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Attempted Stereoselective Syntheses of 3-Hydroxy Fatty Acids

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Master's degree in Chemistry

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Abbreviations

AA – Arachidonic acid

CoA – Coenzyme A

COX – Cyclooxygenase

DCM – Dichloromethane

DHA – Docosahexaenoic acid

DMP – Dess-Martin Periodinane

DMSO – Dimethylsulfoxide

ee – enantiomeric excess

EPA – Eicosapentaenoic acid

Eq – Equivalents

FAS – Fatty acid synthase

HRMS – High Resolution Mass Spectrometry

In situ – in place

In vacuo – Evaporated under reduced pressure

LDA – Lithium Diisopropylamide

LiHMDS – Lithium bis(trimethylsilyl)amide

LOX – Lipoxygenase

M – Molar

MUFA – Mono-unsaturated fatty acid

NADPH – Nicotinamide adenine dinucleotide phosphate

NSAID – Non-steroidal anti-inflammatory drug

PUFA – poly-unsaturated fatty acid

SFA – Saturated fatty acid

TFA – Trifluoroacetic acid

THF – Tetrahydrofuran

TLC – Thin Layer Chromatography

TMSOK – Potassium Trimethylsilanoate

General remarks

All schemes and figures are drawn by me, unless otherwise noted. Chemical structures and schemes are drawn in PerkinElmer's ChemDraw Professional version 21.0.0.28. The text in this document is "Times New Roman", font size 12, line spacing 1.5. References are marked by EndNote 20.5.

Abstract

Syntheses of and with Braun's chiral auxiliary (*R*)-(+)-2-acetoxy-1,2,2-triphenylethanol were explored, for the syntheses long-chain 3(*R*)-hydroxy-oxylipins. The chiral auxiliary was used to stereoselectively synthesize 3-hydroxy-methyl-icosanoate. This was achieved through a novel magnesium-mediated aldol compound of the auxiliary and the aldehyde octadecaenal. This intermediary compound was transesterified to 3-hydroxy-methyl-icosanoate. The stereoselective synthesis of 3-hydroxy-methyl-icosanoate shows acceptable enantiomeric excess by mosher ester analysis. However, absolute conformation was not confirmed. A novel reformatsky reaction protocol for the achiral synthesis of 3-hydroxy-methyl-icosanoate from octadecaenal was also developed. Hydrolysis of 3-hydroxy-methyl-icosanoate to 3-hydroxy-icosanoic acid was not completed due to time constraints.

Both the Swern oxidation and the Dess-Marin oxidation protocols were used for the synthesis of octadecaenal.

The polyunsaturated target compounds (all *Z*)-3(*R*)-hydroxyicosa-5,8,11,14-tetraenoic acid (3-HETE) and (all *Z*)-3(*R*)-hydroxyicosa-5,8,11,14,17-pentaenoic acid (3-HEPE) were not synthesized. (all *Z*)-docosa-4,7,10,13,16,19-hexaenoic acid (DHA) was iodolactionised via the Corey lactonization protocol, then hydrolysed to the γ-diol acid (all *Z*)-4,5-dihydroxydocosa-7,10,13,16,19-pentaenoic acid. Oxidative cleavage to (all *Z*)-octadeca-3,6,9,12,15-pentaenal with sodium periodate or periodic acid did not yield a clean product after purification.

The Braun chiral auxiliary is demonstrated to be a good acetate donor for stereoselective aldol reactions. Alternative synthetic protocols and further studies are required to maximise the potential of this auxiliary.

Sammendrag

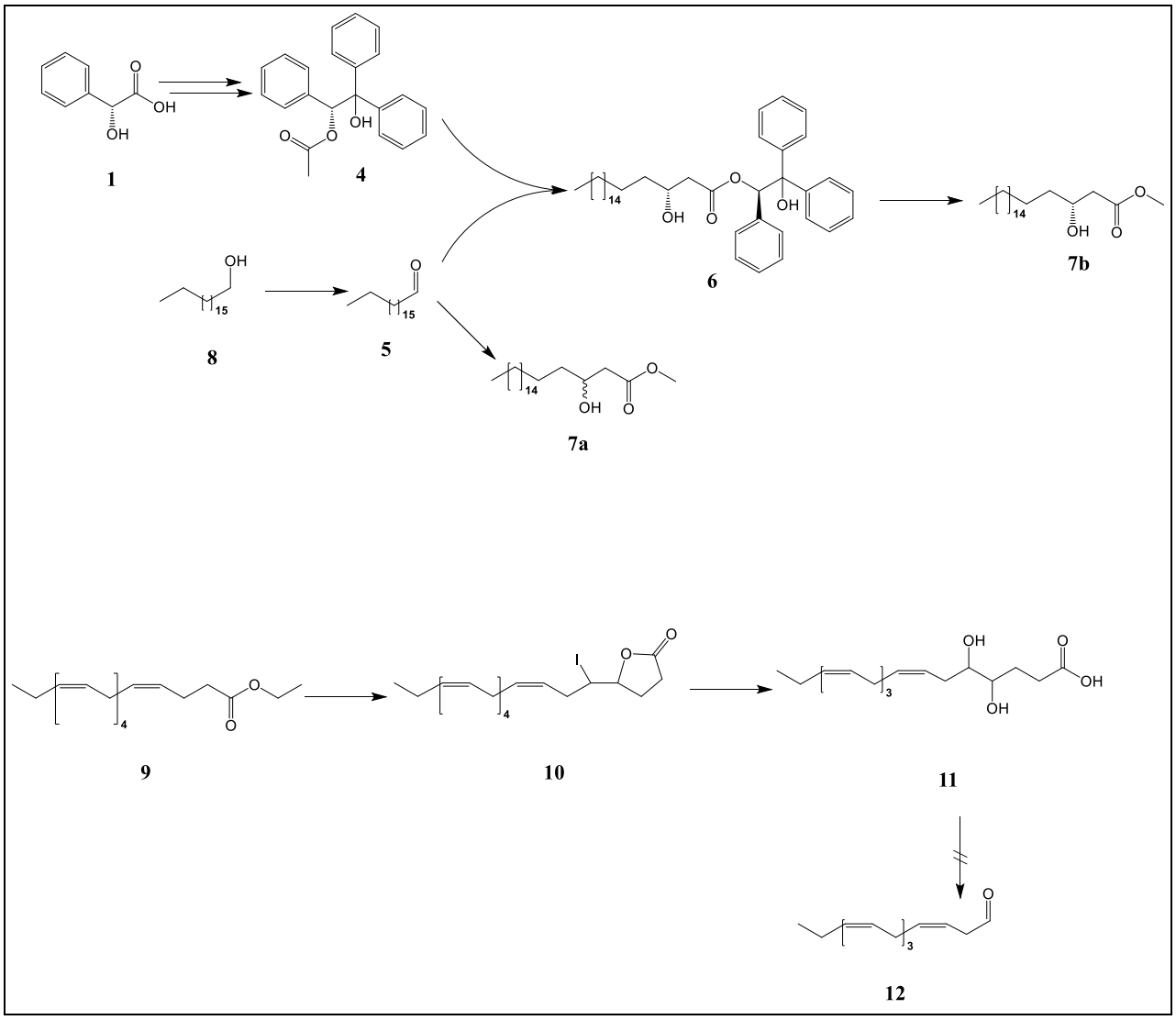
Synteser av og med Brauns kirale hjelpemolekyl (*R*)-(+)-2-acetoksy-1,2,2-trifenyletanol ble utforsket for synteser av langkjedete 3-hydroksy-oksylipiner. Hjelpemolekylet ble brukt for stereoselektiv syntsese av 3-hydroksy-metyl-ikosanoat. Dette ble gjennomført via et nytt magnesium-mediert aldol-produkt av hjelpemolekylet og aldehydet oktadekaenal. Mellomproduktet ble transesterifisert til 3-hydroksy-metyl-ikosanoat. Den stereoselektive syntesen av 3-hydroksy-metyl-ikosanoat viser et akseptabelt enantiomerisk overskudd via mosher ester analyse. Den absolutte konformasjonen ble derimot ikke bekreftet. En ny reformatsky reaksjonsprotokoll ble utviklet for den akirale syntesen av 3-hydroksy-metyl-ikosanoat fra oktadekanal. Hydrolyse av 3-hydroksy-metyl-ikosanoat til 3-hydroksy-ikosansyre ble ikke gjennomført grunnet tidspress.

Både Swern oksidasjonen og Dess-Martin oksidasjonen ble benyttet for syntesene av oktadekaenal.

De flerumettede målmolekylene (alle *Z*)-3(*R*)-hydroksyikosa-5,8,11,14-tetraensyre (3-HETE) og (alle *Z*)-3(*R*)-hydroksyikosa-5,8,11,14,17-pentaensyre (3-HEPE) ble ikke syntetisert. (alle Z)-dokosa-4,7,10,13,16,19-heksaensyre (DHA) ble jodlaktonisert via Coreys jodlaktoniseringsprotokoll, så hydrolysert til γ-diol-syren (alle Z)-4,5-dihydroksydokosa-7,10,13,16,19-pentaensyre. Oksidativ kløyving til (alle Z)-oktadeka-3,6,9,12,15-pentaenal med natrium perjodat og perjodsyre gav ikke et rent produkt etter opprensing.

Brauns hjelpemolekyl er vist som en god acetat-donor for stereoselektive aldol-reaksjoner. Alternative syntesemetoder og videre studier er nødvendig for maksimering av hjelpemolekylets potensiale.

Graphical abstract



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I would like to thank all the people who I have worked alongside in the laboratory and study hall, for making this year a great one.

Lastly, a great thank you to my parents and to my partner, for encouragement, interest, and support.

Ås, August 2023

Olav Peter Arntsen

1 Introduction

1.1 – Aim of thesis

The aim of this thesis is to explore the stereoselective synthesis of long-chain 3-hydroxy fatty acids through the chiral acetate auxiliary (*R*)-*2*-acetoxy-1,1,2-triphenylethanol (**4**). Reaction parameters such as temperature, base and Lewis acid used will be studied. Stereoselective aldol reactions with the chiral auxiliary, presents a convenient synthetic method of creating 3-hydroxy acids and esters.

1.2 – Fatty acids

Fatty acids are organic chain-like compounds largely consisting of carbon, hydrogen, and some oxygen usually in the form of a terminal carboxylic acid. Together with a large variety of compounds, these make up the lipid class of molecules. Fatty acids can be either saturated (SFA), mono-unsaturated (MUFA), or poly-unsaturated (PUFA), referring to the number of double bonds in the carbon chain. Said double bonds can either be the commonly found *Z* or the very unusual *E* isomers. These can also be referred to as *cis* or *trans* double bonds respectively. The patterns of double bonds in unsaturated fatty acids can indicate if it is of plant or animal origin. Most of the common fatty poly-unsaturated fatty acids are not conjugated but rather methylene-interrupted.

These fatty acids can have short chains of 4 carbons, to long chains of 30 carbons. However the most common have a length of 16 or 18 carbons. The more common fatty acids contain an even number of carbons in their chain, owing to the biological fatty acid synthesis, increasing chain lengths in increments of two, with acetyl-Coenzyme A[1]. Although important, free fatty acids usually exist in trace amounts. The most prevalent forms of fat or fatty compounds in biological organisms are triglycerides, a backbone of glycerol with ester linkages to three fatty chains. These triglycerides can be comprised of three identical fatty chains, referred to as simple triglycerides. Usually however, a triglyceride consists of differing fatty acyl chains making it a mixed triglyceride. Vital as a form of energy storage in organisms, triglycerides are incorporated in cell membranes in addition to being structural surface components in skins, leaves and feathers[2]. Fats in the human body also serve the role of transporting the lipophilic vitamins A, D, E and K throughout[3].

1.3 – Poly-unsaturated fatty acids

While saturated fatty acids can make up a large portion of an organism's available fats, the unsaturated fat is often more desirable or reactive. As unsaturated fatty acids are sorted into mono-unsaturated and poly-unsaturated classes, the classification can be further broken down into the position (called omega, as is the Greek letter ω) of the double bond nearest the terminal methyl-group. This system mainly consists of the omega-3 (ω-3), omega-6 (ω-6) and omega-9 (ω-9) types of fatty acyl chains. Shown in Figure 1.3-1 is an overview of the different omega-types of fatty acids, such as (all *Z*)-docosa-4,7,10,13,16,19-hexaenoic acid (**27**) (DHA), (all *Z*)-icosa-5,8,11,14-tetraenoic acid (**28**) (AA) and (9*Z*)-octadec-9-enoic acid (**29**).

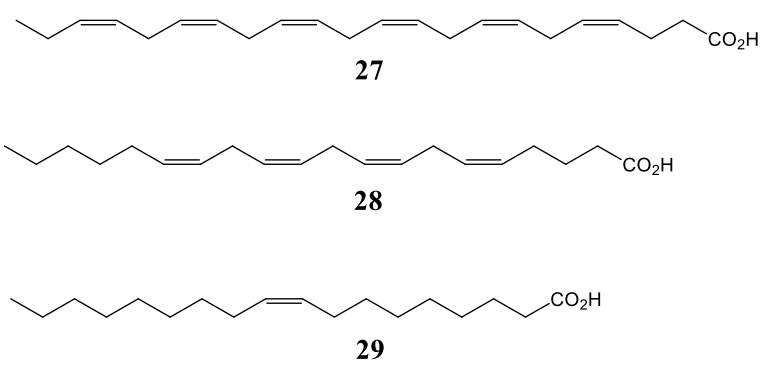


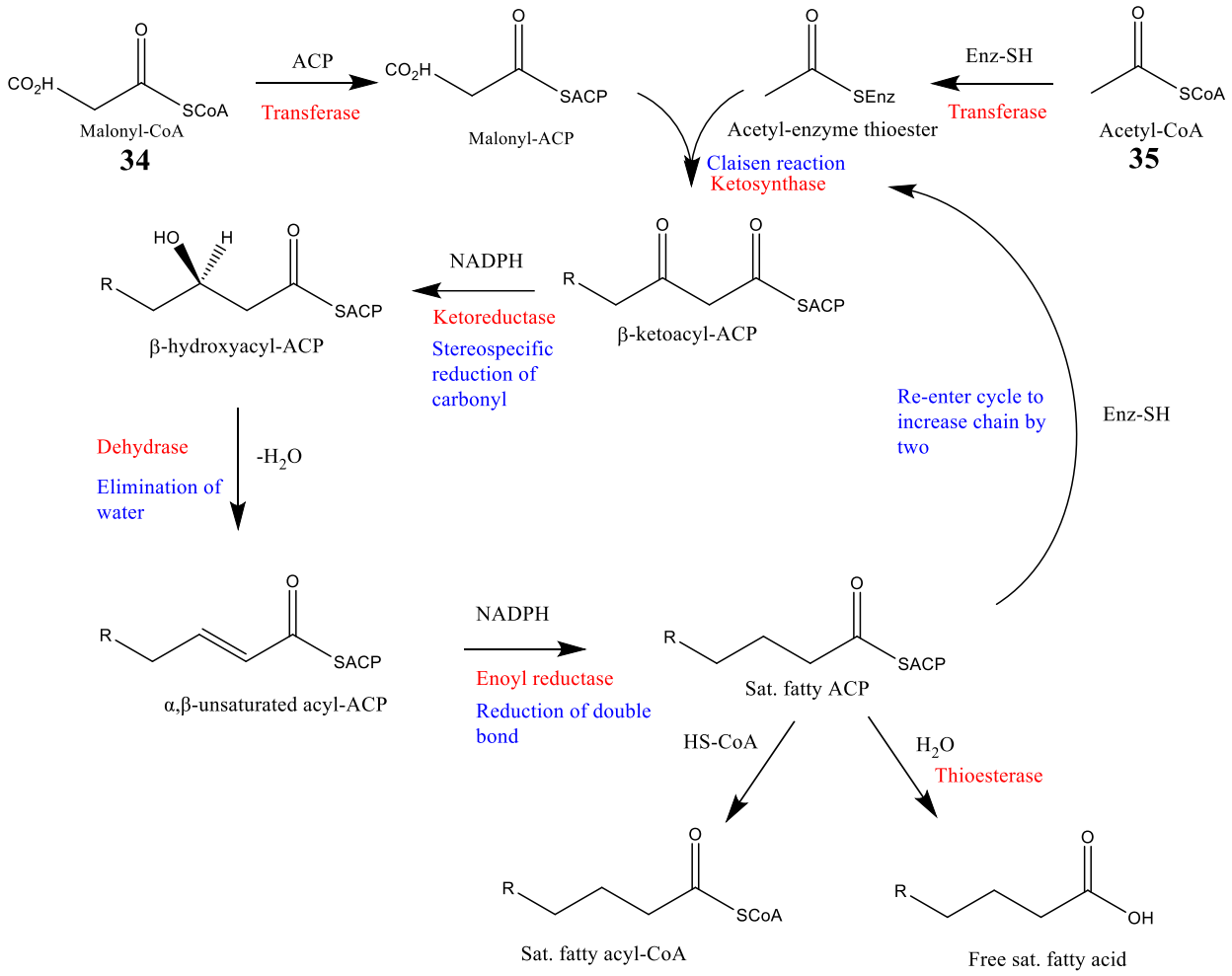
Figure 1.3-1 – Structural overview of DHA **27**, AA **28,** and oleic acid **29**.

The omega-3 class of fats is often widely known to the public as merely "omega-3", recognised for its health effects and consumed as dietary supplements. Important omega-3 fatty acids include DHA **27**, and (all *Z*)-icosa-5,8,11,14,17-pentaenoic acid (EPA) (**32**) due to their wide variety of positive effects such as reducing inflammation and cardiovascular diseases[3]. Another omega-3 fatty acid that is deemed essential is α-linolenic acid (all *Z*)-octadeca-9,12,15-trienoic acid (**33**) (ALA), as the human body is not able to synthesize it enzymatically and thus must come from the diet. In turn, ALA **33** is able to be further synthesized to DHA **27** and EPA **32**[4]. Foodstuffs such as cod liver oil and certain leafy greens are rich in omega-3 fatty acids, making them a ripe resource for obtaining the required omega-3 fats. From a nutritional point, there is an uncertainty in where the potential upper limit of consumption is with regards to omega-3 fatty acids. Despite low levels of omega-3 consumed to have a noticeable positive effect, such as 500mg a day of EPA **32**, increasing the

intake to multiple grams a day seemingly has no downsides other than diminishing returns of higher consumption[3]. Though the effects of PUFAs as the omega-3s come from small dosages, the omega-6 fatty acids are usually consumed in larger dosages. An example is the typical North American diet, which consists of a larger ratio of omega-6 to omega-3 fatty acids, such as 10 or 30:1. Ideally, the ratio consumed would be between equal amounts and 4:1, such as the closer ratio of omega-6 to omega-3 observed in the typical Mediterranean diet. The biological significance of this is the increased prevalence of cardiovascular diseases, and in some cases cancer[3]. This further highlights the importance of the different usual PUFAs and their effect in day-to-day lives of the population.

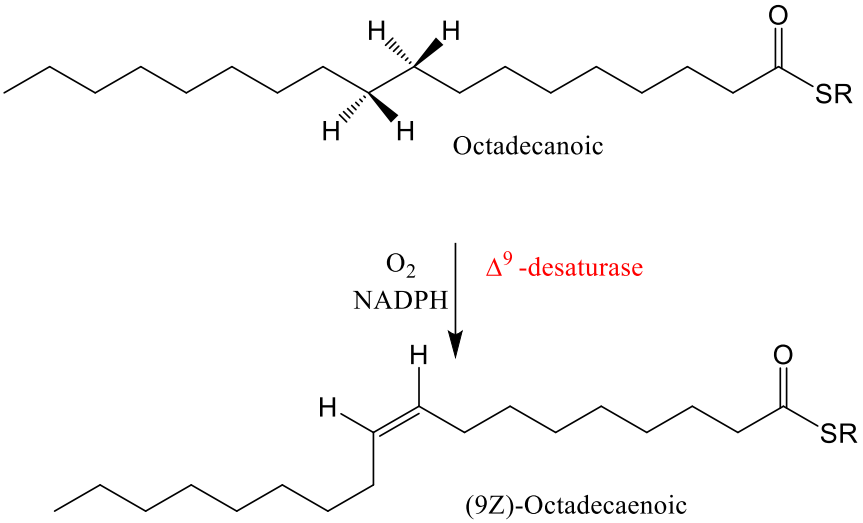
1.4 – Fatty acid biosynthesis

The biosynthesis of fatty acids is done enzymatically in organisms, through a large multifunctional enzyme referred to as fatty acid synthase, FAS. Although there are a few differing factors and characteristics in mammalian FAS and others such as fungal FAS, the end function is largely the same[1]. The biosynthetic process is a repeating one, as the acyl chain of a fatty acid is built in the sequences of two carbons per cycle. Scheme 1.4-1 contains an overview of the FAS-cycle. The starting units Malonyl-CoA (**34**) and Acetyl-CoA (**35**) are converted to thioesters attached to an acyl carrier protein (ACP) or a cysteine residue on the FAS enzyme[4] respectively. Through a Claisen reaction, the two are conjoined into a β-ketoacyl-thioester further joined to the ACP. Stereospecific reduction of the β-carbonyl with NADPH, gives a β-hydroxyacyl-ACP. The hydroxyl-group is eliminated as water giving an α,β-unsaturated acyl-ACP. Further reduction with NADPH removes the formed double bond to give a four-chained saturated fatty acyl-ACP. This can be re-entered to undergo the Claisen reaction with another malonyl-CoA and react through each successive step to increase the fatty chain length by two. When the required chain length is reached, the finished saturated product can be released as a CoA-ester or as a free fatty acid[1, 2].



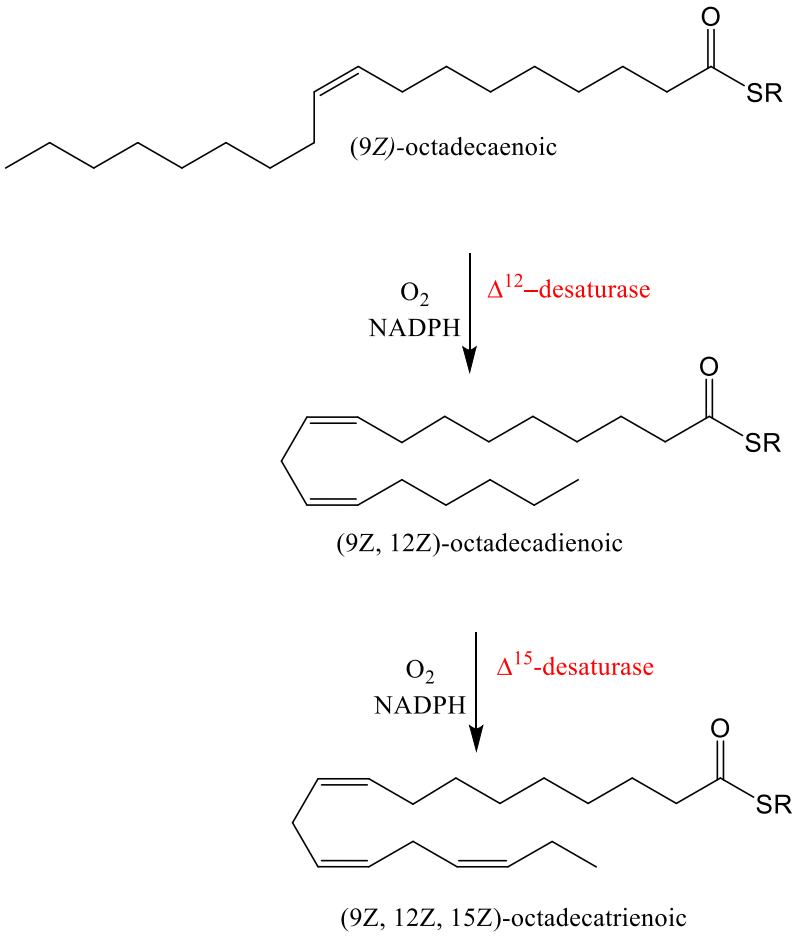
Scheme 1.4-1: Biosynthesis of saturated fatty acids. Enzymes participating in reactions are outlined in red.[1]

As the fatty acid synthase pathway creates mere saturated fatty acids, the biosynthesis of the many unsaturated fatty acids utilizes these products. Although modifications to chain length as brief elongations and β-oxidations, the fatty acids are enzymatically desaturated to produce the required PUFAs. Although the processes of biosynthesis towards PUFAs share many similarities, the domains of plants, fungi and mammals differ. The initial desaturation (Scheme 1.4-2) reactions of a fatty acyl chain are that of a Δ^9 -desaturase, inserting a *Z*-double bond between C9 and C10 while consuming O_2 and NADPH. From this, a non-mammalian organism will insert further double-bonds towards the methyl terminus, synthesizing an omega-6 fatty chain and an omega-3 fatty chain with a third double bond introduced.



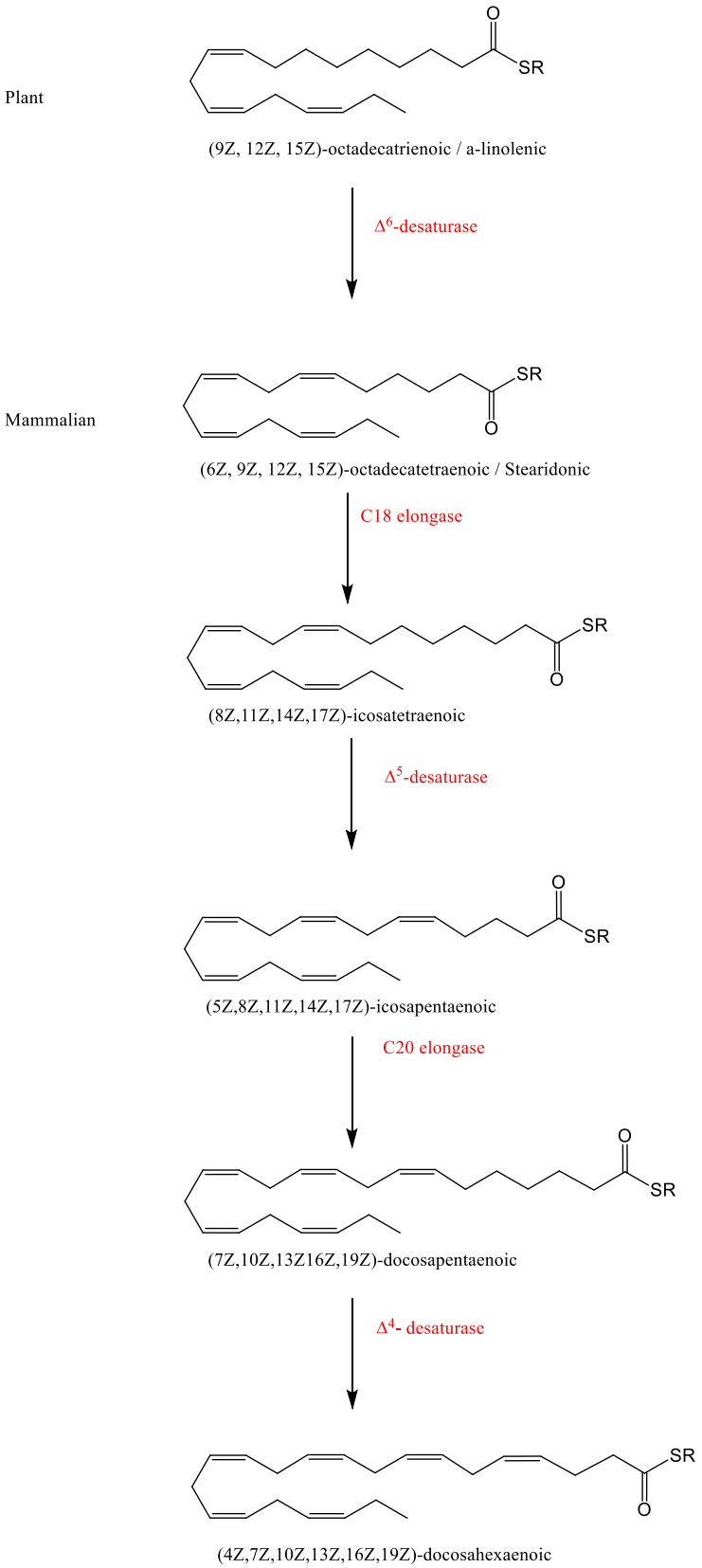
Scheme 1.4-2: The initial desaturation reaction of a fatty acid thioester[1]. From C18:0 to C18:1 ω-9.

In the mammalian domain, new double bonds are inserted though the carboxyl-terminus. The Δ^{12} and Δ^{15} -desaturases responsible for the new double bonds in plant organisms (Scheme 1.4-3), are missing in mammals. Therefore, the essential fatty acids are indeed essential to continued biosynthesis of PUFAs.



Scheme 1.4-3: Desaturation reactions to produce omega-6 and omega-3 fatty acids in plants[1]. The fatty acids are depicted as thioesters coupled to their respective enzyme. Enzymes involved are marked in red.

As seen in Scheme 1.4-3, the biosynthesis to α-linolenic acid (**33**) proceeds though desaturases in plants. When ingested by animals and humans, the omega-3 PUFAs can be further desaturated and elongated to other omega-3 PUFAs as evident in Scheme 1.4-4. Here, the mammalian biosynthesis of DHA (**27**) from **33** is depicted. The same enzymatic machinery is used to synthesize a plethora of other PUFAs, dependent on the initial fatty acid. This is illustrated in Scheme 1.4-4 below.



Scheme 1.4-4: Biosynthesis of DHA (**27**) from α-linolenic acid (**33)**[1]. The fatty acids are depicted as their respective thioesters. Enzymes are marked in red.

Contrary to the important anabolic processes of fatty acid synthesis, the catabolic processes of breaking these molecules down are equally important. As a catabolic process named β-oxidation, it involves the light modification to complete consumption of natural products such as fatty acids. Essentially the reversal of the Claisen and aldol reactions amongst the enzymatic processes of the fatty acid synthesis, a cyclic series of reactions end in the cleavage of a C2 unit as Acetyl-CoA (**35**) [1]. Following the anabolic and catabolic pathways creating PUFAs, further biosyntheses may be performed to create highly specialized PUFAs.

1.5 – Oxylipins

A substantial class of biomolecules are the oxylipins. Despite the name, oxylipins are not determined by the presence of an oxygen in the molecule. Rather, it is the involvement of an oxygenase enzyme such as lipoxygenases or cytochrome P450s in the biosynthesis[5]. Ranging from simpler hydroxy-substituted fatty acids to greatly modified fatty acid derivatives, oxylipins perform important and highly specialized biological roles[1, 2].

Considering the large variety of different oxylipins and their specific targets and roles, an abundance of these originate from the PUFAs DHA (**27**), EPA (**32**) and AA (**28**)[2, 6]. Icosanoids, C20 fatty compounds, are known to be precursors to the pro-inflammatory classes of compounds prostaglandins and leukotrienes[7]. These oxygenated derivatives of AA (**28**) may be multi-hydroxylated and isomerized enzymatically. Such biosyntheses are carried out through lipoxygenases (LOX) and cyclooxygenases (COX). Leukotriene biosynthesis begins though hydroperoxy-icosatetraenoic acids such as 5-hydroperoxy-icosatetraenoic acid (5-HPETE) (**36**). Although the main biosynthetic route is epoxidation to create leukotriene A_4 (**38**), a side reduction of its precursor **36** created the hydroxy acid 5-hydroxyicosatetraenoic acid (5-HETE) (**37**). This is an example of how a versatile precursor such as AA (**28**) may be enzymatically modified to an immense variety of bioactive compounds[2]. This is relevant here because the partial scope of this project is stereoselective synthetic studies of the hydroxy-icosanoid (all *Z*)-3-hydroxy-icosa-5,8,11,14-tetraenoic acid (**14**).

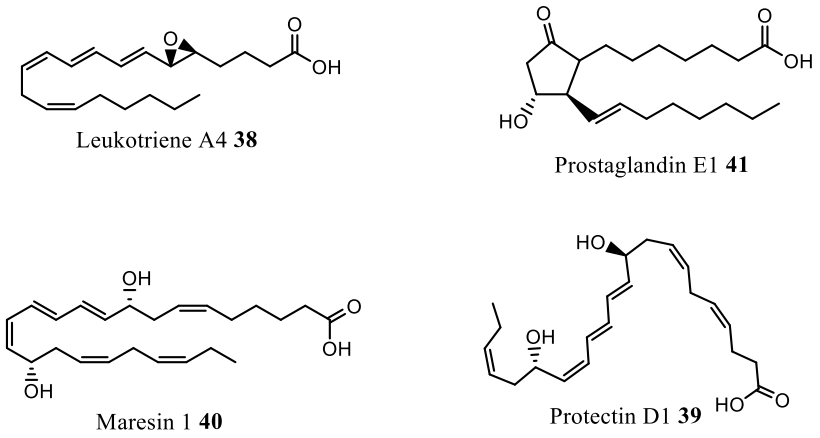


Figure 1.5-1: Molecular structures of pro and anti-inflammatory oxylipins[2].

In addition to the icosanoids outlined above, docosanoids are another large class of oxylipins. Originating from DHA (**27**), the anti-inflammatory lipid mediator classes protectins and maresins can be biosynthesized. DHA (**27**) has in addition been a substrate in stereoselective syntheses discussed in this thesis. The 15-LOX enzyme can stereoselectively hydroxylate DHA (**27**) to protectin D1 **(39**) and maresin 1 (**40**) [2]. This is indicative of the equal importance hydroxy-docosanoids and pro-resolving anti-inflammatory lipid mediators to the pro-inflammatory hydroxy-icosanoids.

Along numerous compounds in the class of oxylipins, the compounds in Figure 1.5-1 have been strongly targeted in total synthetic studies[2]. Following the synthetic availability of a developed total synthesis, the methods may in many cases be improved. Such initiatives for development are fundamental and will be further discussed in the next chapter.

1.6 – 3-hydroxy fatty acids

1.6.1 – Introductory

3-hydroxy fatty acids are lipids with a chiral hydroxy group on the β-carbon from the carboxyl head. They have increased amphiphilic properties[8] and may have saturated, unsaturated, straight or branched acyl chains. As many other hydroxy-oxylipins, this class may be biomarkers as well as building blocks of higher oxylipins and molecular structures[9-11]. On this basis, the 3-hydroxy acids are of biological significance and are the subject of ongoing research[12].

1.6.2 – Biological significance

The biosynthesis of 3-hydroxy fatty acids usually arises from three main pathways. Incomplete fatty acid synthesis or β-oxidation may create these compounds, as the 3-hydroxy moiety is present in these biosyntheses. Lastly, hydroxylation of a fatty acid substrate by a cytochrome P450 enzyme may also yield a 3-hydroxy fatty acid[9]. Although there are many possible 3-hydroxy fatty acids, this thesis will encompass (all *Z*)-3(*R*)-hydroxy-5,8,11,14-icosatetraenoic acid (3*R*-HETE) (**14**) and (all *Z*)-3(*R*)-hydroxy-5,8,11,14,17-icosapentaenoic acid (3*R*-HEPE) (**15**).

In addition to biomolecules such as prostaglandins and leukotrienes, 3-HETE (**14**) originates from the ω-6 C-20:4 polyunsaturated fatty acid arachidonic acid (**28**) [12, 13]. A study conducted by Van Dyk *et al.*[14] where 3-HETE (**14**) was isolated from the yeast *Dipodascopsis uninucleata* by HPLC, as a novel metabolite of exogenously added arachidonic acid (**28**). Further, they discovered the enzymatic production of 3-HETE (**14**) was inhibited by the addition of acetylsalicylic acid, aspirin.

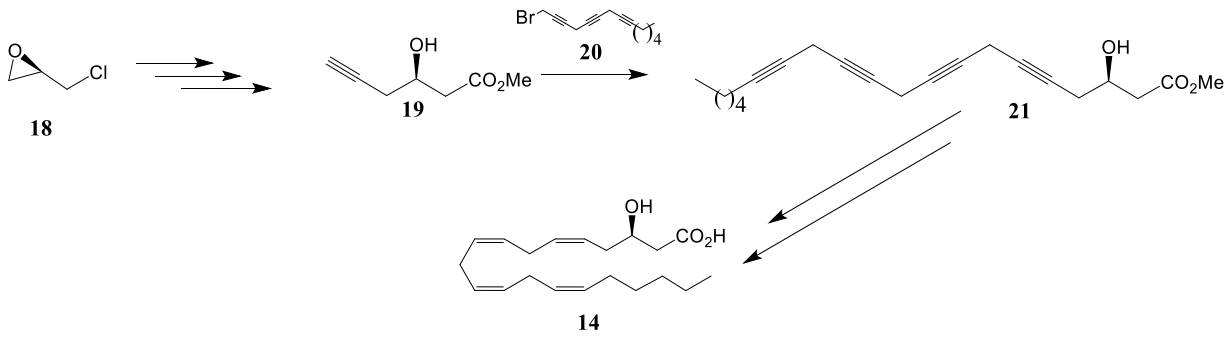
The limited access to the required pure oxylipins in research applications has previously been a detrimental factor to biological studies. However, the fields of chemical synthesis and biochemistry are steadily progressing. Widely available in nature, such oxidised fatty acids as 3-hydroxyoxylipins can be found in plants, bacteria, algae, and animalic and fungal cells[15]. A field which may benefit greatly from additional research on 3-hydroxy oxylipins is in medicine, as there is an occurrence in gram-negative bacteria such as *E. coli*. Specific components of endotoxins in gram-negative bacteria include 3-hydroxy oxylipins, which are responsible for toxic properties of the lipopolysaccharide layer. Thus, these oxylipins can be employed as biomarkers for the estimation of quantities of endotoxins and gram-negative bacteria[9]. The aspirin-mediated inhibition of 3-hydroxy oxylipins has been shown to influence morphogenesis and growth in cultures such as *Candida albicans*, which in turn may be effective in the treatment of vaginal yeast infections[15, 16]. Relations between cellular aggregation and 3-hydroxy oxylipins was highlighted by Kock *et al*[15]. in their study. The increased size of the polar head, from the hydroxyl group in the oxylipin, aids the aggregation of cells on other hydrophilic cell surfaces. This increased ability to form thread- or tube-like micelles enables such cellular aggregation[10, 15]. The developing understanding of the

relationship between bacterial aggregation and 3-hydroxy oxylipins steadily continues, along other fields in which this class of oxylipins are relevant[8].

1.6.3 – Chemical synthesis

There have been stereoselective syntheses developed for several different compounds in the class of 3-hydroxy polyunsaturated fatty acids[17, 18]. However, many methods are cumbersome and expensive. Synthetic pathways to this class of oxylipins have been explored by Gjessing *et al.*[12], Groza *et al.*[16] (Scheme 1.6.3-1) and Bhatt *et al.*[19] (Scheme 1.6.3-2), and to name some.

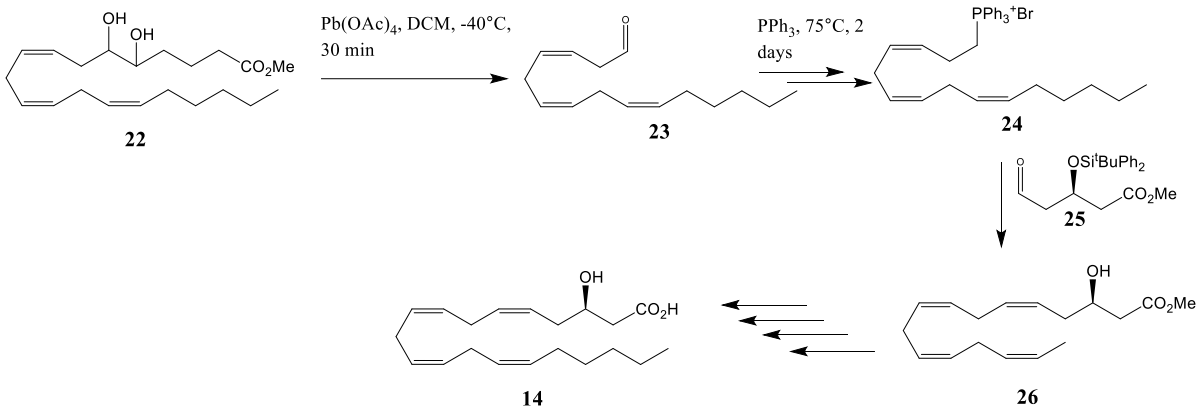
Observing the Schemes 1.6.3-1 and -2, the two synthetic pathways described by Groza *et al.*[16] and Bhatt *et al.*[19] respectively, show two different synthetic doctrines.



Scheme 1.6.3-1: Synthetic alkyne pathway to 3(*R*)-HETE (**14**) by Groza *et al*[16].

The method developed by Groza *et al.*[16], a six-step synthesis from the building blocks (*R*)-(-)-epichlorohydrin (**18**) and a brominated alkyne (**20**). Their protocol involves building the finished product 3(*R*)-HETE (**14**) through cross-coupling reactions and hydrogenation. This is a very gradual synthesis, originating from a small, easily tweakable starting material with little resemblance to the final product. In turn, this allows for more flexibility with chain length, degrees of unsaturation and optional radiolabelling[16]. This protocol has reported good reaction yields. The stereochemical yield is not reported for **14** in this article, but through an enzymatic hydroxylation of carbon 15. This enzyme, 15-LOX, add an exclusively *S*-hydroxyl. Purification through chiral phase HPLC reports the stereogenic purity to be more than 96% for the di-hydroxylated compound[16]. Therefore, the optical purity can be estimated to be approximately as high for their product **14**. Groza *et al.*[16] have presented an

evidently good synthetic protocol to 3(*R*)-HETE (**14**), although the partial hydrogenation steps may not be suitable for all synthetic applications.



Scheme 1.6.3-2: Synthetic pathway to 3(*R*)-HETE (**14**) described by Bhatt *et al*[19].

A more convenient synthetic pathway is choosing a starting material with an appropriate fatty chain backbone, such as the diol methyl ester **22** in Scheme 1.6.3-2. Where possible, this enables easier control of the reaction products at the cost of flexibility. The first diol-cleavage reaction in Scheme 1.6.3-2 above, will be relevant for reported synthetic results later in this thesis (see Section 2.9). The reaction through a Wittig salt is a convenient method of C-C bond creation. However some of the steps in this synthesis require extensive reaction times[19]. Here, Bhatt *et al.*[19] also do not mention enantiomeric excess or stereochemical purity, merely that the synthesis of the respective *R* and *S* enantiomers were satisfactory. As their chiral moiety **25** originates from pure 2-deoxy-D-ribose[19], the configuration is expected to remain intact through to the product **14**. Combining elegant reactions, existing molecular backbones and stereoselectivity, the field of chiral auxiliaries is suitable. This will be discussed below.

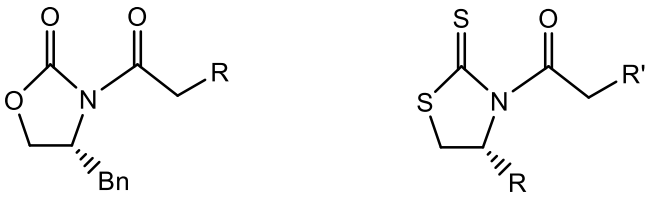
1.7 – Chiral acetate auxiliaries

The synthesis of natural and biologically active compounds often involve chirality and stereogenic centres[20]. Necessitating the ever-lasting need for synthetic methods with good stereoselectivity and ease-of-use, the class of chiral auxiliaries have been developed. A chiral auxiliary is a compound easily covalently bonded to a target molecule, to induce an

enantiomeric excess in or through a stereocenter and subsequently be cleaved off. There are numerous chiral auxiliaries described in the literature, usually fit for a specific field of use[20].

1.7.1 – Evans & Nagao-Fujita

With variations in functional groups, some widely employed auxiliaries are the Evans and Nagao-Fujita n-oxazolidinones[20]. Largely similar, the two auxiliaries **42** and **43** in Figure 1.7.1-1, share the same main molecular skeleton. The differing factor is that Nagao-Fujita replaced the ring-oxygens with sulfur[21]. Functioning as an acetate donor, a secondary alkyl group (R and R' in Figure 1.7.1-1) may also be added into the final product molecule. The auxiliaries are reacted with an aldehyde in the presence of a base and a Lewis acid such as $TiCl_4$ or $MgCl_2$[12, 21-23], the latter of which is present in reactions performed in this thesis. These mildly differing auxiliaries have delivered promising results in the synthesis of 3-hydroxy moieties and fatty acids as described by Gjessing *et al.*[12]. Further research is being done on this. However, another promising natural product-derived auxiliary is the Braun chiral acetate auxiliary **4**.



Evans **42** Nagao-Fujita **43**

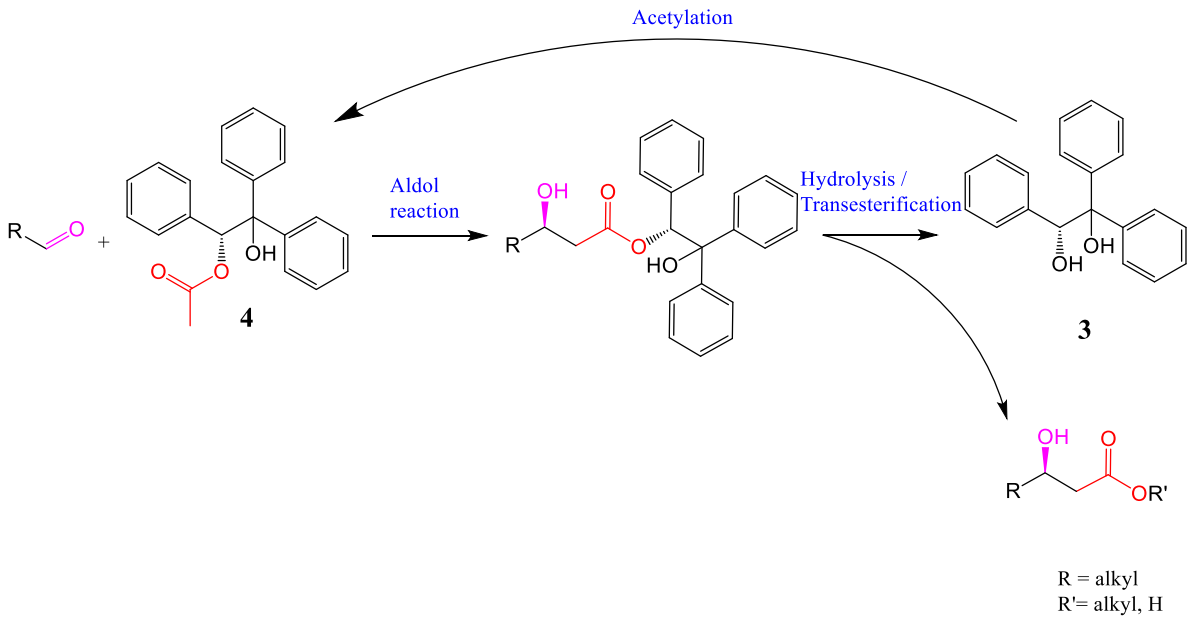
R = alkyl
R' = alkyl, H

Figure 1.7.1-1: The chiral auxiliaries **42** and **43**[21]

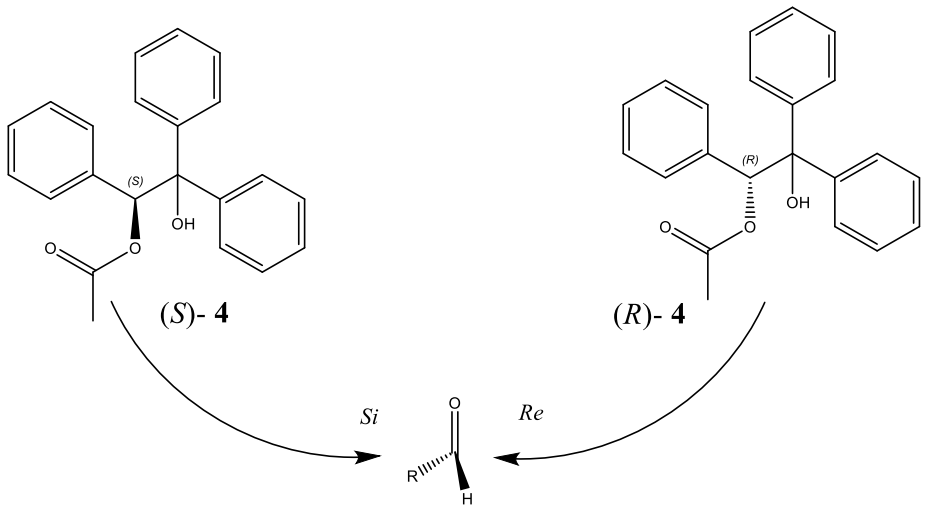
1.7.2 – Braun

Developed by Manfred Braun and Ralf Devant in the 1980s, the chiral acetate auxiliary (R/S)-2-acetoxy-1,1,2-triphenylethanol (**4**) (also referred to as HYTRA[24-27] and the Braun reaction), is a very cheap and easily recoupable acetate donor[28]. Originating from the widely commercially available mandelic acid (**1**), larger-scale synthesis is viable, a synthesis performed in this thesis.

Scheme 1.7.2-1 shows the mode of action of the auxiliary **4**, forming a C-C bond through an aldol reaction, selectively inducing a stereocenter in the adduct compound. The auxiliary **4** will attack the aldehyde from differing sides depending on its configuration, visualized in Scheme 1.7.2-2. It has been shown that (*R*)- **4** will attack mainly from the *Re* side, and (*S*)- **4** mainly attacks from the *Si* side[24]. Basic hydrolysis or transesterification can then be performed to cleave the auxiliary off, leaving the diol **3** and the 3-hydroxy acid or ester. Cleavage via basic hydrolysis shows no chance in stereochemistry in the auxiliary **3**[28]. Coloured in red in Scheme 1.7.2-1 is the acetate group donated by the auxiliary, and the aldehyde in pink. Although less attention received compared to other auxiliaries, the Braun auxiliary has been utilized in the synthesis of many new bioactive compounds[25, 26] and cancer-medicines[29].



Scheme 1.7.2-1: General mode of action of stereoselective 3-hydroxy synthesis though chiral auxiliary **4**[28].



Scheme 1.7.2-2: Auxiliary (*R/S*)- **4** attack from *Si* and *Re* sides of aldehyde[24].

Addition to an aldehyde in the aldol reaction is often performed under cooling, such as -78°C[12] or -115°C[28], through strong bases such as LDA or LiHMDS in a dry, ethereal solvent. Reacting the auxiliary **4** lithium enolate in the aldol reaction has proven to be a viable pathway with acceptable yields. Transmetallation of the lithium enolate with a magnesium halide prior to aldol addition has shown to enhance the stereoselectivity in the reaction[27]. Base selection in the aldol reaction and transmetallation of the resulting enolate will be studied in this thesis.

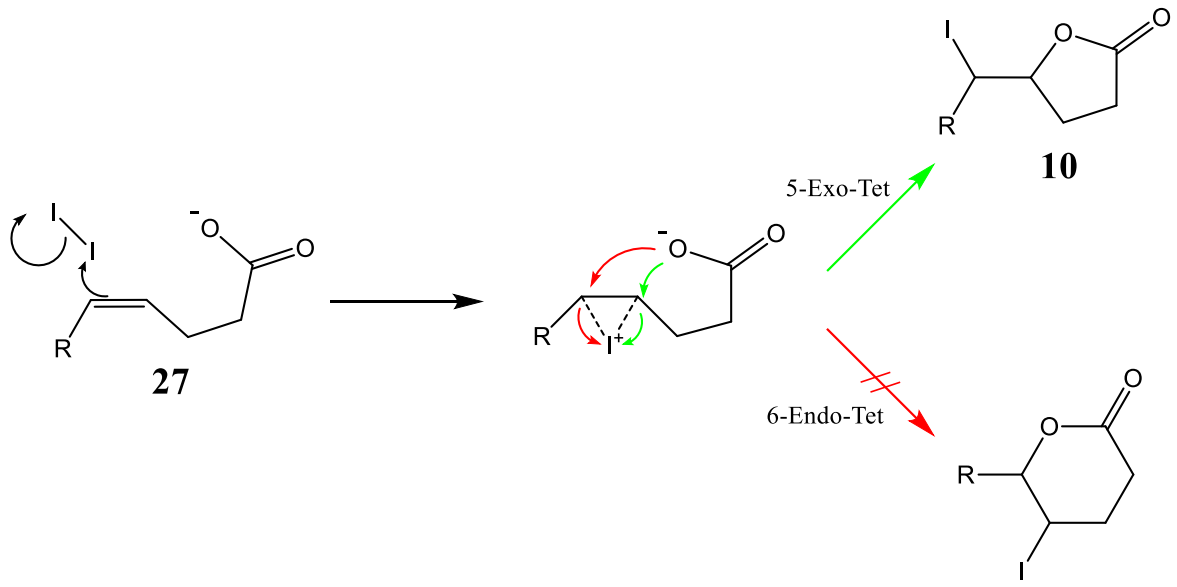
1.8 – Importance of chirality

Accounting for chirality in a compound is an important feature of natural product and drug synthesis. From a synthetic perspective, designing a chiral pathway for the desired chiral product should always be the strife of a chemist. Separating two enantiomers from a racemic mixture is not only difficult, but it may also be inefficient as large amounts of product is forfeit. Even more so if the desired product is only one of the synthesized enantiomers. Therefore, a chiral synthesis is usually the preferred option. Bioactive compounds may also have effect in only a certain stereoisomer[30]. An example of this is the non-steroidal, anti-inflammatory drugs (NSAIDs) such as the common ibuprofen. These NSAIDs may be chiral, as in the active *S*-(+)-enantiomer with the inactive *R*-(-)-enantiomers[30]. Though ibuprofen is served as a racemate, it goes to show some of the real-world application of chirality in molecules.

1.9 – Significant chemical reactions

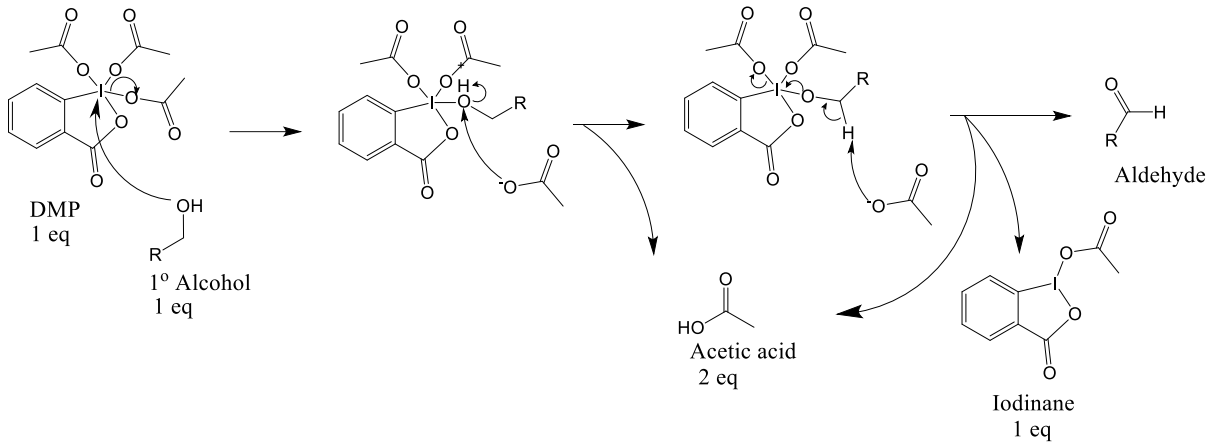
Many of the reactions used during this project are well known in academic chemical literature. In addition to the previously discussed Braun aldol reaction involving the chiral auxiliary **4** (see Section 1.7.2), important reactions preformed in this project were iodolactonisation (see Section 4.10) and DMP-oxidation (see Section 4.4).

A useful reaction for the introduction of functional groups on carbon number 4[31] of DHA (**27**), iodolactonisation is a reaction which adds a halogen and cyclizes a γ-unsaturated acid to a lactone. Although this halide-addition may be performed with other halides such as bromine, iodine is the commonly more versatile halogen to use[32]. Scheme 1.9-1 shows the mechanism behind this reaction as well as Baldwin's[33] rules for ring closure. The reaction is initiated by an attack of the alkene by the I_2, generating the intermediate iodonium ion and HI from the residual iodine and carboxyl proton. The carboxyl anion can then attack the iodonium ion to cyclize the lactone, as indicated by the green arrows in Scheme 1.9-1. This lactonization proceeds in accordance with the mentioned rules for ring close, specifically rule 1 stating "3 to 7-Exo-Tet are all favoured, 5 to 6- Endo-Tet are disfavoured"[33]. As the iodine is substituted to a carbon outside the lactone, it is prefixed Exo, and Tet for tetrahedral systems. The unfavoured 6-Endo-Tet is shown in red arrows in Scheme 1.9-1 and will not proceed.



Scheme 1.9-1: Mechanism of iodolactonisation[32] and Baldwin's[33] rules of ring closure.

Oxidation of alcohols to aldehydes or ketones by the Dess-Martin oxidation protocol offers gentle reaction conditions and simple procedures[34]. The essential reagent, Dess-Martin periodinane (DMP) is a hypervalent iodine compound, which works at room temperature in DCM or chloroform. The reaction mechanism is visualized in Scheme 1.9-2. The alcohol to be oxidised attacks the iodine, displacing one acetyl-group. This now acetate-molecule attacks twice to pick off two protons and displacing another acetyl group. Releasing the alcohol-substrate as an aldehyde, biproducts of this reaction are two equivalents of acetic acid and one equivalent of iodinane.



Scheme 1.9-2: Dess-Martin oxidation.

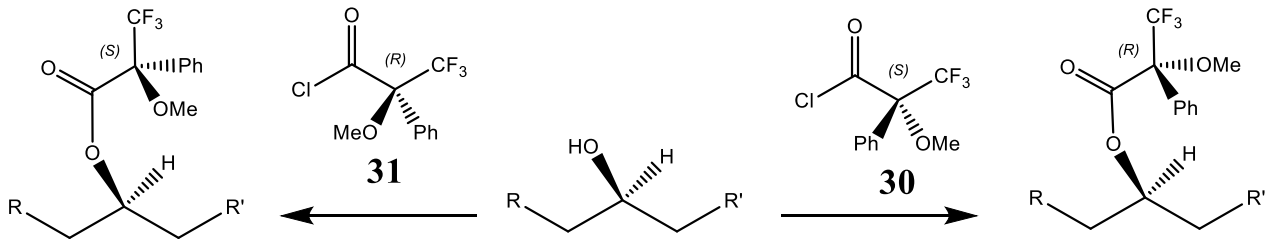
1.10 – Mosher ester theory

The process known as Mosher ester synthesis or Mosher ester analysis, is a useful procedure for the determination of stereochemistry in a secondary alcohol or amine[35]. The diastereomeric compound α-methoxy-α-trifluoromethylphenylacetyl chloride (MTPA-Cl) is reacted in one of its pure enantiomeric forms with the secondary alcohol to be analysed. Developed and reported by Dale and Mosher in 1973[36], with additional modifications along the years, the method relies on NMR analysis of the difference in chemical shifts. The large phenyl-group in the product Mosher ester will proportionally shield or de-shield the protons adjacent to the carbinol. This happens because of the aromatic pi-bond "ring" system in the phenyl depending on the configuration of the ester.

Due to the rules of determining stereocenters, an *R*-MTPA-Cl will become an *S*-Mosher ester once reacted, as the trifluoride group has a lower priority than the COCl. It is imperative for

this to be considered when implementing this method or else the final configuration analysis will be accurate but wrong[35].

In contrast to the binary determination of R or S in the carbinol, Mosher ester synthesis may also be utilized in the partial determination of enantiomeric excess. Although ^{1}H-NMR is often used for this, ^{19}F-NMR can also be recorded to aid this purpose. By comparing the relative intensities of signals in a solution of mixed stereocenters, an estimate of enantiomeric excess can be given.



Scheme 1.10-1: General overview of a Mosher ester synthesis[35].

Scheme 1.10-1 displays the synthesis of a Mosher ester for chiral analysis of an arbitrary aliphatic secondary alcohol. Note that the displayed stereochemistry of the *R* (**31**) and *S* (**30**) MTPA-chlorides is reversed when reacted with the alcohol. For interpretation through proton NMR, the position of the aromatic ring in the Mosher ester moiety induces an upshift or downshift of proton signals. The effect, known as anisotropic magnetic shielding, is a property of the ring's aromaticity, with its pi-bond system above the ring inducing the upshift of protons in its plane. Downshifting of the signal is induced when the affected proton is perpendicular to the ring, as the magnetic field increases the shielding.

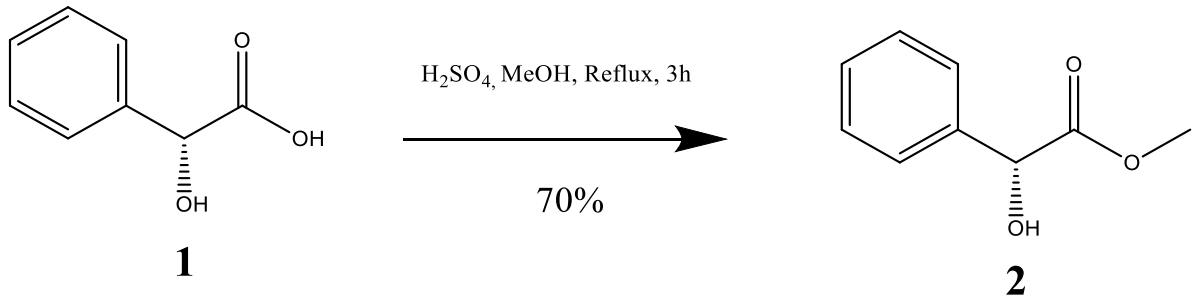
1.11 – Introduction summary

In the above introductory chapter, the biological applications and and synthetic chemistry of fatty acids has been outlined. In the next chapter, specific synthetic experiments to 3-hydroxy fatty acids and supporting compounds will be described and discussed.

2 Results and Discussion

2.1 - Synthesis of (R)-(+)-2-Acetoxy-1,1,2-triphenylethanol (4)

The Braun chiral auxiliary (**4**) is usually a commercially available reagent. However, in the beginning of this project in the fall of 2022, the *R*-enantiomer of **4** was unavailable for purchase. To rectify the situation, it had to be synthesised. *R*-mandelic acid (**1**) and *R*-methyl mandelate (**2**), from Merck and Apollo Scientific respectively, were chosen as starting materials to ensure a simple synthesis.

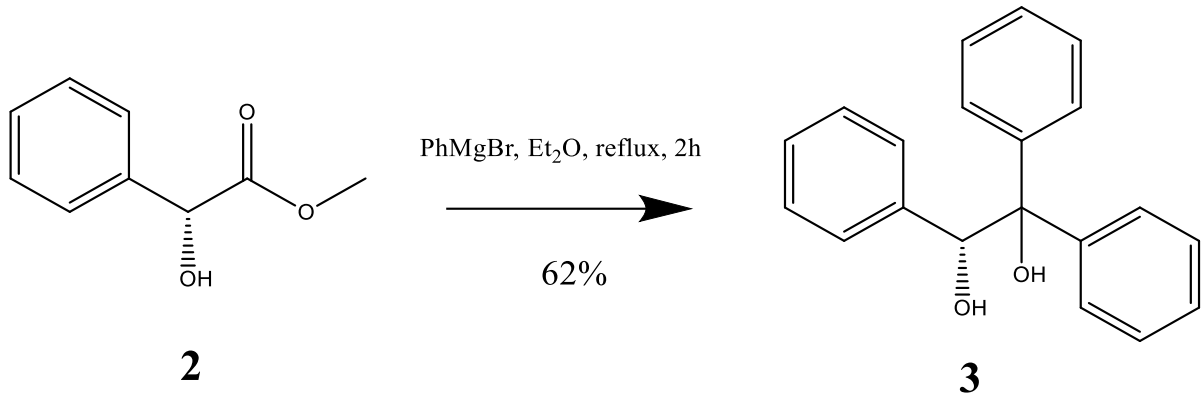


Scheme 2.1-1: Fisher esterification of *R*-mandelic acid (**1**).

The acid **1** was methylated through a Fisher esterification in methanol (Scheme 2.1-1) with a 70% yield, this was slightly lower than a yield of 89% reported in the literature[37].

The spectroscopic data match that of methyl mandelate (**2**). ^{1}H-NMR shows the -O*H*-group as a broad singlet at 3.56ppm, the -O-CH_3 at 3.75ppm, and the -C*H*OH at 5.18ppm. The phenyl-group is seen at 7.42-7.32ppm. ^{13}C-NMR shows the ester group at 174.22ppm, the phenyl group peaks at 138.33-126.70ppm, the carbinol carbon at 73.00ppm and the methyl carbon at 53.11ppm.

The ester **2** was reacted with phenylmagnesium bromide through a Grignard reaction (Scheme 2.1-2) to produce the diol **3**. The synthesis of the chiral auxiliary **4**, was adapted from the procedure described by Ewing *et al*.[38].



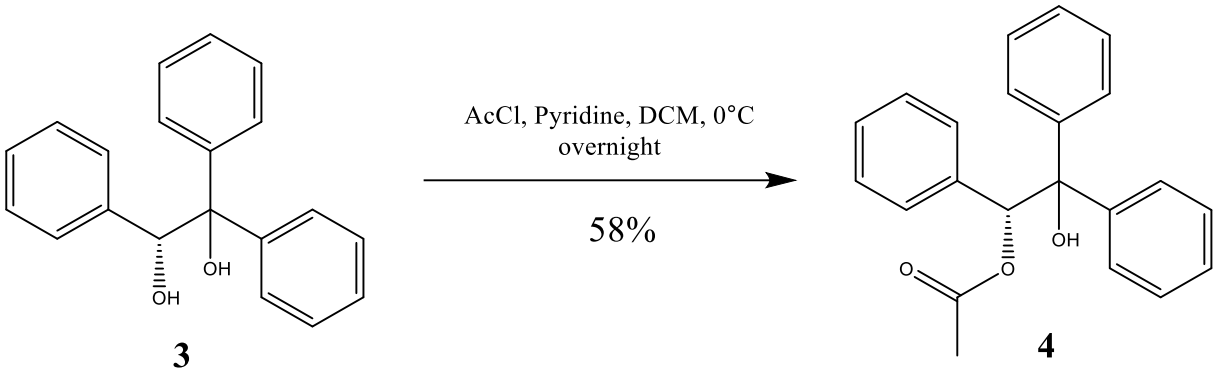
2 **3**

Scheme 2.1-2: Grignard reaction to diol **3**.

This reaction was performed multiple times at an increasing scale. First on a milligram-scale scale with the Grignard reagent phenyl magnesium bromide made *in situ* using bromobenzene, magnesium shavings and methyl mandelate (**2**). Purification by column chromatography with silica gel showed a decomposition of the diol **3** by presumed elimination of the hydroxyl groups. In further purification columns involving the diol **3** or the chiral auxiliary **4**, the silica was deactivated by flushing with 2 to 3% triethylamine in the selected eluent prior to loading the sample on the gel. Following this, the Grignard reaction to the diol **3** was smoothly upscaled 16 times to approximately 4 grams, using commercial methyl mandelate (**2**) and phenylmagnesium bromide-solution. The yield was 62% , compared to the reported yield of 80%, although performed at a smaller scale in the literature[38]. The product **3** was isolated as a white powder.

NMR-spectroscopic data are conclusive that the diol was formed. ^{1}H-NMR show signals for the 15 phenyl-protons in the aromatic area at 7.99-7.05ppm, the carbinol proton as a singlet at 5.64ppm. The two hydroxyl-groups can be seen at 3.14ppm and 2.40ppm, both as singlets. ^{13}C-NMR show the phenyl-carbons at 145.08-126.18ppm. The two carbinols appear as two signals at 80.77ppm and 78.00ppm respectively.

Selective acetylation of the diol **3** (Scheme 2.1-3) was carried out using acetyl chloride, in accordance with the procedure[38]. Purification by recrystallising the crude mixture twice from hot acetone, gave the product **4** as a clean white powder.



Scheme 2.1-3: Acetylation to chiral auxiliary **4**.

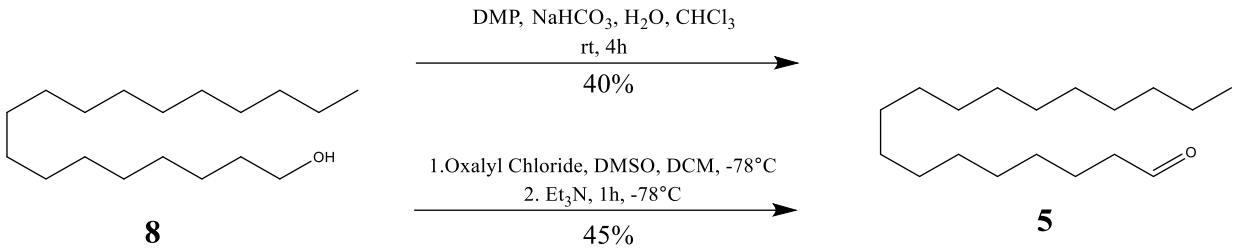
The yield of 58% of **4** is in accordance with the reported yield of 62%[38].

^{1}H-NMR confirms the acetylation to **4**, with the acetyl-protons showing as a singlet at 1.98ppm and integrating to three. The phenyl-protons appear in the region 7.56-7.04ppm, integrating to 15. The carbinol proton shows as a singlet at 6.67ppm, and the tertiary hydroxyl-group shows as a singlet at 2.81ppm.

As the product was washed with water during vacuum filtration, a peak from water-contamination can be observed. ^{13}C-NMR was set to be recorded. However, due to an instrument error, this spectrum was lost. The ^{1}H-NMR spectrum (Figure 5.3-1) is adequate for structural elucidation and confirmation for this compound.

2.2 – Multiple syntheses of octadecanal (5).

The scope of this project was to study the reaction of β-hydroxy-polyunsaturated fatty acids, specifically C18-chains. However, the saturated octadecanal (**5**) was chosen as a model compound to optimise the reaction sequence prior to the introduction of double bonds. For this, 1-octadecanol (**8**) was chosen as a starting material since it is a low-cost compound, and commercially available in larger quantities. It can easily be oxidised to the aldehyde by standard methods. The performed oxidations to **5** were the Swern oxidation[39] and later the Dess-Martin oxidation[34, 40], both of which selectively oxidise the primary alcohol to an aldehyde in moderate and equal yields (Scheme 2.2-1).



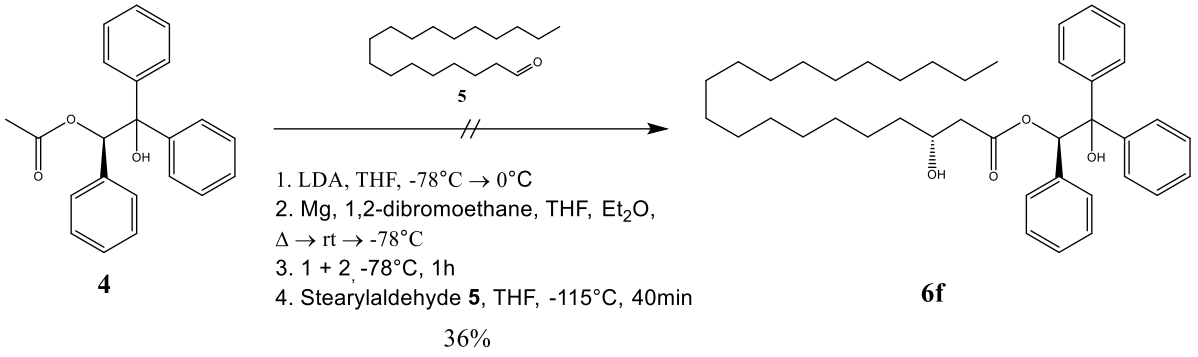
Scheme 2.2-1: Dess-Martin (top) and Swern (bottom) oxidations to octadecanal **5.**

The first synthesis of octadecanal (**5**) was the Swern oxidation. Due to the moderate yield, the Dess-Martin oxidation was tried. The advantages of the Dess-Martin oxidation are a less involved synthesis and no generation of dimethylsulfide as a byproduct. Both reactions were performed multiple times. However, difficulties in the purification step, and potential bad reagents (such as old DMP), may have caused the moderate yields.

NMR-spectroscopic data were identical throughout the two oxidation protocols to **5**. Therefore, only the spectra from the Swern oxidation are reported here to avoid duplicates. ^{1}H-NMR shows the distinct aldehyde-triplet at 9.75ppm. The protons α to the aldehyde shows as a double doublet at 2.40ppm, the β-protons shows as a multiplet at 1.61ppm. The remaining methylene-groups in the chain combine to a large multiplet at 1.24ppm, with the terminal methyl-group as a triplet at 0.87ppm. ^{13}C-NMR shows the distinct aldehyde-peak at 202.85ppm, while the residual carbon resonances are in the aliphatic area from 43.91ppm to 14.10ppm.

2.3 – Attempted syntheses of (*R*)-2-hydroxy-1,2,2-triphenylethyl-(*R*)-3-hydroxyicosanoate (6)

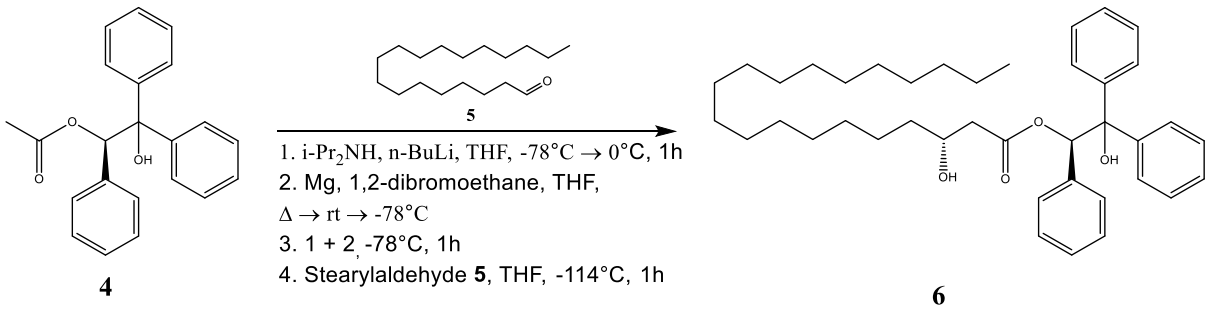
The procedure for this synthesis was adapted from the original article by Braun and Devant[28]. In their article, they introduce (*S*)- and (*R*)-2-acetoxy-1,1,2-triphenylethanol (**4**) as an effective and viable chiral auxiliary for the stereoselective synthesis of 3-hydroxycarboxylic acids. Scheme 2.3-1 shows the initial reaction involving the chiral auxiliary **4** with the saturated fatty aldehyde **5**.



Scheme 2.3-1: Presumed stereoselective aldol reaction to novel compound **6.**

HRMS-analysis (Figures 5.1-4 and 5.1-5 in Section 5.1) show signals for m/z 623.4072, designated as the sodium-adduct of the molecular ion (calculated exact mass: m/z 600.4179). Despite this, it is apparent the molecule/product created and isolated is not the expected compound **6**. The complete MS-spectrum (Figure 5.1-5) show the base peak at m/z 355.1304 and a peak at m/z 313.1199. This does not directly match fragmentation patterns of the proposed structure of **6**.

The NMR-spectra (Figures 5.1-1 through 5.1-3) also show signals which roughly correspond to the proposed molecule **6** but ultimately does not match. The repeated and failed attempts at the transesterification or hydrolysis of **6** (this will be explained in Section 2.4) is partially explained by the compound being something else than anticipated. Structural elucidation by NMR and MS-data is inconclusive, and the unidentified compound is further referred to as **6f**.



Scheme 2.3-2: Successful synthesis of **6.**

Since the initial synthesis resulting in compound **6f** was inconclusive, the synthesis was repeated. This resulted in a different product being synthesized. Scheme 2.3-2 shows the successful synthesis of compound **6**, with some key differences in the reaction conditions.

Instead of utilizing a commercial LDA-solution, it was created fresh the usual way[41] from diisopropylamine and n-butyllithium. In the *in situ* creation of magnesium bromide[42] from dibromoethane and magnesium shavings, only THF was added instead of THF and Et_2O. Crucially, the solution of stearylaldehyde **5** in THF was added exceedingly slowly dropwise. For the synthesis of compound **6f**, the solution with aldehyde **5** was added all at once by canulation using nitrogen gas pressure, a potential route of failure. In the last step of the failed synthesis, cooling to approximately -115°C was achieved by mixing Et_2O with liquid nitrogen. This was changed from Et_2O to EtOH in the successful synthesis of **6**. To avoid enriching the enantiomeric excess by separation of the enantiomers with flash chromatography, or to risk decomposition on the silica, the crude product was not purified. Not knowing the root of why the first aldol reaction created compound **6f**, the crude product with **6** was analysed by NMR immediately following workup of the reaction. A D_2O wash was also performed (see Figure 5.5-2), to verify the peaks of the labile hydroxyl-groups present in the molecule.

^{1}H-NMR analysis and subsequent D_2O-wash show three labile signals, one sharp singlet at 3.34ppm and two broad singlets at 3.22ppm and 2.89ppm respectively. HSQC-NMR show the sharp singlet at 3.34 to be coupled with a carbon resonance, and the two latter singlets as uncoupled. These are designated as the two hydroxyl-groups in the molecule **6**. The three phenyl-groups in the chiral auxiliary are distinctly visible in the range 7.7ppm-7.05ppm. The single proton, which is α to the ester group, in the auxiliary shows as a singlet at 6.73ppm. The C*H*$_2$-group in the γ-position to the ester group shows as a multiplet at 1.62ppm. The residual CH_2-groups in the alkyl chain show in the large multiplet at 1.29ppm, with the terminal CH_3-group as a triplet at 0.89ppm. As it is a crude reaction mixture, there are substantial amounts of starting materials left, evident by the large aldehyde-triplet present at 9.77ppm. Therefore, considerable overlap of signals from the starting materials and the product **6** are present. Identifying the signals from the formed carbinol and subsequent α-protons proved a challenge due to low relative peak size, however a good estimation of their position has been made. The carbinol proton is estimated to show at 3.81ppm as a poorly resolved multiplet. HSQC-NMR shows a coupling between this multiplet and a carbon resonance at 68.2ppm. The two α-protons to the ester-group show as a strongly uneven muliplet at 2.15ppm, as HMBC-NMR shows a coupling between it and the ester-carbon resonance at 172.2ppm. HMBC also shows a coupling between the ester-carbon and the

proton in the auxiliary group at 6.73ppm, indicating a reaction between the chiral auxiliary **4** and the aldehyde **5**.

^{13}C-NMR also suffers from overlapping or close-to-overlapping signals from starting materials and the product **6**. The alkyl chain and terminal methyl-group all show signals in the aliphatic area between 44ppm and 14ppm. The phenyl-groups in the auxiliary-group show in the aromatic region, 145ppm though 126ppm. The intra-auxiliary carbons can be observed at 80.87ppm and 80.39ppm. The ester-carbon shows at 172.2ppm and the adjacent methylene-carbon shows at 44ppm as it couples with their β-position through HMBC. As previously stated, there are high degrees of overlap between the product **6** and the staring material **5**.

2.4 – Attempts at transesterification and hydrolysis of 6f

Following the reaction and purification of compound **6f**, the chiral auxiliary is cleaved off by transesterification or hydrolysis. The reaction conditions of these attempts are listed in table 2.4-1. All reactions were monitored by TLC in 20% EtOAc/Heptane and by NMR. When conventional heating was applied, an oil-bath was used.

Table 2.4-1: Overview of reactions involving compound **6f**

Entry	Reaction	Compound **6f**	Base	Solvent	Heating	Temperature	Time
a	Transesterification	16.6mg	K_2HPO_4 6mg	0.5mL MeOH	Conventional	65 °C	Overnight
b	Transesterification	15mg	K_2HPO_4 6.8mg + NaOMe 7.5mg	10mL MeOH	Conventional /none	65 °C 20h Rt 40h	60h
c*	Transesterification	15.5mg	K_2HPO_4 7mg	5mL MeOH	Conventional	65 °C	40h
d*	Transesterification	15mg	K_2HPO_4 11mg	5mL 1:4 DMSO/MeOH	Conventional	66 °C	Overnight
e*	Transesterification	15.9mg	K_2HPO_4 11mg	6mL 1:1 Toluene/MeOH	Conventional /Microwave	66 °C 100 °C	18h 1h
f*	Transesterification	16mg	LiOMe Excess	6mL MeOH	Microwave	76 °C 100 °C	1.5h 3h
g	Hydrolysis	15mg	LiOH ·H_2O 15.5mg	5mL 4:1 THF/H_2O	None	0 °C	25h
h	Hydrolysis	15mg	LiOH ·H_2O 3.6mg	2mL MeOH	None Microwave None	Rt 100 °C Rt	24h 1.5h 72h
i*	Transesterification	14mg	TMSOK 4.2mg +31.4mg	3mL THF	None	Rt Rt	4h Overnight

* = Reaction performed under anhydrous conditions

The article by Gjessing *et al.*[12] describes the cleavage of the chiral auxiliary by transesterification using K_2HPO_4 in hot methanol as a simple overnight reaction to produce a methyl-ester. This is shown in entry a in Table 2.4-1. Shinada *et al.*[43] also demonstrate transesterification by K_2HPO_4, with encountered hindrances and corresponding reaction conditions. A transesterification with stochiometric amounts of K_2HPO_4 may need heating and prolonged reaction times to proceed.

As no change was seen on TLC for entry a, the reaction was repeated (entry b) with more solvent as compound **6f** is a solid with some solubility issues. Heating at reflux for 20h produced no discernible change by TLC. Sodium methoxide was then added to the mixture, as methoxides are useful nucleophiles for transesterification. The sodium methoxide was added in one go and let stir at room temperature for 40h, owing to its increased strength compared to K_2HPO_4. The reaction was periodically monitored by TLC with no change observed other than some cloudiness of the reaction mixture.

To eliminate any possible interference through the presence of water[44], the transesterification with K_2HPO_4 was performed in anhydrous conditions (entry c). Glassware was meticulously flame-dried and dry solvent were used for anhydrous conditions. Halving the solvent amount to 5mL from 10mL, increases the concentration while just dissolving the compound during heating. The reaction mixture was stirred for 40h, with no notable change observed on TLC.

To eliminate any solvation issues, a 5mL 1:4 solution of DMSO in MeOH was utilized with K_2HPO_4 as the base (entry d). The reaction mixture was heated at 66°C with strong stirring overnight. The MeOH was evaporated *in vacuo*, and the DMSO was removed by water/heptane separation. NMR analysis show no signal for a methyl-ester, in addition to no observable change by TLC.

With the failures to induce a reaction with K_2HPO_4 in MeOH in conventional heating situations, the decision was made to attempt microwave-assisted heating. There are reported situations in which a reaction will not work or requires long reaction times, but achieves fast and good yields with microwave heating[45]. To alter the polarity and solvent system of the reaction, a solution of toluene in MeOH was tried (entry e). Compound **6f** was dissolved in 6mL 1:1 toluene/MeOH and conventionally heated to 66°C with strong stirring overnight. No change was observed by TLC, the reaction mixture was transferred to a microwave vial and

heated at 100°C for 30min, with 1min of pre-stirring. The solution took on a slight red tint, but no change was observable by TLC. A sample of the reaction mixture was analysed by NMR, and a D_2O-wash was performed. No signal indicating a methyl-ester was formed.

Exhausting the options for K_2HPO_4, the base was changed to lithium methoxide in MeOH (entry f). The solution of lithium methoxide was prepared adding a solution of methyllithium (0.1mL) to the reaction mixture (6mL MeOH), cooled on an ice-bath. At least three equivalents of methyllithium were needed, due to the anticipated presence of two hydroxyl-groups being deprotonated in the process. Heating by microwave radiation at 76°C for 1.5h then 3h at 100°C, periodically monitoring the reaction by TLC, also fell short. TLC shows no distinct signs of a clean transesterification taking place. This is backed by NMR analysis, as no methyl-ester signal is present.

As all previous reaction attempts have been transesterifications, a hydrolysis was attempted (entry g). Hydrolysing the compound **6f** to a carboxylic acid would bypass the need to hydrolyse any ester created with transesterification. This alternate route could also include methylation with TMS diazomethane[46], a higher yielding methylation compared to the standard Fisher esterification. Compound **6f** was dissolved in a 5mL solution of 4:1 THF/H_2O. As the solvent system includes water, non-dry THF was also used. 3eq of $LiOH \cdot H_2O$ was added to the reaction solution on an ice-bath and the mixture was stirred overnight. Monitoring by TLC shows new spots with higher R_f-values than the starting material. TLC show staining spots by $KMnO_4$ being UV-active, spots only staining and a spot only UV-active. No additional spots on baseline or broad NMR-signals at high shifts opposes the formation of a carboxylic acid by hydrolysis.

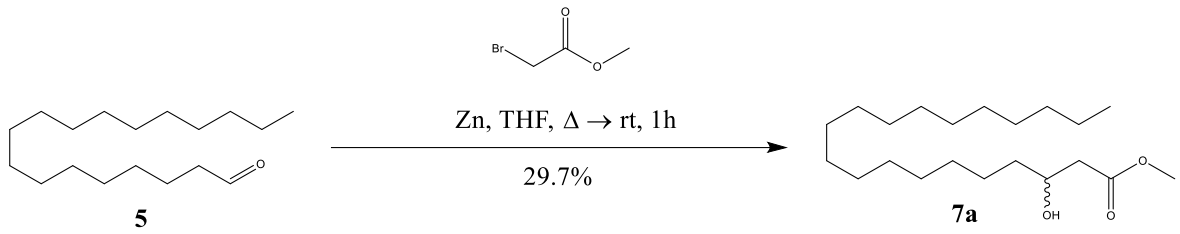
A hydrolysis with $LiOH \cdot H_2O$ in MeOH was attempted (entry h). With the hydroxide monohydrate as a base, non-dry MeOH was the solvent of choice. The compound and base were added to a microwave vial along with a smaller amount of MeOH to test if further increasing the reaction concentration could have a positive outcome. Stirring at room temperature, the compound was slow to dissolve, and the mixture was further stirred for 24h. Monitoring by TLC after 24h show a UV-only spot at a slightly higher R_f than the starting material. The reaction mixture was then heated by microwave radiation at 100°C for 1.5h. Seeing little change by TLC, the vial was stirred at room temperature for an additional three days. Removal of the solvent *in vacuo* left a small amount of yellow liquid. NMR analyses are in total inconclusive, except for a small singlet at ~3.8ppm. The time required for the result indicate that no significant reaction is occurring, and that this method is unviable.

Trimethylsilanoate alkali salts have proven to be a diverse class of reagents. Functioning as a nucleophile in hydrolysis of esters, a mild base and gentle reagent, they can replace harsher reagents or conditions successfully[47]. On this background, potassium trimethylsilanoate (TMSOK) was employed in a transesterification (entry i). TMSOK should give good yields in anhydrous conditions with short reaction times[47], therefore anhydrous conditions were used. Compound **6f** and 1.2eq TMSOK, solved in dry THF was stirred at room temperature for 6 hours, regularly monitored by TLC. With little change observed by TLC, a large excess of 31mg (10eq) TMSOK was added, and the reaction mixture was stirred for another hour. Tinting the solution yellow, the reaction mixture was stirred overnight at room temperature. TLC shows multiple spots with varying UV-activity and oxidative staining. With inconclusive results by TLC and a dwindling batch of compound **6f**, this experiment was a failure.

2.5 – Synthesis of racemic 3-hydroxy-methyl-icosanoate (7a)

As the stereoselective synthesis of **7b** with the chiral auxiliary **4** will yield a product with a certain enantiomeric excess, a racemic mixture of the compound **7a** is needed for chiral analysis.

For this the Reformatsky reaction, developed in 1887 by Reformatsky[48] is a simple and versatile[49] method of creating 3-hydroxy-esters. Utilizing zinc and not magnesium, Reformatsky reagents are less reactive analogues of Grignard reagents. Thus, these reactions can be performed in neutral and non-dry conditions. The ready availability of methyl- and ethyl bromoacetate makes for an enticing pathway to create 3-hydroxy methyl- or ethyl esters. The reaction is not stereoselective and so creates racemic mixtures. However, stereoselective Reformatsky additions have been reported[50]. Although proposed as a synthetic pathway to **7a** by Gensler *et al.*[51], the Reformatsky reaction to **7a** has not previously been reported in the literature. Sailer *et al.*[52] reported multiple syntheses of medium-length (C4-C10) aliphatic 3-hydroxy esters by the Reformatsky reaction, thus their procedure was adapted for this synthesis (Scheme 2.5-1).



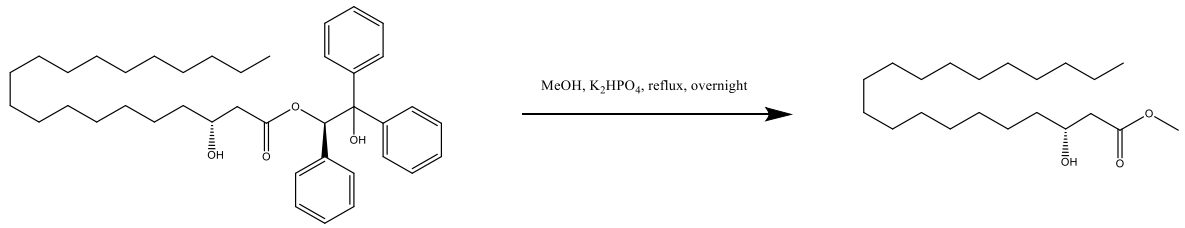
Scheme 2.5-1: Reformatsky reaction to **7a.**

Although prone to create by-products, the Reformatsky reaction between aldehyde **5** and methyl bromoacetate in the presence of zinc, gave the racemic 3-hydroxy methyl ester **7a** in a 29.7% yield.

^{1}H NMR data of **7a** after purification are in accordance with the literature[53]. A strong singlet at 3.71ppm (3H) indicates that the methyl ester is formed. Although no significant signal from the hydroxyl-group is detected, the -C*H*OH proton gives a multiplet (1H) at 4.02-3.97ppm, implying the hydroxyl is present. The α-protons to the ester group give signals for multiple double doublets reminiscent of an asymmetrical quartet (2H) at 2.54-2.37ppm. The α-protons of the hydroxyl group gives a broad multiplet at 1.44-1.41ppm (2H), with the residual protons on the alkyl chain giving a broad very strong signal at 1.25ppm (33H). The peak integrates for 33H where there are only 30 hydrogens, owing to the large peak size and thus magnifying any inaccuracies in the integration process. The terminal methyl group gives a triplet at 0.87ppm (3H).

2.6 – Transesterification of 6 to 3-hydroxy-methyl-icosanoate (7b)

The transesterification and removal of the auxiliary moiety of compound **6** was done as described by Gjessing *et al.*[12], and previously mentioned in Section 2.4, by heating a solution of **6** and K_2HPO_4 in MeOH. Cleaving the chiral auxiliary from the saturated fatty acyl chain of molecule **6** (Scheme 2.6-1), further strengthens this method for use in the Braun reaction.



6 **7b**

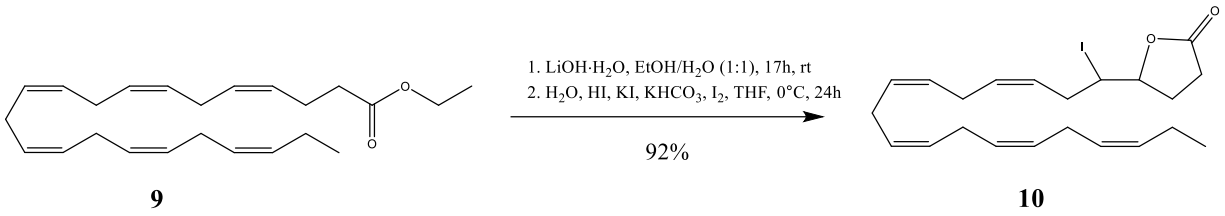
Scheme 2.6-1: Transesterification of **6.**

Previous points discussed in Section 2.4 for compound **6f** regarding purification by flash chromatography were still concerning. This perpetuated the decision to utilize the unpurified compound **6** in this transesterification reaction to the methyl ester **7b**. Reaction monitoring by TLC in 20% EtOAc/Heptane, shows multiple spots staining by $KMnO_4$ which also are UV-active. The largest stain-spot at an R_f of 0.61 is not UV-active, given the lack of UV chromophores, but a substituent group able to be oxidized, in compound **7b**. Minimal available solvents such as heptane in the laboratory, prevented purification of the product compound by flash chromatography. In turn, no total yield was able to be calculated. Given the reaction chance visible by TLC, the auxiliary was cleaved off.

2.7 – Iodolactonisation to 10 from Docosahexanenoic acid ethyl ester (9)

As a saturated fatty chain has been previously briefly explored as a model compound, polyunsaturated fatty chains are of great interest. In the Braun reaction with the chiral auxiliary **4**, the C18:5 ω-3 fatty aldehyde **12** will be used to synthesize 3-hydroxyicosapentaenoic acid **15**. To synthesize the unsaturated fatty aldehyde **12**, docosahexaenoic acid ethyl ester (**9**) was chosen as a convenient starting material.

The first steps of this will be the Corey iodolactonisation protocol[54], described by and adapted from Langseter *et al.*[55] amongst others[56, 57]. Over two overnight steps, the ethyl ester **9** was completely hydrolysed to DHA, and further lactonized to **10** (Scheme 2.7-1).



9 **10**

Scheme 2.7-1: Hydrolysis and lactonization of (all-z)-docosa-4,7,10,13,16,19-hexaenoic acid ethyl ester **9.**

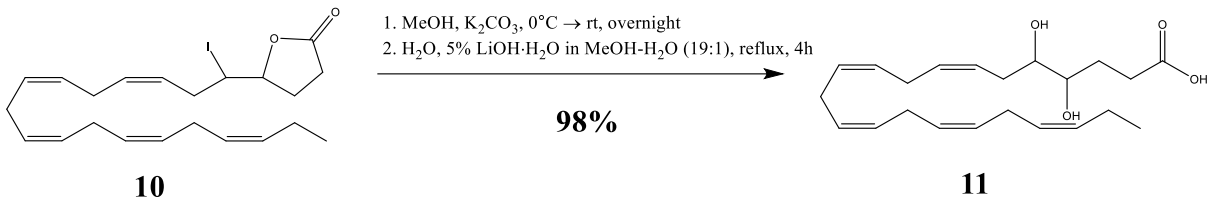
This reaction was performed in total darkness by covering the reaction flask with aluminium foil. Yield of this reaction was good at 92%, which corresponds to reported literature[57].

NMR analysis returned satisfactory data. The ^{1}H-NMR spectrum shows the 10 alkene-protons between 5.60 and 5.28ppm as two multiplets, respectively. The proton on the iodine-substituted carbon shows as a multiplet at 4.26ppm, with the proton α to the iodine and the lactone as a multiplet at 4.12ppm. The methylene-interrupting protons show as a large multiplet at ~2.7ppm. ^{13}C-NMR data are also satisfactory, with the lactone-carbon showing at 176.38ppm and its γ-carbon at 80.88ppm. The alkene-carbons are observed in the area 132.27-126.94ppm. The iodine-substituted carbon is seen at 37.80ppm, showing a strong coupling to the adjacent proton through HSQC-NMR.

The product **10** of this reaction was reacted further without additional purification.

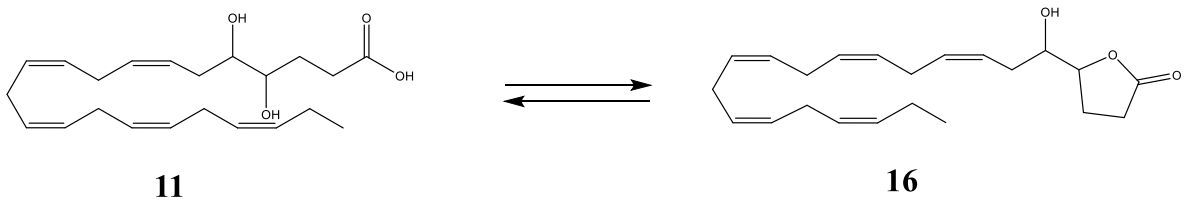
2.8 – Hydrolysis of iodolactone 10 to γ-diol 11

For reacting the synthesized iodolactone **10** to the diol acid **11**, the procedure described by Langseter *et al.*[55] was used. As described in Scheme 2.8-1, the synthesis was performed though an overnight cooling step, followed by refluxing.



Scheme 2.8-1: Synthesis of the diol **11.**

This synthesis was performed three times, where the reaction workup was deemed critical. Immediate cooling following the reflux and careful neutralization of the basic reaction conditions, contrary to acidification, were needed. The lactonization of the diol **11** in acidic conditions to compound **16** (Scheme 2.8-2) has been observed and described by Flock *et al.*[58].



Scheme 2.8-2: Lactonization of a γ-diol carboxylic acid under acidic conditions[58].

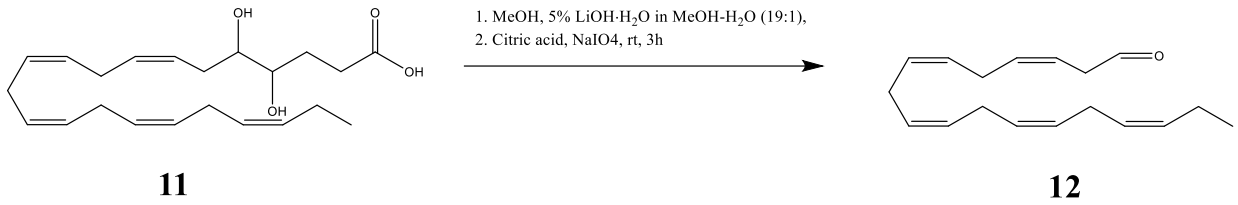
The product of this synthesis was isolated as a dark oil with a good yield of 98%.

^{1}H-NMR of **11** shows the characteristic large broad singlet of a carboxylic acid at 8.82ppm. Another broad singlet is observable at 3.61ppm, characteristic of a hydroxyl group. Some of hydroxylactone **16** is observable in the spectrum as a multiplet at 4.47ppm, from the γ-proton to the lactone in **16**. Alkene-protons are shown in the range 5.59ppm-5.29ppm, indicating that the polyunsaturated fatty chain is intact.

2.9 – Oxidative diol cleavage to aldehyde 12

Given the reversible hydrolactonization of **11**[58] (Scheme 2.8-2), the previous product oil was briefly treated with LiOH·H_2O in MeOH-H_2O, to hydrolyse any hydroxylactone **16** present, prior to oxidative cleavage.

The synthesis of the aldehyde **12** from the diol acid **11** (Scheme 2.9-1) was to be performed by oxidative cleavage with sodium periodate or periodic acid. Sodium periodate ($NaIO_4$) is a useful reagent for cleaving vicinal diols to aldehydes[59], as described in the procedure to **12** by Langseter *et al.*[55]. Periodic acid (H_5IO_6) has been shown to cleave epoxides, forming functional groups such as ketones[60] and acetals [12].



11 **12**

Scheme 2.9-1: Cleavage of the 4,5-diol **11** to form aldehyde **12**.

The synthesis of **12** was performed utilizing both periodic acid and sodium periodate. The aldehyde **12** is earlier reported as a sensitive compound, prone to creation of side-products[58]. This portrays the results of the attempted syntheses of **12**.

Table 2.9: Attempted syntheses of aldehyde **12.**

Entry	Reagent	Amount	Reaction time	Purification
a	Periodic acid	1.5eq	2h	Flash chromatography with 10% EtOAc/Heptane
b	Sodium periodate	1.4eq	3h	Flash chromatography with 5% EtOAc/Heptane

Entry a in Table 2.9, the reaction with 1.5eq of periodic acid was seemingly smooth. Reaction monitoring by TLC showed good separation of the products and starting materials. Although the reaction did not fully go to completion, purification by flash chromatography resulted in a very complex mixture with the components badly separated.

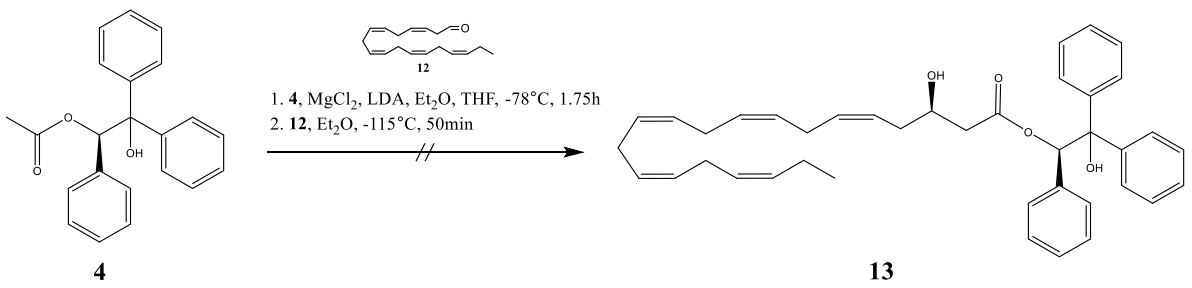
Repetition of this synthesis, entry b in Table 2.9, sodium periodate was used according to the procedure reported by Langseter *et al.*[55]. After initial hydrolysis, the sodium periodate was added, and the reaction mixture was rapidly adjusted to pH 4 by aqueous saturated citric acid. Monitored by TLC, the finished crude reaction mixture was purified by column chromatography in a weaker mobile phase of 5% EtOAc/heptane. Yielding similar results as the previous attempt, a complex mixture of badly separated compounds was gathered where the initial TLC showed merely one- or two compound spots.

Looking at the seemingly crude ^{1}H-NMR spectrum of **12** in Figure 6.10-1, the characteristic aldehyde triplet is present at 9.67ppm, albeit smaller than expected. The triplet integrates to 0.22, calibrated against the terminal methyl-group peak at 0.97ppm, thus the amount of further aldehyde available for reaction is low. The ^{1}H-NMR spectra also show signals for the expected molecular structure of **12**. The alkene protons show as the expected but convoluted multiplets around 5.68-5.32ppm, integrating to 9.53. The methylene-interrupting protons show as a large multiplet at 2.81ppm, integrating to 7.78. The methylene-group α to the terminal methyl group is visible as an apparent pentet at 2.07ppm, with an integral of 2.18. Differing from the expected signals are the methylene protons α to the aldehyde group, where a triplet is the be expected. A doublet shows with peaks at 3.32ppm and 3.21ppm, with an integral of 0.52. This alongside the multiple unelucidated signals and some residual solvent peaks indicates the strange complexity of the eluted mixtures following purification.

With a critical lack of available solvents and time allocated for this project running out, the chromatography fractions containing aldehyde **12** were collected for further reactions without additional purification.

2.10 – Stereoselective aldol reactions of octadecapentaenal 12 to 13

Advancing onto polyunsaturated fatty acid synthesis, the stereoselective Braun aldol reaction to compound **13** was explored. The area of interest for this segment, was to study the addition of magnesium chloride and alternative base systems in the Braun reaction as seen in Scheme 2.10-1.

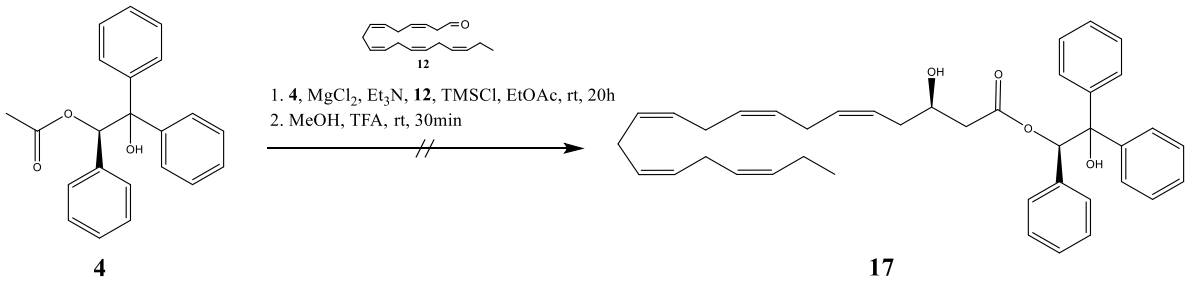


Scheme 2.10-1: Stereoselective aldol reaction with LDA.

The first aldol reaction was performed following the procedure adapted from Braun and Devant [28], transmetallating the lithium enolate of the auxiliary **4** with $MgCl_2$ instead of $MgBr_2$. Contrary to the synthesis of the saturated **6**, the magnesium halide was not made *in situ*, but sourced commercially as a solid.

The impure mixtures of compounds that resulted form the purification attempts of octadecapentaenal (**12**) (see Section 2.9) were used in these aldol syntheses due to time constraints. The presence of a significant aldehyde peak in their respective NMR-spectra (Figure 5.10-1) was estimated to be suffieciently viable to observe a reaction. This is a major source of uncertainty in the results in this thesis.

Following reaction procedure, the crude reaction mixture was quickly woked up and analysed by NMR, recording ^{1}H, ^{13}C, COSY, HSQC and HMBC. In the crude proton-NMR spectrum, the starting materials can be observed. The aldehyde peak is removed, though no further signs of the aldol reaction are present. The acetyl group of the auxiliary **4** remains a large singlet at 1.99ppm. There is also a substantial amount of residual EtOAc present, further cluttering the spectra. No carbinol signal or couplings akin to the stereoselective aldol of the saturated **6** in Section 2.3, are observed. To further mediate the estimated reaction failure, an attempted transesterification of this reaction product produced no observable reaction. Therefore, the aldol reaction did no go through, likely due to the very impure addition of aldehyde **12**. Following a second attempted synthesis such as described in Section 2.9, another reaction protocol was attempted.



Scheme 2.10-2: Stereoselective aldol with Et_3N and protective group.

Evans *et al.*[23] have developed a method for stereoselective synthesis of 3-hydroxy-compounds. This involves their *N*-acyloxazolidinone chiral auxiliary **42** [22] in a magnesium mediated aldol reaction with Et_3N and protection of the hydroxyl group with TMSCl. Formulated as a one-pot reaction, the components are added and stirred at room temperature for an extended period. The enolization, coordination of magnesium chloride and subsequent protection of the formed alcohol take place simultaneously. The product compound is then deprotected by addition of a few drops of TFA. With good reported yields and ee-values[23], the extrapolation of this method for our auxiliary **4** and aldehyde **12** may enlighten new synthetic approaches. This method is illustrated in Scheme 2.10-2.

Testing these reaction parameters as described, the reactants were added to the EtOAc solvent system and let stir overnight. The uncertainty in the quality and presence of the aldehyde **12** from its respective complex product mixture, as described in Section 2.9, was ever-present for this experiment. The reaction mixture was purified though silica gel twice though the procedure. Following the last purification through column chromatography, the resulting fractions were collected. NMR-analysis was performed through ^{1}H, ^{13}C, COSY, HSQC and HMBC experiments. Interpretation of the resulting spectra reveal no significant reaction to have taken place. Given the impurity of the aldehyde **12**, this specific reaction can only be regarded as an estimation of the procedure's viability. Additional parallels and potentially further reaction optimization are therefore due.

2.11 – Mosher ester synthesis

(*S*)-MTPA-Cl was used for the mosher ester synthesis and chiral derivatisation. The reaction products 3-hydroxy-methyl-icosanoate (racemic **7a** and stereoselective **7b**) were derivatised. The procedure was followed as described by Gjessing *et al.*[12].

With the intent of analysis by chiral column HPLC, only the *R*-Mosher ester of the two compounds were created. The mosher ester offers a way of attaching a UV-active chromophore, from the phenyl-group in the mosher ester, to use a UV-detector in the HPLC. Comparison of the retention times of the stereoselective **7b** with the racemic **7a** can give

information about the stereochemistry and enantiomeric excess of **7b**. As the time allocated for this project was running out, this was unfortunately not possible to perform. Instead, analysis by ^{19}F-NMR was carried out. Interpreting the ^{19}F-NMR spectra (see Figures 5.7-1 and -2 in Section 5.7), the stereoselective **7b** shows two singlets of differing intensities. The smaller singlet at -71.24ppm is integrated to 1, while the larger singlet at -71.65ppm integrates to 5.5. This is to be expected, as the -CF_3 group in the mosher ester is exposed to different environments depending on the stereochemistry of the carbinol. From this, an approximate calculation of enantiomer excess indicates the stereoselectively synthesized **7b** has an ee-value of approximately 84.6%.

Regarding the racemic **7a**, the same mosher synthesis and subsequent NMR analysis was performed. As it is a racemate, the ee-value should theoretically be approximately 50%, which would be evident by two similarly sized singlets in the ^{19}F spectrum. This is not the case, as can be seen in Figure 5.7-1 in Section 5.7. At a different chemical shift, a triplet with sequentially decreasing peak height is showing at -71.33ppm. Integrating this triplet to 1, the larger singlet remaining at the same shift of -71.66ppm, integrates to 2.78. This relation calculates to an ee-value of 73.58%. The root cause of this is unknown, as it does not match the ideal theoretical outcome of 50%. Although the mosher ester synthesis gave an approximate outlook on the enantiomeric excess of the 3-hydroxy methyl ester **7b**, the absolute conformation has not been able to be confirmed.

2.12 – Brief summary and future prospects

Though the compounds 3-HETE (**14**) and 3-HEPE (**15**) were not synthesized, the synthetic study of (R)-(+)-2-acetoxy-1,2,2-triphenylethanol (**4**) was partially successful. The stereoselective synthesis of 3-hydroxy-methyl-icosanoate (**7b**) using **4**, shows it is a viable option for such saturated substrates in addition to the explored unsaturated alkyl chain substrates. The hydrolysis of **7b** to yield the hydroxy-acid and further syntheses of analogues with different chain lengths is in due for future studies.

Here, the focus was on the *R*-enantiomer of the chiral auxiliary **4**. For future work, the syntheses described herein should be repeated with the *S*-enantiomer of **4** for comparison along with studies of yields and enantiomeric excesses.

As the oxidative cleavage reactions of the γ-diol acid **11** to the aldehyde **12** did not work given two attempts, the synthesis of **12** from DHA-EE (**9**) should go through another approach. Involving an additional reaction and lower total yields, the protocol by Gjessing *et al*.[12] should give satisfactory results.

For chiral analysis, a system for separation of mosher ester diastereomers with chiral column HPLC should be a target for development.

The Braun chiral auxiliary **4** shows promising results both herein and in the literature, with regards to applications in synthesis and cost-effectiveness. Further studies are therefore required to develop alternative reaction conditions. In turn, this may increase the scalability and viability of larger industrial syntheses involving the auxiliary **4**.

3 Conclusion

In this thesis, both successful and non-successful synthetic approaches to 3-hydroxy fatty acids have been described.

The synthetic studies regarding the chiral acetate auxiliary (*R*)-(+)-2-acetoxy-1,2,2-triphenylethanol (**4**), were partially successful. A novel aldol product **6** between the chiral auxiliary **4** and octadecanal (**5**) was synthesized, then transesterified to 3-hydroxy-methyl-icosanoate (**7b**). A simple non-stereoselective reformatsky reaction to 3-hydroxy-methyl-icosanoate (**7a**) was also developed. Although absolute conformation was not determined, Mosher ester analysis by ^{19}F-NMR determined an acceptable enantiomeric excess of **7b**. This has potential to be improved upon in the future. The experiments that did not go through as anticipated, still aided to further the understanding of their respective reactions. One of which being the oxidative cleavage of diol **11** to aldehyde **12** with regards to handling sensitivity and generation of complex mixtures during purification. The syntheses to the aldol product (all *Z*)-2-hydroxy-1,2,2-triphenylethanol-3-hydroxyicosa-5,8,11,14,17-pentaenoate (**13**) were unsuccessful. Time allocated for this project did not allow for a synthesis to (all *Z*)-3(*R*)-hydroxyicosa-5,8,11,14-tetraenoic acid (3*R*-HETE) (**14**) to be developed.

As indicated above, further synthetic studies to unsaturated 3-hydroxy fatty acids through magnesium-mediated aldol reactions of the chiral auxiliary **4** and **12** should be possible. Given another reliable synthetic pathway to **12**, the Braun chiral auxiliary **4** is a good chiral acetate donor for the further synthesis of polyunsaturated 3-hydroxy fatty acids. Optimising the aldol reaction with the right base, temperature and Lewis acid should prove worthwhile.

4 Materials and methods

NMR analyses were run on a Bruker Ascend 400 instrument, at 25°C in $CDCl_3$ as a solvent and at 400MHz (^{1}H NMR) or 100MHz (^{13}C NMR). Software used to process spectra was Bruker TopSpin.

All $CDCl_3$ for NMR was passed through a small plug of basic alumina to remove any traces of water or DCl prior to making the NMR-sample.

Thin-layer chromatography was performed using Merck TLC silica gel 60 F_{254} plates and checked by UV-light at 254nm or stained by $KMnO_4$.

Purification of compounds by column chromatography was performed using Merck silica gel 60 (0.040-0.063mm) as a stationary phase.

Unless noted otherwise, all silica gel chromatography columns were treated with triethylamine prior to loading sample.

Optical rotation measurements were run on a PerkinElmer Model 341 polarimeter at 589nm and 20°C in $CHCl_3$.

Microwave irradiation heating was performed in a Biotage initiator+.

IR spectroscopy analysis was performed on an Agilent Technologies 500 Series FTIR.

All water used was Merck Milli-Q deionised water dispensed through a Merck Q-Pod and a Millipak 0.22µm filter.

4.1 – Synthesis of (*R*)-(-)-Methyl mandelate (2)

Following the procedure as described by Basavaiah and Peddinti[37], in a round bottom flask 4.57g (0.030mol) (*R*)-mandelic acid **1** was dissolved in 30mL methanol (0.74mol, 25eq) and three drops of conc. sulphuric acid was added. The solution was heated to reflux for three hours, left to cool to room temperature and subsequently concentrated *in vacuo* to a thick clear oil. To the oil was added 25mL diethyl ether and the solution was transferred to a separatory funnel. The product was washed with saturated K_2CO_3 followed by brine, and the phases were separated. The organic phase was dried with $MgSO_4$, fitted with a septum, and let sit overnight. Concentration *in vacuo* yielded 3.6g (70%) methyl mandelate **2** (0.021mol). $[\alpha]^{20}_D$ = -168.3

Data

R_f=0.5 (40% EtOAc / hexanes, $KMnO_4$ stain, silica plate).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42-7.32 (m, 5H), 5.18 (s, 1H), 3.75 (s, 3H), 3.56 (broad s, 1H)

^{13}C NMR (100MHz, $CDCl_3$): δ 174.22, 138.33, 128.72, 128.61, 126.70, 73.00, 53.11

4.2 – Synthesis of (*R*)-(+)-1,1,2-triphenyl-1,2-ethanediol (3)

To an oven-dried 250mL three-neck round-bottom flask fitted with a magnetic stir-bar and rubber septa, 3.99g (24mmol) (*R*)-Methyl mandelate **2** was dissolved in 25mL dry diethyl ether. Then, 28mL (84mmol, 3.5eq) 3M in diethyl ether phenylmagnesium bromide solution was added, and the reaction mixture was heated to reflux for two hours. Following reflux, the mixture was cooled to room temperature, cooled over an ice-bath, and subsequently quenched dropwise with ~38mL saturated aqueous ammonium chloride-solution. The flask was stoppered and stored in a refrigerator over the weekend. Having separated into two phases, the upper ether layer was decanted, and the remaining thick mass was dissolved in aqueous NaCl-solution. The aqueous phase was extracted with ethyl acetate, and the organic phases were combined. Removal of solvents *in vacuo* left the crude product as a thick red oil. Purification by flash chromatography (66% EtOAc/Heptane, silica gel) yielded 4.3g (14.8mmol, 62%) of product **3** as a white powder. $[\alpha]^{20}_{D} = +201.8^{\circ}$

Data

R_f = 0.18 (66% EtOAc/heptane, silica gel, $KMnO_4$ stain)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99-7.28 (m, 5H), 7.18-7.05 (m, 10H), 5.64 (s, 1H), 3.12 (s, 1H), 2.40 (s, 1H).

^{13}C NMR (100MHz, $CDCl_3$): δ 145.08, 143.35, 138.77, 134.90, 129.92, 129.03, 128.47, 128.07, 127.73, 127.64, 127.48, 127.40, 126.99, 126.75, 126.18, 80.77, 78.00.

4.3 – Synthesis of (*R*)-(+)-*2*-acetoxy-1,1,2-triphenylethanol (4)

2.31g (7.9mmol) of **3** was added to a flame-dried 100mL round-bottom flask, with a magnetic stir-bar and rubber septum. The powder was dissolved in 50mL dry dichloromethane (DCM), and the flask was cooled on an ice-bath. When cool, there was added 0.91mL (11.27mmol, 1.42eq) dry pyridine and 0.64mL (8.96mmol, 1.13eq) acetyl chloride. After stirring for a few minutes, the reaction mixture was let warm to room temperature, turning the solution to a bright pink colour. After stirring overnight, the crude product was concentrated *in vacuo*. Following recrystallisation from acetone, the auxiliary **4** was vacuum filtered to a white powder and washed with ice-cold water, thus yielding 1.5g (4.6mmol, 58%). $[\alpha]^{20}_{D}$ = +211.7 °

Data

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56-7.29 (m, 5H), 7.17-7.04 (m, 10H), 6.67 (s, 1H), 2.81 (s, 1H), 1.96 (s, 3H)

4.4 – Synthesis of octadecanal (5) – Dess Martin oxidation

Following the general procedure described by Levya-Gutierrez and Wang [40].

To a 250mL round-bottom flask with a stir-bar, 1g 1-octadecanol (3.69mmol) was dissolved in 100mL chloroform. To the solution, 1.75g Dess-Martin periodinane (DMP) (4.12mmol, 1.11eq), 50μL H_2O (0,0027mmol, 0,000752eq) and a spatula of $NaHCO_3$ were added. The flask was stoppered, and the suspension was stirred at room temperature for 4h. Additional 3.6g (8,48mmol, 2.3eq) DMP was added, and the reaction mixture was left to stir over the weekend. The solvent was then removed *in vacuo*, and the crude product purified using flash chromatography without prior treatment with triethylamine. The reaction yielded 403mg, 40%, of aldehyde **5**.

Data

$\mathbf{R_f}$ = 0.4 (10% EtOAc/heptane, Silica gel, $KMnO_4$ stain)

^1H and ^{13}C spectroscopic data are listed in Section 4.5 below.

4.5 – Synthesis of octadecanal (5) – Swern oxidation

Following the procedure by Matuszewska *et al*.[39] all glassware was flame dried, fitted with rubber septums and under nitrogen atmosphere.

A 100mL round-bottom flask was fitted with a magnetic stir-bar, cooled to -78℃ with a dry ice/acetone bath and injected with 15mL dry DCM followed by 0.6mL (6.98mmol, 1eq) oxalyl chloride. To this mixture, a solution of 15mL dry DCM, with 1.0mL (14.07mmol, 2eq) of dry DMSO made in a 25mL pear shaped flask, was added dropwise via a syringe. The solution was left to stir for 15 minutes. 1.89g (6.98mmol) 1-octadecanol was added to a 100ml three-neck round-bottom flask and dissolved in 30mL dry DCM. The alcohol-solution was added dropwise via a syringe to the main cooled solution and left to stir for another 15 minutes. A solution of 4.7mL (33.67mmol, 4.8eq) dry triethylamine in 10mL dry DCM was added dropwise with a syringe, and the reaction mixture was stirred for 1 hour at -78℃. The mixture was allowed to warm to room temperature before evaporation of the solvent *in vacuo*.

The crude product was dissolved in 30mL DCM and washed with aqueous 5% citric acid, saturated $NaHCO_3$ and brine. The organic phase was dried with $MgSO_4$, and solvent removed *in vacuo*. Purification by column chromatography twice yielded 850mg, 45%, of aldehyde **5.**

Data

R_f = 0.31 (10% EtOAc/heptane, Silica gel, $KMnO_4$ stain).

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.75-9.74 (t, J = 1.84Hz, 1H), 2.40 (dd, J = 1.84, 7.32, 2H), 1.63-1.58 (m, 2H), 1.32-1-17 (m, 28H), 0.88-0.85 (t, J = 6.64, 3H)

^{13}C NMR (100MHz, $CDCl_3$): δ 202.85, 43.91, 33.60, 31.93, 29.93, 29.69, 29.66, 29.64, 29.58, 29.49, 29.43, 29.36, 29.17, 22.69, 22.08, 15.35, 14.10

4.6 – Aldol reaction of octadecanal (5) and chiral auxiliary 4 to unidentified compound 6f

Per the article from Braun & Devant[28].

To an oven-dried 100mL round-bottom flask fitted with a magnetic stir-bar, 380mg (1.1mmol) of chiral auxilliary **4** was added. The flask was fitted with rubber septa, then 10mL dry THF was added, and the powder allowed to dissolve. The flask was cooled to -78℃ with a dry-ice/acetone bath. After cooling, 2.5mL (2.5mmol, 2.3 eq) of 1M solution of LDA in THF/Hexanes was added dropwise to the solution. Following the addition, the flask was placed on an ice-bath and warmed to 0℃.

To an oven-dried 250mL three-neck round-bottom flask fitted with a magnetic stir-bar, 60mg (2.46mmol) of magnesium turnings was added and the flask fitted with rubber septums. To the flask, 20mL of dry THF was added, followed by the addition of 0.21mL (2.43mmol, 1.98eq) 1,2-dibromoethane. With a condenser attached, heat was applied to start the reaction and a reflux. After a complete reaction, the flask was cooled to -78℃ with a dry-ice/acetone bath, and 45mL dry diethyl ether was added. After cooling, the solution of chiral auxiliary **4** and LDA in THF was transferred and stirred for an hour at -78℃. The reaction mixture was further cooled to -115℃ using diethyl ether and liquid nitrogen. A solution of aldehyde **5** (0.445g, 1.66mmol, 1.5eq) in THF was added by cannulation, and the reaction mixture was stirred at -115℃ for 40 minutes. Following removal from cooling bath, the reaction was quenched with aqueous sat. NH_4Cl and extracted with chloroform. Purification by flash chromatography (26% EtOAc/heptane, silica gel) yielded 240mg (0.39mmol, 36%) of the compound **6f** (mp– 217 - 218℃). $[\alpha]^{20}_D = +63.9^\circ$

4.8 – Synthesis of (*R*)-2-hydroxy-1,2,2-triphenylethyl-3-hydroxyicosanoate (6)

All glassware was oven-dried overnight and fitted with magnetic stir-bars.

To a 5mL glass vial, 0.17mL (1.21mmol, 2.46eq) diisopropylamine and 0.73mL THF were added. The vial was cooled to -78°C on a dry-ice/acetone bath, and 0.78mL (1.24mmol) of a 1.6M n-Butyllithium solution was added dropwise. After stirring for a few minutes, the solution was warmed to 0°C on an ice-bath and stirred until needed.

In a glass vial, 166mg (0.49mmol) of chiral auxiliary **4** was dissolved in 1.7mL THF and cooled to -78°C. The LDA-solution was added dropwise via syringe and the reaction mixture was stirred for a few minutes before warming to 0°C on an ice-bath, turning a faint yellow colour.

To a round-bottom flask, 24mg (0.98mmol, 2eq) of magnesium shavings were added along with 3.3.mL THF. Attaching a condenser to the flask, 0.09mL (0.19mg, 1.01mmol, 2.06eq) of dibromoethane was added in one go and the solution was briefly heated with a heat-gun to kickstart the reaction to magnesium bromide. Following a short reflux and cooling to room temperature, 6.6.mL Et_2O was added.

Cooling the $MgBr_2$-solution back down to -78°C, the LDA/auxiliary-solution was added dropwise and stirred for an hour. The reaction mixture was then cooled to approximately -114°C in an EtOH/liquid nitrogen bath. A solution of 203mg (0.75mmol, 1.53eq) stearylaldehyde **5** in 1.7mL THF was then added very slowly dropwise with strong stirring, being further stirred at temperature for 40 minutes. Maintaining cooling by liquid nitrogen was ceased and the reaction mixture was gradually warmed with the EtOH-bath for 30 minutes. The reaction mixture was removed from the cooling-bath and warmed to room temperature before quenching with saturated aqueous NH_4Cl. The reaction mixture was extracted three times with DCM. The organic phase was washed with brine, dried with $MgSO_4$ and evaporated *in vacuo*. The crude product was left as an off-white solid (356mg).

Data

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71-7.05 (m, 15H), 6.73 (s, 1H), 3.81 (m, 1H), 3.20 (s, 1H), 2.88 (s, 1H), 2.16-2.12 (m, 2H), 1.64-1.61 (m, 2H), 1.26 (m, 30H), 0.88 (t, 3H)

^{13}C NMR (100MHz, $CDCl_3$): δ 172.16, 145.29, 144.95, 143.53, 142.79, 139.01, 135.96, 128.56, 128.44, 128.20, 127.87, 127.70, 127.54, 127.15, 126.43, 126.30, 80.87, 80.39, 68.14, 68.12, 44.02, 32.04, 29.81, 29.77, 29.69, 29.54, 29.47, 29.28, 22.80, 22.20, 21.28, 14.23

4.9 – Synthesis of racemic 3-hydroxy-methyl-icosanoate (7a)

To a round-bottom flask fitted with a reflux condenser and a magnetic stir-bar, 8mL non-dry THF was added. The THF was heated to near-reflux on a water-bath, with the subsequent rapid addition of zinc granules (68mg, 1.03eq), a solution of aldehyde **5** (134mg, 0.5mmol, in 2mL dry THF) and methyl bromoacetate (0.095mL, 1mmol, 2eq). With all reagents added, the solution was strongly heated and stirred, and let reflux for 1 minute before removal of the water bath. With gradual cooling to room temperature, the solution was stirred for 1 hour to become a grey colour. The solvents were evaporated *in vacuo* and the crude product was taken up in EtOAc. In a separatory funnel, the solution was washed with water, filtered to remove residual solid particles, and washed again with water. The organic phase was washed twice with 1M aq. HCl, followed by brine. The organic phase was dried with $MgSO_4$, filtered and solvents removed *in vacuo*. Purification by column chromatography without prior triethylamine treatment (gradient, 10% → 20% EtOAc/heptane, silica gel) yielded 51mg of pure 3-hydroxy-methyl-icosanoate **7a** as an off-white solid (0.148mmol, 29.7%).
$[\alpha]^{20}_{D=}$ -0.004˚

Data

R_f = 0.27 (20% EtOAc/heptane, silica gel plate, $KMnO_4$ stain).

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.02-3.97 (m, 1H), 3.71 (s, 3H), 2.83-2.82 (d, J = 4Hz, 1H), 2.54-2.37 (dq, J = 24, 16, 16, 2H), 1.44-1.41 (m, 2H), 1.25 (m, 30H), 0.89-0.86 (t, J = 6.4, 3H)

^{13}C NMR (100MHz, $CDCl_3$): δ 173.54, 68.04, 51.74, 41.08, 36.53, 31.93, 29.70, 29.66, 29.59, 29.57, 29.52, 29.36, 25.48, 22.69, 14.12

4.10 – Synthesis of 5-((all *Z*)-1-Iodooctadeca-3,6,9,12,15-pentaenyl)dihydro-2(3H)-furanone (10)

In a 500mL round-bottom flask covered in aluminium foil, DHA ethyl ester **8** (10.01g, 28.07mmol) and $LiOH \cdot H_2O$ (5.8g, 140mmol, 5eq) were dissolved in 60mL EtOH-H_2O (1:1). The mixture was stirred for 17h until full conversion to DHA **9**. The flask was cooled on an ice-bath and 90mL H_2O was added. Then, 20mL 57% HI was added, followed by saturated $KHCO_3$-solution to pH 9, and 3g (18mmol, 0.64eq) KI. A solution of 21.3g (84mmol, 2.9eq) I_2 in 70mL THF was added dropwise with stirring. The mixture was let stir in the dark for 18h while cooled. The reaction was quenched with 100mL saturated aqueous $Na_2S_2O_3$ and solid NaCl was added to saturation. The product was extracted with heptane thrice, and the extract was washed with brine twice. The organic phase was dried with $MgSO_4$, and the solvent removed *in vacuo*. The product **10** was left as a yellow oil (11.8g, 25.9mmol, 92%) and further reacted without additional purification.

Data

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.6-5.53 5.41-5.28 (m, 10H), 4.28-4.24 (m, 1H), 4.14-4.09 (m, 1H), 2.86-2.80 (m, 10H), 2.76-2.68 (m, 1H), 2.60-2.51 (m, 1H), 2.45-2.36 (m, 1H), 2.13-2.03 (m, 3H), 0.99-0.95 (t, J=7.5, 3H)

^{13}C NMR (100MHz, $CDCl_3$): δ 176.38, 132.27, 131.82, 129.01, 128.82, 128.64, 128.14, 128.06, 127.57, 127.23, 126.94, 80.88, 37.90, 34.80, 28.71, 27.51, 26.11, 25.90, 25.87, 20.78, 14.50

4.11 – Synthesis of (All *Z*)-4,5-dihydroxydocosa-7,10,13,16,19-pentaenoic acid (11)

The iodolactone **10** (2.646g, 5.7mmol) was taken up in 40mL methanol and subsequently cooled on an ice-bath. Following cooling, 1.726g (12.48mmol, 2.19eq) K_2CO_3 was added, and the reaction mixture was stirred overnight at room temperature, covered in aluminium foil. 3.6mL H_2O then was added, followed by a 5% (w/v, 1.5g) solution of $LiOH \cdot H_2O$ in 19:1 MeOH-H_2O (30mL). The solution was refluxed for 4 hours, and immediately cooled on an ice-bath. The reaction mixture was acidified to pH 6 with aqueous 1M HCl-solution and solid NaCl was added to saturation. The mixture was extracted four times with EtOAc, the organic phase was washed with brine and subsequently dried with $MgSO_4$. Following removal of the solvent *in vacuo*, the crude oil was purified by column chromatography (gradient 50% EtOAc/Heptane → ~10% HOAc/EtOAc, silica gel) to yield 1.83g (5mmol, 87%) of diol **11**.

Data

R_f = 0.0, 50% EtOAc/heptane

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.82 (broad s), 5.59-5.29 (m, 10H), 4.49-4.45 (m, .4H). 3.48 (broad s, 1H), 2.84-2.81 (m, 7H), 2.63-2.61 (m, 1H), 2.59-2.52 (m, 1H), 2.50-2.47 (m, 2H), 2.40-2.38 (m, 3H), 2.06-2.03 (m, 13H), 0.98-0.94 (t, J = 5.2, 3H)

^{13}C NMR (100MHz, $CDCl_3$): δ 178.17, 177.51, 132.21, 132.18, 131.98, 130.72, 128.81, 128.74, 128.63, 138.54, 138.48, 128.16, 138.10, 128.07, 127.98, 127.71, 127.14, 125.62, 124.40, 82.06, 73.27, 31.54, 29.84, 29.41, 28.74, 25.88, 25.80, 25.77, 25.74, 25.68, 24.24, 21.17, 20.69, 14.41

4.12 – (All *Z*)-octadeca-3,6,9,12,15-pentaenal (12)

9.26g of the diol acid **11** was added to a round-bottom flask. To this, 5g $LiOH \cdot H_2O$ in 100mL $MeOH/H_2O$ (19:1) was added and the mixture was stirred at room temperature for one hour. Then, 70mL H_2O was added, followed by 7.64g (35mmol, 1.4eq) of $NaIO_4$. Immediately following the addition, the reaction mixture was acidified to pH 4 with aqueous saturated citric acid. The reaction mixture was strongly stirred for approximately 3 hours before extraction with heptane. The organic phases were combined, washed twice with brine, dried with $MgSO_4$ and solvents removed *in vacuo* to a yellow oil. The crude oil was attempted purified by column chromatography without triethylamine treatment (silica gel, 5% EtOAc/heptane).

Data

R_f = 0.33 (silica gel, 5% EtOAc/Heptane, $KMnO_4$ stain)

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.67 (t, J=1.88, 1H), 5.68-5.57 (m, 1H), 5.41-5.32 (m, 9H) 2.83-2.81 (m, 8H), 2.09-2.04 (p, J=7.52, 2H), 0.99-0.95 (t, J=7.52, 3H)

4.13 – Stereoselective aldol reaction between aldehyde 12 and chiral auxiliary 4

4.13.1 –$MgCl_2$/LDA

To an oven-dried glass vessel fitted with a magnetic stir-bar, 167mg (0.5mmol) of chiral auxiliary **4** was added and dissolved with 3.3mL dry THF in 6.6mL dry Et_2O. The vessel was stoppered, and the mixture was cooled to -78°C with a dry-ice/acetone bath. 1.7mL LDA-solution (1.24mol, 2.48eq) prepared fresh from diisopropylamine and n-BuLi was added dropwise and subsequently 101mg of $MgCl_2$ (1.06mmol, 2.12eq) was added in one go. The reaction mixture was stirred at -78°C for 1h and 45min before cooling to approximately -115°C with an ethanol/liquid nitrogen bath. A solution of aldehyde **12** (approximately 161mg, 0.62mmol, 1.24eq) in 1mL dry Et_2O was added dropwise via syringe and the reaction mixture was stirred for 50min. The reaction vessel was taken off the cooling bath, quenched with sat. aq. NH_4Cl and extracted with DCM. The organic phases were combined, dried with $MgSO_4$, and concentrated *in vacuo*.

4.13.2 – $MgCl_2$/Et_3N/TMSCl

Adapted from the procedure by Evans *et al.*[23]

To a round-bottom flask, 664mg (2mmol) of the chiral auxiliary **4** was added together with 26mg (0.2mmol, 0.1eq) of $MgCl_2$ and a magnetic stir-bar. Approximately 620mg (ap. 2.4mmol, 1.2eq) of an impure mixture of aldehyde **12** was added. 4mL EtOAc and 6mL THF were added followed by 0.56mL (4mmol, 2eq) of dry triethylamine and 0.38mL (3mmol, 1.5eq) of chlorotrimethylsilane. The flask was stoppered with a rubber septum and let stir at room temperature for 20h. The reaction mixture was then pushed through a short plug of silica gel with 200mL Et_2O. The solvent was evaporated *in vacuo* to an orange solid. The product was taken up in 17mL methanol, then a stir-bar and 4 drops of trifluoroacetic acid were added. The solution was stirred for 30 min. The solvent was evaporated *in vacuo* to give a thick yellow oil. The product was purified by column chromatography (gradient, 0 → 5% EtOAc/Heptane, silica gel)

4.14 – Attempts at transesterification and hydrolysis of unidentified compound 6f

This general procedure was used for all attempts at reacting the compound (see table 2.5-1).

~15mg of compound **6f** was added to an appropriate reaction vessel. To this, a magnetic stir-bar and the solvent system was added. When the compound **6f** was dissolved, the base was added, the reaction vessel stoppered with a septum or cap, and stirred at room temperature or heated by the appropriate method. For conventional heating, a rubber septum was used for stoppering and an oil-bath heated by a hot-plate with a coupled thermometer for precise temperature control was used. Microwave radiation heating was carried out in the appropriate microwave vial size to the solvent amount, and the vial was capped by crimping. The reactions were monitored by TLC in with silica plates and 20% EtOAc/heptane. Spotting of the crude reaction mixtures was done by canulation.

4.15 – Transesterification of 6 to 3-hydroxy-methyl-icosanoate (7b)

To a small oven-dried round-bottom flask fitted with a magnetic stir-bar, 14.8mg (0.024mmol) of **6** and 6.2mg (0.035mmol, 1.45eq) of K_2HPO_4 were added. 2.5mL of MeOH was added and the flask was stoppered. The reaction mixture was heated in an oil-bath at 74°C overnight. The MeOH was then removed *in vacuo*. The crude reaction product was then extracted with EtOAc, dried with $MgSO_4$, evaporated *in vacuo*. The compound was purified by column chromatography (2.5% EtOAc/heptane, silica gel).

Data

R_f = 0.62 (20% EtOAc/heptane).

4.16 – Mosher ester analysis

Mosher ester syntheses was carried out according to procedure by Gjessing *et al.*[12], which is based on the work by Hoye *et al.*[35]. Each to a separate 5mL glass vial, fitted with magnetic stir-bars, the substrate was added; racemic **7a** (10mg, 0.029mmol), Compound **6** (20mg, 0,033mmol), Methyl ester **7b** (20mg, 0.058mmol). 1mL $CHCl_3$ was added to the vial through a small plug of basic alumina to remove any traces of water present. 16µL (0.2mmol) dry pyridine and 23µL (0.12mmol) (S)-MTPA-Cl were added. The vial was stoppered with a rubber septum and stirred for two hours at room temperature. 1mL water was added and the reaction mixture was extracted twice with 3mL Et_2O. The ether layers were dried with $MgSO_4$, filtered, and evaporated *in vacuo*. ^{19}F NMR was recorded.

Data

^{19}F NMR (376.46 MHz, $CDCl_3$): **7a**: δ -71.33- -71.45 (decreasing t, 1), -71.66 (s, 2.78) **7b**: δ -71.24 (s, 1), -71.66 (s, 5.50)

5 Spectroscopic data

5.1 – (*R*)-Methyl mandelate (2)

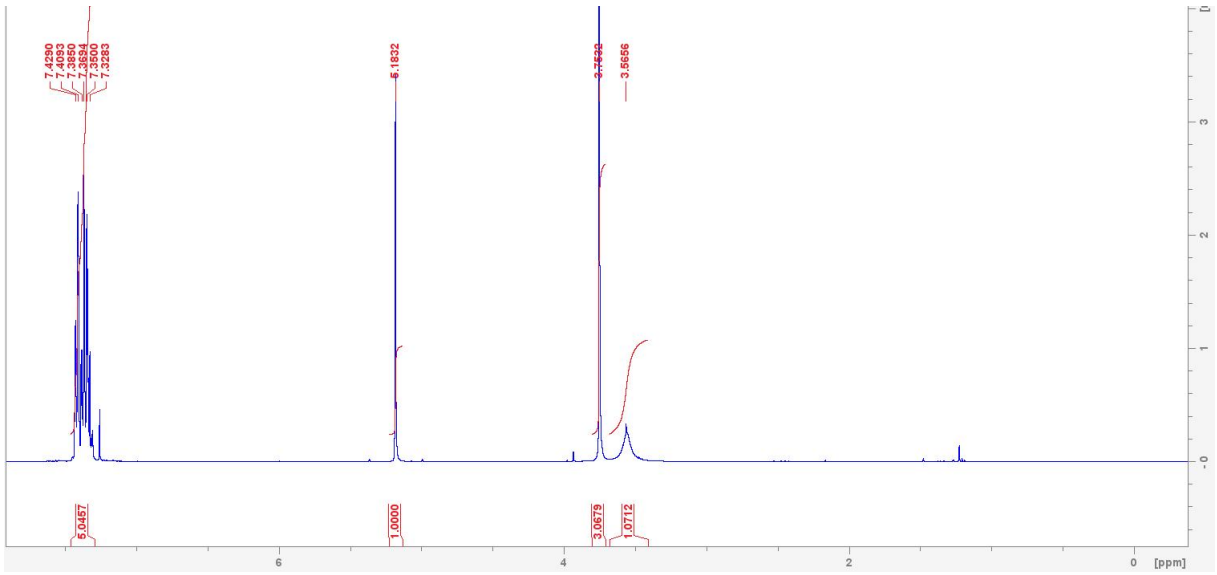


Figure 5.1-1: ^{1}H-NMR of (*R*)-Methyl mandelate **2**

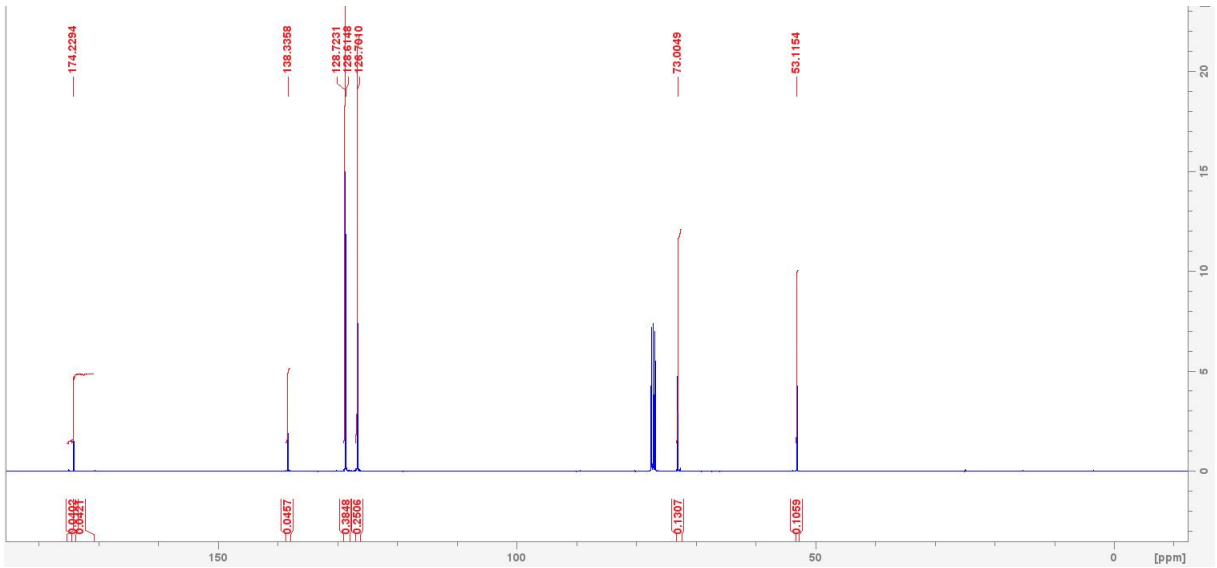


Figure 5.1-2: ^{13}C-NMR of (*R*)-Methyl mandelate **2**

5.2 – (R)-(+)-1,1,2-triphenyl-1,2-ethanediol (3)

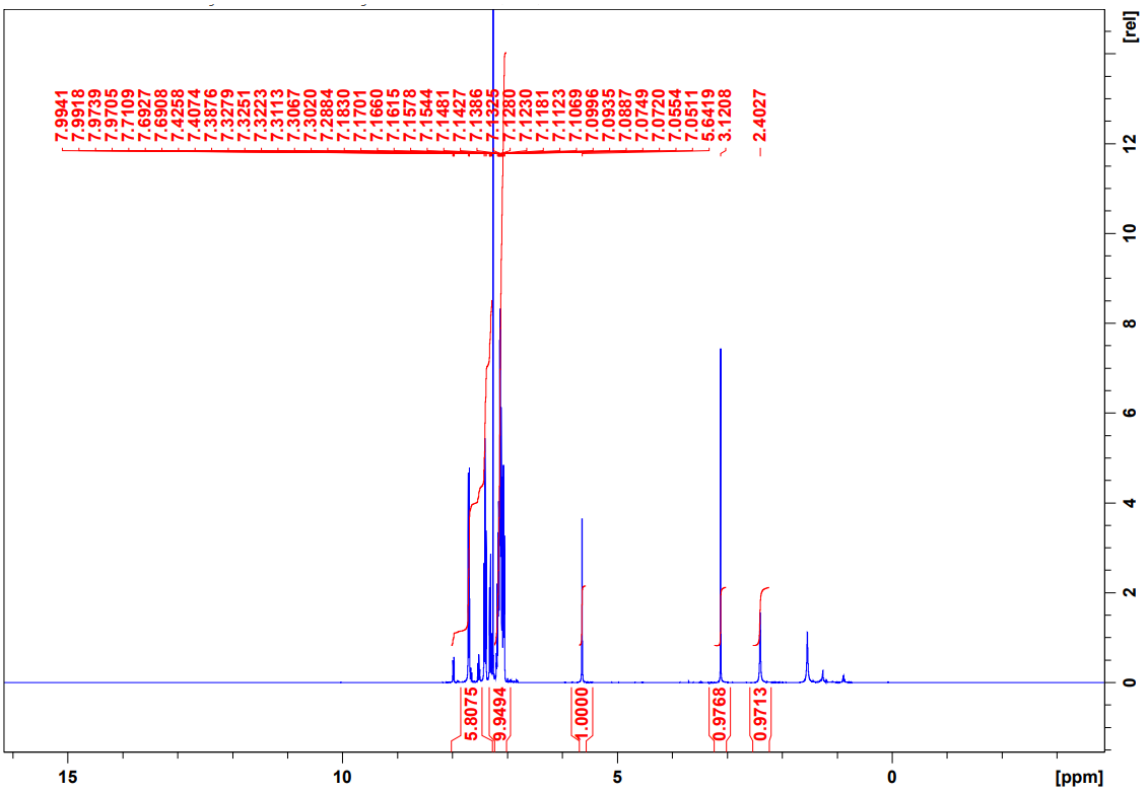


Figure 5.2-1: ^{1}H-NMR of **3**

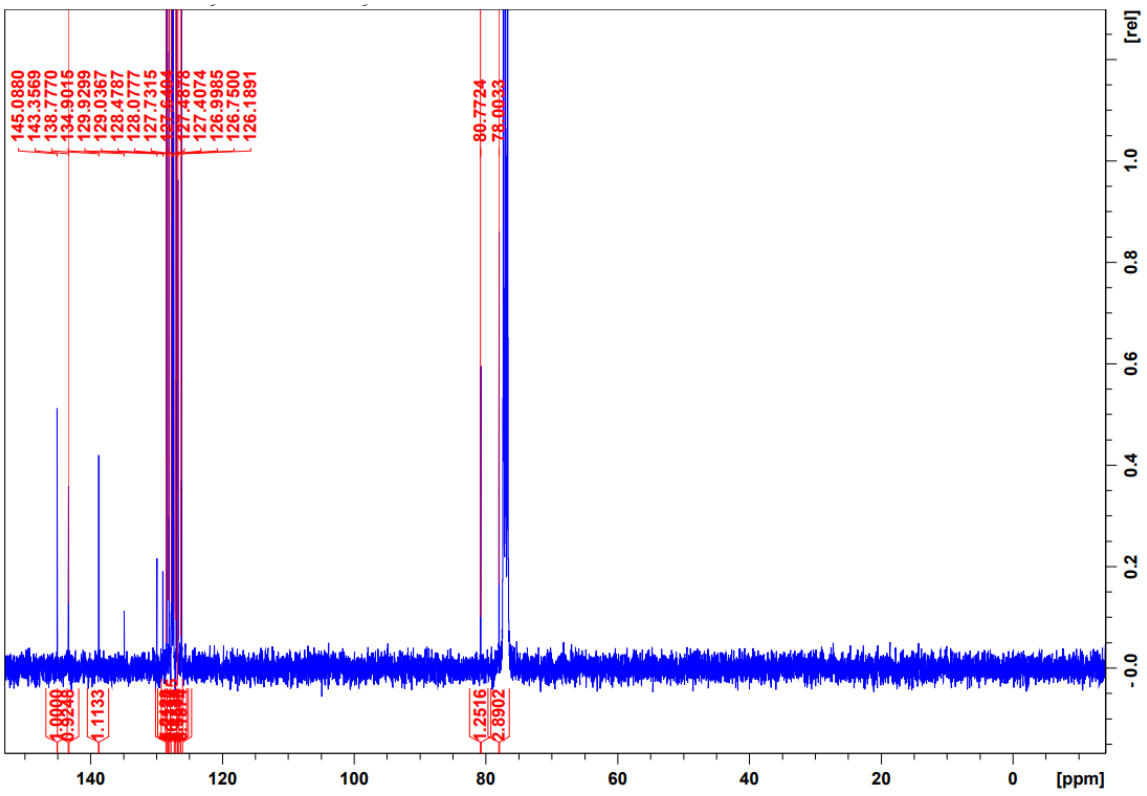


Figure 5.2-2: ^{13}C-NMR of **3**

5.3 – (*R*)-(+)-*2*-acetoxy-1,1,2-triphenylethanol (4)

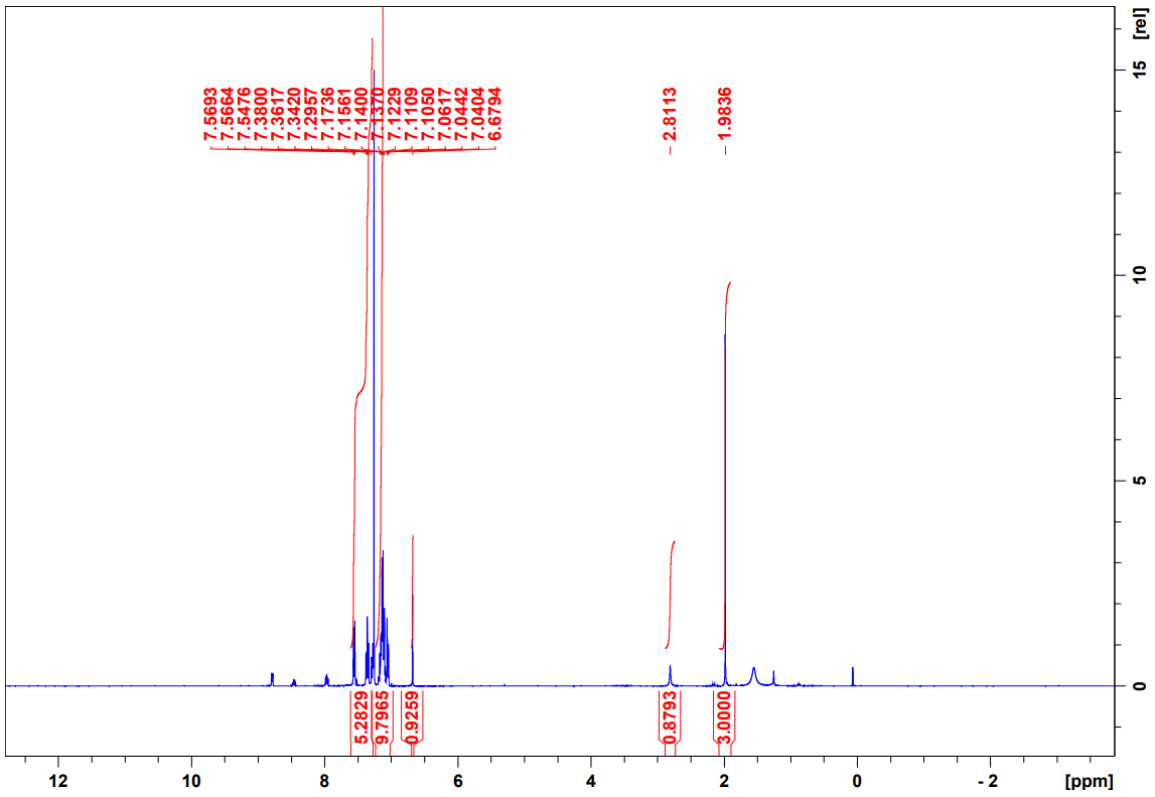


Figure 5.3-1: ^{1}H-NMR of chiral auxiliary **4**

5.4 – Octadecanal (5)

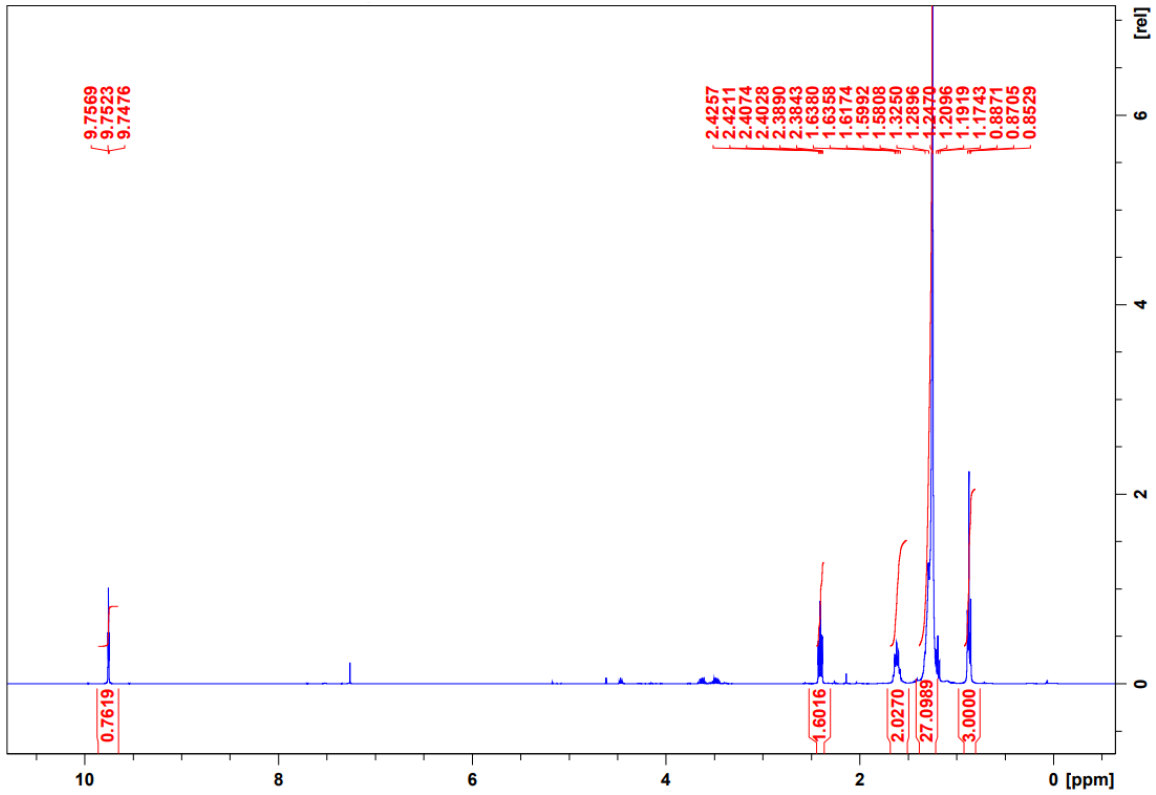


Figure 5.4-1: ^{1}H-NMR of octadecanal **5**

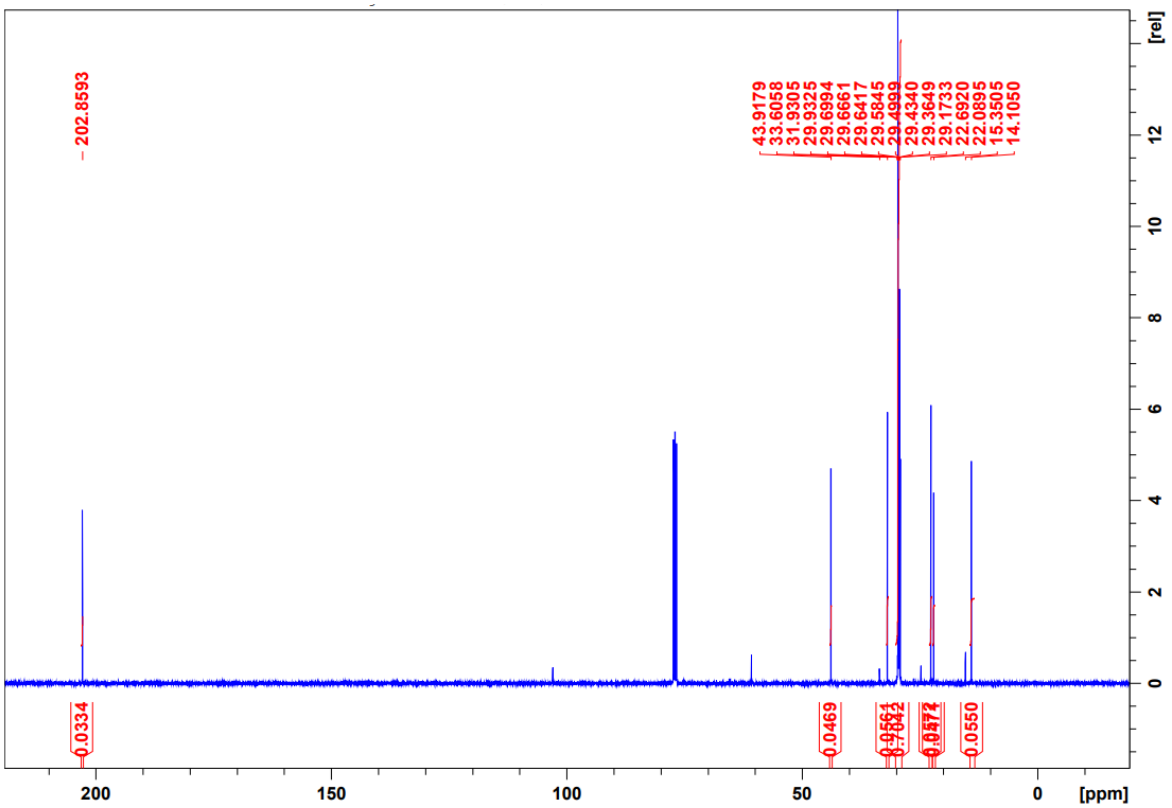


Figure 5.4-2: ^{13}C-NMR of octadecanal **5**

5.5 – (*R*)-2-hydroxy-1,2,2-triphenylethyl-3-hydroxyicosanoate (6)

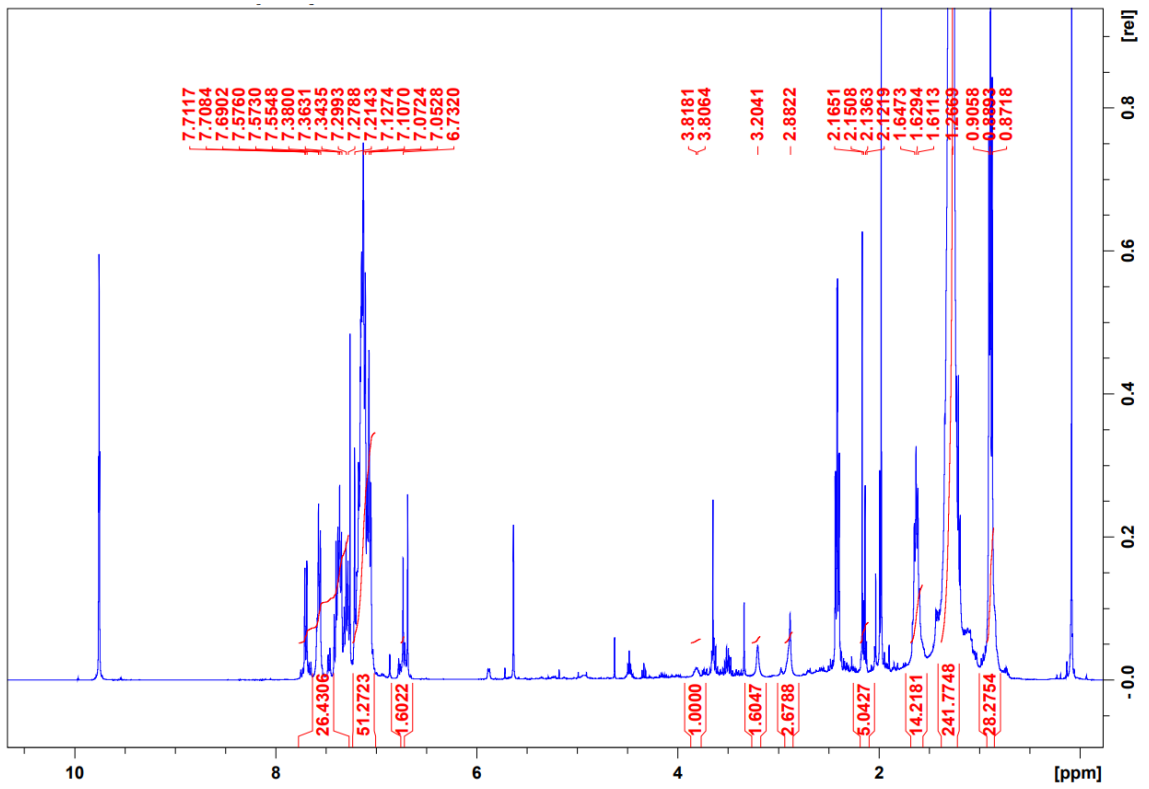


Figure 5.5-1: ^{1}H-NMR of crude aldol reaction mixture to compound **6**

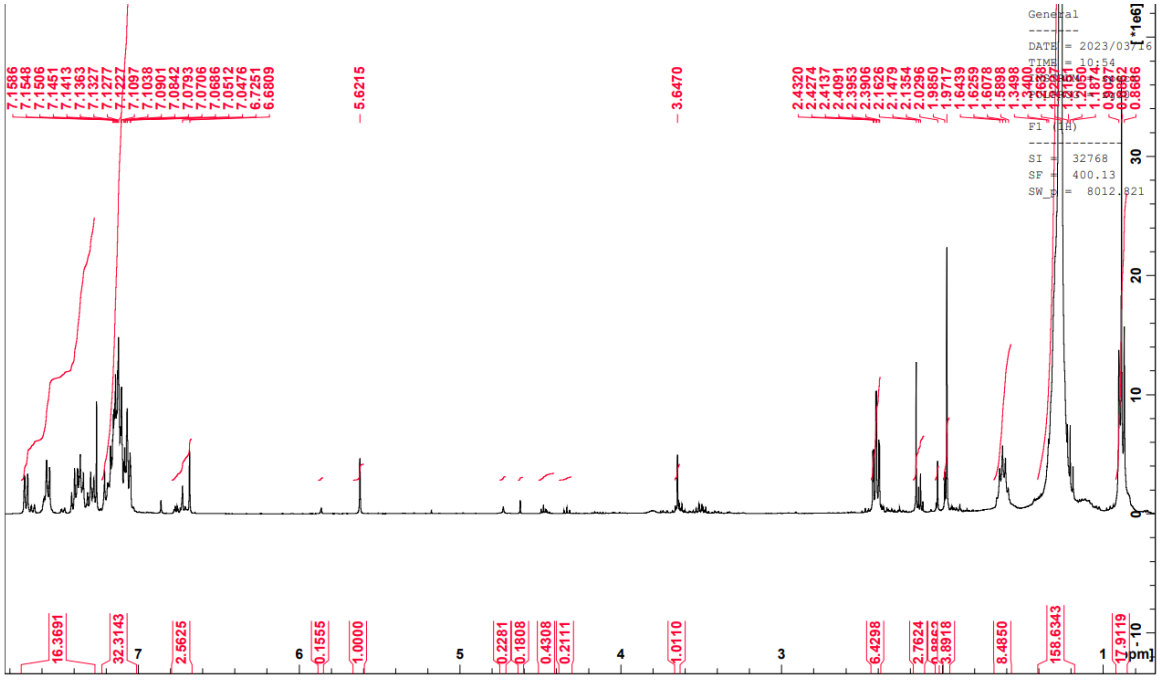


Figure 5.5-2: D_2O-wash of crude aldol reaction mixture to compound **6**

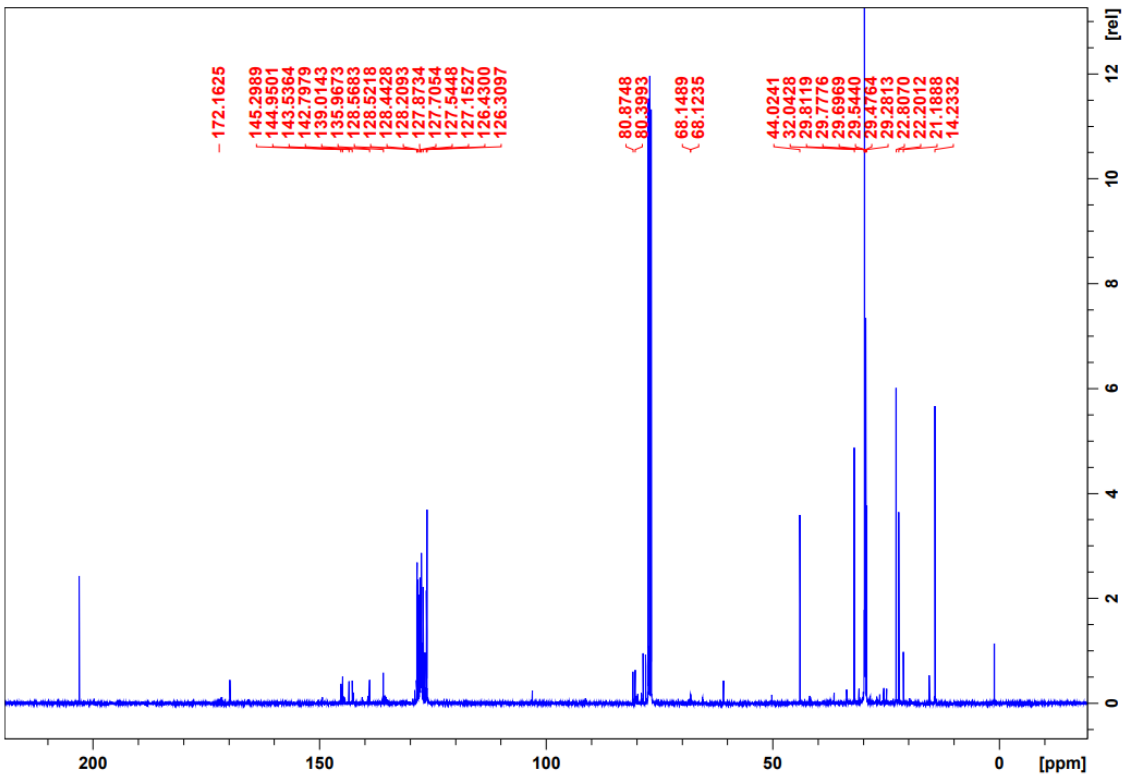


Figure 5.5-3: ^{13}C-NMR of crude aldol reaction mixture to compound **6**

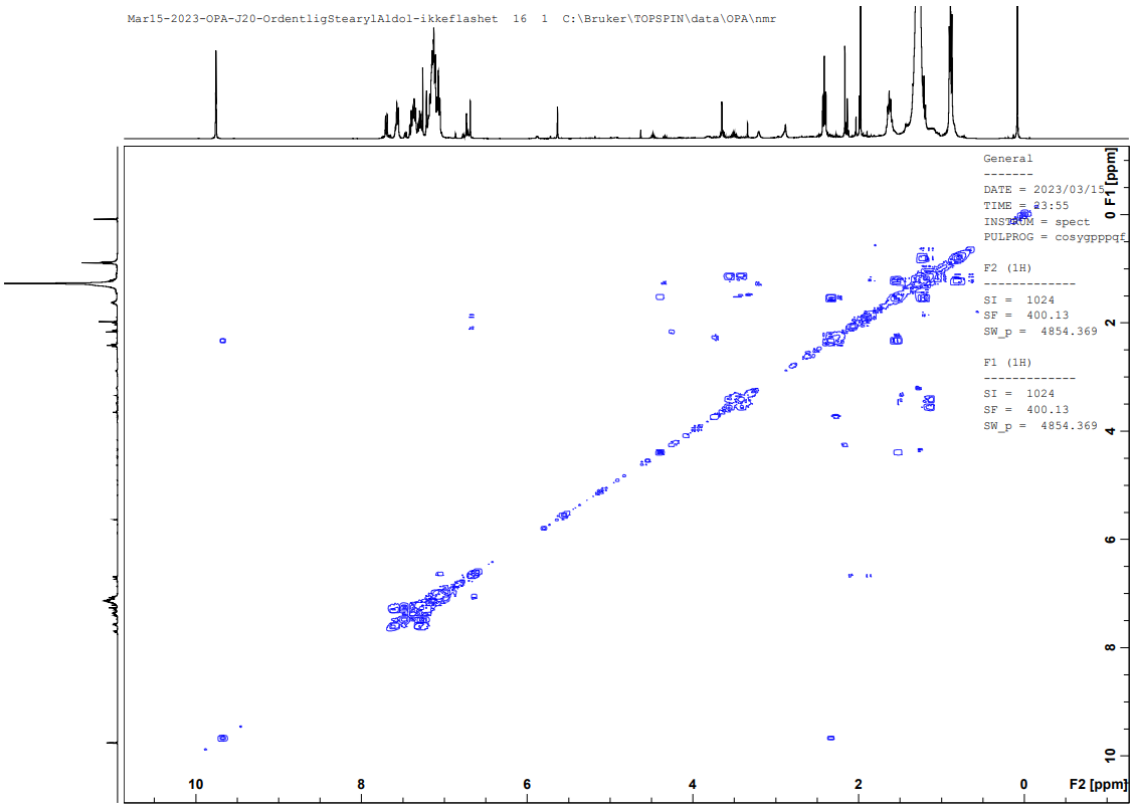


Figure 5.5-4: COSY-NMR of crude aldol reaction mixture to compound **6**

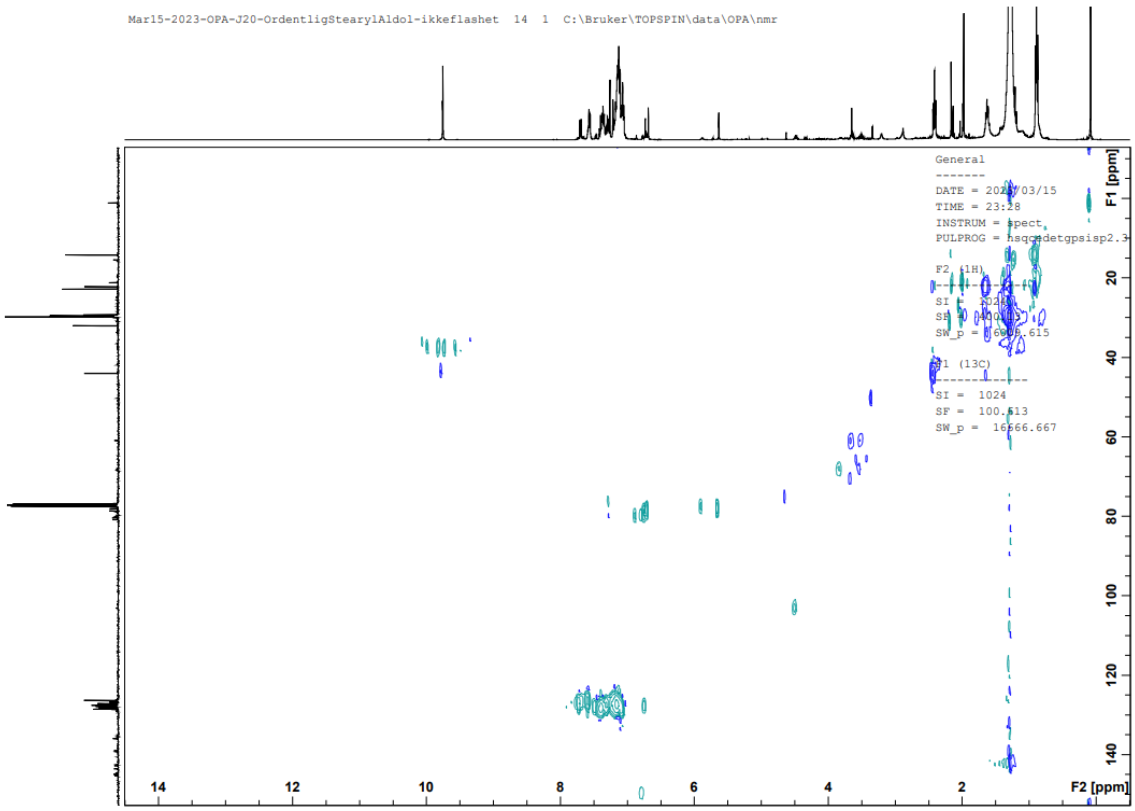


Figure 5.5-5: HSQC-NMR of crude aldol reaction mixture to compound **6**

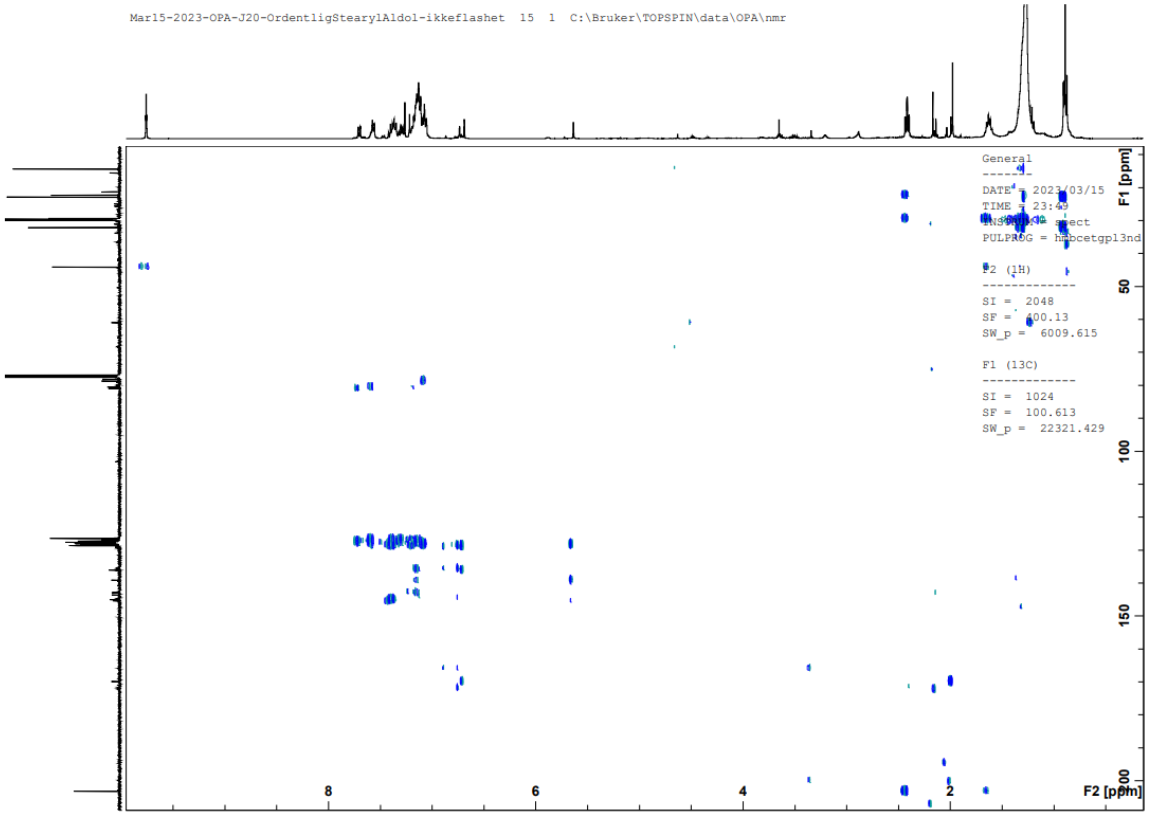


Figure 5.5-6: HMBC-NMR of crude aldol reaction mixture to compound **6**

5.6 – Compound 6f

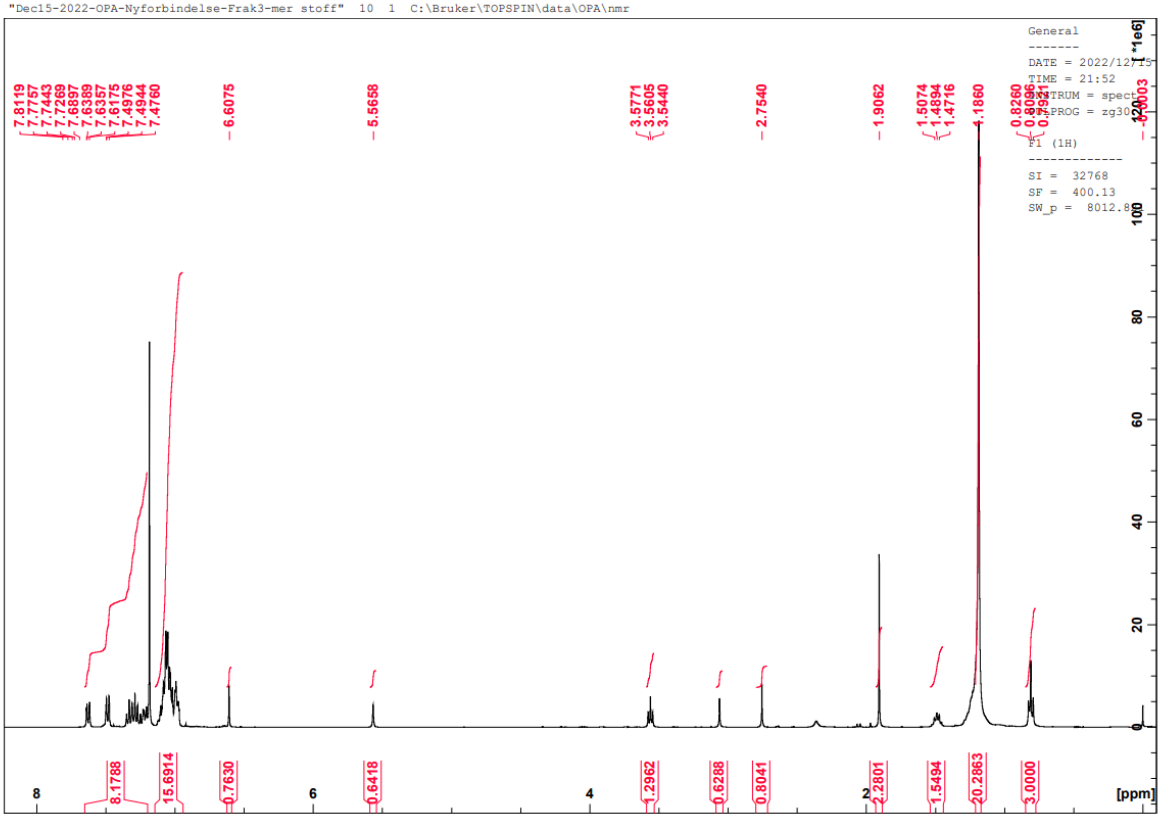


Figure 5.6-1: ^{1}H NMR-spectrum of compound **6f**

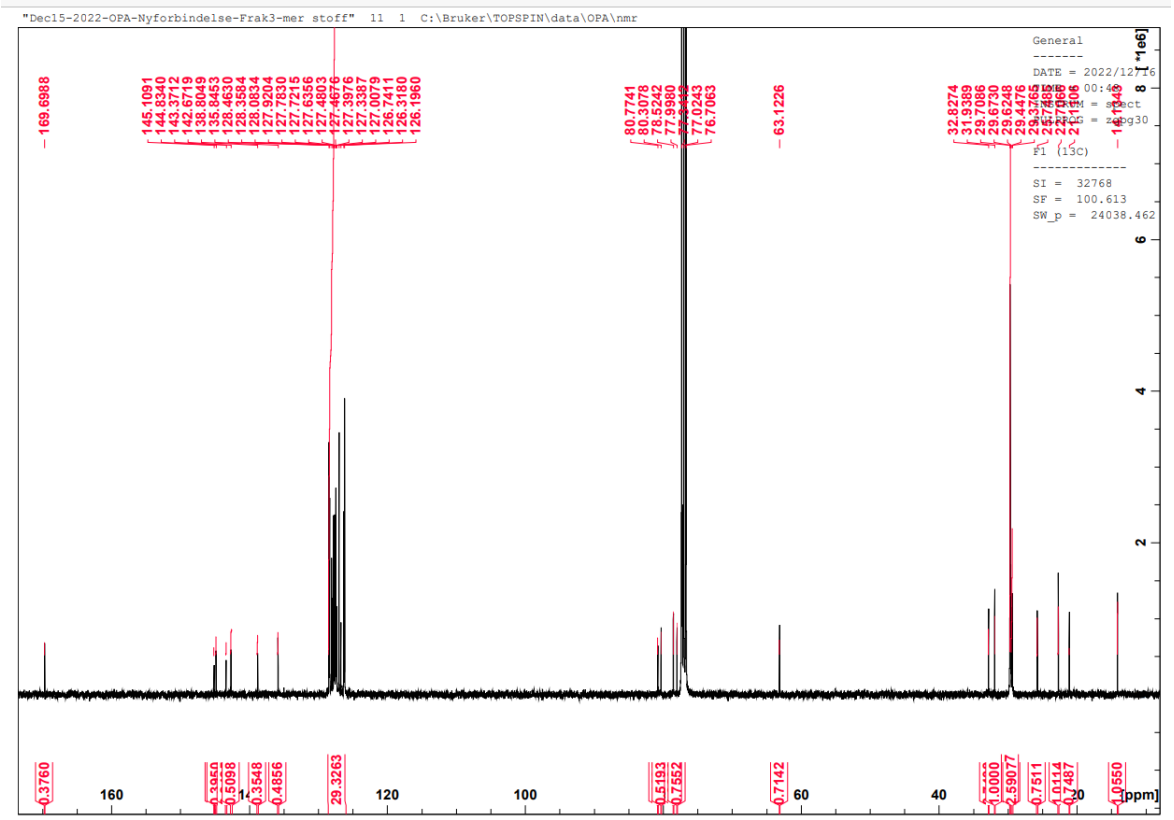


Figure 5.6-2: ^{13}C NMR-spectrum of compound **6f**

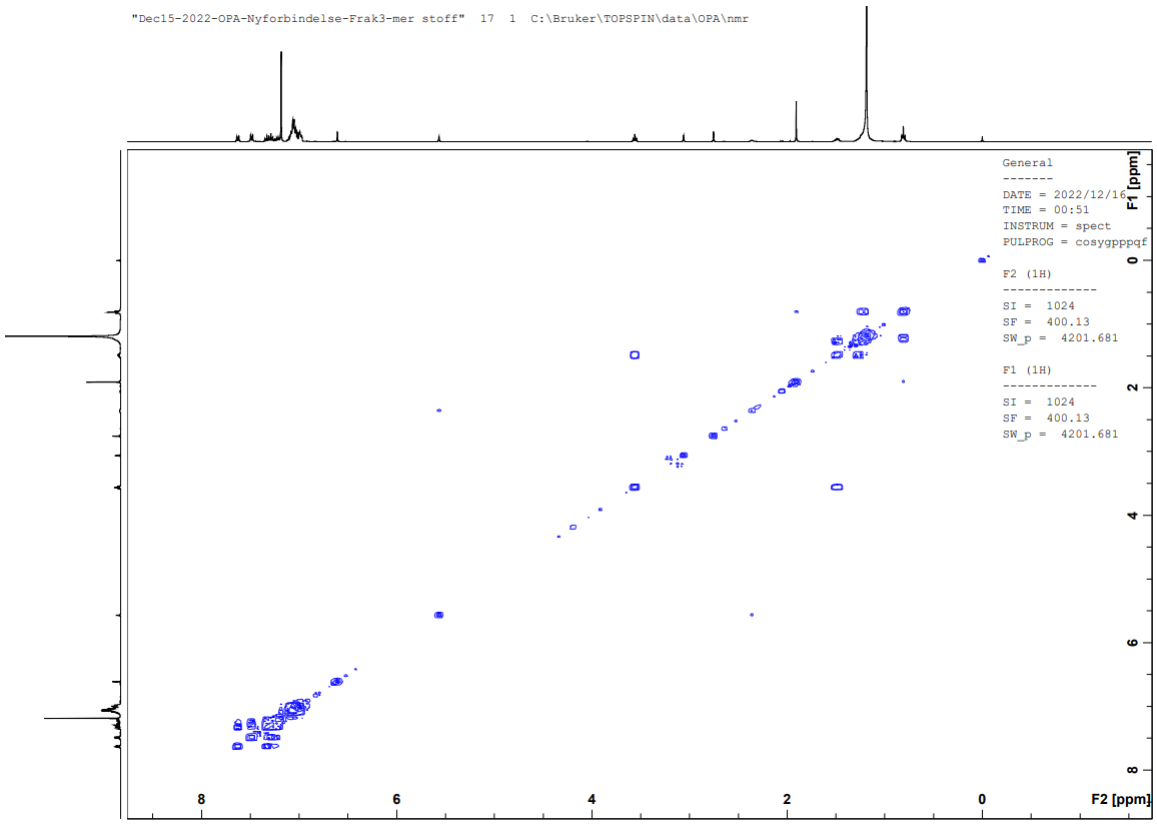


Figure 5.6-3: COSY NMR-spectrum of compound **6f**

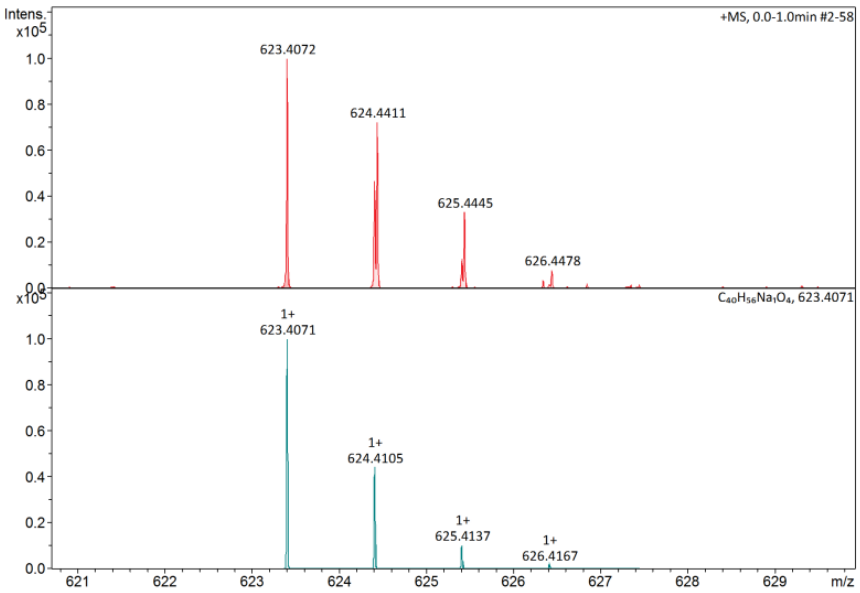


Figure 5.6-4: Mass spectrum of compound **6f,** molecular ions

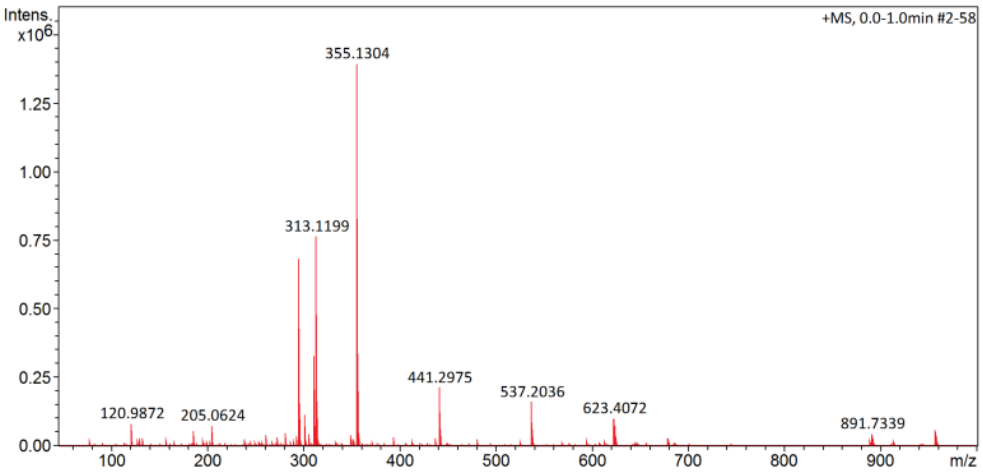


Figure 5.6-5: Full mass spectrum of compound **6f**

5.7 – *R*-Mosher esters of 7a and 7b

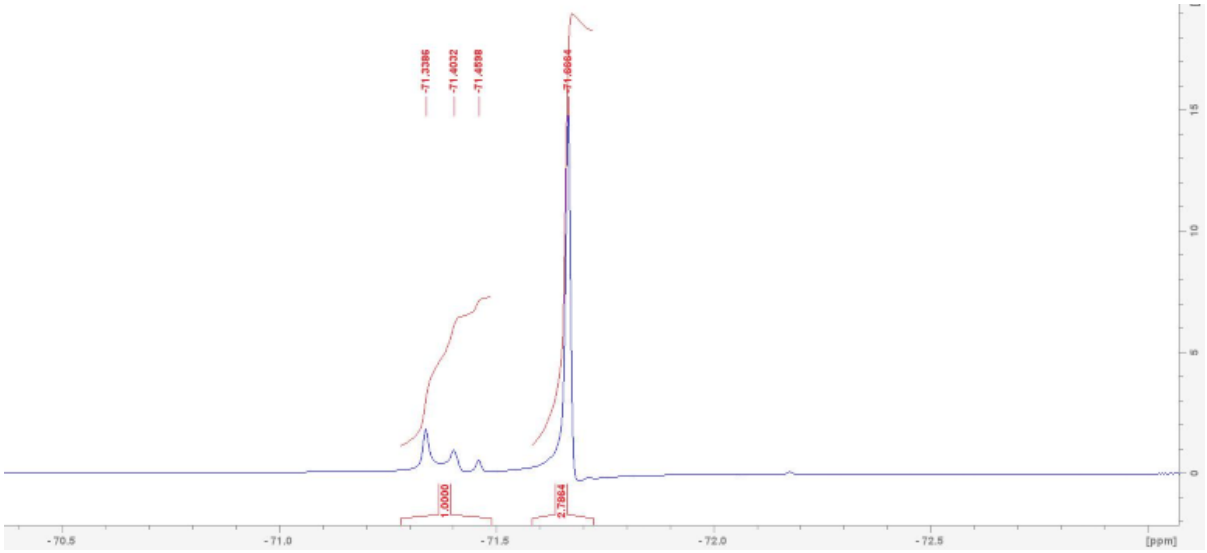


Figure 5.7-1: *R*-Mosher ester derivatised racemic **7a**

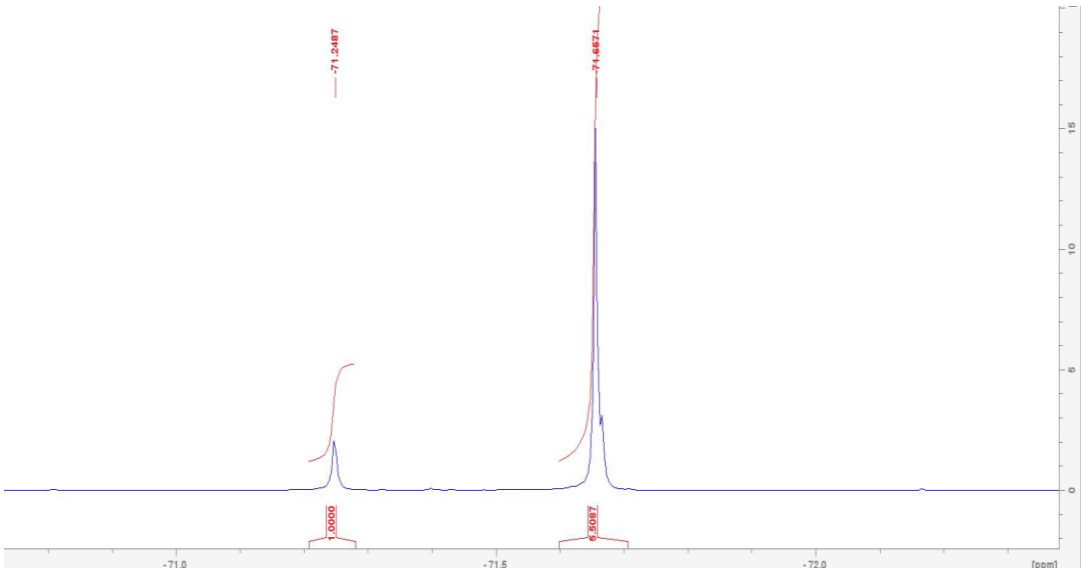


Figure 5.7-2: ^{19}F-NMR of *R*-Mosher ester derivatised **7b**

5.8 – Iodolactone 10

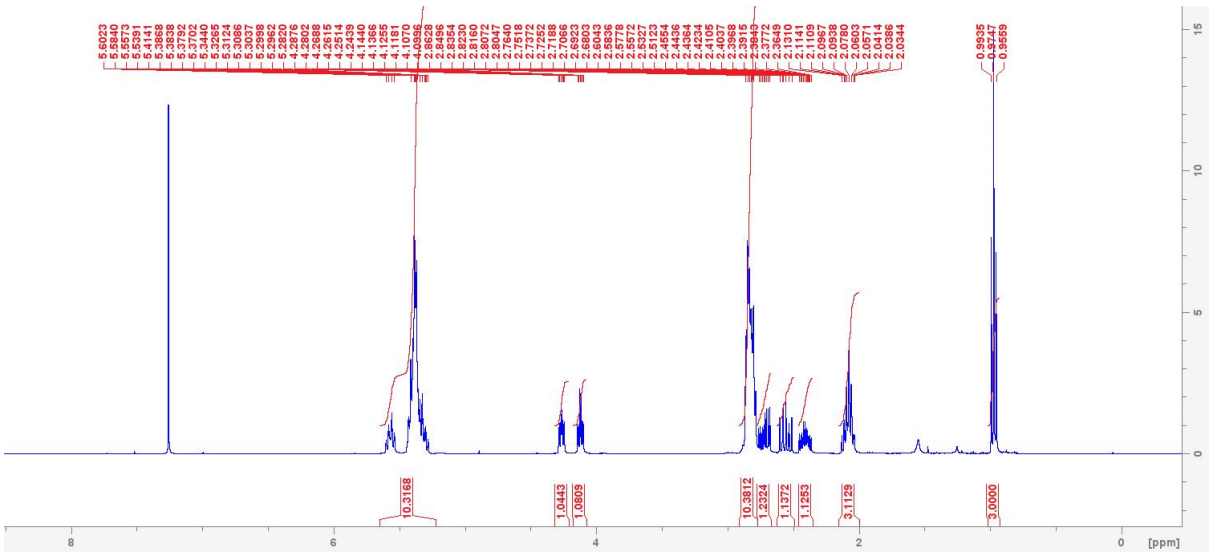


Figure 5.8-1: ^{1}H-NMR of iodolactone **10**

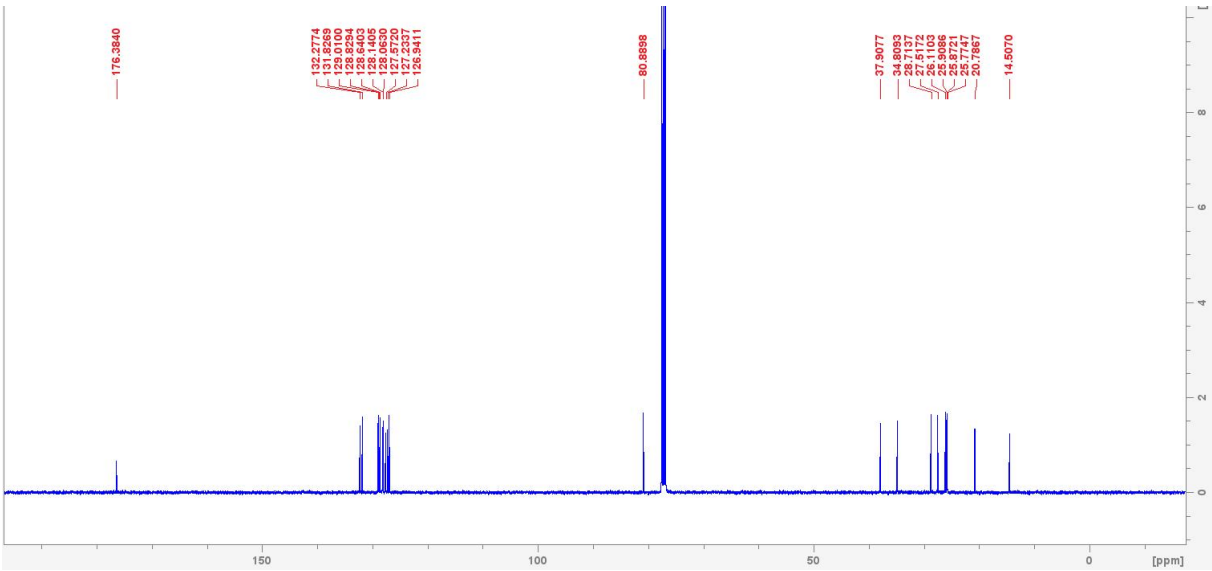


Figure 5.8-2: ^{13}C-NMR of iodolactone **10**

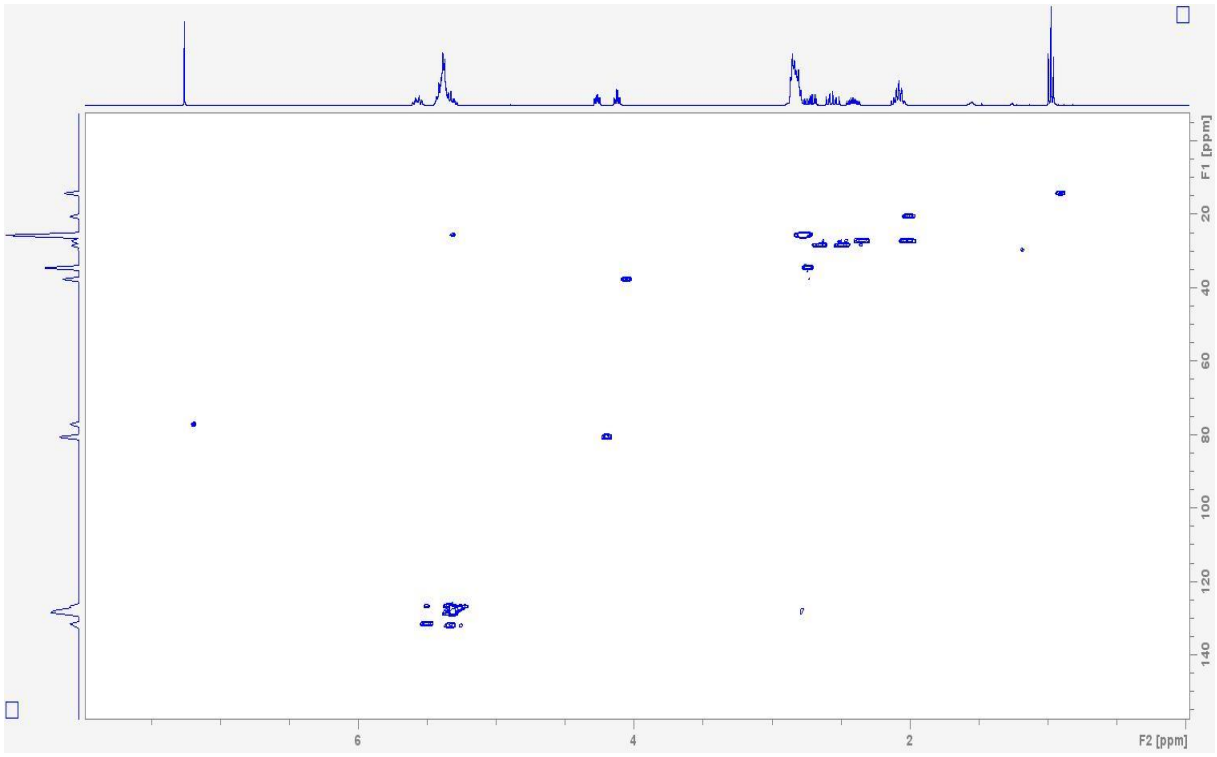


Figure 5.8-3: HSQC-NMR of iodolactone **10**.

5.9 – Diol acid 11

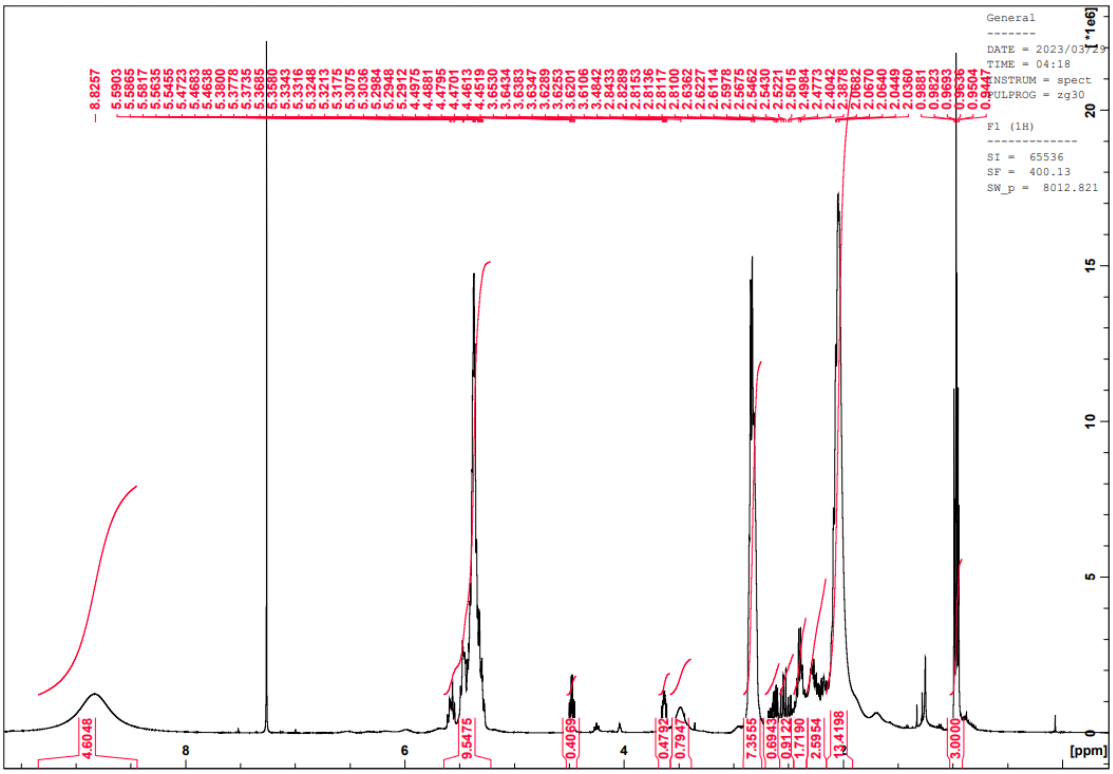


Figure 5.9-1: ^{1}H-NMR of diol acid **11**

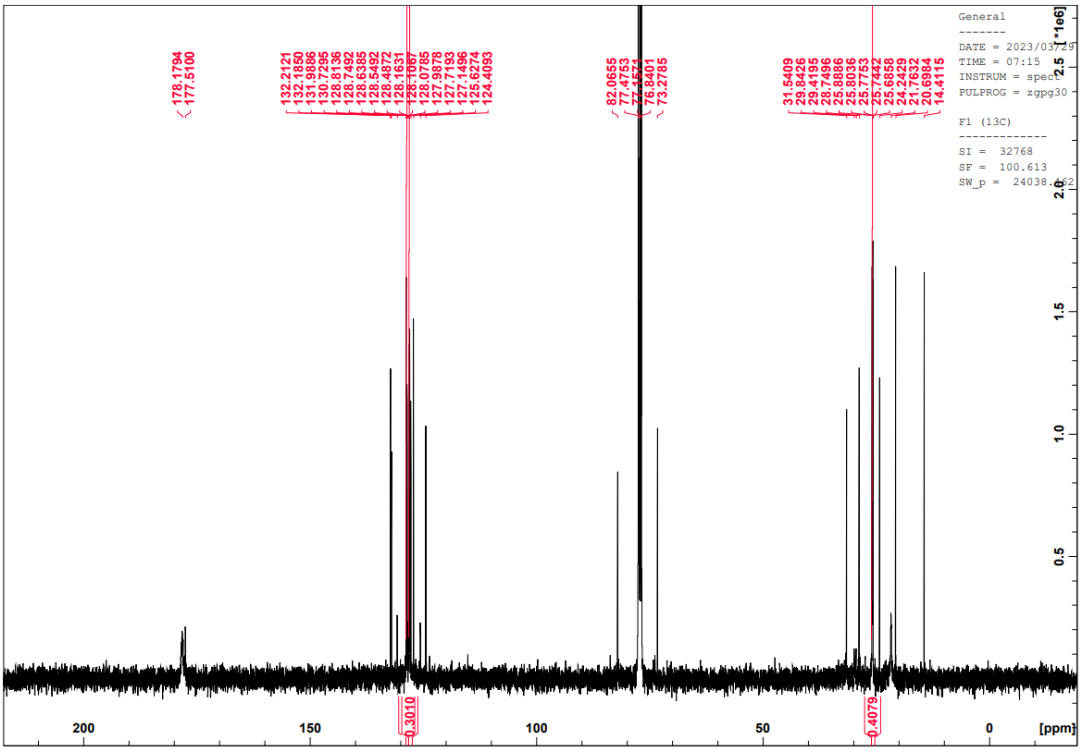


Figure 5.9-2: ^{13}C-NMR of diol acid **11**

5.10 – Octadecapentaenal (12)

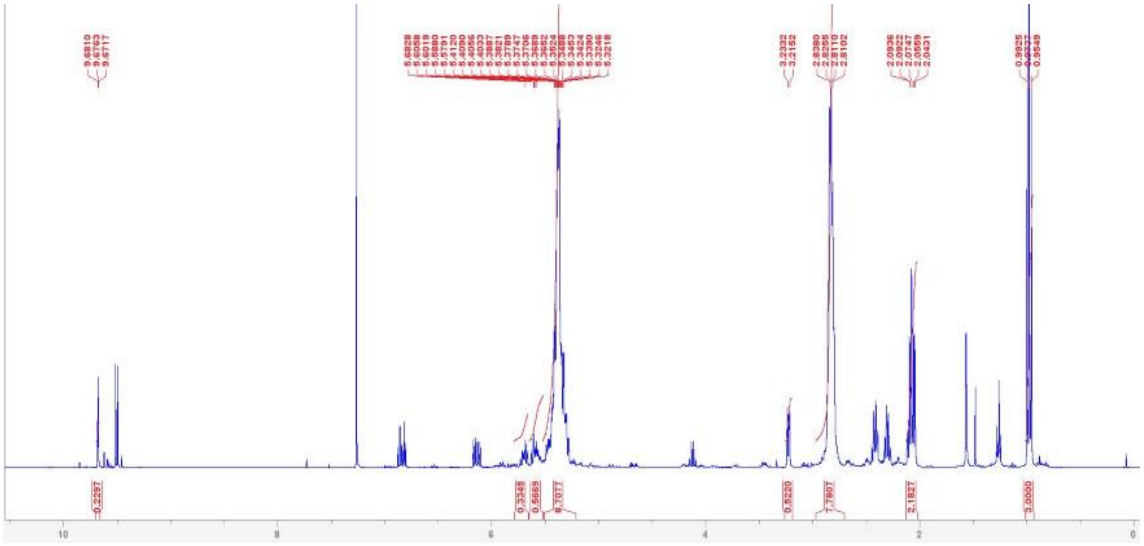


Figure 5.10-1: ^{1}H-NMR of the complex mixture containing octadecapentaenal **12**.

5.11 – Racemic 3-hydroxy-methyl-icosanoate (7a)

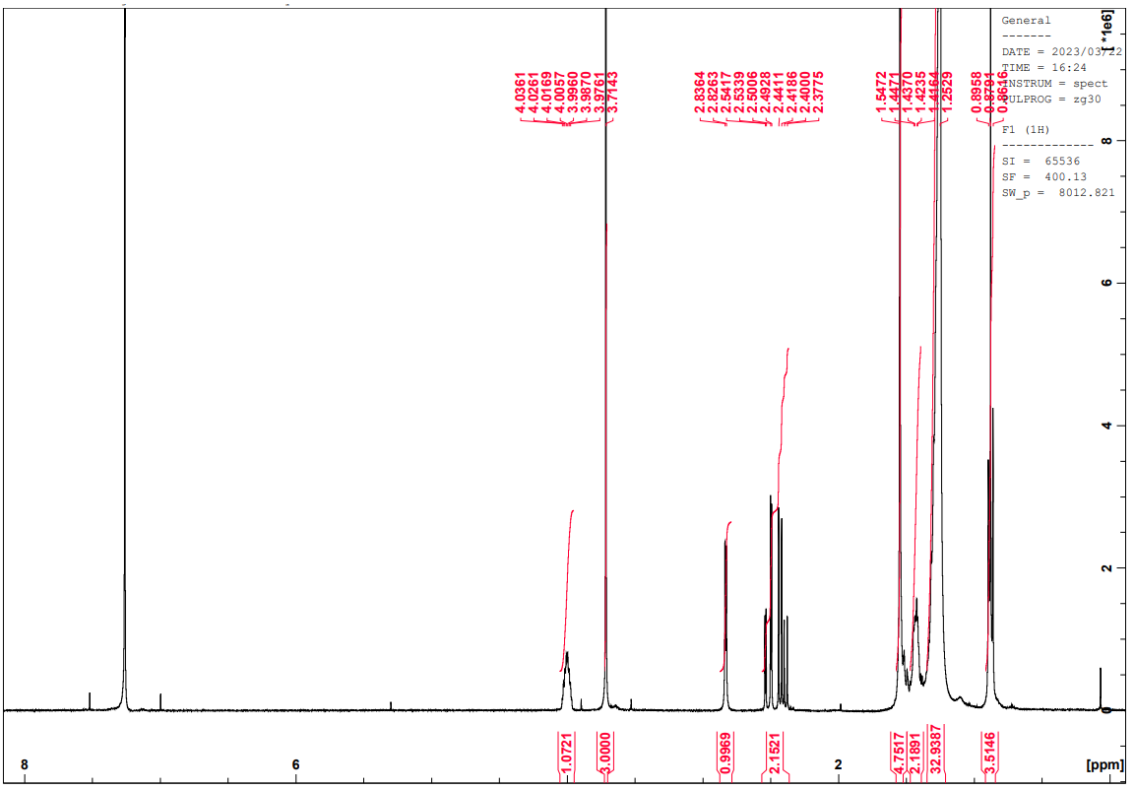


Figure 5.11-1: ^{1}H-NMR of 3-hydroxy methyl ester **7a** through the Reformatsky reaction

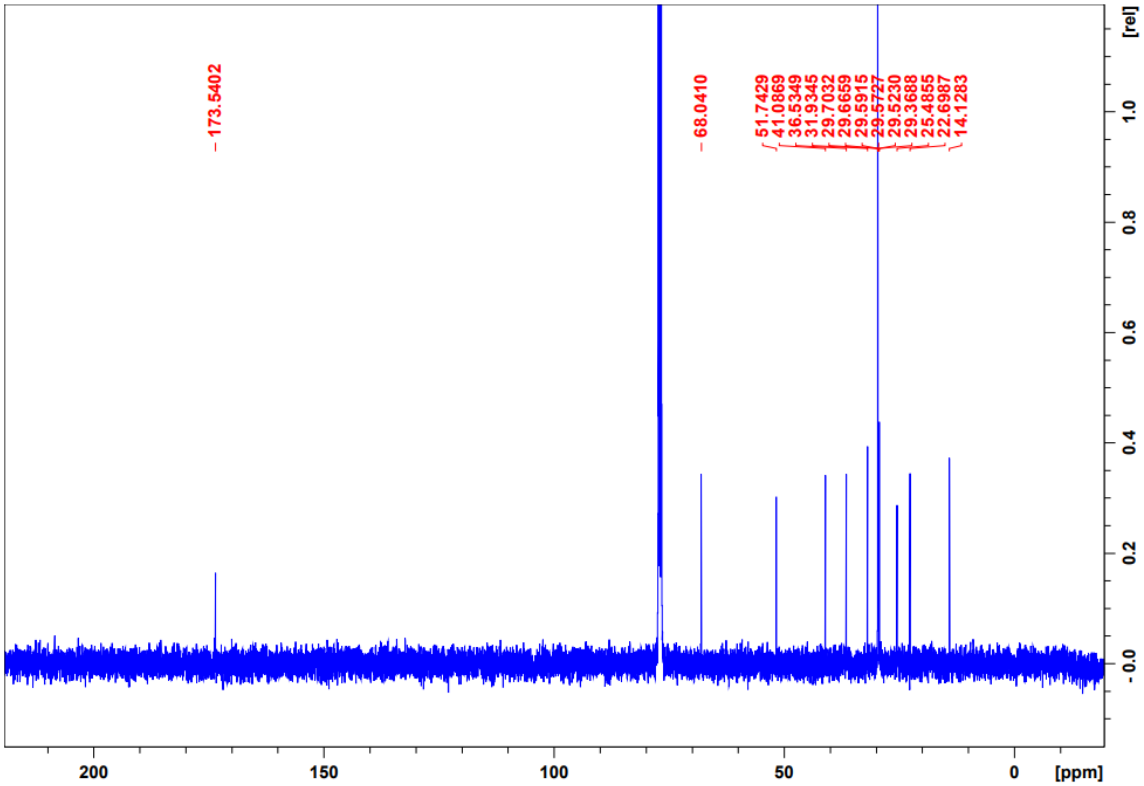


Figure 5.11-2: ^{13}C-NMR of 3-hydroxy methyl ester **7a** through the Reformatsky reaction.

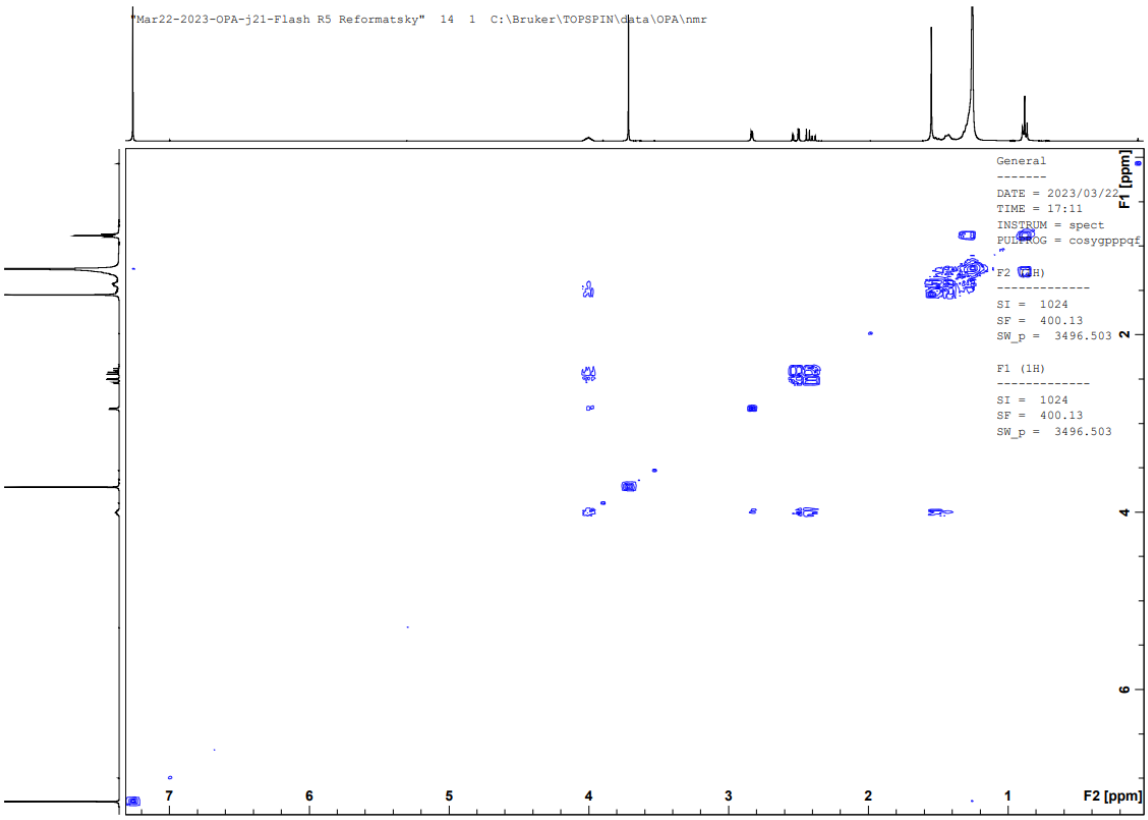


Figure 5.11-3: COSY-NMR of 3-hydroxy methyl ester **7a** through the Reformatsky reaction

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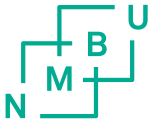

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