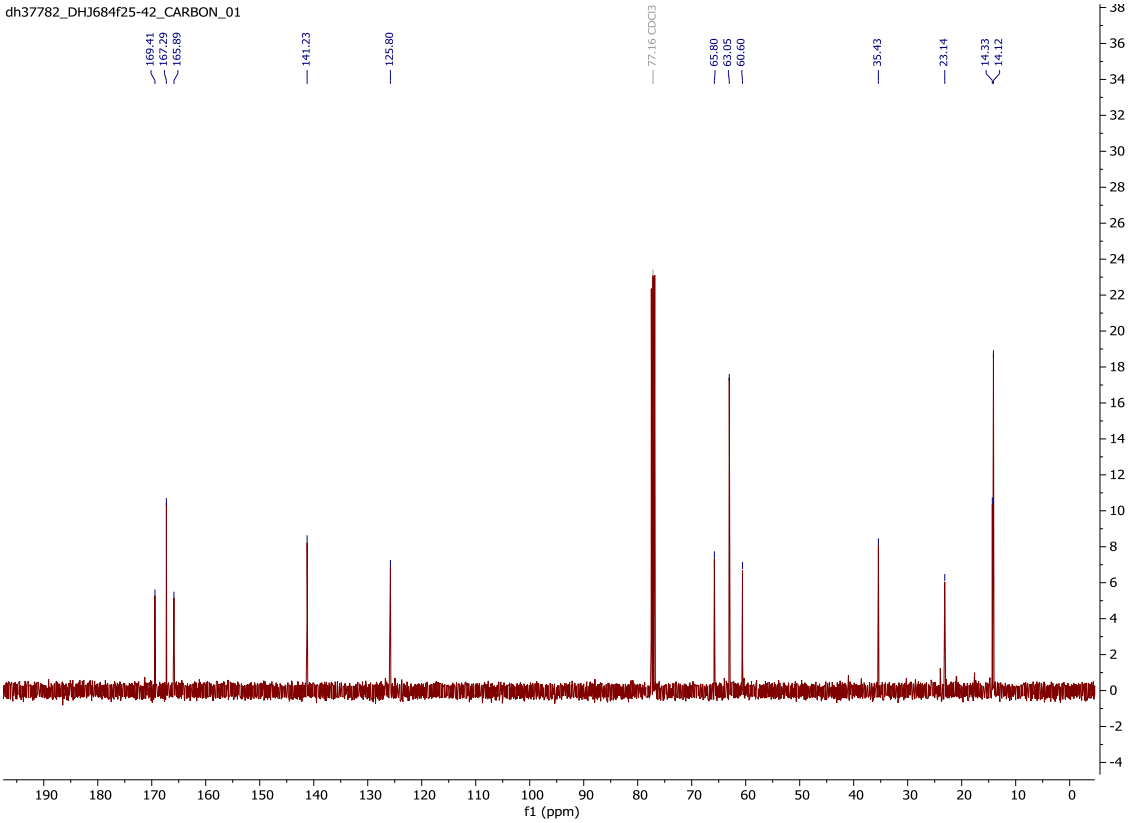
1-Oxo-5-pentylcyclohept-3-ene-3,4-dicarboxylic acid anhydride 217



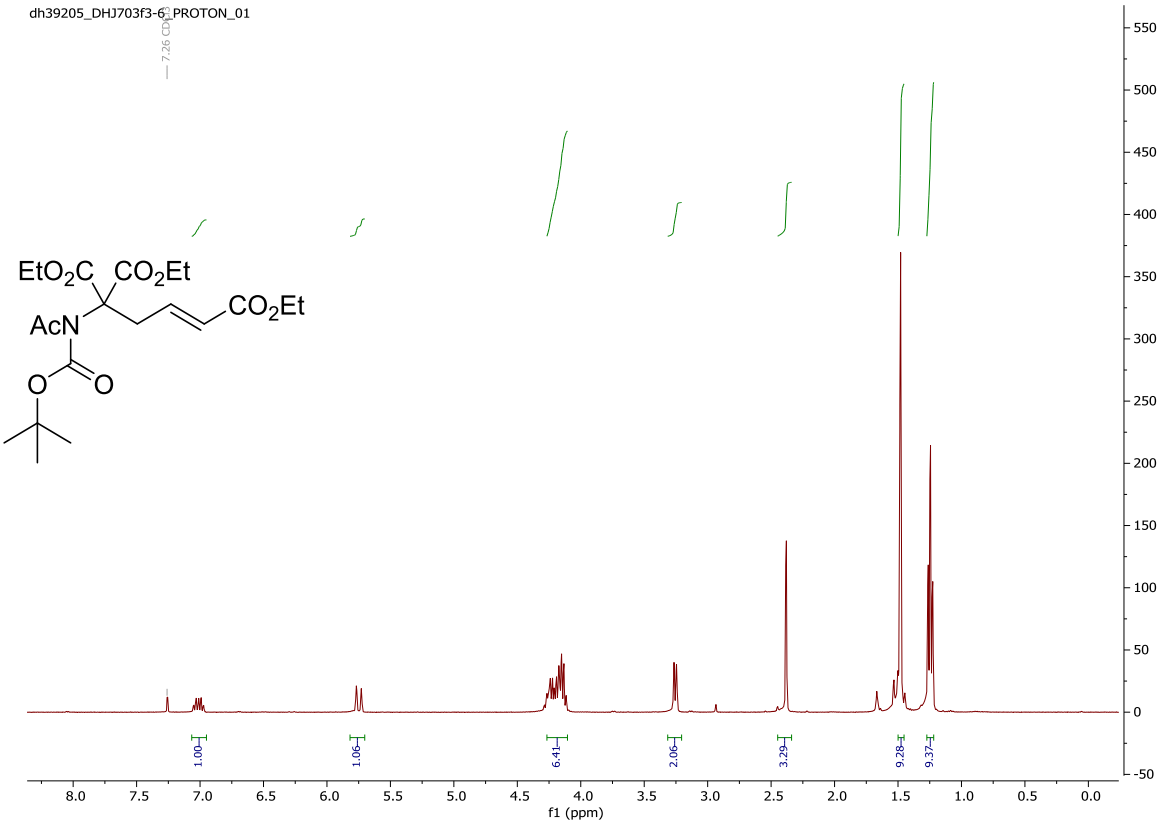


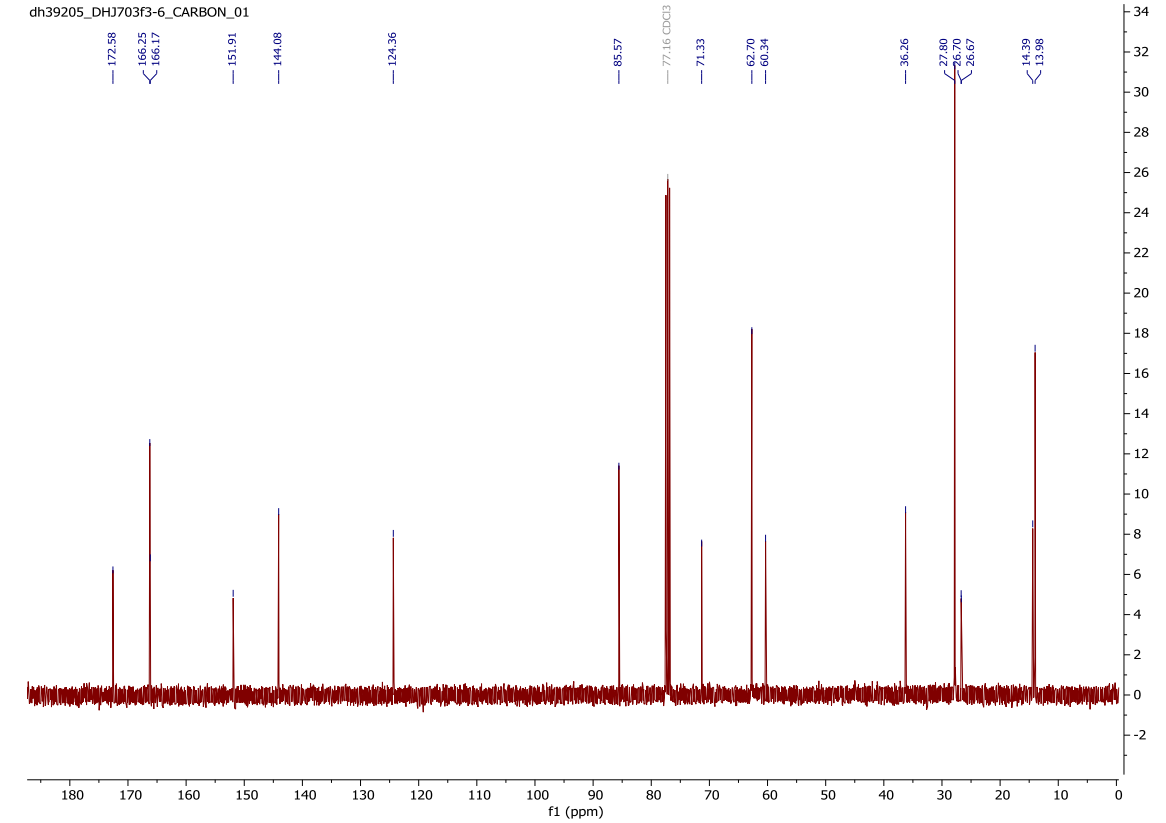
Triethyl (*E*)-1-acetamidobut-3-ene-1,1,4-tricarboxylate S18



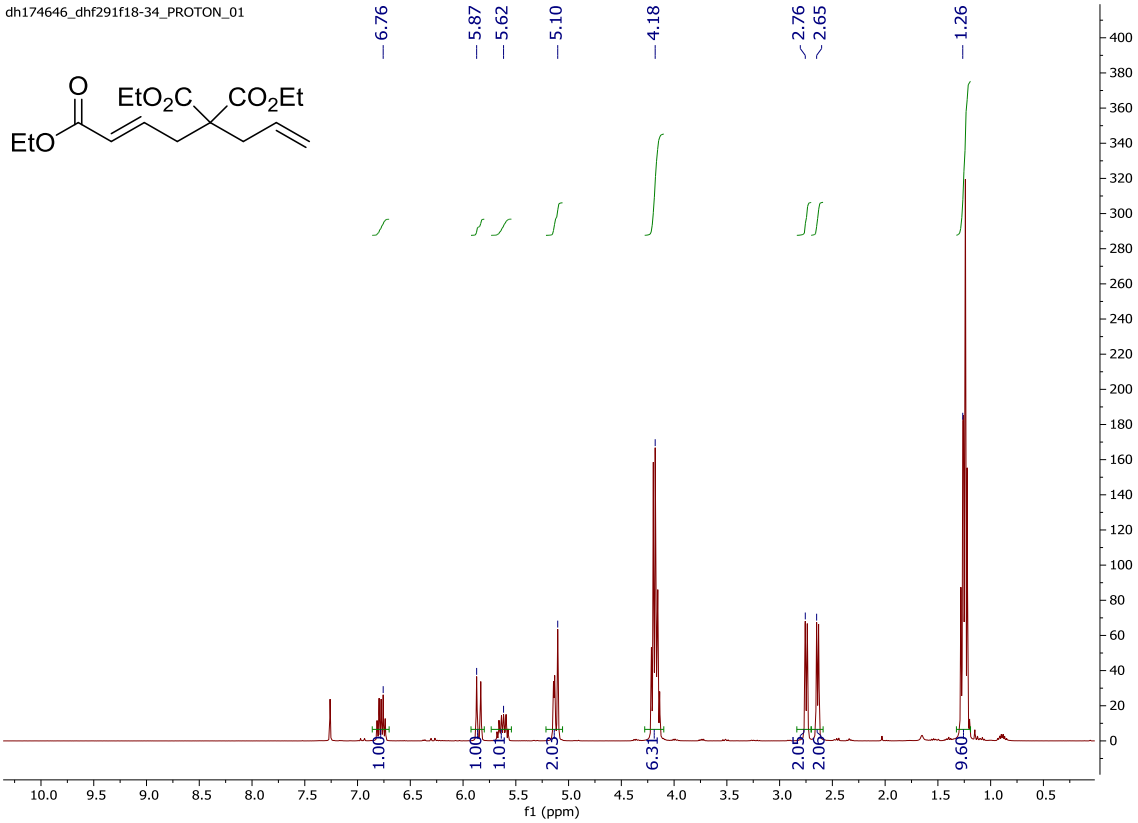


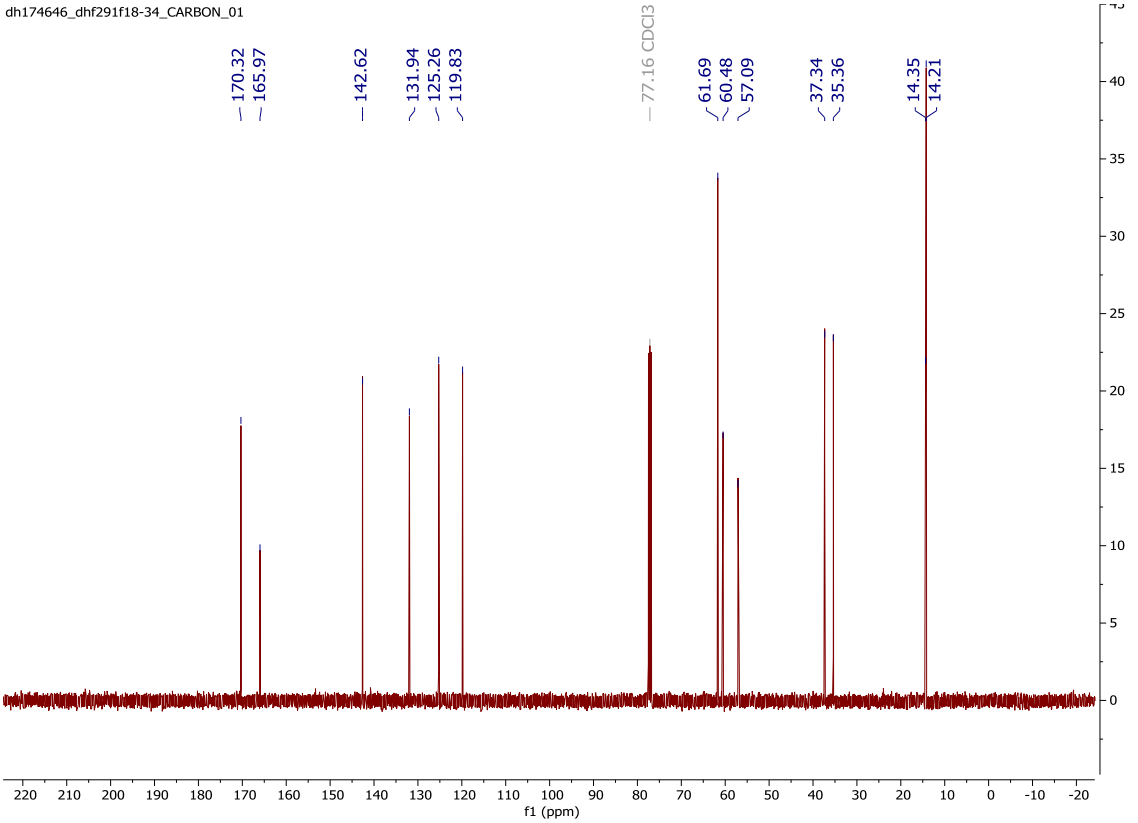
Triethyl (*E*)-1-(*N*-(tert-butoxycarbonyl)acetamido)but-3-ene-1,1,4-tricarboxylate S19



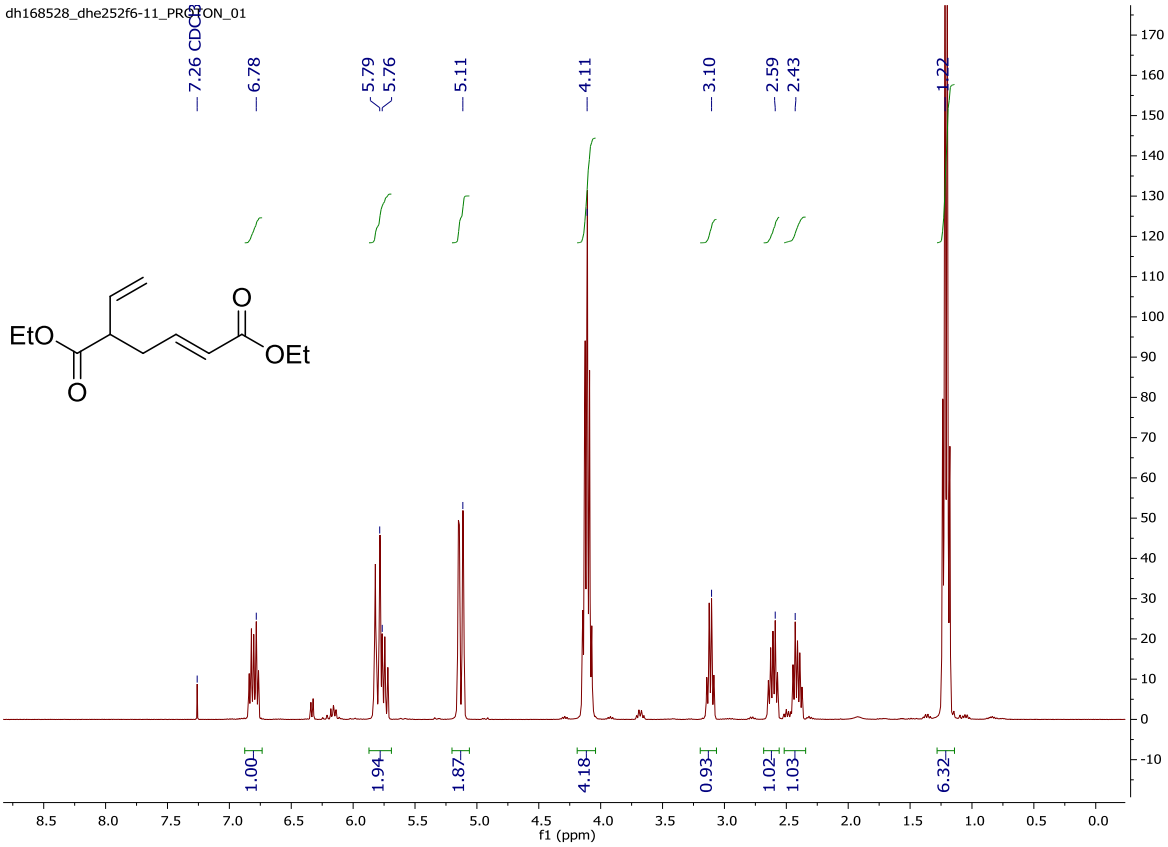


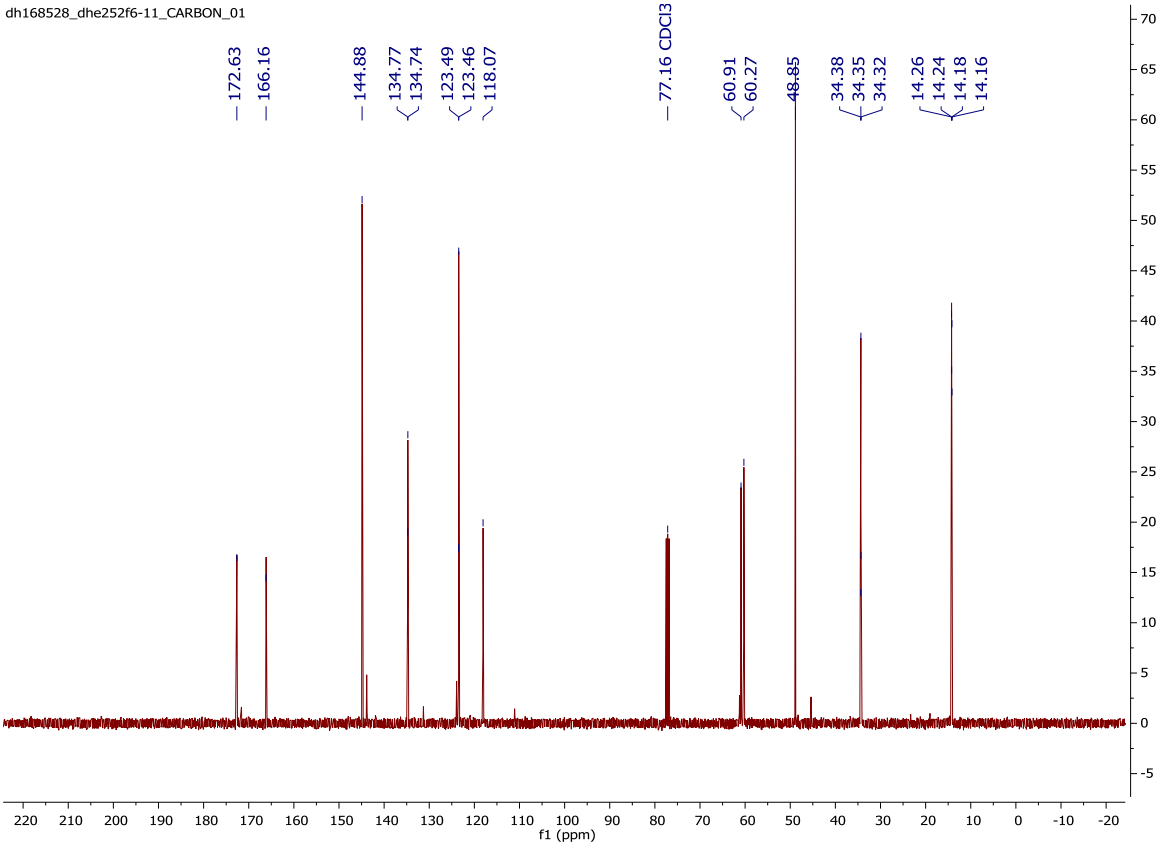
Ethyl 5,5-bis(ethoxycarbonyl)-2,7-octadienoate S20



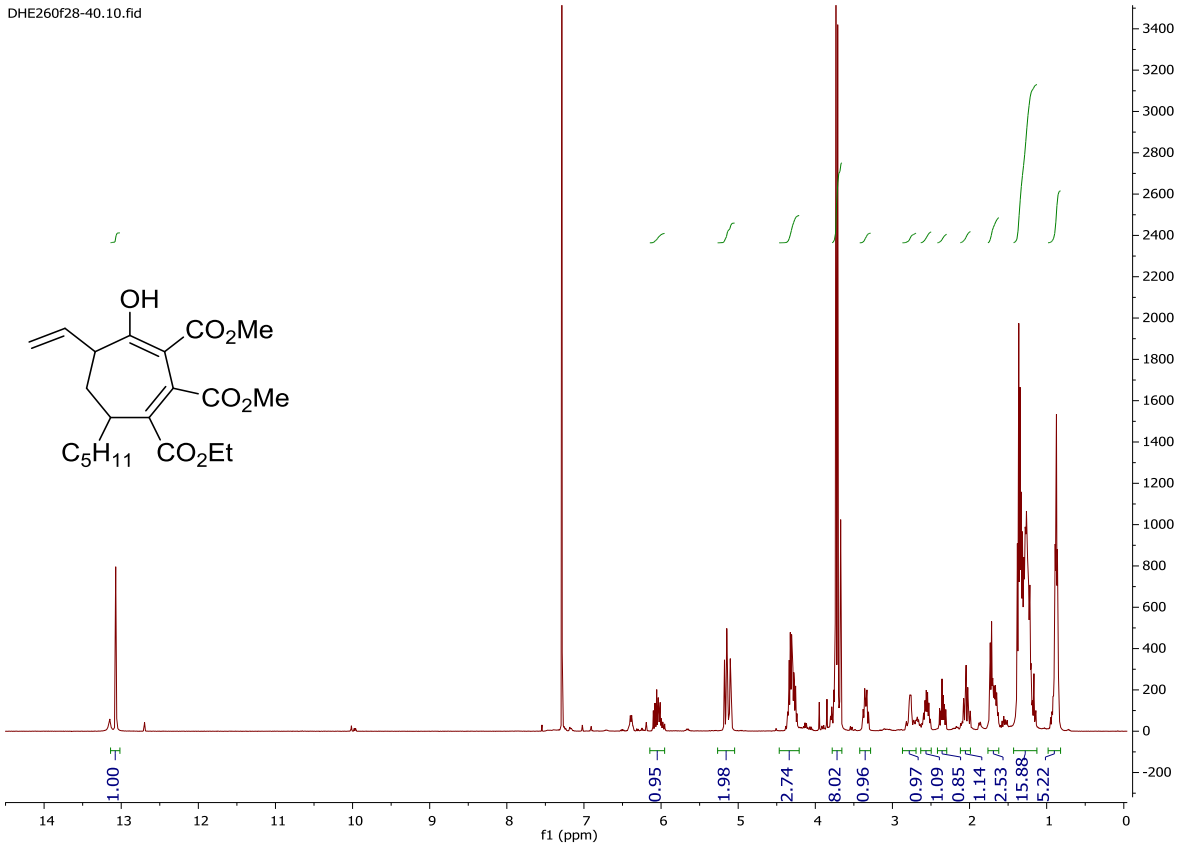


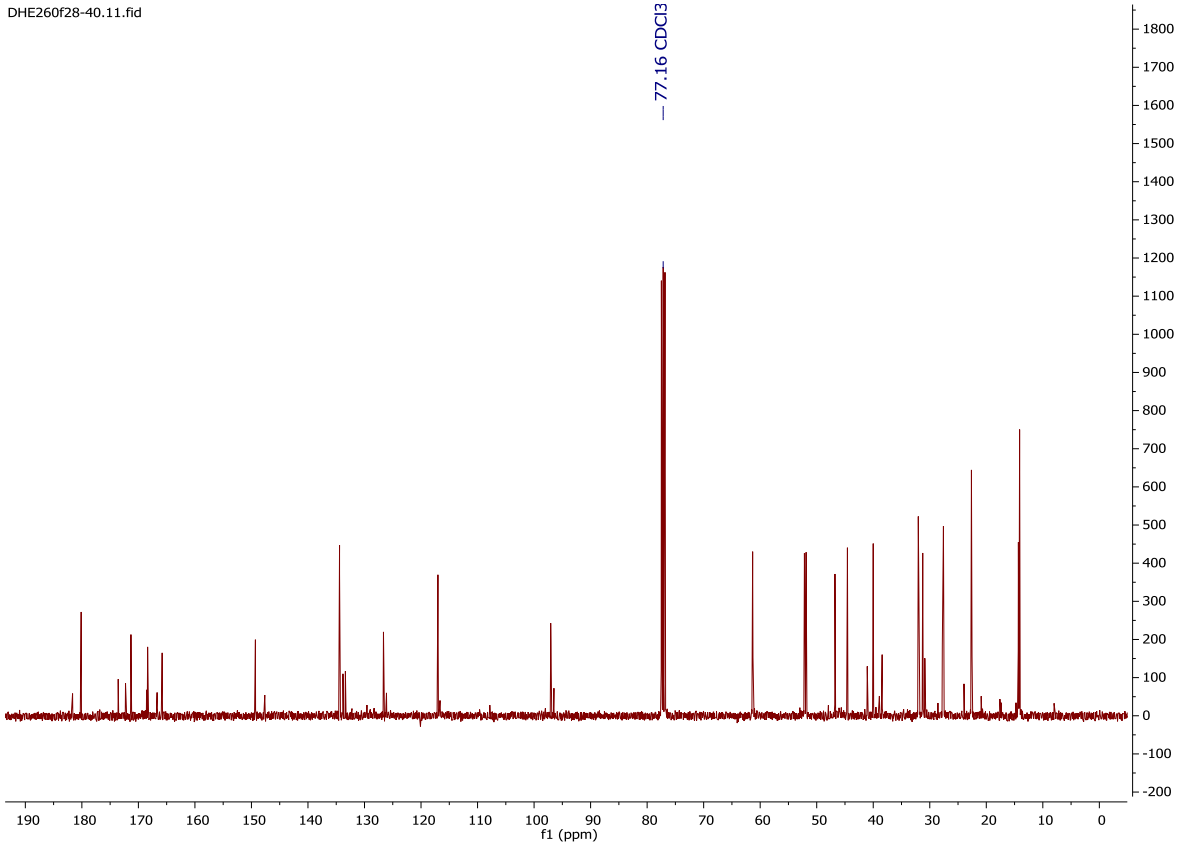
Diethyl (*E*)-5-vinylhex-2-enedioate 251



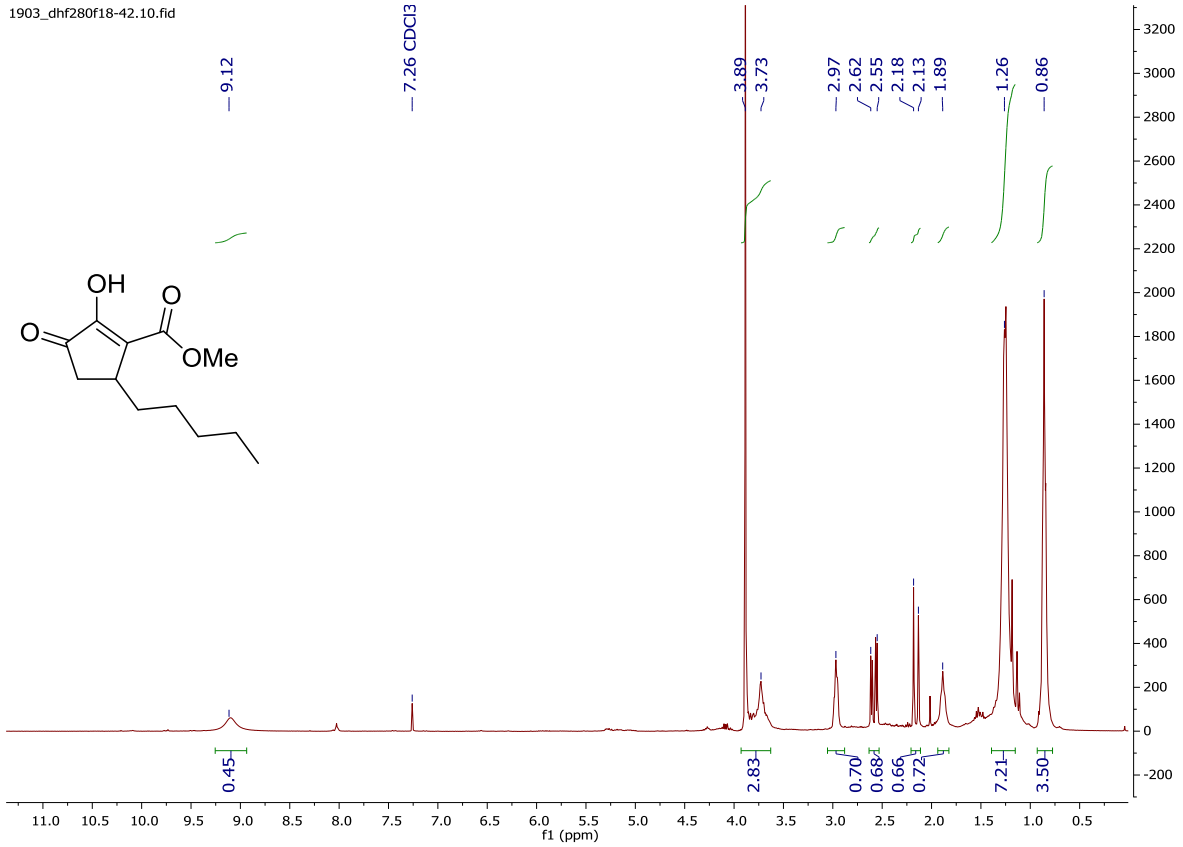


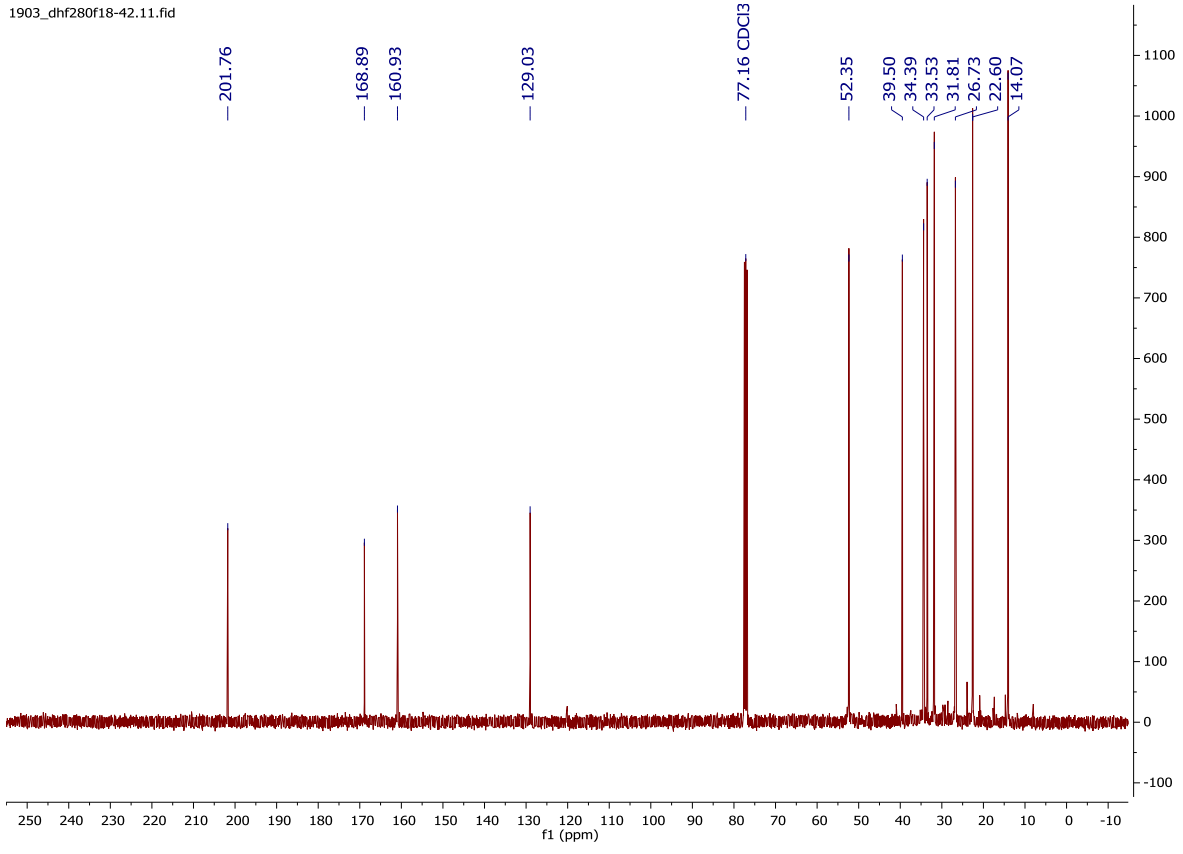
1-Ethyl 2,3-dimethyl 4-hydroxy-7-pentyl-5-vinylcyclohepta-1,3-diene-1,2,3-tricarboxylate S23



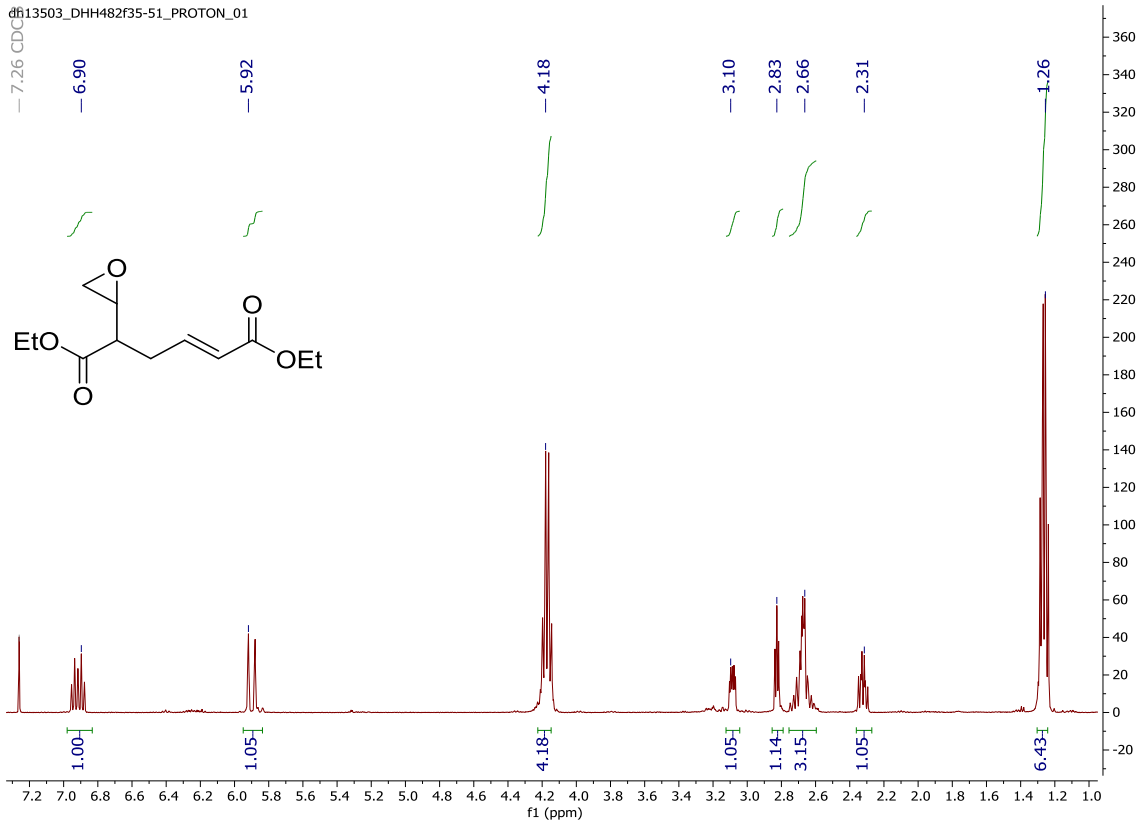


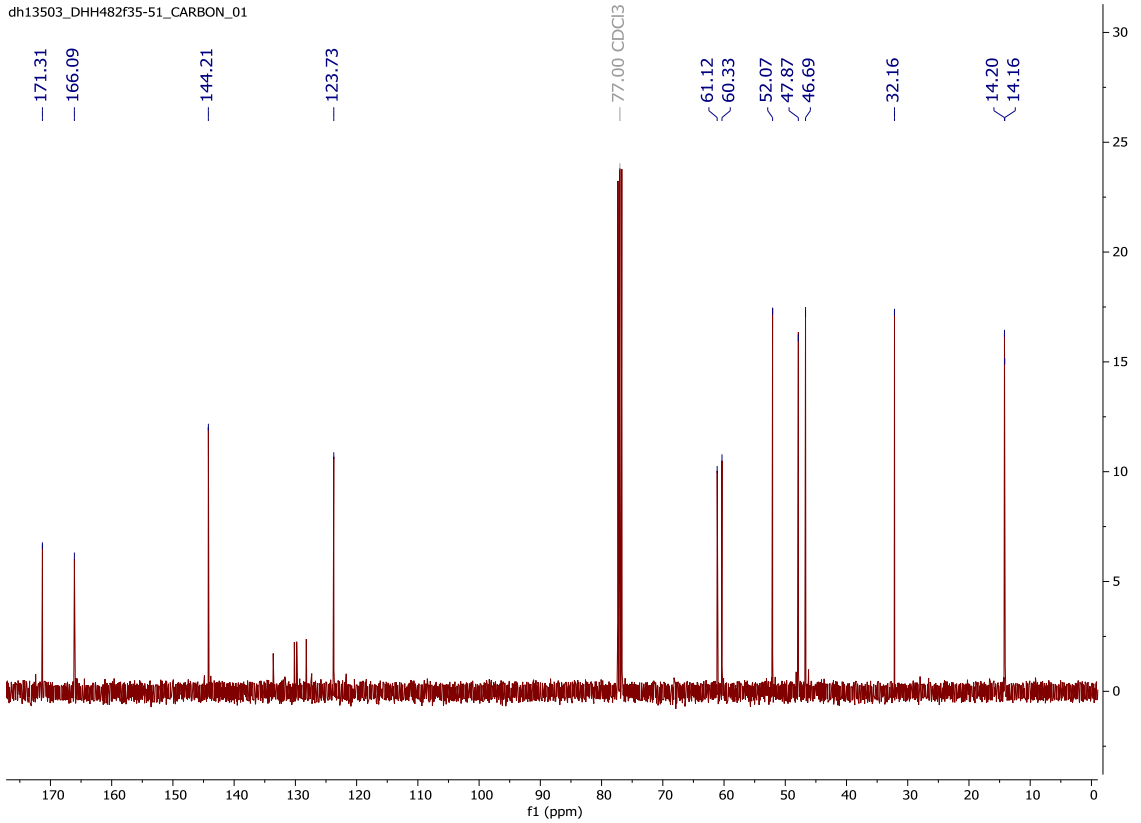
Methyl 2-hydroxy-3-oxo-5-pentylcyclopent-1-ene-1-carboxylate 249



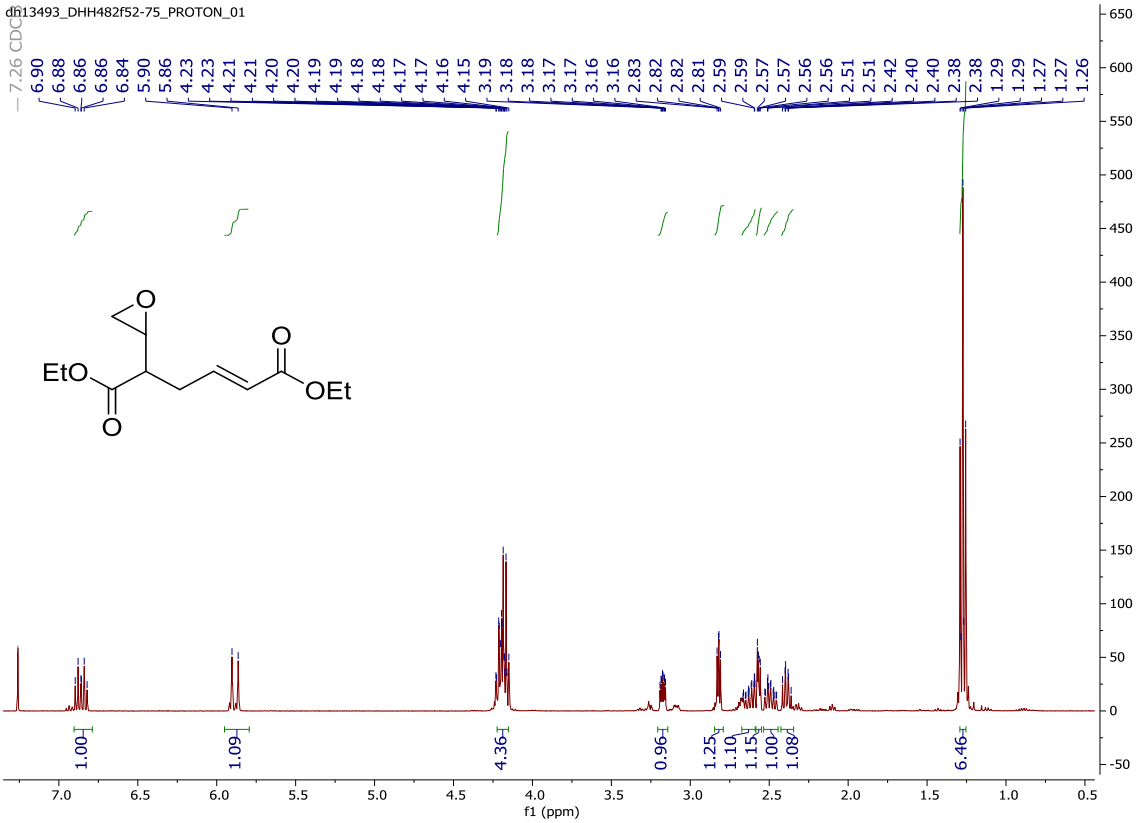


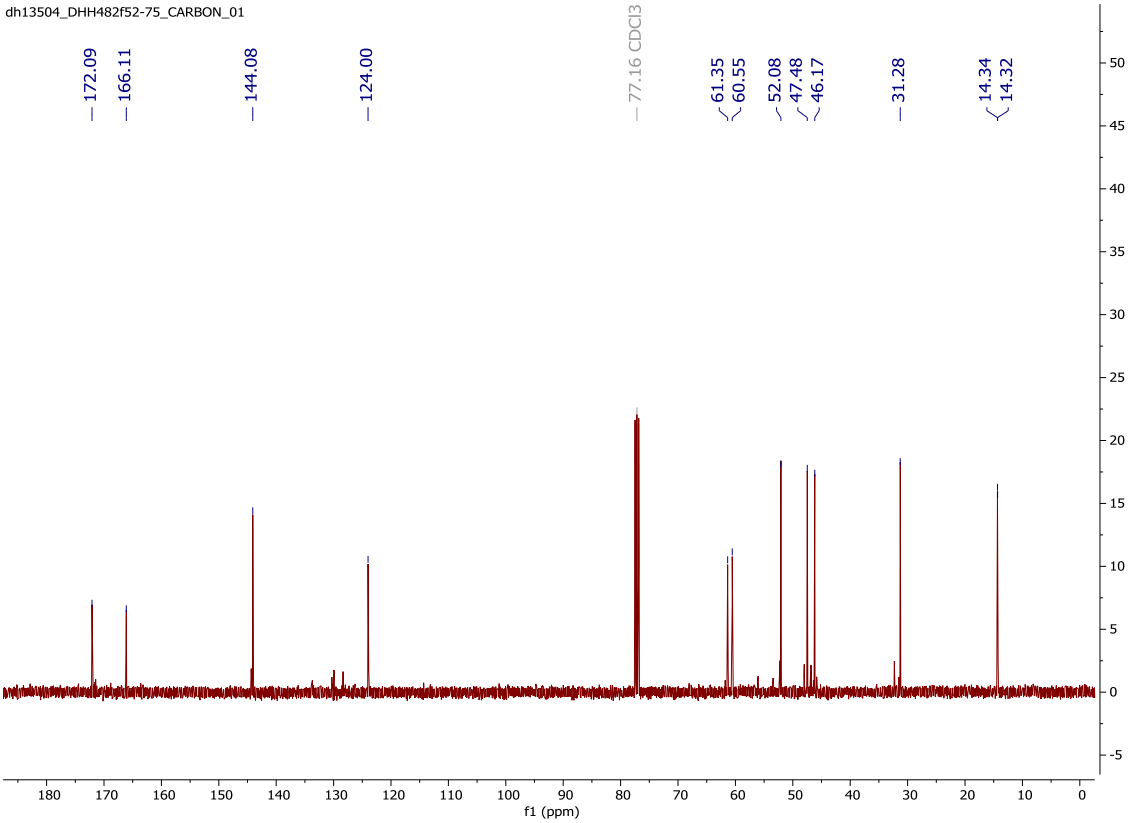
Diethyl (*E*)-5-(oxiran-2-yl)hex-2-enedioate 252 (Diasteromer A)



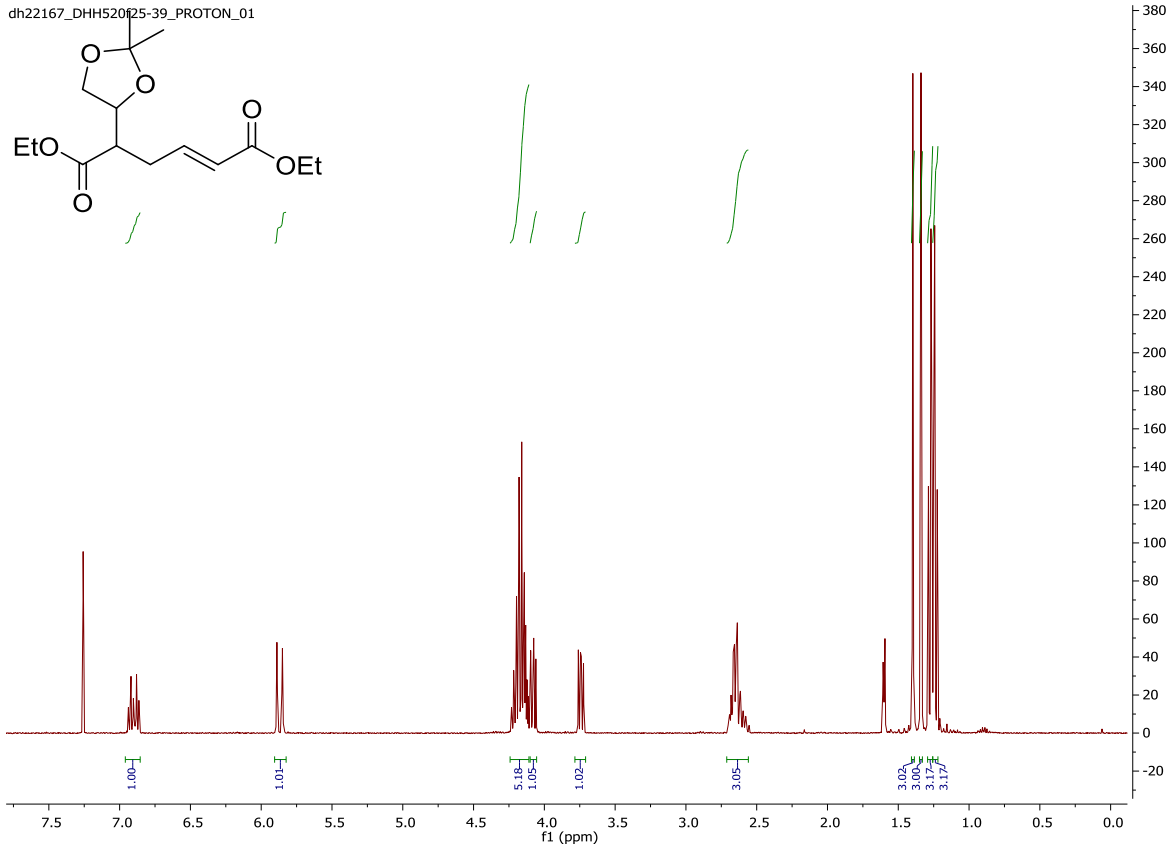


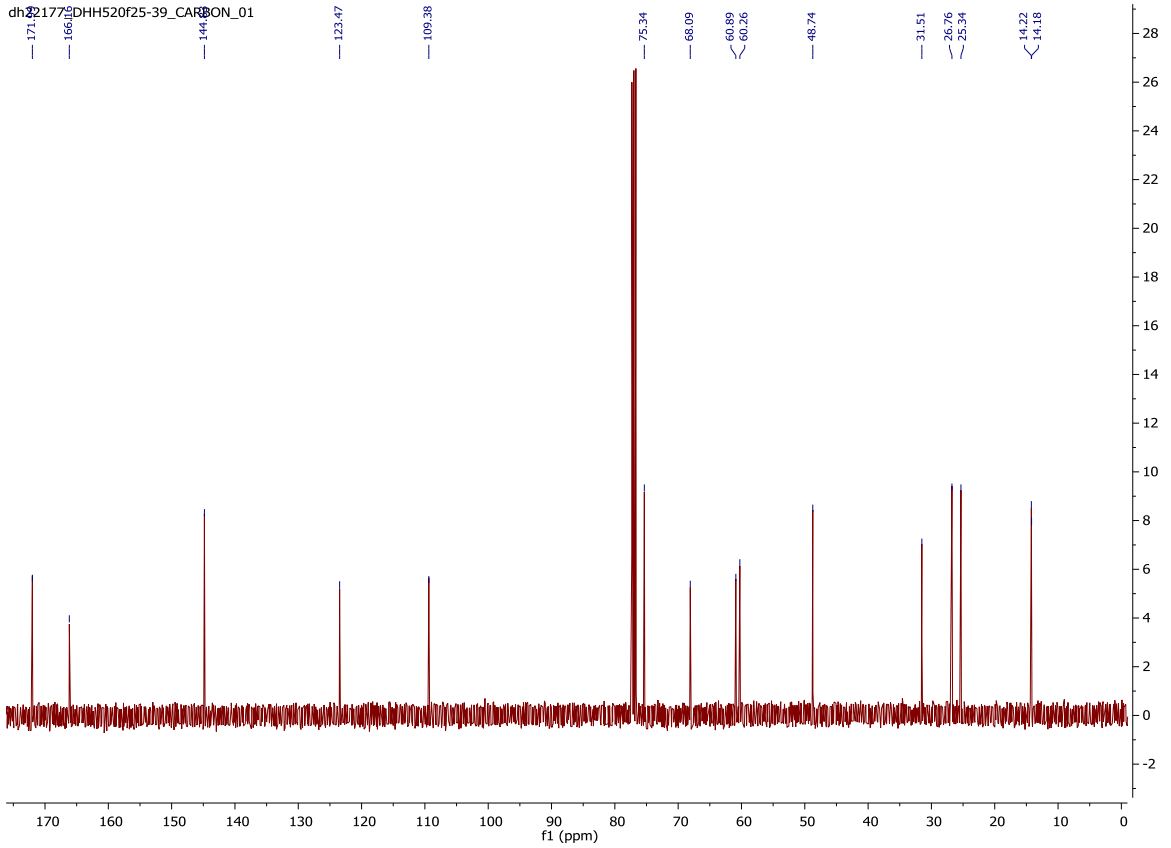
Diethyl (*E*)-5-(oxiran-2-yl)hex-2-enedioate 252 (Diasteromer B)



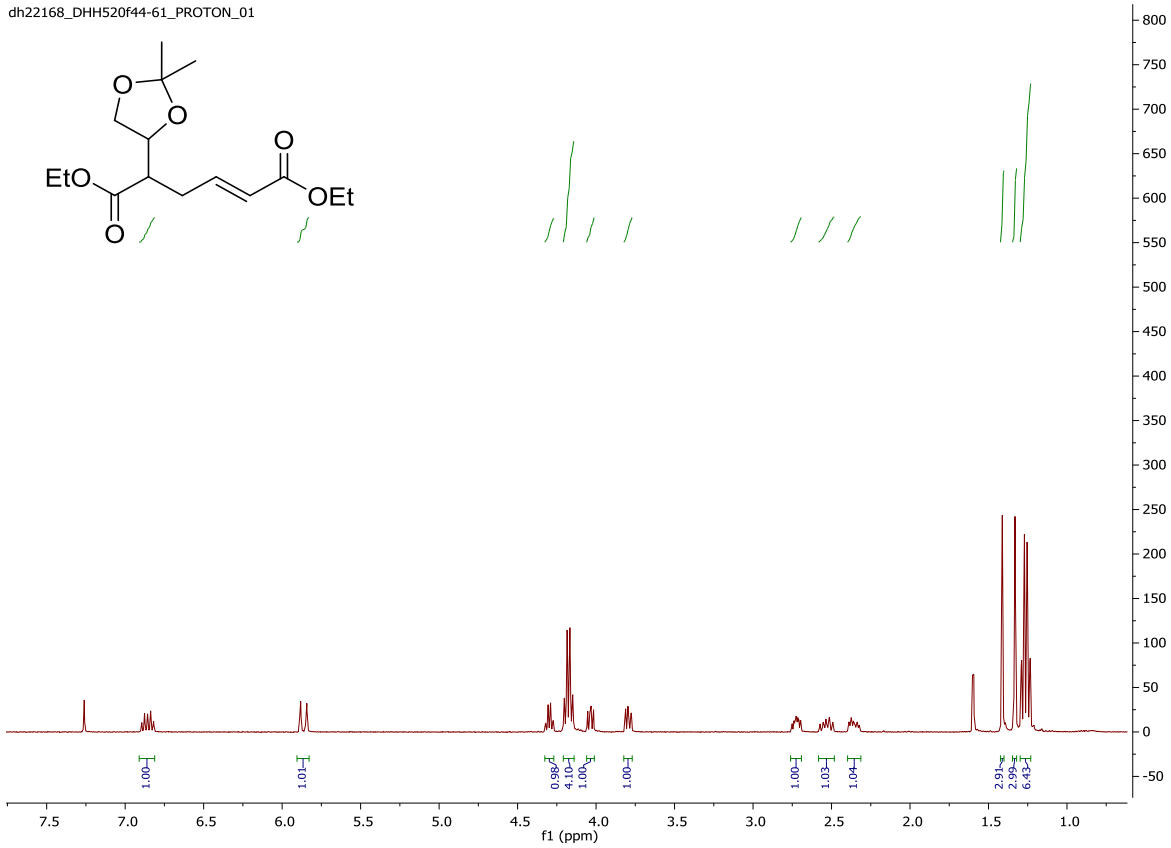


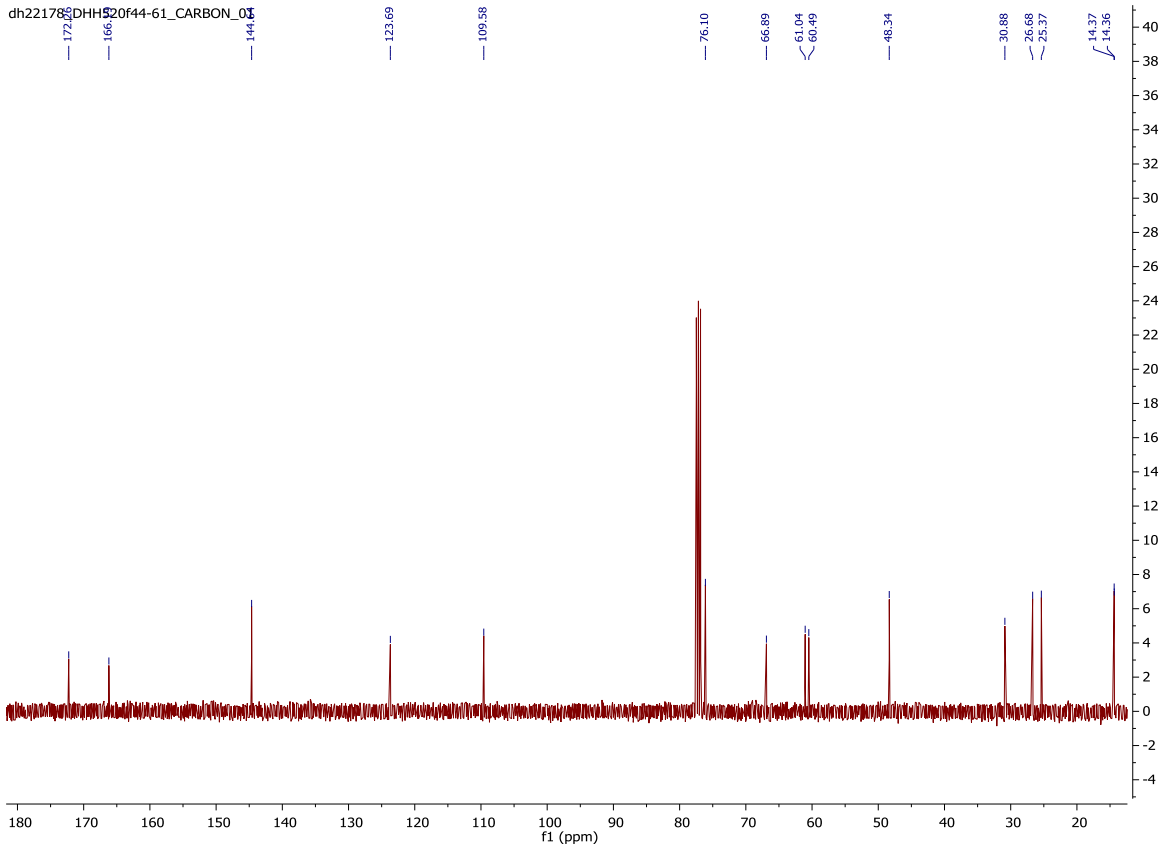
Diethyl (E)-5-(2,2-dimethyl-1,3-dioxolan-4-yl)hex-2-enedioate 253 (Diasteromer A)



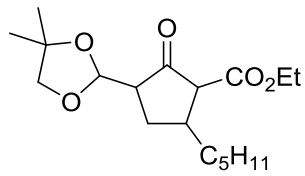


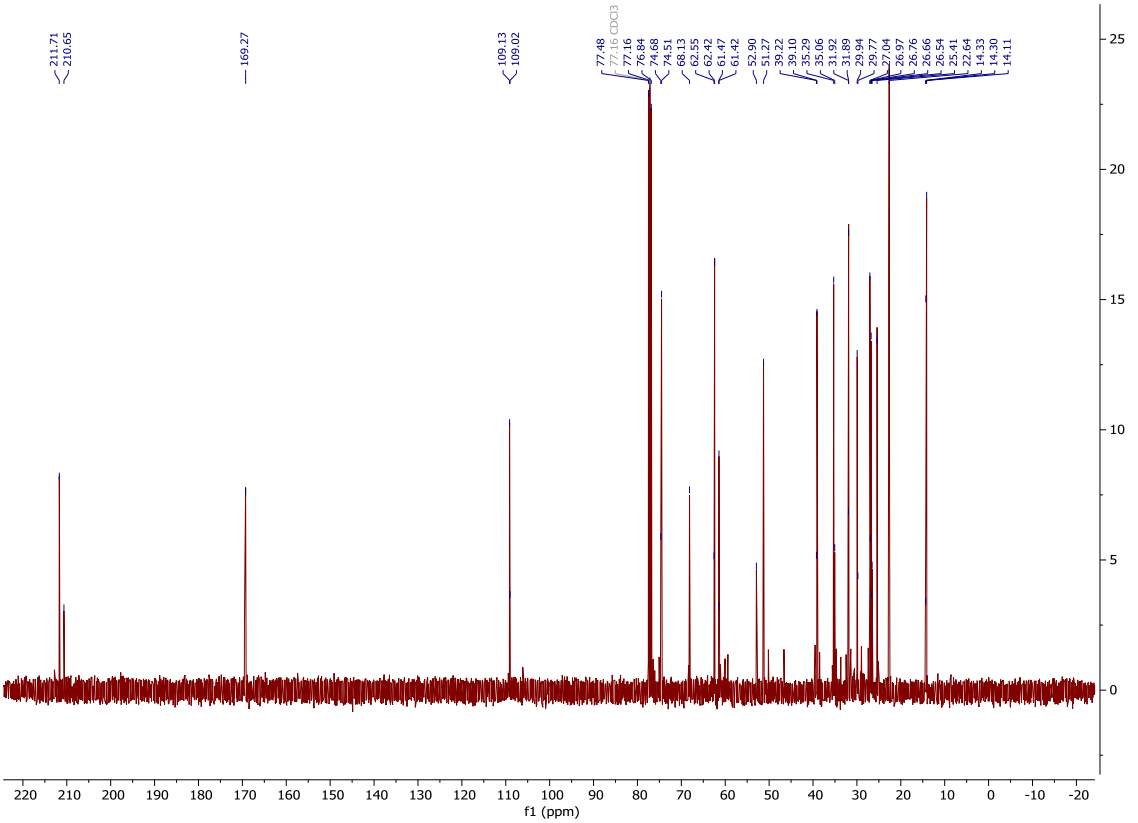
Diethyl (E)-5-(2,2-dimethyl-1,3-dioxolan-4-yl)hex-2-enedioate 253 (Diastereomer B)



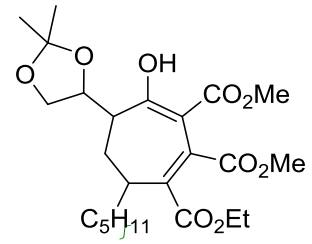


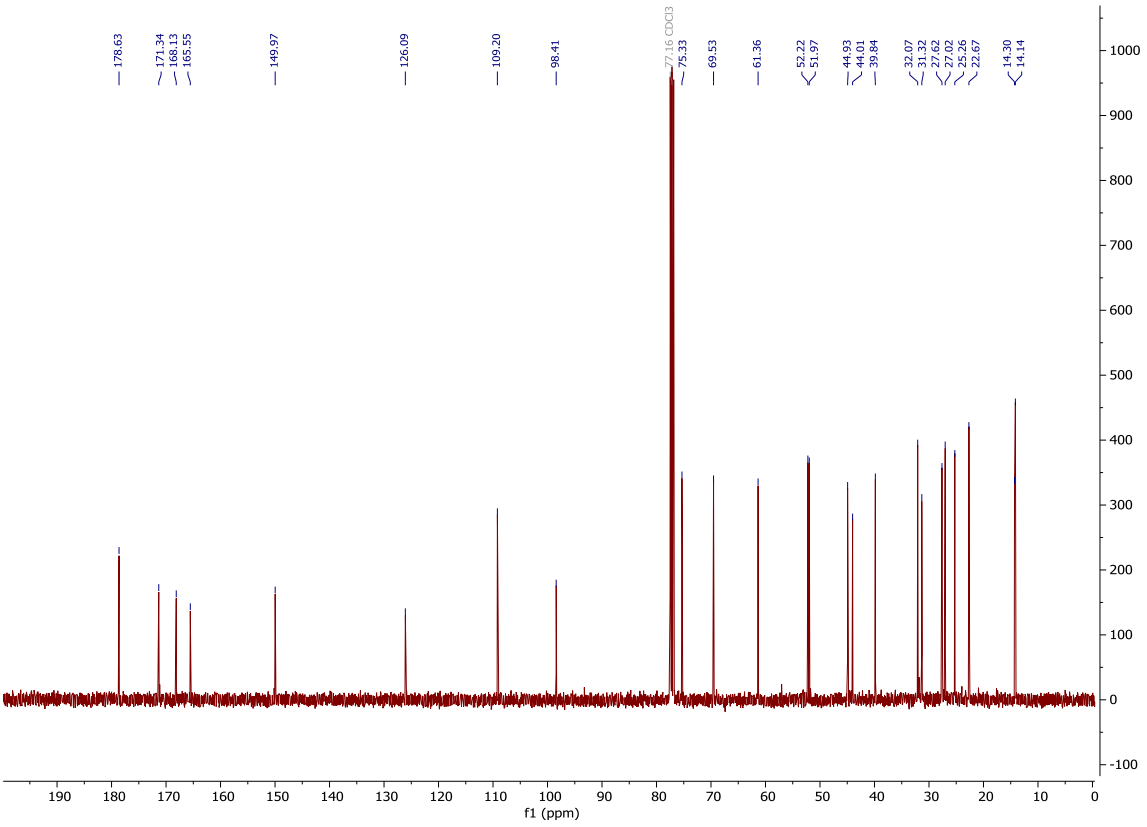
Ethyl 3-(2,2-dimethyl-1,3-dioxolan-4-yl)-2-oxo-5-pentylcyclopentane-1-carboxylate 254



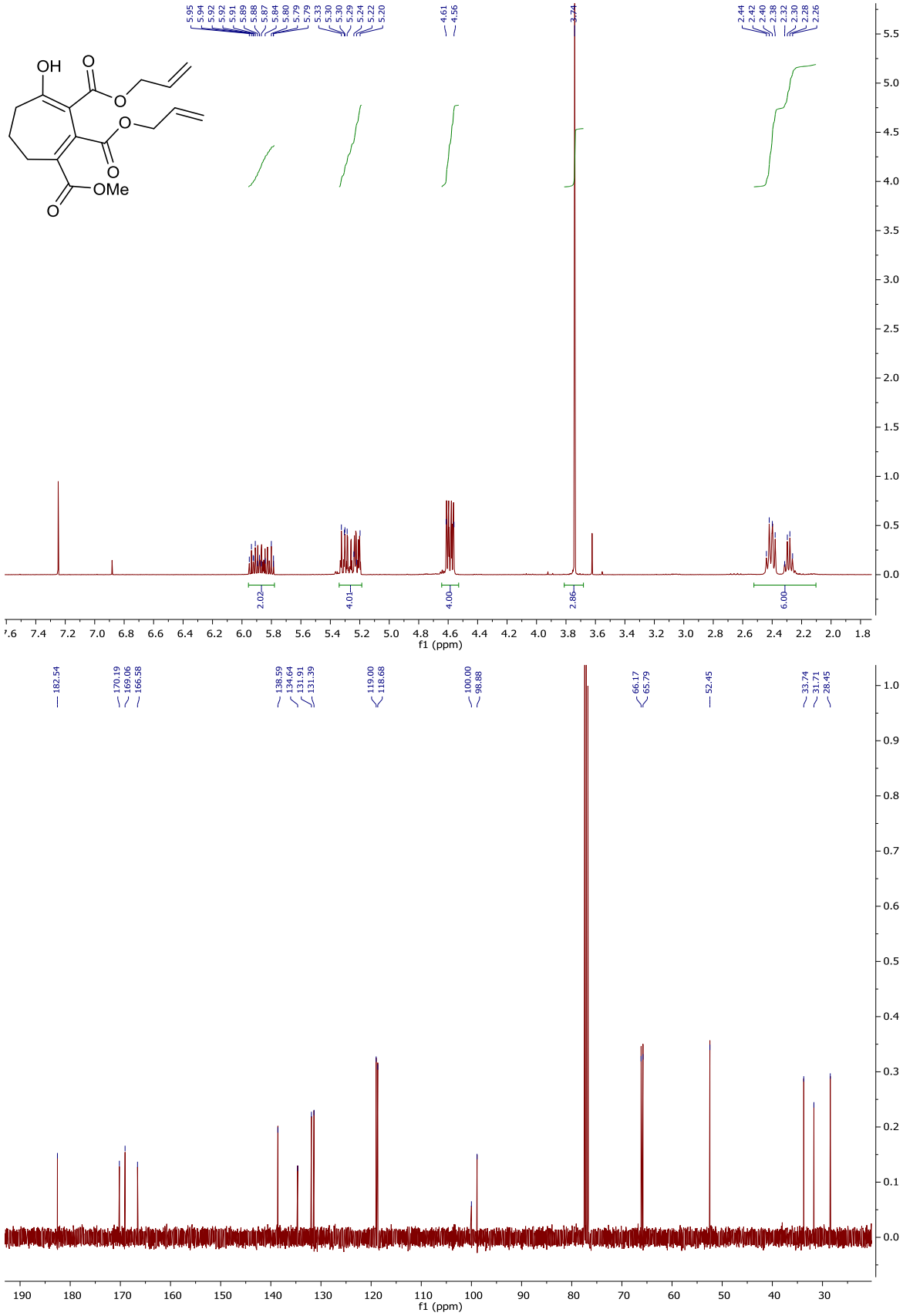


1-Ethyl 2,3-dimethyl 5-(2,2-dimethyl-1,3-dioxolan-4-yl)-4-hydroxy-7-pentylcyclohepta-1,3-diene-1,2,3-tricarboxylate 255

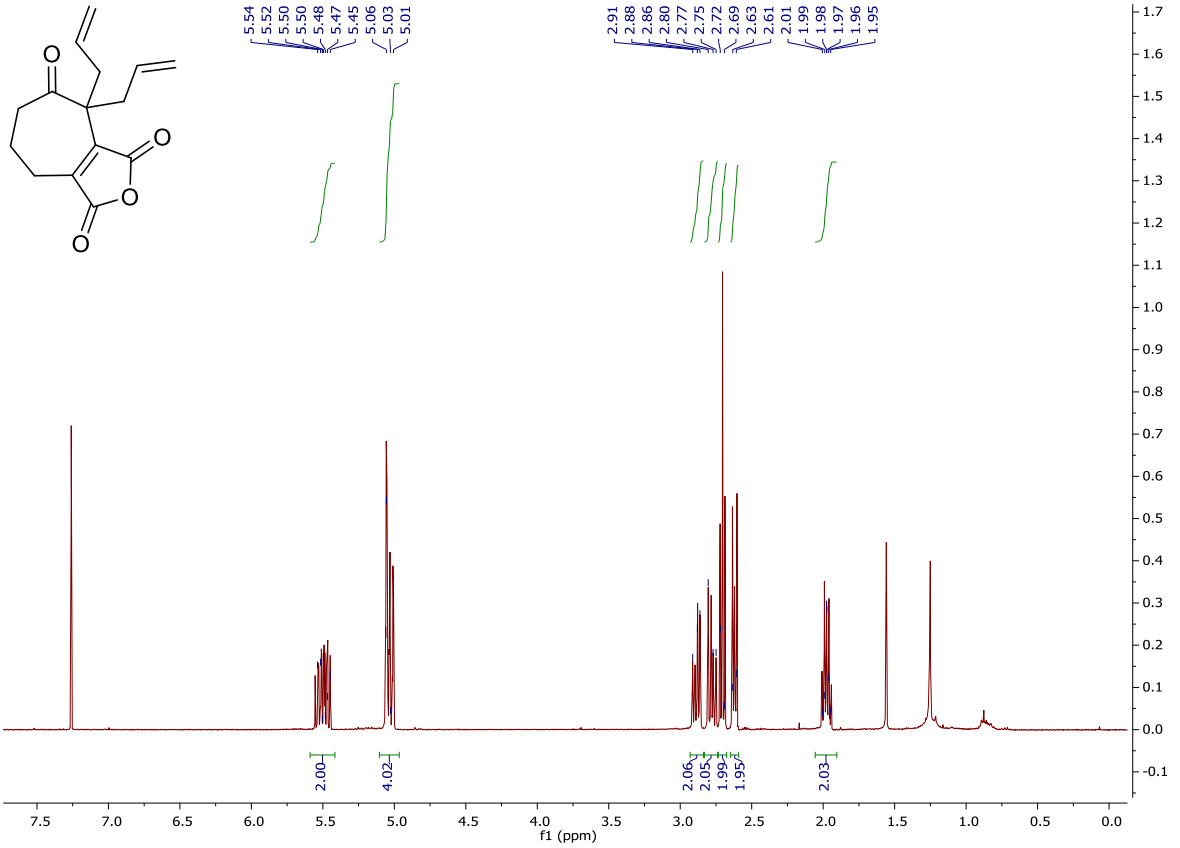


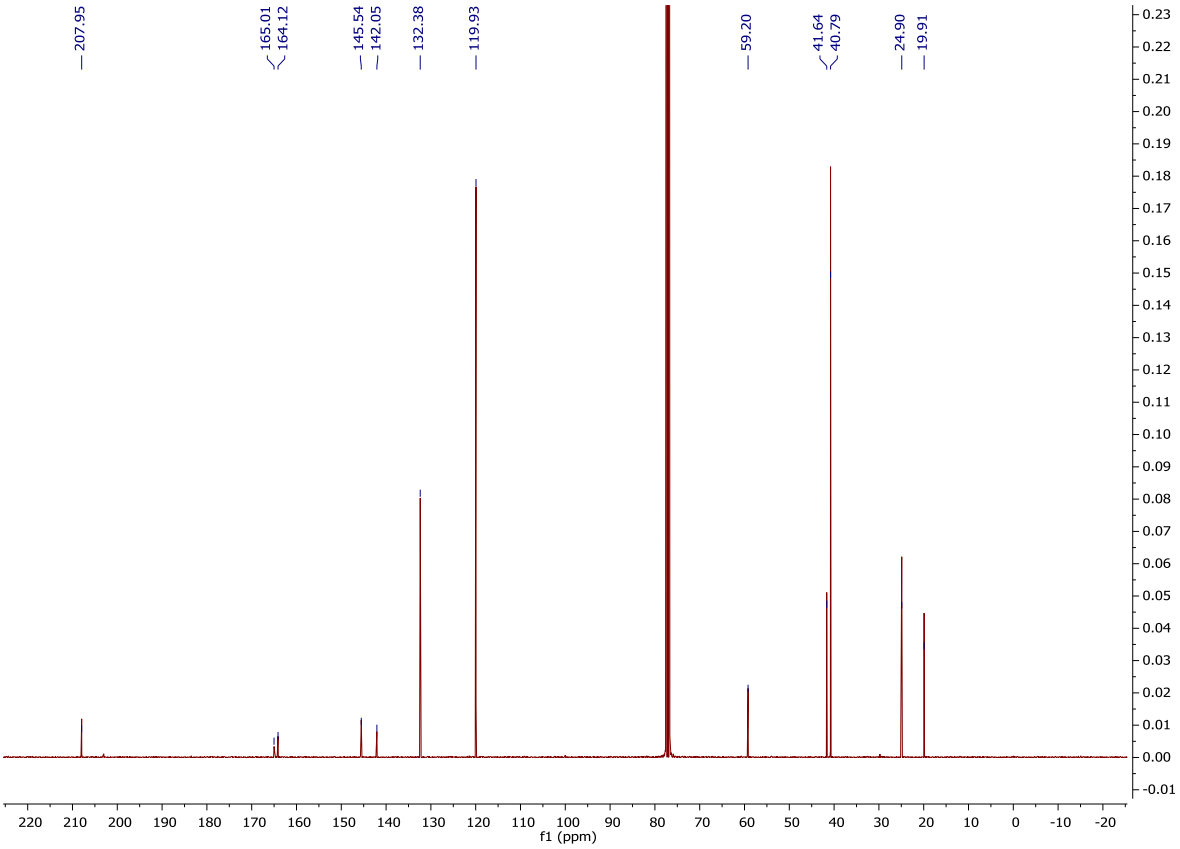


2,3-Diallyl 1-methyl 4-hydroxycyclohepta-1,3-diene-1,2,3-tricarboxylate 265 (†)

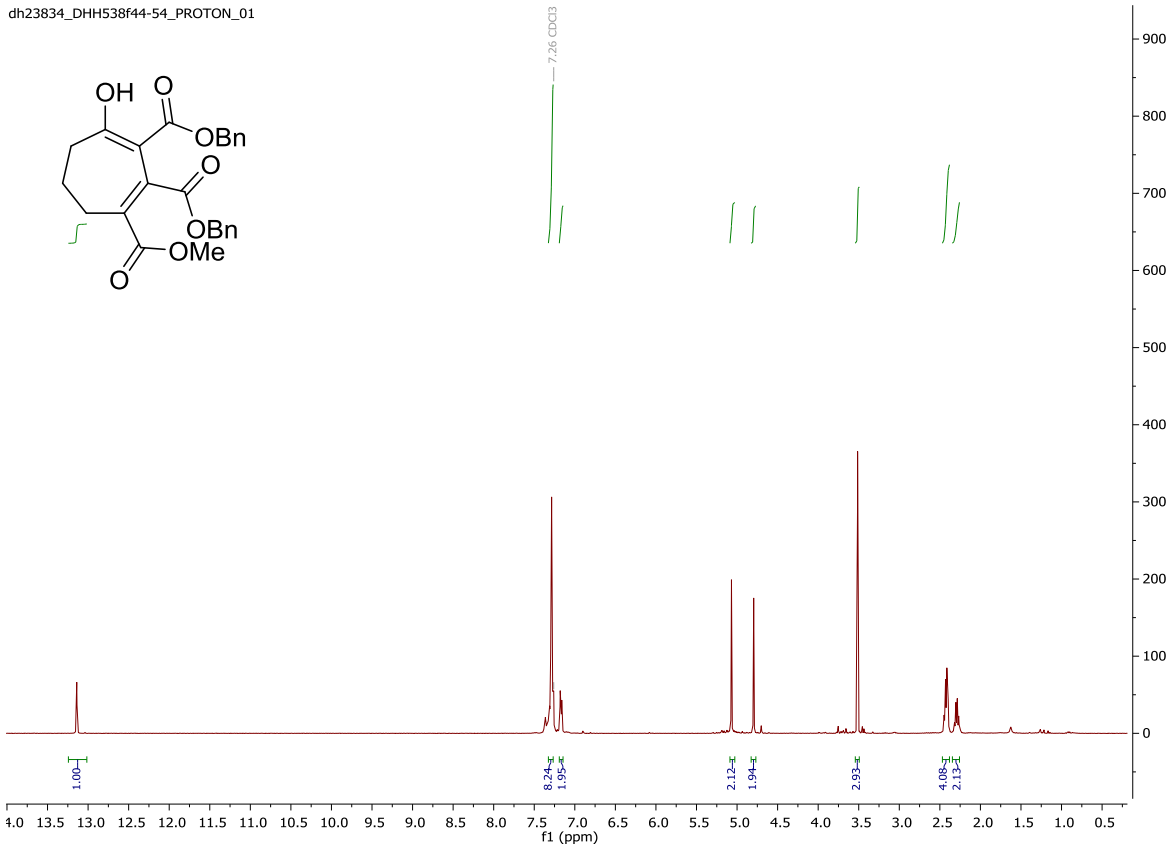


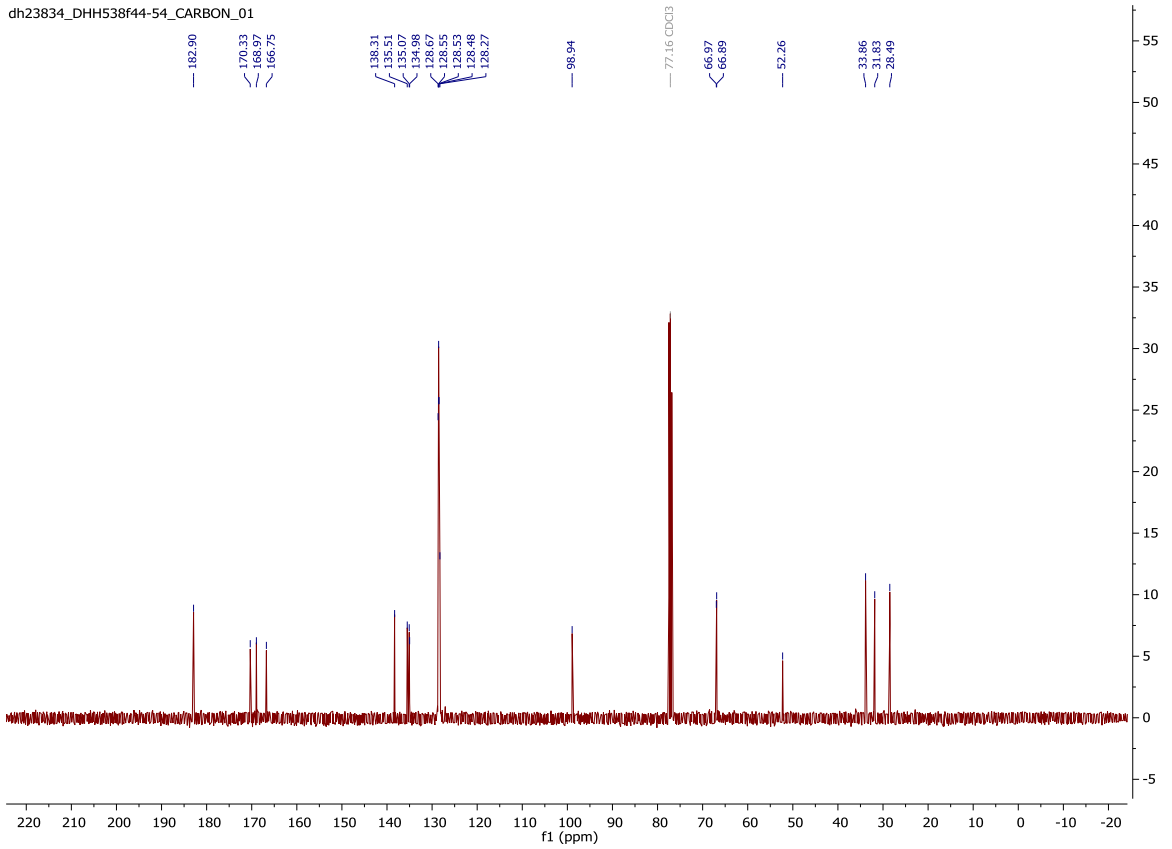
1-Oxo-2,2-diallyl-cyclohept-3-ene-3,4-dicarboxylic acid anhydride 266 (†)



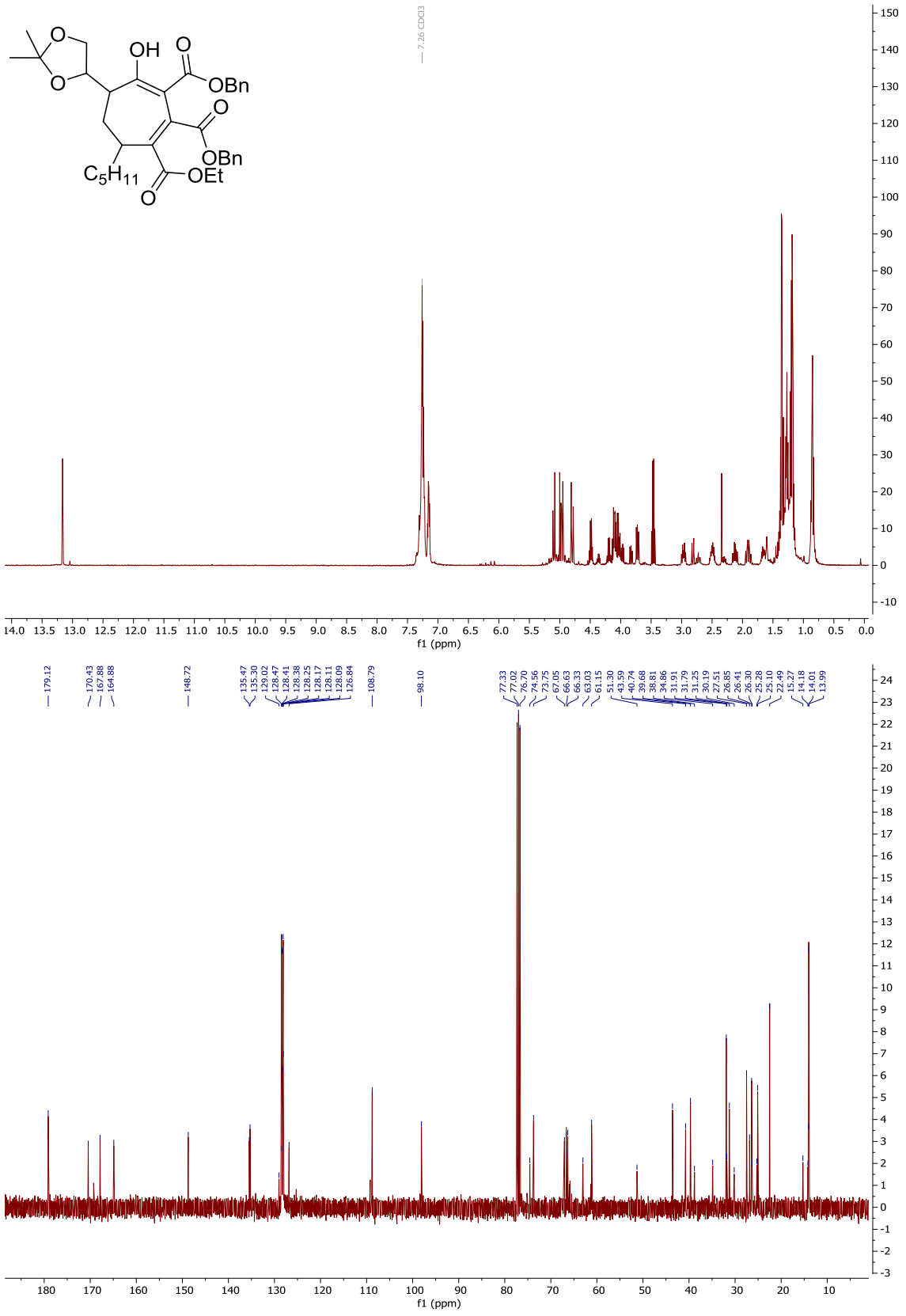


2,3-Dibenzyl 1-methyl 4-hydroxycyclohepta-1,3-diene-1,2,3-tricarboxylate 268



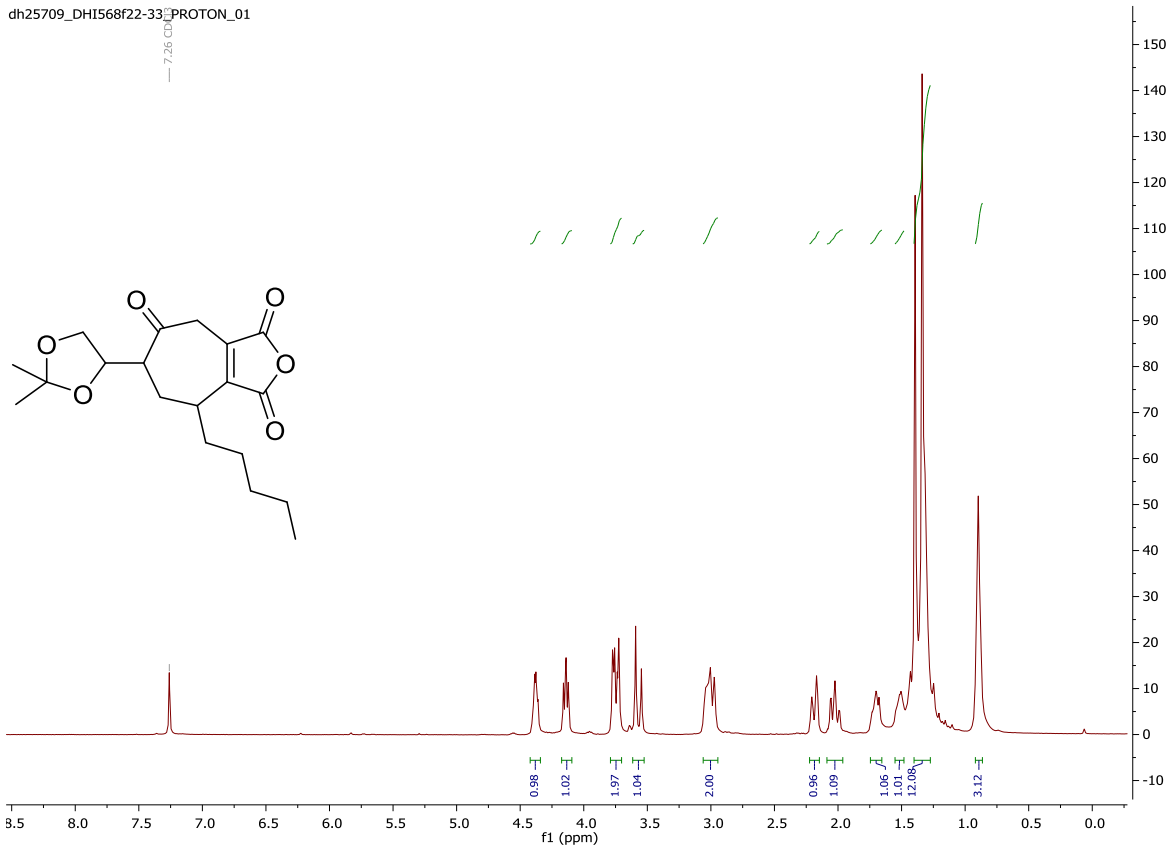


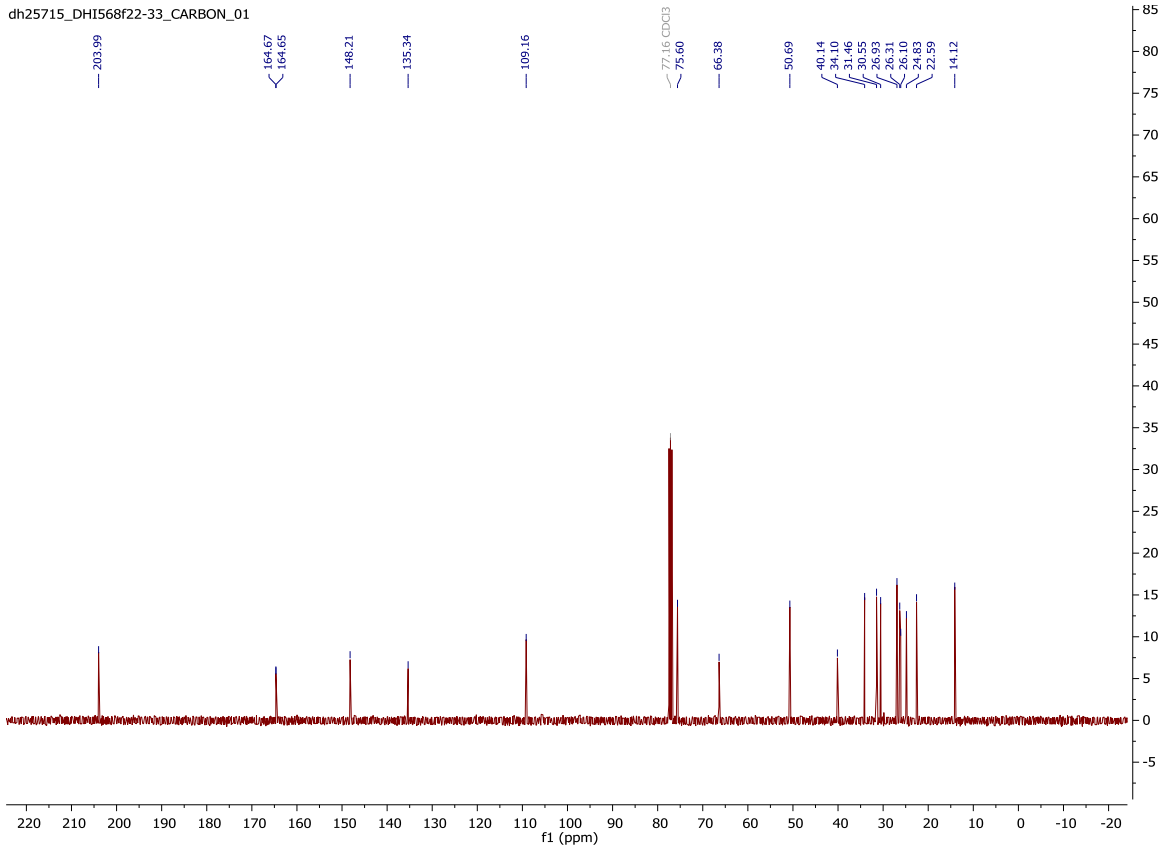
2,3-Dibenzyl 1-ethyl 5-(2,2-dimethyl-1,3-dioxolan-4-yl)-4-hydroxy-7-pentylcyclohepta-1,3-diene-1,2,3-tricarboxylate 269



1-Oxo-2-(2,2-dimethyl-1,3-dioxolan-4-yl)-4-pentyl-cyclohept-5-ene-5,6-dicarboxylic acid anhydride

270





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10. Publications from PhD studies

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The cycloaspeptides: uncovering a new model for methylated nonribosomal peptide biosynthesis†

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The cycloaspeptides are bioactive pentapeptides produced by various filamentous fungi, which have garnered interest from the agricultural industry due to the reported insecticidal activity of the minor metabolite, cycloaspeptide E. Genome sequencing, bioinformatics and heterologous expression confirmed that the cycloaspeptide gene cluster contains a minimal 5-module nonribosomal peptide synthetase (NRPS) and a new type of *trans*-acting *N*-methyltransferase (*N*-MeT). Deletion of the *N*-MeT encoding gene and subsequent feeding studies determined that two modules of the NRPS preferentially accept and incorporate *N*-methylated amino acids. This discovery allowed the development of a system with unprecedented control over substrate supply and thus output, both increasing yields of specific metabolites and allowing the production of novel fluorinated analogues. Furthermore, the biosynthetic pathway to ditryptophenaline, another fungal nonribosomal peptide, was shown to be similar, in that methylated phenylalanine is accepted by the ditryptophenaline NRPS. Again, this allowed the directed biosynthesis of a fluorinated analogue, through the feeding of a mutant strain. These discoveries represent a new paradigm for the production of *N*-methylated cyclic peptides *via* the selective incorporation of *N*-methylated free amino acids.

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Introduction

Cycloaspeptides are bioactive cyclic pentapeptides which were originally identified from an unclassified *Aspergillus* species in 1987.[1] Cycloaspeptides A–C **1–3** contain anthranilic acid (position p1), L-alanine (p2), L-phenylalanine (p3), L-leucine (p4) and L-tyrosine (p5) residues, and differ only in the *N*-methylation pattern of the phenylalanine and tyrosine moieties (Chart 1). A later screen of the *Aspergillus* genus failed to identify any cycloaspeptide producers, but multiple cycloaspeptide-producing *Penicillium* species have since been identified.[2–4] Cycloaspeptide D **4**, which contains a valine at p4, was isolated along with cycloaspeptide A **1** from *Penicillium ribeum* and *P. algidum*, in work which also identified these cycloaspeptides as being active against the malarial parasite *Plasmodium falciparum*.[3,4]

In 2006, cycloaspeptide E **5** was isolated as a minor metabolite from several *Penicillium* species and one *Trichothecium* species, and reported to exhibit insecticidal activity against lepidoptera.[5] **5** differs from **1** in having phenylalanine instead of tyrosine at p5 (Chart 1), and was proposed to have a neurotoxic mode of action. There is significant interest in natural products with insecticidal properties due to the huge economic and social burden created by insect damage to crop species worldwide.[6]

Further research into the bioactivity of cycloaspeptide E **5** has been hampered by the fact that yields are only approximately 2% of the main metabolite; cycloaspeptide A **1**, meaning that purification for bioactivity screening is problematic and the natural titres are too low to be commercially viable. Most recently cycloaspeptides F **6** and G **7** from the entomopathogenic fungus *Isaria farinosa*, have been isolated and characterized. These compounds have cytotoxic properties against tumour cell lines.[7]

In order to investigate cycloaspeptide biosynthesis, with the key aim of improving the yields of **5**, we set out to discover and manipulate the cycloaspeptide biosynthetic pathway, using two publicly available cycloaspeptide producers, *Penicillium soppii* (CBS 869.70) and *Penicillium jamesonlandense* (CBS 102888).[2]

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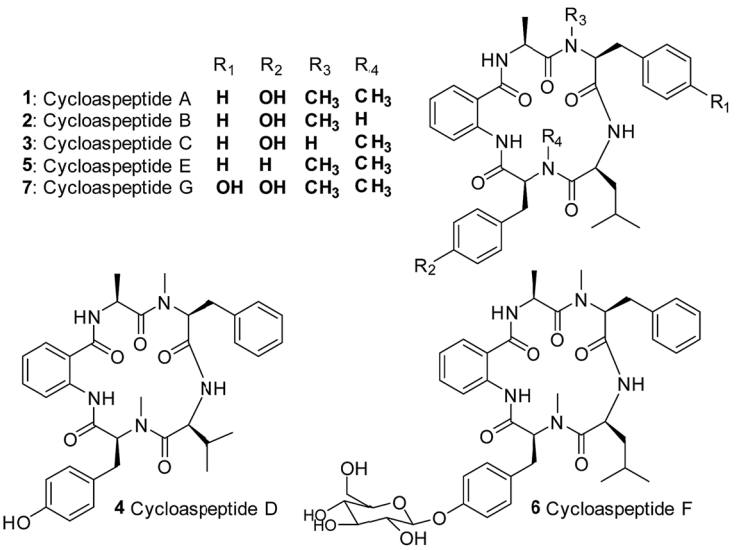
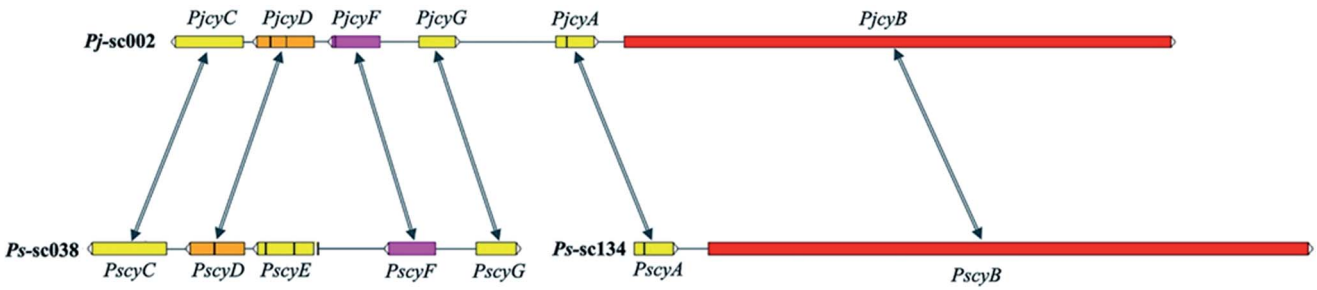


Chart 1 Structures of the cycloaspeptides – isolated from various filamentous fungi.

Our original working hypothesis was that the cycloaspeptides would fit the accepted paradigm for methylated cyclic peptide biosynthesis, and as such would consist of a traditional 5-module NRPS containing the two requisite *N*-methyltransferase (*N*-MeT) domains. We reasoned that substrate promiscuity of the NRPS could account for the range of cycloaspeptides reported, whereas we considered a RiPP system unlikely due to the fidelity of ribosomally synthesised peptides to their encoded precursor peptide sequence. A combination of approaches including bioinformatic analyses, heterologous expression, gene deletions and feeding experiments have demonstrated that indeed, an NRPS is responsible for the biosynthesis of the cycloaspeptides. However, rather than containing *N*-Met domains, the NRPS requires a pathway-specific *trans*-acting methyltransferase to provide the necessary substrates and preferentially incorporates methylated amino acids at two positions. This discovery unlocked a new avenue for pathway engineering through synthetic biology, by allowing unprecedented control over substrate availability.

Results and discussion

P. soppii and *P. jamesonlandense* were cultured on a range of solid media and screened by LCMS for the production of



P. jamesonlandense	*P. soppii*	Predicted gene function	% Identity
PjcyA	*PscyA*	N-methyltransferase (N-MeT)	91.35 %
PjcyB	*PscyB*	NRPS	89.26 %
PjcyC	*PscyC*	Aminotransferase	88.58 %
PjcyD	*PscyD*	Transcription factor	85.05 %
-	*PscyE*	Dehydrogenase	-
PjcyF	*PscyF*	Amino acid transporter	92.86 %
PjcyG	*PscyG*	Amidase	89.76 %

Fig. 1 Cycloaspeptide gene clusters from *P. jamesonlandense* (top) and *P. soppii* (bottom). The *N*-methyltransferase (*PjcyA* and *PscyA*) and NRPS (*PjcyB* and *PscyB*) genes are adjacent to one another in both species. Additional genes identified at the same locus in *P. jamesonlandense*, which could potentially be involved in secondary metabolite biosynthesis, such as an amino acid transporter and transcription factor were identified on a separate scaffold in *P. soppii*. Arrows denote homologous genes; vertical black bars within genes denote introns.

cycloaspeptides. A compound with the correct mass for cycloaspeptide A **1** could be reliably detected by LCMS, from both species and on all media after 2 weeks. This was confirmed by purification and NMR analysis (Table S8†). *P. soppii* was also grown in three different liquid media (CYB, MEB and YEB), and again **1** could be detected in all, with the highest concentrations being achieved with CYB (Fig. S21†). A compound with the correct mass for cycloaspeptide E **5** could be detected in certain cultures, but only in trace amounts and not reliably. Analysis using the highly sensitive UPLC-Orbitrap mass analyser system confirmed that the accurate masses matched the chemical formulae of the predicted compounds (Fig. S22 and Table S6†). The sensitivity of this system also allowed the detection of a compound with the correct predicted formula to be cycloaspeptide G **7**, a compound which has not been detected in a *Penicillium* species before.

Cycloaspeptide A **1** could also be easily detected by LCMS in MEB cultures of *P. jamesonlandense* (Fig. S23, S26 and Table S7†). There were also compounds present with the correct masses to be cycloaspeptides D **4**, E **5** and G **7** (Table S7†). Unfortunately, the yields of **5** were too low to purify for NMR and structural validation.

Paired-end genomic sequence data was generated for both species and assembled to produce draft genomes (Table S2†), which were analysed using AntiSMASH[8] to identify putative biosynthetic gene clusters (BGCs). A total of 82 and 83 BGCs were detected for *P. soppii* and *P. jamesonlandense* respectively, demonstrating the rich metabolic potential of these species (Table S3†).

P. soppii contains 20 NRPS clusters whereas *P. jamesonlandense* contains 17, with 8 such clusters being common to both species. The domain architecture of each predicted NRPS was analysed using the NCBI conserved domain database (CDD) (Table S4†). Only one was found to contain the five modules that would be expected for pentapeptides such as the cycloaspeptides (Fig. S7†). Unexpectedly this NRPS lacked any integral *N*-methyltransferase (*N*-MeT) domains, which appeared inconsistent with cycloaspeptide biosynthesis, as *N*-methylation, if present, is normally performed by such domains embedded within the NRPS. However, an adjacent gene present in both species is predicted to encode a methyltransferase, so might perform this activity *in trans*. Additionally, the NRPS appears to contain a final condensation-like (CT) domain, where rather than having the highly conserved HHXXXDXXS/T motif found in condensation domains, it has the modified motif SHXXXDXXS/T (5377SHAQYDGVS5385). CT domains are known to catalyse the macrocyclisation of cyclic peptides in filamentous fungi[9] – a role analogous to that of the final thiolesterase in bacterial cyclic peptide biosynthesis.

In *P. jamesonlandense*, adjacent to the NRPS and putative *N*-MeT encoding genes are various additional predicted genes that could potentially be involved in cycloaspeptide production. These encode an amino acid transporter, a Zn_2Cys_6 transcription factor and an aminotransferase. In *P. soppii* however, these genes are translocated on another scaffold (sc038, Fig. 1). It is still possible that they are involved in cycloaspeptide biosynthesis, as split secondary metabolite clusters have been observed in fungi multiple times.[10] However, it is also possible that the NRPS and *N*-MeT are the only proteins required for the production of cycloaspeptides.

Bioinformatic analysis was conducted for the adenylation (A) domains of the NRPS. This focused on the predicted presence of an anthranilic acid adenylating domain, due to their distinct nature.[11] The online NRPS predictor tool[12] was used to generate the 10 amino acid code for each domain (Table 1), and these

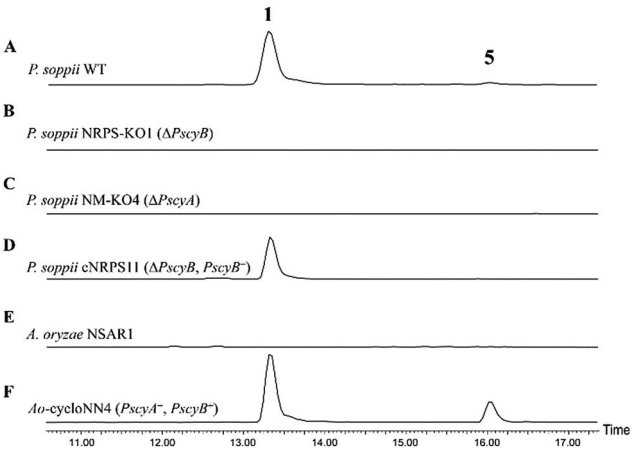


Fig. 2 ES+ extracted ion chromatograms for **1** ($[M + H]^+$: 642.7) and **5** ($[M + H]^+$: 627.7) in various fungal cultures: (A) wild-type *P. soppii* produces both **1** and **5**; (B) *P. soppii* Δ*PscyB* strain, NRPS-KO1, produces no cycloaspeptides; (C) *P. soppii* Δ*PscyA* strain, NM-KO1, produces no cycloaspeptides; (D) NRPS-KO1 complemented with a wild-type copy of the cycloaspeptide NRPS (Δ*PscyB*, *PscyB*+), demonstrates a restoration in cycloaspeptide biosynthesis; (E) wild-type *A. oryzae* NSAR1; (F) strain Ao-cycloasNN4, which is an *A. oryzae* NSAR1 transformant co-expressing *PscyA* and *PscyB*, produces both cycloaspeptide A and cycloaspeptide E.

Table 1 The 10 amino acid codes for the *P. soppii* cycloaspeptide NRPS (*PscyB*) A domains, analysed using the online tool NRPSpredictor2 [12]

Module	Pos1 (235)	Pos2 (236)	Pos3 (239)	Pos4 (278)	Pos5 (299)	Pos6 (301)	Pos7 (322)	Pos8 (330)	Pos9 (331)	Pos10 (517)	Predicted substrate
1	G	V	I	F	I	A	A	G	I	K	Ant
2	D	V	F	F	V	V	G	V	L	K	Ala
3	D	A	Y	A	V	G	G	I	C	K	Phe
4	D	L	M	L	V	G	A	V	I	K	Leu
5	D	A	Y	T	S	G	G	I	C	K	Tyr/Phe

were compared with those of known fungal NRPS enzymes.[11] The presence of a glycine in the first position of the 10 amino acid code for domain 1, rather than the aspartic acid typically seen, is indicative of domain 1 being an anthranilic acid adenylating domain. This is consistent with the fact that all known fungal anthranilic acid A-domains are positioned within the first module of the synthetase.[11] It then follows that the second module incorporates an alanine, the third a phenylalanine, the fourth a leucine, and the fifth, either a tyrosine (to produce **1**) or a phenylalanine (to produce **5**). Evidence supporting this assignment is the similarity of the third and fifth modules, which are predicted to incorporate Phe and Phe/Tyr respectively. In *P. soppii* these domains have 80% sequence similarity when comparing the 10 amino acid code, and 85% sequence similarity when comparing the 34 extracted residues. Interestingly, it was not possible to identify homologous gene clusters in publicly available fungal genomes, even when specifically searching *Aspergillus*, *Penicillium*, *Trichothecium* or *Isaria* genomes for homologues of *PscyA* and *PscyB*. This suggests that although the cycloaspeptides have been reported from various fungal genera, the gene cluster is not particularly wide-spread and cycloaspeptide biosynthesis may be limited to a fairly small fungal clade, or clades.

To allow an investigation of gene function in *P. soppii*, a protoplast-based transformation system was developed, using either hygromycin or geneticin resistance as selectable markers. This transformation system was optimized using eGFP as a reporter gene (Fig. S9†) and a bipartite knock-out strategy[13] was then used to disrupt specific genes. The disruption of either the NRPS (*PscyB*) or the *N*-MeT (*PscyA*) led to a complete loss of production of both cycloaspeptide A **1** and E **5**, (Fig. 2A–C) confirming that both genes are required for cycloaspeptide biosynthesis and that **1** and **5** are synthesized by the same pathway.

The disruption of the transcription factor (*PscyD*) led to a marked reduction in cycloaspeptide yields, suggesting that although it is translocated in *P. soppii*, it is still involved in the regulation of cycloaspeptide biosynthesis (Fig. S24†).

To further confirm the identity of the gene cluster and conclusively demonstrate that the loss of production was not an indirect consequence of the transformation system, strain NRPS-KO1 (Δ*PscyB*) was complemented by transformation with the plasmid pTYGen–*N*-MeT–NRPS which contains *PscyA* and *PscyB* under the control of P*adh* (the alcohol dehydrogenase promoter from *A. oryzae*) and P*tub* (the tubulin promoter from *P. soppii*) respectively. Selection of geneticin resistant

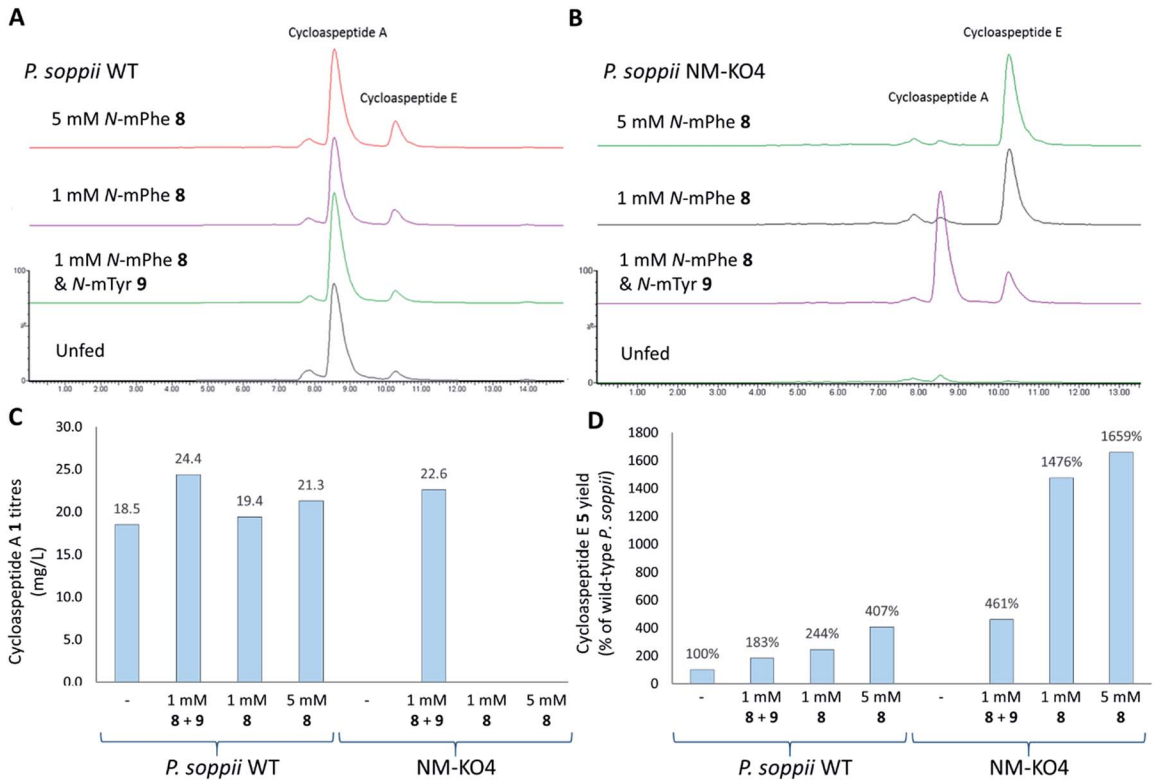


Fig. 3 Feeding *P. soppii* cultures with *N*-methylated phenylalanine **8** and *N*-methylated tyrosine **9** was shown to impact cycloaspeptide biosynthesis, increasing titres in wild-type *P. soppii* and restoring cycloaspeptide production in an *N*-MeT knock-out strain (NMKO4: Δ*PscyA*). (A) ES+ extracted ion chromatograms for cycloaspeptide A **1** ($[M + H]^+$: 642.7) and cycloaspeptide E **5** ($[M + H]^+$: 627.7). Wild-type cultures, from bottom to top: unfed control. Fed with 1 mM each of **8** and **9**. Fed with 1 mM **8**. Fed with 5 mM **8**. (B) NMKO4 (Δ*PscyA*) cultures, from bottom to top: unfed control. Fed with 1 mM each of **8** and **5**. Fed with 1 mM **8**. Fed with 5 mM **8**. (C) Absolute titres of **1** (mg L^{-1}) in various fed cultures. Some differences were observed for the fed wild-type cultures and complete restoration was seen for the *N*-MeT knock-out strain. (D) Relative titres of **5** (% of wild-type) in the various cultures demonstrating an increase in **5** with feeding, particularly in the knock-out strain NM-KO4. Quantification was not possible for **5** due to a lack of cycloaspeptide E standard, so relative titres were calculated.

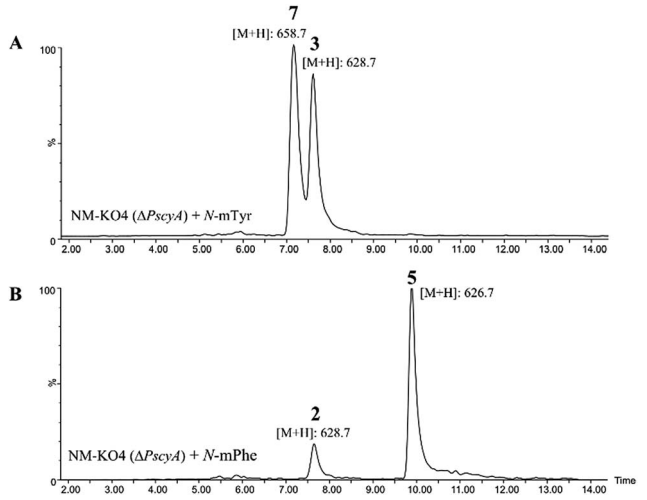


Fig. 4 (A) ES+ extracted ion chromatogram for masses of 628.7, which corresponds to cycloaspeptide C **3** ($[M + H]^+$) and 658.7 which corresponds to cycloaspeptide G **7** ($[M + H]^+$); (B) ES+ extracted ion chromatogram for masses of 628.7 which corresponds to cycloaspeptide B **2** ($[M + H]^+$) and 626.7 which corresponds to compound cycloaspeptide E **5** ($[M + H]^+$). These compounds are not present in NM-KO4 (Δ*PscyA*), unless cultures are fed with methylated amino acids.

transformants led to the restoration of cycloaspeptide production (Fig. 2D).

Heterologous production of both **1** and **5** was achieved in *Aspergillus oryzae* NSAR1 *via* the co-expression of *PscyA* and *PscyB* from a multi-gene expression vector with arginine prototrophy selection (Fig. 2E and F).[14] This demonstrates that *PscyA* and *PscyB* are the only two structural genes required for cycloaspeptide biosynthesis. Interestingly, the ratio of cycloaspeptide A **1** to E **5** appeared to differ in *A. oryzae* when compared to the natural producers, with cycloaspeptide E **5** being easily detectable in initial screens of the *A. oryzae* transformants. An apparent decrease in fitness and growth rate was observed in *A. oryzae* transformants producing cycloaspeptides, and production was not stable. A loss of production occurring after subculturing prevented quantification of titres. In an attempt to push the ratio of 5 : 1 further in favour of cycloaspeptide E **5** biosynthesis, a full A domain swap was conducted. The seemingly promiscuous A domain from module 5 (Tyr/Phe) was replaced with a second copy of the A domain from module 3 (Phe). This engineered NRPS was expressed both in *A. oryzae* and the *P. soppii* Δ*PscyB* strain but the NRPS was dysfunctional, with no cycloaspeptides being detected in either strain (data not shown).

To ascertain the order of events in the cycloaspeptide biosynthetic pathway, LCMS chromatograms for the *N*-MeT knock-out strains were searched for any putative un-methylated intermediates. The absence of any such compounds suggested that rather than the *N*-methylations serving to decorate the product of the NRPS, the *N*-MeT may act first, providing methylated amino acids for incorporation by the NRPS. To test this, *N*-MeT knock-out strain 4 (NM-KO4) was fed with 1 mM of both *N*-methylated tyrosine (*N*-mTyr **8**) and *N*-methylated phenylalanine (*N*-mPhe **9**). This fully restored cycloaspeptide biosynthesis (Fig. 3B), demonstrating that the NRPS accepts free methylated amino acids, a feature not previously observed in non-ribosomal peptide systems in either fungi or bacteria.

In further experiments, wild-type *P. soppii* and NM-KO4 cultures were fed with either a 1 : 1 mixture of *N*-mPhe **8** and *N*-mTyr **9** (1 mM each), or with *N*-mPhe alone (either 1 mM or 5 mM final culture concentration) (Fig. 3). Increases in the cycloaspeptide yields when the WT strain was fed with the *N*-methylated amino acids suggests that substrate availability is a limiting factor in this system (Fig. 3C). The yields of the cycloaspeptides in the fed NM-KOS cultures were most striking. In addition to producing wild-type yields of cycloaspeptide A **1**, cultures fed with both *N*-mPhe **8** and *N*-mTyr **9** produced over four times more cycloaspeptide E **5** than the unfed wild-type strain (Fig. 3D). When supplemented with either 1 mM or 5 mM *N*-mPhe **8** cycloaspeptide E **5** titres were increased further, to approximately 14.5 and 16.5 times respectively (Fig. 3D).

Such increased yields allowed purification of cycloaspeptide E **5** for structural confirmation by NMR (Table S9 and Fig. S23 and S24†). In addition to **5**, a compound could be detected in NM-KO4 cultures fed with *N*-mPhe **8** that has the expected mass and UV to be cycloaspeptide B **2** (Fig. 4). **2** was identified from the original *Aspergillus* sp. NE-45,[1] and has a methylated phenylalanine at p3, but an unmethylated tyrosine at p5. This compound has not been reported in *Penicillium* species before.

A minor metabolite with the correct accurate mass to be cycloaspeptide G **7**, which has methylated tyrosine at both p3 and p5, was detected in wild-type *P. soppii* cultures using an UPLC-Orbitrap mass analyser. To determine whether the titres of this compound could be increased, NM-KO4 cultures were fed with *N*-mTyr alone.

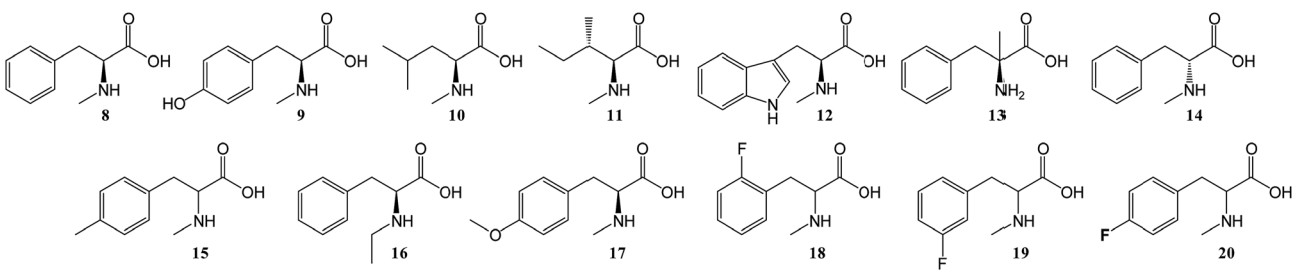
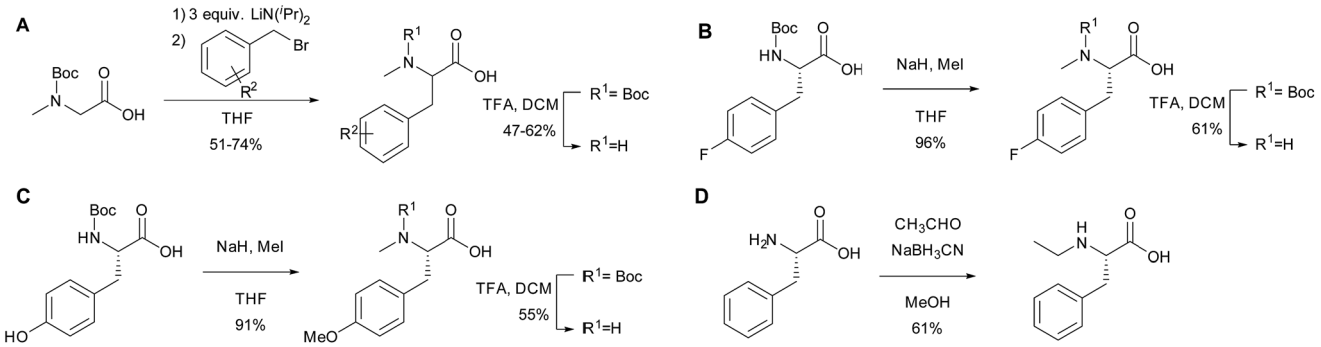


Chart 2 Various amino acid analogues fed to *P. soppii* NM-KO4 (Δ*PscyA*).



Scheme 1 The synthesis of amino acid analogues.

This resulted in the easy detection of **7** in all fed cultures using standard LC-MS methods (Fig. 4). Feeding with *N*-mTyr also led to the production of a compound not detected in the wild-type cultures which has the expected mass to be cyclo-aspeptide C **3. 3** has un-methylated phenylalanine at p3 and *N*-mTyr at p5. As with **5**, this compound has not been previously observed in *Penicillium* strains. These results indicate that the third and fifth modules of the NRPS have a strong preference for methylated amino acids, but also the ability to accept and incorporate un-methylated amino acids.

To further investigate the substrate selectivity of the NRPS, a range of alternative amino acids were fed to strain NM-KO4. *N*-Methyl-leucine **10**, *N*-methyl-isoleucine **11**, *N*-methyl-tryptophan **12**, α-methyl-phenylalanine **13** and *N*-methyl-D-phenylalanine **14** were obtained commercially (Chart 2). Racemic *p*-methyl-*N*-mPhe **15**, and a range of fluorinated *N*-mPhe analogues (with the fluorine at the *ortho*, *meta* and *para* positions **18–20**) were synthesised by alkylation of the Boc–*N*-methylglycine dianion with the appropriate benzyl bromides, followed by Boc deprotection with trifluoroacetic acid (TFA)

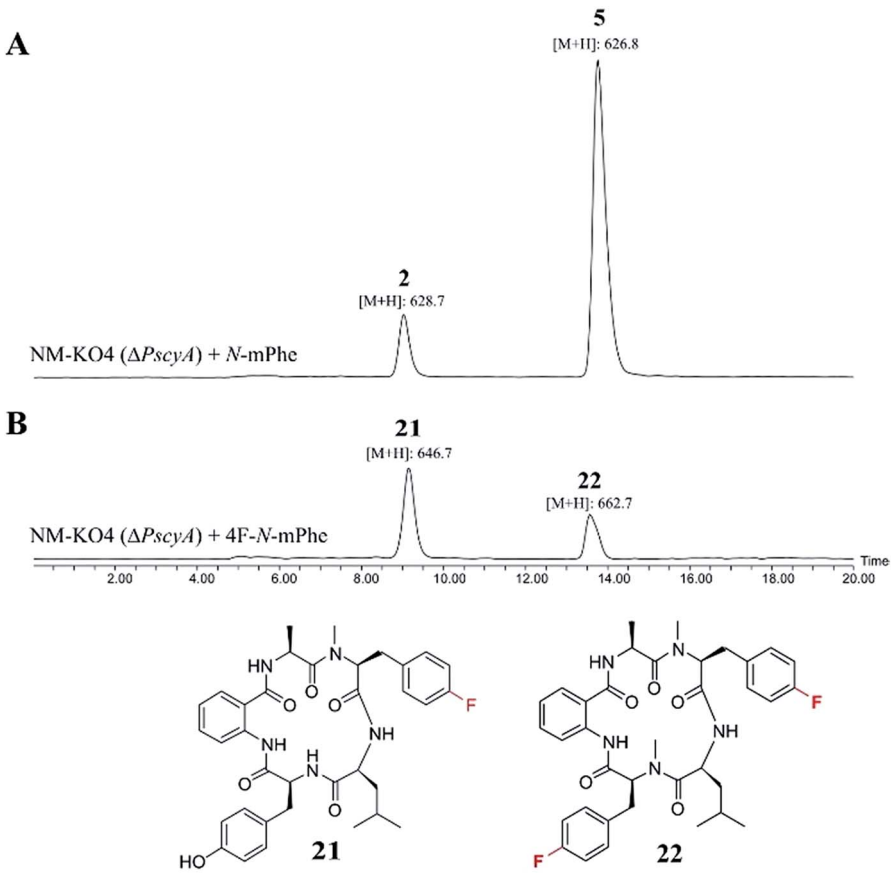


Fig. 5 ES+ extracted ion chromatograms ($[M + H]^+$) for cycloaspeptides B **2** (*m*/*z*: 628.7) and E **5** (*m*/*z*: 626.7), and their fluorinated counterparts **21** (*m*/*z*: 646.7) and **22** (*m*/*z*: 662.7). (A) NM-KO4 (Δ*PscyA*) cultures fed with *N*-mPhe **8**, produced **2** and **5**. (B) Cultures fed with 4F–*N*-Met–Phe **20**, produced **21** and **22**.

(Scheme 1A). *N*,*O*-Dimethyl-L-Tyr **17** was synthesised by double deprotonation of Boc-L-tyrosine with sodium hydride, followed by dimethylation with methyl iodide (Scheme 1C). Boc deprotection was again performed with TFA. *N*-Ethyl-L-Phe **16** was synthesised by reductive amination of L-phenylalanine with acetaldehyde and sodium cyanoborohydride (Scheme 1D).

These compounds were either fed alone (5 mM) or in combination with *N*-mPhe **8** (at a ratio of 5 : 1–5 mM : 1 mM). The only cultures to produce novel compounds in the initial screens were those supplemented with fluorinated phenylalanine analogues. The synthesis of 4F–*N*-mPhe **20** was therefore scaled up (see ESI†) and an LCMS analysis identified compounds with the correct masses to be fluorinated analogues of cycloaspeptide E **5**, and cycloaspeptide B **2** (Fig. 5). 4F–cycloaspeptide E **22** was purified and characterized using proton, carbon and fluorine NMR (Table S10 and Fig. S28–S30†). Purified **5** and **22** were screened for anti-lepidopteran activity using the tobacco budworm (*H. virescens*) as an industrially relavent target organism, but no activity was observed against this species following injection with either compound (Table S12†).

A search of the NCBI database for any potential *PscyA* homologues identified an *N*-MeT from *A. flavus* with 39% sequence identity to *PscyA* at the protein level (Table S5†). Investigating the genomic context of this gene determined that it is part of a BGC containing a 2 module NRPS and cytochrome P450, which has been identified as the ditryptophenaline **23** gene cluster by Watanabe and coworkers.[15] **23** is a dimeric diketopiperazine consisting of two cyclic Trp:*N*-Met-Phe dipeptides.

An *A. flavus* strain with the ditryptophenaline *N*-MeT disrupted (Δ*dtpB*) was kindly supplied by Prof. Kenji Watanabe of the University of Shizuoka, and feeding studies were conducted. Analogous to the cycloaspeptide system, feeding cultures with *N*-mPhe **8** fully restored ditryptophenaline **23** production, demonstrating the acceptance of **8** by the ditryptophenaline NPRS (*dtpA*). Furthermore, the production of a fluorinated ditryptophenylene analogue **24** was again achieved by feeding cultures with 4F–*N*-mPhe **20** (Fig. 6). Interestingly, feeding with *N*-mTyr **9** did not result in the production of hydroxylated analogues, demonstrating that the ditryptophenaline NRPS exhibits lower promiscuity than the cycloaspeptide NRPS. The

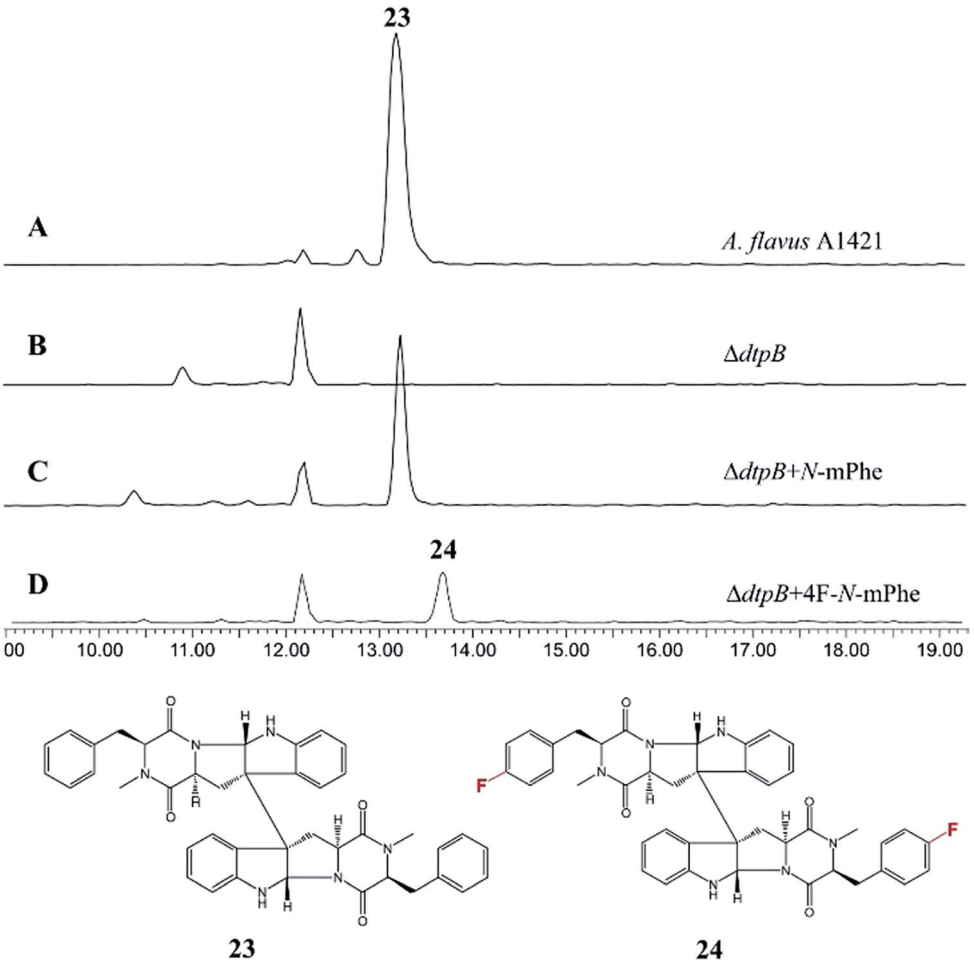


Fig. 6 HPLC ES+ spectrum for the crude extracts of various *A. flavus* cultures: (A) *A. flavus* strain A1421 produces ditryptophenaline **23**; (B) a Δ*dtpB* strain can no longer produce **23**; (C) feeding the Δ*dtpB* strain with *N*-methylated phenylalanine restores the production of ditryptophenaline **23**. (D) Feeding the Δ*dtpB* strain with a fluorinated phenylalanine analogue (4F–*N*-mPhe **20**) led to the production of a fluorinated ditryptophenaline analogue **24**.

rapid discovery of a second NRPS which accepts methylated amino acids suggests that these systems represent a new fungal route to methylated peptide natural products, rather than the cycloaspeptide system being unique.

Conclusions

The gene cluster responsible for the biosynthesis of the cycloaspeptides has been identified in two psychrotolerant *Penicillium* species: *P. soppii* and *P. jamesonlandense*. Heterologous expression in *A. oryzae* has demonstrated that the minimal gene set required to produce both cycloaspeptide A **1** and cycloaspeptide E **2** is a 5-module NRPS and a new type of pathway specific *N*-methyltransferase (*N*-MeT). Gene knock-outs and feeding studies have demonstrated that two modules of the NRPS preferentially accept and incorporate *N*-methylated amino acids, which are provided by the pathway specific *N*-MeT, a system not previously seen in secondary metabolism. Selective amino acid *N*-methyltransferases are very rare in either primary or secondary metabolism. The only known enzyme to catalyse a single *N*-methylation of a proteogenic amino acid is glycine *N*-methyltransferase from primary metabolism. The most similar known examples from fungal secondary metabolism are 4-dimethylallyltryptophan *N*-methyltransferase, which is the second pathway-specific enzyme in the production of ergot alkaloids,[16] and a methyltransferase from the ergothioneine pathway which trimethylates histidine.[17]

Deletion of the cycloaspeptide *N*-Met allowed the supply of methylated amino acids to the NRPS to be controlled, and this was exploited to direct biosynthesis towards specific minor metabolites, accomplishing the original aim of increasing cycloaspeptide E **5** yields. Feeding cultures with a fluorinated phenylalanine analogue also led to the production of novel fluorinated cycloaspeptides. The ditryptophenaline pathway from *Aspergillus flavus* was quickly confirmed as being a second example of such a system, suggesting that as more NRPS gene clusters are identified and characterised, further examples will be uncovered. Again, feeding a *N*-Met knock-out strain allowed the production of an unnatural ditryptophenaline analogue.

The preferential acceptance of methylated amino acids by an NRPS combined with the ability to remove the natural substrate supply provides a unique biotransformation opportunity over traditional feeding of modified amino acids. Firstly, methylated amino acids fed into the system have little competition from unmethylated cytoplasmic amino acids. Secondly, the incorporation will be more efficient because synthetic methylated amino acids are not at risk of being consumed by other cellular processes such as protein production.

The ability to produce natural product analogues is valuable due to the potential for altered bioactivities or pharmacokinetic properties. *N*-Methylated peptides are particularly desirable as they are known to often display improved stability over their non-methylated counterparts. Currently medicinal chemistry employs expensive and toxic reagents to synthesise *N*-methylated peptides. Consequently, new approaches for *de novo* biosynthesis of *N*-methylated peptides could be an attractive alternative to chemical synthesis. The ability to generate fluorinated natural product analogues is particularly relevant for natural product research due to their extreme rarity in nature (less than 0.005% of identified natural products contain fluorine[18]) combined with the various benefits observed with compounds containing one or more fluorine atom,[19,20] demonstrated by the fact that 15–20% of pharmaceuticals now contain at least one fluorine atom.[18] Such compounds could also be more amenable to semi-synthetic derivatization, which is a major route to drug development.

Using A-domains which accept methylated amino acids in domain swaps could also be used to introduce methylated amino acids, or unnatural analogues such as the fluorinated amino acids into other NRP natural products of agricultural of pharmaceutical interest. Indeed, there have been some major advances in NRPS structural biology and engineering recently,[21–23] which means that the prospect of swapping in domains/modules to introduce *N*-methylated amino acids into existing NRPs could become feasible. Detailed *in vitro* studies of the *N*-MeT enzyme and the *N*–Me amino acid activating A-domains and modules, including crystallography, could help to elucidate where the selectivity and specificity of such systems lie, and the roles that the individual NRPS domains play in controlling the output of such systems. In the longer term, such an understanding could help guide engineering to alter the substrate specificity of these systems, enabling methylation and incorporation of a wider range of *N*-methylated amino acids. Also, further genome sequencing and mining could lead to the discovery of other *N*-MeT and NRPS that incorporate different *N*-methylated amino acids in nature. Taken together, such studies could provide a set of *N*-Met enzymes and NRPS domains/modules for engineering the *de novo* biosynthesis of *N*-methylated peptide 'non-natural' products through the assembly of novel chimeric NRPS.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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