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Synthetic studies towards the oxylipin 3-(*R*)-HEPE

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Organic synthetic chemistry
Faculty of Chemistry, Biotechnology and Food Science

Once, contented
by being worthy of your destiny
you shall know: This was my will,
All that happens to me happens justly.
Then say, when the green woods
of your joy for life has been wandered through:
I want nothing different,
I wish nothing changed.

Amor Fati, André Bjerke

Acknowledgments

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Ås, May 2017

Fredrik Garnås Rylandsholm

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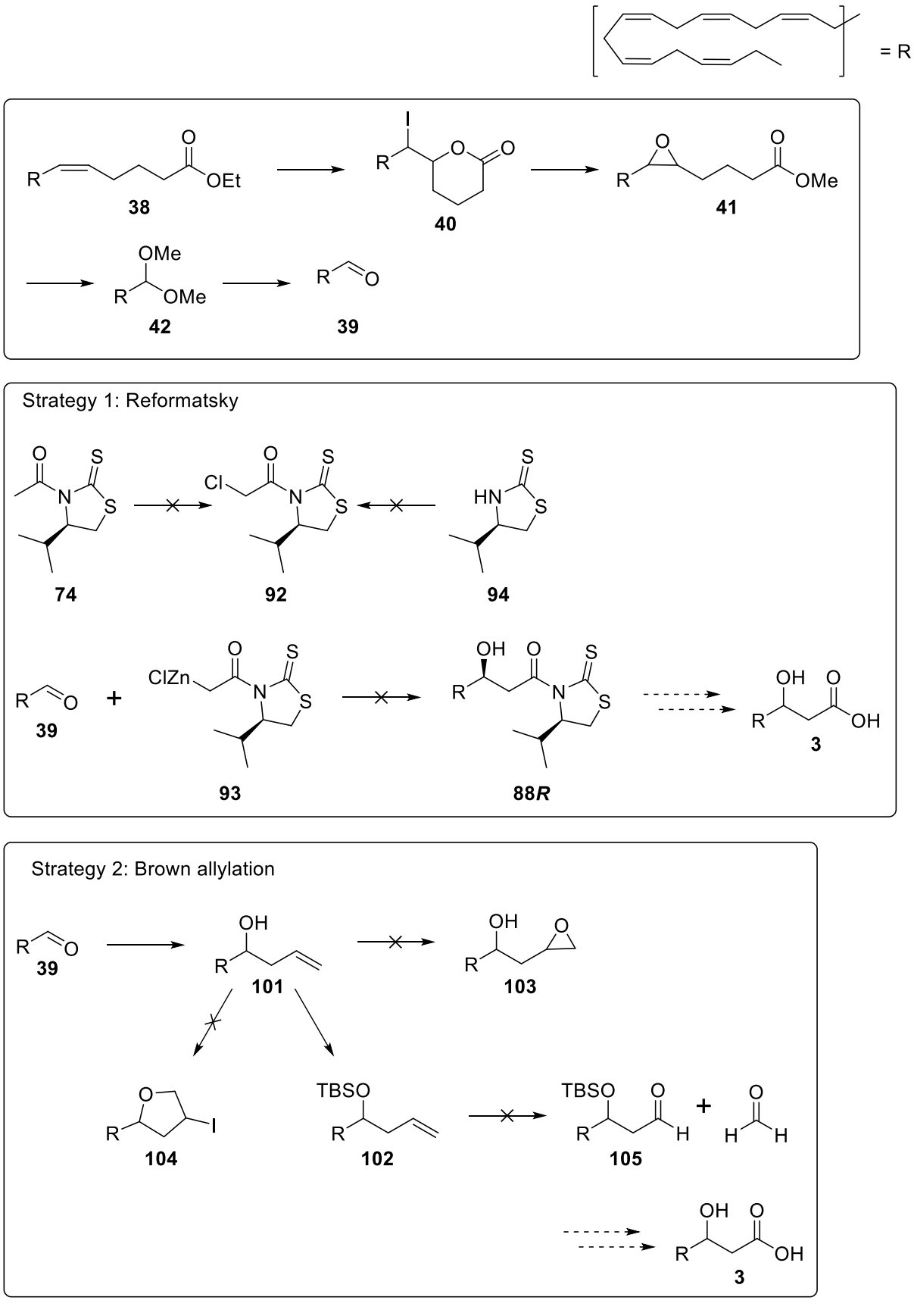
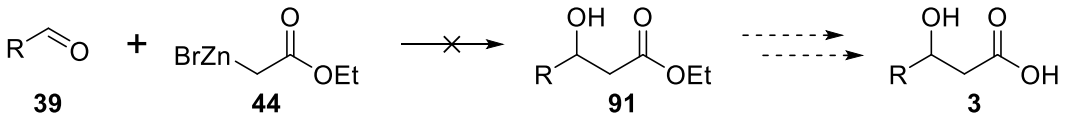
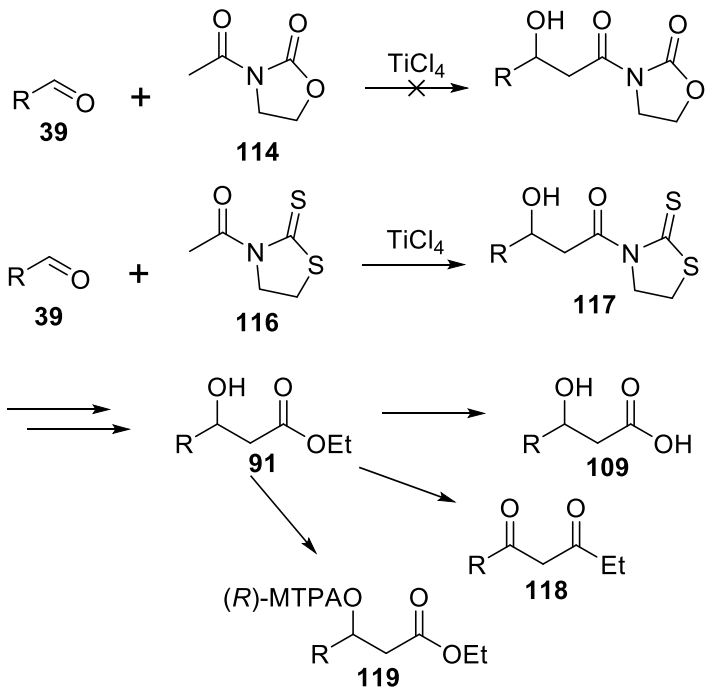
Graphical abstract



Strategy 3: Racemic Reformatsky



Startegy 4: Racemic Aldol



Abstract

As a consequence of the increased interest in natural products from fungal origin, it has been discovered that 3-(*R*)-hydroxilipins play a crucial role in how the yeast *Candida albicans* develop. The yeast is behind more than 70% of candidemia isolates in Norway, it is the fourth most common pathogen isolated from blood cultures in the United States of America, and rank among the ten most common pathogens in Europe. Efficient methods of synthesising the target of this project, the hydroxylipin 3-(*R*)-HEPE (**3**), and thereby enabling biological testing, is deemed important in figuring out more efficient ways to treat fungal infections.

It was postulated that the target material could be synthesised through a Reformatsky reaction. The crucial auxiliary **92** could however not be produced in the laboratory, so the strategy was abandoned.

A non-chiral Grignard reaction was chosen as a means to extrapolate a protocol from the aldehyde produced from DHA-EE (**39**) to 3-HEPE (**109**). The protocol could then be used on the product from an asymmetric Brown allylation reaction, to create the target material. Though several strategies from the Grignard product **101** was attempted, no way could be found, and the strategy was abandoned.

The racemic mixture of 3-HEPE (**109**) was necessary to check the exact stereochemistry of the asymmetric product. The non-chiral version of the Reformatsky reaction, without any auxiliary, was chosen. The product from the reaction proved hard to purify, and spectral data indicated that many very similar by-products were created.

As the asymmetric aldol reaction had proven to be an efficient method towards 3-(*R*)-HEPE (**3**), the non-chiral version with a non-chiral auxiliary was chosen. The cheaper oxazolidinone **114** did not produce the aldol product with $TiCl_4$ as chelating agent, but the corresponding, more expensive thiazolidinethione **116** worked under the same conditions. The ethyl ester of 3-HEPE (**91**) was then synthesised without any major obstacles. The ethyl ester **91** was split into three portions: One portion was used to create the target material **109**, which could not be completely purified due to lack of time and small amounts of material; another portion was used to create the β-keto ester **118** for biological testing; the third portion was used to create

the Mosher's ester **119**, which was used to confirm the exact stereochemistry of 3-(*R*)-HEPE (**3**).

Sammendrag

Som en konsekvens av økt interesse innenfor naturprodukter fra sopp, har det blitt oppdaget at 3-(*R*)-hydroksylipiner spiller en viktig rolle i hvordan gjærsoppen *Candida albicans* utvikler seg Gjærsoppen står bak omtrent 70% av alle candidemi-isolater på norske sykehus, er den fjerde oftest isolerte patogenet i Amerikas forente stater, og er listet som et av de ti mest vanlige patogener i Europa. Effektive metoder for å syntetisere målmolekylet for dette prosjektet, hydroksilipinet 3-(*R*)-HEPE (**3**), og dermed tilrettelegge for biologiske tester, regnes som viktig for å finne bedre måter å bekjempe soppinfeksjoner.

Det ble foreslått at målmolekylet kunne syntetiseres gjennom en Reformatsky-reaksjon. Den nødvendige hjelpegruppen **92** kunne ikke syntetiseres, til tross for flere forsøk, så en ny strategi måtte velges.

Valget falt på en akiral Grignard-reaksjon, som skulle brukes for å lage en protokoll fra aldehydet av DHA-EE (**39**) til 3-HEPE (**109**). Protokollen skulle så kunne benyttes til en asymmetrisk Brown allylerings-reaksjon for å danne målmolekylet **109**. Selv om flere strategier fra Grignard-produktet **101** mot 3-HEPE (**109**) ble forsøkt, ble det ikke funnet en god metode. Strategien ble derfor valgt bort.

For å bekrefte den eksakte stereokjemien til en asymmetrisk reaksjon, skal også den tilsvarende rasemiske blandingen syntetiseres. En akiral Reformatsky-reaksjon, uten hjelpegruppe, ble valgt. Resultatet av reaksjonen viste seg å være vanskelig å rense, samt at spektraldata indikerte at biprodukter som liknet på Reformatsky-produktet ble dannet.

Siden den asymmetriske aldol-reaksjonen hadde vist seg å fungere på systemet, ble en akiral versjon med en akiral hjelpegruppe valgt. Det billigere oksasolidinonet **114** viste seg å ikke hjelpe i reaksjonen med $TiCl_4$ som kileringsreagens, men det korresponderende, dyrere tiazolidinetionet **116** fungerte under de samme betingelsene. Etylesteren av 3-HEPE (**91**) ble syntetisert uten særlige problemer. Etylesteren **91** ble splittet i tre porsjoner: Én ble brukt til å danne målmolekylet **109**, men med begrenset tid og mengde materiale ble det ikke funnet en god måte å rense det på; neste porsjon ble brukt til å danne β-ketoesteren **118** til biologiske tester; den siste porsjonen ble brukt til å danne den korresponderende Mosher-esteren (**119**). Sistnevnte ble brukt til å stadfeste absoluttkonfigurasjonen til 3-(*R*)-HEPE (**3**).

Contents

Abbreviations

3-(*R*)-HEPE	(3*R*, all-*Z*)-3-hydroxyicosa-5,8,11,14,17-pentaenoic acid
AA	Arachidonic acid
BINAL-H	1,1'-bi-2,2'-naphtol-lithium aluminium hydride complex
CDI	1,1′-Carbonyldiimidazole
COX	Cyclooxygenase
CYP	Cytochrome P450
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DHA	Docosahexaenoic Acid, ((all-*Z*)-4,7,10,13,16,19-docosahexaenoic acid)
DIPEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMP	Dess-Martin Periodinane, 1,1,1-Triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1H)-one
ee	Enantiomeric excess
EPA	Eicosapentaenoic Acid, ((all-*Z*)-5,8,11,14,17-eicosapentaenoic acid)
EDTA	Ethylenediaminetetraacetate
HETE	(5*S*,6*E*,8*Z*,11*Z*,14*Z*)-5-Hydroxyicosa-6,8,11,14-tetraenoic acid
LDA	Lithium Diisopropylamine
LOX	Lipoxygenase
LT	Leukotrienes
MPM	Methylbenzyl
MTPA	Mosher's acid, α-methoxy-α-(trifluoromethyl) phenylacetic acid
PG	Prostaglandins
PGI	Prostacyclins
PMB	*para*-methoxy benzyl
PUFA	Polyunsaturated Fatty Acid
TBAF	Tetrabutylammonium flouride
TBHP	*Tert*-butyl hydroperoxide
TBSOTf	*Tert*-butyldimethylsilyl Trifluoromethanesulfonate
TBDPS	*Tert*-butyldiphenylsilyl
TMS	Trimethylsilyl
TX	Thromboxanes
Vanadyl acac	Vanadyl acetoacetonate

1. Introduction

1.1 General background and goal

During the last century, natural products from mammalian cells and marine environments have been extensively studied.[1-2] However, less attention has been given to fungal compounds. The yeast *Candida albicans* and its morphogenesis, meaning structural development, has recently been shown to be dictated by 3-(*R*)-hydroxylipins.[3-4] 3-(*R*)-hydroxylipin inhibitors have therefore been proposed as a lead compound for developing new drugs against vaginal candidasis.[4] Our group has worked on polyunsaturated fatty acids, such as (all-*Z*)-4,7,10,13,16,19-docosahexaenoic acid (DHA, **1**) and (all-*Z*)-5,8,11,14,17-eicosapentaenoic acid (EPA, **2**),[5-10] and metabolites derived from them. DHA (**1**) is the starting material for the hemi-synthesis of (3*R*, all-*Z*)-3-hydroxyicosa-5,8,11,14,17-pentaenoic acid (3-(*R*)-HEPE, **3**), and synthesising this natural product is this project's target.

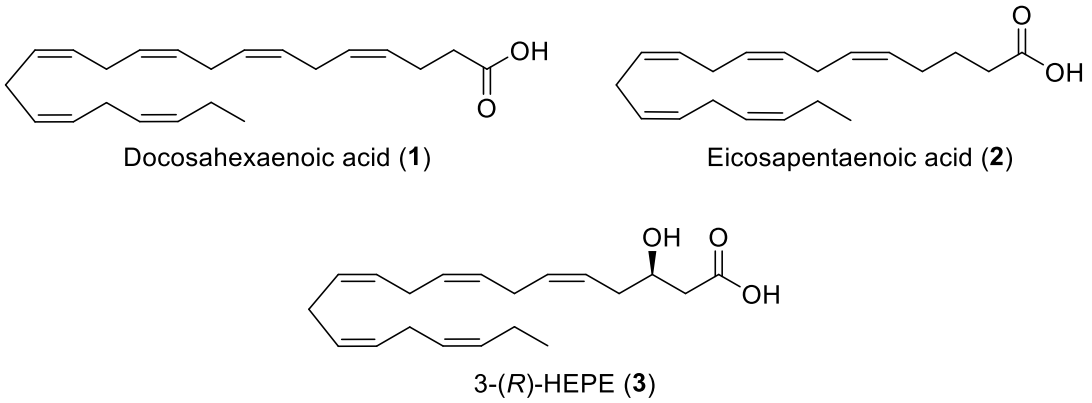


*Figure 1: The structures of DHA (**1**), EPA (**2**), and 3-(R)-HEPE (**3**).*

1.1.1 Natural products

Natural products chemistry is the study of chemicals produced by living organisms. Natural products are typically divided into primary and secondary metabolites. Primary metabolites are directly involved in normal growth, development, and reproduction, making them key components in primarily maintaining normal, physiological processes. Meanwhile, secondary metabolites are often more specialised compounds found only in a small selection of species or cells.[11] With their specialised properties, secondary metabolites are good candidates for clinical drugs.

Currently, one third of clinically-used drugs originate from natural products.[12] These drugs are either native-, precursors of-, or modified natural products. Natural products span from

simple compounds to complex structures, such as proteins. Well-known examples include morphine (**4**), penicillin G (**5**), salicylic acid (**6**), and botulinum toxin A (**7**).

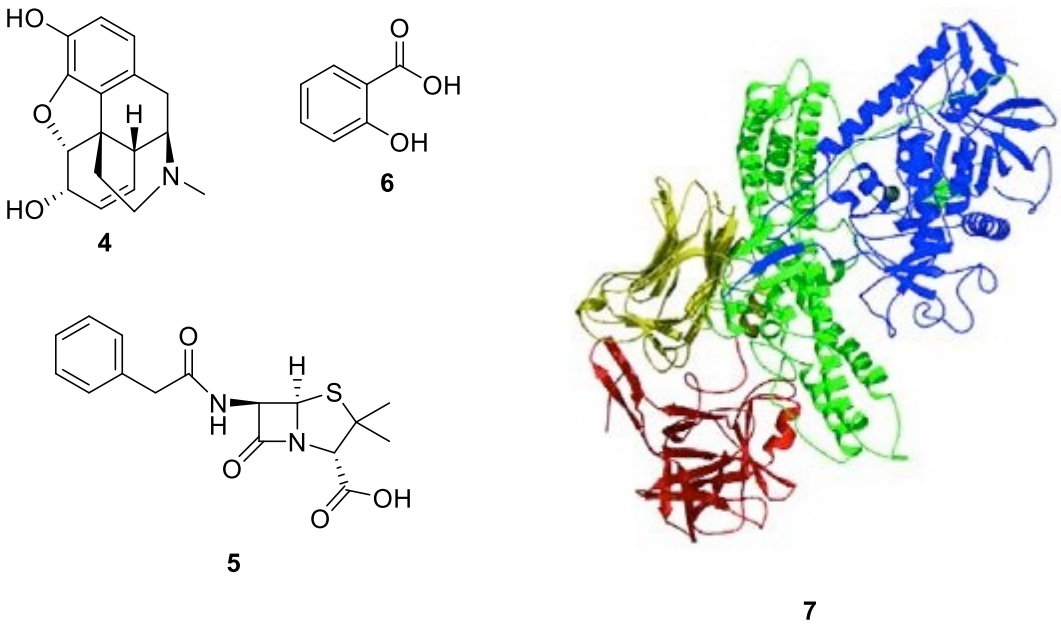


Figure 2: Structures of compounds 4-7. Botulinum toxin A structure from reference 13.

There are several ways to mass produce natural products and their derivatives. Morphine (**4**) is produced commercially by extraction from the opium poppy[14], penicillin G (**5**) from the *Penicillium* fungi[15], and Botulinum toxin A (**7**) from the *Clostridium botulinum* bacteria,[16] the latter two by fermentation.

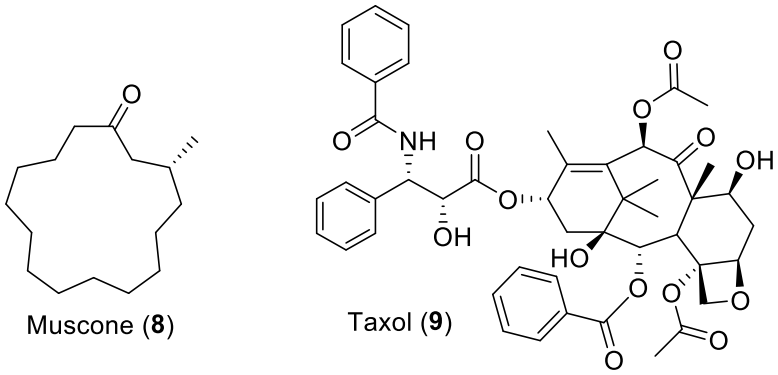
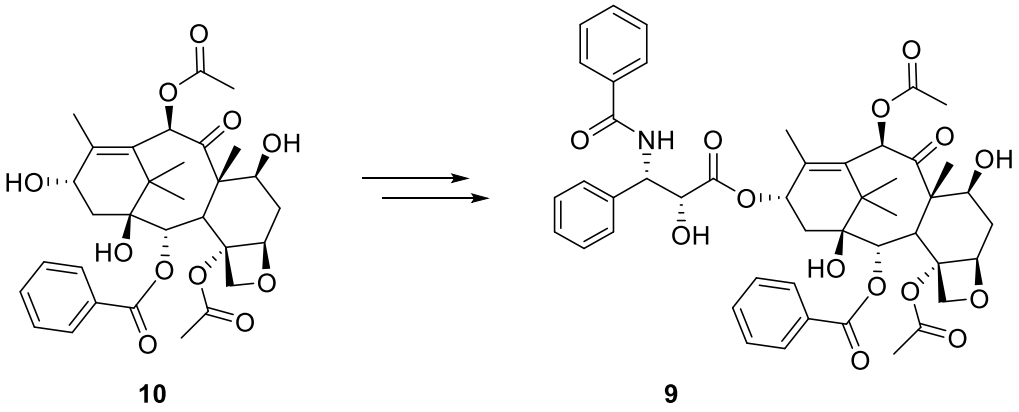


*Figure 3: The structure of muscone (**8**) and taxol (**9**); two natural products that are synthetically manufactured, due to their presence in miniscule amounts in endangered species. [17-22]*

For other natural products, extraction is unfeasible. The species they derive from can be listed as endangered animals or plants, or the natural product is present in such minuscule amounts that any commercial production would be impractical.[23] Muscone (**8**), a noticeably important chemical in both perfume- and medicinal industry, is found in the endangered musk deer.[18-22]

The anticancer therapeutic drug taxol (**9**) is found in the bark of the Pacific yew (*Taxus brevifolia*).[17] Extraction requires removing the bark, killing the tree in the process. Therefore, efficient hemi-syntheses of taxol (**9**) were found necessary for industrial large-scale production, to overcome natural limitations. The industrial production of taxol (**9**) is a hemi-synthesis from the natural product baccatin III (**10**).[24]



10 **9**

*Scheme 1: The hemisynthetic route to taxol (**9**) from the natural product baccatin III (**10**).[24]*

As with both muscone (**8**) and taxol (**9**), laboratory syntheses are the only viable option in making the required amounts of many natural products; both in the early stages of profiling and clinical tests, and in the commercial production of the drugs. The complex structures of many other natural products also make total synthesis impractical or expensive. Instead, a hemi-synthesis or a synthesis of structurally simpler analogues are chosen. The latter solution is preferred, as natural products only can be viewed as prototypical drug candidates, while their analogues can be designed to yield higher efficacy than the original.[25]

Sometimes natural products are inadequate in their original form, so they are modified into more biochemically active derivates. For instance, salicylic acid (**6**) is extracted from the willow tree (*Salix alba*), and then acetylated into aspirin (**11**). Although salicylic acid (**6**) is the active form of the drug, aspirin (**11**) more easily enters the blood stream.

As many natural products stem from fatty acids, they are a point of interest. Even though several fatty acids are found throughout most species as components of cell membranes or used as energy storage, chemists are more interested in the bioactive fatty acids.

1.1.2 Fatty acids

There are more than 1 000 known, natural fatty acids, where about 20-50 are of common interest.[26] Most natural fatty acids, whether they are saturated or unsaturated, are straight-chained compounds with an even-number of carbon atoms. Chain lengths can be as short as three carbon atoms or as long as 80, but lengths between 12 and 22 are commonplace. It is noteworthy that uneven-numbered chains do occur, as in C_{17} heptadecanoic acid (**12**), and branched chains are found in fatty acids such as isopalmitic- (**13**) or isononadecanoic (**14**) acid. Furthermore, fatty acids can also be cyclic, as in sterculic- (**15**) or chauloorgic acid (**16**).

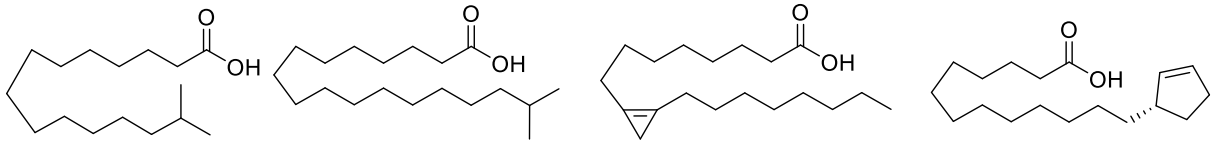


Figure 4: The structure of a variety of unusual lipids.

If only one unsaturated centre is present, it is normally a *Z* double bond, either 9 carbon atoms from the carboxylic acid (Δ9) or the terminal methyl (n-9 or ω-9).[26]

Most fatty acids do not contain extra functional groups, other than the carboxylic acid. However, some acids contain a fluoro group or oxygen based functionalities such as hydroxy, epoxy, keto, or ether groups (most commonly methoxy or furanyl groups).[26]

1.1.3 Oxygenated fatty acids

Ricinoleic acid (12*R*-hydroxyoleic acid, **17**), the main component (90%) in castor oil (*Ricinus communis* seed oil), is the best known natural hydroxy acid. Both the acid and the oil are widely used in cosmetics, lubricants, and as a drying oil (dehydrated castor oil, DCO).

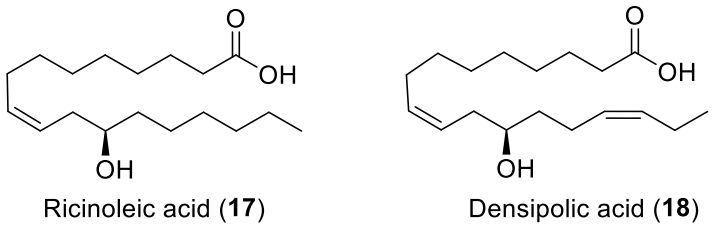
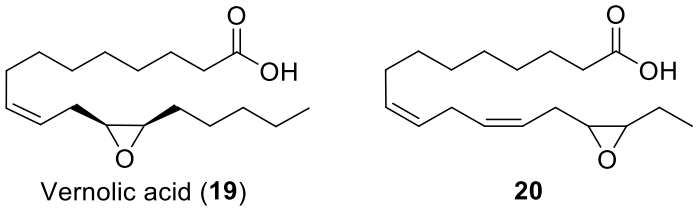


*Figure 5: Structure of the hydroxy acid rinoleic acid (**17**), and the hydroxy PUFA densipolic acid (**18**).*

Many epoxy acids are known. Vernolic acid (**19**) is the most famous, discovered in 1954. It is abundant in different seeds. Consequently, *Vernonia anthelimintica* (70-75% Vernolic acid (**19**)) and *Euphorbia lagascae* (60-65% Vernolic acid (**19**)) are pursued as commercial crops.

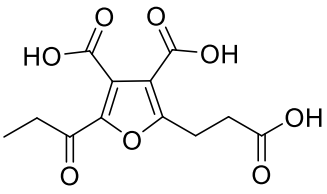
On prolonged seed storage, unsaturated acids may become oxidised into optically active forms via enzymatic oxidation.



Vernolic acid (**19**) **20**

*Figure 6: Structure of the epoxy acid vernolic acid (**19**), and an epoxy PUFA.*

Natural furanoid acids have been identified at low levels in fish oils. The proportion of acids, including breakdown products from vegetable sources, are increased upon fasting. Short-chained urofuranic acids (from urea), have been identified in animal blood and urine. [26]



The urafuranoic acid **21**

*Figure 7: The structure of the urafuranoic acid **21**.*

Keto acids often come as α-, β-, or γ-keto acids. Especially the α-keto acids are important in enzymatic transamination (α-ketoglutarate (**22**)),[27] the Krebs cycle (oxaloacetic acid (**23**)), and as an energy source and for fatty acid synthesis in the liver.[28]

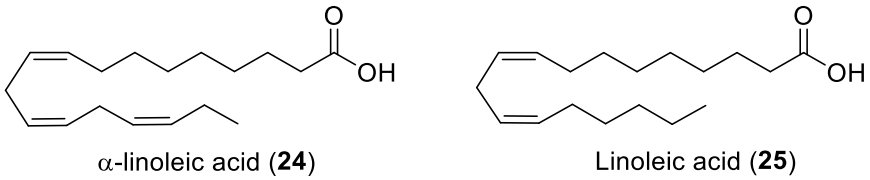
α-ketoglutarate (**22**) Oxaloacetic acid (**23**)

*Figure 8: Structure of the two α-keto acids α-ketoglutarate (**22**) and oxaloacetic acid (**23**).*

1.1.4 Polyunsaturated fatty acids (PUFAs)

PUFAs are usually arranged with all-*Z* skipped double bonds and methylene-groups between them.[26] PUFAs are divided into families, where they are grouped together with the most biosynthetically similar fatty acids. The two major families are based upon the ω-3 or ω-6

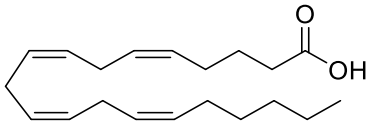
acids, α-linoleic acid (**24**) and linoleic acid (**25**), respectively. The prominent PUFAs have 2-6 double bonds.



α-linoleic acid (**24**) Linoleic acid (**25**)

*Figure 9: structure of the ω-3 and ω-6 acids, α-linoleic acid (**24**) and linoleic acid (**25**).*

C_{18} PUFAs are universally found in most vegetable oils, while C_{16}, C_{20} (such as EPA (**2**)), and C_{22} (such as DHA (**1**)) acids are found in fish oils and the lipids of mammals. As such, arachidonic acid (AA ,**26**) is only a minor compound in fish oils but show up in phospholipids in eggs and livers from other animals. Though uncommon in the plant kingdom, AA (**26**) is expressed in mosses, ferns, and some algae and fungi. [26] AA (**26**) is also a PUFA of considerable importance as the precursor of many important C_{20} metabolites such as prostaglandins, thromboxanes, and leukotrienes.



Arachidonic acid (**26**)

Figure 10: Structure of the C_{20} arachidonic acid.

1.1.5 Eicosanoids

Eicosanoids is a term covering polyunsaturated fatty acid derivatives originating from C_{20} fatty acids.[29] Functioning as signalling molecules, they are important in a variety of diverse physiological and pathological systems, such as inflammation, regulation of cell growth, controlling blood pressure, immune responses, inflammation, cell proliferation, and angiogenesis.[30]

The eicosanoids are generated through three different enzymatic pathways, governed by three classes of enzymes: Cyclooxygenase (COX 1 and COX 2), lipoxygenase (LOX) and epoxygenases, known as cytochrome P450 (CYP) isoforms.[31] Important metabolites produced by these enzymes are leukotrienes (LT), prostaglandins (PG), including prostacyclins (PGI), and thromboxanes (TX).[30]

The prostaglandins have their name because they were first found in the prostate glands and seminal plasma of humans.[32-34] Likewise, thromboxanes were found in platelets (thrombocytes) and leukotrienes in leukocytes. Scientists first thought they were all only present in the tissue they were originally discovered, but they are now known to be present in every tissue in animals.

The eicosanoids are denoted as a four-character abbreviation composing of: The eicosanoid's two-letter abbreviation, as described above; an A-B-C-… sequence letter; a number (subscript or plain script) signifying the number of double bonds. Examples include PGE_1 (Prostaglandin E_1, **27**), PGI_2 (Prostacyclin I_2, **28**), TXA_2 (Thromboxane A_2, **29**), and LTB_4 (Leukotriene B_4, **30**).

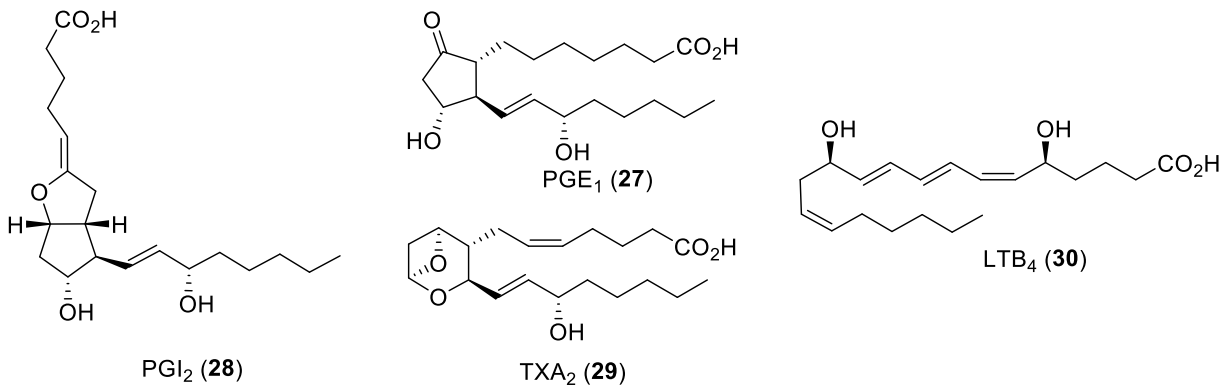


Figure 11: The structure of a variety of eicosanoids.

As with other chemicals, prostaglandins have different effects in different tissues, depending on the receptors in that particular tissue. The prostaglandins can act both as paracine (locally active) and autocrine (acting on the same cell from which it is synthesised) factors. The thromboxanes are vasoconstriction mediators, and the prostacyclins are active in the inflammation resolution phase. The prostaglandins are synthesised in the early stages of inflammation and are essential for controlling blood flow and stimulating neutrophil influx to the tissue. The other oxygenated lipid mediators take over during the later stages of inflammation.[35]

von Euler observed that prostaglandins could affect the contraction and relaxation of muscles.[33] In 1971 it was discovered that aspirin-like drugs could inhibit the biosynthesis of prostaglandins.[36] Following this research, several prostanoids are sold commercially as

pharmaceuticals. PGE_2 (**31**) is sold as a pharmaceutical to induce labour under different brand names such as cervidil, prepidil, and prostin E_2; $PGF_{2\alpha}$ (**32**), known pharmaceutically as dinoprost, is used for the same purpose, and also as an abortifacient;[37] latanoprost (**33**) is used to control the glaucoma progression;[38] PGI_2 (**28**), marketed as epoprostenol, reduces blood pressure and inhibits platelet aggression.

1.1.6 Candida albicans

The yeast *Candida* is a part of the normal flora on the human mucous.[39] More than 80 different *Candida* species exist, where nine can give sickness in people. *Candida albicans* is the most common among them, behind about 70% of candidemia (hospital-acquired bloodstream infections) isolates in Norway. In the United States of America, candidemia is the fourth biggest pathogen isolated in blood cultures,[40-41] and it ranks among the ten most common pathogens in Europe.[42]

Figure 12: A colony of Candida albicans *with yeast-like cells on the edges and filaments in the middle.*[43]

Most common are oral or genital infections.[39] The infection does not spread between people, usually not even between sexual partners upon genital infection. However, mothers can infect their children upon birth if the infection is vaginal, as infants have underdeveloped immune systems. Infants are often infected with oral yeast, which is normally called thrush. Infants with thrush can infect their mothers' breast buds through breast-feeding.[44]

Equal to infants, other people with reduced immunity resistance are particularly prone to become infected by *C. albicans*; including people under antibiotic- or steroid treatment, sick with diabetes or HIV, or pregnant women. In these people, *C. albicans* can spread from the mouth to the stomach, and from there reach the blood stream to infect vital organs such as the lungs, kidneys, or the brain. If the infection is untreated, it can threaten a weakened host's life.

Several creams and medicaments are available to treat both outbursts and chronical infections,[39] but with increased need of new and improved methods for defeating infections, we must look to *C. albicans*' biochemistry. The target molecule for this thesis, 3-(*R*)-HEPE (**3**), is believed to be vital for *C. albicans*' morphogenesis.[4] Therefore, developing a remedy based on the target molecule is of interest towards treating *Candida* infections.

1.1.7 Morphogenesis

Development biology describe how plants and animals develop and grow,[45] including their morphogenesis. Morphogenesis specifically tells us about how cell cultures distribute in the growth phase. In small cell cultures, such as yeasts or tumours, the morphogenesis may be induced by substrates produced by other organisms or by mechanical stress, among diverse factors.[46]

Introduced factors that largely increase the danger of *C. albicans* spreading include central venous catheters (allows the yeast directly into the blood stream), application of broad-spectrum antibacterials (allows for fungal overgrowth), and trauma or gastrointestinal surgery (disrupts the mucosal barriers, the body's natural defence).[47]

C. albicans coexist in three different states: a budding yeast, parallel-walled filaments (hypha), and elongated ellipsoid cells with constrictions at the septa (pseudohyphae).[48-50] Slight changes in temperature, pH, CO_2, or nutrients can cause change between the different states.[51] All three states also have their own advantages and drawbacks.[50, 52-53] The yeast is effortlessly carried within the bloodstream, as clumps of stem-cells can switch off their cell-to-cell adhesion, making it possible to migrate to other areas and thereby spread the infection. Hyphae cells are more invasive, and are thought to be important in tissue penetration, attack on organs, cell survival, and avoiding the body's cleaners, the macrophages. Following, the yeast and hyphae are both observed during infection, but the role of the pseudohyphae is so far unknown, as it has not been observed in patient samples.[54-55]

C. albicans has a set of proteins called adhesins, that are specialised to mediate adherence to other *C. albicans* cells, abiotic surfaces, and host cells.[46] These proteins are important in the starting phase of the biofilm production. The biofilm can be produced on both biologic cell surfaces, such as mucosal cell surfaces, and abiotic surfaces, such as catheters and dentures.[56]

The biofilm is formed gradually: Yeast cells adhere to the surface, hyphae form, extracellular matrix material accumulates, and finally new yeast cells disperse from the biofilm complex.[57] When the biofilm is fully evolved, several factors come in to increase the resistance to internal and external impact, for instance physical shield, enzymes, …[46]

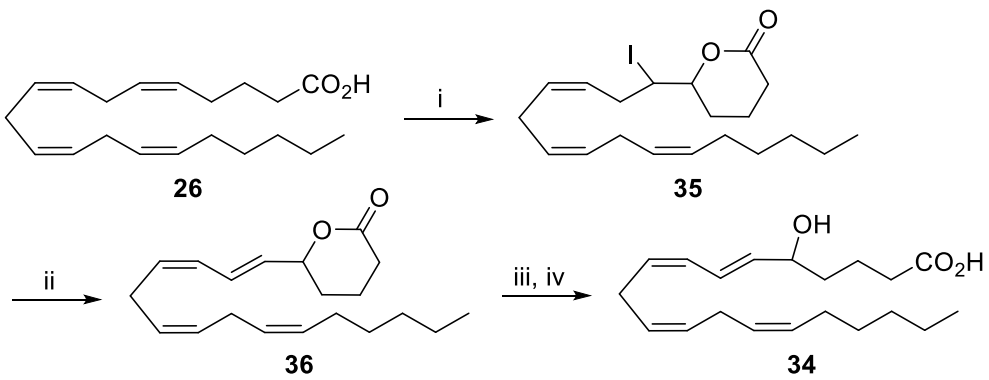
As a pathogen, *C. albicans* use two different strategies:[46] Induced endocytosis or active penetration. The former use specialised proteins (invasins) expressed on the cell surface to bind to host ligands and thereby get engulfed in the host cell. In contrast, hyphae actively penetrate barriers.

1.2 Chemical background

1.2.1 Hemi-synthesis of PUFAs

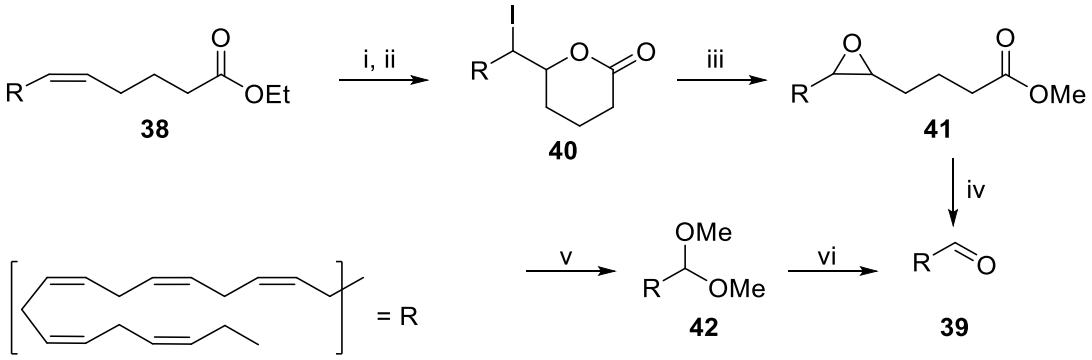
PUFAs are fairly complex molecules with respect to regio- and stereoisomers of the double bonds. PUFA metabolites even more so. Therefore, it is more practical, and increasingly efficient, to use available PUFAs, such as AA (**26**), EPA (**2**), DHA (**1**), … The core structure is kept, but the structure is elongated or degraded, while keeping all, or some, of the double bonds unaltered.

Corey *et al*. developed the iodolactonisation protocol in their hemi-synthesis of (±)-5-HETE (**34**).[58] In their protocol, the iodolactone of AA (**35**) was treated with DBU in benzene to eliminate iodine and provide the tetraene lactone (**36**). The lactone **36** was then transformed into the methyl ester of 5-HETE (**37**) with Et_3N in MeOH. Hydrolysis in basic environment yielded the target molecule **34**.



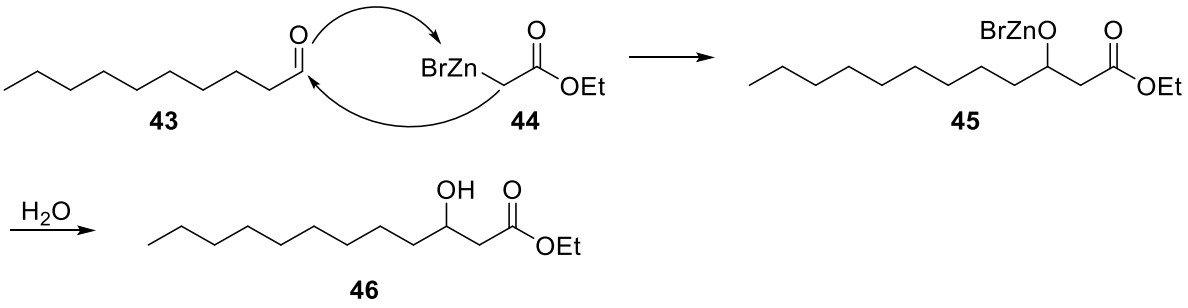
*Scheme 2: The synthesis of (±)-5-HETE (**34**).[58] Reagents and conditions: (i) KI, I_2, $KHCO_3$, H_2O, THF, 0°C; (ii) DBU, PhH; (iii) Et_3N, MeOH; (iv) LiOH, DME, H_2O.*

An alternative iodolactinisation protocol can forge the ethyl ester from DHA (**38**) into the corresponding aldehyde (**39**), as seen in Scheme 3.[59-60] The iodolactone achieved from DHA (**40**) is treated with potassium carbonate in methanol, creating the corresponding epoxy methyl ester (**41**). Oxidative cleavage of the epoxide (**41**), employing periodate in dry ethanol, yield the aldehyde (**39**). As the aldehyde (**39**) is very sensitive, the epoxide (**41**) can instead be treated with periodate in wet MeOH, yielding the protected aldehyde; the acetal (**42**).



*Scheme 3: The synthesis of the DHA aldehyde **39**.[59-60]Reagents and conditions: (i) LiOH, EtOH/H_2O (1:1, v/v), rt; (ii) HI, KI, $KHCO_3$, I_2, THF, H_2O, 0^O-4^O C; (iii) K_2CO_3, MeOH, rt; (iv) H_5IO_6, Et_2O; (v) H_5IO_6, MeOH, rt 6h; (vi) Dioxane, Formic acid, rt 1.5 h.*

1.2.2 Reformatsky



*Scheme 4: A representation of the Reformatsky reaction between the aldehyde **43** and the Reformatsky reagent **44**.[61] First, the carbanion next to the zinc performs a nucleophilic attack on the carbonyl, followed by two reactions where the zinc ends up at the oxygen. When water is added, the reaction is quenched, and the preferred alcohol (**46**) is formed.*

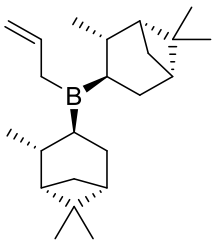
The Reformatsky reaction is very reminiscent of the Grignard reaction, in that a metal is dissolved in ether to form a reactive product, such as the Reformatsky reagent **44**. Apart from the different metal in complex, the Reformatsky reaction is notably milder, and more selective towards aldehydes or ketones, without forming enolates.[62] The Reformatsky reaction is less nucleophilic than the Grignard, so reactions with esters do not occur, contrary to the Grignard.

The Reformatsky reaction has been known for about 130 years, but the stereoselective version is not widely used. However, recently a micro review paper has been published, indicating the reaction's value in asymmetric reactions.[63]

A potential problem with the Reformatsky reaction is that excess zinc can work as a Lewis acid. Any problems met could potentially be fixed by work-up with a buffer to strictly control pH, such as phosphate buffer, and a chelating agent that bind any excess zinc, such as ethylenediaminetetraacetate (EDTA).

1.2.3 Brown allylation

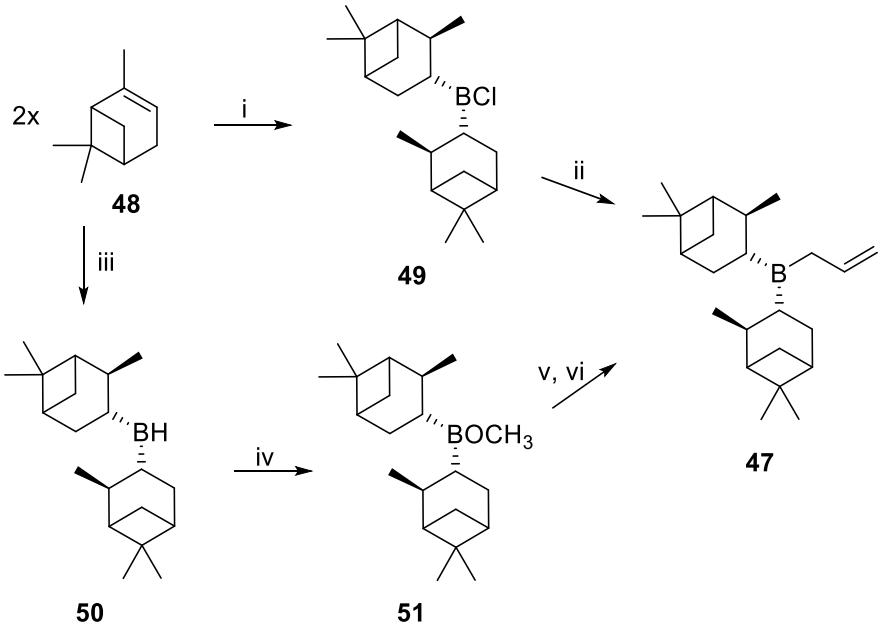
When H. C. Brown published his Ph.D. thesis in 1938, he started a long career studying boranes for organochemical purposes.[64] As boron reagents were quite expensive at the time, and only produced two places in the world, the interest and practicality of the reagent was relatively low for quite some time.



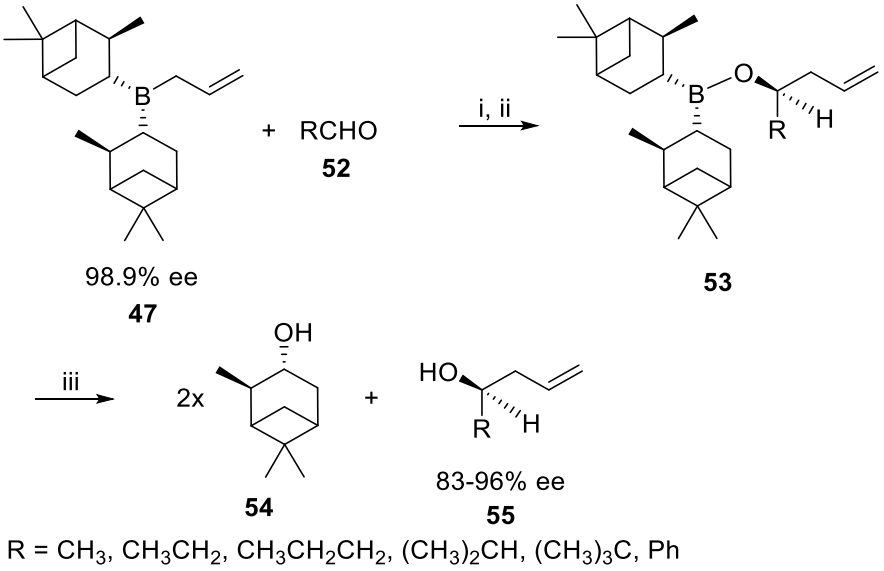
(+)-B-Allyldiisopinocampheylborane (**47**)

Figure 13: One diastereomer of the chiral reagent reported by Brown & Jadhav in 1983.[65]

The interest picked up after a while, and around 1980, several publications reported diverse chiral bromo-reagents for asymmetric allylation.[65-66] In 1983, Brown and Jadhav reported a chiral borane auxiliary for asymmetric allylation (Figure 13).[65] With commercially available reagents, simple preparation, and a convenient one-pot allylation reaction, the proposed auxiliary is highly preferable. To prepare *B*-allyldiisopinocampheylborane (**47**), α-pinene (**48**) is first treated with a boron donor, followed by allyl magnesium bromide in a Grignard reaction (as depicted in Scheme 5). Brown and his colleague reported that the auxiliary **47** was able to produce a secondary homoallylic alcohol from various aldehydes, with a purity in the range of 83-96% enantiomeric excess (*ee*), without depending on the aldehyde's nature. Brown continued to work on the chiral auxiliary throughout the 80s.[67-68]



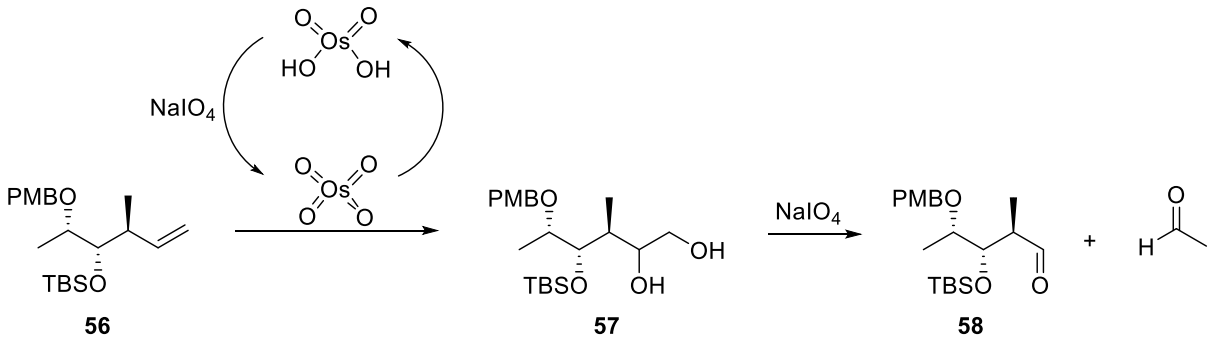
Scheme 5: Preparation of B-*allyldiisopinocampheylborane (**47**).[65] Reagents and conditions: (i) $H_2BCl{\cdot}OEt_2$, Et_2O, 0^OC; (ii) Allyl MgBr, -78^OC; (iii) $H_3B{\cdot}SMe_2$, THF, 0^OC, 72 h; (iv) MeOH, 0^OC, 1 h; (v) Allyl MgBr, -78^OC-25^OC; (v) 25^OC, 1h.*



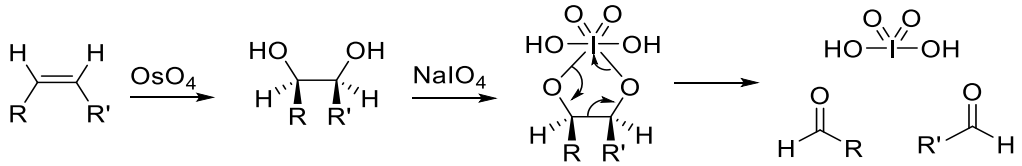
Scheme 6: Asymmetric brown allylation.[65] Reagents and conditions: (i) -78^OC. 1 h; (ii) -78^OC-25^OC; (iii) $NaOH/H_2O_2$.

1.2.4 Oxidation

An oxidative cleavage of a double bond can be performed by a Lemieux-Johnson oxidation. Here, two steps are carried out in a one-pot manner. Osmium tetroxide reacts with an olefin and create a dihydroxide (Scheme 7), which is cleaved by periodate to make two aldehydes (Scheme 8).[69] Excess periodate regenerate osmium tetroxide, allowing the strong oxidant to be present in catalytic amounts.



*Scheme 7: Lemieux-Johnson oxidative cleavage of an olefin (**57**) in a reaction where ozonolysis have no effect.[70] Reagents and conditions: OsO_4 (cat.), $NaIO_4$ (excess), 2,6-lutidine, Dioxane/H_2O, 1 h.*



Scheme 8: Oxidative cleavage using periodate as an oxidative agent.[71-72]Scheme supplementary to Scheme 7.

A problem with the Lemieux-Johnson oxidation reaction is low yields due to many by-products.[70] The yields can be improved by adding non-nucleophilic bases, such as 2,6-lutidine, in small amounts. For the reaction described in Scheme 7, the yield was improved from 44% to 83% by utilising this exact base.

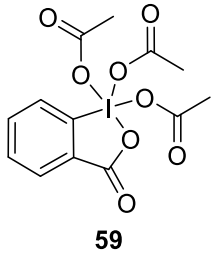
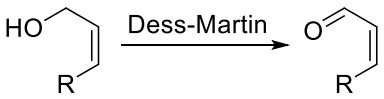


*Figure 14: Dess-Martin Periodinane (DMP, **59**). An iodine(V)-complex for mild oxidation.*

There are many diverse oxidation reactions to convert a secondary alcohol into a ketone. When performing an oxidation on a large and complex system, both selectivity and sensitivity must be addressed. DHA-derivates are relatively sensitive systems, so harsh oxidation techniques are disadvantageous. For this reason, the classic Jones oxidation with chromium is omitted. A good choice is therefore Dess-Martin oxidation: A mild technique employing Dess-Martin Periodinane (DMP, **59**) as the oxidising agent.[62] The iodine(V)-complex will oxidise alcohols into carbonyls, even alcohols that are prone to other reactions, for instance elimination. As an example, few other oxidising agents can yield the *cis*-α,β-unsaturated

aldehyde from a *cis*-allylic alcohol, without producing the *trans*-isomer, or some other by-product.



Scheme 9: Dess-Martin oxidation of a cis-allylic alcohol to a cis-α,β-unsaturated aldehyde.[62] Dess-Martin is one in a few oxidation methods that can perform this reaction, without producing the trans-isomer, or other by-products.

1.2.5 Aldol condensation

Since its discovery in the last half of the 19th century,[73-74] the aldol condensation reaction has become a convenient carbon-carbon bond-forming reaction. Base or acid in catalytic amounts is used to transform a carbonyl group in a ketone or aldehyde into the corresponding enolate. The enolate in turn executes a nucleophilic attack on an electrophilic carbonyl. Through a three-step mechanism, the aldol product is produced.

As the aldol condensation produces a β-hydroxy group, the reaction can be utilised to produce a chiral centre. The key step in the asymmetric aldol condensation is the Zimmermann-Traxler intermediate state.[75] A double six-membered ring intermediate state is utilised to achieve the preferred configuration on the β-hydroxy group (See Figure 15).

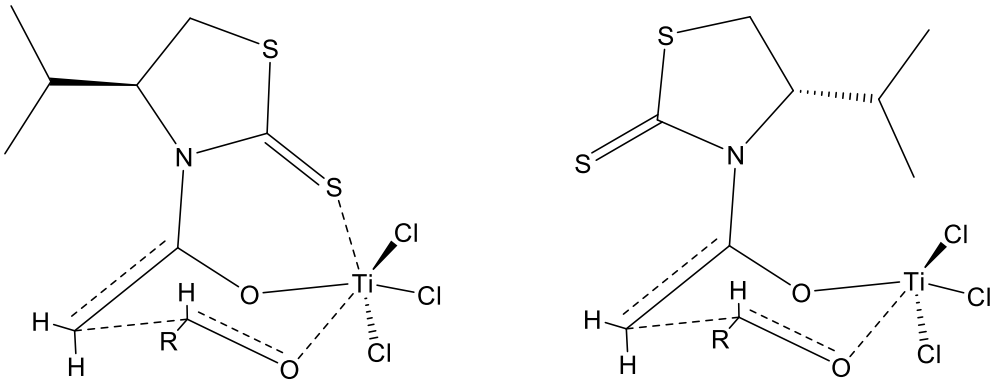
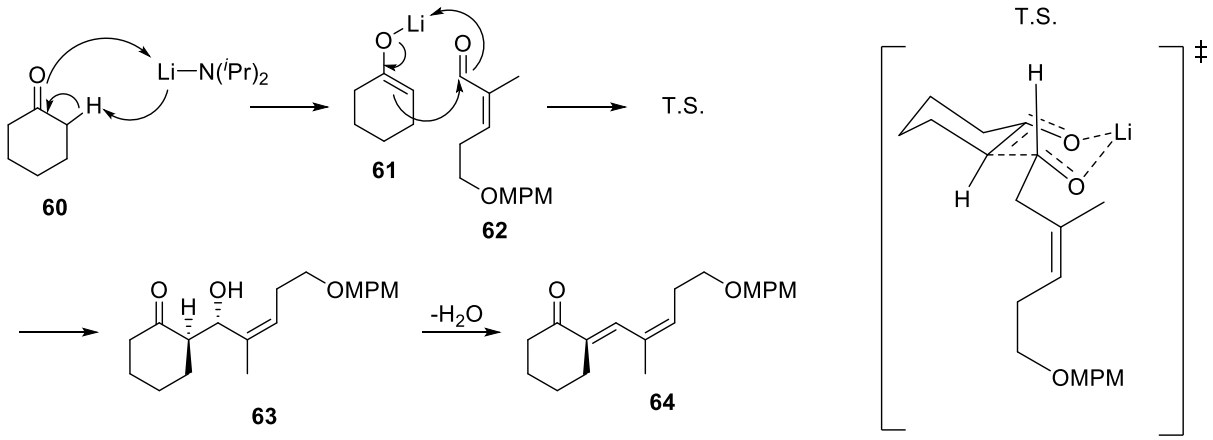


*Figure 15: A closed Zimmermann-Traxler projection (left) of the intermediate state of the aldol condensation between a generic aldehyde and the Evans auxiliary **2b**, and an open Zimmermann-Traxler projection (right) of the same groups.[75]*

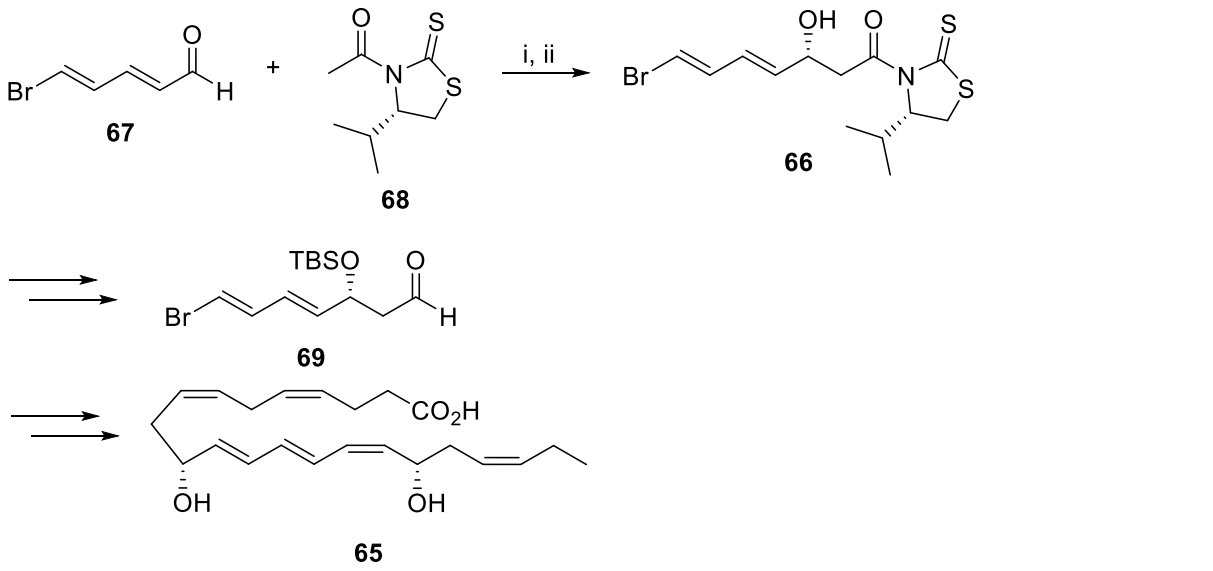
During the last half of the 20th century, different groups started investigating the stereochemical outcome of the aldol reaction.[76-77] It was found that the *E*- and *Z*-enolates give the *anti*- and the *syn*-diastereomer, respectively. More recently, several chiral auxiliaries have

been developed for highly selective syntheses.[78-79] Evan's oxazolidinones and Crimmin's thiazolidinethiones are the most noteworthy.



Scheme 10: Formation of an anti-aldol from a trans-enolate.[80-81]

Aursnes *et al.* used a thiazolidinethione auxiliary in their total synthesis of protectin D1 (**65**), to achieve a diastereomeric ratio of 15.3:1 of the aldol product **66**.[82]



*Scheme 11: The use of a thiazolidinethione auxiliary in the total synthesis of protectin D1 (**65**).*[82] *Reagents and conditions: (i) $TiCl_4$, DCM, -78°C; (ii) DIPEA.*

The reagents can also react with themselves in a self-condensation reaction, creating non-favoured by-products.

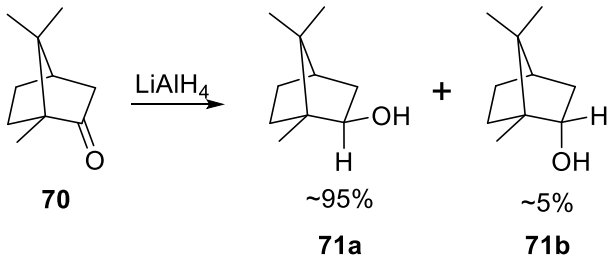
1.2.6 Asymmetric synthesis

While normal reaction conditions will yield the racemic mixture of the products from any stereogenic centre, the asymmetric compounds are oftentimes preferred. That is, the

enantiotopic or diastereotopic compound. More so than the chemist, nature is enantiomerically pure in its synthesis.[11] Therefore, the receptors accepting the chemicals will reject the opposite, or "wrong", enantiomer; often because of steric hindrance. For preliminary medicinal tests, the enantiomeric excess (*ee*) will usually need to be better than 95%. Hence, chemical purity and stereochemical integrity is more important than yields when first exploring new compounds.

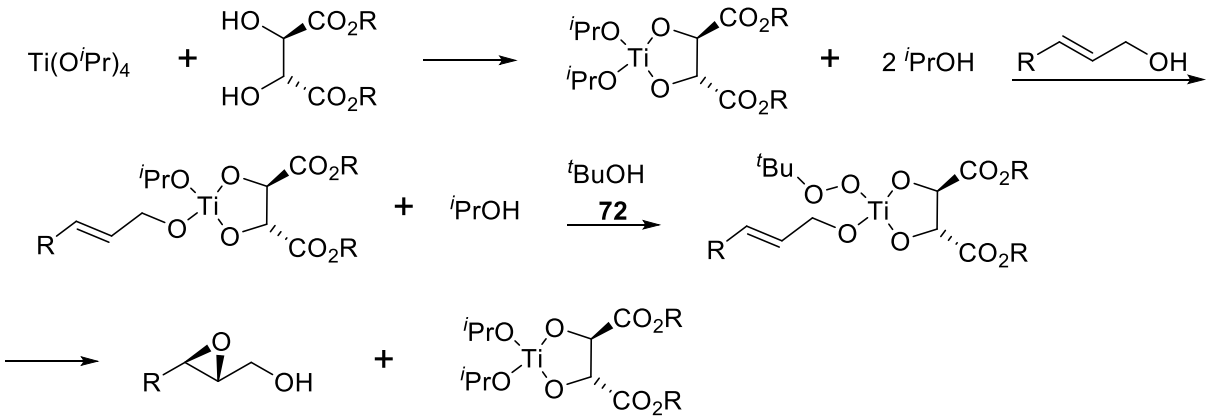
Four main strategies are utilised in asymmetric synthesis: Substrate control, reagent control, use of a chiral catalyst, and use of a chiral auxiliary.[62, 83]

In substrate control, the substrate's stereochemistry controls the formation of new stereocentres. This is perhaps the most limited method, as very specific substrates are required. Camphor (**70**) is a good example of substrate control: A six-membered ring is held together with a "bridge" that induce diastereotopic faces.[84-85] An "outside" face (*exo*) which is less hindered than the "inside" face (*endo*). Therefore, the carbonyl will have diastereotopic faces, and the reaction will be forced into the less hindered transition state. The reaction described in Scheme 12 is diastereoselective, meaning that one form is favoured above the other.



*Scheme 12: Reduction of Camphor (**70**).[84-85] An example of substrate control, where the hindered face of the starting material dictates the stereochemistry of the main product.*

For reagent control, a chiral reagent perform an asymmetric reaction on a prochiral centre in the substrate. [62, 83] A non-chiral reagent is first placed in a chiral environment, forcing the previously non-chiral reagent to become chiral, as seen in Scheme 13.



Scheme 13: Sharpless asymmetric epoxidation.[86-87] An example of reagent control where the oxidation agent, tert*-butyl hydroperoxide (TBHP, **72**), is placed in a chiral environment, thereby forcing a specific stereochemistry upon the product.*

The third method is to employ a chiral catalyst.[62, 83] BINAL-H (**73**) is such a catalyst, able to reduce α,β-unsaturated carbonyl group-containing compounds with almost complete enantioselectivity.[88] For a chiral catalyst to work properly, the transition state must be highly-ordered, and the faces of the substrate must be enantiotopic.[62]

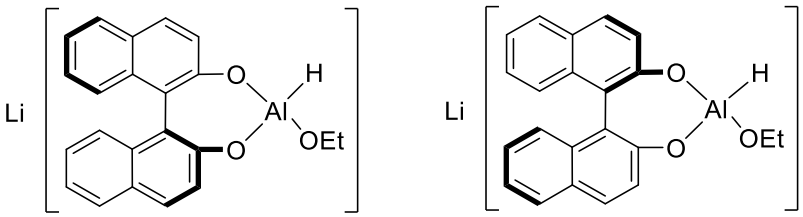


*Figure 16: Both stereoisomers of BINAL-H (**73**), a chiral reducing catalyst.[88]*

The last approach is to use a chiral auxiliary.[83] An auxiliary is selected based upon several factors. For a given reaction, the auxiliary should be: enantiomerically pure; available in both enantiomeric forms; easy to produce in high yields; easy to both introduce in high yields and remove selectively in high yields; be reusable; give good control of diastereoselectivity of the substrate.[62] Two examples of auxiliaries with these properties are Evans oxazolidinones and Crimmins thiazolidinethiones.

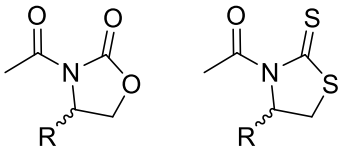
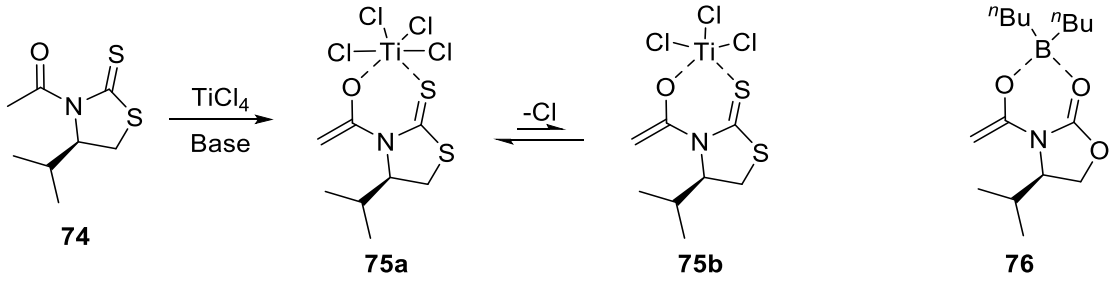


Figure 17: General representations of Evans oxazolidinones and Crimmins thiazolidinethiones.[79, 89-91]

Evans *et al*.,[90] developed the oxazolidinone auxiliary, which was further developed by Nagao *et al*.,[91] and Crimmins *et al.*[79, 89] to the thiazolidinethione. The idea was that with a chelating metal and a base, the auxiliary would make an enolate that could perform a nucleophilic attack on a carbonyl. The stereochemistry of the following aldol product would be dictated by the size and type of R-group positioned on the fourth position on the heterocyclic ring.



*Scheme 14: The preparation of an enolate (**75**) from an Evans auxiliary (**74**). The corresponding Crimmins auxiliary enolate (**76**) is prepared in the same manner, but $TiCl_3$ is exchanged by $B(n\text{-}Bu)_2$, bound to the oxygen.*

Zimmermann and Traxler suggested, for the Ivanov and the Reformatsky reactions, that the transition state is highly ordered, dictating the stereochemistry of the product;[92] the transition state involves a low-energy, six-membered ring. With a closed Zimmermann-Traxler transition state, the stereochemistry of an aldol reaction could be dictated in the same manner. Contrary to this, an open transition state does not favour one stereoisomer over the other.

1.2.7 Analysis with Mosher's reagent (77)

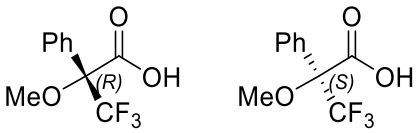


Figure 18: The R *and* S *enantiomers of Mosher's acid (**78**). The stereochemistry is inversed in the acid chloride (**77**).*

In 1968, Mosher and co-workers started employing α-methoxy-α-(trifluoromethyl) phenylacetic acid (MTPA, **78**) to determine enantiomeric excess of chiral molecules.[93] MTPA (**78**) is usually referred to as Mosher's acid (**78**), while the corresponding acyl chloride **77** is known as Mosher's reagent (**77**). The latter is used to prepare esters or amides from primary or secondary alcohols or amines. The resulting ester is therefore called Mosher's ester. As there is no α-proton in the acid, racemisation is impossible, showing the great advantage of the reagent: complete reagent control, giving only one diastereomer.

As the reagent contains both hydrogen and fluoride, ^{1}H NMR and ^{19}F NMR can both be used to determine % *ee*. Numbers obtained from ^{1}H NMR of the Mosher's ester are found to be consistent with the data obtained from chiral HPLC (<1% error),[94] making it a practical method without the need of specialised equipment.

An advantageous quality of the Mosher's reagent (**77**), is that it can differentiate between the *S* and *R* configurations of alcohols;[93] both between alcohols from natural sources and alcohols synthesised in the lab, including when there is no available reference material. One hydrogen-group attached to (*R*)-(+)-MTPA (**78a**) ester moves exclusively upfield in NMR, while the other hydrogen-group moves exclusively downfield (See Figure 19).[95] The opposite is true for the same groups coupled with the (*S*)-(+)-MTPA (**78b**). The described effect is a consequence of the significant difference between the substituents of Mosher's reagent (**77**): the electron withdrawing α-trifluormethyl group moves NMR shifts downfield and the cloud of π-electrons in the α-phenyl group moves NMR shifts upfield.

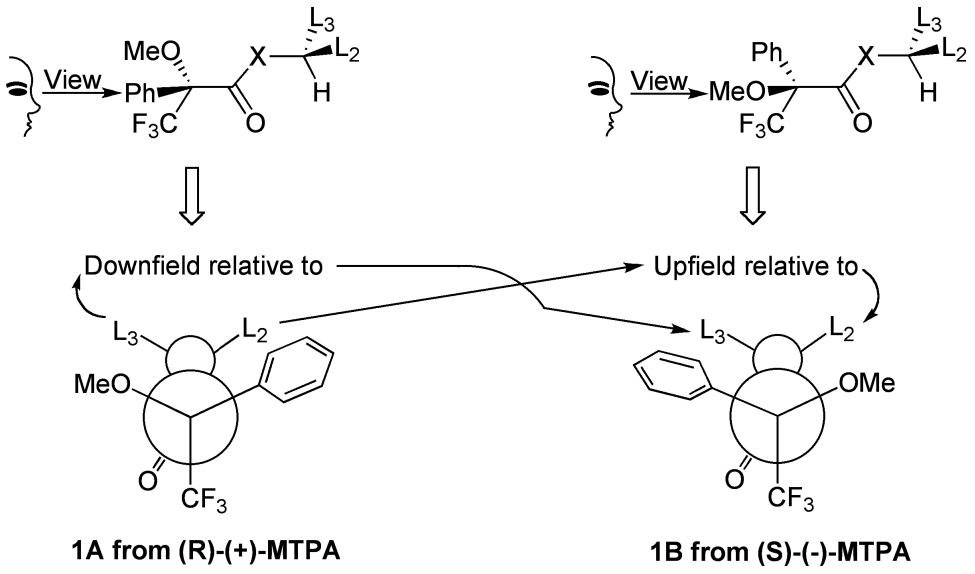


Figure 19: A representation of how the chemical shifts move relative to each other between the R *and* S *diastereomers of the Mosher's esters. Figure from reference [93].*

To fully determine the absolute configuration of an asymmetric alcohol or amine, several steps are taken.[93] Firstly, the esters from both Mosher's acids are prepared, and NMR spectra, such as ^{1}H-, COSY-, and TOCSY NMR, are collected. These spectra assign every proton to their chemical shift, and the difference in chemical shifts (Δδ) is calculated. With the knowledge of how the substituents of Mosher's reagent affect the hydrogen-groups in the

ester, the absolute configuration of the alcohol or amine can be determined. Alternatively, one of the Mosher's esters are prepared from one enantiomer of the alcohol or amine, and the Mosher's ester of the racemic mixture is made as a reference.

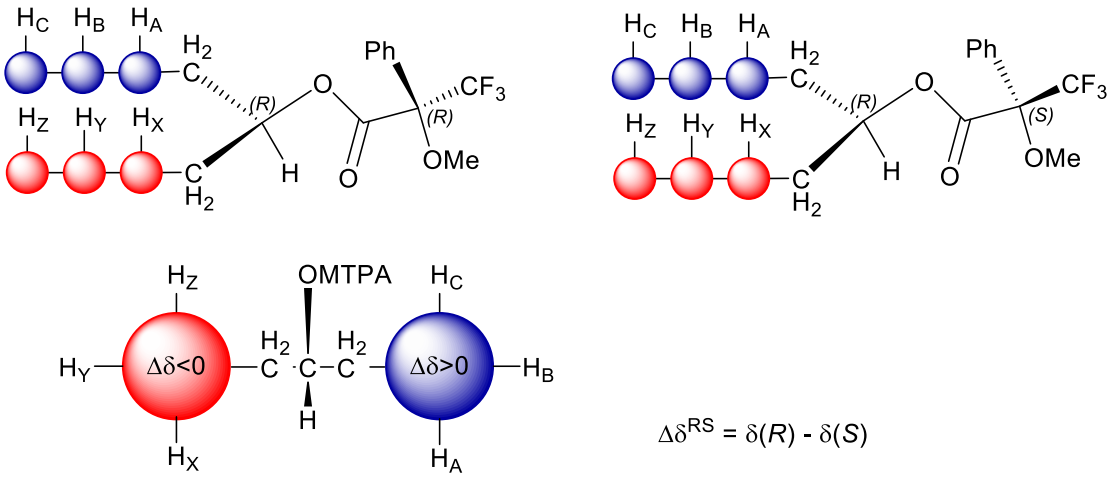
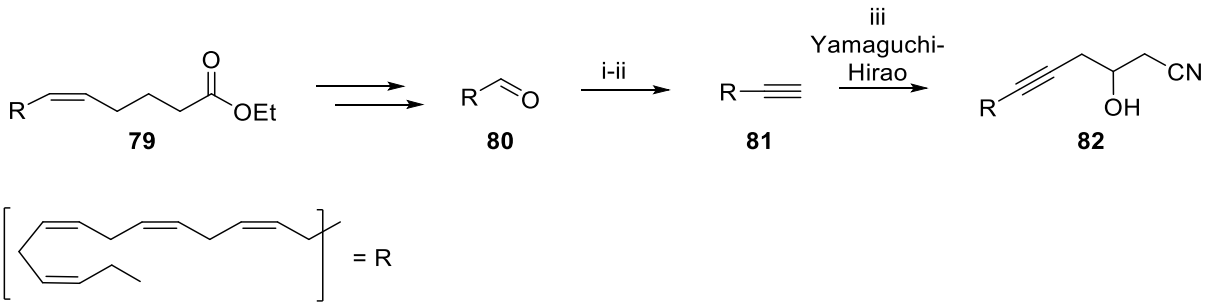


Figure 20: Empirical model to determine the absolute configuration of secondary alcohols. Figure considerably based on reference [96].

1.3 Other strategies towards 3-(R)-HEPE (3)

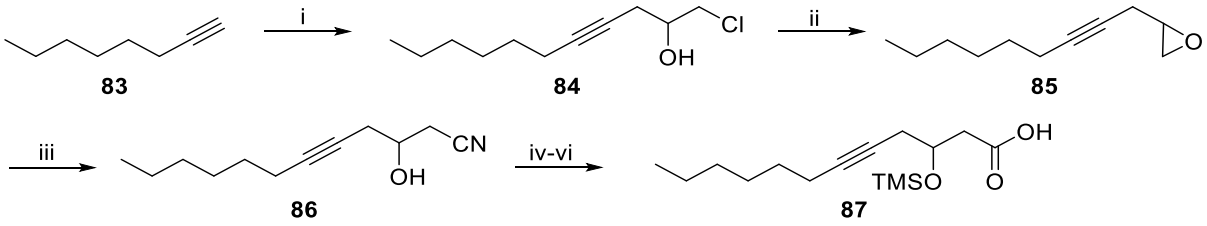
Our group has worked towards the synthesis of 3-(*R*)-HEPE (**3**) for two years.[97] With the target material finally synthesised,[98-99] the other strategies towards the target molecule are described below.

1.3.1 Yamaguchi-Hirao alkylation



Scheme 15: Graphical summary of Haukebø's project. Reagents and conditions: (i) CBr_4, PPh_3, DCM, 0°C-rt, overnight; (ii) n-BuLi, Et_2O, -78°C-rt, 2.5 h; (iii) n-BuLi, $BF_3{\cdot}Et_2O$, (±)-epichlorohydrin, -78°C, 3 h.[97]

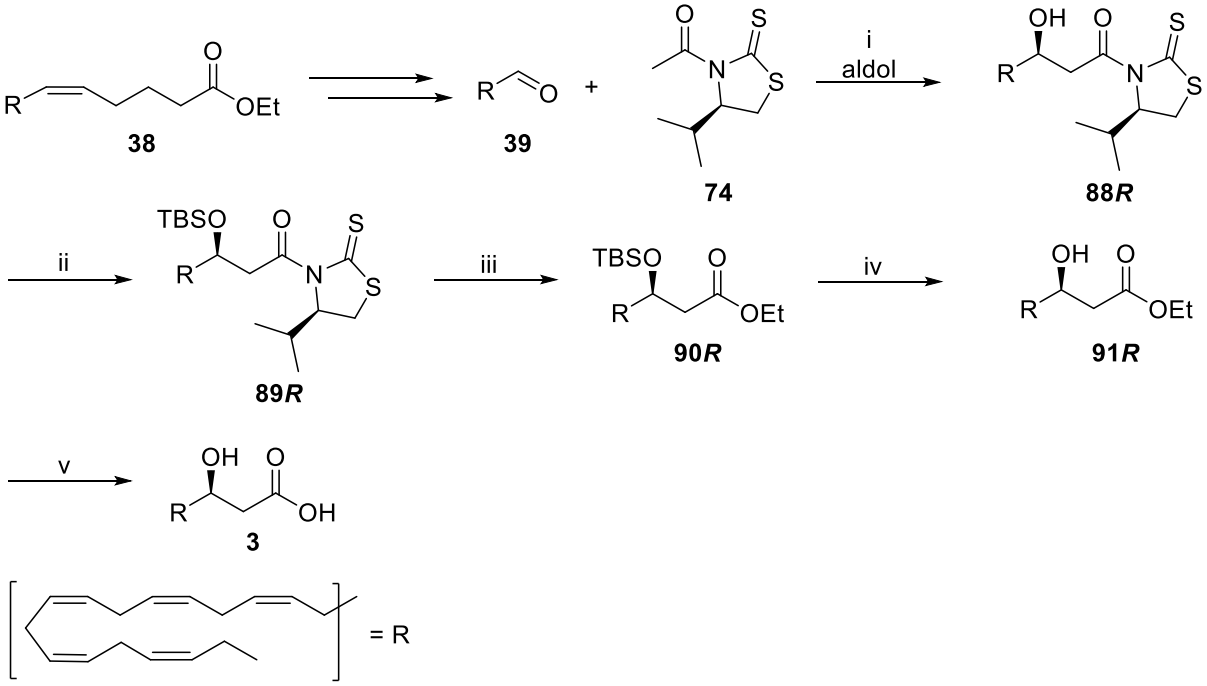
The aldehyde **80** was synthesised from EPA-EE (**79**).[97] The aldehyde (**80**) underwent a Corey-Fuchs reaction to form the alkyne **81**. A Yamaguchi-Hirao alkylation was used to transform the alkyne to the nitrile **82**.



Scheme 16: Graphical summary of Haukebø's test system. Reagents and conditions: (i) n*-BuLi, $BF_3{\cdot}Et_2O$, (±)-epichlorohydrin, -78^{O}C, 45 min; (ii) KO^{t}Bu, Et_2O, 0^{O}C; (iii) KCN, EtOH, reflux; (iv) Imidazole, TBSCl, DMF, 0^{O}C-rt, overnight; (v) DIBAL-H, Rochelle salt, hexane, -78^{O}C-rt; (vi) cyclohexene, NaH_2PO_4, $NaClO_2$, ^{t}BuOH/H_2O (5:1), 0^{O}C, 3 h.*[97]

A model system from 1-oktyn (**83**) was used to extrapolate the way from EPA-EE (**79**) towards the target molecule 3-(*R*)-HEPE (**3**). The chlorohydrin **84** was produced as a racemic mixture. Several functional group interchanges followed, to produce the TBS-protected acid **87**. The plan was to finish the model system in two steps and use the protocol to transform the chlorohydrin **82** to the target molecule, but there was no time.[97]

1.3.2 Aldol condensation



Scheme 17: Graphical summary of Gjessing's project. Reagents and conditions: (i) $TiCl_4$, DIPEA, DCM, -78^{O}C, 1 h; (ii) 2,6-lutidine, TBSOTf, DCM, -78^{O}C, 2 h; (iii) K_2CO_3, EtOH, 0^{O}C-rt, 24 h; (iv) TBAF, THF, 0^{O}C, 7 h; (v) LiOH, THF/EtOH/H_2O (2:2:1), 0^{O}C-rt, 2.5 h.[98]

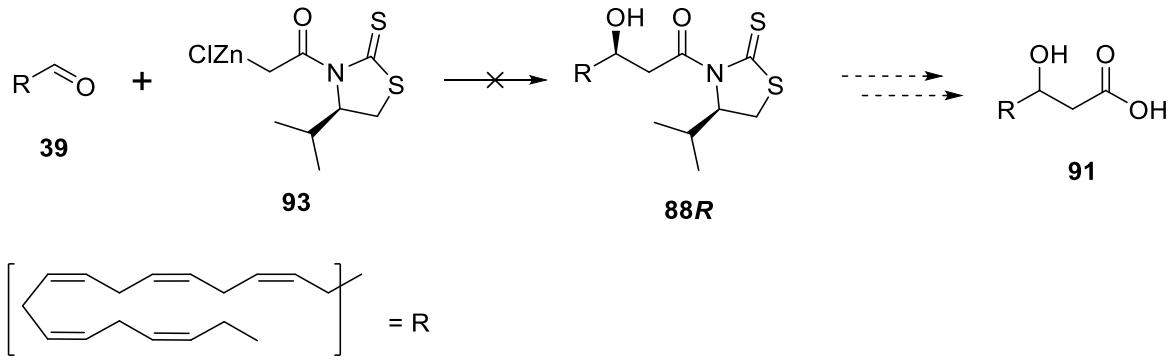
The aldehyde **39** was synthesised from DHA-EE (**38**).[98] Simultaneously, the Crimmins auxiliary **74** was synthesised. The two products were combined in an aldol condensation reaction under low temperatures, yielding the two diastereomers **88*S*** and **88*R*** in a ratio 1:8. The diastereomers could be separated on a silica column, whereupon the major product's

secondary alcohol was protected with TBSOTf to produce **89*R***. The auxiliary was then cleaved off, yielding 90, followed by the cleavage of the protection group to yield **91*R***. The ester was hydrolysed to the corresponding acid to produce the target molecule 3-(*R*)-HEPE (**3**).[98]

Late in the thesis project, it was found that hydrolysis of the aldol product **88*R*** directly into the ethyl ester **91*R*** was possible, saving both time and material.

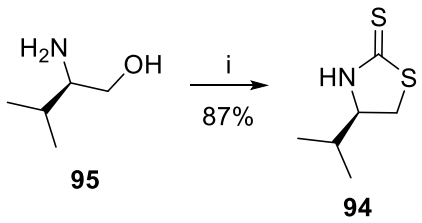
2. Results and Discussion

2.1 Attempt at synthesis of the auxiliary **92** for the Reformatsky reaction



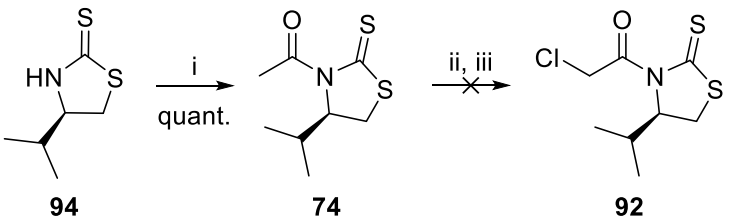
Scheme 18: The initial Reformatsky strategy.

The synthesis of the thiazolidinethione **94** was performed according to literature, with higher yields than recorded: 87% compared to 77%.[100] In ^{1}H NMR, **94** has a distinct, broad peak at 8.00 ppm, corresponding to the hydrogen connected to the nitrogen.



*Scheme 19: Preparation of thiazolidinethione **94**. Reagents and conditions: (i) CS_2, KOH, EtOH/H_2O (1:1 v/v), reflux, 67 h.*

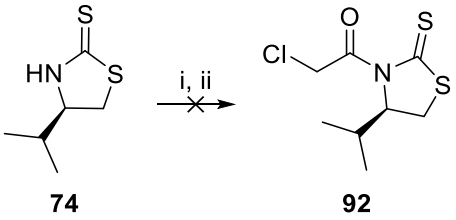
Several experiments were performed to see if the reaction time could be cut down from the 72 hours given in the literature.[100] The results after 24 h (no visible traces of product), 46 hours (74% yield), and 67 hours (87% yield), showed that three days reaction time is preferred regarding yield. To receive these results, the equivalents was increased for both the base (KOH, 2 → 4 equivalents) and sulphur donor (CS_2, 2 → 4 equivalents). The result was a significant increase in yield (30% in first attempt → 87% in final). However, more experience with the system, and bigger scale could also be factors explaining the increased yield.



94 **74** **92**

*Scheme 20: Preparation of thiazolidinethione **74** and attempt at **92**. Reagents and conditions: (i) NaH, acetyl chloride, THF, 0^{O}C-rt, 5h; (ii) DIPEA, TMSCl, DCM, 0^{O}C; (iii)* N*-chlorosuccimide, 0^{O}C-rt, 4 h.*

The thiazolidinethione **74** was also synthesised with a high yield: quantitative, comparable to the literature.[91, 100] Here, the broad peak at 8.00 ppm was gone, but a new singlet with an area corresponding to 3 H appeared at 2.78 ppm, indicating the new methyl close to the new carbonyl group.



74 **92**

*Scheme 21: Attempt at creating the thiazolidinethione **92**. Reactions and conditions: (i) Base (1.1 eq. MeLi, 3.6 eq. LDA, 1.1 eq. K^{t}BuO, or 1.1 eq. BuLi), THF, -78^{O}C; (ii) Chloroacetyl chloride, -78^{O}C-rt.*

The targeted auxiliary **92** could not be produced in our lab. The initial strategy was to go directly from the thiazolidinethione **74** to the auxiliary **92** using the method for the similar oxazolidinone **96** reported by Njiojob *et al*.[75] BuLi as base gave no positive results. Instead, MeLi was utilised as base. This gave something looking like the target molecule, but an extra unexplainable triple triplet, with an area of 1 H could be found in the ^{1}H NMR spectrum at 3.80 ppm. It was decided not to use extra time on figuring out what the exact product was. Next, lithium diisopropyl amine (LDA) was used as base. The product this time was also similar to the target molecule, but the chloride had been replaced by diisopropyl amine (See appendix, multiplets at 1.35-1.20 and 1.18-1.14 ppm in ^{1}H NMR, with area of 7 H each). The method was also attempted with K^{t}BuO as base, with no success. From comparing all the spectra, it was concluded that the target auxiliary **92** had indeed been formed, but that the α hydrogen was so reactive that excess base at once destroyed the auxiliary, giving either the starting material or the modified auxiliary as the product. To review the reaction, the exact molarity of the flask of BuLi was controlled by Gilman & Cartledge's method of titration.[101] The reaction was, however, still not successful.

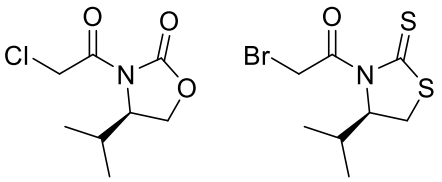
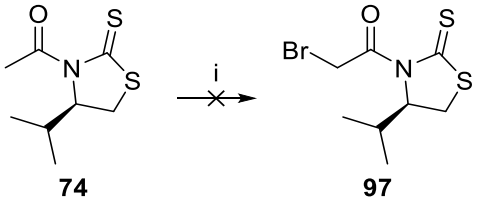


Figure 21: The oxazolidinone created by Njiojob et al,[75] *and the reminiscent thiazolidinethione* ***96****.*

A new strategy was to go from **94** through **74** to the target molecule **92** or the related **97**.[102] The result was a complex mixture with mono- and di-halogenated **92** or **97**, respectively, as well as backward synthesised **94**.



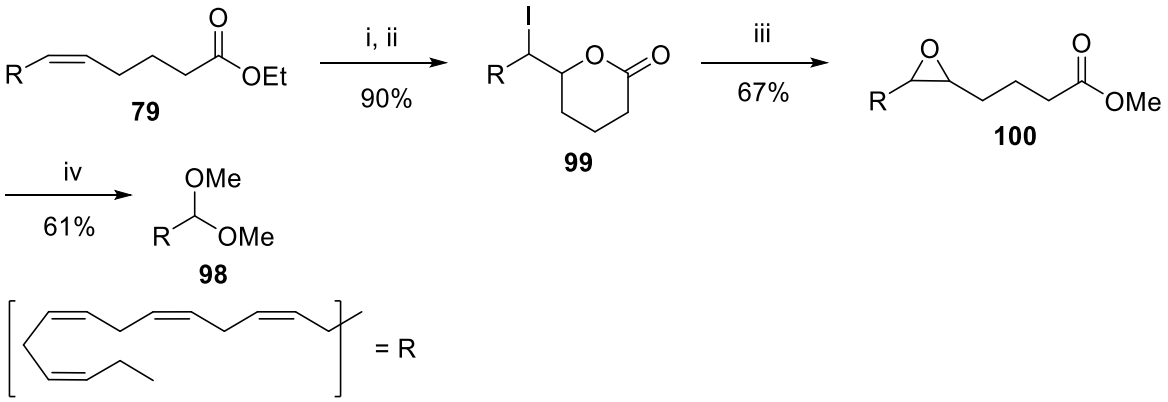
Scheme 22: Attempt at creating the thiazolidinethione ***96****. Reagents and conditions: (i)* Br_2*, DCM, rt.*

After several failed attempts of creating the auxiliary **92**, the asymmetric Reformatsky strategy was abandoned.

2.2 Synthesis of the acetal 80 from EPA-EE (79)

Protocols developed by Flock *et al.* were used for the full syntheses from both EPA-EE (**79**) and DHA-EE (**38**) to their corresponding acetals **98** and **42**, respectively.[60] All data for the reactions fitted the already published data.

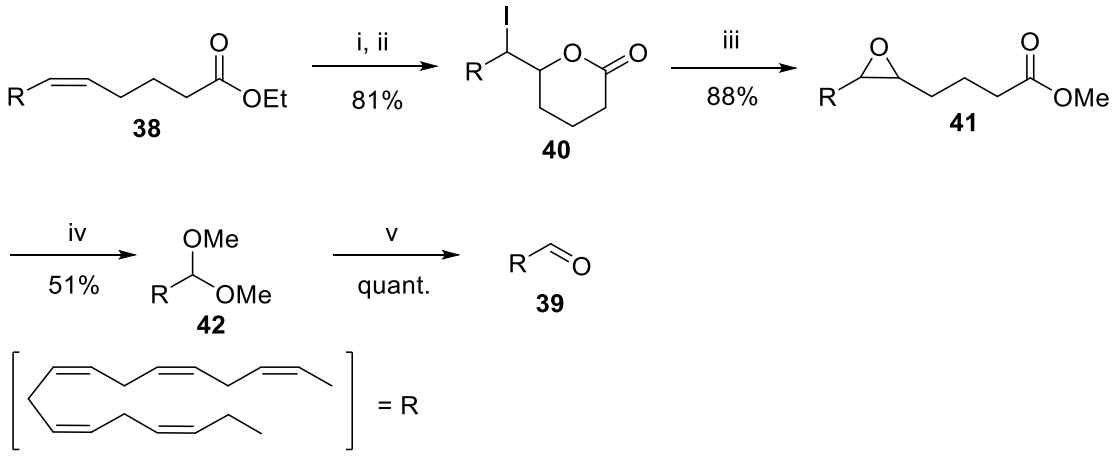
The acetal from EPA-EE (**98**) was first synthesised as a test system, simply because there was more of the EPA-EE (**79**) than of the DHA-EE (**38**) in the storage.



*Scheme 23: Preparation of the acetal **98** from DHA-EE (**79**). Reagents and conditions: (i) LiOH, EtOH/H_2O (1:1, v/v), rt; (ii) HI, KI, $KHCO_3$, I_2, THF, H_2O, 0^O-4^O C; (iii) K_2CO_3, MeOH, rt; (iv) H_5IO_6, MeOH, rt 6h; (v) Dioxane, Formic acid, rt 1.5 h.*

The iodolactone **99** was prepared over two steps, with an overall yield of 90%, compared to 95%[60] and 98%[103] in the literature. The next step to the epoxide **100** gave a yield of 67%, compared to 91% in the literature.[60] The stable acetal **98** was made with a higher yield than the reference material, namely 61% compared to 45%.[60]

2.3 Synthesis of aldehyde 39 from DHA-EE (38)



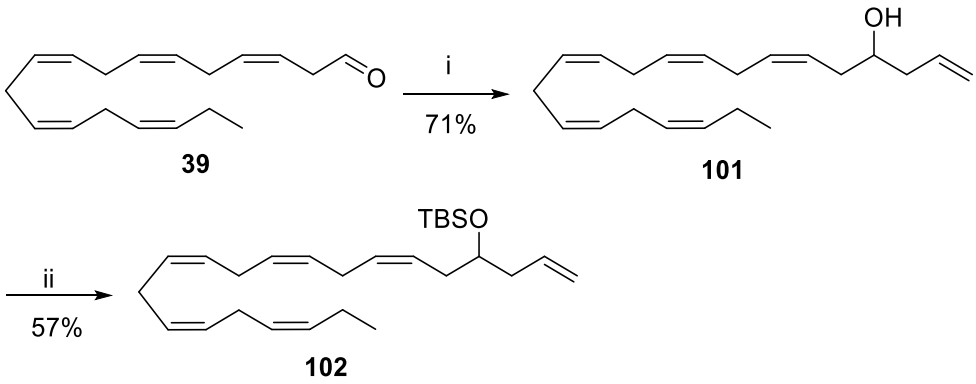
*Scheme 24: Preparation of the aldehyde **39** from DHA-EE (**38**). Reagents and conditions: (i) LiOH, EtOH/H_2O (1:1, v/v), rt; (ii) HI, KI, $KHCO_3$, I_2, THF, H_2O, 0^O-4^OC; (iii) K_2CO_3, MeOH, rt; (iv) H_5IO_6, MeOH, rt, 6h; (v) Dioxane, Formic acid, rt, 1.5 h.*

The iodolactone **40** was prepared over two steps, with an overall yield of 81%, compared to 95%[60] and 97%[103] in the literature. The next step to the epoxide **41** gave a yield of 88%, compared to 93% in the literature.[60] The stable acetal **42** was made with a higher yield than the reference material, namely 51% compared to 46%.[60] The aldehyde **39** was very sensitive, and had to be made freshly. With a reaction time of 1.5 h, quantitative yield could be obtained of **39**, but with the doubled reaction time, only 39% yield.

2.4 Grignard reaction – Precursor for Brown allylation

After the auxiliary **92** was deemed unstable, a new strategy was made: To go through a stereoselective Brown allylation, using a chiral reagent, to form the target molecule. First, the reaction was to be carried out as a racemic mixture. Both as a test system, and so that the products from the asymmetric and racemic reactions could be tested and compared by chiral LC. The racemic mixture was obtained by a Grignard reaction, with decent yields (71%), where the biggest obstacle was relatively small differences in elution time between the

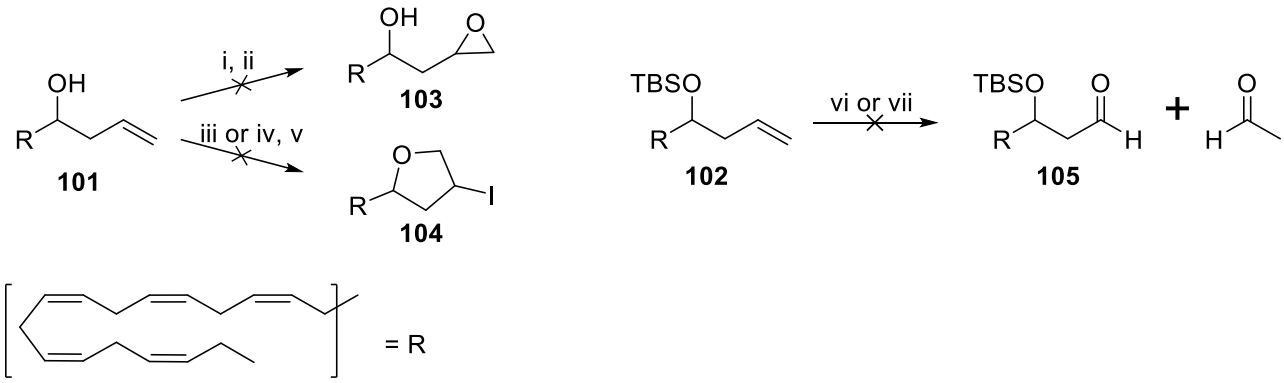
aldehyde **39** and the Grignard product **101** through the flash column; very small fractions had to be collected to obtain pure product. The aldehyde had a clear triplet at 9.69 ppm. In the Grignard product, this peak was gone, and a multiplet of 1 H between 5.94-5.77 ppm, corresponding to the hydrogen in the new homoallylic double bond, closest to the hydroxyl group, had appeared. The hydroxyl group could be found as a broad singlet at 1.66 ppm.



Scheme 25: The Grignard reaction, and the following TBS-protection. Reagents and conditions: (i) Allyl magnesium bromide, THF, -78^{O}C-rt, 1.5 h; (ii) 2,6-lutidine, TBSOTf, DCM, 0^{O}C-rt.

Protection of the β-hydroxy group was also carried out without problems, with a fair yield of 57%. The TBS protection groups are easily distinguished in ^{1}H NMR as a singlet with area 9 H at 0.84 ppm, and a singlet with area 6 H at 0.00 ppm.

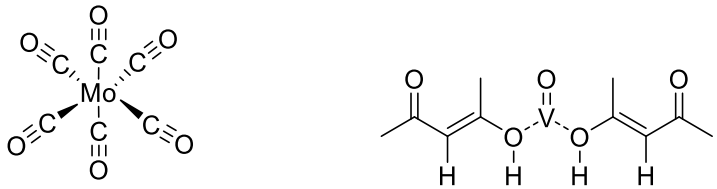
2.5 Attempt at further reaction upon the Grignard product



*Scheme 26: Attempts at further reactions on the Reformatsky products **101** and **102**. Reagents and conditions: (i) Catalyst (Molybdene (CO)$_6$ (**106**) or Vanadyl acetoacetonate (Vanadyl acac, **107**)), DCM, -72^{O}C; (ii) TBHP, -72^{O}C-rt; (iii) I$_2$, ^{t}BuNH$_2$, DCM/toluene, rt; (iv) K$_2$CO$_3$, DCM, rt; (v) I$_2$, 0^{O}C-rt; (vi) OsO$_4$, 2,6-lutidine, NaIO$_4$, DCM, 0^{O}C-rt; (vi) OsO$_4$, 2,6-lutidine, NaIO$_4$, H$_2$O, Dioxane, 0^{O}C-rt.*

The next step was to create the terminal epoxide **103**, that could later undergo oxidative cleavage, similar to Scheme 8. The reaction was attempted using different conditions, with

107 as catalyst. To try out the conditions, they were also reacted on a test system: 3-methyl-3-buten-1-ol (**108**). The conditions were not successful in either attempts. However, ^{1}H NMR indicate that **107** was indeed successful at catalysing minuscule amounts of the reaction.



Molybdenum hexacarbonyl (**106**) Vanadyl acetoacetonate (**107**)

*Figure 22: The two catalysts **106** and **107** used for epoxidation of the homoallyl **101**.*

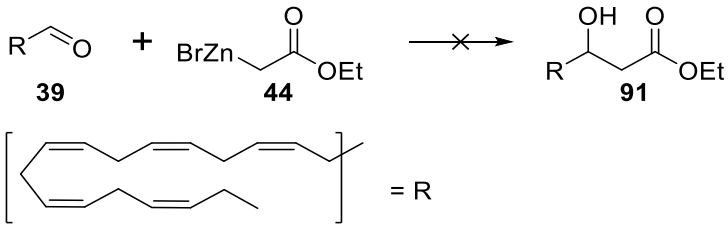
Initial attempts at the Lemieux-Johnson reaction from the homoallyl **102** to the aldehyde **105** revealed that the reaction is not selective enough to be used. The double bond in the active site sits isolated compared to the five allylic double bonds, additional to less steric hindrance. Even with these differences, and a small difference in electron density, ^{1}H NMR show that the reaction attacks multiple double bonds, to create several new products in a non-favoured way.

Initial attempts at making the iodo ether **104** using only I_2 or also adding ^{t}Bu amine were not successful.

After the Grignard product could not extrapolate an efficient strategy towards 3-*R*-HEPE (**3**), the second strategy was abandoned.

2.6 Racemic Reformatsky

To analyse the enantiomeric excess and specific stereochemistry of the final product, both the racemic mixture, and at least one of the asymmetric products must be made. It was decided that another member of the group would make 3-(*R*)-HEPE (**3**), and this project would focus on producing the racemic mixture of 3-HEPE (**109**). The Reformatsky strategy was chosen, to go back to the original track.



39 **44** **91**

Scheme 27: The racemic Reformatsky reaction. Reactions and conditions: (i) Et_2O, -30^{O}C-rt.

Initial attempts at *in situ* Reformatsky were not successful, probably because zinc dust was used instead of granular zinc. Zinc dust is completely oxidised, making it hard to produce any Zn^0 that could be used to make the Reformatsky reagent. Granular zinc was used to form the Reformatsky reagent **44**, which could be stored for up to a week in the fridge. The reagent **44** was attached to the aldehyde **39** to form **91**. The latter was not purified on silica gel, as the slightly acidic pH was suspected to eliminate the β-hydroxy group as water. The yield was instead regarded as quantitative in solution for further reactions.

Protection using *tert*-butyldiphenylsilyl chloride (TBDPSCl) was not successful. There are three suggestions as to why: The Reformatsky product **91** was not purified, and impurities can make the protection with silyl groups difficult; The hydrogen in the β-hydroxy group is strongly bounded to the carbonyl oxygen in the ester, making a hydrogen-bond that is hard to break; The TBDPSCl favours primary alcohols, while tertiary alcohols are least favoured.[104] The triflate of the same group could be used, as triflates are more reactive, due to the nucleofuge triflate, but TBDPSTf is very expensive.

The formation of the β-keto ester was also not successful. Instead a complex mixture of products resulted. The suggestions above as to why the protection would not work also apply here, but the idea now was that maybe the Reformatsky reaction was not complete.

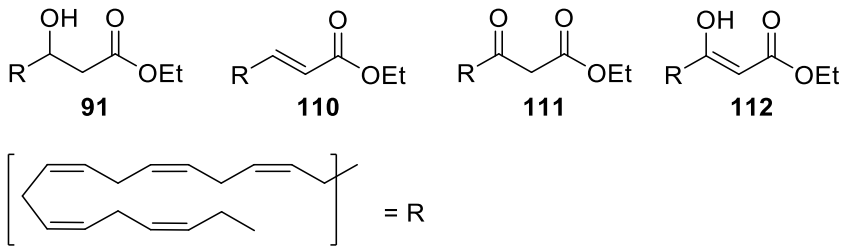


Figure 23: The different products found with the data available after the Reformatsky reaction.

The Reformatsky product was attempted purified, after the silica gel was deactivated with Et_3N. Even though all base was washed out with *n*-hexane, as checked by TLC, the fractions that were separated on the column came out as mixtures of seemingly decomposed material. The general skeleton of the molecule could be seen in both ^1H NMR and ^{13}C NMR, but after checking the molecule on IR and UV, and fine search through the NMR spectra, it was concluded that the mixture probably composed of H_2O-eliminated product (**110**), β-keto ester (**111**) and its enolate (**112**), and the Reformatsky product (**91**). Unfortunately, the different products could not be separated. HRMS found only the β-keto ester (**111**) and its enolate

(**112**), but as not all the pure DHA-derivates from other reactions could be found on HRMS, this method is not regarded as conclusive.

Table 1: Spectral data indicating which by-products are present after the Reformatsky reaction. The spectrums are not included in the report.

Spectral data indicating by-products					
Structure	^{1}H NMR	^{13}C NMR	IR	UV	HRMS
91	11.93 ppm		3506 cm^{-1}		
110		91.8 ppm		249 nm	
111		194.6 ppm			MW = 344.50
112	11.93 ppm	91.8 ppm	3506 cm^{-1}	249 nm	MW = 344.50

The peak at 11.93 ppm in ^{1}H NMR disappears after shaking with D_2O, showing that alcohol is present. The reason why it comes so high is probably a strong hydrogen-bond to the carbonyl oxygen close to it.

The Reformatsky reaction was attempted one more time, so that the product could be thoroughly cleaned before further synthesis, but the resulting dark oil substantiated the suggestion from the former attempt.

2.7 Aldol

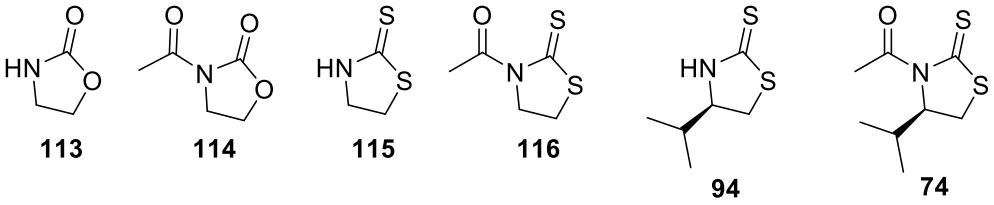


Figure 24: Some of the auxiliaries produced for the different strategies.

As the asymmetric Aldol strategy had satisfactory results,[98-99] it was decided to follow the exact same steps to produce the racemic mixture of **109**. The chiral auxiliary **74**, however, was switched out in favour of a non-chiral oxazolidinone (**114**). The precursor of oxazolidinone **114**, oxazolidinone **113**, is commercially available and cheaper compared to producing thiazolidinethione **116**. The auxiliary **114** was attempted made through two different paths; the same path as **74** from **94**, and a second pathway utilising CDI. With 1.1 equivalents of reagents, none of the methods gave full conversion. Neither CDI gave full

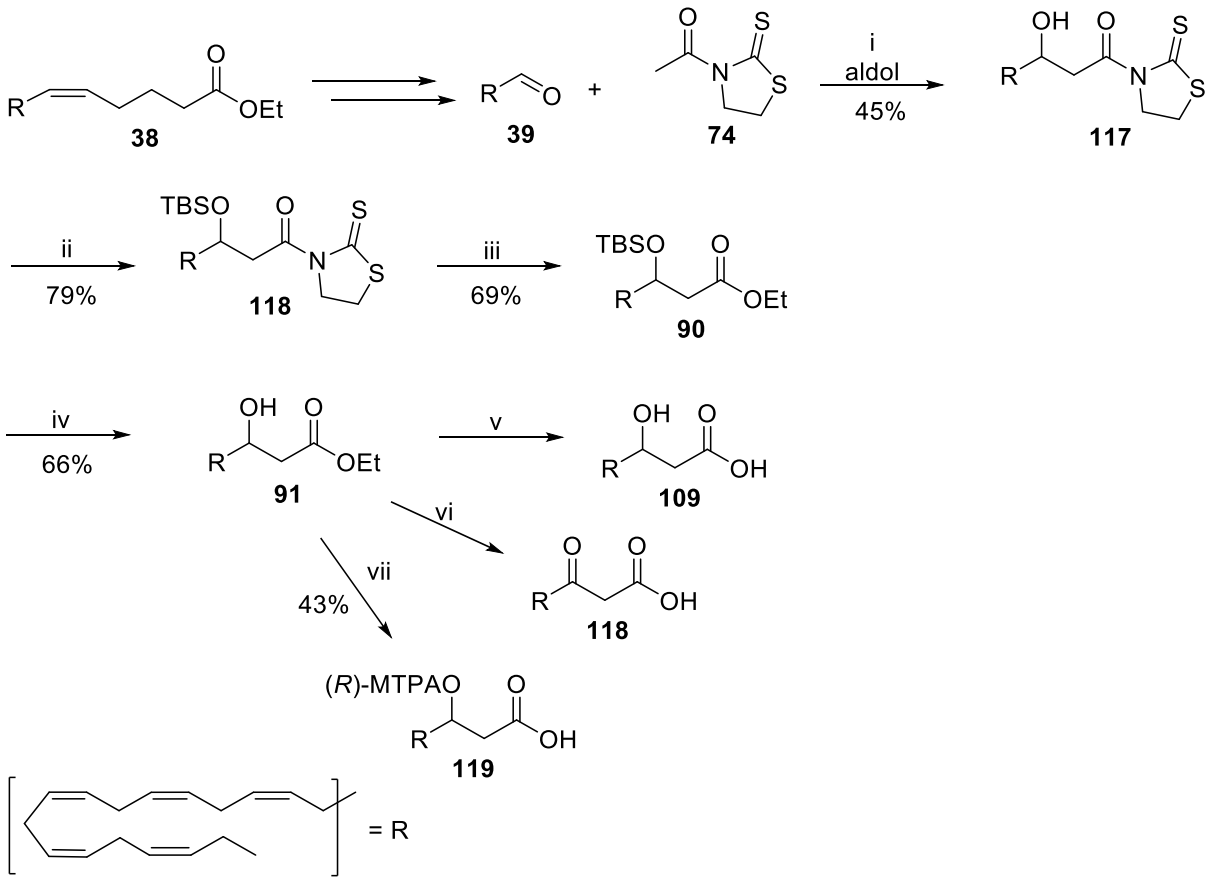
conversion with 2 equivalents, contrary to the original method. To sum up: even though the reaction for making the auxiliaries **74** and **114** are similar, the difference between the soft sulphur and hard oxygen show. While the reaction with sulphur give full conversion with 1.1 equivalents of reagents, the same reaction with oxygen "only" give 87% yield with 2 equivalents.

The aldol reaction between the aldehyde **39** and the oxazolidinone **114** with $TiCl_4$ as chelating agent was not successful. Most of the auxiliary **114** could be recovered after the reaction, and most of the aldehyde **39** seemed to have undergone a self-condensation reaction; the triplet at 9.69 ppm in ^{1}H NMR had converted into a doublet at 9.44 ppm. The collected data indicates that the enolate from the auxiliary **114** is not produced. Evans *et al*. describe boron as a chelating agent for oxazolidinone auxiliaries.[105] They mention that titanium is a good chelating agent for sulphur, while boron is better for oxygen. The O-B bond is notably shorter than the O-Ti bond, 1.4 Å and 1.9 Å, respectively.[106-107] The next step would therefore be to attempt the aldol reaction with di-*n*-BOTf as the chelating reagent, to see if the aldol strategy could be utilised with the cheaper auxiliary (**114** compared to **116**).

As the oxazolidinone enolate could not be achieved, the thiazolidinethione **116** was made, with decent yields: 65% for thiazolidinethione **115**, and quantitative for thiazolidinethione **116** on the first attempt. Regarding their chemical shifts, the characteristic peaks in ^{1}H NMR are the same as with thiazolidinethiones **94** and **74**. Without the ^{i}Pr group, however, there are now two CH_2 groups situated on the heterocyclic ring, appearing as two coupling double doublets. In thiazolidinethione **115** they appear at 3.99 and 3.57 ppm in ^{1}H NMR, and their corresponding carbons at 51.4 and 33.9 ppm in ^{13}C NMR. The carbonyl group appear at 202.2 ppm in ^{13}C NMR. When **115** is acetylated into the thiazolidinethione **116**, the conversion is easy to follow in NMR; the two double doublets appear as triplets at 4.57 and 3.29 ppm in ^{1}H NMR, and their corresponding carbons at 55.8 and ~28 ppm in ^{13}C NMR. The new carbonyl group appear at 171.6 ppm in ^{13}C NMR.

The aldol reaction itself was performed after the protocol of Tungen *et al*.,[108] with alterations found by laboratory tests.[98-99] For the purpose of comparison, the racemic reaction was performed exactly as the asymmetric, as far as practically possible. So even though tests by TLC showed that starting material (**39**) was still present, the reaction was stopped after one hour at -78^{O}C, yielding 45% aldol product **117**. For comparison, the asymmetric reaction gave

30% major product (**88*R***) and 15% minor product (**88*S***).[98-99] To further explore the non-chiral aldol reaction, it could be attempted at 0^{O}C as the stereochemistry does not need to be as carefully controlled.



Scheme 28: Graphical summary of the aldol reaction strategy. Reagents and conditions: (i) $TiCl_4$, DIPEA, DCM, -78^{O}C, 1 h; (ii) 2,6-lutidine, TBSOTf, DCM, -78^{O}C, 2 h; (iii) K_2CO_3, EtOH, 0^{O}C-rt, 24 h; (iv) TBAF, THF, 0^{O}C, 7 h; (v) LiOH, THF/EtOH/H_2O (2:2:1), 0^{O}C-rt, 2.5 h; (vi) DMP, DCM, rt, 2 h; (vii) Pyridine, (S)-MTPACl, DCM, rt, 2 h.

Next, the β-hydroxy group was TBS-protected, with an easy purification and a yield of 79% of **117**. The auxiliary (**113**) was cleaved off, with a yield of 69% **90**. The β-hydroxy group was de-protected with a yield of 66% **91**, without the need of purification after work-up. Each of the four products are easily distinguished by TLC, as can be seen in Table 2.

Table 2: The R_f-values of the aldol products.

Product	R_f-value (DCM)
Aldol (**117**)	0.07
TBS-protected aldol (**118**)	0.78
TBS-protected EE (**90**)	0.70 (n-hexane/EtOAc 7:3 v/v)
3-HEPE EE (**91**)	0.16

The ethyl ester of 3-HEPE (**91**) was split into three groups: One for making 3-HEPE (**109**), one for making the β-keto ethyl ester (**118**) and -acid (**120**), and one for making the corresponding Mosher's ester (**119**).

3-HEPE (**109**) was prepared by hydrolysing the ethyl ester **91**, but could not be adequately purified; both eluents and a silyl-group could be found in the spectrum, both before and after purification, additionally to other small impurities. With very limited time and material (<6 mg), an efficient purification method was not found. The acid from the β-keto ethyl ester (**120**) was not synthesised, as the same problems would probably be met there.

2.8 Mosher's ester and asymmetric resolution

The Mosher's ester from the racemic 3-HEPE EE (**91**) was synthesised on a low scale (10 mg) with a yield of 43%. The crude product could not be used to determine the symmetry of Gjessing's major product (**88*R***),[98-99] so it had to be cleansed on a silica column. The purification did however give an enrichment of the *R*,*S* diastereomer, changing the ratio to 1:1.5 of the *R*,*R* diastereomer compared to the *R*,*S* diastereomer. TLC indicated that the two diastereomers could be separated, as they came out with R_f-values of 0.64 and 0.58 (DCM). Which diastereomer corresponded to the different R_f-values was not checked.

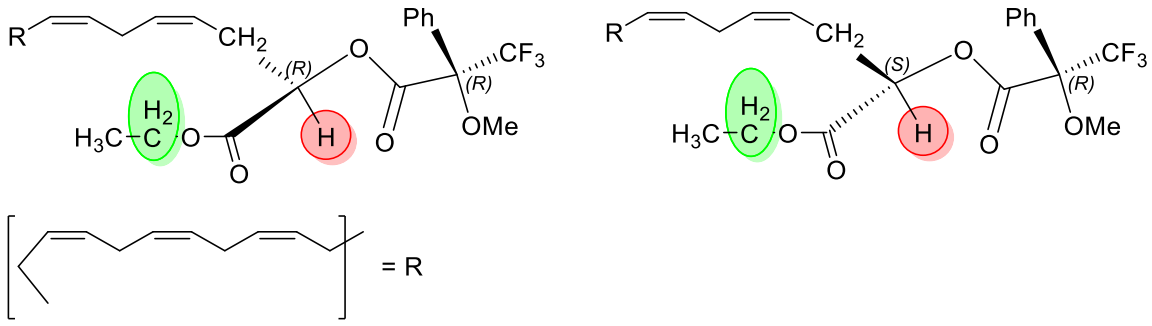


Figure 25: The R,R *(left) and* R,S *(right) Mosher's esters (**119**). The carbinol hydrogen circled in red moved in ^{1}H NMR from 4.07 ppm in the ethyl ester **91**, to merge with the multiplet at 5.45 – 5.23 ppm in the Mosher's ester **119**. The carboxy group circled in green splits from a quartet at 4.17 ppm in ^{1}H NMR, into two separate quartets at 4.12 and 4.05 ppm. The carboxy group in the (*R,R*)-diastereomer comes closer to the phenyl group, while the carboxy group in the (*R,S*)-diastereomer comes closer to the influence of the trifluoromethyl group. The chemical shift for the (*R,R*)-quartet will therefore be upfield compared to the (*R,S*)-quartet, following the description is section 1.2.7.*

The effect of the Mosher's reagent was clear: The carbinol peak in ^{1}H NMR moved from 4.07 ppm to merge with the multiplet at 5.45 – 5.23 ppm, corresponding to the protons on the double-bonds; the most distinguishable feature was that the quartet at 4.17 ppm with an area

corresponding to 2 H in ^{1}H NMR, belonging to the carboxy group on the ethyl ester, split into two quartets at 4.12 and 4.05, with areas 1:1.5 compared to each other (area of 2 H in total). With the *S*-enantiomer of the Mosher's reagent, the ^{1}H NMR shifts for the *R*,*R* diastereomer should be downfield compared to the corresponding shifts in the *R*,*S* diastereomer.

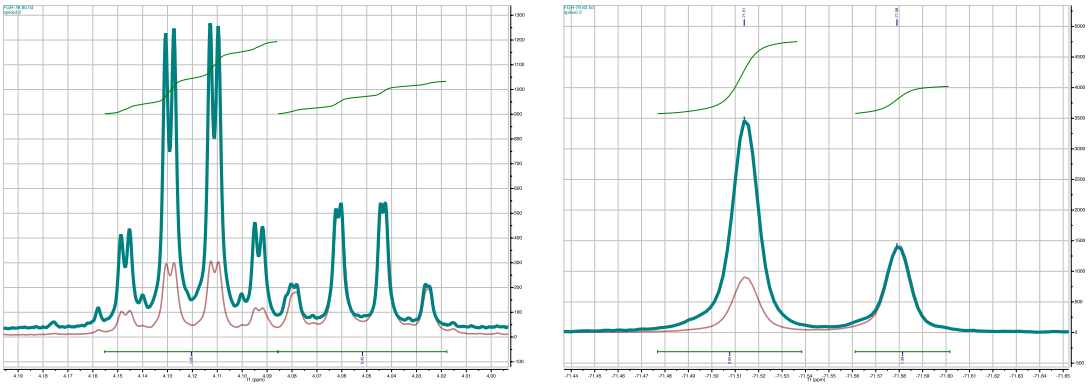


*Figure 26: Section of the ^{1}H NMR (left) and ^{19}F NMR (right) of the Mosher's ester **119**. The lower line is before spiking with* R,R-*Mosher's ester (**119R**) made by Gjessing,[98-99] and the tallest line is after spiking.*

In ^{19}F NMR, the Mosher's ester (**119**) came out as two singlets at -71.51 and -71.58 ppm, with comparable areas of 1:1.5, respectively. When trifluoroacetic acid was added, the two singlets switched places between them, while the relationship remained identical.

Gjessing's corresponding *R*,*R*-Mosher's ester (**119*R***):[98-99] The Mosher's ester from the ethyl ester **91*R*** was added to spike the sample, and only the area of the most downfield peak increased (quartet at 4.12 ppm in ^{1}H NMR, and singlet at -71.51 ppm in ^{19}F NMR). It was then concluded that the major product from Gjessing's asymmetric aldol reaction indeed gave 3-(*R*)-HEPE (**3**).

2.9 Conclusion and further work

As for auxiliaries, it can be stated that the difference between hard oxygen and soft sulphur is much greater than anticipated. Coupled with a sensitive system, such as the DHA-analogues, this difference is particularly evident. Some experiments could be performed to find the limitations of each version of the auxiliaries, and the borderline between their applications. Prominently, repeating the aldol reaction between the aldehyde **39** and the auxiliary **114** or **121** using a boron chelating agent instead of a titanium chelating agent.

Another suggestion for performing the aldol reaction with the oxazolidinone **114** is to attempt another base. A base stronger than 2,6-lutidine could be attempted to make the enolate. The fine line between making the enolate and destroying the aldehyde could be explored with a number of different bases.

For the aldol reaction, the *S* enantiomer of 3-HEPE (**109**) should also be synthesised, using the mirror image of auxiliary **74**. This is useful for both the full identification of 3-HEPE, and to see if 3-(*S*)-hydroxy acids have the same or similar effects as 3-(*R*)-hydroxy acids in *Candida albicans*.

The Reformatsky reaction is another reaction that could be further explored. The spectra from the two steps attempted, Reformatsky reaction and oxidation of the Reformatsky product, indicate that the reaction was to some extent successful. Alternating the work-up could improve the results.

As the oxazolidinone **121** was made with good yields, it would also be interesting to attempt to use it in an asymmetric Reformatsky reaction to produce the (*S*,*S*)-diastereomer, towards 3-(*S*)-HEPE (**122**).

3. Experimental

General methods

NMR spectra were recorded on a Bruker AscendTM 400 at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR. All samples were dissolved in deuterated chloroform ($CDCl_3$). Chemical shifts (δ) are given in ppm and the coupling constants (*J*) in Hz.

Mass spectra were recorded at 70 eV on Waters Prospec Q spectrometer using EI as the method of ionisation. The values were reported in the unit of mass to charge ratio (m/z). The mass spectra are reported in the appendix.

For TLC, Merck C-60 F_{254} silica gel plates were used and developed using UV-light or a suitable stain; mostly $KMNO_4$-solution, but anis aldehyde was also used. Flash chromatography was performed using a glass column packed with Merck 60 silica gel (40-63 µm particles) as stationary phase. All liquid phases are specified in the experimental procedures.

Melting points were measured using a Kofler bench.

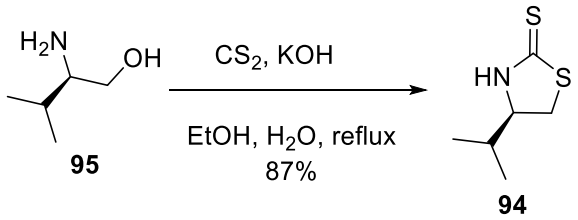
IR spectra (4000-600 cm^{-1}) were recorded on an Agilent Technologies 5500 series FTIR with and ATR-cell (diamond).

UV data were collected on a Biochrom Libra S32 PC.

Optical rotations were measured using a mL cell with a 1.0 dm path length on a Perkin Elmer 341 polarimeter using $CHCl_3$.

All reactions were performed in a flamed and degassed RBF, unless water was one of the reagents, or one of the reagents were hydrous. All reactions were performed under a nitrogenous atmosphere. All reactions involving EPA- or DHA-analogues were protected against light exposure.

3.1 Synthesis of (*R*)-4-isopropylthiazolidine-2-thione (**94**)



To a solution of (*R*)-2-amino-3-methylbutan-1-ol (**95**, 2.1 g, 20 mmole, 1 eq.) in EtOH (6.2 mL) was added carbon disulphide (4.9 mL, 81 mmole, 4.1 eq.). A solution of KOH (4.5 g, 80 mmole, 4.0 eq.) in EtOH/H_2O (1:1 v/v, 24 mL) was added dropwise at room temperature over 30 min. The now red solution was stirred at reflux for 67 hours under a nitrogen atmosphere. The flask was cooled, the volatiles removed *in vacuo*, and the mixture slowly acidified with 0.5 M HCL (130 mL). The aqueous solution was extracted with DCM (3 x 50 mL) and the combined organic extracts were dried (Na_2SO_4). Concentration *in vacuo* yielded a yellow oil, which was purified by flash chromatography (silica gel, *n*-hexane/EtOAc 4:1→7:3 v/v) to give the thiazolidinethione **94** (2.8 g, 17 mmole, 87%) as a colourless crystalline solid.

Data:

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (bs, 1H), 4.06 (td, *J* = 8.2, 6.5 Hz, 1H), 3.51 (dd, *J* = 11.2, 8.3 Hz, 1H), 3.33 (dd, *J* = 11.1, 8.4 Hz, 1H), 1.98 (dq, *J* = 13.4, 6.7 Hz, 1H), 1.02 (dd, *J* = 13.9, 6.8 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 201.1, 70.0, 36.0, 32.0, 18.8, 18.2.

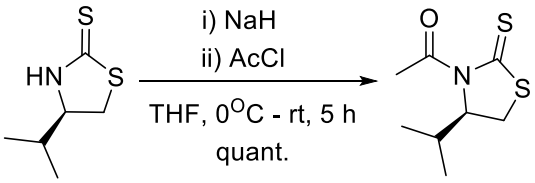
IR: 3182, 2964, 2869, 1661 cm^{-1}

Melting point: 71-73^{O}C

$[\alpha]_D^{20}$: +33.3^{O} ($CHCl_3$, c 0.78)

R_f: 0.53 (*n*-hexane/EtOac 7:3 v/v)

3.2 Synthesis of (*R*)-1-(4-isopropyl-2-thioxothiazolidin-3-yl)ethan-1-one (**74**)



94 **74**

To a suspension of 60% NaH (0.15 g, 3.7 mmole, 1.13 eq.) in dry THF (5 mL) was added a solution of **94** (0.53 g, 3.3 mmole, 1 eq.) in dry THF (5 mL). The mixture was stirred at 0°C for 10 min, before acetyl chloride (0.25 mL, 3.6 mmole, 1.10 eq.) was injected into the mixture. After 10 minutes at 0°C, the mixture was heated to room temperature, and left to stir for 5 h. 5% HCl (5 mL) was added, and the aqueous phase was extracted with EtOAc (3 x 30 mL). The organic phases were combined, washed with brine (20 mL), dried (Na_2SO_4), and concentrated *in vacuo* to give **74** (0.65 g, 3.2 mmole, quant.) as a yellow oil.

Data:

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.21 – 5.12 (m, 1H), 3.51 (dd, *J* = 11.5, 7.9 Hz, 1H), 3.03 (dd, *J* = 11.5, 1.3 Hz, 1H), 2.78 (s, 3H), 2.44 – 2.31 (m, 1H), 1.26 (s, 2H), 1.07 (d, *J* = 6.8 Hz, 4H), 1.03 – 0.95 (m, 4H), 0.93 – 0.79 (m, 1H).

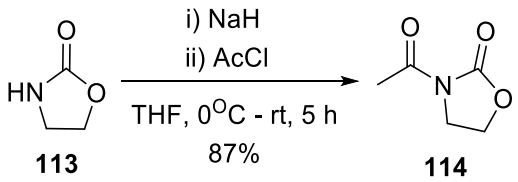
^{13}C NMR (101 MHz, $CDCl_3$) δ 203.2, 170.7, 71.2, 30.7, 30.4, 26.9, 19.0, 17.7.

IR: 2984, 1700 cm^{-1}

$[\alpha]_D^{20}$: -398.9° ($CHCl_3$, c 0.53)

R_f: 0.85 (*n*-hexane/EtOAc 1:9 v/v)

3.3 Synthesis of 3-acetyloxazolidin-2-one (**114**) I



To a suspension of 60% NaH (0.92 g, 23 mmole, 2.0 eq.) in dry DCM (10 mL) was added a solution of **113** (1.0 g, 11 mmole, 1 eq.) in dry DCM (10 mL). The mixture was stirred at 0°C for 10 min, before acetyl chloride (1.6 mL, 23 mmole, 2.0 eq.) was injected into the mixture. After 30 minutes at 0°C, the mixture was heated to room temperature. and left to stir overnight. 5% HCl (10 mL) was added, and the aqueous phase was extracted with EtOAc (3 x 30 mL). The organic phases were combined, washed with brine, dried (Na_2SO_4), and concentrated *in vacuo* to give a yellow oil. The oil was purified by column chromatography (silica gel, *n*-hexane/EtOAc 3:1 v/v) to yield **114** (1.3 g, 10 mmole, 87%) as a crystalline white solid.

Data:

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.41 (s, 2H), 4.02 (s, 2H), 2.53 (s, 3H).

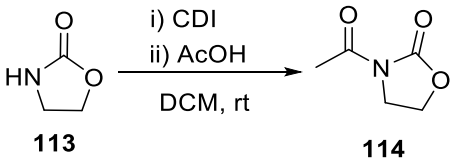
^{13}C NMR (101 MHz, $CDCl_3$) δ 170.45, 77.32, 77.01, 76.69, 61.94, 42.36, 23.22.

IR: 2992, 2924, 1778, 1694 cm^{-1}

Melting point: 75-76°C

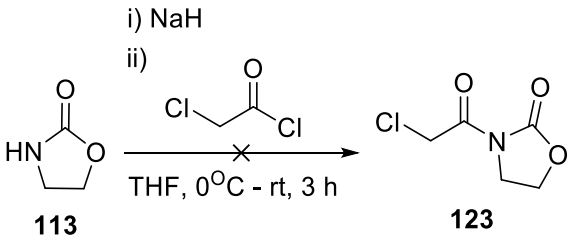
R_f: 0.08 (*n*-hexane/EtOAc 3:1 v/v)

3.4 Synthesis of 3-acetyloxazolidin-2-one (**114**) II



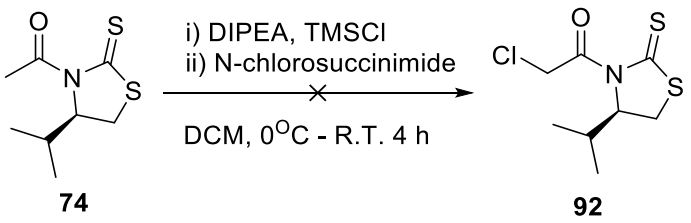
To a stirred solution of CDI (4.0 g, 25 mmole, 2.0 eq.) in dry DCM (10 mL) was added acetic acid (1.4 mL, 25 mmole, 2.0 eq.) dropwise. The mixture was stirred at room temperature for 1 h, before **113** was added, and the mixture was stirred overnight. 5% HCl was added until pH ≈ 1. The two phases were separated, and the aqueous phase was extracted with DCM (3 x 20 mL). The organic phases were combined, washed with brine (20 mL), dried (Na_2SO_4), and concentrated *in vacuo* to yield a mixture of unreacted **113** and the target material **114**. The oil was not purified.

3.5 Attempt at synthesis of 3-(2-chloroacetyl)oxazolidin-2-one (**123**)



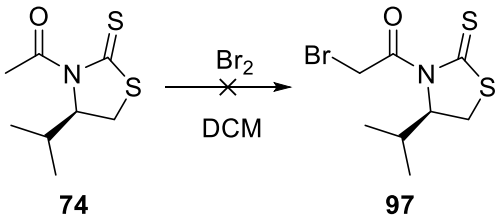
To a suspension of 60% NaH (0.11 g, 2.7 mmole, 1.14 eq.) in dry THF (4 mL) was added a solution of **113** (0.21 g, 2.4 mmole, 1 eq.) in dry THF (6 mL). The mixture was stirred at 0°C for 10 min, before chloroacetyl chloride (0.25 mL, 3.6 mmole, 1.10 eq.) was injected into the mixture. After 10 minutes at 0°C, the mixture was heated to room temperature and left to stir for 3 h. 5% HCl (2 mL) was added, then $NaHCO_3$ until basic pH. The aqueous phase was extracted with EtOAc (20 mL). The organic phase was washed with brine (20 mL), dried (Na_2SO_4), and concentrated *in vacuo* to give unreacted **113**.

3.6 Attempt at synthesis of (*R*)-2-chloro-1-(4-isopropyl-2-thioxothiazolidin-3-yl)ethan-1-one (**92**) I



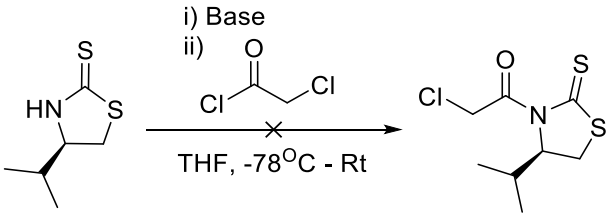
To a solution of **74** (0.23 g, 1.1 mmole, 1 eq.) in DCM (2.3 mL) at 0°C, was added DIPEA (0.25 mL, 1.5 mmole, 1.27 eq.) followed by TMSCl (0.18 mL, 1.4 mmole, 1.2 eq.). The latter resulted in formation of a mist, which dispersed within ten minutes. The mixture was maintained at 0°C for 30 min, before *N*-chlorosuccinimide (0.18 g, 1.4 mmole, 1.20 eq.) was added in small portions. The resulting mixture was stirred at room temperature for 4 h. Within ten minutes of removing the mixture from the ice bath, the mixture turned from yellow to orange to brown. The mixture was concentrated *in vacuo* to give a brown oil. The oil was washed with water (15 x 40 mL). The aqueous phases were combined and extracted with EtOAc (30 mL). The organic phases were combined, dried (Na_2SO_4), and concentrated *in vacuo* to give **94** as an impure, brown oil.

3.7 Attempt at synthesis of (*R*)-2-chloro-1-(4-isopropyl-2-thioxothiazolidin-3-yl)ethan-1-one (**97**)



Two parallels of **74** (0.21 g, 1.0 mmole, 1 eq.) in DCM (3 mL) were added Br_2 (0.05 mL, 1.0 mmole, 1 eq.). The mixtures were stirred overnight (a: packed in aluminium foil; b: not protected from light). The reaction was quenched with $Na_2S_2O_3$ (20 mL). The aqueous phase was then extracted with DCM (3 x 10 mL). The organic phases were combined, dried (Na_2SO_4), and concentrated *in vacuo* to give a complex mixture of products.

3.8 General procedure for attempt at synthesis of (*R*)-2-chloro-1-(4-isopropyl-2-thioxothiazolidin-3-yl)ethan-1-one (**92**) II



94 **92**

To a stirred solution of **94** (1.00 eq.) in anhydrous THF (to concentration 0.15 M) at -78°C was added a solution of Base (1.1 eq. MeLi, 3.6 eq. LDA, 1.1 eq. K^{t}BuO, or 1.1 eq. BuLi) over 15 min. After 30 min, chloroacetyl chloride (1.01 eq.) was added, and the solution was stirred for 30 min, then heated to room temperature over 30 min. The reaction was left to stir for 6-18 hours at room temperature, before it was quenched with a saturated aqueous solution of NH_4Cl. The aqueous solution was extracted with EtOAc. The organic phases were combined, dried (Na_2SO_4), and concentrated *in vacuo*, to give unreacted **94**.

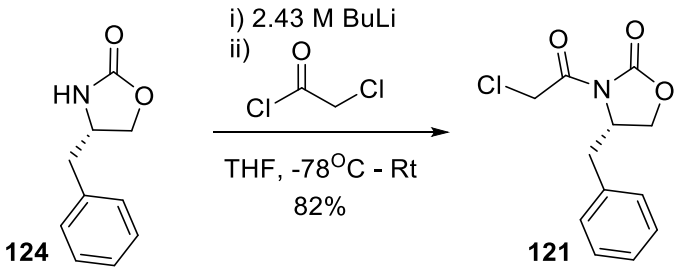
Data:

Of spectrum with base = LDA.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.15 – 3.89 (m, 1H), 3.43 (s, 0H), 3.34 (dd, *J* = 10.7, 8.1 Hz, 0H), 3.08 (dd, *J* = 10.8, 9.2 Hz, 0H), 1.87 (dt, *J* = 13.4, 6.7 Hz, 0H), 1.32 (d, *J* = 6.8 Hz, 1H), 1.17 (dd, *J* = 6.7, 3.1 Hz, 1H), 0.99 (d, *J* = 6.7 Hz, 1H), 0.91 (d, *J* = 6.7 Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.7, 164.9, 162.4, 82.8, 49.2, 46.0, 43.1, 37.6, 32.9, 20.7, 20.2, 19.6, 18.9.

3.9 Synthesis of (*S*)-4-benzyl-3-(2-chloroacetyl)oxazolidin-2-one (**121**)



To a stirred solution of **124** (0.53 g, 3.0 mmole, 1 eq.) in anhydrous THF (20 mL) at -78^{O}C was added a titrated 2.4 M solution BuLi (1.4 mL, 3.4 mmole, 1.13 eq.) over 15 min. After 30 min, chloroacetyl chloride (0.24 mL, 3.0 mmole, 1.01 eq.) was added, and the solution was stirred for 30 min, then heated to room temperature over 30 min. The reaction was left to stir for 6 hours at room temperature, before it was quenched with a saturated aqueous solution of NH_4Cl (30 mL). The aqueous solution was extracted with EtOAc (3 x 20 mL). The organic phases were combined, dried (Na_2SO_4), and concentrated *in vacuo* to yield **121** (0.62 g, 3.9 mmole, 82%) as a crystalline white solid.

Data:

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.08 (m, 6H), 4.65 – 4.59 (m, 1H), 4.23 – 4.09 (m, 2H), 3.70 – 3.58 (m, 3H), 3.23 (dd, *J* = 13.4, 3.3 Hz, 1H), 2.76 (dd, *J* = 13.4, 9.4 Hz, 1H), 1.80 – 1.72 (m, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 206.9, 202.4, 166.1, 153.2, 134.6, 129.3, 129.0, 127.5, 67.0, 55.4, 43.8, 37.6, 30.9.

IR: 3031, 2964, 2924, 1773, 1711 cm^{-1}

UV: 252.8, 258.7 nm

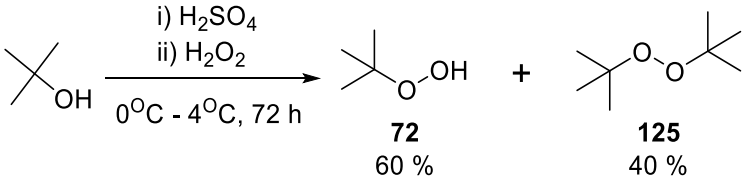
$[\alpha]_D^{20}$: +35.45^{O}C

Melting point: 79-81^{O}C

R_f: 0.48 (*n*-hexane/EtOAc 1:4 v/v)

3.10 Synthesis of Tert-butyl hydroperoxide (**72**)

The method is published by Milas & Surgenor.[109]



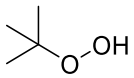
72 60 %

125 40 %

To a 70% solution of H_2SO_4 (140 g, 1.00 mole, 1 eq.) at 0^OC was added ^{t}BuOH (74 g, 1 mole, 1 eq.). When all ^{t}BuOH had dissolved, 30% H_2O_2 (113 g, 1.00 mole, 1 eq.) was added over 40 min. The ice-bath was removed, and the mixture was placed in a cold room (4^OC) to stir for 72 h. At this point, the mixture had separated into two layers. The organic layer was removed, neutralised with concentrated K_2CO_3 (100 mL), washed with water (200 mL), and dried ($MgSO_4$) to yield 49 g crude product. ^{1}H NMR showed that the product consisted of 60% **72** and 40% di-^{t}Bu peroxide (**125**). The mixture was added to a dry and degassed RBF, and dry toluene (100 mL) was added to make a 3.3 M solution of **72**.

3.11 Anhydrous TBHP from 70% TBHP (**72**) in water

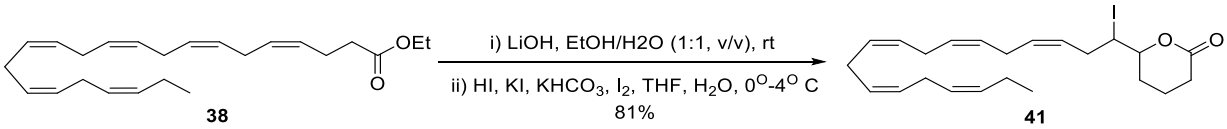
The method is published by Hill, Rossiter, & Sharpless.[110]



72

To a 250 mL separatory funnel was added TBHP-70 (100 mL, Sigma Aldrich) [70% TBHP, 30% water], followed by toluene (115 mL). The solution was swirled (shaking may cause an emulsion to form). The aqueous layer was separated, and the organic layer transferred to a 500 mL two-necked flask, equipped with a Dean-Stark trap (10 mL side arm filled with toluene), a reflux condenser, and a thermometer. After addition of a stirring magnet, the solution was refluxed using a heating mantle (caution: prevent overheating the TBHP by avoiding high power settings and by not allowing the solvent level to drop below the top of the mantle). After 4 h, only 0.5 mL water had been trapped at 89^{O}C. The Dean-Stark trap was isolated with aluminium foil, and the solution was heated. Water began to condense from 92^{O}C, and after about 1 h, the pot had reached 108^{O}C. Now about 6.5 mL of water had condensed off, and only toluene was trapped. Another 6.5 mL of toluene was tapped off, to ensure that all water was gone. After cooling, the pot solution was transferred to a 250 mL RBF, flushed with nitrogen, and stored in the fridge.

3.12 Synthesis of 6-((3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-1-iodooctadeca-3,6,9,12,15-pentaen-1-yl)tetrahydro-2H-pyran-2-one (**40**)



38 **41**

A mixture of the ethyl ester of DHA (**38**, 11 g, 33 mmole, 1.0 eq.) and $LiOH{\cdot}H_2O$ (6.3 g, 0.15 mole, 4.6 eq.) in EtOH/H_2O (1:1 v/v, 60 mL) was stirred at room temperature overnight. H_2O (90 mL) was added to the mixture, and it was cooled to $0^{O}C$. 57% aqueous HI (4.5 g, 0.23 mole, 7.0 eq.) was added, followed by KI (3.0 g, 18 mmole, 0.6 eq.), saturated aqueous solution of K_2CO_3 and $LiOH{\cdot}H_2O$ until pH ≈ 8.5. A solution of I_2 (23 g, 90 mmole, 2.7 eq.) in THF (70 mL) was added dropwise over 30 min. The flask was flushed with nitrogen and placed in a cold room ($4^{O}C$) to stir for 18 h. The reaction was quenched by adding a saturated aqueous solution of $Na_2S_2O_3$ (100 mL). Solid NaCl was added to saturation and the product was extracted with *n*-hexane (3 x 50 mL). The combined extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give **41** (12 g, 26 mmole, 81%) as a yellow oil.

Data:

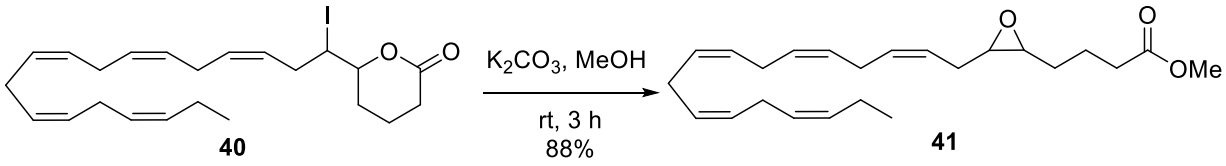
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.58 (dtt, *J* = 10.3, 7.2, 1.5 Hz, 1H), 5.49 – 5.26 (m, 8H), 4.28 (ddd, *J* = 7.7, 6.8, 3.0 Hz, 1H), 4.13 (td, *J* = 7.5, 3.1 Hz, 1H), 3.81 – 3.68 (m, 1H), 2.93 – 2.79 (m, 9H), 2.73 (ddd, *J* = 18.0, 10.6, 4.8 Hz, 1H), 2.57 (ddd, *J* = 17.9, 10.6, 8.2 Hz, 1H), 2.48 – 2.36 (m, 1H), 2.16 – 2.02 (m, 3H), 1.89 – 1.83 (m, 1H), 0.98 (t, *J* = 7.5 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 176.2, 132.0, 131.6, 128.8, 128.6, 128.4, 127.9, 127.8, 127.3, 127.0, 126.7, 80.7, 37.7, 34.6, 28.5, 27.3, 25.9, 25.7, 25.6, 25.6, 20.6, 14.3.

IR: 3014, 2964, 1784, 1656 cm^{-1}

R_f: 0.28 (*n*-hexane/EtOAc 3:1 v/v)

3.13 Synthesis of methyl 4-(3-((2*Z*,5*Z*,8*Z*,11*Z*,14*Z*)-heptadeca-2,5,8,11,14-pentaen-1-yl)oxiran-2-yl)butanoate (**41**)



To a solution of **40** (11 g, 23 mmole, 1 eq.) in MeOH (119 mL) was added K_2CO_3 (6.3 g, 35 mmole, 1.5 eq.). The solution was left to stir for 3 hours at room temperature. Water (40) mL was added, and the polar phase was extracted with *n*-hexane (3 x 40 mL). The non-polar phases were combined, dried ($MgSO_4$), and concentrated *in vacuo* to give **41** (7.4 g, 20 mmole, 86%) as a yellow oil.

Data:

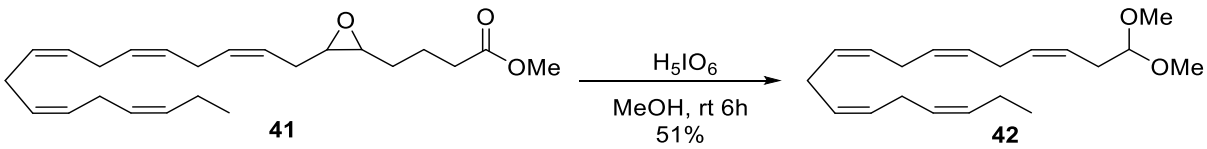
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.59 – 5.25 (m, 10H), 3.71 (s, 3H), 2.99 (td, *J* = 6.2, 5.6, 3.3 Hz, 2H), 2.92 – 2.77 (m, 8H), 2.56 – 2.37 (m, 3H), 2.31 – 2.20 (m, 1H), 2.08 (pd, *J* = 7.5, 1.3 Hz, 2H), 2.01 – 1.88 (m, 1H), 1.88 – 1.75 (m, 1H), 1.58 (s, 1H), 1.34 – 1.23 (m, 1H), 0.98 (t, *J* = 7.5 Hz, 3H), 0.88 (d, *J* = 7.1 Hz, 0H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.19, 132.03, 130.66, 128.58, 128.49, 128.34, 127.94, 127.81, 127.70, 126.98, 124.17, 56.61, 55.95, 51.73, 30.99, 26.19, 25.80, 25.64, 25.61, 25.53, 23.33, 20.55, 14.26.

IR: 3014, 2964, 1739, 1656 cm^{-1}

R_f: 0.63 (DCM)

3.14 Synthesis of (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-1,1-dimethoxyoctadeca-3,6,9,12,15-pentaene (**42**)



To a stirred solution of **41** (7.4 g, 20 mmole, 1 eq.) in MeOH (120 mL) was added periodic acid (5.0 g, 22 mmole, 1.1 eq.). The solution was left to stir for 6 hours at room temperature. Water (100 mL) was added, and the solution extracted with *n*-hexane (3 x 50 mL). The organic phases were combined, washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. The resulting yellow oil was filtrated on silica gel (*n*-hexane/EtOAc 95:5 v/v) to give **42** (3.0 g, 9.9 mmole, 51%) as a colourless oil.

Data:

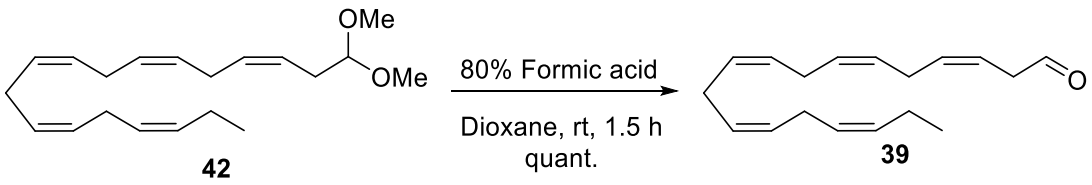
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.56 – 5.26 (m, 10H), 4.40 (t, *J* = 5.7 Hz, 1H), 3.35 (s, 6H), 2.92 – 2.77 (m, 8H), 2.48 – 2.37 (m, 2H), 2.14 – 2.01 (m, 2H), 0.99 (t, *J* = 7.6 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 132.0, 130.3, 128.6, 128.3, 128.0, 128.0, 127.9, 127.0, 123.9, 104.1, 53.0, 31.0, 25.8, 25.6, 25.6, 25.5, 20.6, 14.3.

IR: 3014, 2964, 2830, 1656 cm^{-1}

R_f: 0.30 (DCM)

3.15 Synthesis of *Synthesis* of (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-octadeca-3,6,9,12,15-pentaenal (**39**)



To a stirred solution of **42** (320 mg, 1.05 mmole, 1 eq.) in Dioxane (3 mL) was added 80% aq. Formic acid (4 mL). The solution was stirred for 1.5 hour at room temperature. Water was added, and the solution was extracted with hexane (2 x 20 mL). The organic phases were combined, neutralised with saturated aqueous solution of $NaHCO_3$ (25 mL), washed with brine (25 mL), and dried ($MgSO_4$). The solution was concentrated *in vacuo* to yield **39** (272 mg, 1.05 mmole, quant.) as a colourless oil.

Data:

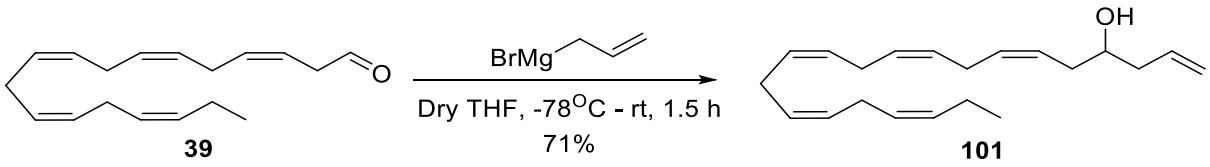
^{1}H NMR (400 MHz, $CDCl_3$) δ 9.69 (s, 1H), 5.77 – 5.66 (m, 1H), 5.66 – 5.55 (m, 1H), 5.49 – 5.26 (m, 8H), 3.24 (dt, *J* = 7.2, 1.7 Hz, 2H), 2.93 – 2.77 (m, 8H), 2.09 (pd, *J* = 7.4, 1.3 Hz, 2H), 1.55 (s, 1H), 0.98 (t, *J* = 7.5 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 199.3, 133.1, 132.1, 128.4, 127.8, 127.8, 127.1, 118.7, 42.5, 26.0, 25.7, 25.6, 25.5, 20.6, 14.3.

IR: 3014, 2964, 2724, 1728 cm^{-1}

R_f: 0.71 (DCM)

3.16 Synthesis of (6*Z*,9*Z*,12*Z*,15*Z*,18*Z*)-henicosa-1,6,9,12,15,18-hexaen-4-ol (**101**)



39 **101**

To a stirred solution of **39** (0.47 g, 1.8 mmole, 1 eq.) in dry THF (20 mL) at -78^{O}C was added a 1 M solution of Allyl Magnesium Bromide (3.3 mL, 3.3 mmole, 1.8 eq.) dropwise. The reaction was stirred at -78^{O}C for 30 min, then heated to room temperature over 1 h. The mixture was stirred overnight, then quenched with saturated aqueous solution of NH_4Cl (30 mL). The aqueous phase was extracted with diethyl ether until all product was in the organic phase, as checked by TLC. The organic phases were combined, washed with Brine (40 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The resulting oil was purified by column chromatography (Silica gel, DCM) to yield **101** (0.39 g, 1.3 mmole, 71%) as a colourless oil.

Data:

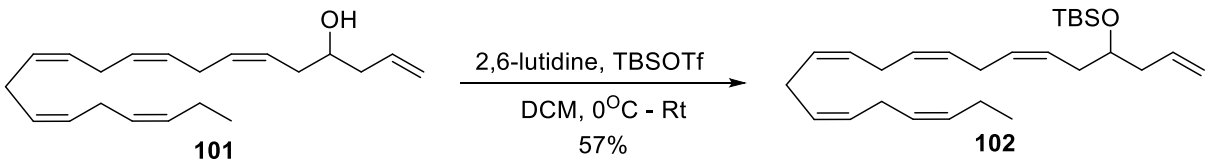
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.94 – 5.77 (m, 1H), 5.64 – 5.26 (m, 10H), 5.22 – 5.10 (m, 2H), 3.71 (dtd, *J* = 7.6, 5.7, 4.5 Hz, 1H), 2.95 – 2.77 (m, 8H), 2.39 – 2.14 (m, 4H), 2.09 (pd, *J* = 7.4, 1.4 Hz, 2H), 1.66 (s, 1H), 0.99 (t, *J* = 7.5 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 134.7, 132.0, 131.0, 128.6, 128.3, 128.3, 128.0, 127.9, 127.8, 127.0, 125.5, 118.1, 70.4, 41.3, 34.7, 25.8, 25.7, 25.6, 25.5, 20.6, 14.3.

IR: 3344 (broad), 3075, 3014, 2964, 1644 cm^{-1}

R_f: 0.32 (*n*-hexane/EtOAc 95:5)

3.17 Synthesis of tert-butyl(((6*Z*,9*Z*,12*Z*,15*Z*,18*Z*)-henicosa-1,6,9,12,15,18-hexaen-4-yl)oxy)dimethylsilane (**102**)



To a solution of **101** (0.22 g, 0.74 mmole, 1.0 eq.) in DCM (7.4 mL) at 0°C was added 2,6-lutidine (0.26 mL, 2.2 mmole, 3.0 eq.) and TBSOTf (0.22 mL, 0.97 mmole, 1.3 eq.). The reaction was stirred at 0°C – room temperature overnight. The reaction was quenched with NH_4Cl (30 mL). The aqueous phase was extracted with DCM (3 x 20 mL). The organic phases were combined, dried (Na_2SO_4), and concentrated *in vacuo* to yield a yellow oil. The oil was purified by column chromatography (silica gel, *n*-hexane/EtOAc 95:5 v/v), to yield **102** (0.17 g, 1.1 mmole, 57%) as a colourless oil.

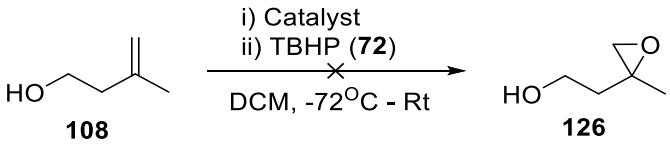
Data:

^{1}H NMR (400 MHz $CDCl_3$) δ 5.95 – 5.75 (m, 1H), 5.52 – 5.27 (m, 10H), 5.11 – 5.01 (m, 2H), 3.76 (p, *J* = 5.9 Hz, 1H), 2.93 – 2.77 (m, 8H), 2.31 – 2.15 (m, 4H), 2.15 – 2.04 (m, 2H), 1.00 (t, *J* = 7.5 Hz, 3H), 0.94 – 0.86 (m, 10H), 0.13 – -0.00 (m, 7H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 135.28, 131.97, 129.38, 128.53, 128.25, 128.20, 128.10, 128.04, 127.85, 127.00, 126.25, 116.81, 71.97, 41.58, 34.85, 25.86, 25.84, 25.64, 25.61, 25.53, 20.55, 18.10, 14.26, -4.48, -4.53.

R_f: 0.79 (DCM)

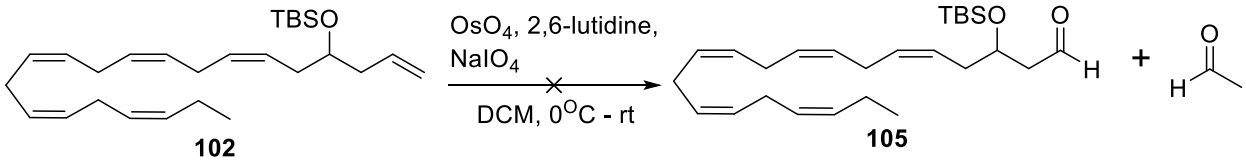
3.18 Attempt at synthesis of 2-(2-methyloxiran-2-yl)ethan-1-ol (**126**)



108 **126**

To a solution of **108** (0.51 g, 5.9 mmole, 1 eq.) in DCM (10 mL) at 0°C was added catalyst (Molybdene $(CO)_6$ or Vanadyl acac, 31 mg, 0.12 mmole, 2 mole%), then 3.3 M TBHP (**72**, 2.2 mL, 7.1 mmole, 1.2 eq.). After 20 min, the mixture was heated to room temperature and left to stir overnight. The mixture was washed with water (40 mL), brine (40 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Product was not found.

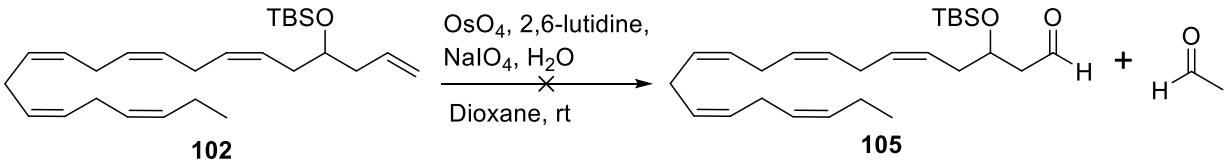
3.19 Attempt at synthesis of (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-3-hydroxyicosa-5,8,11,14,17-pentaenal (**105**) I



102 **105**

To a solution of **102** (70 mg, 0.17 mmole, 1 eq.) in DCM (10 mL) at 0°C was added 2,6-lutidine (0.02 mL, 0.20 mmole, 1.2 eq.), sodium periodate (40 mg, 0.19 mmole, 1.1 eq.), and OsO_4 (0.03 mL, 0.01 mmole, 3 mole%). The mixture was stirred at 0°C for 1 h, then heated to room temperature, and stirred for 24 h. The reaction was quenched with NH_4Cl (40 mL). The phases were separated, and the aqueous phase was extracted with DCM (3 x 20 mL). The organic phases were combined, dried (Na_2SO_4), and concentrated *in vacuo* to yield impure **102**.

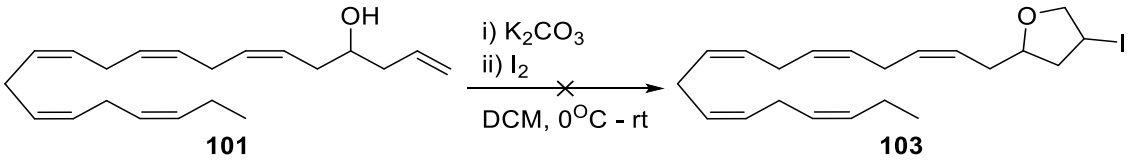
3.20 Attempt at synthesis of (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-3-hydroxyicosa-5,8,11,14,17-pentaenal (**105**) II



102 **105**

To a stirred solution of **102** (64 mg, 0.15 mmole, 1 eq.) in dioxane (3.2 mL) was added, sequentially, 2,6-lutidine (0.04 mL, 0.31 mmole, 2 eq.), OsO_4 (0.02 mL, 0.00 mmole, 2 mole%), water (1 mL), and sodium periodate (0.13 g, 0.62 mmole, 4 eq.). The reaction was stirred at room temperature for 2.5 h, before it was added water (20 mL) and DCM (40 mL). The phases were separated, and the aqueous phase was extracted with DCM (3 x 20 mL). The organic phases were combined, dried (Na_2SO_4), and concentrated *in vacuo*. The reaction cleaved **102** at several double bonds.

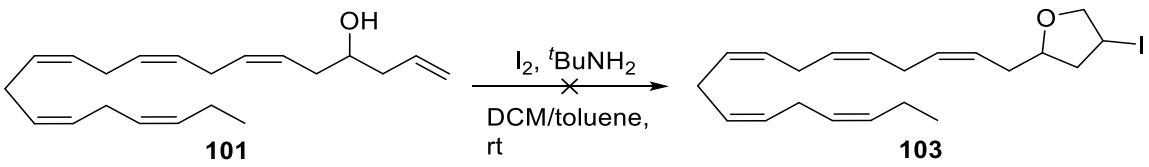
3.21 Attempt at synthesis of 2-((2*Z*,5*Z*,8*Z*,11*Z*,14*Z*)-heptadeca-2,5,8,11,14-pentaen-1-yl)-4-iodotetrahydrofuran (**103**)



101 **103**

To a solution of **101** (70 mg, 0.23 mmole, 1 eq.) in DCM (12 mL), was added K_2CO_3 (94 mg, 0.68 mmole, 2.9 eq.), and the mixture was cooled to $0^{O}C$. I_2 (80 mg, 0.32 mmole, 1.4 eq.) in DCM (7.5 mL) was added, and the solution was stirred at $0^{O}C$ for 1 h, before it was heated to room temperature and stirred overnight. DCM (40 mL) was added to the reaction. The organic phase was washed with $Na_2S_2O_3$ (30 mL), brine (30 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The concentrate was purified by column chromatography (silica gel, *n*-hexane→DCM→EtOAc) to yield relatively pure **101**.

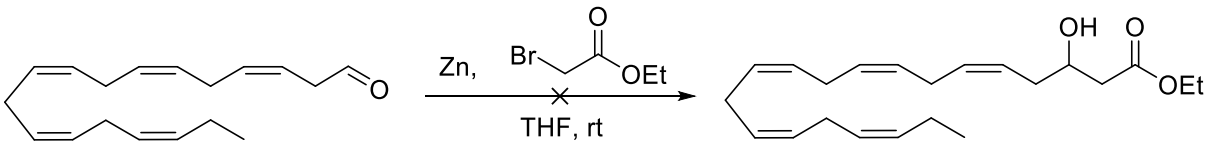
3.22 Attempt at synthesis of 2-((2*Z*,5*Z*,8*Z*,11*Z*,14*Z*)-heptadeca-2,5,8,11,14-pentaen-1-yl)-4-iodotetrahydrofuran (**103**) II



101 **103**

^{t}BuNH$_2$ (0.06 mL, 0.53 mmole, 4 eq.) and I_2 (68 mg, 0.27 mmole, 2 eq.) were stirred in toluene for 15 min, then added to a stirred solution of **101** (40 mg, 0.13 mmole, 1 eq.) in DCM (0.65 mL). The reaction was stirred for 18 h, before DCM (50 mL), water (20 mL), 1.6 M HCl (10 mL), and saturated aquatic solution of $Na_2S_2O_4$ (10 mL) were added. The two phases were separated, and the aqueous phase was extracted with DCM (3 x 50 mL). The organic phases were combined, dried ($MgSO_4$), and concentrated *in vacuo* to yield pure **101**.

3.23 Attempt at synthesis of ethyl (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-3-hydroxyicosa-5,8,11,14,17-pentaenoate (**91**) I

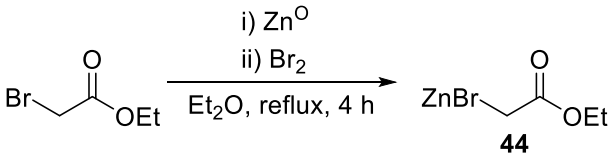


39 **91**

To a stirred solution of ethyl bromoacetate (451 mg, 2.70 mmole, 2 eq.) in THF (5 mL) was added zinc powder (265 mg, 4.05 mmole, 3 eq.). After 20 min, **39** (349 mg, 1.35 mmole, 1 eq.) was added dropwise. The solution was stirred for 19 hours at room temperature, then quenched with water (20 mL). The aqueous solution was extracted with Et_2O (2 x 40 mL). The organic phases were combined, washed with 0.5 M HCl (40 mL), then brine (40 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The resulting oil was purified by column chromatography (silica gel, *n*-hexane → DCM → EtOAc) to yield inseparable fractions, and residues of **39**.

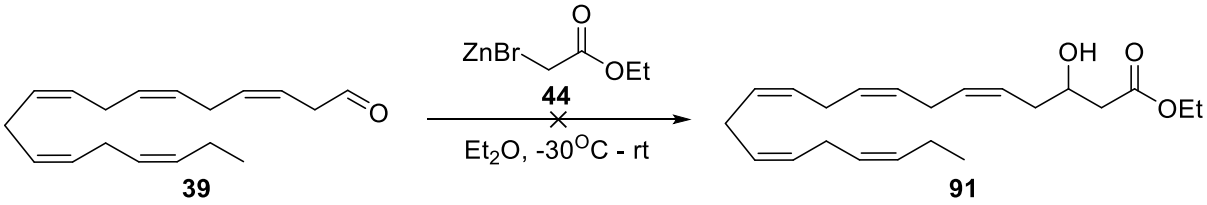
3.24 Synthesis of Reformatsky reagent (**44**)

The method is by Greszler, Malinowski, & Johnson.[111]



To a flamed and degassed two-necked flask was added granular zinc (0.81 g, 12 mmole, 2 eq.) and Et_2O (15 mL). The flask was fitted with a condenser and purged with nitrogen. Br_2 (0.04 mL, 0.80 mmole, 13 mole%) was added dropwise over 2 min with stirring. The suspension was heated to reflux in a water bath, before ethyl bromoacetate (1.6 mL, 6.2 mmole, 1 eq.) was added dropwise over 15 min. The mixture was stirred for 4 hours at reflux, then cooled to room temperature. The reagent **44** was stored as 0.35 M in a fridge under nitrogen for up to a week.

3.25 Attempt at synthesis of ethyl (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-3-hydroxyicosa-5,8,11,14,17-pentaenoate (**91**) II

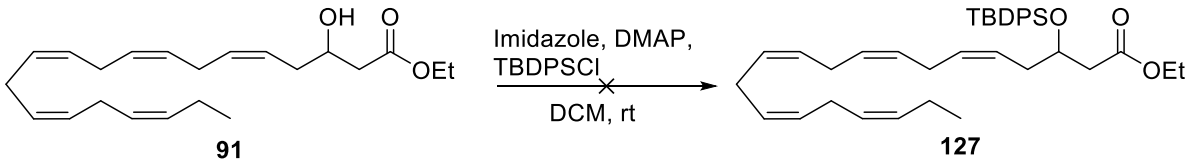


A flamed and degassed RBF was charged with the Reformatsky reagent **44** (10 mL, 3.5 mmole, 2.5 eq.). The suspension was diluted with Et_2O (8 mL) and cooled to -30^{O}C in an acetone/dry-ice bath (bath temperature monitored with a thermometer). The aldehyde **39** (0.37 g, 1.4 mmole, 1 eq.) was dissolved in Et_2O (12 mL) in a second RBF, and cooled to -30^{O}C in the same bath, before it was added to the Reformatsky reagent. Additional Et_2O (8 mL) was used to transfer residues of **39**. The reaction was allowed to heat to room temperature overnight, before it was worked-up using either Method A or Method B.

Method A The reaction was quenched with 0.5 M HCl (20 mL). The aqueous phase was extracted with Et_2O (3 x 25 mL). The organic phases were combined, washed with brine (40 mL), dried (Na_2SO_4), and concentrated *in vacuo* to yield an impure oil. The oil was purified by column chromatography (silica gel, DCM) to yield an inseparable and complicated mixture of **91**, the de-hydroxygenated product (**110**), and a conjugated system (**110** or **112**).

Method B The reaction was quenched with phosphate buffer (pH = 7.29, 25 mL), and added EDTA (1.2 g, 4.0 mmole, 2.8 eq.). The phases were separated, and the aqueous solution was extracted with DCM (3 x 20 mL). The organic phases were combined and washed with brine (40 mL). A solid emulsion formed throughout both phases at once, after the brine was added. To separate the emulsion into two phases, additional DCM was added, and a glass pipette was used to disrupt the emulsion. When the phases were separated, the organic phase was dried (Na_2SO_4) and concentrated *in vacuo* to yield the enolate of the β-keto ester (**112**).

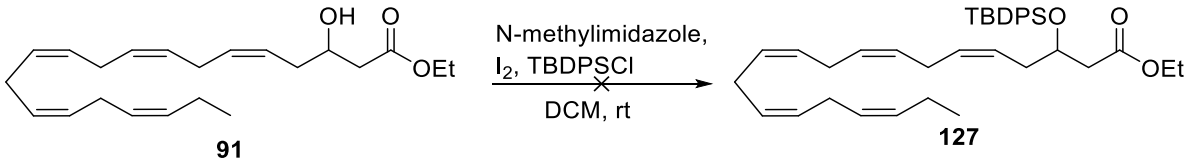
3.26 Attempt at synthesis of ethyl (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-3-((tert-butyldiphenylsilyl)oxy)icosa-5,8,11,14,17-pentaenoate (**127**) I



91 **127**

To a flamed and degassed RBF was added the Reformatsky product **91** (0.49 g, 1.4 mmole, 1.0 eq.), DCM (10 mL), imidazole (0.38 g, 5.6 mmole, 3.9 eq.), and DMAP (12 mg, 0.10 mmole, 7 mole%). In a second flamed and degassed RBF was diluted TBDPSCl (0.07 mL, 1.4 mmole, 1.01 eq.) in DCM (4 mL), before it was added to the mixture. The mixture was then stirred overnight, before the solvent vas removed *in vacuo*. The resulting brown oil was purified by flash column (silica gel, *n*-hexane → EtOAc) to yield an impure product, different from the target molecule.

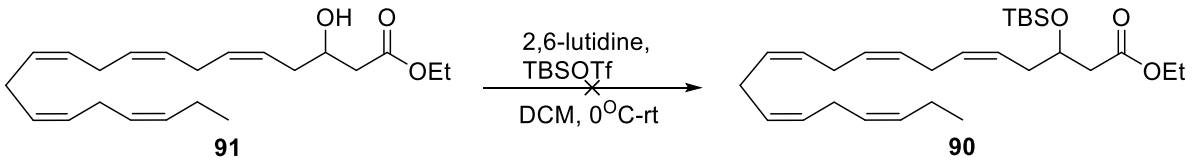
3.27 Attempt at synthesis of ethyl (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-3-((tert-butyldiphenylsilyl)oxy)icosa-5,8,11,14,17-pentaenoate (**127**) II



91 **127**

To a solution of the Reformatsky product **91** (89 mg, 0.26 mmole, 1 eq.) in DCM (3 mL), was added *N*-methylimidazole (0.06 mL, 0.77 mmole, 3.0 eq), I_2 (23 mg, 0.77 mmole, 3.0 eq.), and TBDPSCl (0.07 mL, 0.28 mmole, 1.1 eq.). The mixture was left to stir at room temperature overnight. The resulting oil was added aqueous solution of $Na_2S_2O_3$ (10 mL), and the aqueous solution was extracted with EtOAc (30 mL). The organic phase was dried (Na_2SO_4) and concentrated *in vacuo* to yield a complicated mixture.

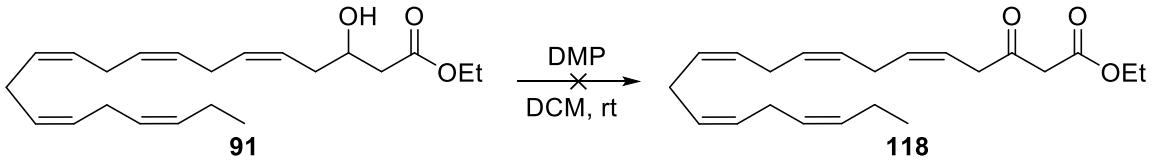
3.28 Attempt at synthesis of ethyl (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-3-((tert-butyldimethylsilyl)oxy)icosa-5,8,11,14,17-pentaenoate (**90**)



91 **90**

To a solution of the Reformatsky product **91** (0.23 g, 0.68 mmole, 1.0 eq.) in DCM (7 mL) at 0^{O}C was added 2,6-lutidine (0.24 mL, 2.0 mmole, 3.0 eq.) and TBSOTf (0.20 mL, 0.88 mmole, 1.3 eq.). The mixture was stirred at 0^{O}C- room temperature overnight, before the reaction was quenched with saturated aqueous solution of NH_4Cl (30 mL). The aqueous phase was extracted with DCM (3 x 30 mL). The organic phases were combined, dried (Na_2SO_4), and concentrated *in vacuo*. The resulting orange oil was purified by column chromatography (silica gel, *n*-hexane/EtOAc 95:5 v/v) to yield an impure product, different from the target molecule.

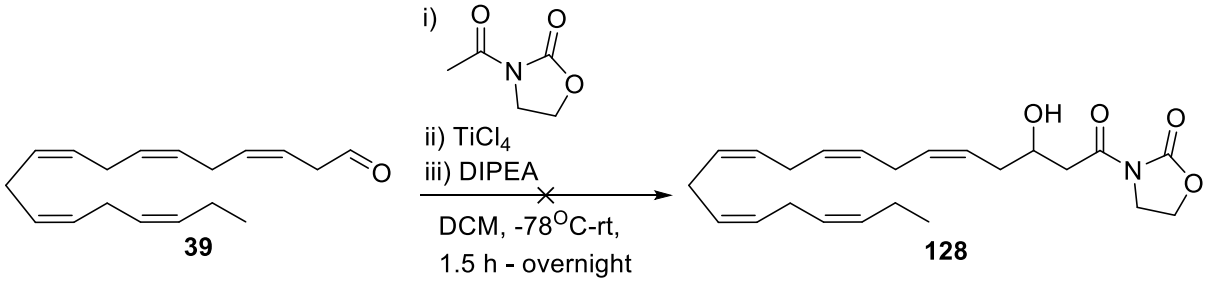
3.29 Attempt at synthesis of ethyl (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-3-oxoicosa-5,8,11,14,17-pentaenoate (**118**)



91 **118**

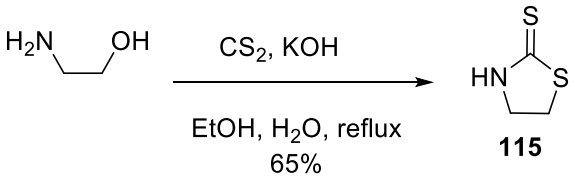
The Reformatsky product **91** (0.23 g, 0.68 mmole, 1.0 eq.) was dissolved in wet DCM (10 mL) and placed under powerful stirring. DMP (0.29 g, 0.68 mmole, 1.0 eq.) was dissolved in wet DCM (10 mL) and added to the stirred solution. The mixture was stirred at room temperature for 2.5 h, before 20% $Na_2S_2O_3$ (20 mL) was added. The phases were separated, and the organic phase was washed with 3% NaOH (20 mL). The aqueous phases were combined and extracted with Et_2O (3 x 20 mL). The organic phases were combined and washed with first 20% $Na_2S_2O_3$ (20 mL), then 3% NaOH (20 mL), and lastly brine (20 mL). The organic phase was then dried (Na_2SO_4) and concentrated *in vacuo*. The resulting orange oil was purified by column chromatography (silica gel, *n*-hexane/EtOAc 3:1 v/v) to provide a complicated by-product.

3.30 Attempt at synthesis of 3-((5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-3-hydroxyicosa-5,8,11,14,17-pentaenoyl)oxazolidin-2-one (**128**)



To a solution of the auxiliary **114** (0.51 g, 3.9 mmole, 1.11 eq.) in dry DCM (40 mL) at -78^{O}C was added dropwise a 1 M solution of $TiCl_4$ (3.9 mL, 3.9 mmole, 1.11 eq.). The mixture was stirred for 5 min, while the colour went from blank to orange to cream-yellow. DIPEA (0.82 mL, 4.7 mmole, 1.33 eq.) in dry DCM (10 mL) was added dropwise. The mixture was stirred at -78^{O}C for 1 hour. The colour changed from yellow to a very dark red. The freshly made aldehyde **39** (0.92 g, 3.6 mmole, 1.00 eq.) in dry DCM (10 mL) was added dropwise over 1.5 h. The mixture was stirred at -78^{O}C for (a: 1.5 h; b: overnight) before the reaction was quenched with half saturated NH_4Cl (40 mL) and heated to room temperature. The phases were separated, and the aqueous phase was extracted with DCM (2 x 30 mL). The organic phases were combined, dried (Na_2SO_4), and concentrated *in vacuo* to yield an opaque, orange oil. The oil was purified by column chromatography (silica gel, *n*-hexane/EtOAc 3:1 v/v) to yield unreacted **39** (0.43 g, 1.7 mmole, 47%), **114** (0.41 g, 3.2 mmole, 81%), and varying impure fractions.

3.31 Synthesis of thiazolidine-2-thione (**115**)



To a solution of 2-aminoethanol (2.0 g, 33 mmole, 1 eq.) in EtOH (10 mL) was added carbon disulphide (8.0 mL, 0.13 mole, 4 eq.). A solution of KOH (2.3 g, 41 mmole, 4.9 eq.) in EtOH/H_2O (1:1 v/v, 40 mL) was added dropwise over 30 min. The now red solution was then stirred at reflux for 90 hours under a nitrogen atmosphere. The flask was then cooled, the volatiles removed *in vacuo*, and the mixture slowly acidified with 0.5 M HCL (200 mL). The aqueous solution was extracted with DCM (3 x 50 mL) and the combined organic extracts were dried (Na_2SO_4). Concentration *in vacuo* yielded an orange solid, which was purified by column chromatography (silica gel, *n*-hexane/EtOAc 4:1→7:3 v/v) to give the thiazolidinethione **115** (2.6 g, 22 mmole, 65%) as a crystalline white solid.

Data:

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (s, 1H), 3.99 (dd, *J* = 8.3, 7.3 Hz, 2H), 3.57 (dd, *J* = 8.3, 7.3 Hz, 2H).

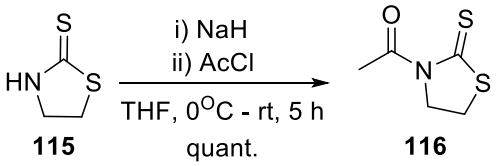
^{13}C NMR (101 MHz, $CDCl_3$) δ 202.20, 51.39, 33.91.

IR: 3148, 2964, 2874 cm^{-1}

Melting point: 113-115^{O}C

R_f: 0.06 (*n*-hexane/EtOAc 7:3)

3.32 Synthesis of 1-(2-thioxothiazolidin-3-yl)ethan-1-one (**116**)



115 **116**

To a suspension of 60% NaH (0.38 g, 9.5 mmole, 1.10 eq.) in dry THF (7 mL) was added a solution of **115** (1.0 g, 8.6 mmole, 1 eq.) in dry THF (7 mL). The mixture was stirred at 0°C for 10 min, before acetyl chloride (0.67 mL, 9.4 mmole, 1.10 eq.) was injected into the mixture. After 10 minutes at 0°C, the mixture was heated to room temperature, and left to stir for 5 h. 5% HCl (3.8 mL) was added, and the aqueous phase was extracted with EtOAc (3 x 20 mL). The organic phases were combined, washed with brine (10 mL), dried (Na_2SO_4), and concentrated *in vacuo* to give **116** (1.4 g, 8.6 mmole, quant.) as a yellow oil.

Data:

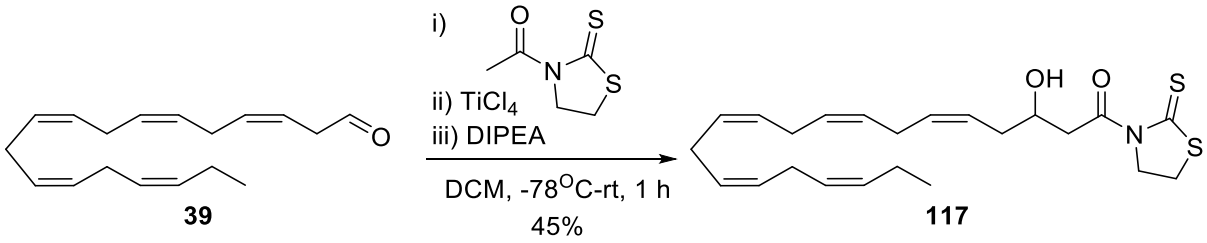
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.57 (t, *J* = 7.5 Hz, 2H), 3.29 (t, *J* = 7.5 Hz, 2H), 2.77 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 202.11, 171.60, 55.78, 28.35, 27.14.

IR: 2924, 2852, 1700 cm^{-1}

R_f: 0.30 (*n*-hexane/EtOAc 7:3)

3.33 Synthesis of (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-3-hydroxy-1-(2-thioxothiazolidin-3-yl)icosa-5,8,11,14,17-pentaen-1-one (**117**)



To a solution of the auxiliary **116** (0.47 g, 2.9 mmole, 1.69 eq.) in dry DCM (20 mL) at -78^{O}C was added dropwise a 1 M solution of $TiCl_4$ (2.2 mL, 2.2 mmole, 1.25 eq.). The mixture was stirred for 5 min, while the colour went from blank to orange to cream-yellow. DIPEA (0.45 mL, 2.6 mmole, 1.50 eq.) in dry DCM (5 mL) was added dropwise. The mixture was stirred at -78^{O}C for 1 h. The colour changed from yellow to a very dark red. The freshly made aldehyde **39** (0.45 g, 1.7 mmole, 1 eq.) in dry DCM (5 mL) was added dropwise over 1 h. The mixture was stirred at -78^{O}C for 1 h, before the reaction was quenched with half saturated NH_4Cl (40 mL) and heated to room temperature. The phases were separated, and the aqueous phase was extracted with DCM (2 x 30 mL). The organic phases were combined, dried (Na_2SO_4), and concentrated *in vacuo* to yield an opaque, orange oil. The oil was purified by column chromatography (silica gel, *n*-hexane/EtOAc 9:1 v/v) to yield first the auxiliary **3S** (0.10 g, 0.63 mmole, 22%), then the aldol product **117** (0.33 g, 0.78 mmole, 45%) as a yellow oil.

Data:

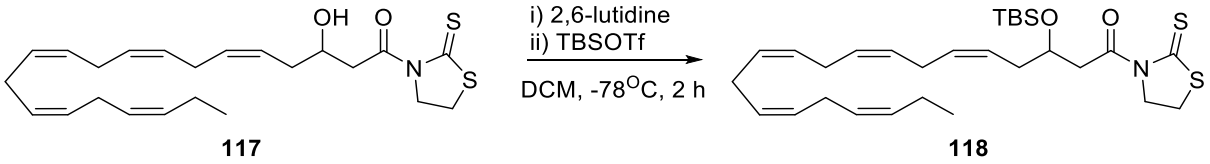
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.57 – 5.23 (m, 10H), 4.57 (td, *J* = 7.5, 3.6 Hz, 2H), 4.21 – 4.05 (m, 1H), 3.51 (dd, *J* = 17.7, 2.7 Hz, 1H), 3.34 – 3.20 (m, 3H), 2.91 (s, 1H), 2.87 – 2.73 (m, 9H), 2.34 (qd, *J* = 6.5, 1.2 Hz, 2H), 2.12 – 1.98 (m, 2H), 0.96 (t, *J* = 7.5 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 201.82, 174.02, 132.09, 131.21, 128.64, 128.46, 128.38, 128.10, 127.99, 127.94, 127.10, 125.02, 67.98, 55.75, 45.30, 34.41, 28.46, 25.89, 25.76, 25.73, 25.64, 20.65, 14.39.

IR: 3523 (broad), 3008, 2964, 1689 cm^{-1}

R_f: 0.07 (DCM)

3.34 Synthesis of (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-3-((tert-butyldimethylsilyl)oxy)-1-(2-thioxothiazolidin-3-yl)icosa-5,8,11,14,17-pentaen-1-one (**118**)



117 **118**

To a solution of the aldol product **117** (0.32 g, 0.76 mmole, 1.0 eq.) in DCM (30 mL) at -78^{O}C was added 2,6-lutidine (0.27 mL, 2.3 mmole, 3.0 eq.) dropwise. After 10 min, TBSOTf (0.21 mmole, 0.92 mmole, 1.2 eq.) was added dropwise, and the mixture was stirred at -78^{O}C for 2 h. The reaction was quenched with saturated NH_4Cl (40 mL). The two phases were separated, and the aqueous phase was extracted with DCM (3 x 30 mL). The organic phases were combined, dried (Na_2SO_4), and concentrated *in vacuo*. The resulting yellow oil was purified by column chromatography (silica gel, *n*-hexane/EtOAc 3:1 v/v) to yield **118** (0.32 g, 0.61 mmole, 79%) as a yellow oil.

Data:

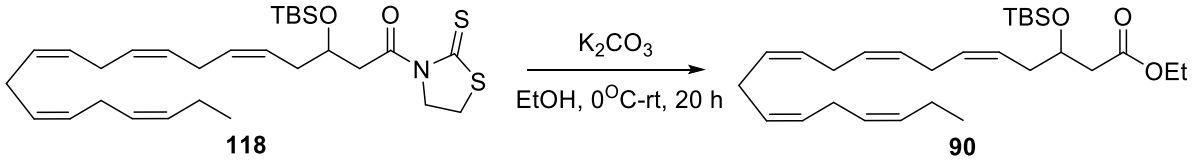
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.52 – 5.25 (m, 10H), 4.60 – 4.43 (m, 2H), 4.35 (dtd, *J* = 7.6, 5.9, 4.5 Hz, 1H), 3.47 – 3.34 (m, 2H), 3.34 – 3.18 (m, 2H), 2.90 – 2.73 (m, 8H), 2.32 (td, *J* = 6.2, 1.2 Hz, 2H), 2.13 – 2.01 (m, 2H), 0.97 (t, *J* = 7.5 Hz, 3H), 0.07 (d, *J* = 15.0 Hz, 7H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 201.57, 173.06, 132.21, 130.65, 128.76, 128.48, 128.27, 128.24, 128.08, 127.22, 125.40, 69.28, 56.17, 45.98, 35.74, 28.59, 26.02, 25.99, 25.87, 25.83, 25.74, 20.76, 18.19, 14.48, -4.21, -4.63.

IR: 3014, 2958, 2857, 1700 cm^{-1}

R_f: 0.78 (DCM)

3.35 Synthesis of ethyl (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-3-((tert-butyldimethylsilyl)oxy)icosa-5,8,11,14,17-pentaenoate (**90**)



118 **90**

To a solution of **118** (0.10 g, 0.19 mmole, 1.0 eq.) in absolute ethanol (4 mL) at 0^{O}C was added K_2CO_3 (39 mg, 0.28 mmole, 1.5 eq.). The mixture was stirred at this temperature for 2 h, then heated to room temperature and stirred for 22 h. The reaction was quenched with saturated aqueous solution of NH_4Cl (15 mL). The phases were separated, and the aqueous phase was extracted with DCM (3 x 15 mL). The organic phases were combined, washed with 1 M KOH (10 mL), brine (10 mL), dried (Na_2SO_4), and concentrated *in vacuo* to yield **90** (60 mg, 0.13 mmole, 69%) as a colourless oil.

Data:

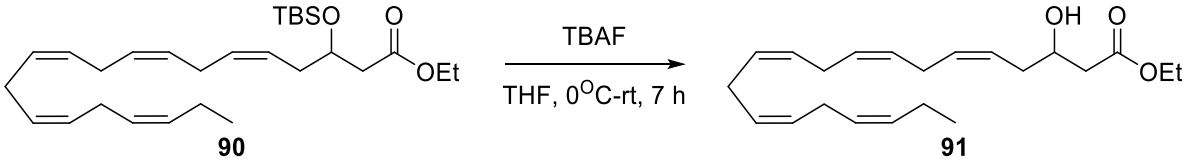
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.52 – 5.25 (m, 10H), 4.23 – 4.05 (m, 3H), 2.89 – 2.76 (m, 8H), 2.42 (dd, *J* = 6.3, 2.5 Hz, 2H), 2.30 (ddd, *J* = 9.4, 4.6, 2.6 Hz, 2H), 2.13 – 2.00 (m, 2H), 1.25 (t, *J* = 7.1 Hz, 3H), 0.97 (t, *J* = 7.5 Hz, 3H), 0.86 (s, 10H), 0.06 (d, *J* = 11.9 Hz, 7H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.99, 132.21, 130.46, 128.76, 128.48, 128.45, 128.23, 128.19, 128.04, 127.19, 125.45, 69.50, 60.49, 42.51, 35.65, 25.93, 20.74, 18.16, 14.46, 14.38, -4.28, -4.76.

IR: 3014, 2964, 2857, 1733 cm^{-1}

R_f: 0.70 (*n*-hexane/EtOAc 7:3 v/v)

3.36 Synthesis of ethyl (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-3-hydroxyicosa-5,8,11,14,17-pentaenoate (**91**)



90 **91**

To a solution of the protected ethyl ester **90** (0.12 g, 0.26 mmole, 1 eq.) in THF (5 mL) at 0°C was added a solution of 1 M TBAF in THF (1.3 mL, 0.40 mmole, 5 eq.). The mixture was stirred at 0°C for 5.5 h, before the reaction was quenched with phosphate buffer (pH=7.3, 5 mL). Brine (10 mL) and EtOAc (10 mL) was added, and the phases separated. The aqueous phase was washed with EtOAc (2 x 10mL). The organic phases were combined, dried (Na_2SO_4), and concentrated *in vacuo*. The resulting oil was purified by column chromatography (silica gel deactivated by 2% Et_3N in *n*-hexane, *n*-hexane/EtOAc 7:3 v/v) to yield the ethyl ester **91** (58 mg, 0.17 mmole, 66%) as a colourless oil.

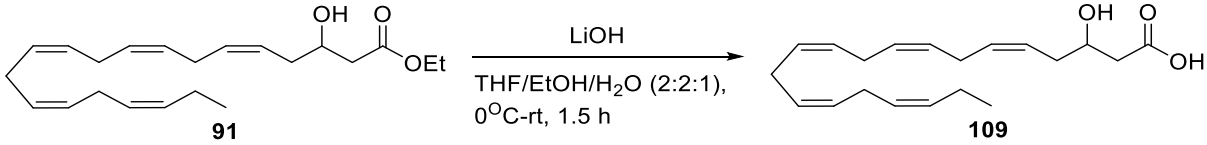
Data:

^{1}H NMR (400 MHz, CDCl3) δ 5.59 – 5.25 (m, 10H), 4.17 (q, *J* = 7.1 Hz, 2H), 4.07 (dtd, *J* = 9.6, 6.4, 3.4 Hz, 1H), 2.92 (s, 1H), 2.89 – 2.76 (m, 8H), 2.56 – 2.39 (m, 2H), 2.39 – 2.22 (m, 2H), 2.13 – 2.00 (m, 2H), 1.56 (s, 1H), 1.28 (t, *J* = 7.1 Hz, 3H), 0.98 (t, *J* = 7.5 Hz, 3H).

^{13}C NMR (101 MHz, CDCl3) δ 173.06, 132.24, 131.27, 128.78, 128.60, 128.52, 128.19, 128.03, 128.00, 127.19, 125.07, 100.16, 68.04, 60.92, 40.81, 34.51, 25.98, 25.85, 25.82, 25.74, 20.75, 14.47, 14.37.

R_f: 0.16 (DCM)

3.37 Synthesis of (all-*Z*)-3-hydroxyicosa-5,8,11,14,17-pentaenoic acid (**109**)



91 **109**

To a solution of the ethyl ester **91** (9.8 mg, 0.03 mmole, 1 eq.) in THF/EtOH/H_2O (3 mL, 2:2:1 v/v) at 0^{O}C, was added LiOH·H_2O (42 mg, 0.99 mmole, 35 eq.). The mixture was heated to room temperature and stirred for 1.5 h. The solvents were removed *in vacuo*, and thereafter dissolved in EtOAc (3 mL), before it was acidified with aqueous NaH_2PO_4 (1.5 mL). The phases were separated, and the aqueous phase was extracted with EtOAc (2 x 3 mL). The organic phases were combined, dried (Na_2SO_4), and concentrated *in vacuo* to yield the target material 3-HEPE (**109**, 13 mg, 0.04 mmole, 141%) as an impure, colourless oil. After purification by column chromatography (silica gel, 5% MeOH in DCM), the oil became less pure.

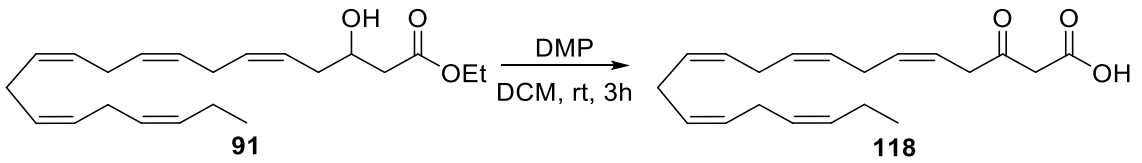
Data:

^{1}H NMR (400 MHz, CDCl3) δ 7.03 – 6.12 (m, 5H), 5.63 – 5.48 (m, 2H), 5.48 – 5.24 (m, 9H), 4.16 – 4.01 (m, 2H), 4.01 – 3.89 (m, 2H), 3.78 – 3.60 (m, 1H), 2.83 (td, *J* = 8.0, 6.8, 3.8 Hz, 8H), 2.64 (s, 2H), 2.62 – 2.51 (m, 1H), 2.46 (dd, *J* = 16.6, 8.9 Hz, 1H), 2.31 (dp, *J* = 21.5, 7.2 Hz, 2H), 2.17 (s, 4H), 2.12 – 2.03 (m, 8H), 2.03 – 1.89 (m, 2H), 1.89 – 1.78 (m, 2H), 1.25 (s, 8H), 0.97 (t, *J* = 7.5 Hz, 3H).

^{13}C NMR (101 MHz, CDCl3) δ 211.16, 132.23, 131.51, 128.78, 128.65, 128.53, 128.15, 128.01, 127.91, 127.18, 124.82, 108.14, 69.92, 68.02, 67.89, 53.90, 31.89, 29.37, 29.30, 25.96, 25.82, 25.80, 25.72, 24.10, 20.73, 14.45.

R_f: 0.10 (DCM)

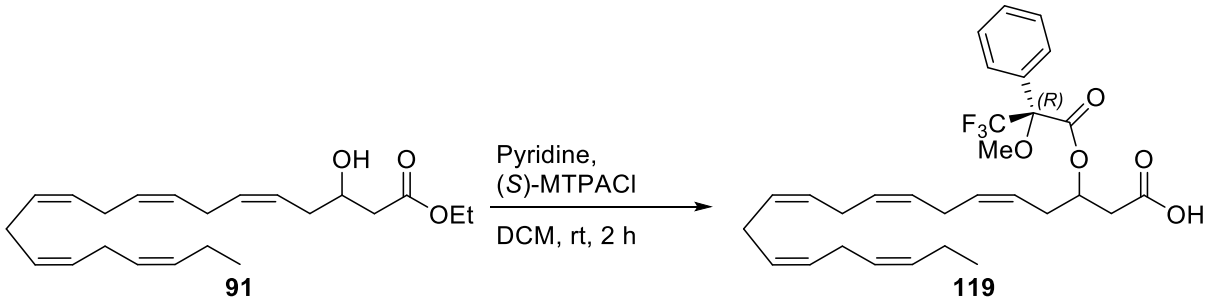
3.38 Synthesis of (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-3-oxoicosa-5,8,11,14,17-pentaenoic acid (**118**)



91 **118**

The ethyl ester **91** (12 mg, 0.03 mmole, 1 eq.) was dissolved in wet DCM (0.5 mL) and set under powerful stirring. DMP (15 mg, 0.03 mmole, 1 eq.) was dissolved in wet DCM (0.5 mL) and added to the stirring solution. The mixture was stirred at room temperature for 2 h, before 20% $Na_2S_2O_3$ (2 mL) was added. The phases were separated, and the organic phase was washed with 3% NaOH (2 mL). The aqueous phases were combined and extracted with DCM (2 x 3 mL). The organic phases were combined, washed with 20% $Na_2S_2O_3$ (2 mL), 3% NaOH (2 mL), and brine (2 mL), then dried (Na_2SO_4), and concentrated *in vacuo*. The concentrate was purified by column chromatography (layers from bottom to top: silite, silica gel, silite, KF, silite, *n*-hexane/EtOAc 7:3 v/v) to yield the β-hydroxy ester **118** (4.4 mg, 0.03 mmole, 37%) as an impure, colourless oil. Due to time limitations, we were not able to purify the product. Thus, no spectra are reported.

3.39 Synthesis of (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-3-(((*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)icosa-5,8,11,14,17-pentaenoic acid (**119**)



To a solution of **91** (12 mg, 0.03 mmole, 1 eq.) in dry DCM (0.50 mL), was added pyridine (0.01 mL, 0.11 mmole, 3.10 eq.) and (*S*)-MTPACl (0.01 mL, 0.07 mmole, 1.5 eq.). The mixture was stirred at room temperature for 2 h, before water (2 mL) and DCM (5 mL) were added. The phases were separated, and the aqueous phase was extracted with DCM (3 x 5 mL). The organic phases were combined, dried (Na_2SO_4), and concentrated *in vacuo*. The concentrate was purified by column chromatography (silica gel, *n*-hexane/EtOAc 40:1 v/v) to yield a mixture of the two diastereomers of **119** (8.1 mg, 0.02 mmole, 43%), enriched to the relation 1:1.5 of *R*,*R* compared to *R*,*S*.

Data:

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 – 7.47 (m, 2H), 7.39 (dd, *J* = 5.1, 2.0 Hz, 3H), 5.60 – 5.45 (m, 2H), 5.45 – 5.23 (m, 9H), 4.12 (qd, *J* = 7.2, 1.4 Hz, 1H), 4.09 – 4.02 (m, 1H), 3.53 (dq, *J* = 2.6, 1.2 Hz, 3H), 2.88 – 2.78 (m, 7H), 2.68 – 2.58 (m, 2H), 2.58 – 2.44 (m, 2H), 2.40 (q, *J* = 7.2 Hz, 1H), 2.12 – 2.02 (m, 2H), 1.54 (s, 1H), 1.21 (dt, *J* = 15.1, 7.2 Hz, 3H), 0.97 (t, *J* = 7.5 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.18, 169.93, 165.98, 132.39, 132.31, 132.25, 129.74, 128.88, 128.81, 128.61, 128.60, 128.51, 128.09, 128.00, 127.67, 127.63, 127.58, 127.18, 123.36, 123.07, 73.14, 72.97, 61.11, 61.02, 55.61, 38.37, 38.34, 31.68, 31.47, 25.92, 25.85, 25.82, 25.73, 20.75, 14.45, 14.28, 14.24.

^{19}F NMR (376 MHz, $CDCl_3$) δ -71.51, -71.58.

R_f: 0.64, 0.58 (DCM)

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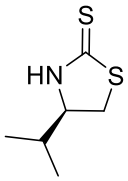
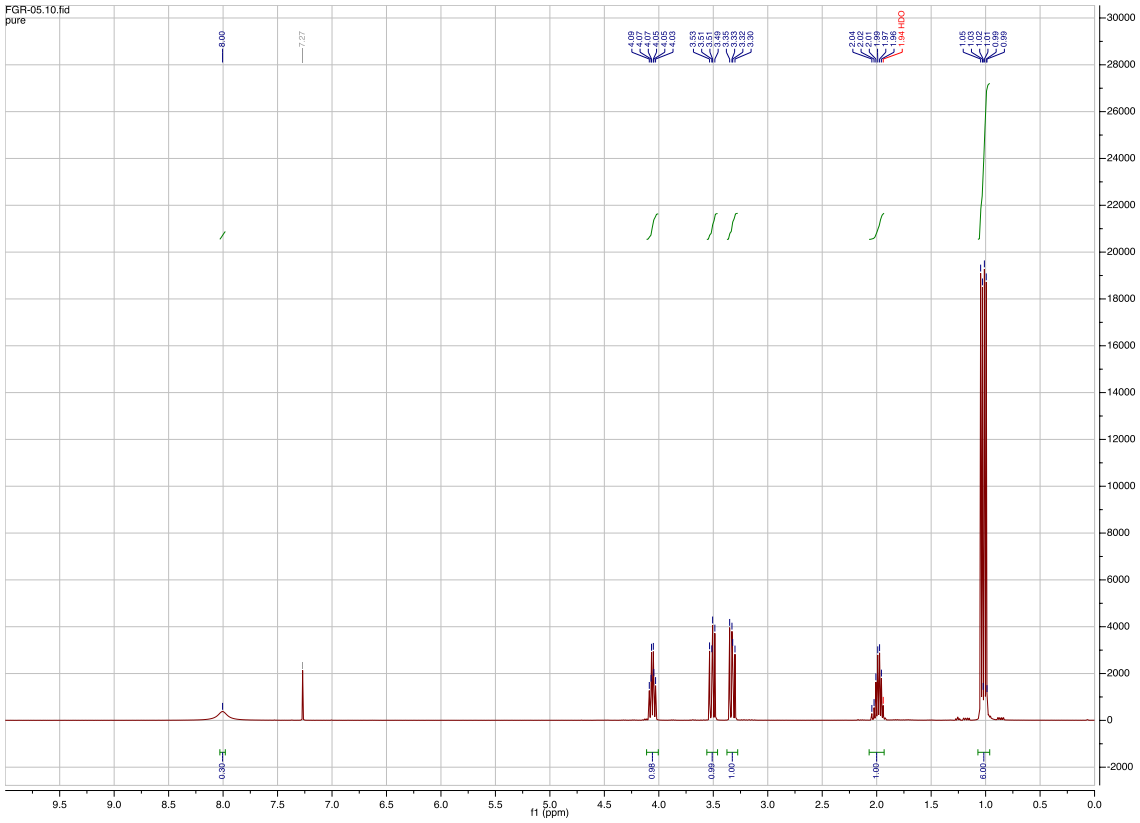
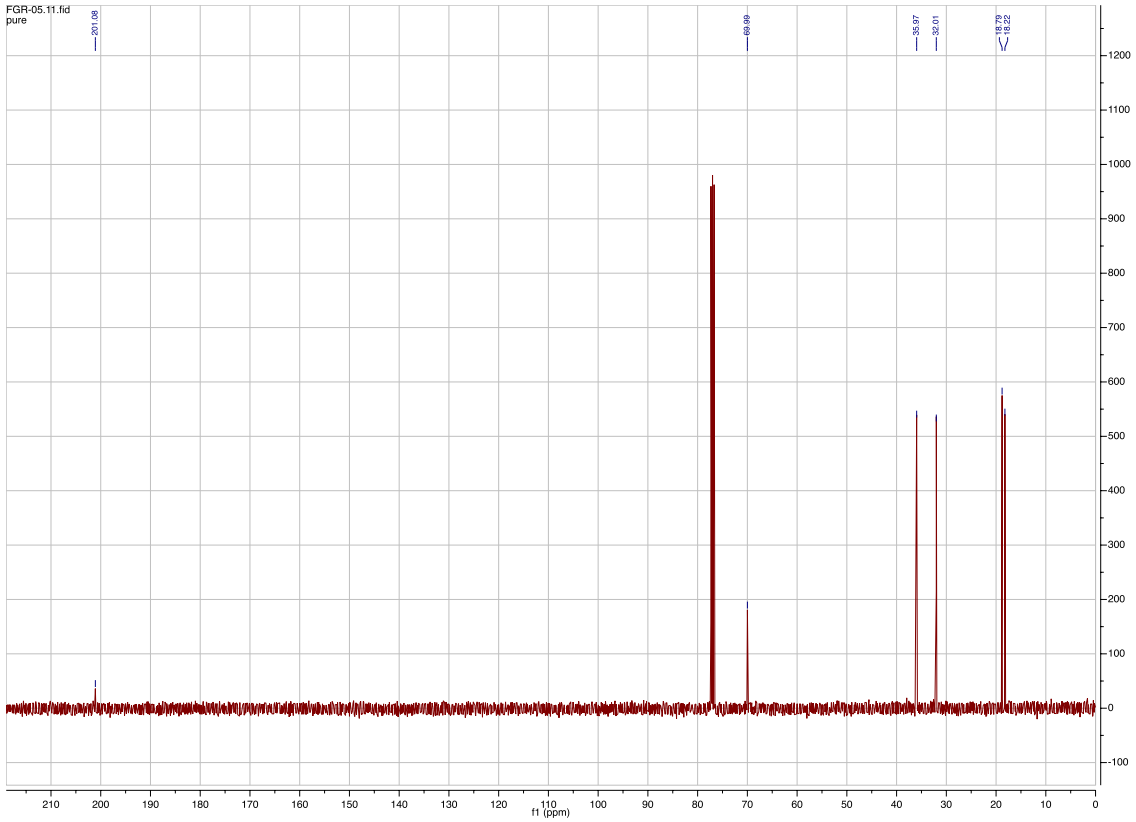
Appendix - Spectra

(*R*)-4-isopropylthiazolidine-2-thione (**94**):

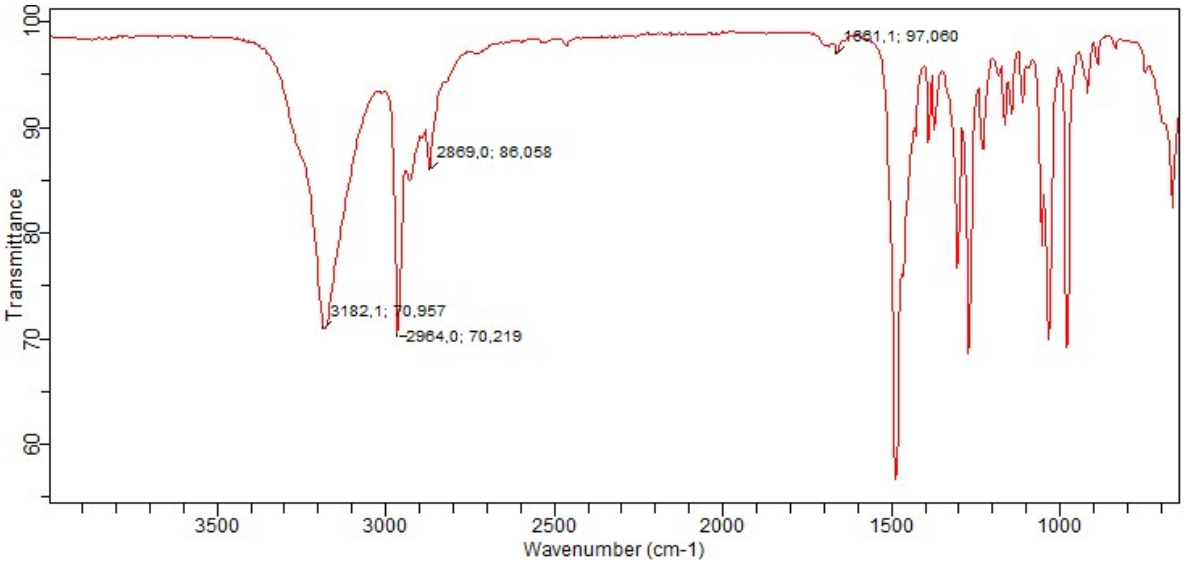




*Spectrum 1: ^{1}H NMR spectrum of the thiazolidinethione **94**.*

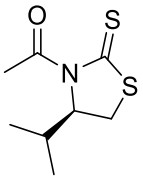


*Spectrum 2: ^{13}C NMR spectrum of the thiazolidinethione **94**.*

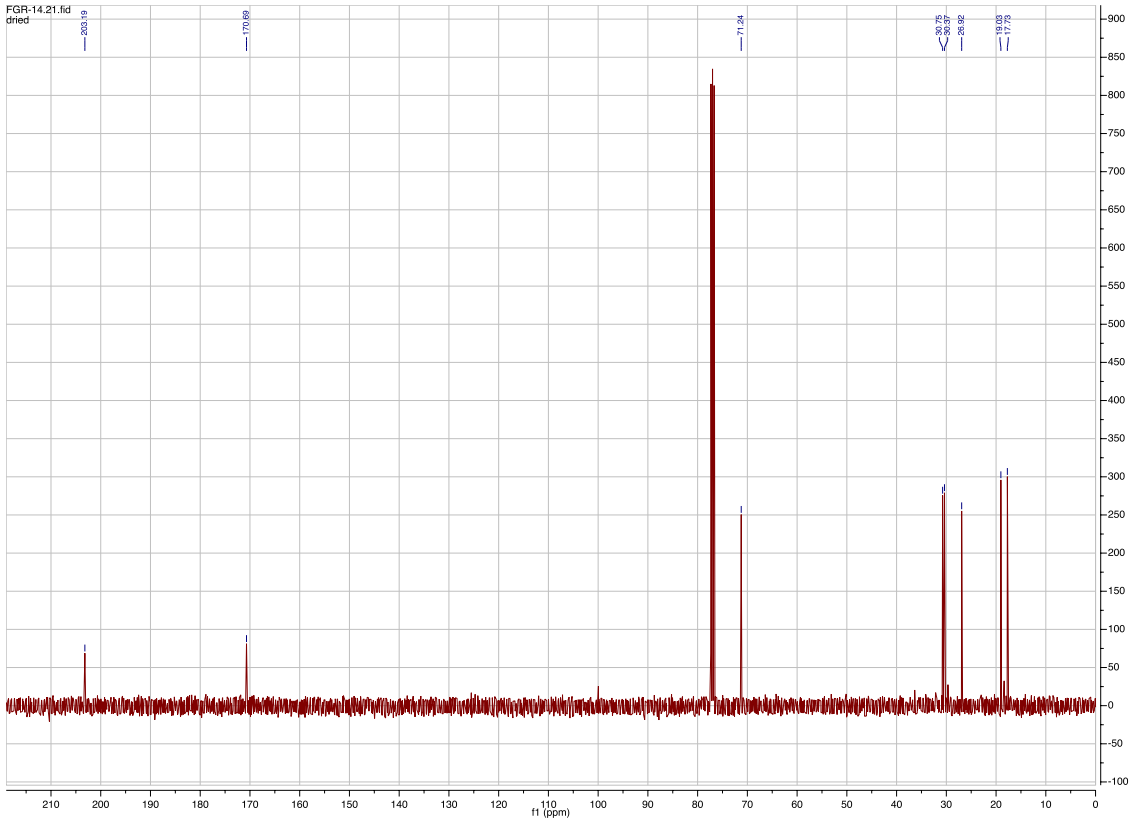


*Spectrum 3: IR spectrum of the thiazolidinethione **94**.*

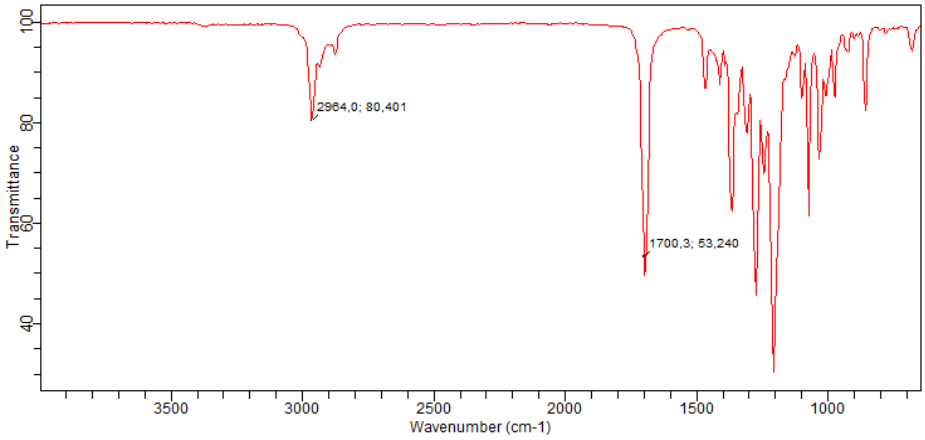
(*R*)-1-(4-isopropyl-2-thioxothiazolidin-3-yl)ethan-1-one (**74**):



*Spectrum 4: ^{1}H NMR spectrum of the thiazolidinethione **74**.*

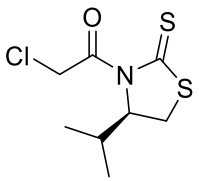


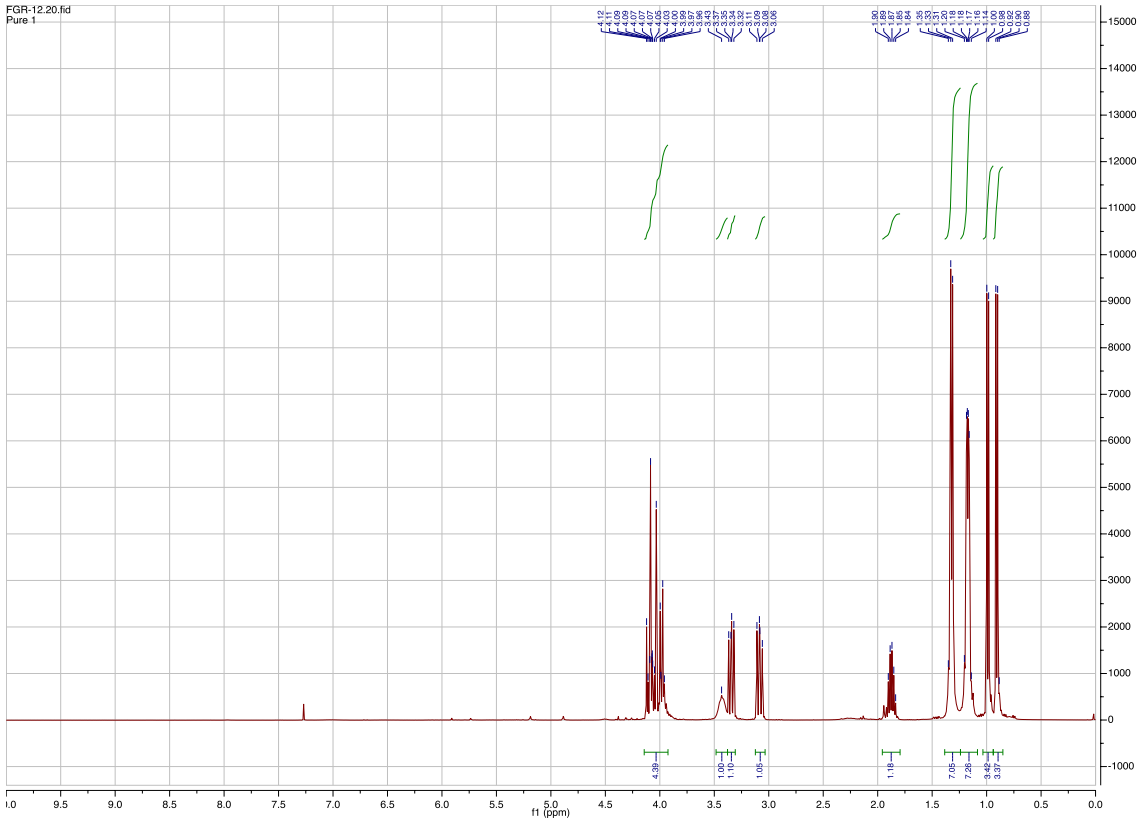
*Spectrum 5: ^{13}C NMR spectrum of the thiazolidinethione **74**.*



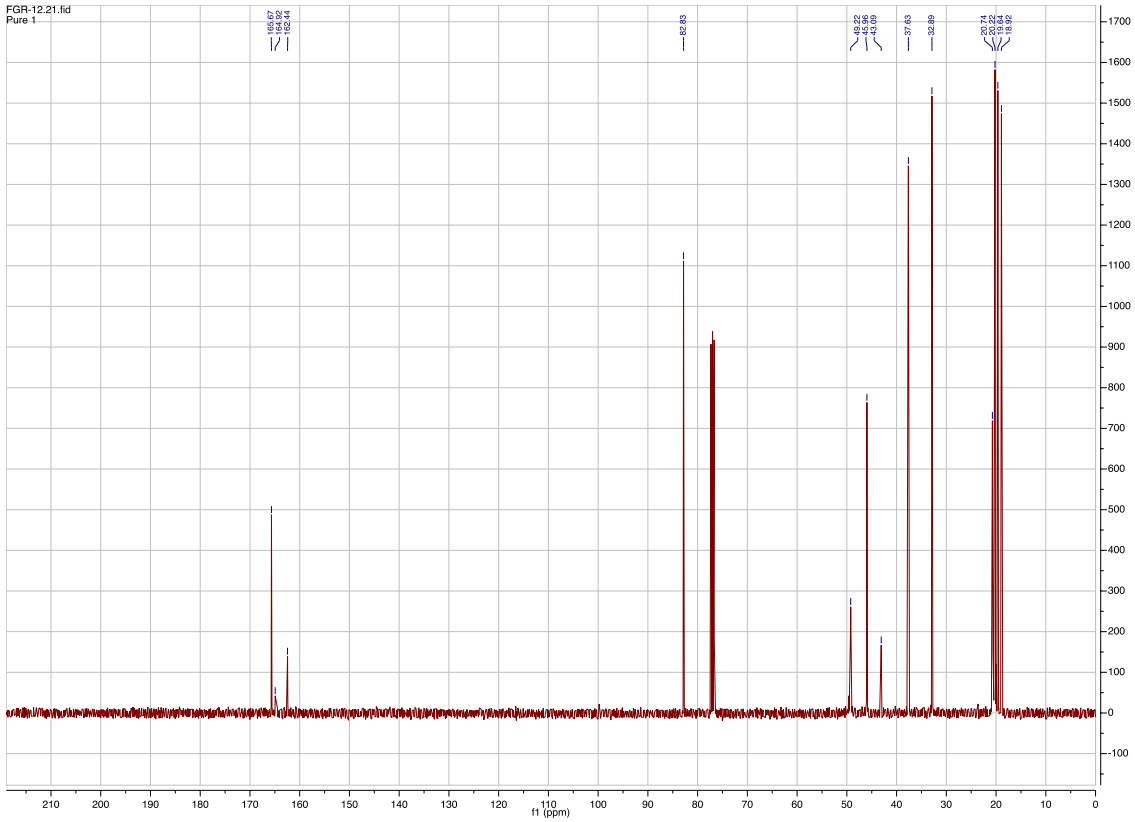
*Spectrum 6: IR spectrum of the thiazolidinethione **74**.*

(*R*)-2-chloro-1-(4-isopropyl-2-thioxothiazolidin-3-yl)ethan-1-one (**92**):



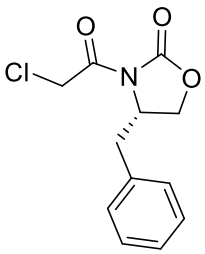


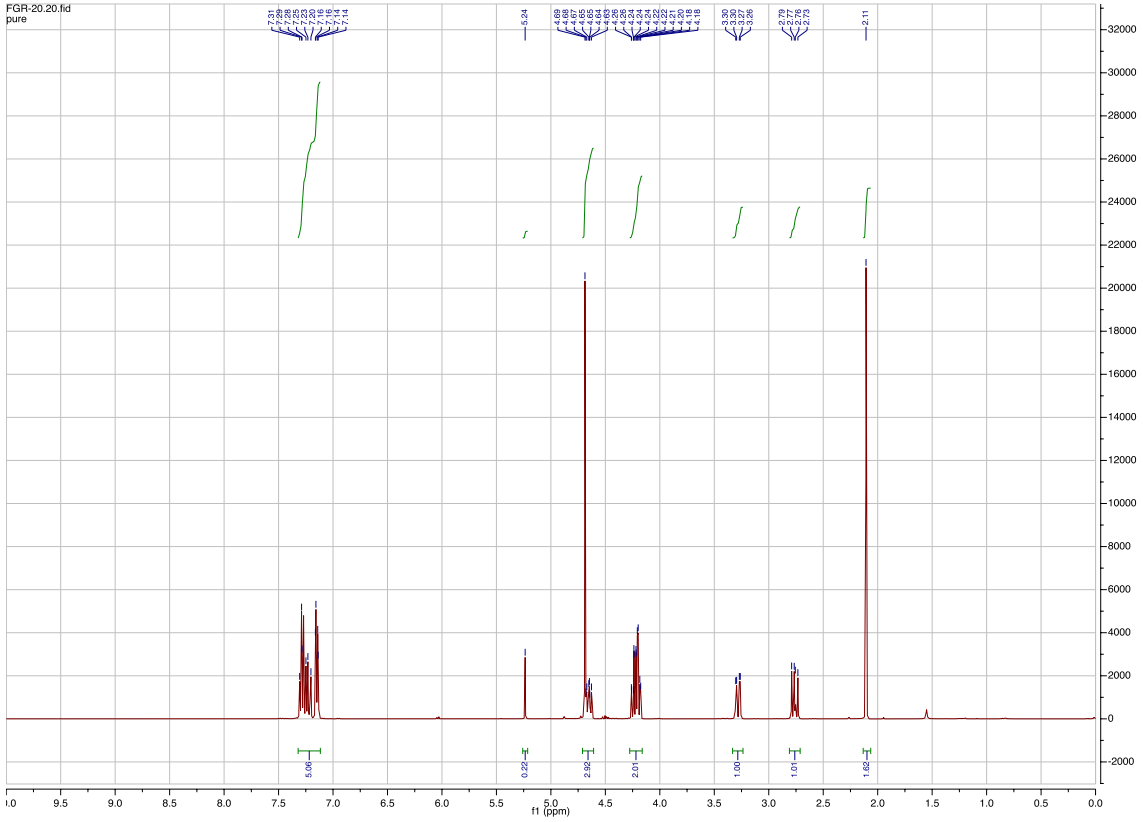
*Spectrum 7: ^{1}H NMR spectrum of the thiazolidinethione **92**, where Cl is exchanged with Et_2N.*



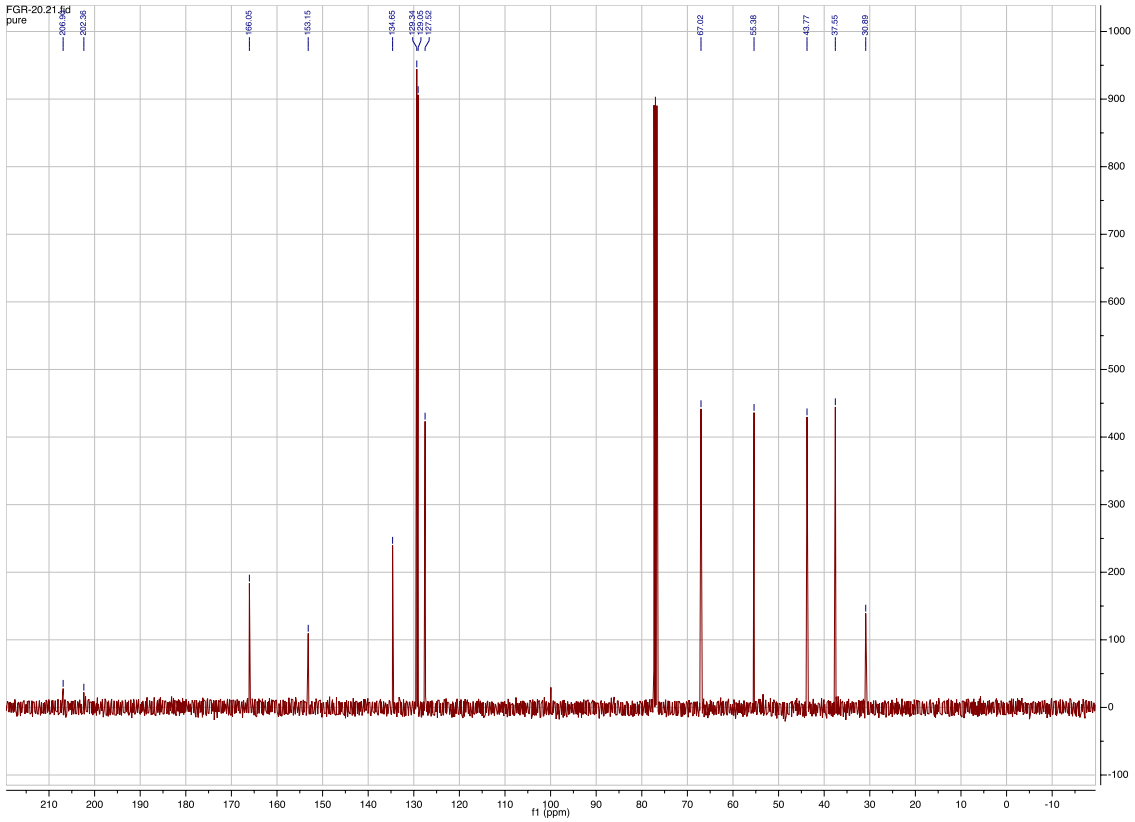
*Spectrum 8: ^{13}C NMR spectrum of the thiazolidinethione **92**, where Cl is exchanged with Et_2N.*

(*S*)-4-benzyl-3-(2-chloroacetyl)oxazolidin-2-one (**121**):

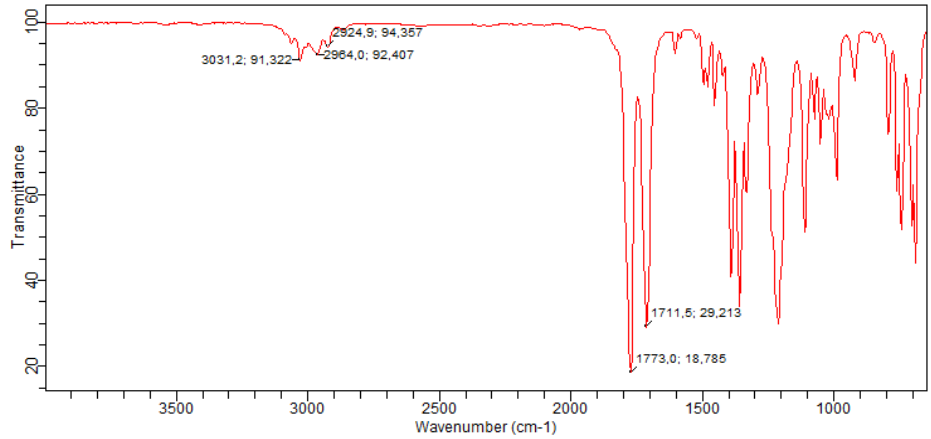




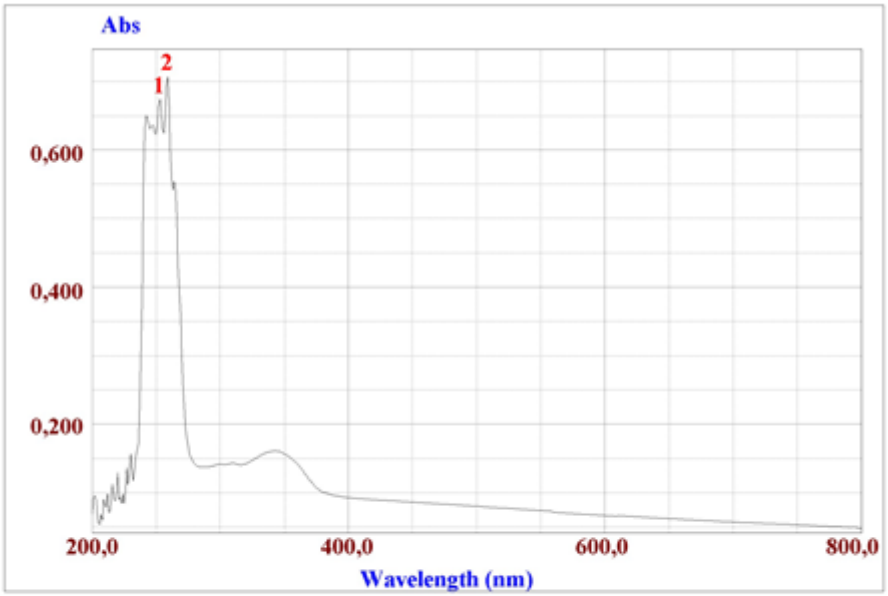
*Spectrum 9: [1]H NMR spectrum of the oxazolidinone **121**.*



*Spectrum 10: ^{13}C NMR spectrum of the oxazolidinone **121**.*



*Spectrum 11: IR spectrum of the oxazolidinone **121**.*



No.	Peak Type	Position	Height
1	Peak	252,8	0,673
2	Peak	258,7	0,707

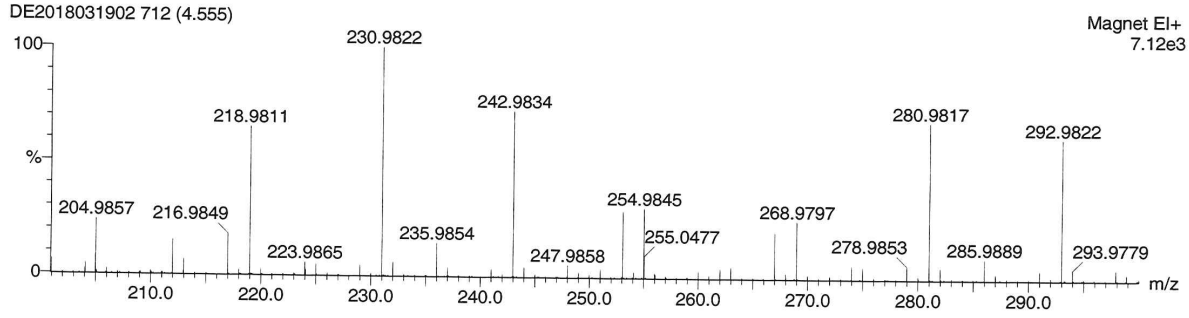
*Spectrum 12: UV spectrum of the oxazolidinone **121**.*

Elemental Composition Report **Page 1**

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

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

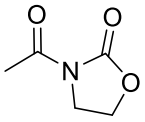
FOR - C
DE2018031902 712 (4.555)

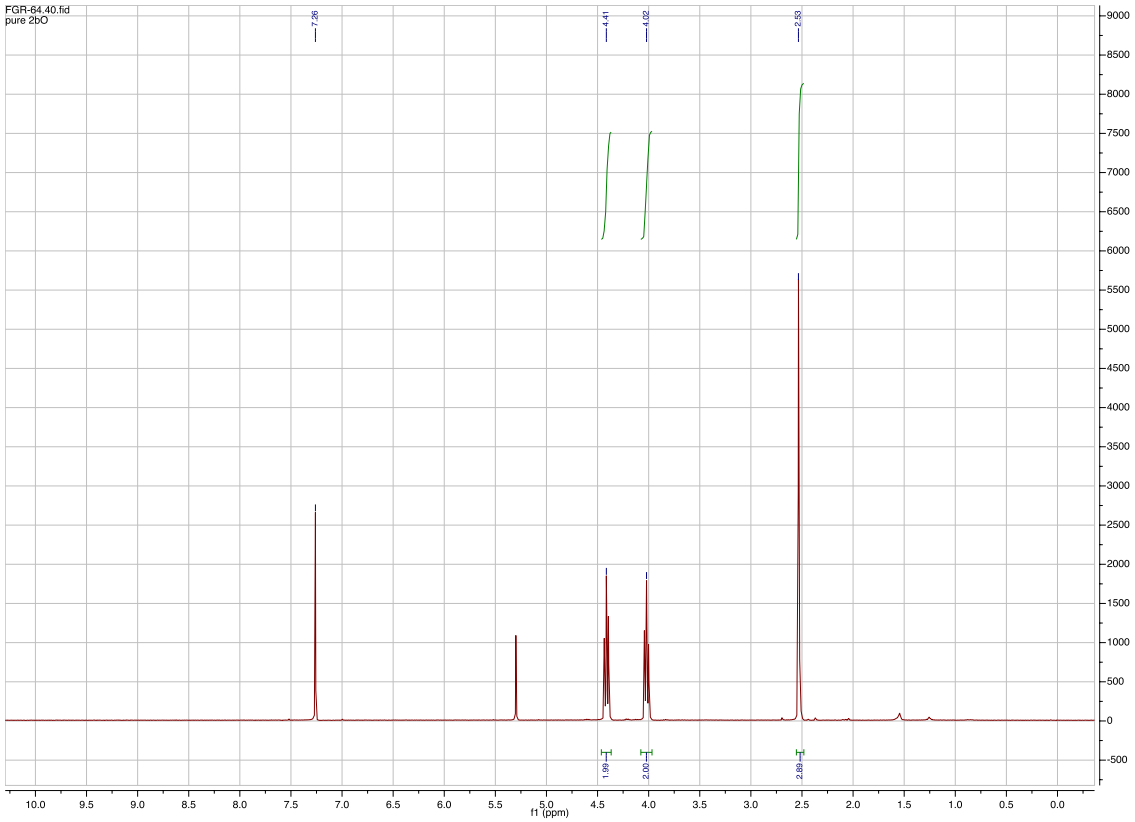


Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
253.0511	253.0506	0.5	2.1	7.0	1	C12 H12 N O3 Cl
	253.0501	1.0	4.0	11.5	2	C15 H9 O4
	253.0528	-1.7	-6.6	16.0	3	C18 H7 N O

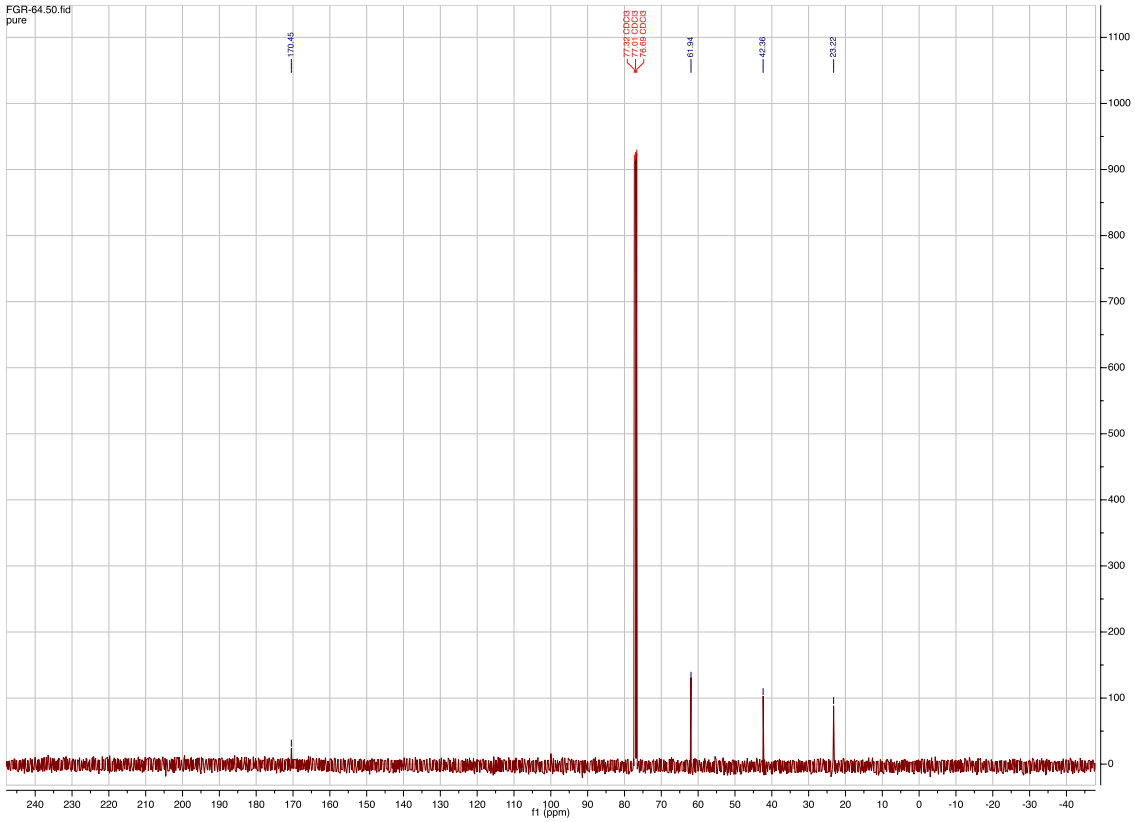
*Spectrum 13: HRMS spectrum of the oxazolidinone **121**.*

3-acetyloxazolidin-2-one (**114**):

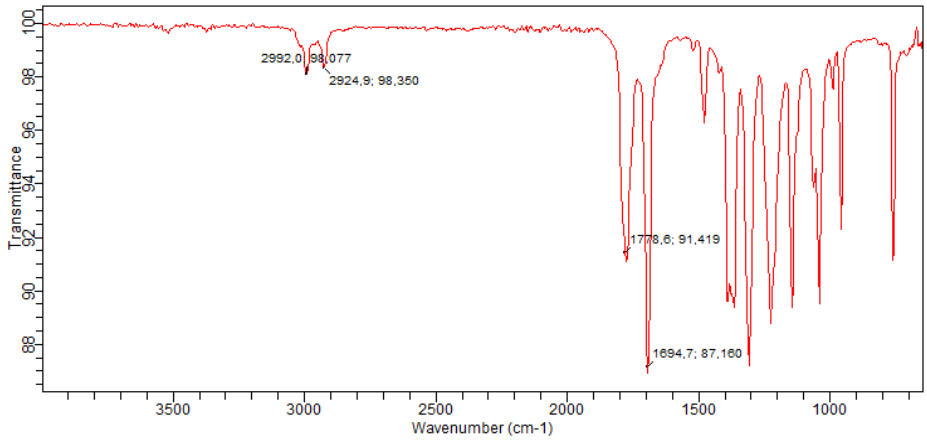




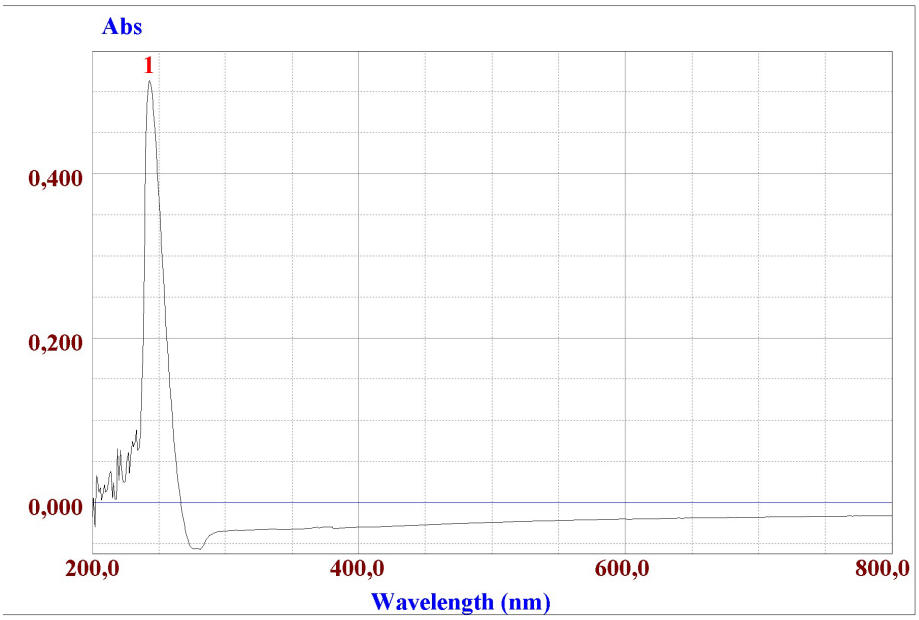
*Spectrum 14: [1]H NMR spectrum of the oxazolidinone **114**.*



*Spectrum 15: ^{13}C NMR spectrum of the oxazolidinone **114**.*



*Spectrum 16: IR spectrum of the oxazolidinone **114**.*



No.	Peak Type	Position	Height	Area	Start	End
1	Peak	242,9	0,514			

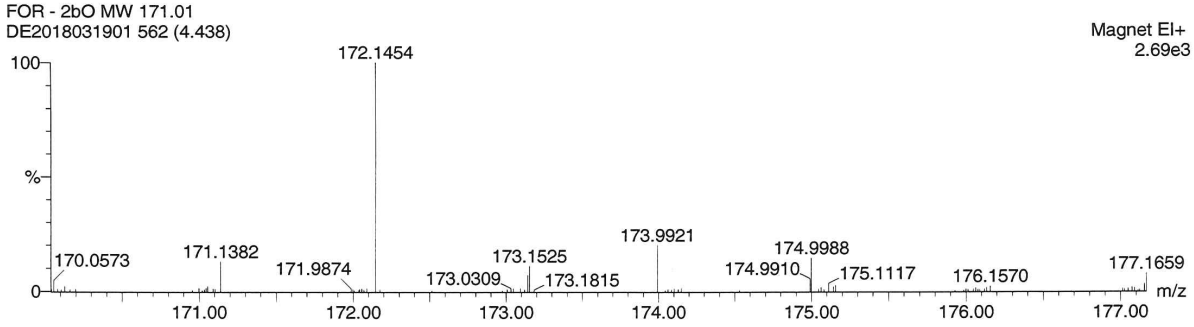
*Spectrum 17: UV spectrum of the oxazolidinone **114**.*

Elemental Composition Report **Page 1**

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

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

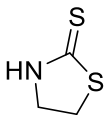
FOR - 2bO MW 171.01
DE2018031901 562 (4.438)

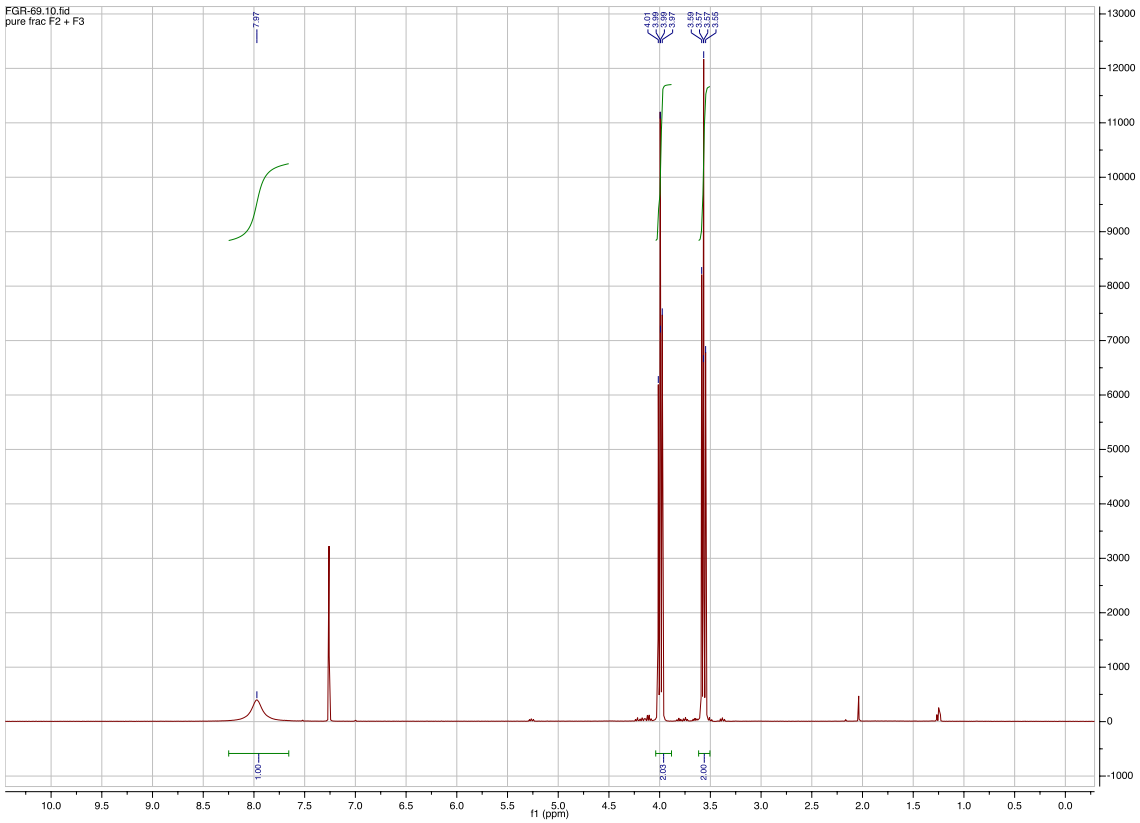


Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
172.1454	172.1463	-0.9	-5.4	1.0	1	C10 H20 O2

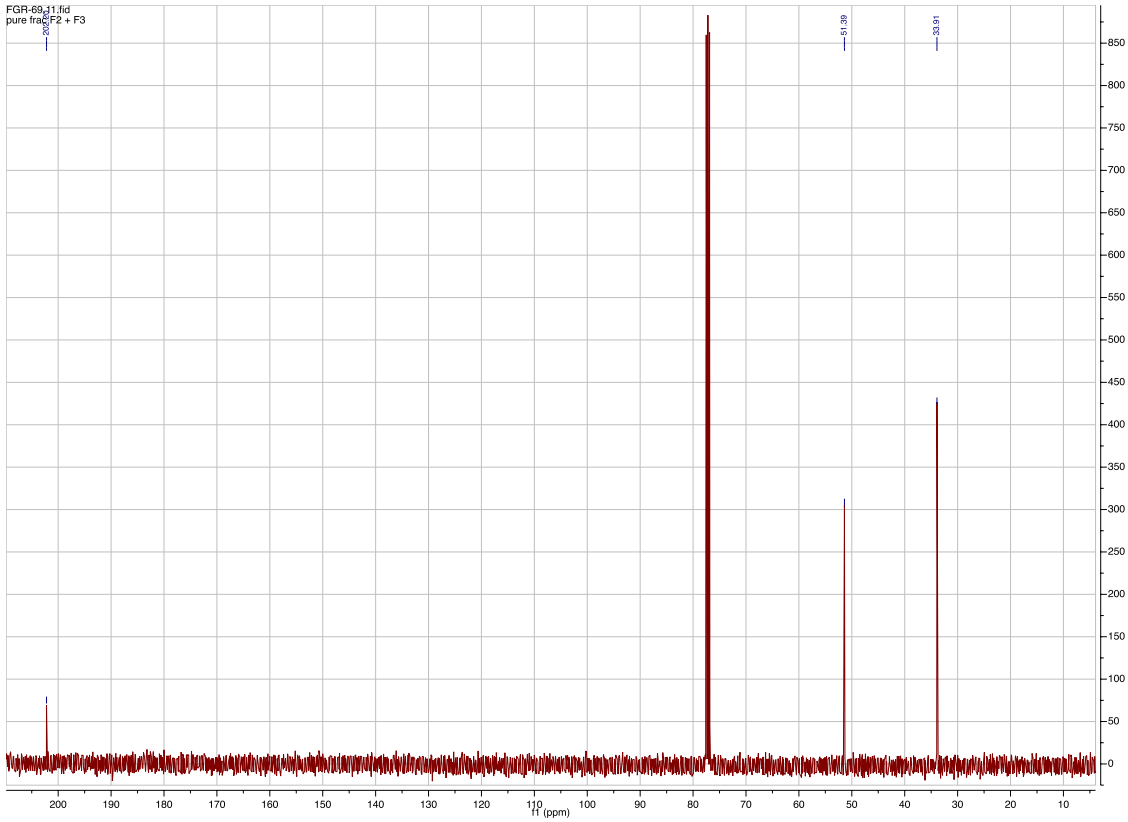
*Spectrum 18: HRMS spectrum of the oxazolidinone **114**.*

Thiazolidine-2-thione (**115**):

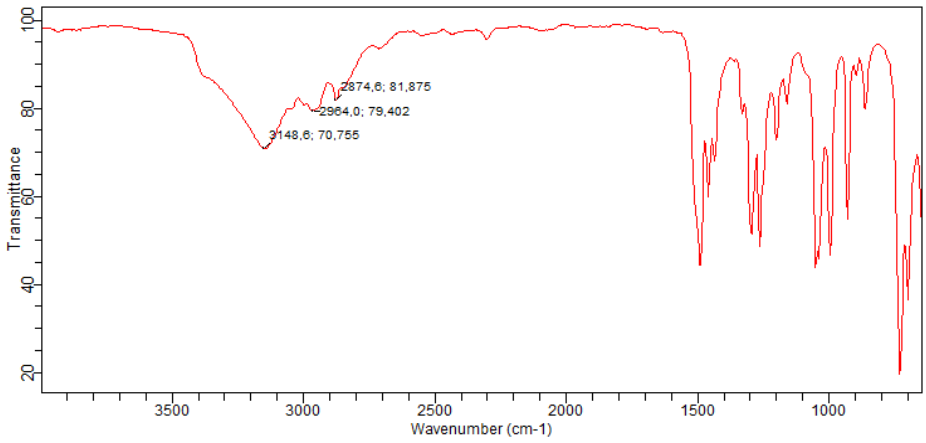




*Spectrum 19: [1]H NMR spectrum of the thiazolidinethione **115**.*

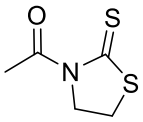


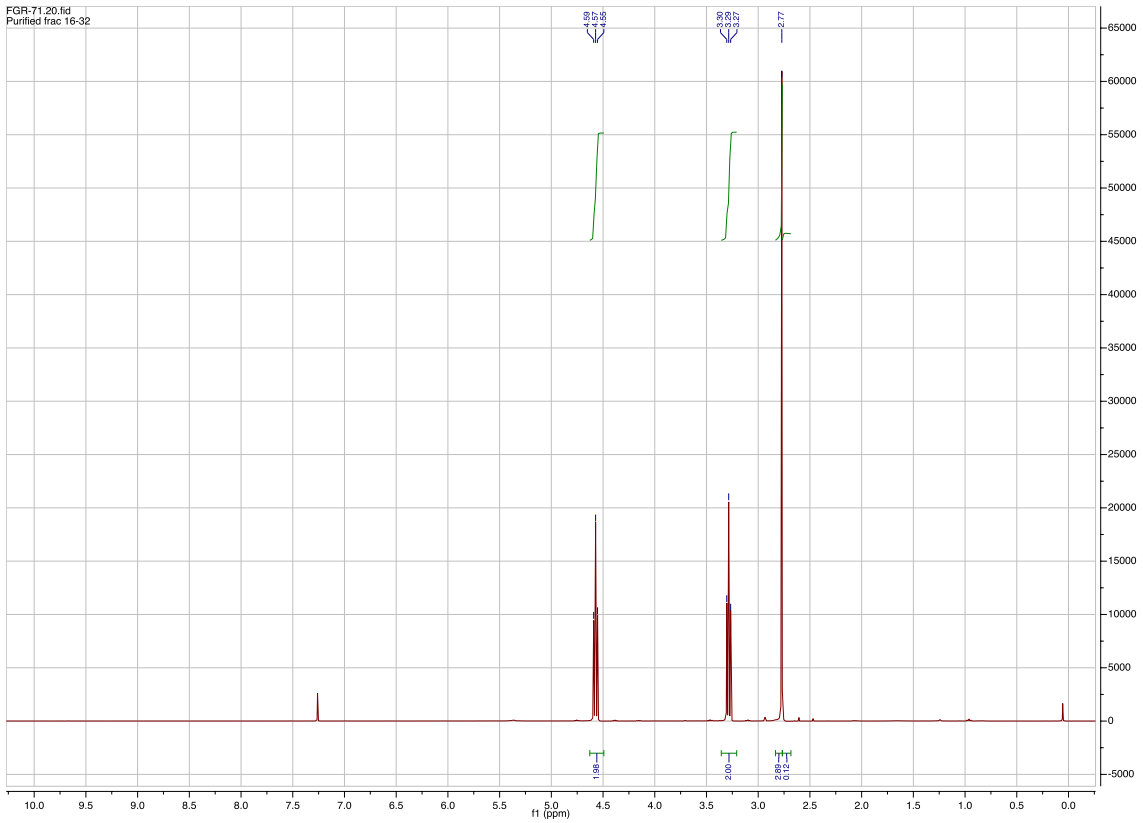
*Spectrum 20: ^{13}C NMR spectrum of the thiazolidinethione **115**.*



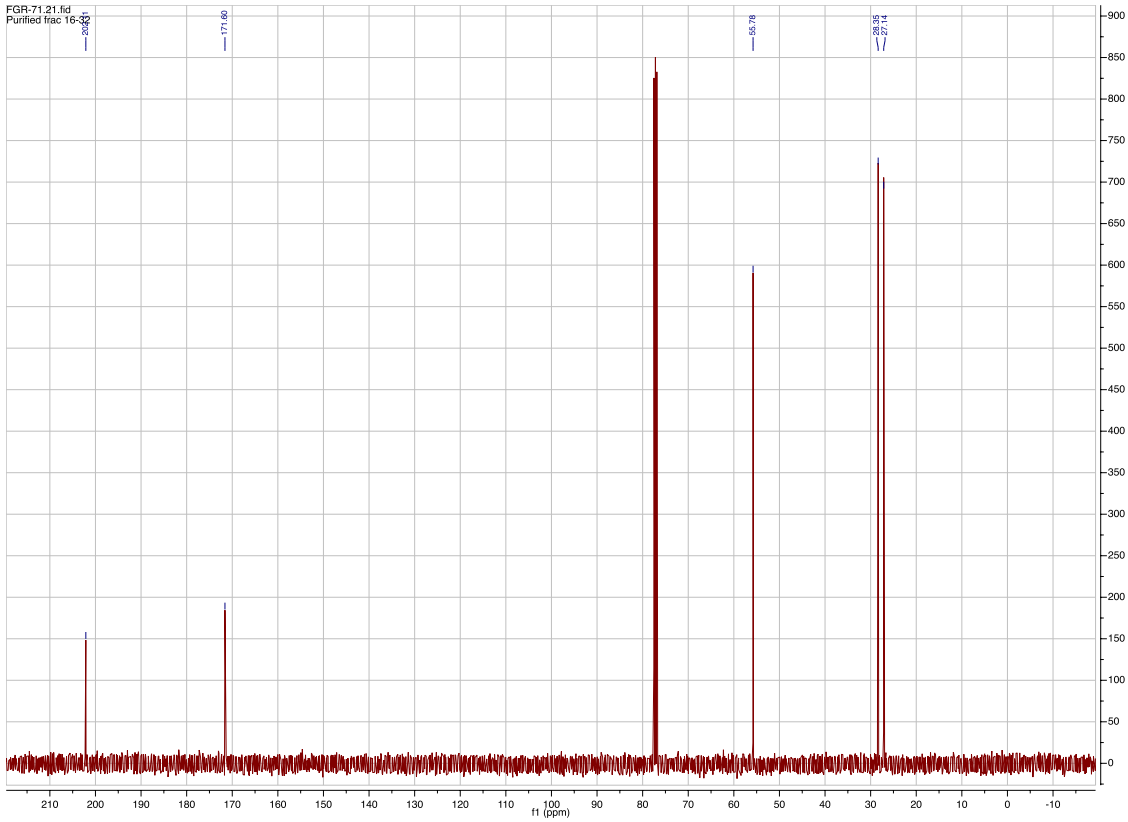
*Spectrum 21: IR spectrum of the thiazolidinethione **115**.*

1-(2-thioxothiazolidin-3-yl)ethan-1-one (**116**):

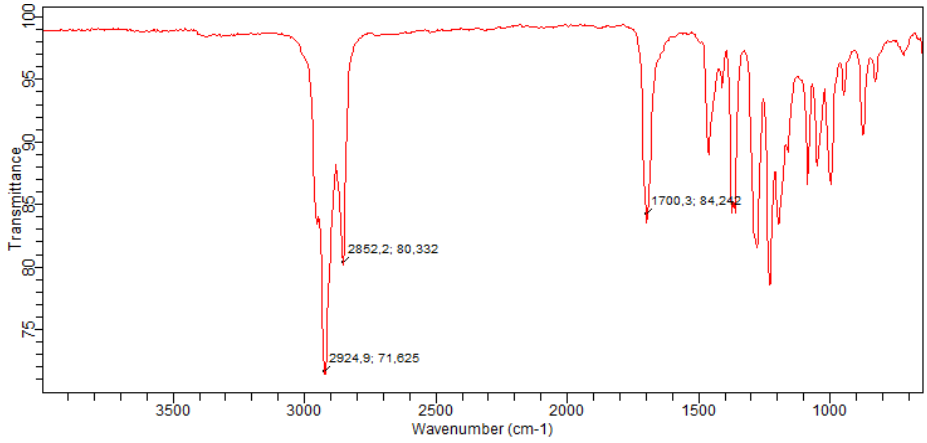




*Spectrum 22: ^{1}H NMR spectrum of the thiazolidinethione **116**.*

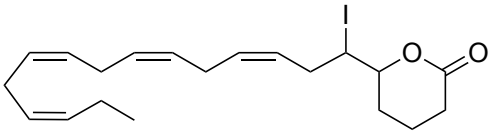


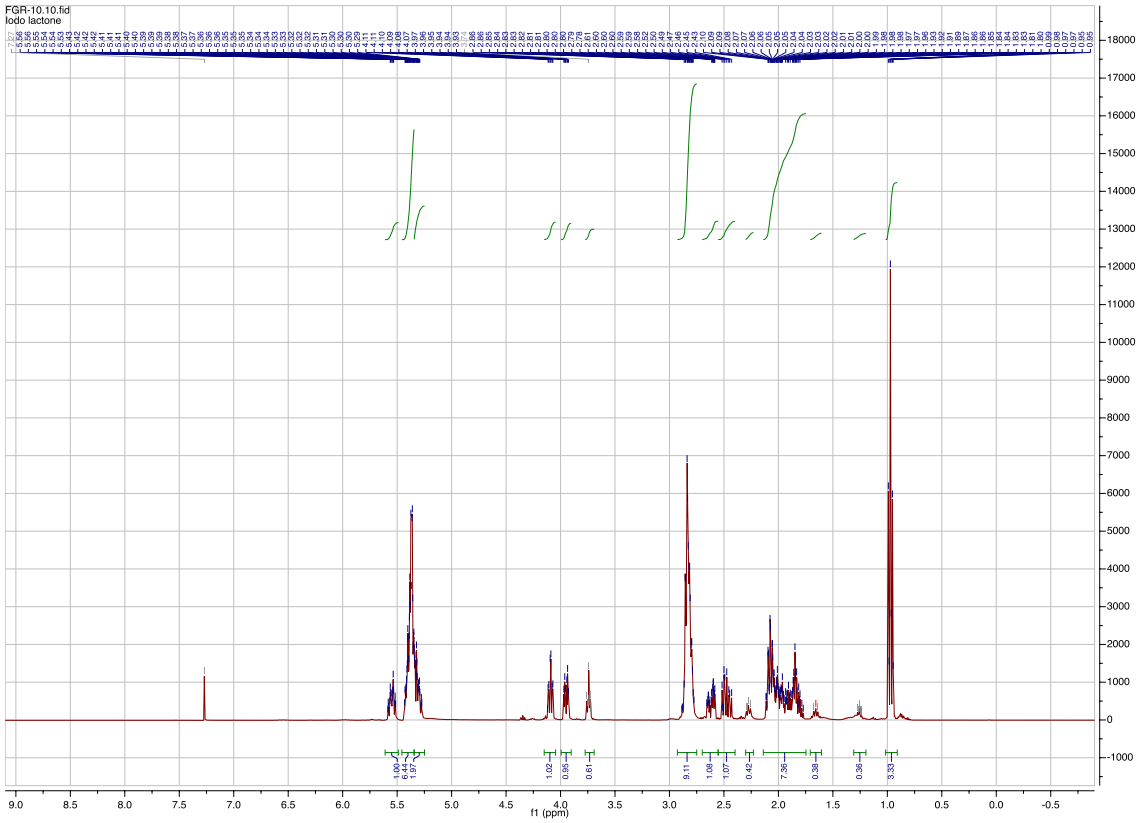
*Spectrum 23: ^{13}C NMR spectrum of the thiazolidinethione **116**.*



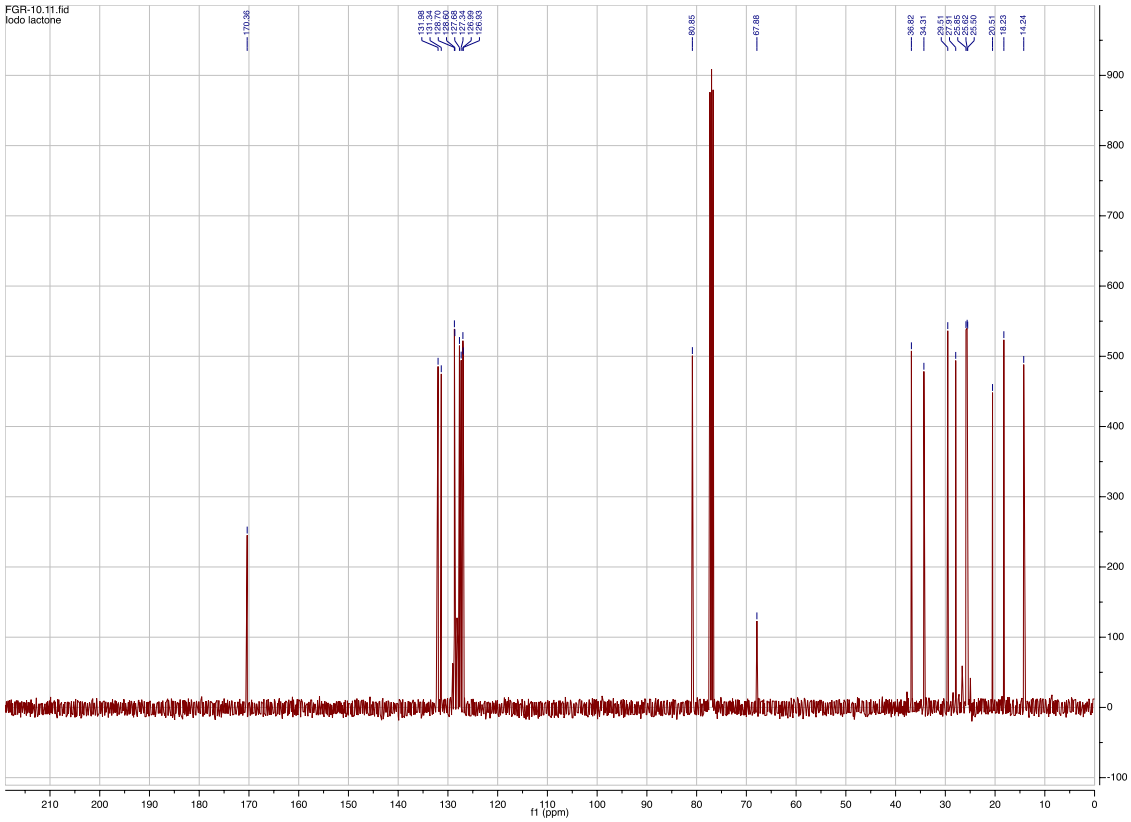
*Spectrum 24: IR spectrum of the thiazolidinethione **116**.*

6-((all-*Z*)-1-iodopentadeca-3,6,9,12-tetraen-1-yl)tetrahydro-2H-pyran-2-one (**99**):



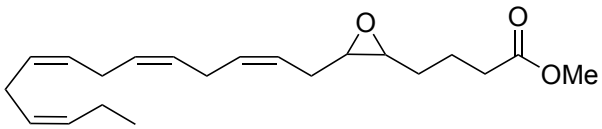


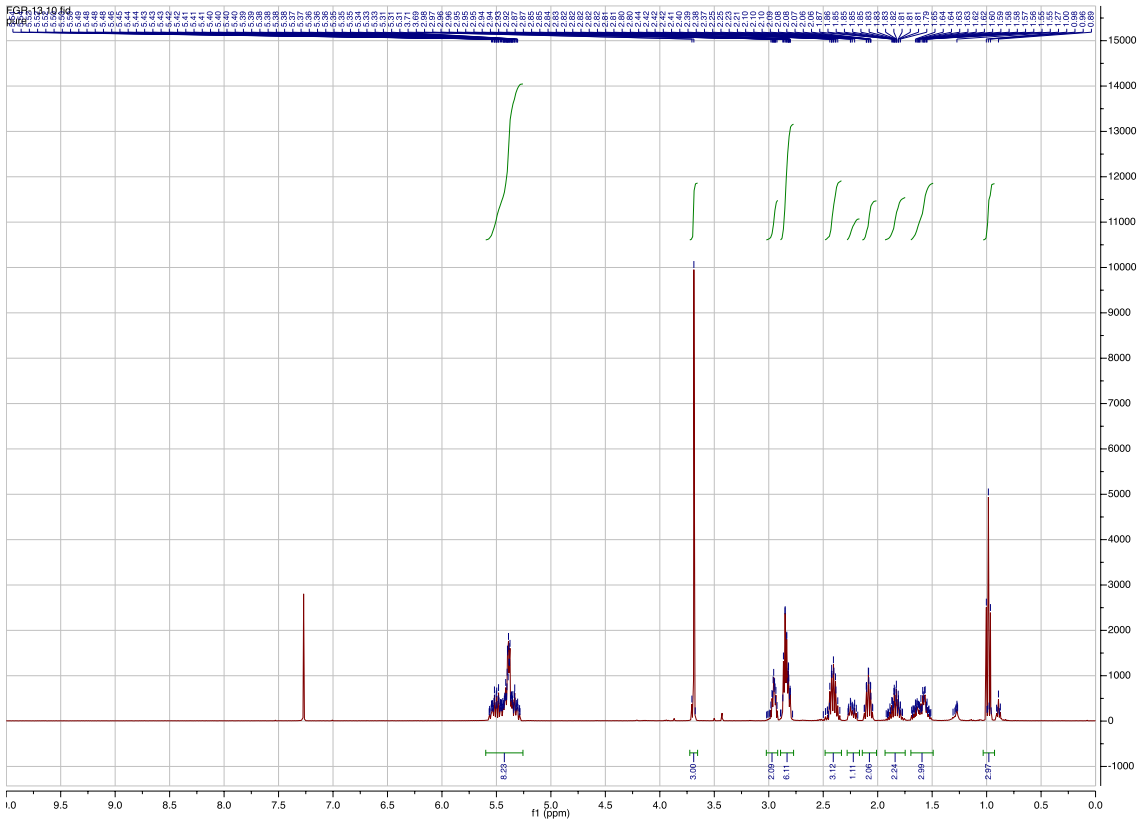
*Spectrum 25: [1]H NMR spectrum of the iodolactone **99**.*



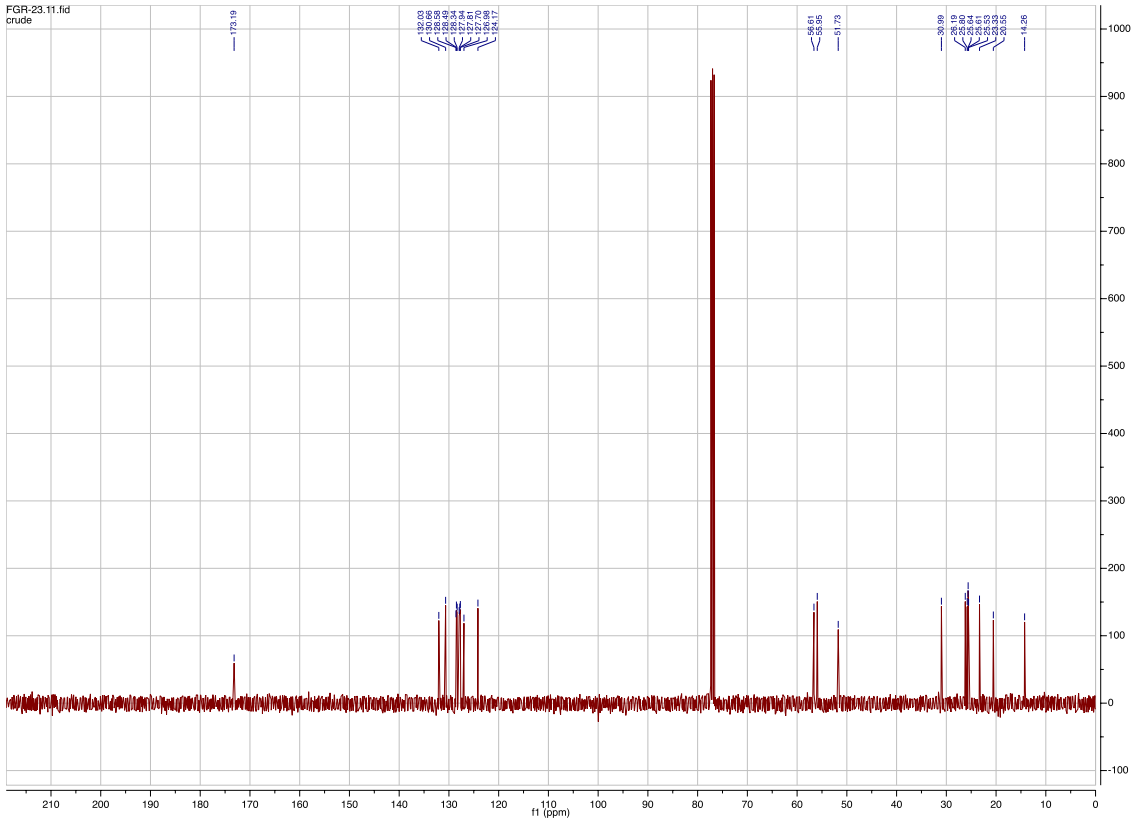
*Spectrum 26: ^{13}C NMR spectrum of the iodolactone **99**.*

Methyl 4-(3-((all-*Z*)-tetradeca-2,5,8,11-tetraen-1-yl)oxiran-2-yl)butanoate (**100**):



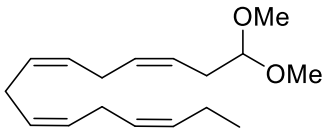


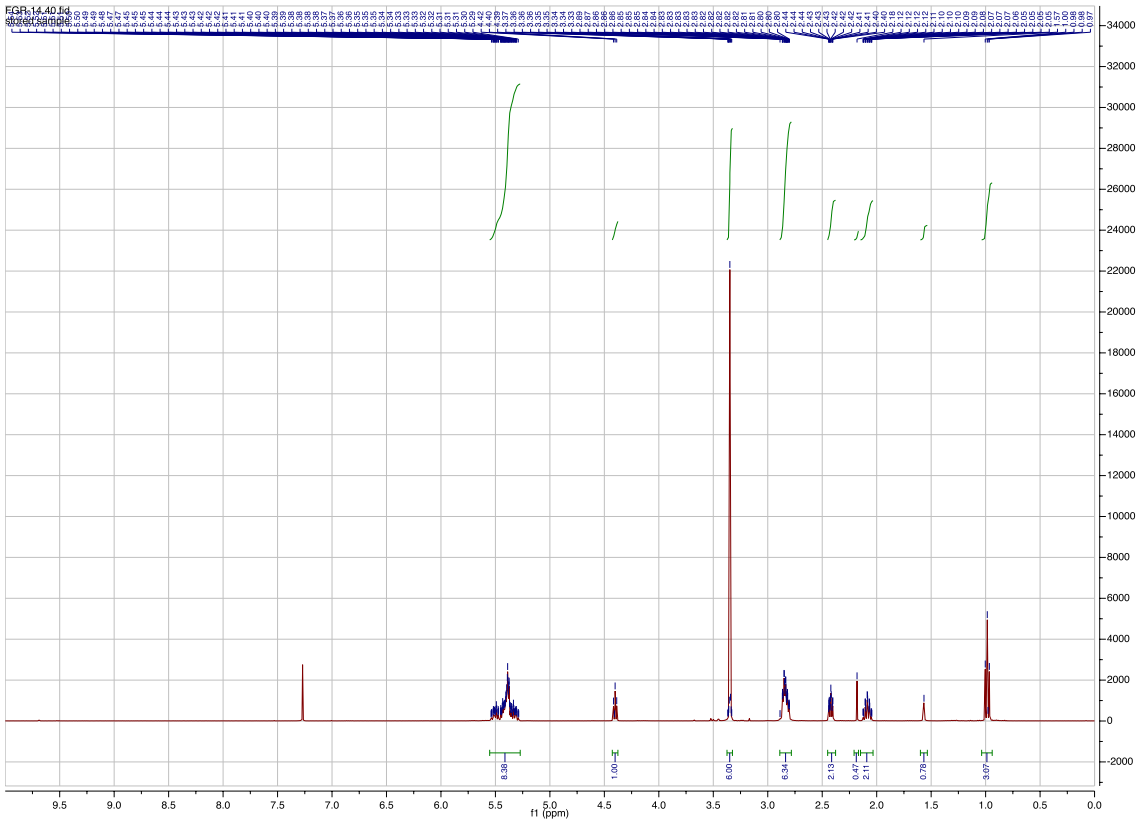
*Spectrum 27: ^{1}H NMR spectrum of the epoxide **100**.*



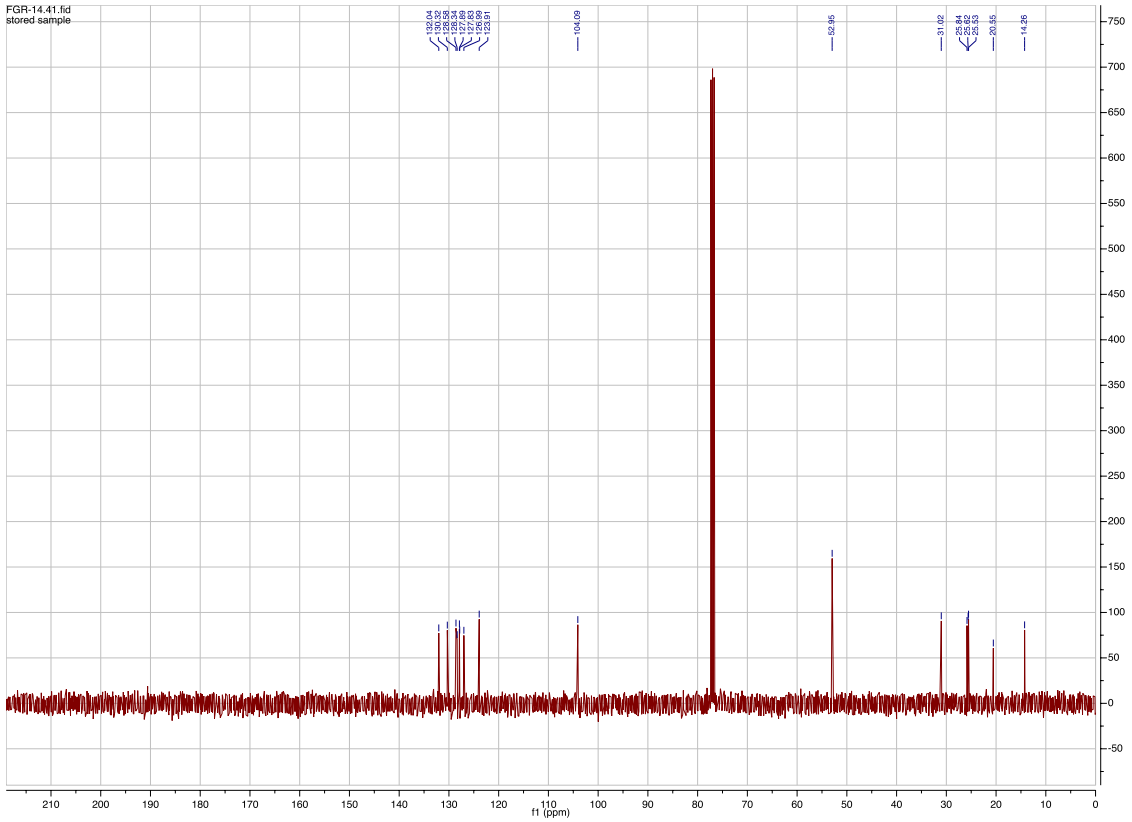
*Spectrum 28: ^{13}C NMR spectrum of the epoxide **100**.*

(all-*Z*)-1,1-dimethoxypentadeca-3,6,9,12-tetraene (**98**):



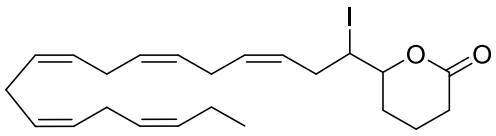


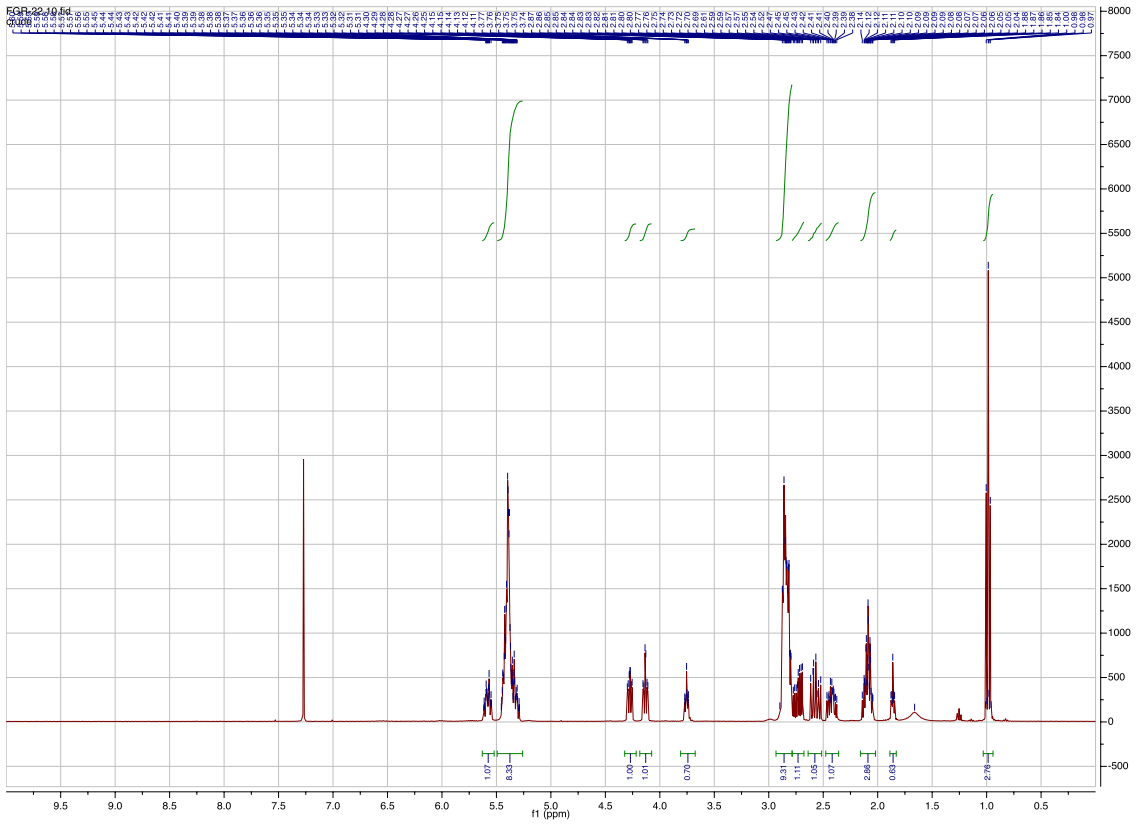
*Spectrum 29: ^{1}H NMR spectrum of the acetal **98**.*



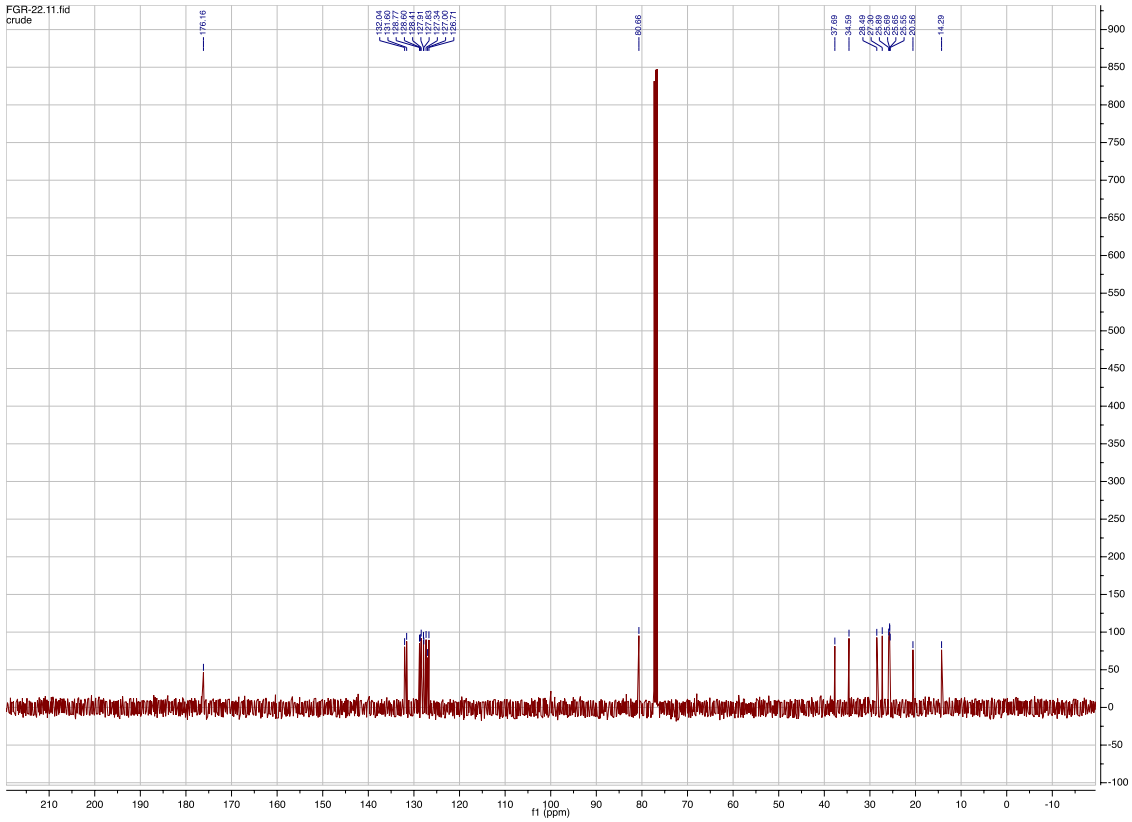
*Spectrum 30: ^{13}C NMR spectrum of the acetal **98**.*

6-((all-*Z*)-1-iodooctadeca-3,6,9,12,15-pentaen-1-yl)tetrahydro-2H-pyran-2-one (**40**):

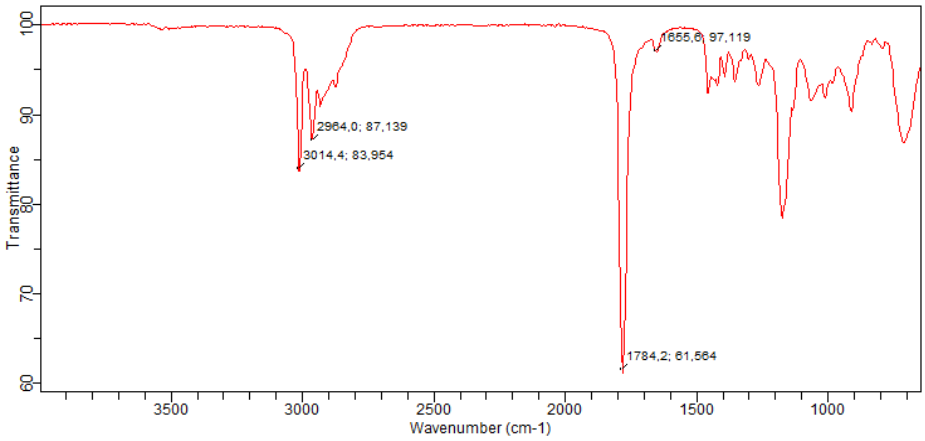




*Spectrum 31: [1]H NMR spectrum of the iodolactone **40**.*

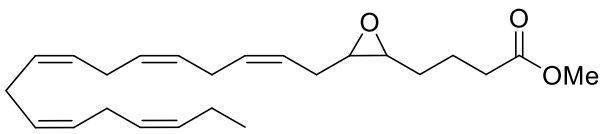


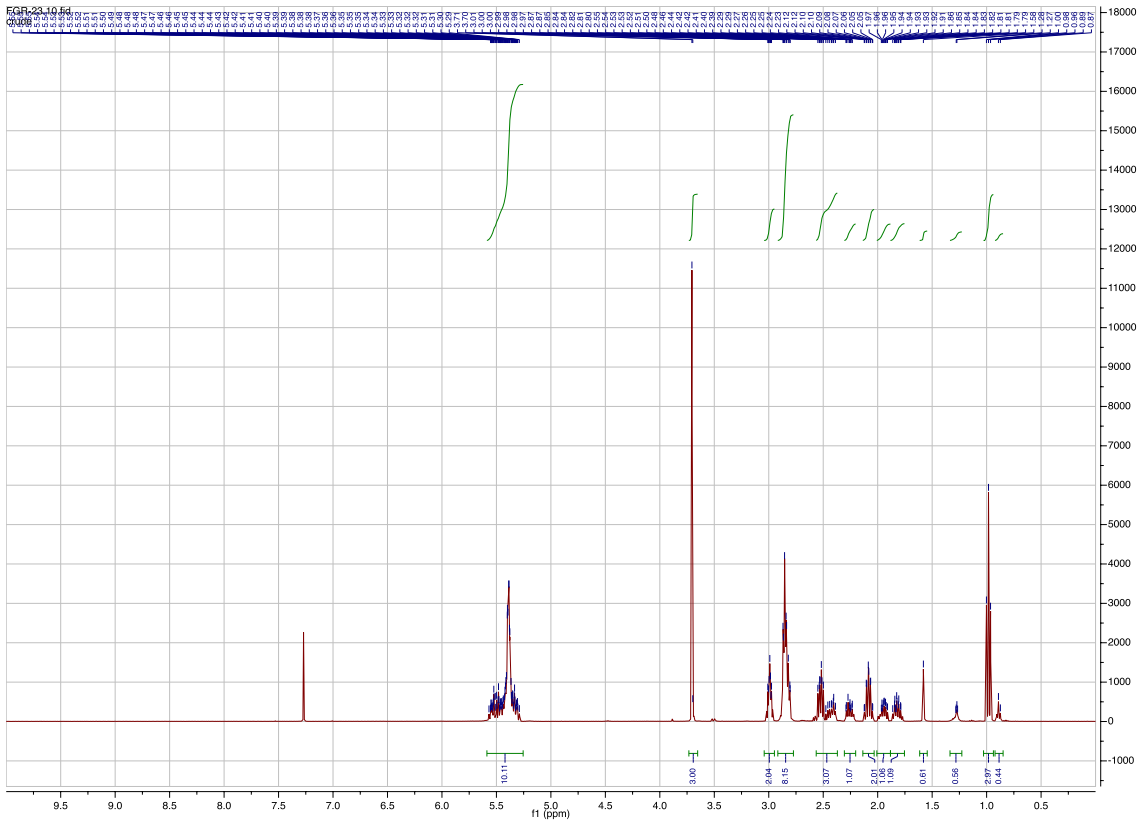
*Spectrum 32: ^{13}C NMR spectrum of the iodolactone **40**.*



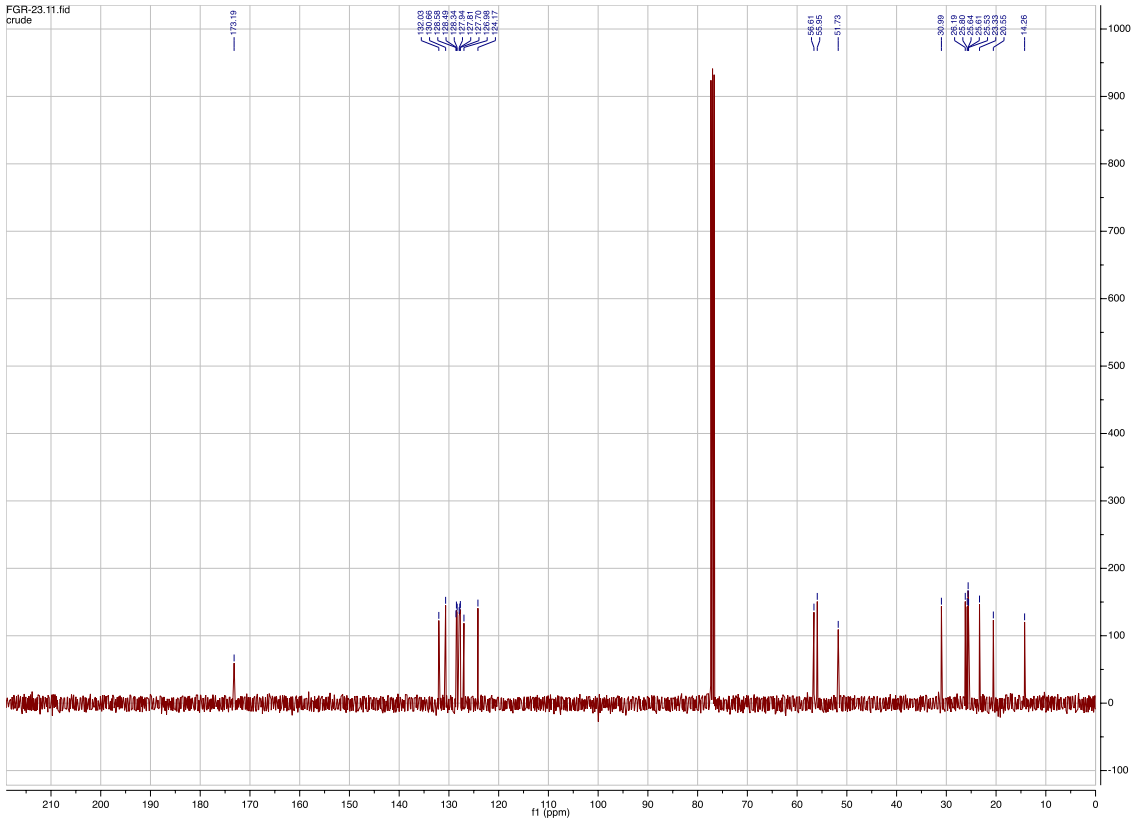
*Spectrum 33: IR spectrum of the iodolactone **40**.*

4-(3-((all-*Z*)-heptadeca-2,5,8,11,14-pentaen-1-yl)oxiran-2-yl)butanoate (**41**):

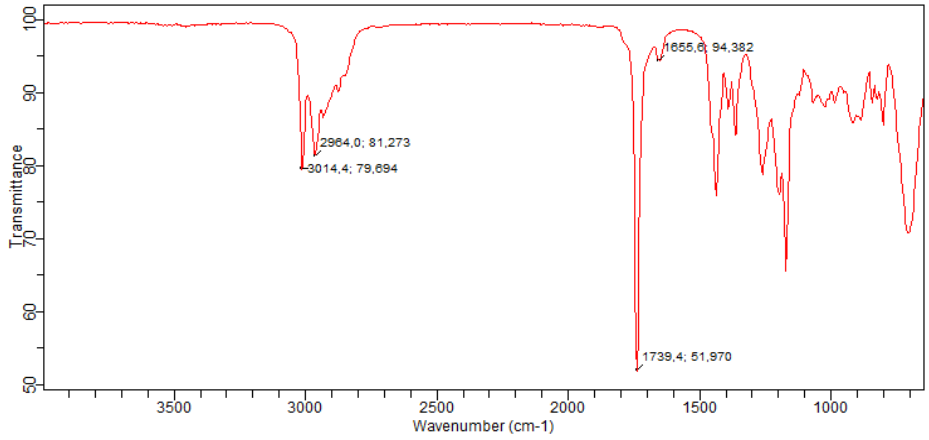




*Spectrum 34: [1]H NMR spectrum of the epoxide **41**.*

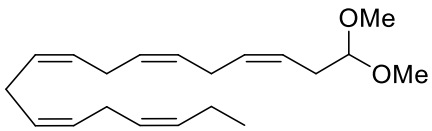


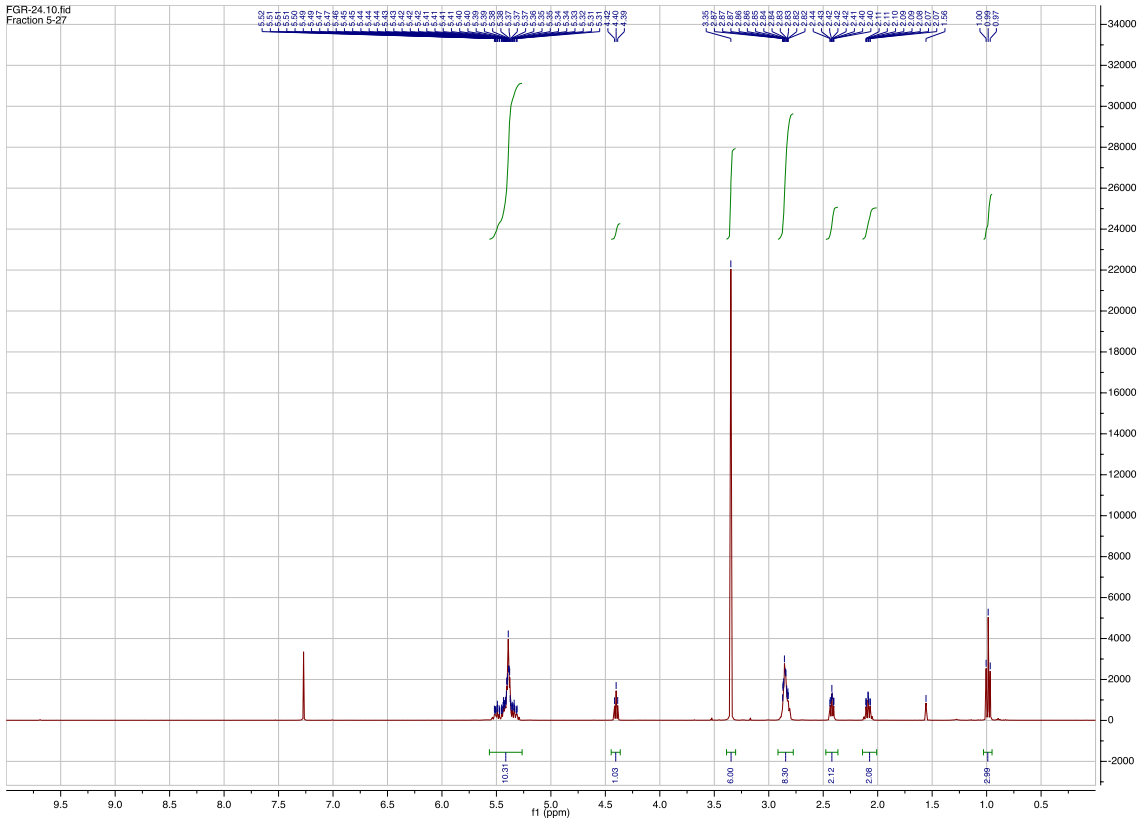
*Spectrum 35: ^{13}C NMR spectrum of the epoxide **41**.*



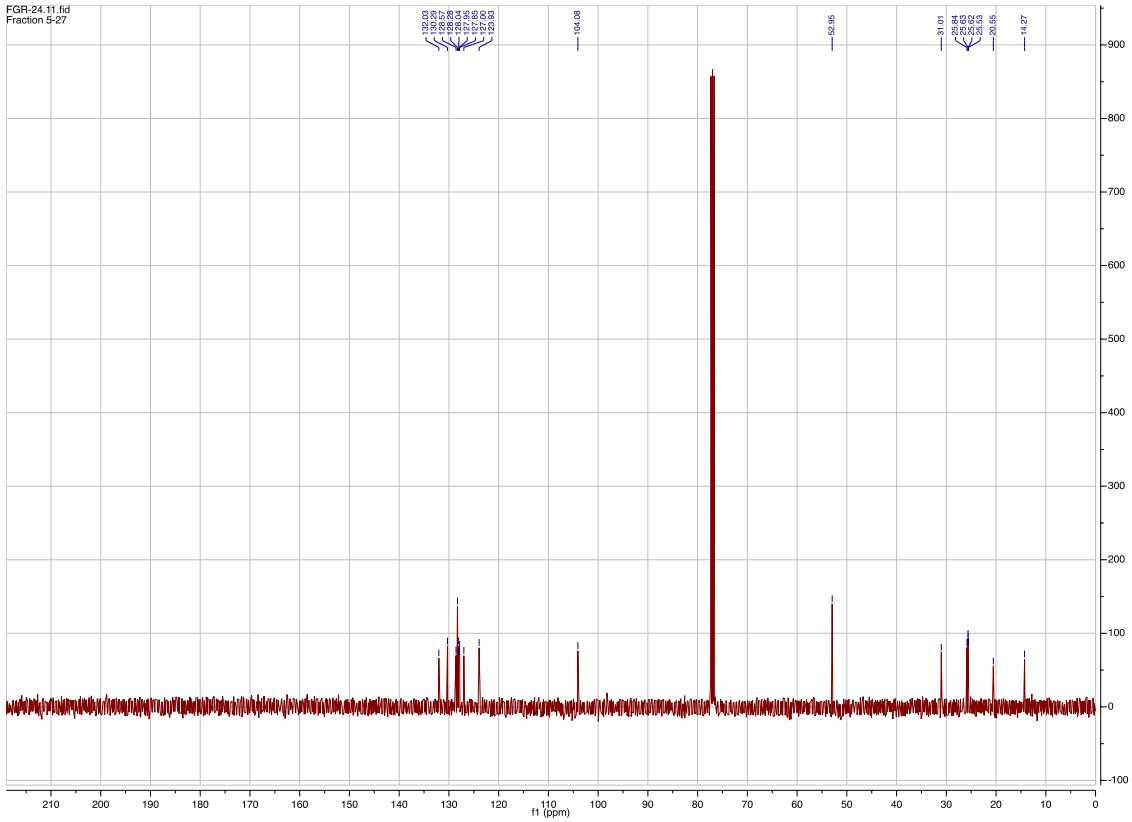
*Spectrum 36: IR spectrum of the epoxide **41**.*

(all-*Z*)-1,1-dimethoxyoctadeca-3,6,9,12,15-pentaene (**42**):

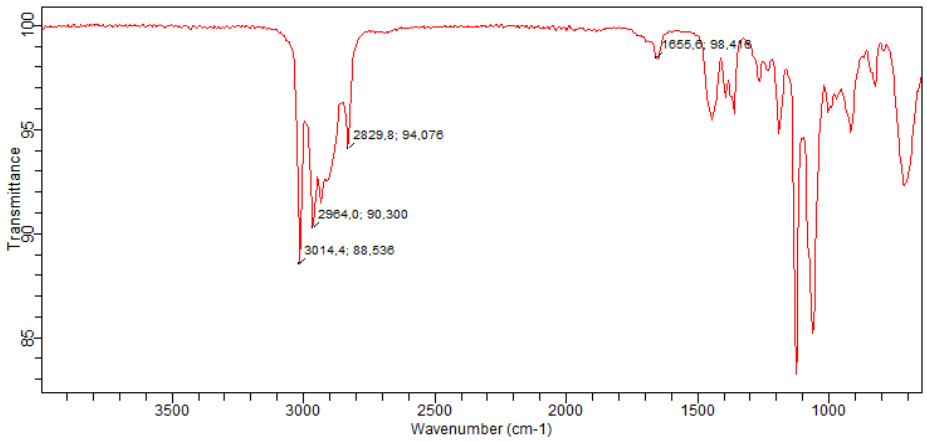




*Spectrum 37: ^{1}H NMR spectrum of the acetal **42**.*

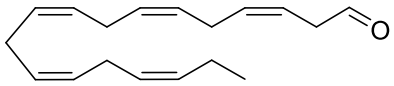


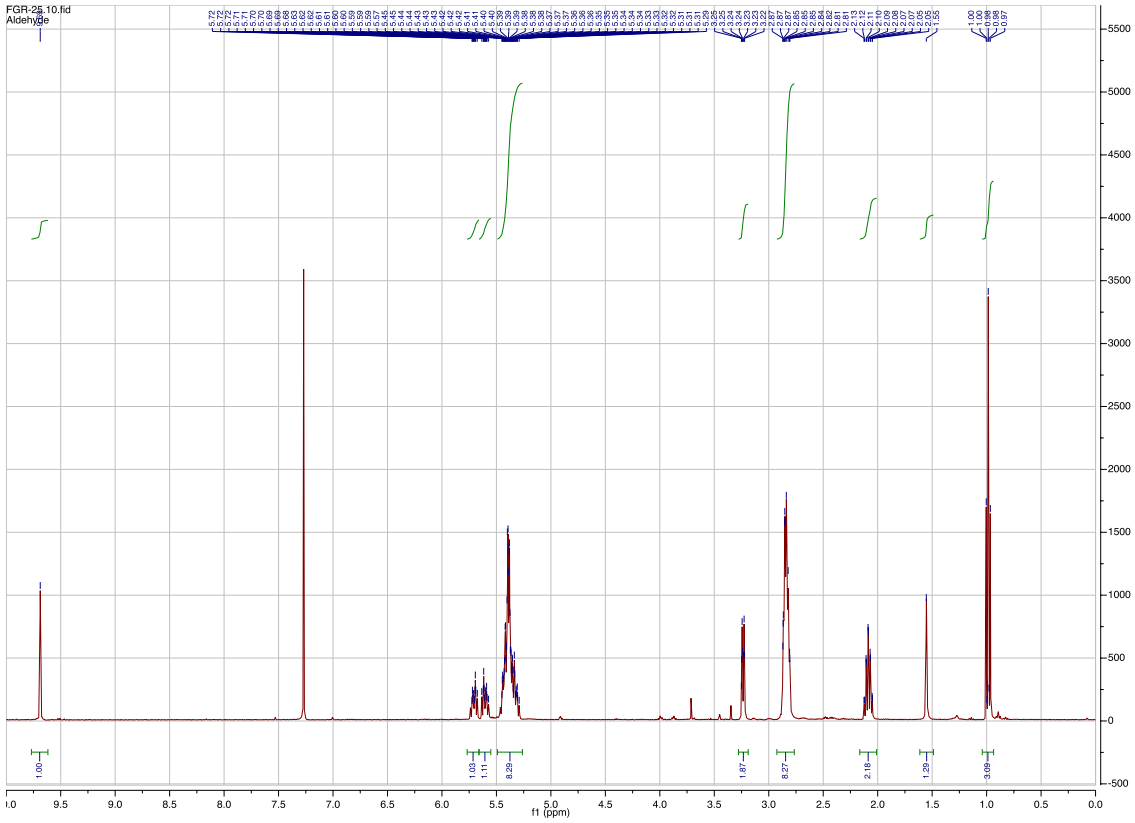
*Spectrum 38: ^{13}C NMR spectrum of the acetal **42**.*



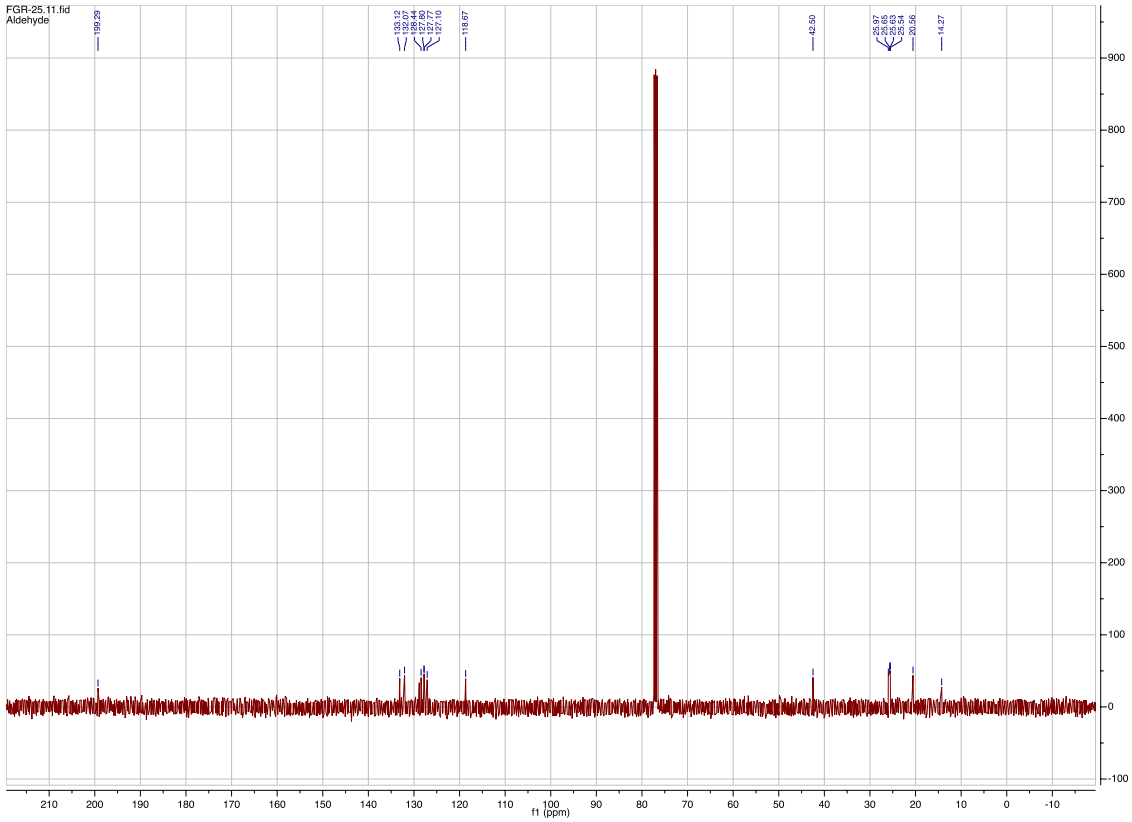
*Spectrum 39: IR spectrum of the acetal **42**.*

(all-*Z*)-octadeca-3,6,9,12,15-pentaenal (**39**):

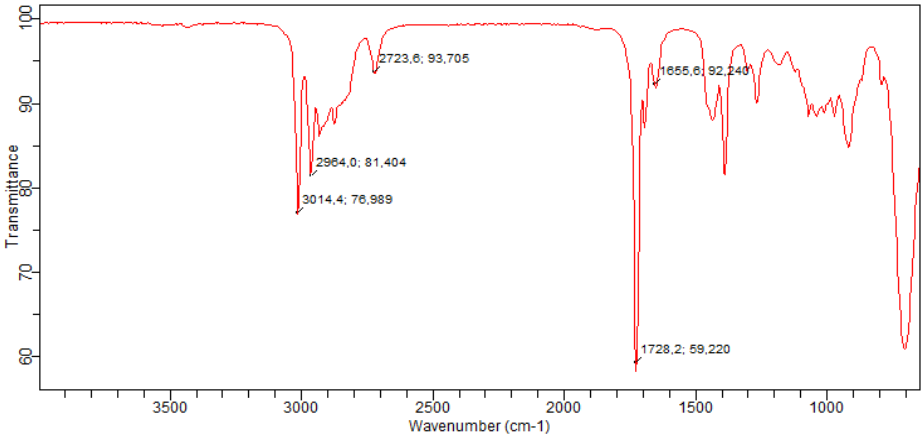




*Spectrum 40: ^{1}H NMR spectrum of the aldehyde **39**.*

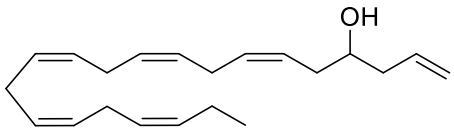


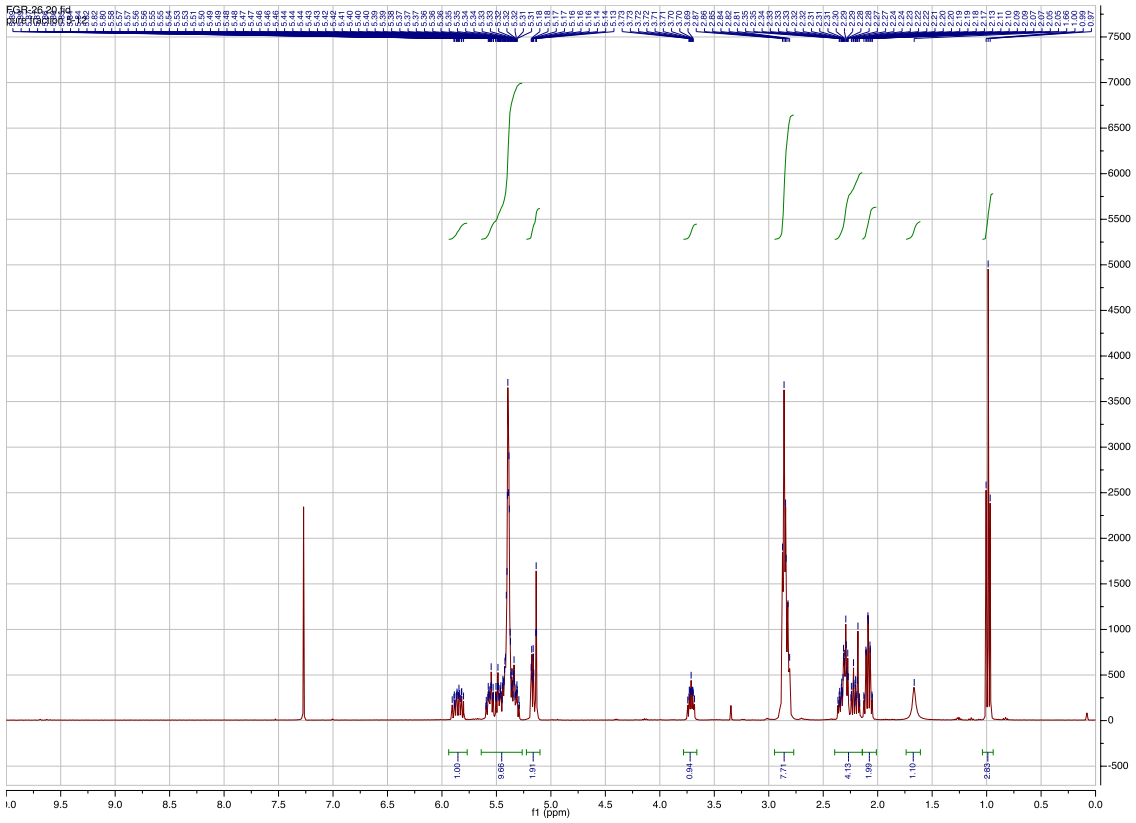
*Spectrum 41: ^{13}C NMR spectrum of the aldehyde **39**.*



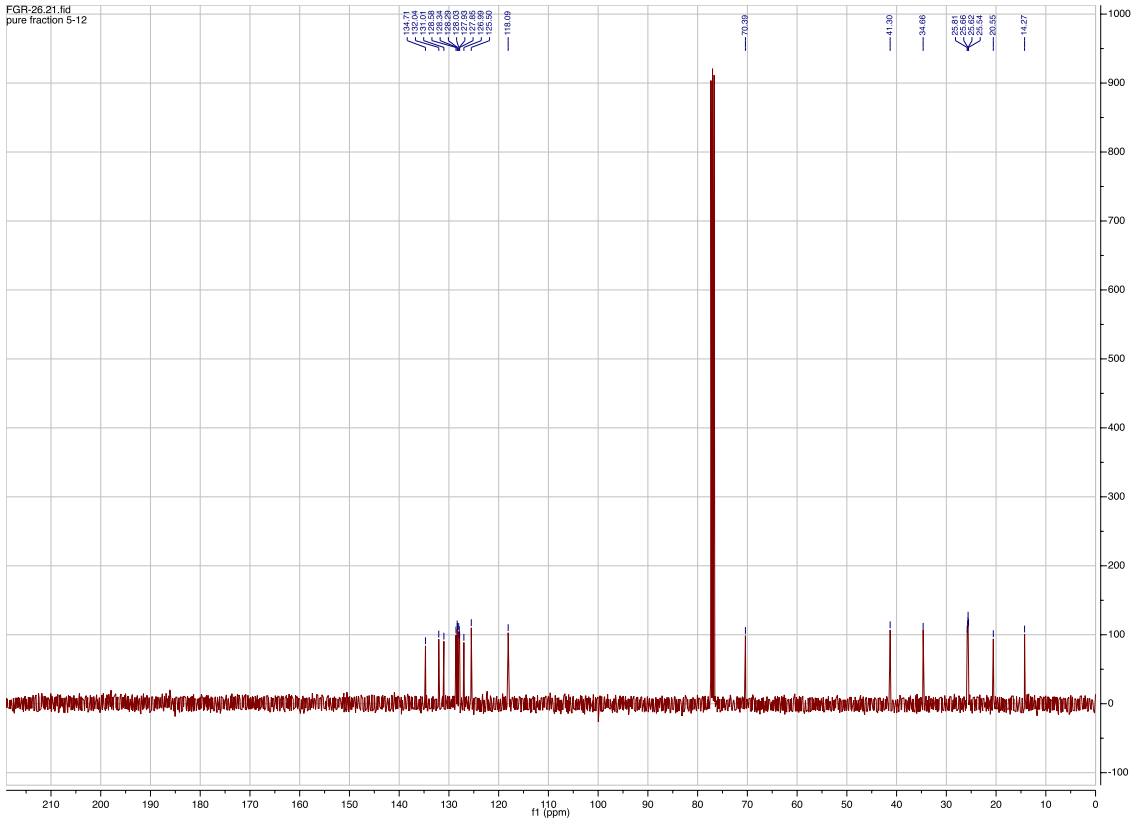
*Spectrum 42: IR spectrum of the aldehyde **39**.*

(all-*Z*)-henicosa-1,6,9,12,15,18-hexaen-4-ol (**101**):

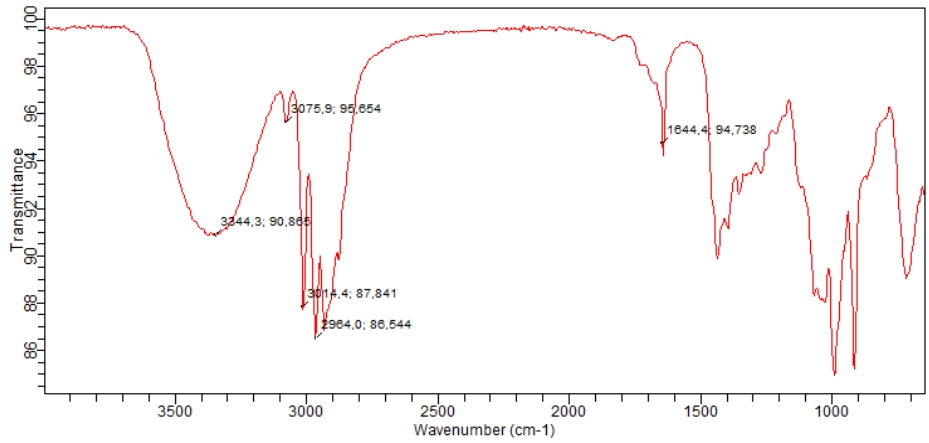




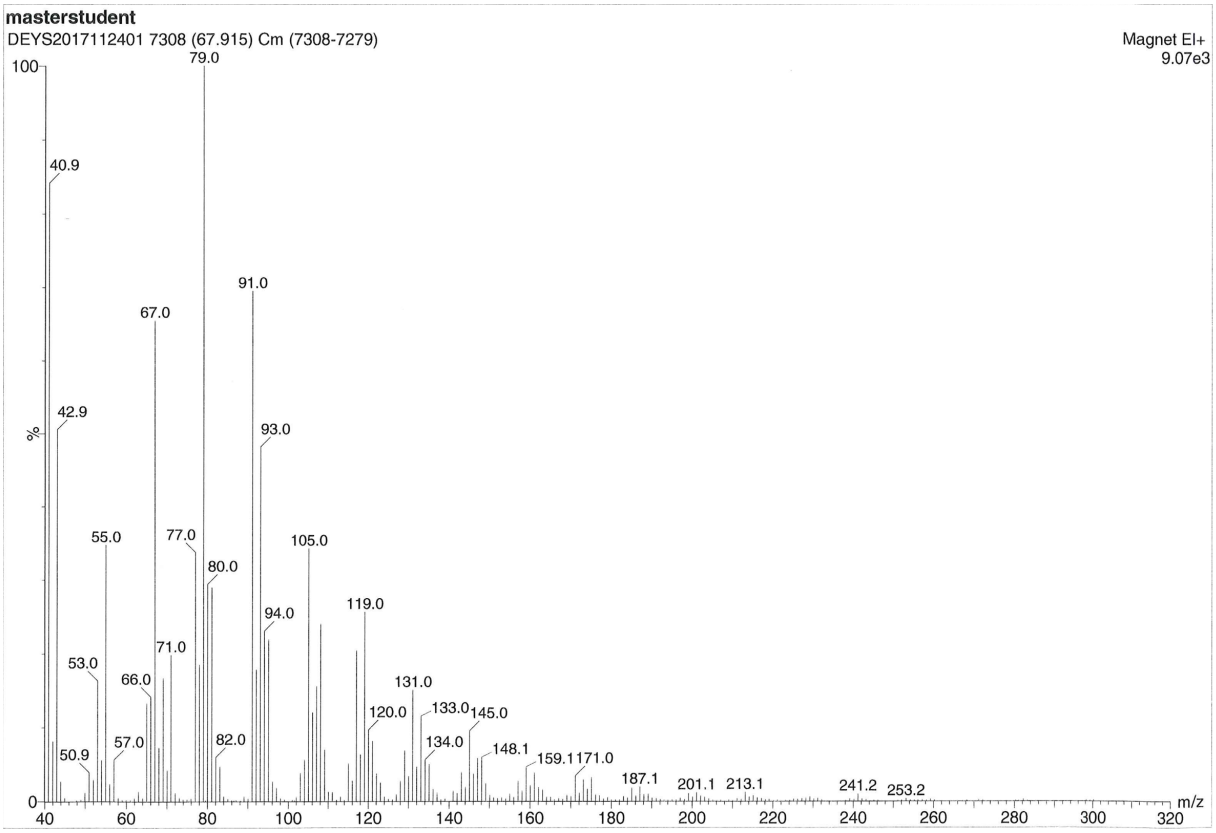
*Spectrum 43: ^{1}H NMR spectrum of the bis-homoallyl **101**.*



*Spectrum 44: [13]C NMR spectrum of the bis-homoallyl **101**.*

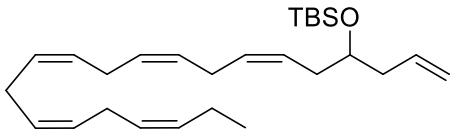


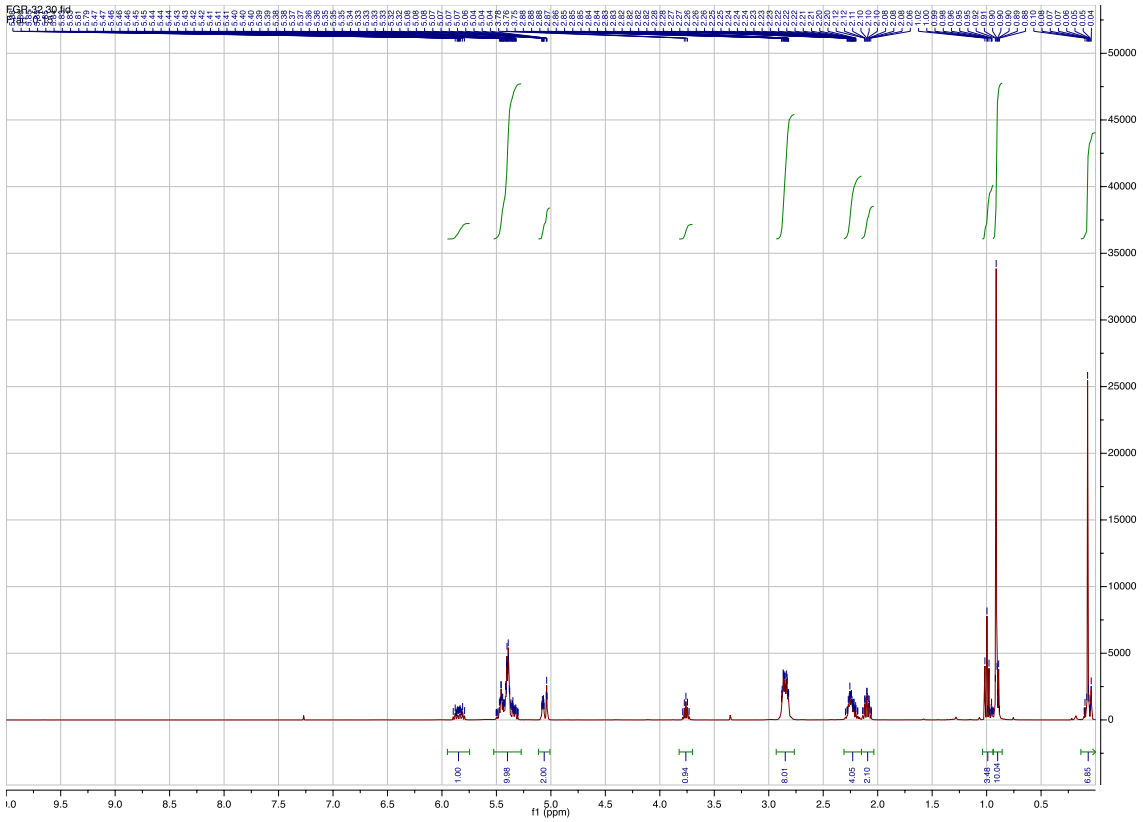
*Spectrum 45: IR spectrum f of the bis-homoallyl **101**.*



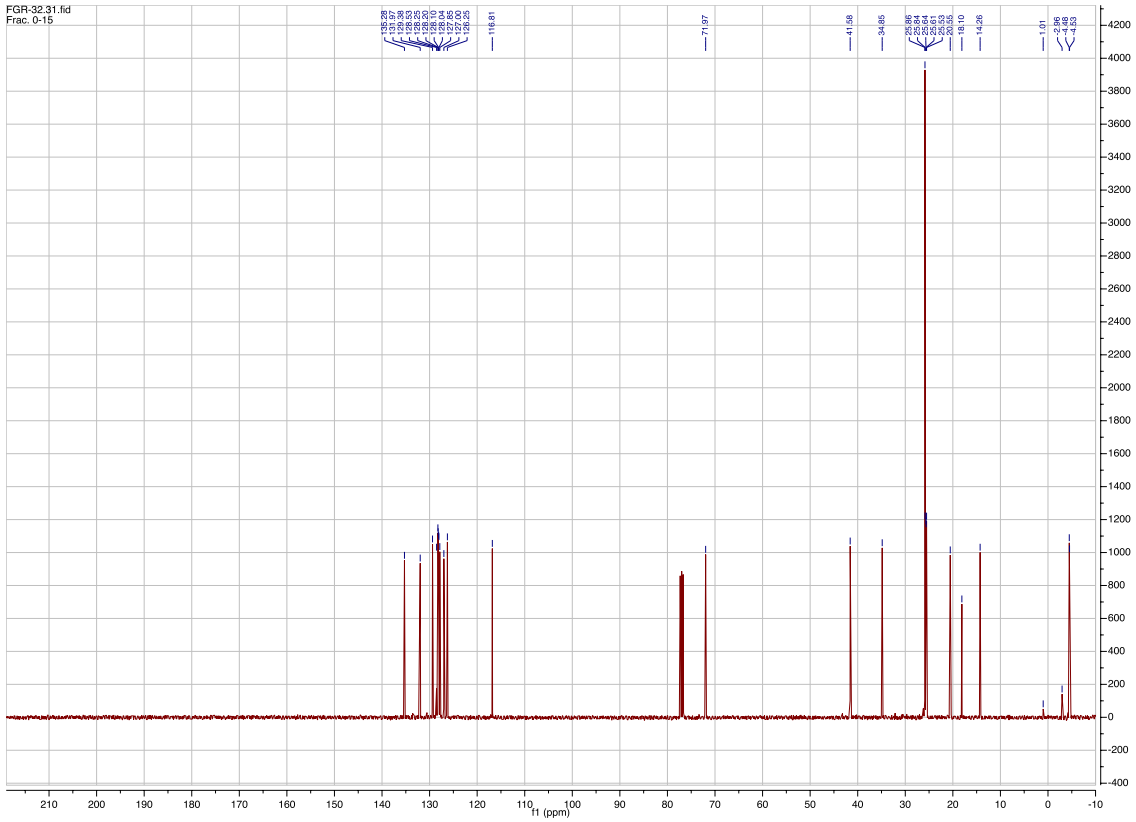
*Spectrum 46: LC-MS spectrum of the bis-homoallyl **101**.*

Tert-butyl(((all-*Z*)-henicosa-1,6,9,12,15,18-hexaen-4-yl)oxy)dimethylsilane (**102**):



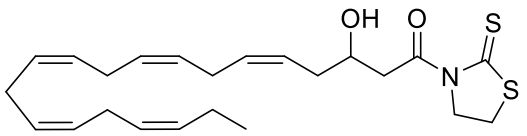


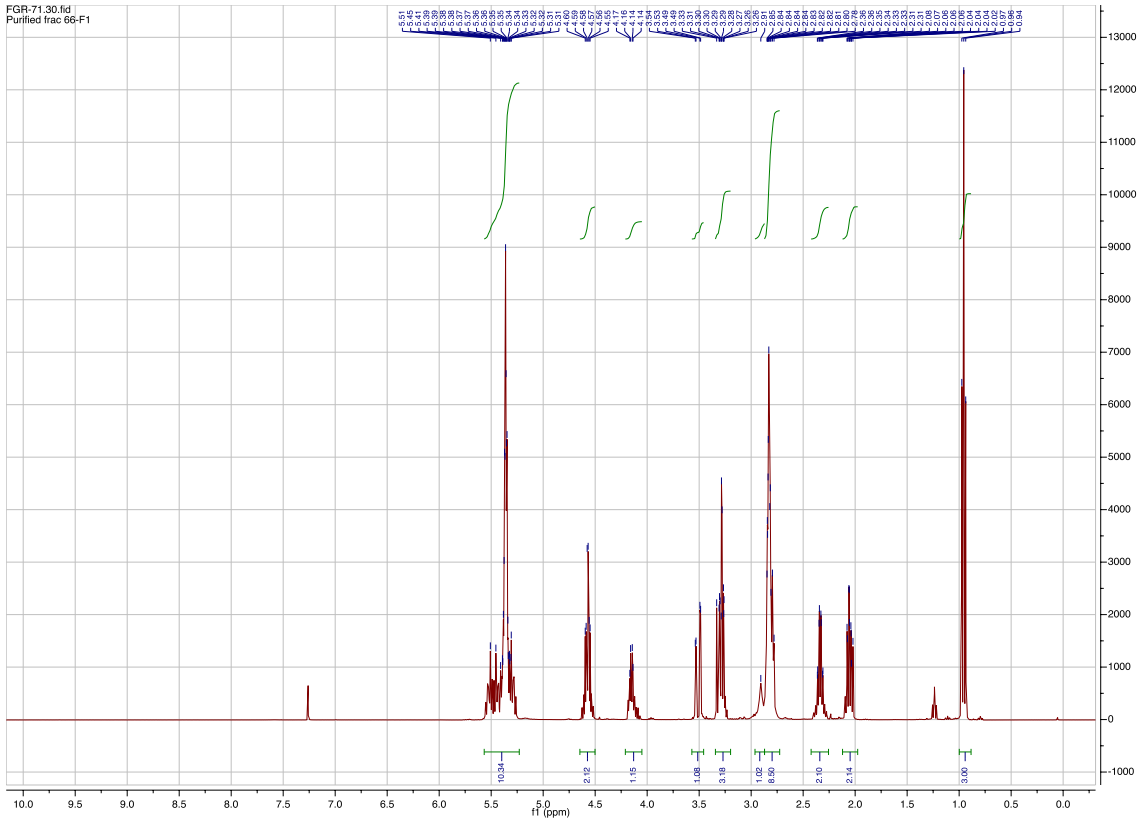
*Spectrum 47: ^{1}H NMR spectrum of the TBS-protected bis-homoallyl **102**.*



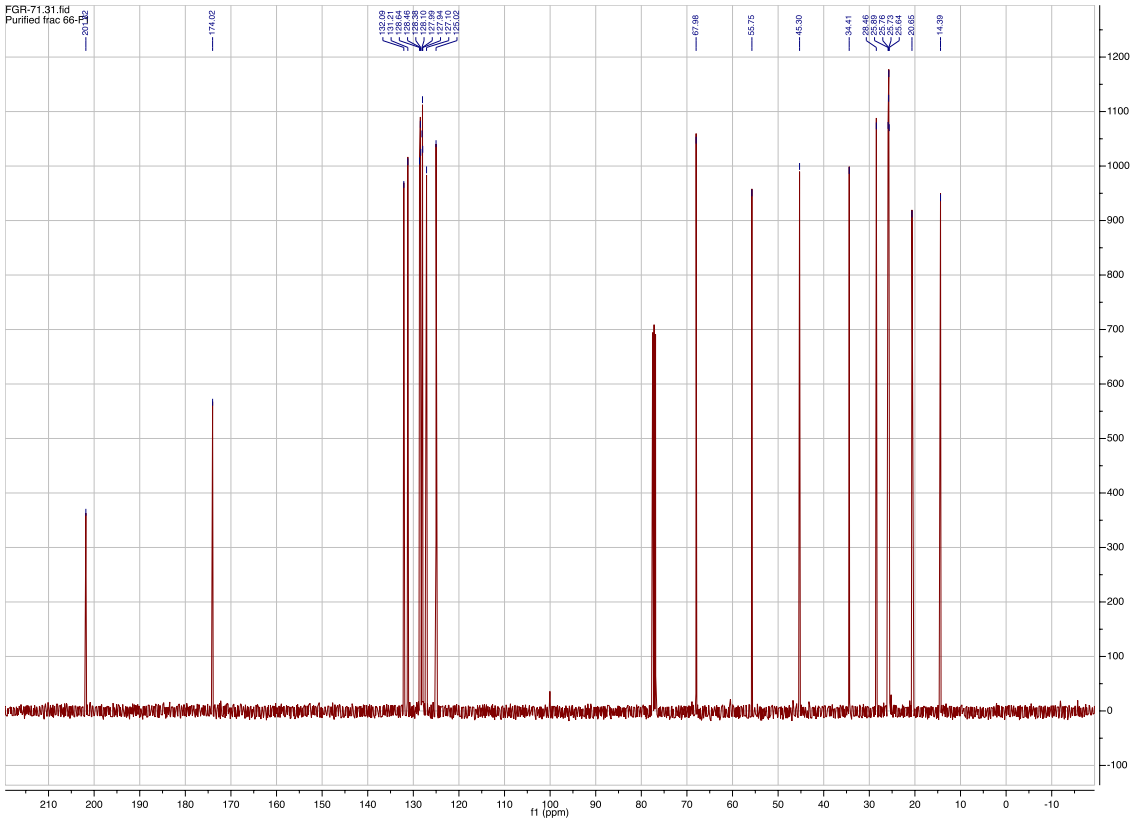
*Spectrum 48: ^{13}C NMR spectrum of the TBS-protected bis-homoallyl **102**.*

(all-*Z*)-3-hydroxy-1-(2-thioxothiazolidin-3-yl)icosa-5,8,11,14,17-pentaen-1-one (**117**):

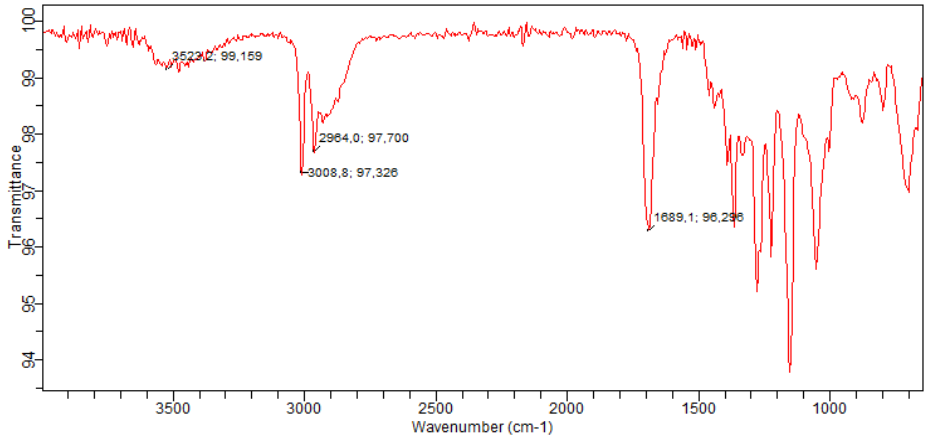




*Spectrum 49: [1]H NMR spectrum of the aldol product **117**.*



*Spectrum 50: ^{13}C NMR spectrum of the aldol product **117**.*

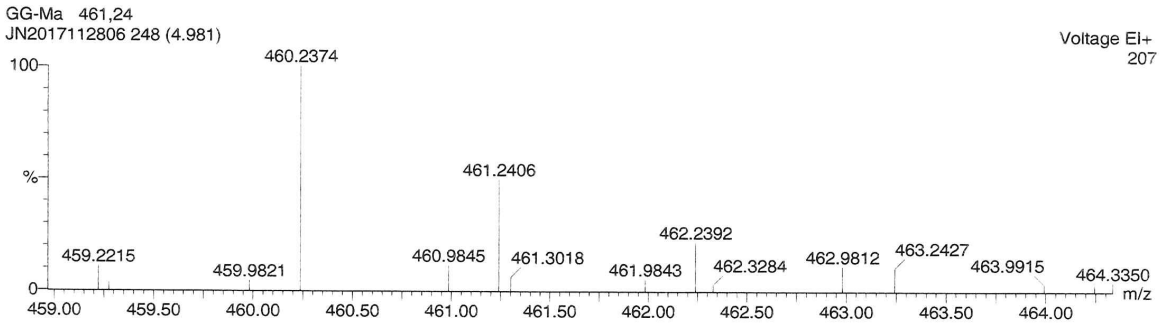


*Spectrum 51: IR spectrum of the aldol product **117**.*

Elemental Composition Report **Page 1**

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

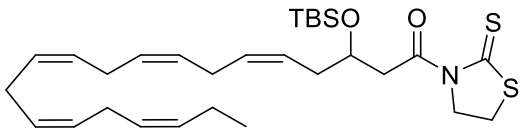
Monoisotopic Mass, Odd and Even Electron Ions
32 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

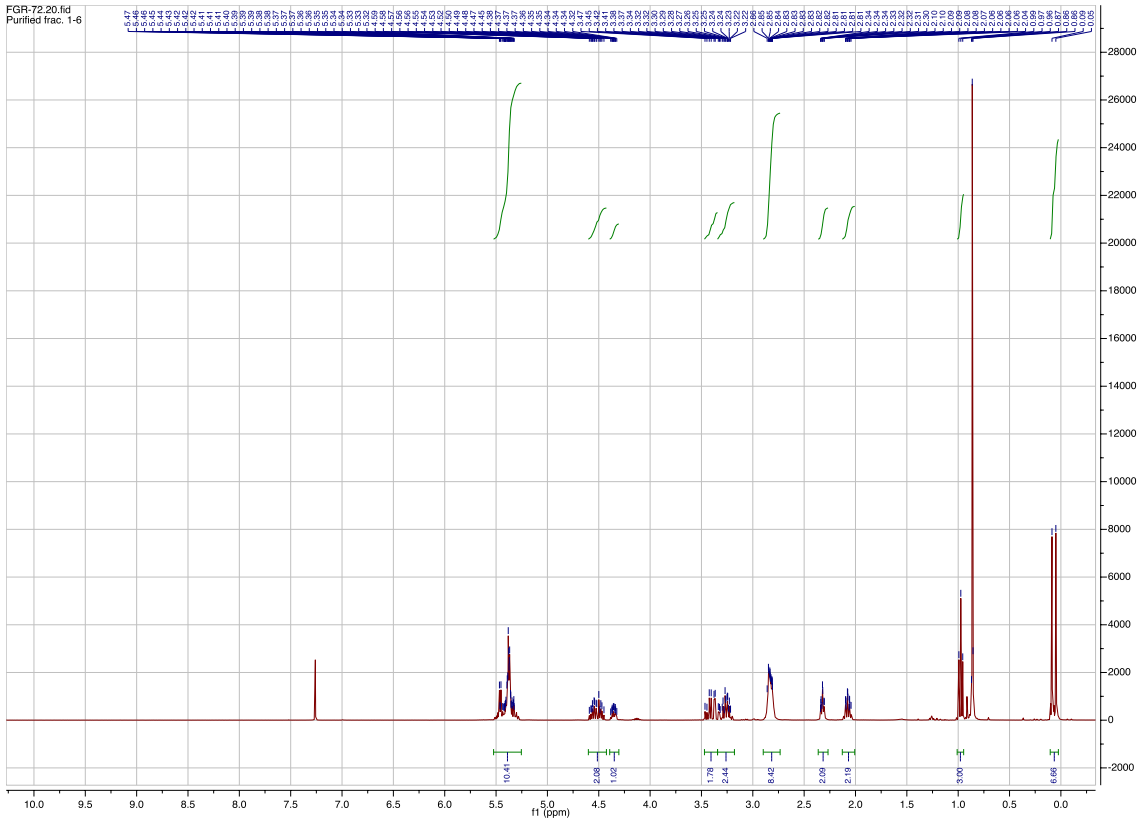


Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
461.2406	461.2422	-1.6	-3.5	8.0	1	C26 H39 N O2 S2

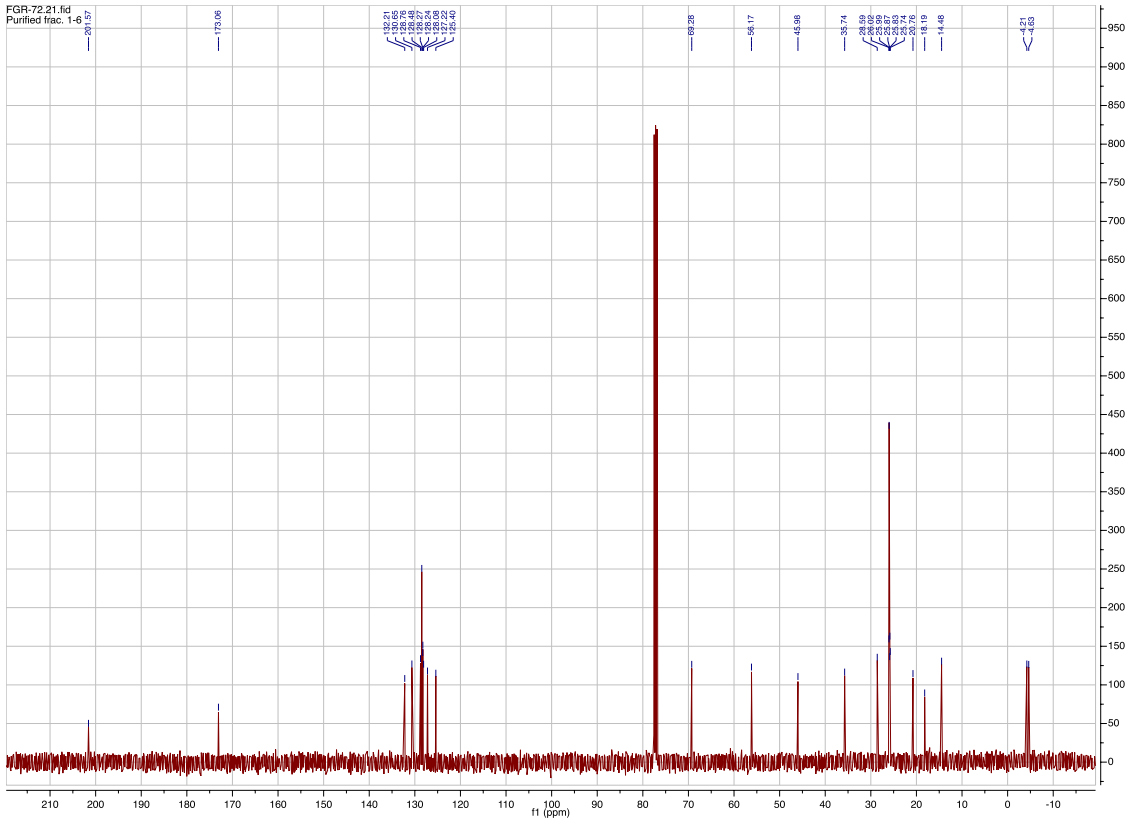
Spectrum 52: HRMS spectrum of the aldol product ***117****.*

(all-*Z*)-3-((tert-butyldimethylsilyl)oxy)-1-(2-thioxothiazolidin-3-yl)icosa-5,8,11,14,17-pentaen-1-one (**118**):

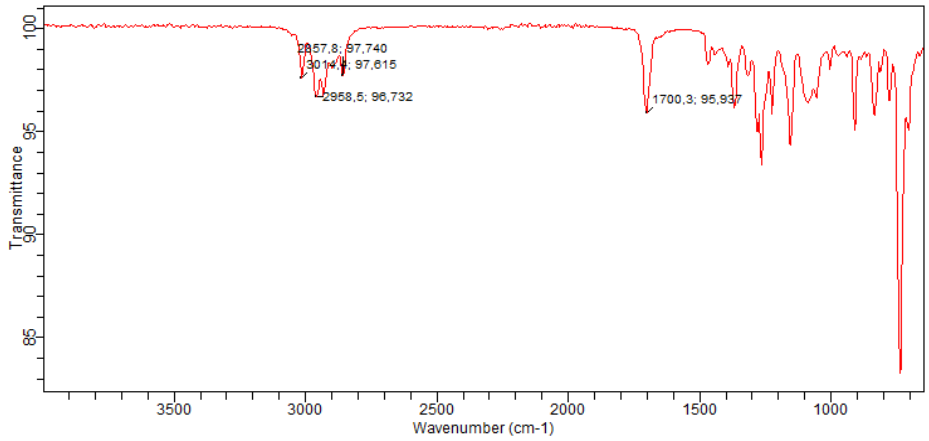




*Spectrum 53: [1]H NMR spectrum of the TBS-protected aldol product **118**.*

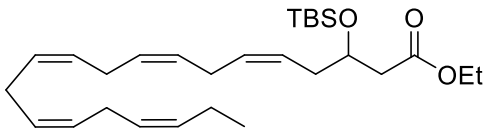


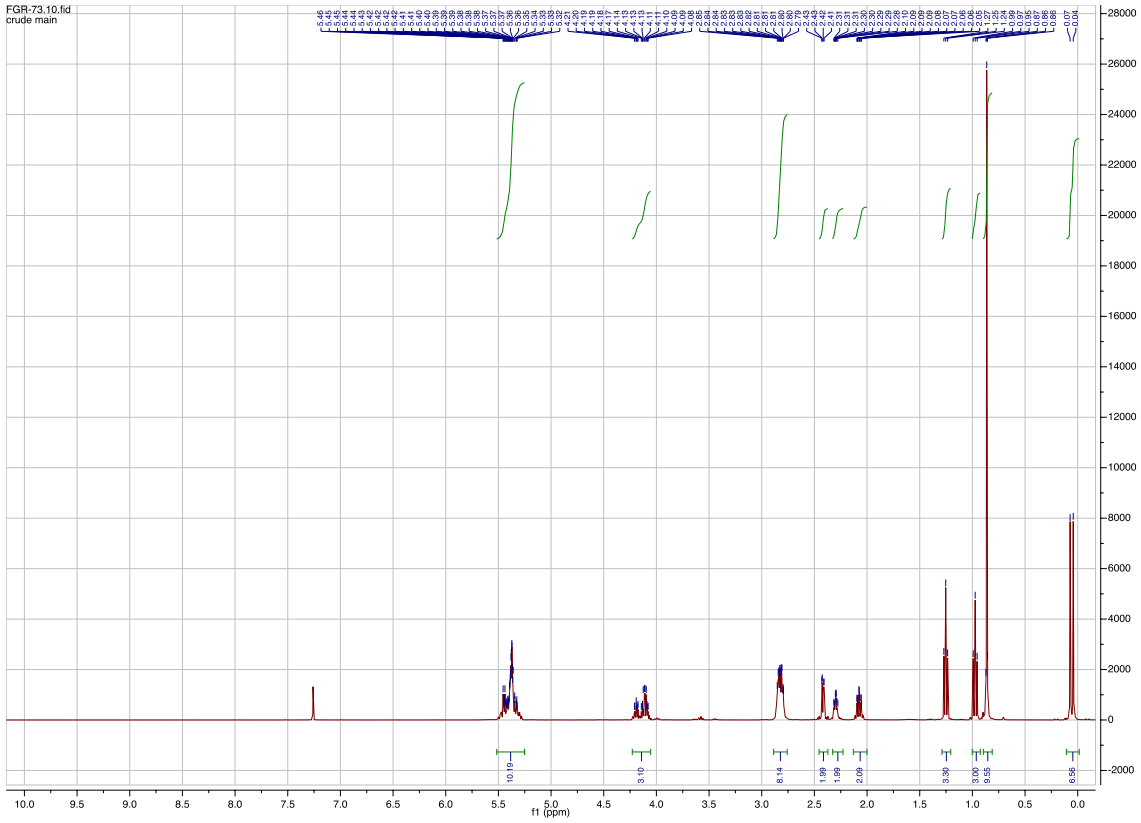
*Spectrum 54: ^{13}C NMR spectrum of the TBS-protected aldol product **118**.*



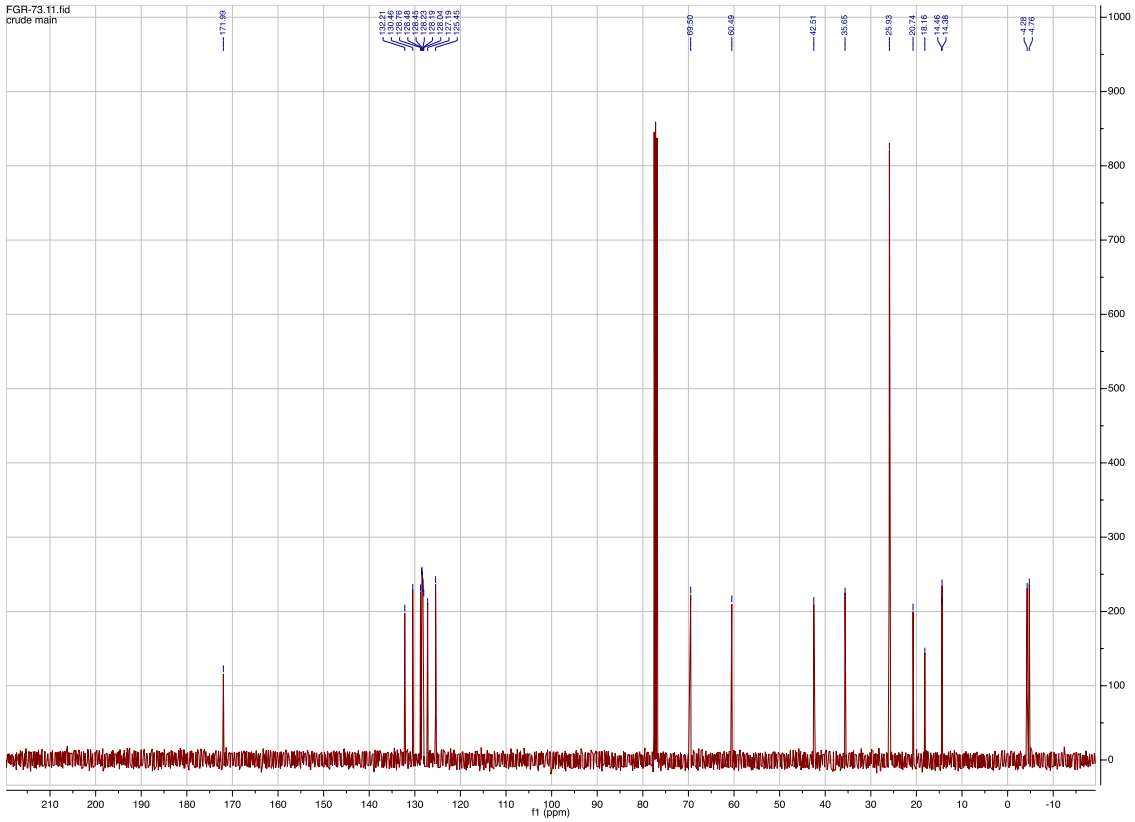
*Spectrum 55: IR spectrum of the TBS-protected aldol product **118**.*

Ethyl (all-*Z*)-3-((tert-butyldimethylsilyl)oxy)icosa-5,8,11,14,17-pentaenoate (**90**):

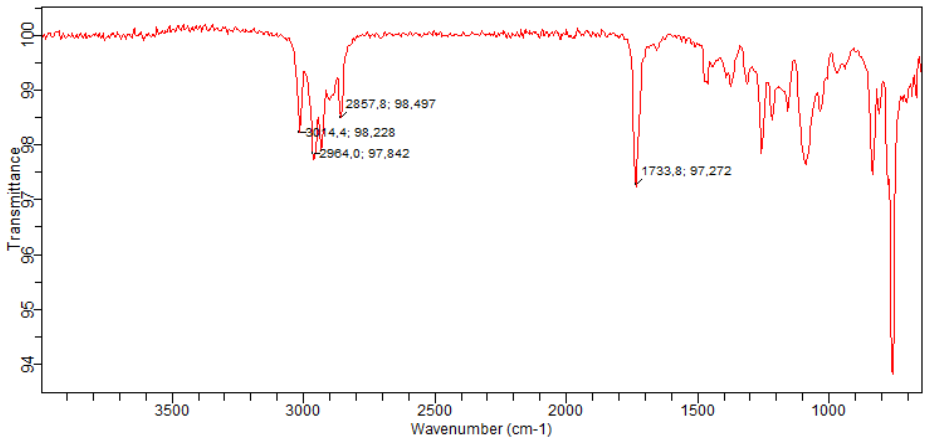




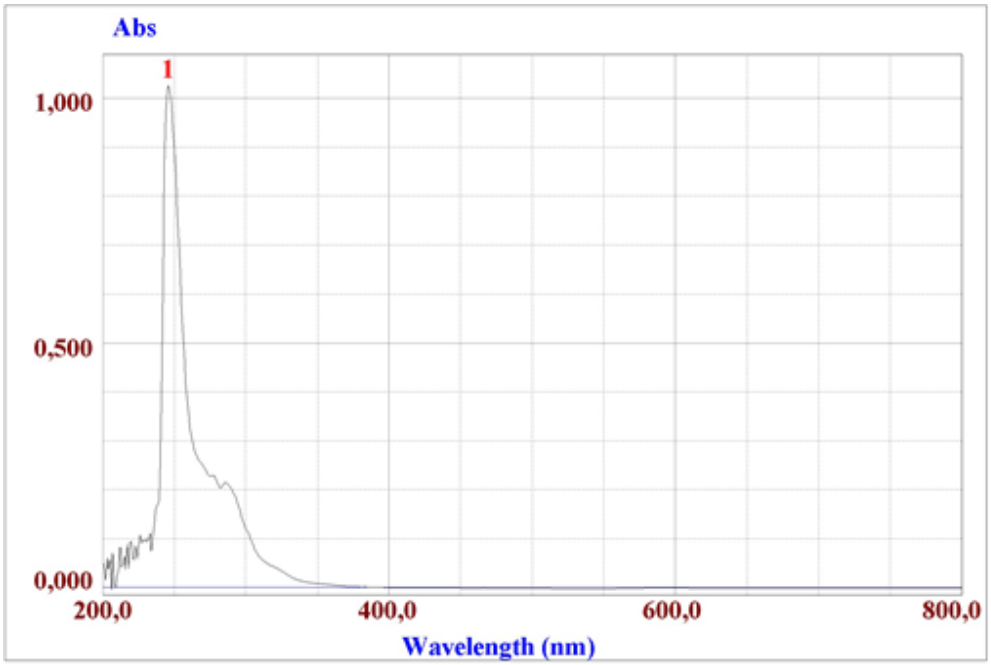
*Spectrum 56: ^{1}H NMR spectrum of the TBS-protected EE **90**.*



*Spectrum 57: [13]C NMR spectrum of the TBS-protected EE **90**.*

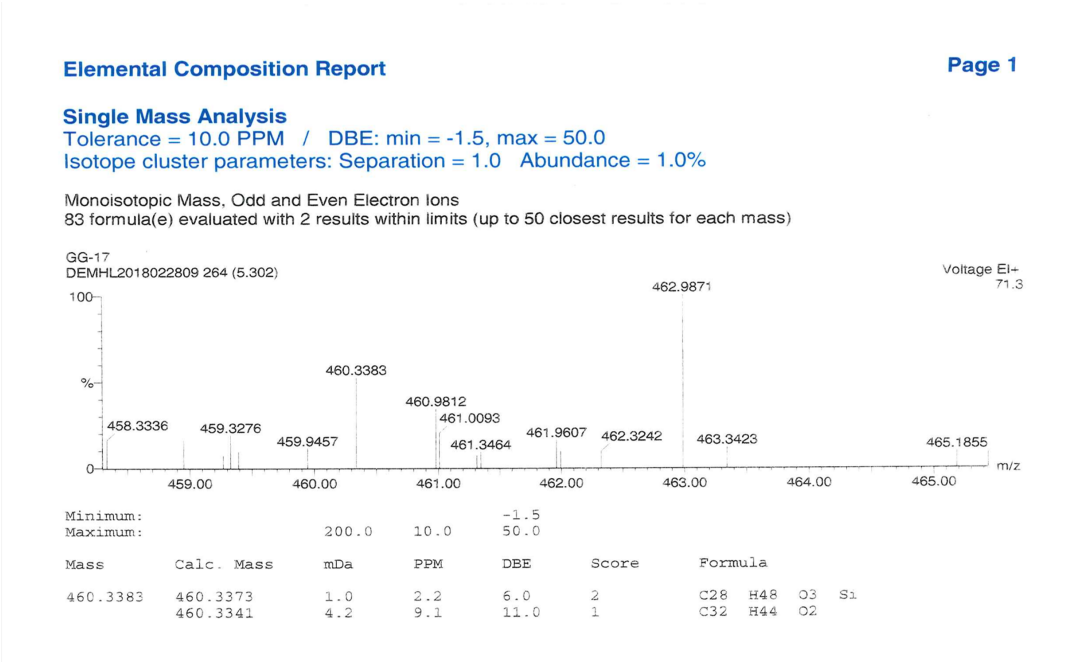


*Spectrum 58: IR spectrum of the TBS-protected EE **90**.*



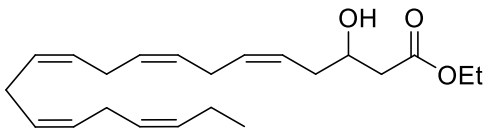
No.	Peak Type	Position	Height
1	Peak	245,8	1,027

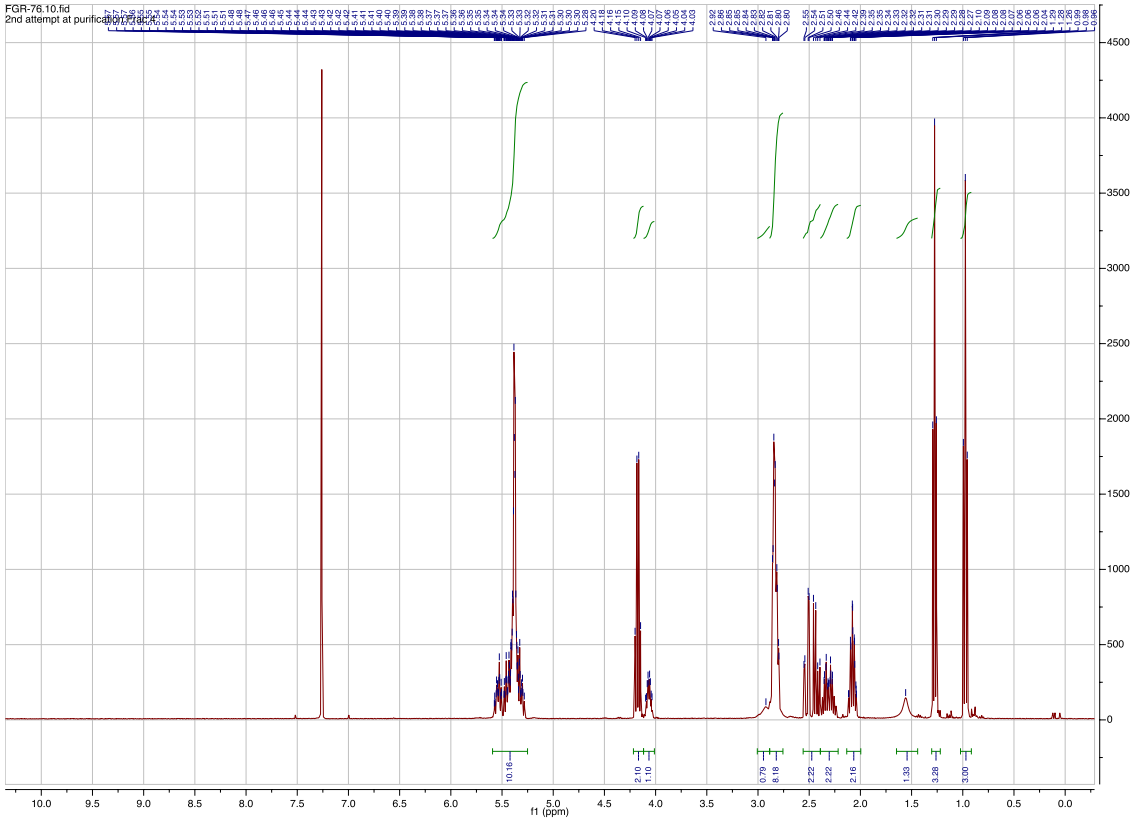
*Spectrum 59: UV spectrum of the TBS-protected EE **90**.*



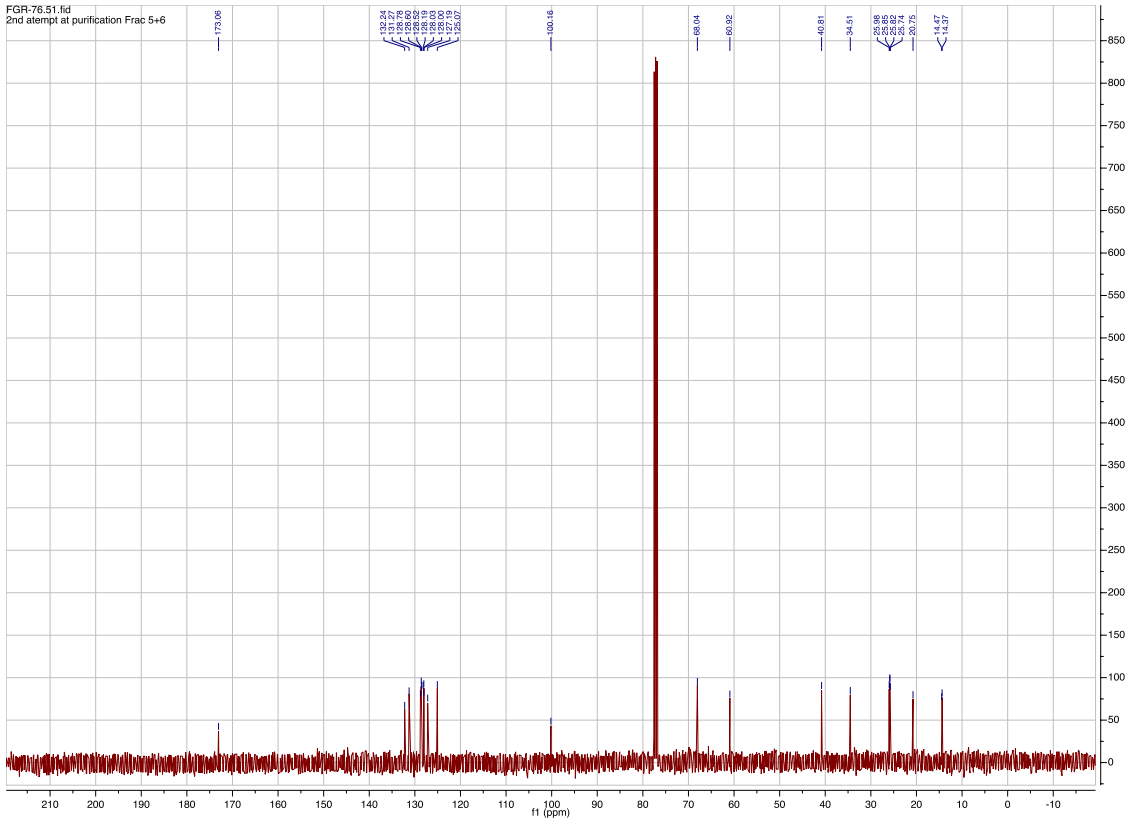
*Spectrum 60: HRMS spectrum of the TBS-protected EE **90**.*

(all-*Z*)-3-hydroxyicosa-5,8,11,14,17-pentaenoate (**91**):

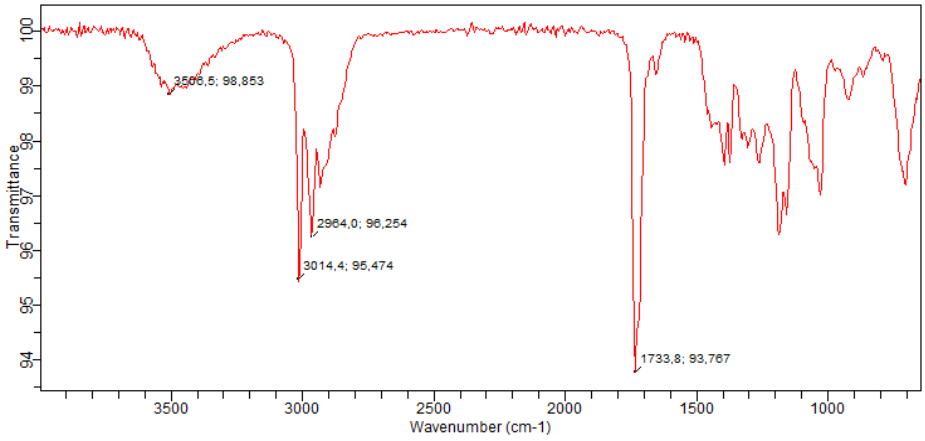




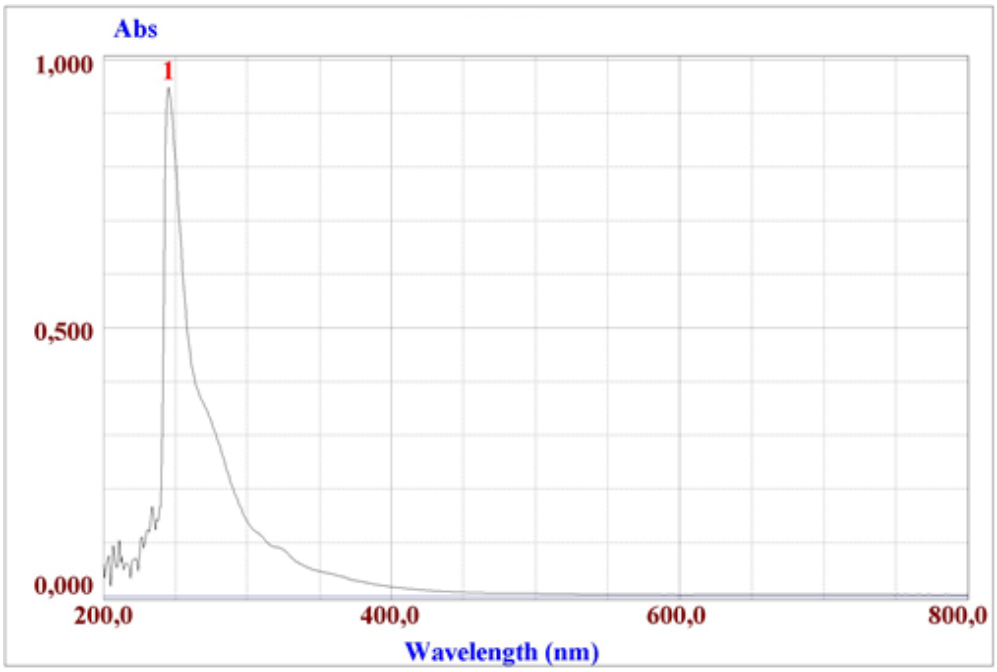
*Spectrum 61: ^{1}H NMR spectrum of the EE **91**.*



*Spectrum 62: ^{13}C NMR spectrum of the EE **91**.*



*Spectrum 63: IR spectrum of the EE **91**.*



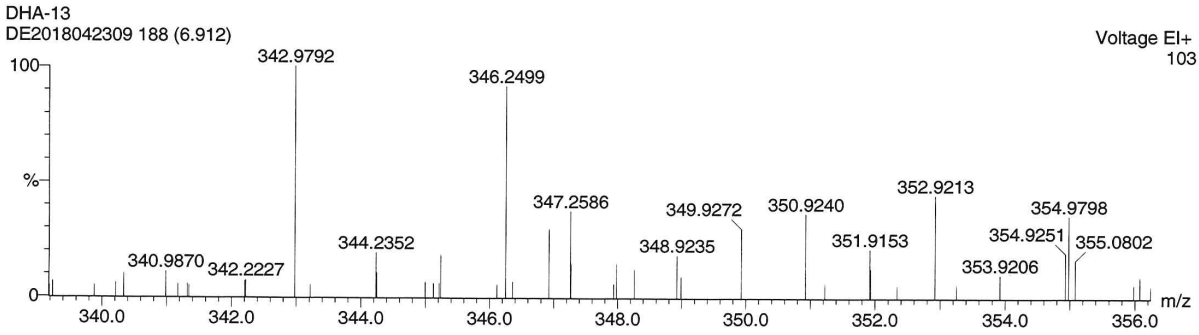
No.	Peak Type	Position	Height	Area	Start	End
1	Peak	245,3	0,949			

*Spectrum 64: UV spectrum of the EE **91**.*

Elemental Composition Report **Page 1**

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

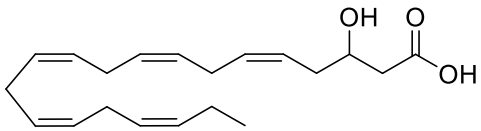
Monoisotopic Mass, Odd and Even Electron Ions
31 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

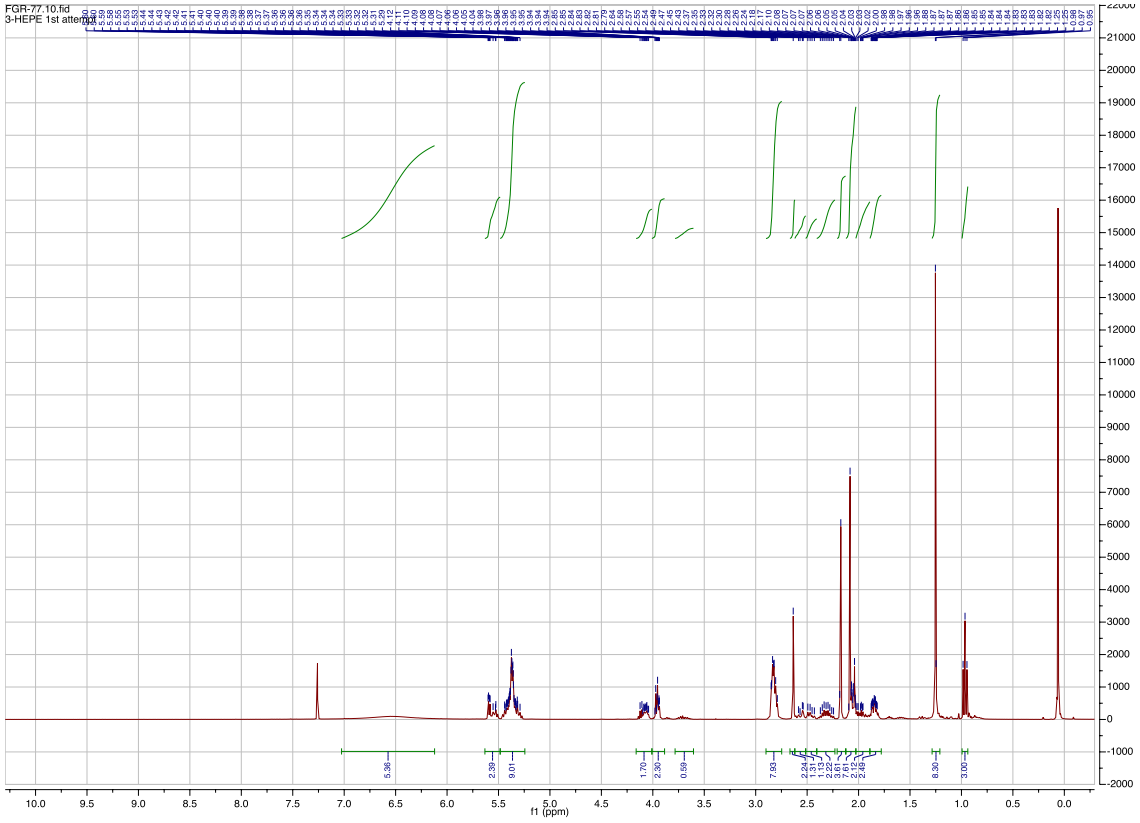


Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
346.2499	346.2508	-0.9	-2.6	6.0	1	C22 H34 O3

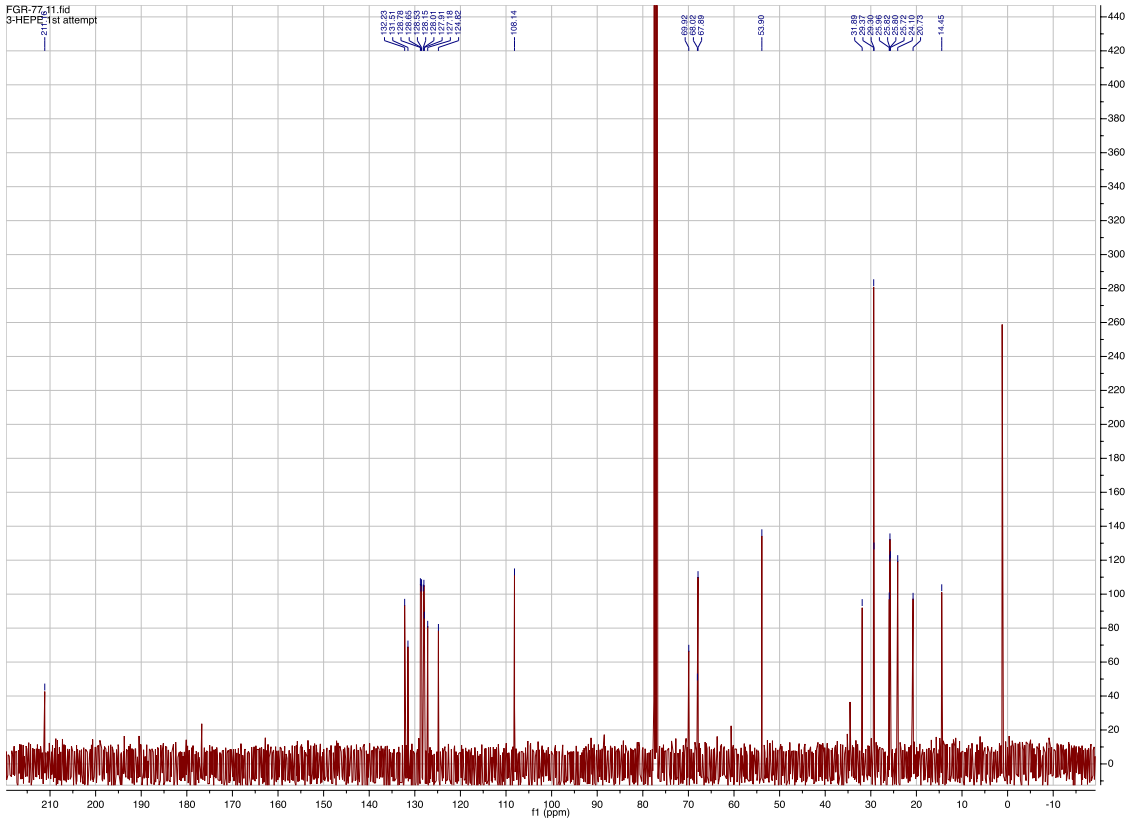
*Spectrum 65: HRMS spectrum of the EE **91**.*

(all-*Z*)-3-hydroxyicosa-5,8,11,14,17-pentaenoic acid (**109**):

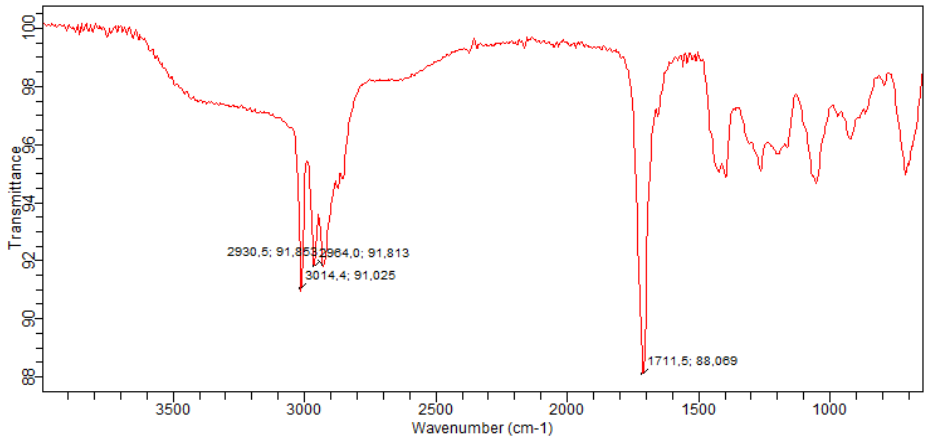




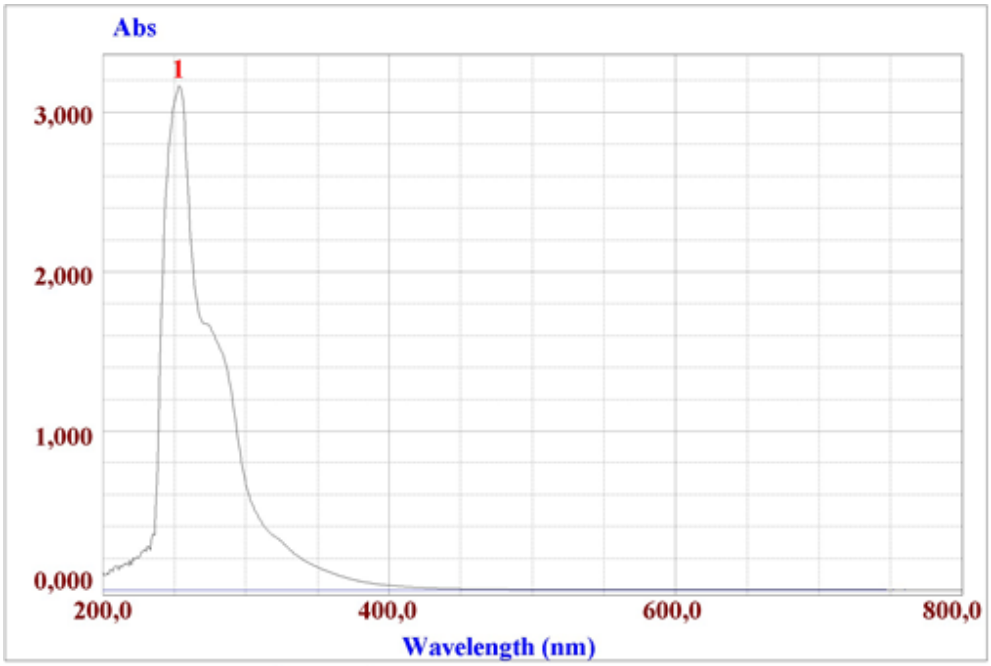
*Spectrum 66: ^{1}H NMR spectrum of the carboxylic acid **109**.*



*Spectrum 67: ^{13}C NMR spectrum of the carboxylic acid **109**.*



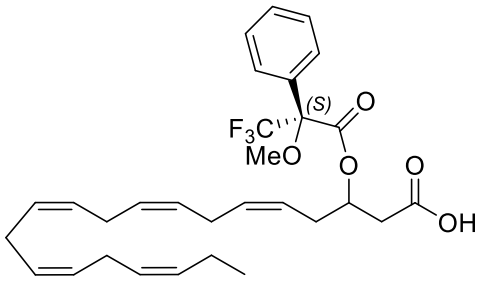
*Spectrum 68: IR spectrum of the carboxylic acid **109**.*

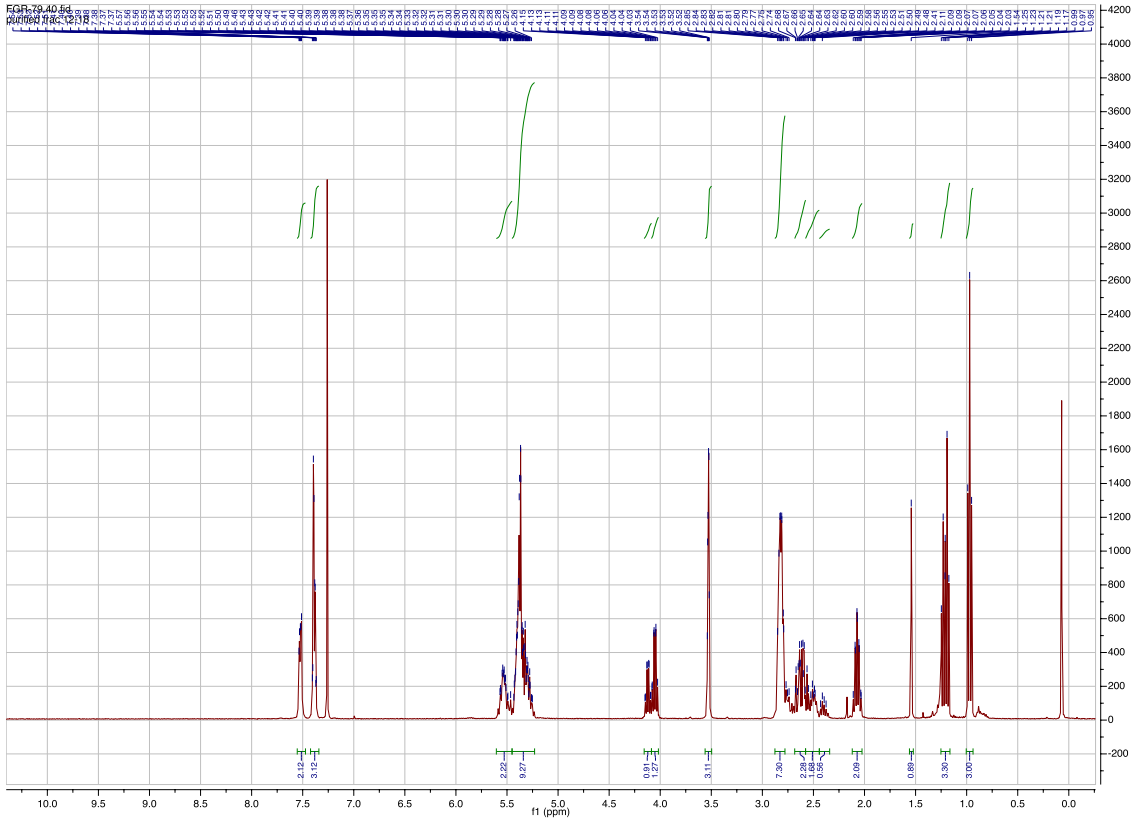


No.	Peak Type	Position	Height
1	Peak	253,4	3,169

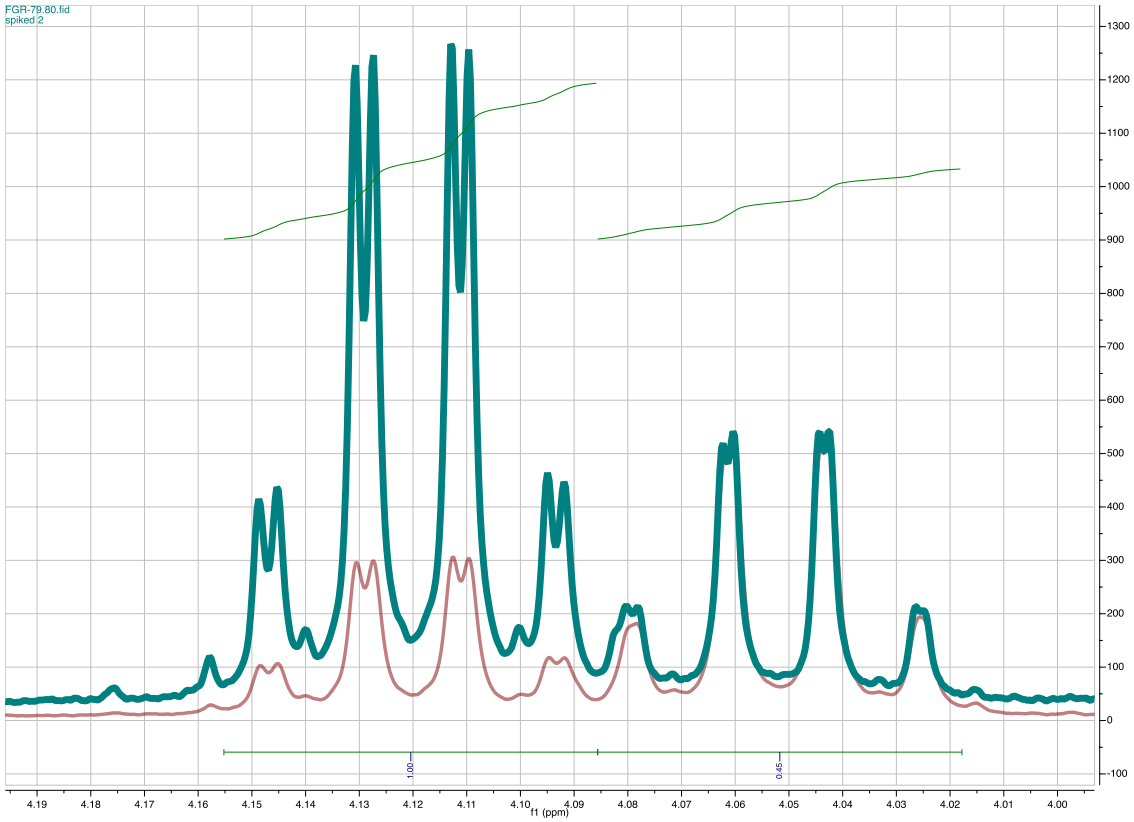
*Spectrum 69: UV spectrum of the carboxylic acid **109**.*

(all-*Z*)-3-(((*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)icosa-5,8,11,14,17-pentaenoic acid (**119**)

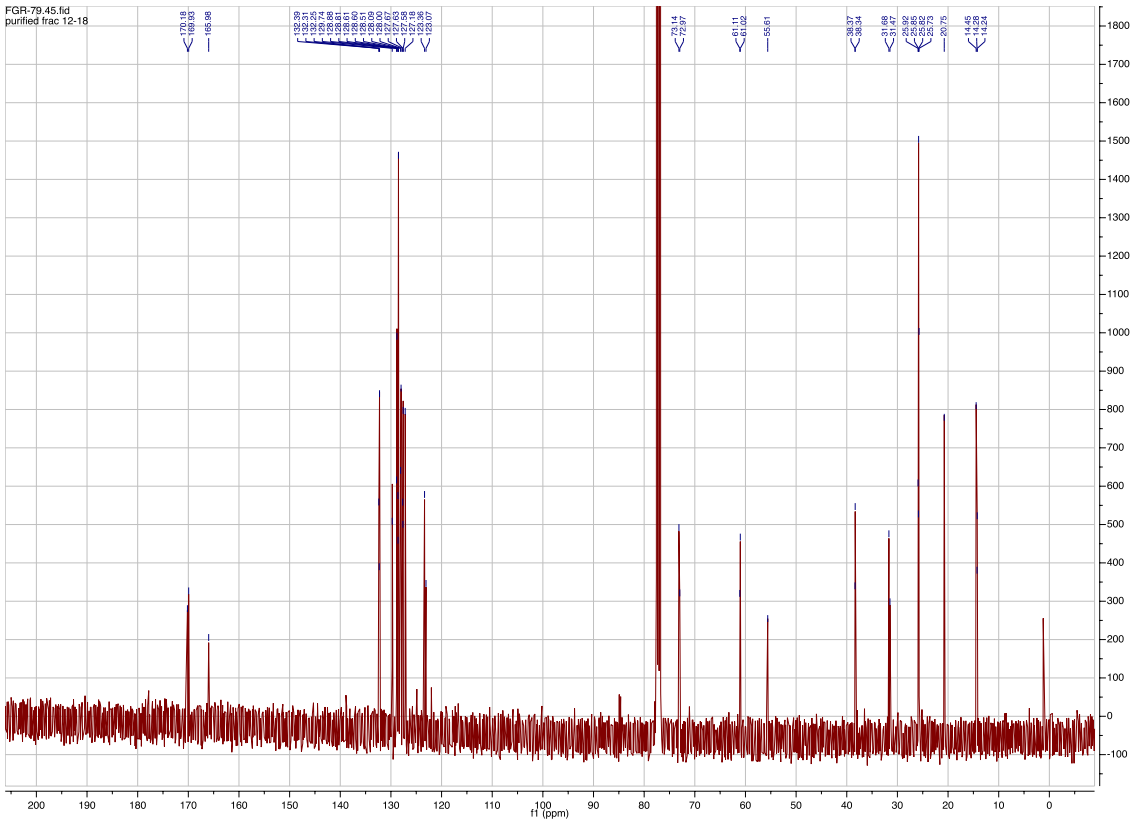




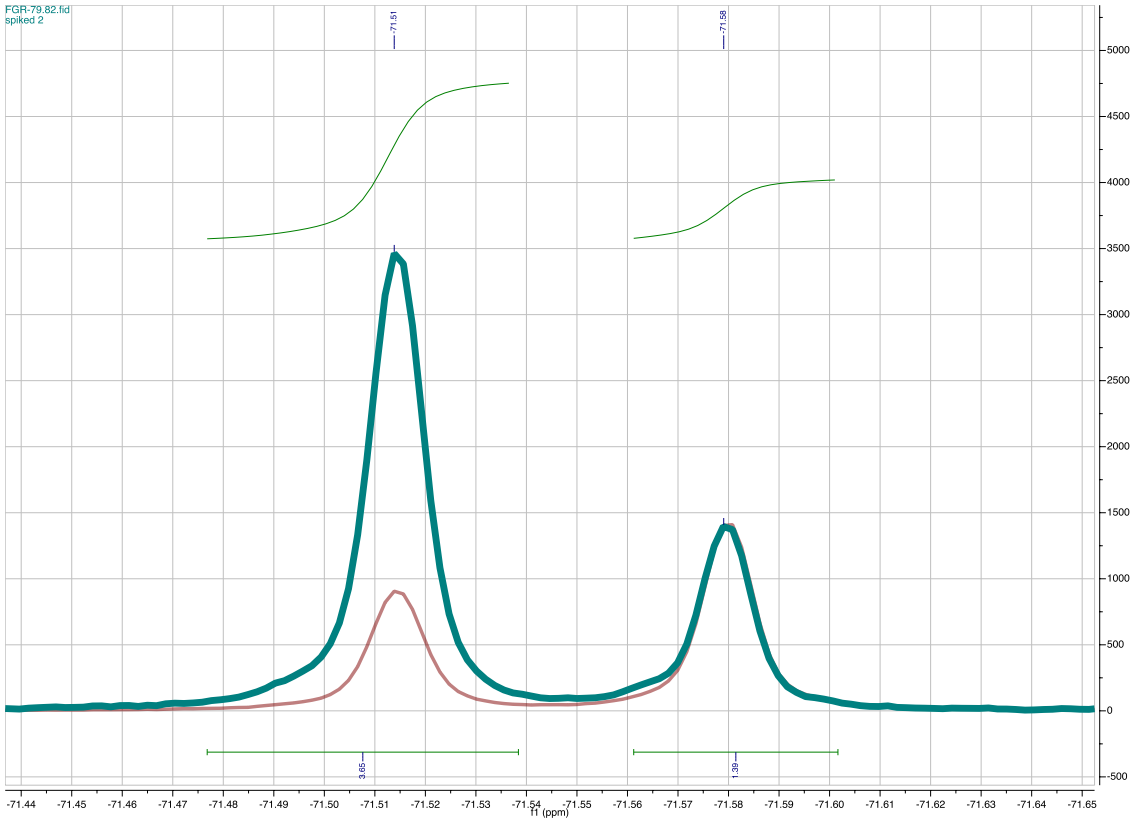
*Spectrum 70: ^{1}H NMR spectrum of the Mosher´s ester **119**.*



*Spectrum 71: Expanded ^{1}H spectrum of the Mosher´s ester **119**. The left quartet corresponds to the* R,R *diastereomer, while the right corresponds to the* R,R *diastereomer. The bottom line is the Mosher´s ester from the racemic mixture, while the top line is the Mosher´s ester from the asymmetric aldol reaction.*



*Spectrum 72: ^{13}C NMR spectrum of the Mosher´s ester **119**.*



*Spectrum 73: Expanded ^{19}F spectrum of the Mosher´s ester **119**. The left peak corresponds to the* R,R *diastereomer, while the right corresponds to the* R,S *diastereomer. The bottom line is the Mosher´s ester from the racemic mixture, while the top line is the Mosher´s ester from the asymmetric aldol reaction.*

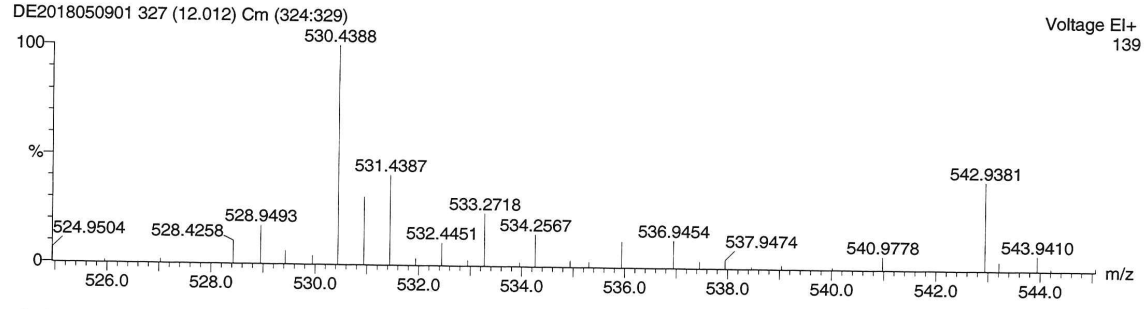
Elemental Composition Report **Page 1**

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

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

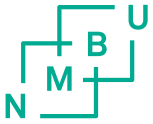
DHA-M
DE2018050901 327 (12.012) Cm (324:329)

Voltage EI+
139



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
534.2567	534.2593	-2.6	-4.9	11.0	1	C30 H37 O5 F3
	534.2534	3.3	6.1	20.0	2	C37 H33 F3

*Spectrum 74: HRMS spectrum of the Mosher´s ester **119**.*



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