Synthesis of saturated, mono- and polyunsaturated methoxylated ether lipids

Svanur Sigurjónsson

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Synthesis of saturated, mono- and polyunsaturated methoxylated ether lipids

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Dissertation submitted in partial fulfillment of a
*Philosophiae Doctor de*gree in Chemistry

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Abstract

Methoxylated ether lipids (MELs) are a subset of 1-*O*-alkyl-*sn*-glycerols that are found ubiquitously in nature in very low concentrations. The MELs occur in significantly higher amounts in the liver oil of shark and related cartilaginous fish species. These compounds have not been explored in a meaningful capacity and their function and physiological effects are therefore completely unknown. They can be described as a glycerol molecule ether-linked with a fatty hydrocarbon tail which can be saturated, monounsaturated, or polyunsaturated, possessing the methoxyl group. A remarkable omega-3 polyunsaturated DHA-like MEL derivative had been discovered in shark liver oil, and the main focus in the work described in this thesis was on its highly successful asymmetric synthesis. This synthetic task may be divided into two challenging parts. Firstly, the design and synthesis of a double-chiral synthon, regarded as the "head piece" of the MEL structure, and the control of its diastereomeric purity. Secondly the synthesis of the omega-3 polyunsaturated fatty chain regarded as the "tail" of the MEL structure and the considerable effort that went in to synthesizing and purifying compounds with up to six all-*cis* skipped double bond structures. The methodology developed in this work was used to synthesize the total of ten saturated, monounsaturated, and polyunsaturated MEL derivatives of which six are present in shark liver oil. A literature survey on the nature of skipped poly-yne/ene synthesis is contemplated in much detail in this thesis, both from the historical and experimental point of view.

Some preliminary studies done mostly in the 1970's showed interesting biological effects, such as anti-tumour, anti-bacterial and immune stimulating properties. These studies were all conducted with isolated mixtures of compounds or synthesized compounds with a mixture of diastereomers. Their low concentration in nature makes them difficult and arduous to isolate and study. This thesis describes a new synthetic strategy which allows access to any imaginable individual MEL compound in high diastereomeric purity which can hopefully rekindle interest in these mysterious compounds.

Útdráttur

Metoxýleruð eter lípíð (MEL) tilheyra undirflokki 1-*O*-alkýl-s*n*-glýseróla og finnast hvarvetna í náttúrunni í afar lágu magni, en koma fyrir í mun ríkari mæli í lifrarlýsi hákarla og annarra brjóskfiska. Þessi efni eru ekki mikið rannsökuð og virkni þeirra og tilgangur algerlega óþekktur. Þau hafa til að bera mettaðar eða ómettaðar kolvetniskeðjur (fituhalinn) settar metoxyl-hópnum, og eru þær tengdar sem eter inn á glýseról hluta sameindarinar (hausinn). Mjög áhugaverð MEL afleiða hefur fundist og verið greind í hákarlalýsi og hefur hún sömu kolefniskeðju og hin mikilvæga ómega-3 fjölómettaða fitusýra DHA. Megináhersla þessarar ritgerðar og rannsóknanna að baki henni fól í sér efnasmíðina á þessari heillandi ómega-3 fjölómettuðu MEL afleiðu og tókst hún með miklum ágætum. Henni má skipta í tvo meginþætti. Annars vegar efnasmíðin á svokölluðum haus-hluta þessara MEL efna með tvö hendin kolefni og rúmefnafræðilegur hreinleika hans; hins vegar efnasmíði fjölómettuðu kolvetniskeðjunnar með sex tvítengi og stjórnun á *cis*-skipaðri rúmefnafræði þeirra. Aðferðafræðinni var beitt til efnasmíða á samtals tíu MEL afleiðum, mettuðum, einómettuðum og fjölómettuðum og hafa sex þeirra fundist í hákarlalýsi. Í ritgerðinni eru efnasmíðar á fjölómettuðum alkýn og alken keðjum skoðaðar og tilraunum til smíða á slíkum efnum lýst í sögulegu samhengi.

Nokkrar rannsóknir gerðar á MEL efnablöndum framkvæmdar á áttunda áratuginum sýndu fram á margvíslega lífvirkni, á borð við krabbameinsdrepandi, bakteríudrepandi og ónæmisvekjandi áhrif. Hinn lági styrkur þessara efna í náttúrunni gerir það að verkum að erfitt er að nálgast þau á hreinu formi til að rannsaka nánar. Þessi ritgerð lýsir nýrri synþetískri nálgun sem gerir efnasmíðar á þessum efnum mögulegar í háum efna- og rúmefnafræðilegum hreinleika. Þetta hefur vonandi það í för með sér að aukinn áhugi vakni aftur á þessum athyglisverðu efnum og þau þá rannsökuð meira og ýtarlegar.

This work is dedicated to my wife Lena Rós Jónsdóttir

And my children Leila and Grjóti

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Abbreviation

ALA	α-Linolenic acid
ARA	Arachidonic acid
ATR	Attenuated total reflection
CAL-B	*Candida antarctica* lipase B
DAGE	Diacyl glyceryl ethers
DBN	1,5-Diazabicyclo[4.3.0]non-5-ene
DBU	1,8-Diazabicyclo[5.4.0] undec-7ene
DCM	Dichloromethane
de	Diastereomeric excess
DHA	Docosahexaenoic acid
DIBAL-H	Diisobutylaluminium hydride
diglyme	Diglycol methyl ether
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DPA	Docosapentaenoic acid
eda	Ethylenediamine
EDCI	1-Ethyl-3-(3-dimethyl aminopropyl)carbodiimide
EE	Ethyl esters
ee	Enantiomeric excess
EL	Ether lipid
EPA	Eicosapentaenoic acid
FGI	Functional group interconversion
FT	Fourier transform
GC	Gas chromatography
GLA	γ-Linolenic acid
GLC	Gas-liquid chromatography
HKR	Hydrolytic kinetic resolution
HMPA	Hexamethylphosphoramide
HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectrometry
IR	Infrared
IUPAC	International Union of Pure and Applied Chemistry
LA	Linoleic acid
LC	Liquid chromatography
m-CPBA	meta-Chloroperoxy-benzoic acid
MEL	Methoxylated ether lipid
MS	Mass spectrometry
NaHMDS	Sodium bis(trimethyl)amide

NMR	Nuclear magnetic resonance
PAF	Platelet activating factor
PUFA	Poly unsaturated fatty acid
r.t.	Room temperature
RP	Reverse-phase
SDA	Stearidonic acid
Sia	Siamyl
sn	Stereospecific numbering
SPM	Specialized pro-resolving mediators
TAG	Triacylglycerol
TBAB	Tetrabutylammonium bromide
TBACl	Tetrabutylammonium chloride
TBAF	Tetrabutylammonium fluoride
TBDPS	tert-Butyldiphenylsilyl
TBS/TBDMS	tert-Butyldimethylsilyl
TES	Triethylsilane
TFA	tri-Fluoroacetic acid
THF	Tetrahydrofuran
THP	Tetrahydropyranyl
TLC	Thin-layer chromatography
TMDSO	Tetramethyldisiloxane
TMS	Trimethylsilane
UV	Ultraviolet

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First of all, I would like to thank my supervisor Guðmundur G. Haraldsson for this opportunity and the amazing experience I have acquired throughout these years. Furthermore, I would like to thank him for his support, guidance, friendship, advice and especially for introducing my now wife into my life. This truly has been a life-changing event for me.

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1 Scope of this work

Methoxylated ether lipids (MELs) are relatively unexplored compounds found in nature. The objective of this work was to synthesize a few different derivatives and then submit them to biological screening tests to gain more information about their biological effects. Only the synthetic part of the project has been completed and is described in this thesis. Prior to this synthetic work, a liver oil sample from sharks or dogfish of the order of Squaliformes, most likely a mixture of the liver oil of three species, Portuguese dogfish (*Centroscymn-us coelolepis*), Black dogfish (*Centroscyllium fabricii*) and Leafscale gulper shark (*Centrophorus squamosus*) was obtained. The liver oil was examined as a part of a M. Sc. project[1] and from this sample a fraction of MELs was isolated and then investigated further. Using LC-MS methods seven different MEL components were identified. MELs **1**-**3** are well known and the most prevalent MELs known to be present in shark liver oil. MEL **4** is also known to be present as is the remarkable DHA-like MEL **5**, the main synthetic challenge of this work. In addition to these, possible MELs having the 18:1 and 18:3 structures for their respective carbon chains were detected and MELs **6**-**9** were all likely or potential structures for these compounds. Finally, MEL **10** was thought to be of interest from a curiosity point of view as a structural analogue to the n-3 polyunsaturated DHA-like MEL **5**. All these compounds, seen in Figure 1.1, were synthesized during this work, where the main synthetic challenges proved to involve the stereochemistry of the chiral centres[2] and the synthesis and purification of the poly-ene framework for the DHA-like MEL **5**.[3] The MS/MS fragmentation spectra of all the synthesized structures were compared to the equivalent natural compounds from the original shark and dogfish sample in an attempt to confirm the natural structure of each compound.

Chapter 2 introduces the main underlining concepts of natural lipids, ether lipids and finally methoxylated ether lipids. Chapter 3 provides an overview of polyenoic synthesis in a historic perspective. The focus is on methylene interrupted (skipped) poly-ene chains which is the natural order in which polyunsaturated fatty acids (PUFAs) are structured. In this chapter the main strategies and methods involved in their synthesis as well as their known drawbacks and difficulties are detailed. In Chapter 4 the MEL syntheses that have been reported in the literature are shortly summarized and then the main synthetic challenge of this project, the synthesis of the DHA-like MEL **5** is described.[3] Chapter 5 is devoted to the total syntheses of all the other MELs that were performed for this project,[4, 5] as well as the syntheses of a few natural PUFAs, mainly stearidonic acid (SDA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). This chapter also includes the MS/MS fragmentation comparisons between the synthetic and natural MEL compounds. Finally, Chapter 6 covers in detail the main challenges in the poly-ene synthesis and the steps taken to address them. This includes the diastereomeric purity of the samples,[2] the optimization of the poly-yne semi-hydrogenation reactions and the purification of the resulting products.

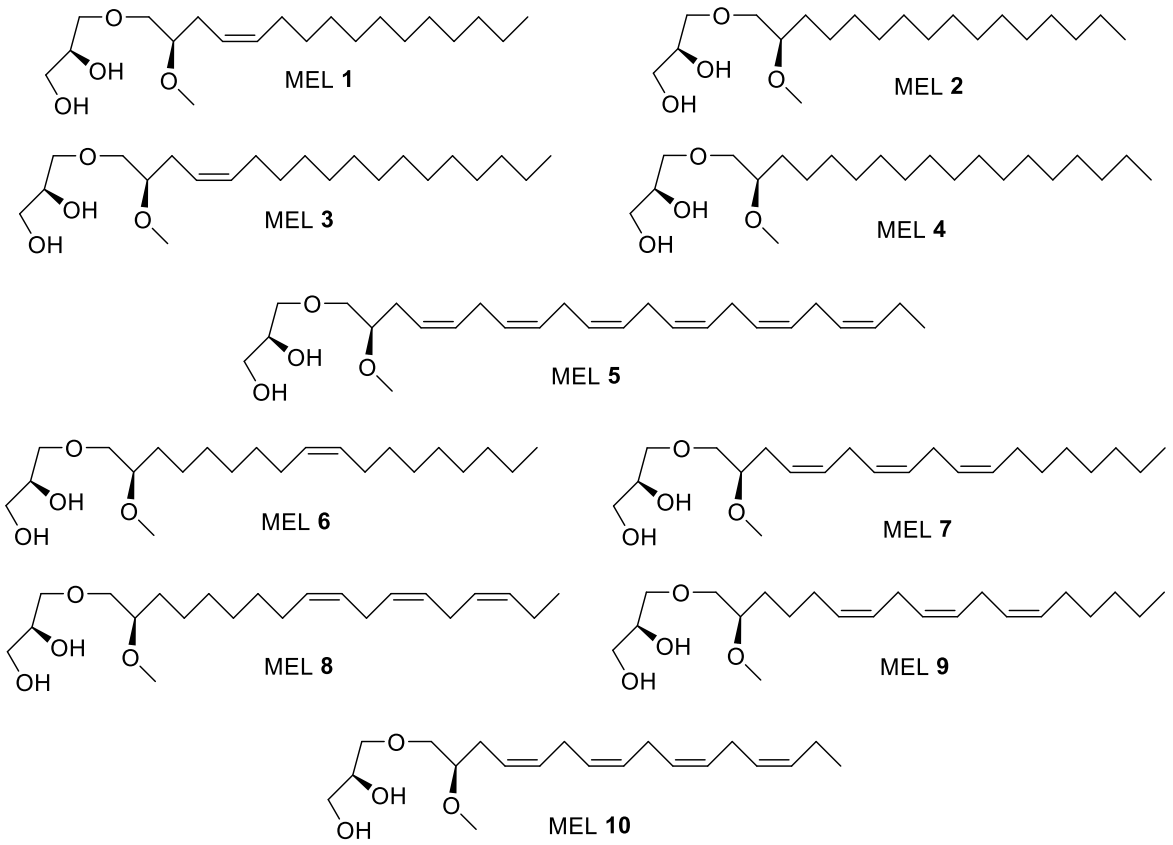


Figure 1.1 All the MEL compounds synthesized for this project.

All compounds synthesized as part of this project and described in this thesis are labelled with a bold number from **1**-**92**. Compounds described as a part of others work and not synthesized for this project are labelled in the same way except with the chapter number in front (example: **3.1-3.130**).

2 Introduction

Lipids in nature are biomolecules that contain hydrophobic hydrocarbon chains. Lipids fulfill many important structural and functional roles in living cells. Many types of lipids exist in organisms such as sterols, certain vitamins, sphingolipids, waxes, and numerous derived signal molecules. The focus in this introduction in Chapter 2 will be on fatty acids and their glycerol derivatives, glycerolipids, which make up the bulk of natural lipids.

Chapter 2 is divided into the four following sections. Section 2.1 will outline the basics of fatty acids and glycerolipids, describe the different types, their nomenclature and stereochemistry. Section 2.2 will cover the role and importance of omega-3 PUFAs. Section 2.3 briefly covers the glycerolipid subgroup, the 1-*O*-alkyl-*sn*-glycerols sometimes just called ether lipids (ELs). Finally, Section 2.4 will then cover in some detail the subgroup of ether lipids, the methoxylated ether lipids (MELs) which syntheses are the main subject of this thesis.

2.1 Fatty acids and glycerolipids

Fatty acids are carboxylic acids with an aliphatic hydrocarbon chain. In nature the number of carbon atoms in the carbon chain is usually an even number between 12 and 22, though shorter, odd number of carbons, super-long and branched chains also exist in abundance. Fatty acids are systematically named according to the IUPAC system of nomenclature but both systematic names as well as common names usually with historic origin are widely used. The carbon next to the carboxylic acid functional group is denoted as the alpha (α) carbon and the CH_3 carbon at the other end is denoted as the omega (ω) carbon. This is illustrated in Figure 2.1.

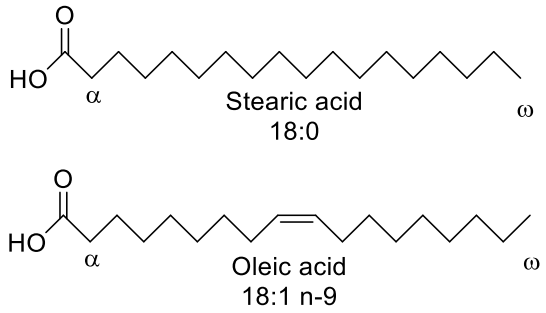


Figure 2.1 Stearic acid as an example of a saturated fatty acid and oleic acid as an example of an unsaturated fatty acid, denoting the alfa and omega end.

A very important feature of fatty acids is their unsaturation. Fatty acids can be saturated, having no carbon-carbon double bond in the hydrocarbon chain or unsaturated, where there are one or more double bonds in the chain. These double bonds generally have the *cis* configuration and appear at certain positions in the chain. Fatty acids with more than one double bond are called polyunsaturated fatty acids (PUFAs). Polyunsaturation on natural

fatty acids generally follows the same structure, where each double bond is separated by a methylene (CH_2) group, sometimes called skipped or methylene interrupted double bonds. The position of the double bonds on the carbon chain is an important feature as well and the fatty acid is given an omega number based on the position of the double bond closest to the omega end. Fatty acids are commonly classified in terms of their omega number (n-3, n-6, n-9, etc.). Short hand notation of fatty acids can then generally describe the most common and important natural fatty acids, X:Z n-y, where X is the number of carbons, Z is the number of double bonds and n-y is the omega number. This is illustrated in Figure 2.2 for arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[6]

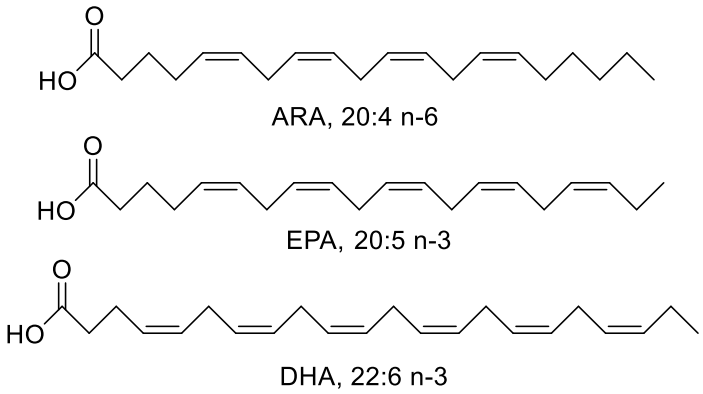


Figure 2.2 The most important PUFAs and their shorthand notation. Arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

The glycerol molecule is a small polyol compound abundant in all living organisms and serves as the backbone in all glycerolipids. The glycerol molecule is a prochiral compound. When one of the terminal alcohol groups are changed, the molecule becomes chiral and can be assigned an *R* or *S* configuration. To differentiate between these two terminal alcohols the stereospecific numbering system (*sn*) is used. If depicted as a Fischer projection where the secondary hydroxyl group in the middle is pointing to the left-hand side the three alcohol groups on the glycerol can be numbered 1 to 3, where *sn*-1 is the pro-*S* alcohol on the top, *sn*-2 is the secondary alcohol in the middle and *sn*-3 is the pro-*R* alcohol on the bottom.[7] This is illustrated in Figure 2.3.

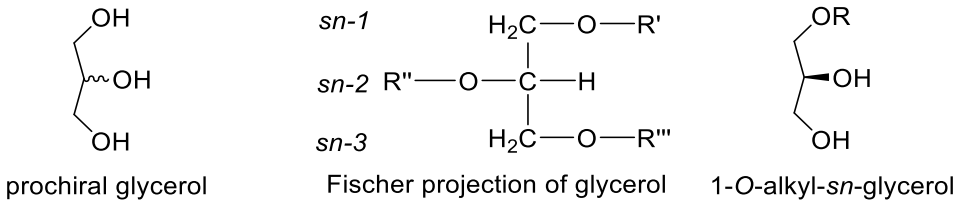


Figure 2.3 The prochiral glycerol molecule, Fischer projection of a glycerol showing the stereospecific numbering (*sn*) system and an example of the natural *sn*-1 configuration of an ether lipid.

Glycerolipids are composed of a glycerol molecule connected to at least one fatty acid with an ester link or a fatty chain with an ether linkage. To this group belong all glycerophospholipids, which are the main building blocks of cell membranes, and triacyl glycerols (TAGs). In nature fat is stored as TAGs where three fatty acids are attached to a glycerol moiety. Glycerophospholipids similarly consist of a glycerol backbone connected

to two fatty acids with the third group being a hydrophilic phosphate group, usually modified with choline, ethanolamine, serine, or inositol. Another subgroup of glycerolipids would be the 1-*O*-alkyl-*sn*-glycerols, sometimes just called ether lipids (ELs), glyceryl ethers or alkyl glycerols, where one of the fatty chains are connected to the glycerol by an ether linkage. Alkyl glycerols in nature are generally of the *sn*-1 type while phospholipids have the phosphate group in the *sn*-3 position, depicted in Figure 2.4.

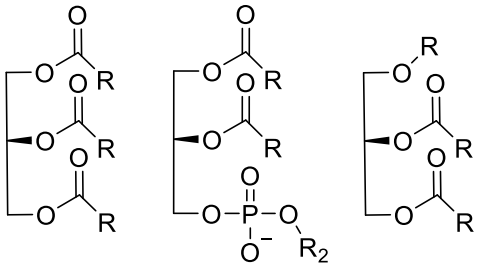


Figure 2.4 General structures of TAGs, phospholipids and alkyl glycerols. R = any alkyl chain. R_2 = any phospholipid modifying group

2.2 The Omega-3 polyunsaturated fatty acids

Linoleic acid (LA, 18:2 n-6) and alfa-linolenic acid (ALA, 18:3 n-3) are essential fatty acids for humans and other animals as they must be ingested from food and cannot be synthesized in their body. In animals these fatty acids serve as precursors to the more polyunsaturated fatty acids (PUFAs) such as ARA, EPA and DHA in the omega-6 and omega-3 enzymatic pathways.[8] These PUFAs then serve as precursors to eicosanoids which are signaling molecules and mediators in cells and have been found to influence immensely important and diverse bodily processes such as stimulating or reducing inflammation, allergy, fever and more immune responses as well as regulating cell growth, blood pressure, pain perception and regional flow of blood to tissues. The conversion of ALA in the body to the longer n-3 PUFAs, EPA and DHA, seems to be very low in humans, especially to DHA. The conversion to DHA in the body seems to be only enough to keep tissue functions while elevated EPA and DHA levels in tissues can be reached through diet or supplementation.[9]

Health effects of omega-3 PUFAs in the diet have been a subject of research for a long time. Scientific publications researching the effect of dietary EPA and DHA are in abundance and have established numerous beneficial health benefits, such as, significantly reducing cardiovascular events.[10] Meta-analysis of randomized controlled trials indicates that EPA and DHA reduce systolic and diastolic (if >2g/day) blood pressure especially among hypertensive individuals.[11] Prescription of >4g/day of omega-3 PUFAs has also been shown to lower triglycerides in individuals with hypertriglyceridemia, especially those with severe triglyceride elevations.[12] High levels of these PUFAs are found in the brain and numerous clinical trials have pointed to the beneficial effects of dietary EPA and DHA on reducing depression and slowing cognitive decline in normal adults as well as improving cognitive impairments in people with mild Alzheimer´s disease symptoms.[13] DHA deficiency in newborns is also associated with visual impairment and delayed cognitive development.[14] In addition to all these claimed health effects, the exact physiological effect on the cell membranes in which EPA and DHA are found in

phospholipids is not very well established. A few studies have probed for some answers and polyunsaturated phospholipids have been found to facilitate membrane deformation and fission[15] as well as being enriched especially in the *sn*-2 position in plasmalogens and ether lipids which also have special physiological effects in cell membranes.[16]

Both EPA and DHA are important precursors to many interesting oxidated signaling molecules called eicosanoids, further classified as specialized pro-resolving mediators (SPMs), converted in the body by enzymatic manipulation. These mediators have been classified further as lipoxins[17] derived from arachidonic acid (ARA), resolvins[18] derived from EPA, n-3 Docospentaenoic acid (DPA, 22:5 n-3) and DHA, protectins[19] derived from n-3 DPA and DHA and finally, maresins[20] derived from DHA. Some examples are provided in Figure 2.5.

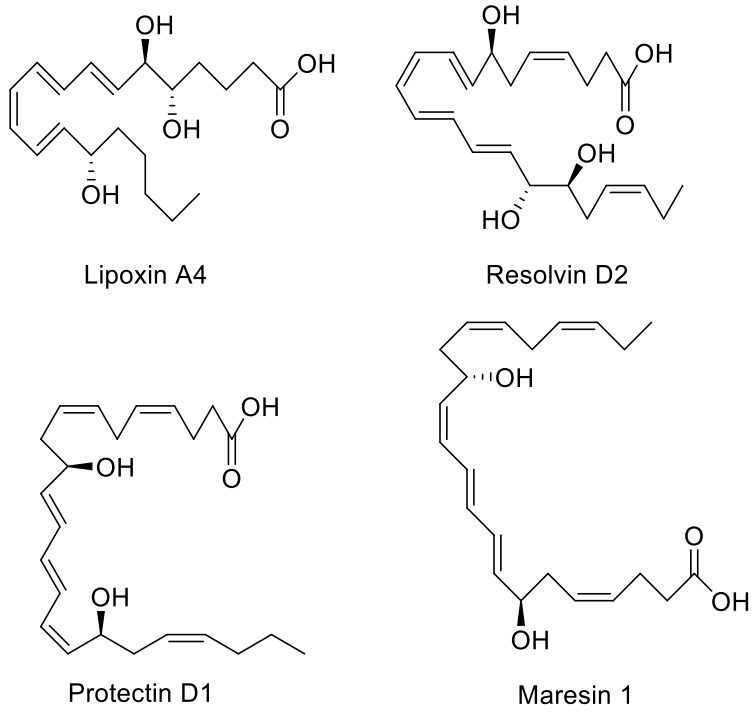


Figure 2.5 Examples of lipoxin, resolvin, protectin and maresin molecules.

These PUFA derived SPMs have been studied and reviewed much in the last two decades and while there is still a lot unknown, immense progress has been made about the knowledge of their structure, stereochemistry, mechanism of action, metabolism, receptor interactions and *in vivo* effects. Many interesting properties have already been associated with these molecules such as to inhibit and resolve inflammation in a range of disease models, control pain perception and stimulate tissue regeneration.[20] Also, inhibiting influenza virus replication and improving survivability and pathology of severe influenza in mice[21], counteract obesity-induced adipose tissue inflammation[22], implicated as attractive means to treating arthritis[23], reprogram host immune responses[24] and many more. Finally, enriched marine oil supplements have been found to increase specialized pro-resolving mediators in blood.[24] The ubiquitous presence and immense effect of SPMs in the human body should reinforce the idea of the apparent health benefits of PUFAs in the diet and have made for an exciting field of research with huge drug development opportunities.

2.3 1-O-alkyl-*sn*-glycerols

Ether lipids (ELs) are found in Archaea (with *sn*-3 proclivity opposite of other organisms) strictly anaerobic bacteria, protozoans, and metazoans (animals) but they are interestingly almost completely absent in fungi or plants.[25] ELs of the type 1-*O*-alkyl-*sn*-glycerols were first discovered in the unsaponifiable fraction of a starfish in 1915.[26] These are glycerol-based molecules where the acyl group in the *sn*-1 position on the glycerol is replaced by an alkyl group. These molecules have been found to constitute, as diacyl glyceryl ethers (DAGEs), a large percentage of the liver oil of many cartilaginous fish, sometimes up to an astounding 89%.[27] Alkylglycerols are mostly found to have an alkyl chain composition consisting of an even number of carbons ranging from 14-22, saturated or monounsaturated. Many types of shorter, polyunsaturated, branched, and odd-numbered chains have been reported but usually in trace amounts or only found in certain species. Alkylglycerols 16:0, 18:0 and 18:1 n-9 are by far the most prevalent types and usually account for more than 80% of all alkylglycerols in shark liver oil.[28] They have been named chimyl-, batyl- and selachyl alcohols, respectively, as based on the chondrichthyan fish species where they are commonly found, ratfish (Chimaeras), rays (Batoidea) and sharks (Selachii) (see Figure 2.6). These molecules have been demonstrated to be ubiquitously found in most animal tissues, marine or land, where they are usually more commonly found as phospholipids.[29, 30] The discovery of the platelet activating factor (PAF) which is an alkyl ether phospholipid (see Figure 2.6) with diverse physiological functions especially in inflammation,[31] showed for the first time how these molecules had important functions in the body.

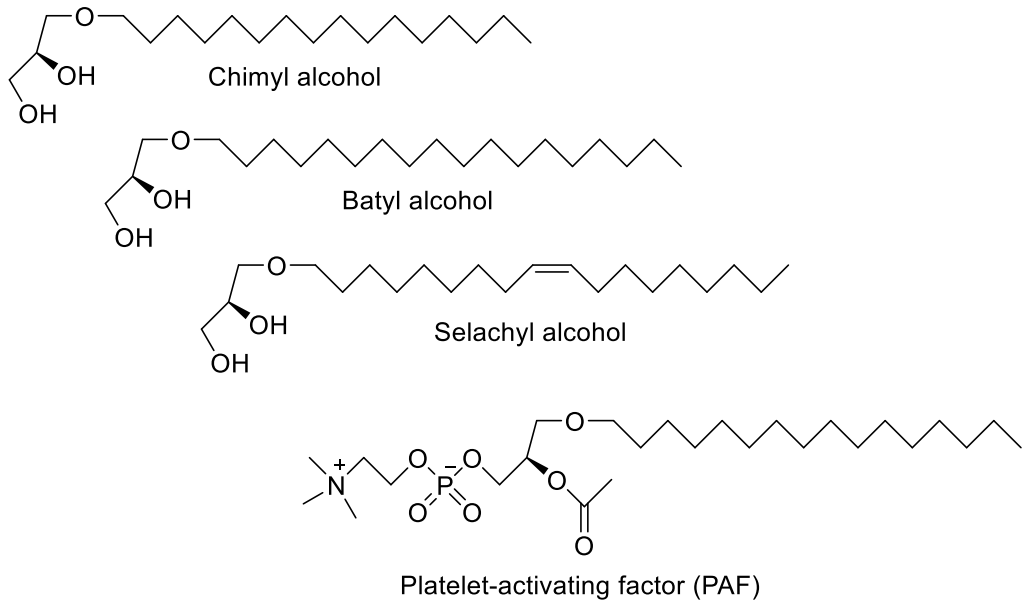


Figure 2.6 The most common alkylglycerol types and the much-studied PAF.

The apparent health benefits of shark liver oil, which is rich in 1-O-alkyl-*sn*-glycerols, has been witnessed in Iceland and Scandinavia for centuries as the oil has been used for preventative measures as well as directly against many ailments such as indigestion, gastric ulcers, colon inflammation, scrofula, arthritis and on open wounds.[32] In more recent decades the 1-O-alkyl-*sn*-glycerols and/or shark liver oil have been found to exert some biological effects such as lowering radiotherapy-induced injuries[33], reducing tumor growth

and metastasis[34-36], immunostimulant properties[37] and facilitating movement of drugs through the blood brain barrier[38]. Later Deniau et al. demonstrated how these anti-tumor capabilities were tied specifically to the unsaturated ELs which exerted much higher growth depression and lowering of metastasis than the saturated types.[39, 40]

The *O*-alk-1´-enyl types are similar molecules where there is a *cis* double bond on the first carbon adjacent to the ether bond making it effectively a vinyl-ether bond. These molecules appear as neutral alkyl-diacyl glycerols or as alkyl-acyl phospholipids and have been given the names neutral plasmalogens and plasmalogens, respectively, (see Figure 2.7). Plasmalogens are a major constituent in mammalian cell-membranes, and can make up to 20% of the phospholipid mass in humans.[41] Plasmalogens have very different chemical and physical properties from other ether lipids and have been studied extensively in the last few decades.[25] Initially their function was believed to be mainly connected to their antioxidant capabilities as the vinyl bond is very labile to reactive oxygen species but they also have very different physical roles in cell membranes compared to alkyl phospholipids as they induce closer packing, decreased fluidity and increase order in the membrane.[42] In cell membranes alkenyl phospholipids are concentrated in cell membrane rafts and are particularly enriched in certain tissues in the nervous, immune and cardiovascular systems.[43]

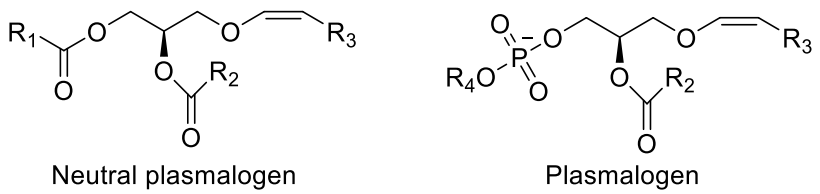


Figure 2.7 Neutral plasmalogen and plasmalogen examples, R_1, R_2, R_3 = any fatty chain, R_4 = any phospholipid modifying group

Different plasmalogen levels between healthy tissues and tissues in pathological condition have been found, especially in brain tumors. Their deficiency has also been observed in Alzheimer patients and other neurological conditions[43] as well as affecting membrane traffic and function.[44] Two diseases connected to impaired ether lipid synthesis are known in humans, Zellweger syndrome and Rhizomelic chondrodysplasia punctate (RCDP) which patients have very short life expectancy.[45] Ether lipid deficient mice exhibited numerous and serious health problems[46] as well as behavioral phenotypes mimicking human psychiatric disorders.[47] Human longevity and ageing have also been connected to certain ether lipid profiles.[48] The direct effects of ether lipids and especially plasmalogens have thus been observed in numerous studies as well as possible indirect effects connected to their enriched PUFA content.

The still unclear molecular and bio-functional roles of plasmalogens as well as unknown sub-cellular distribution and metabolism connections to other lipids such as sphingolipids and cholesterol make it hard to understand causality in the relationship between these molecules and diseases. Alkyl glycerols and alkenyl glycerols have very different distribution in cells. Alkyl glycerols are more common than alkenyl glycerols in neutral lipid fragments and alkenyl glycerols are much more abundant in phospholipid fragments.[29] Biosynthesis of these compounds are very well studied and both are tied to peroxisomes in cells.[42] Both types are usually also enriched in PUFAs such as DHA and ARA in the *sn*-2 position which then serve as an important reservoir for eicosanoids. All

this should demonstrate the important functions of these ether lipid molecules which are far from fully elucidated and that should generate some interest as well in yet another sub-type of ELs, the methoxylated ether lipids (MELs) which are much less common than the other two ELs but are also ubiquitously found in animal tissues and which properties and function are entirely a mystery.

2.4 Methoxylated ether lipids

Much of the work on the methoxylated ether lipids (MELs) was pioneered by Hallgren and coworkers who initially discovered them, synthesized them, as well as determined their structure and configuration, some biological activities, and their distribution across some marine and terrestrial tissues. MELs have recently been reviewed partly by Carballeira[49] (2002) and, more recently, in great detail by Magnússon and Haraldsson[28] (2011). Following is a short summary covering the main aspects of the MELs.

MELs are a sub-group of alkylglycerols, where the O-alkyl chain possesses a methoxyl group located on the second carbon from the ether linkage. Alkyl and alkenyl glycerols display greatly differing physiological properties as phospholipids and fulfill some very important biological roles and have therefore been extensively studied. The MELs on the other hand are only found as a minor or trace component in lipid fractions of various tissues and their physiological effects in membranes are completely unknown.

MELs have the same general structure as other glyceryl ethers in animals, their O-alkyl chain positioned on the *sn*-1 carbon of the glycerol backbone, implying an (*S*)-configuration of the glycerol. The chiral second carbon on the O-alkyl chain possessing the methoxyl group has the (*R*)-configuration.[50] Their structures are revealed in Figure 2.8.

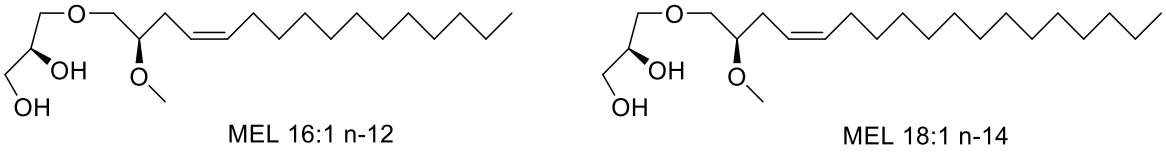


Figure 2.8 The most common methoxylated alkylglycerol types, 16:1 and 18:1.

The MELs were first discovered and isolated in the unsaponifiable fraction of the liver oil of Greenland shark in 1967 by Hallgren and Stallberg.[51] About 4% of the total glyceryl ethers in the oil were these novel MELs. The novel structures discovered then were the MELs 16:0, 18:0, 16:1 and 18:1. Using mass degradation spectra the authors further confirmed that the unsaturated types were comprised of a *cis*-configured double bond on the 4-position on the O-alkyl chain. Other liver oils of cartilaginous fish species have been found to have usually around 0.4-2.5% MELs in the alkylglycerol fraction with some exceptions much higher, reaching up to 14.8%.[28]

A series of MEL compounds were identified in the following years, ranging from a C14 to C22, among which the remarkable polyunsaturated DHA-like 22:6 n-3 MEL **5** (see Figure 2.9), first discovered in 1971.[52] MEL **5** has since then been found in appreciable amounts in some shark or ratfish liver oils (6.5-18% of the MEL fraction).[53] All the other MELs discovered by then were saturated or monounsaturated. Hayashi and Takagi further

identified polyunsaturated types, 18:3 and 22:5, but did not elucidate their exact structure.[53]

Of the MELs discovered vast majority in each sample are usually the 16:0, 16:1 and 18:1 species. These three types are usually around 90% of the total MEL fraction.

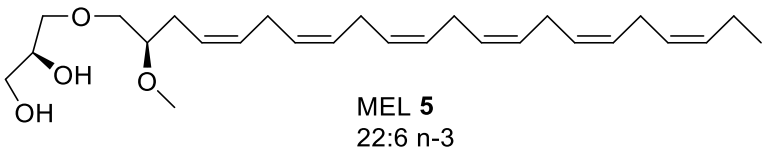


Figure 2.9 The interesting DHA-like all-*cis* 1-O-(*R*)-2-methoxydocosahexaen-4,7,10,13,16,19-yl-*sn*-glycerol, MEL **5**.

In 1974 MELs were reported in trace amounts and up to 0.5% of the total neutral lipids or phospholipids fractions of a few marine animal tissues: herring fillets, Baltic herring fillets, mackerel fillets, marine crayfish, fresh-water crayfish, shrimps, sea mussels and cod liver oil, generally more in the phospholipid fraction. Interestingly, MELs measured to be 25-37% of the alkylglycerol fraction and 10-20% of the phospholipid fraction of these tissues or oils, which is much more in terms of percentage than in the shark oils.[54] MELs were also reported in mammalian tissues: cow's milk, sheep's milk and human colostrum, milk, red bone marrow, blood plasma and uterine carcinoma were studied and trace amounts of MELs were found in all tissues, so it was concluded that these compounds are of common occurrence in animals, but in very low concentrations.[30] Interestingly, the DHA-like MEL **5** comprised a surprisingly high percentage of the MELs in human blood cells (7.5-9.6%).[30] MELs were generally found in considerably higher amounts in marine animal tissues.

Where the biosynthesis of normal alkylglycerols and plasmalogens have been studied in detail the biosynthesis of MELs has never been established. Magnússon and Haraldsson[28] have put forth a likely biosynthetic pathway supported by the findings of hydroxyl-substituted alkylglycerols in shark liver oil.[55] It was initially proposed that plasmalogens were biosynthesized from a 2-hydroxy alkyl intermediates but a preliminary rat study did not support that hypothesis.[55] Now the biosynthesis of ether lipids and plasmalogens are very well established and alkylglycerols are first made into phospholipids and then plasmalogens via 1'-desaturase.[41] The hydroxyl alkylglycerols then remain as likely precursors to the MELs. A possible mode of function for MELs is inhibiting the 1'-desaturase and then disrupting plasmalogen synthesis in the cell causing its cytotoxicity.[56]

A flurry of biological studies in the 1970s by Hallgren and coworkers displayed a variety of biological activities, such as anti-bacterial,[56] anti-fungal,[57] anti-tumor[56, 58-61] and immune-stimulant activities.[62] This sparked some initial interest in these unusual natural compounds but since then they have been only sporadically studied with the last tumor-effect studies done around 2000.[58-60] These studies were all performed with a mixture of alkylglycerols or a mixture of MELs isolated from shark liver oil, or a synthetic stereoisomeric mixture except one[58] which is the only isomerically pure study that has been performed to this author's best knowledge. Owing to the low concentration levels in nature, the tedious isolation process and the unknown absolute configuration at times, the proper screening of the effects of these compounds has been rather difficult. It has therefore been impossible to draw any concrete conclusions regarding the properties of the

MELs as well as make direct comparisons. A convenient asymmetric synthetic strategy could change that.

The synthetic history of the MELs has been reviewed by Magnússon and Haraldsson where synthetic strategies and methodology details are provided.[28] It is rather short and was started with the initial discovery be Hallgren and Ställberg in 1967 who discovered and synthesized MEL 16:0 as a mixture of the four stereoisomers.[51] Almost a decade later in 1975 Ställberg finished the synthesis of the 16:1 MEL also as a mixture of the four stereoisomers.[63] In 1990 the first enantioselective synthesis was completed and all four isomers of 16:0 were synthesized and compared to the natural compound which confirmed the absolute configuration as *S* for the glycerol moiety, making it *sn*-1 and the *R* configuration for the carbon constituting the methoxyl group.[50] Two decades later in 2010 the first enantiomerically pure unsaturated MEL 16:1 was synthesized by Magnússon and Haraldsson.[64] They used the commercially available starting materials (*R*)-solketal and (*R*)-epichlorohydrin to accomplish the right stereochemistry which was then compared to and observed to match with the established value in the literature. Since then the only examples of stereospecific MELs synthesized in the literature are of the non-natural MEL 19:1 derived from oleic acid[65] and two alkyne analogues with the methoxyl group not located on the second carbon.[66]

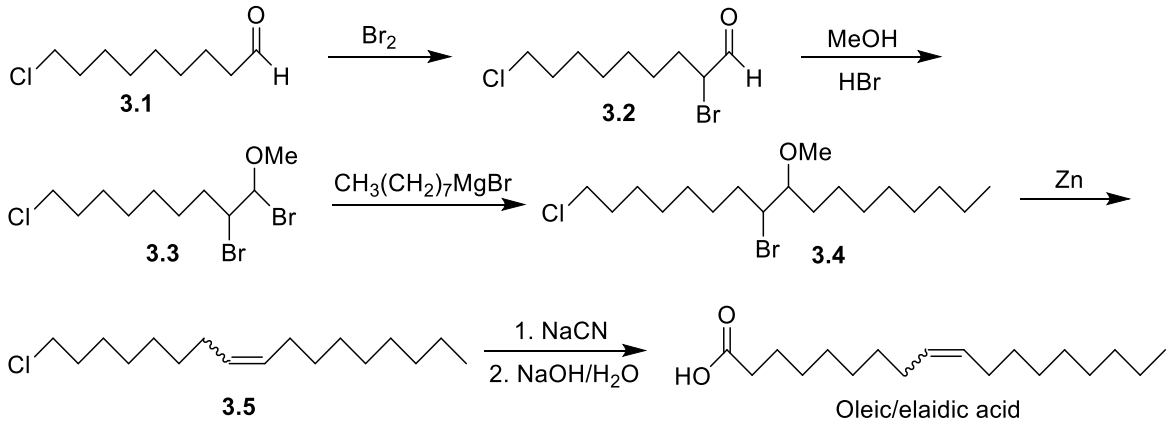
After the total synthesis of enantiopure MEL 16:0 was achieved in 1990 and MEL 16:1 in 2010 not many studies of their biological activity have been carried out and these compounds perhaps fallen in the shadow of their much more abundant cousin the plasmalogens which experienced a huge spike in interest, and publications naming plasmalogens has increased steadily. No natural polyunsaturated MEL has ever been synthesized and studied in any biological studies (only as a minor fraction in the shark liver oil mixtures), and their availability greatly limits the possibilities to research these interesting compounds. Studies on alkyl ether lipids (ELs) have shown that their anti-tumor effects only appeared or were enhanced by unsaturation.[39] The MELs could possibly also be of special interest because of their apparent anti-bacterial effect since finding new natural anti-bacterial compounds has become highly sought after since the rise of antibiotic-immune bacteria has become a global concern.

Unsaturation in ELs has been connected to increased anti-cancer activity compared to their saturated counterparts, but no comparable research has been made for the MELs. Mode of action and general function of the MELs is virtually unknown. However, the fact of their ubiquitous presence in animal tissues and the complex nature of functions, and interactions of glycerolipids with PUFAs, as well as the already demonstrated bioactivity of MELs should spark some interest in learning more about these mysterious molecules.

3 Polyenoic Synthesis

Lipids have immensely important structural and functional roles in living organisms and have therefore been desired targets for organic synthesis for decades. In plants and animals, PUFAs are of special interest. These acids usually have *cis* configured methylene skipped double bonds in their carbon chain and the synthesis of this system is the focus of this chapter. This review of skipped poly-ene synthesis is of special relevance to the work described in chapters 4 and 5 as finding an efficient strategy to synthesize this system was imperative. Reviews have been published largely covering this topic. Polyene acid review from 1999 by Vatale.[67] A review of skipped 1,4-di-ynes synthesis by Tedechi et al.[68] from 2003 and 1,4-di-ene synthesis by Durand et al.[69] from 2000. Finally, a review of alkyne reductions by Oger et al.[70] from 2013.

The first reported synthesis of an unsaturated fatty acid was of the monounsaturated oleic acid, reported by Noller and Bannerot in 1934.[71] The synthesis is depicted in Scheme 3.1 and began with the two-step conversion of the 9-chlorononanal **3.1** through the monobromo derivative **3.2** to the dibromo derivative **3.3**. The subsequent Grignard reaction with octanyl magnesium bromide furnished the bromo-methoxy **3.4**. The double bond in the oleic acid was then formed by a zinc mediated reductive elimination of the 9-bromo-10-methoxy derivative **3.4** producing the double bond as a thermodynamic mixture of the *cis* and *trans*-isomers **3.5**, 34% and 66% respectively.



Scheme 3.1 The first reported total synthesis of a monounsaturated fatty acid, a mixture of oleic and elaidic acids.[71]

Then through a series of crystallizations involving lead acetate or lithium hydroxide the two isomers were separated. This kind of methodology was not very efficient and impossible to use efficiently for polyenoic syntheses. For those purposes, stereoselective techniques were needed. These techniques emerged in the 1950s and 1960s and can generally be sorted as the acetylenic or Wittig approach (see Figure 3.1). The acetylenic approach involves the sequential syntheses of the 1,4-diyne (1,4-diacetylene) moiety to produce a skipped poly-yne compound which is then stereoselectively semi-hydrogenated into an all-*cis* skipped poly-ene. The details of the development of the acetylenic approach are described in Section 3.1 and the following stereoselective semi-hydrogenation of poly-

ynes is then discussed in Section 3.2. The Wittig approach employs sequential Wittig reactions reacting together an ylide and an aldehyde to stereoselectively produce a *cis* alkene. The *cis* compound is then chemically altered to form a new terminal aldehyde or ylide to allow it to couple to another Wittig reagent. The details of the development of the Wittig approach in skipped poly-ene synthesis are provided in Section 3.3. Finally, a few alternative strategies and methods are then covered in Section 3.4.

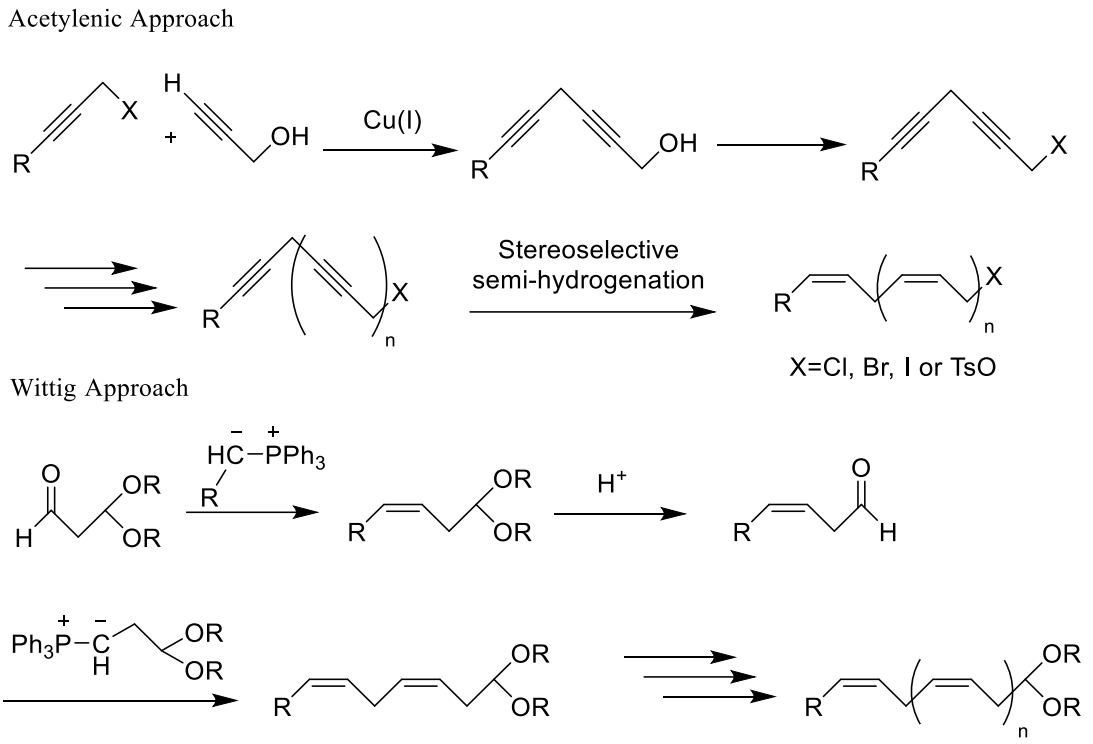
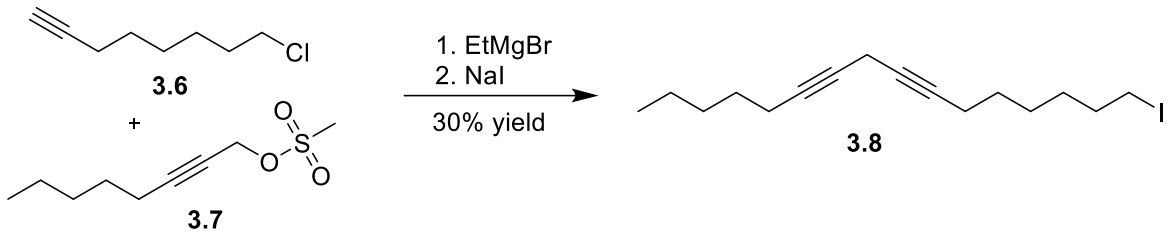


Figure 3.1 The two main approaches to methylene skipped poly-ene synthesis, the acetylenic (top) and Wittig (bottom) approaches.

3.1 The Acetylenic approach

The acetylenic approach involves the formation of a skipped poly-yne which is then stereoselectively semi-hydrogenated to the corresponding all-*cis* poly-ene. By 1950s for the first time the stereoselective synthesis of *cis* double bonds became available to chemists using palladium on calcium carbonate for catalytic semi-hydrogenation of acetylenes. The first synthesis of linoleic acid (LA) was reported by Raphael and Sondheimer in 1950 who employed this technique to yield a product that was deemed to be 63% LA in purity by crystalizing and weighing the bromination product.[72] In this paper, Raphael and Sondheimer also described the synthesis of the 1,4-di-yne, or 1,4-diacetylene, which until then had barely been reported synthesized except at high temperatures and in very low yields. The authors mentioned the instability and reactivity of the 1,4-di-yne system, which makes it hard to work with and is probably one of the main drawbacks of the acetylene approach in poly-ene synthesis. The authors described their 1,4-di-yne synthesis such that they slowly added dropwise the Grignard reagent of 8-chloro-oct-1-yne **3.6** into a solution of the propargyl methanesulfonate (mesylate) **3.7** in ether and refluxed for 14 hours (see Scheme 3.2). This was to prevent extra alkylation on the labile methylene

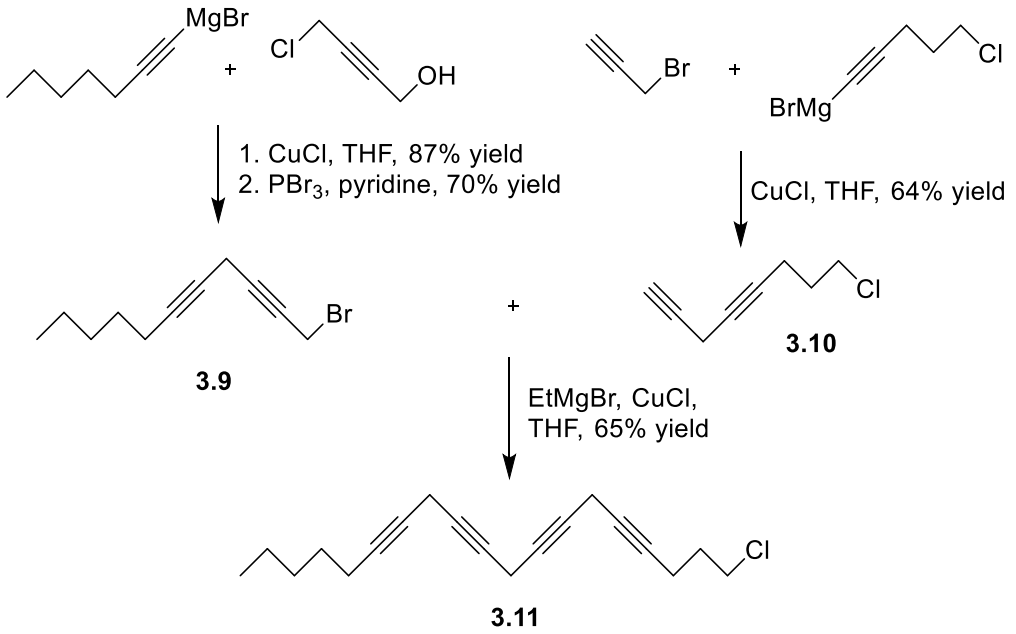
group. After obtaining the crude di-yne product still contaminated with the mesylate reagent it was submitted to a Finkelstein reaction with NaI and finally, the resulting iododi-yne **3.8** was isolated in 30% yield.



Scheme 3.2 The initial Grignard type reaction producing an 1,4-diyne.[72]

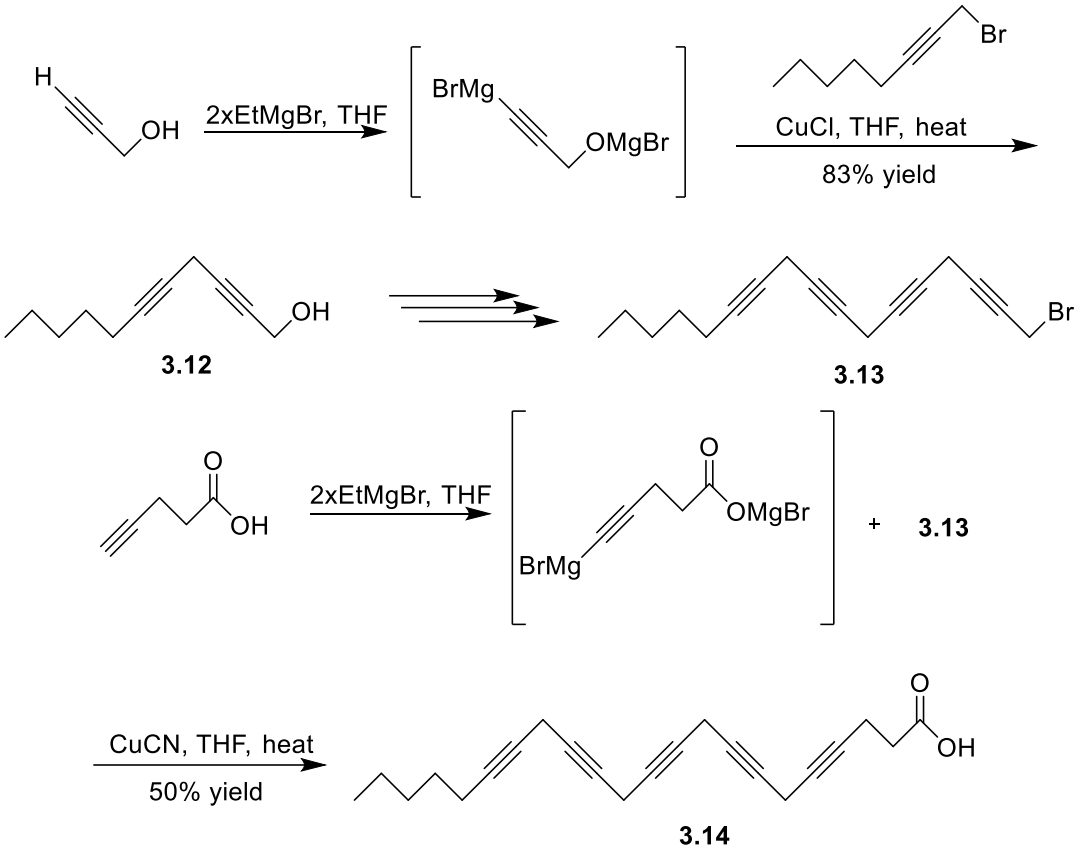
Back in 1936, Danehy et al. had discovered that cuprous salt was necessary to facilitate a reaction between a Grignard agent and an allyl bromide.[73] Then in 1956 Nigam and Weedon greatly improved the 1,4 di-yne synthesis.[74] They added copper(I) salt and changed the mesylate to a bromide leaving group. This enabled them to synthesize α-linolenic acid (ALA) for the first time by also using the Lindlar catalyst. Four years earlier Lindlar published his paper on a palladium catalyst deposited on calcium carbonate and poisoned with lead acetate, later named the Lindlar catalyst, which dramatically improved the semi-hydrogenation of acetylenes.[75] And, in the early 1960s at least three research groups were utilizing these improvements to synthesize arachidonic acid (ARA, 20:4 n-6) which at the time was thought to be the most important PUFA in humans.

Two of the groups[76, 77] used almost the same synthetic strategy, where they made use of the improved Grignard coupling of terminal acetylenes with propargyl halides, usually bromides. The syntheses were convergent by producing two di-yne synthons **3.9** and **3.10** which were in turn coupled together to form the ARA tetra-yne precursor **3.11**. This is illustrated in Scheme 3.3. Yields varied greatly between reactions (26-87%) and results were sometimes contradictory regarding the need to use protecting groups for the alcohol substrates or not. These new tetra-ynes were then hydrogenated with the Lindlar catalyst in good yields (69-80%), where the handling of the poly-yne substrate probably affected the yield. At this time researchers measured conjugation by UV and *trans* double bonds by IR to estimate impurities. Both were present in the products from these papers, but no mention was made of over-hydrogenated products.



Scheme 3.3 The synthesis of the ARA tetra-yne precursor by Ege et al. depicting the improved copper catalyzed Grignard type reactions.[77]

At the same time the third group (Osbond and coworkers), designed a synthetic strategy covering the syntheses of a few omega-6 PUFAs, including the PUFAs ARA and n-6 DPA (22:5 n-6).[78] Their strategy is demonstrated in Scheme 3.4 for the synthesis of the penta-yne precursor **3.14** involved in their n-6 DPA synthesis. By simply using what they called "di-Grignard complex" of propargyl alcohol and then different acetylenic carboxylic acids they could synthesize di-, tri-, tetra- and penta-ynoic acids corresponding to different natural omega-6 PUFAs. By subsequent additions of a di-Grignard propargyl alcohol unit and then bromination of the resulting alcohol, e.g., **3.12**, methylene skipped poly-yne chains up to tetra-ynes **3.13** were built. The final acetylene was added to their penta-yne structure by the Grignard reaction with the di-Grignard complex of 4-pentynoic acid.

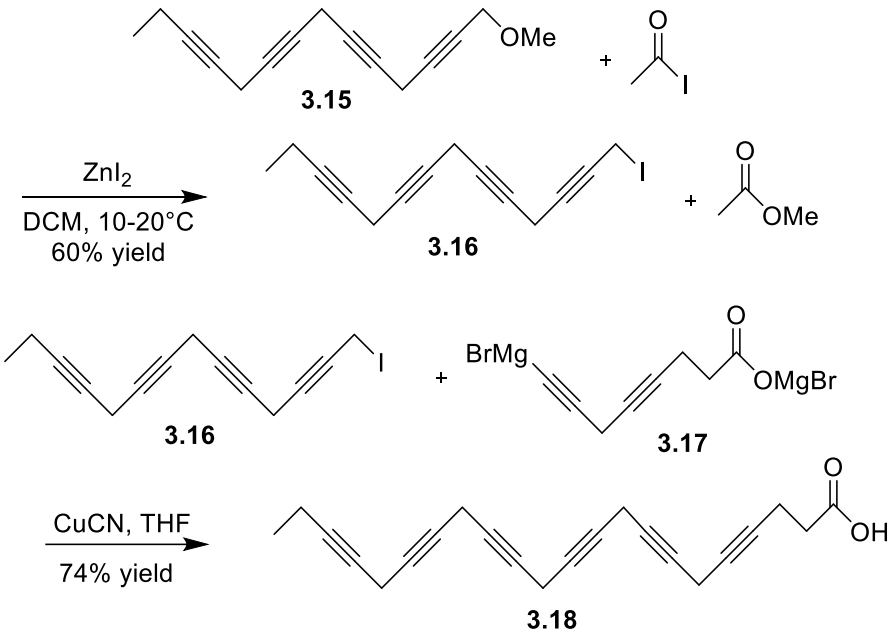


Scheme 3.4 The synthesis of n-6 DPA penta-yne precursor by Osbond et al. utilizing the di-Grignard complexes method both for the propargyl alcohol and acetylenic acid.[78]

The instability and reactivity of the poly-yne compounds had been noticed years earlier and by the additional fourth or fifth acetylene the structures become increasingly unstable, and the yields often reflected that. Yields were generally between 70-80% for both the coupling and the bromination reactions. The coupling of the last di-Grignard agent, the acid, offered yields between 50-75% depending on the substrates. For the **3.14** synthesis the yields for the last bromination, acetylenic acid coupling and the semi-hydrogenation were reported, 36%, 50% and 85%, respectively, with no further purification done to the final product. This methodology, using readily available substrates, introducing Cu(I) salts as catalysts, and using acetylenic acids to introduce the acid group as well as an acetylene to the structure in one step, greatly improved unsaturated fatty acid synthesis in general. The main drawback being the linearity of the synthesis. Using this method, Osbond and coworkers managed to synthesize LA, γ-linolenic acid (GLA, 18:3 n-6), ARA and n-6 DPA.[78]

This methodology was used for the next decades, either with the di-Grignard substrates or with the alcohols protected, usually as tetrahydropyranyl (THP) ethers. By early 1970s Kunau et al. had managed to improve the methodology further by demonstrating a new substitution reaction, where poly-yne propargyl methyl ethers, e.g. **3.15**, could be converted to propargyl iodides, e.g. **3.16**, using zinc iodide and acetyl iodide in very good or excellent yields, even for tetra-ynes.[79] Propargyl iodides gave also higher yields in the subsequent condensation reaction with an acetylene. In addition to this they prepared the di-Grignard reagents of di-yne carboxylic acids, e.g. **3.17** which reacted in higher yields than the mono-yne acids. This correlates with the work described in this thesis where

generally significantly higher yields were obtained when coupling a di-yne head group with a poly-yne propargyl bromide compared to the use of a mono-yne head group. This will be discussed further in chapter 4.2. These improvements enabled the authors to produce PUFAs with up to 6 double bonds in higher yields than ever before.[80] This approach is shown in Scheme 3.5 for the synthesis of a hexa-yne **3.18, a** precursor to DHA.



Scheme 3.5 Synthesis of the hexa-yne precursor of DHA by Kunau et al. utilizing the propargyl iodide and di-yne acid strategy.[80]

Limited technology to accurately measure low amounts of *trans* isomers in their final products as well as over-hydrogenated products has clearly inflated the yields reported for the semi-hydrogenation reactions of poly-ynes in the past. These byproducts are to be expected in various amounts and usually no steps apparently taken to separate them, as they are usually impossible to separate by normal silica chromatography. Evidence of this can be seen in many of the papers, for example van der Steen et al. in 1963[81] reported the synthesis of DHA, by the semi-hydrogenation of the corresponding hexa-yne acid precursor. They obtained an oil in 77% yield presumed to be DHA, and with spectroscopic methods resembled the then recently isolated DHA. But, when attempting to confirm the structure with mass spectrometry methods the parent peak corresponded to a penta-enoic acid, indicating that their reaction produced mainly over-hydrogenated product. Two years later they did manage to identify the right acid using mass spectra after purifying the acid with counter-current distribution in 45% yield and 94% purity according to GC.[82]

Also in the early 1960s[77] authors attempted to explain the increased IR absorption band at 967 cm^{-1} in their synthesized products as not belonging to *trans*-isomers but rather some other absorption inherent in the structure, but we now know that´s not the case for fatty acids. These examples show how the expected products in these poly-yne semi-hydrogenations were not in any way pure products. Instead, they were heavily contaminated with over-hydrogenated or *trans*-isomer byproducts. This has been corroborated by many authors (see next three sections) in recent years and was very noticeable during this work (see Chapter 4). Majority of authors who mention possible byproducts did report *trans*-isomers present in the products but usually only in very low

amounts. This was in most cases only checked by IR spectroscopy or ^{13}C-NMR which do not always give accurate *trans* measurements as will be discussed in Chapter 6.3.

The next revolution in the acetylenic approach was the discovery of the very mild conditioned copper mediated couplings of propargyl halides and terminal acetylenes in the presence of a mild base in the 1990s to form the desired 1,4-diyne building blocks. This eliminated the need for a strong organometallic base for the deprotonation of the terminal acetylene. Much earlier, in 1965, some authors described the reaction of propargyl halide with a terminal acetylene in the presence of copper(I) salts and an amine base producing a 1-alkyne-3-allene, except in the case of t-butyl amine which produced the 1,4-diyne in low or fair yield. This would be one of the earliest cases where the 1,4-diyne motif was demonstrated without a strong organometallic base.[83] Again in 1978 some authors described the coupling of propargyl halide and a terminal acetylene in the presence of copper(I) iodide and the amidine bases DBN or DBU in polar solvents. The authors also mentioned the formation of an allene as a side-product in all reactions and sometimes as the sole product, but limited the use of this method on reactants with only one acetylene in the structure.[84]

Then in 1992 Jeffery at al. described a new method where the coupling of propargyl halide and terminal acetylene was accomplished in the presence of copper(I) iodide, sodium carbonate and tetra-n-butylammonium chloride in DMF or acetonitrile. This method offered the 1,4-diynes in good yields, usually 70-80%.[85] Practically at the same time Lapitskaya et al. published their version of this reaction. The coupling took place in the presence of copper(I) iodide, sodium iodide and potassium carbonate in DMF, where DMF seemed to be vital. Good to excellent yields (66-92%) were obtained for various substrates and alcohols, esters and epoxides were easily tolerated.[86] In the years that followed, the former method by Jeffery was found to be lacking for certain substrates by some authors[87, 88] but both methods are in use to the present day. Figure 3.2 provides a comparison of the two methods. In 2003 Spinella and Caruso published results where the Lapitskaya method was improved even further. By exchanging the potassium carbonate for cesium carbonate increased yields were obtained, sometimes drastically, for all kinds of substrates, 70-95% instead of 42-85%.[88, 89] Allene side-products were still formed in this reaction, around 7-14% according to Parrain and Santelli when exploring conditions using 4-chlorobut-2-yn-1-ol and 3-methoxy-propyne as reagents.[88]

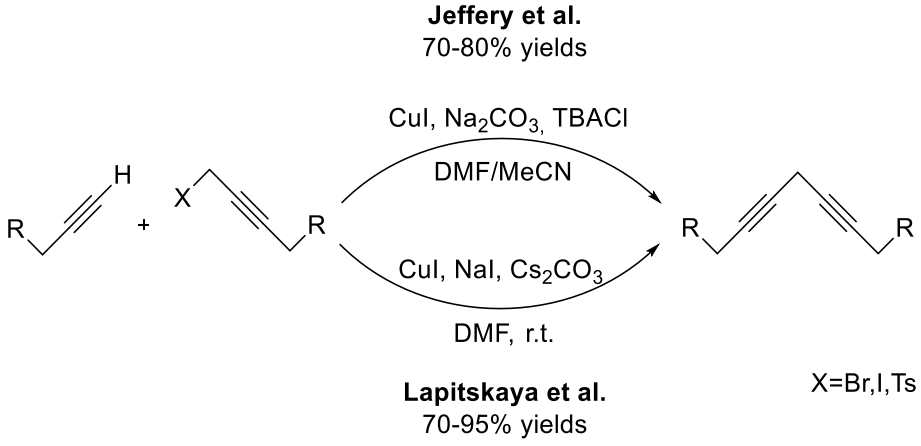


Figure 3.2 The improved copper catalyzed couplings of acetylenes with propargyl halides, showing reaction conditions for both the Jeffery et al. and Lapitskaya et al. approaches.

Today, using the improved version of the reaction, allenes are observed in low or trace amounts except in rare cases where they are formed in greater amounts but can usually be separated by normal silica chromatography. Due to the mild conditions the reaction is applicable to various kinds of substituents on the propargyl or acetylene units. This enables the sequential additions of propargyl units where the labile skipped poly-yne fragment is very well tolerated, generally in good or very good yields. This has made the acetylenic approach a competitive option in the synthesis of highly unsaturated skipped poly-enes. The following semi-hydrogenation of the resulting skipped poly-ynes introduces new problems and is detailed in the next section.

3.2 Semi-hydrogenation of skipped poly-ynes

Throughout this work, the appearance of semi-hydrogenation byproducts was unavoidable. The semi-hydrogenation methods tried, Brown, Rosenmund and Lindlar catalysts all had obvious signs of over-hydrogenation in the ^{1}H-NMR spectra and a distinguishable *trans* band was clearly visible in the IR spectra. These hydrogenation byproducts can be very difficult to separate from the desired poly-ene compounds. Figure 3.3 illustrates the possible products of a semi-hydrogenation reaction.

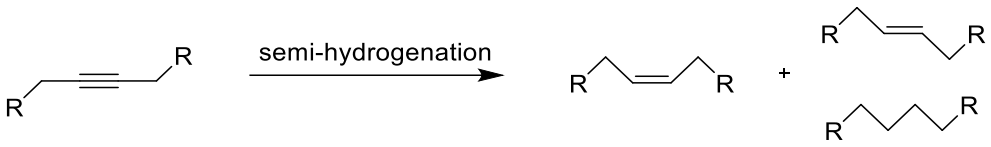


Figure 3.3 The main three possible products of a semi-hydrogenation reaction, the desired *cis*-alkene product and the two byproducts, a *trans*-alkene and an over-hydrogenated alkane.

Combing through the literature to figure out the ideal way to semi-hydrogenate skipped poly-ynes resulted in more questions than answers. A few different methods have been employed in the semi-hydrogenation of skipped poly-enes, namely: Lindlar catalyst, Rosenmund catalyst, Brown catalyst, hydroboration reactions and to a much lesser extent a few other methods listed in Table 3.1. Seemingly no obvious ideal way exists for the semi-hydrogenation of skipped poly-ynes. Multiple authors have demonstrated these methods but always under varying reaction conditions. The results are sometimes quite contradictory in many ways and the production of semi-hydrogenation byproducts also often widely ignored or estimated with questionable clarity. In the next sub-sections, every method reported that has been employed for the semi-hydrogenation of skipped poly-ynes is shortly reviewed. A summary of the semi-hydrogenation explorations made during this work is detailed in Chapter 6.2.

The stereoselective semi-hydrogenation of alkynes to *cis*-alkenes has been a valuable synthetic tool since the emergence of the Lindlar catalyst in the 1950s and since then multitudes of other methods, especially in recent years have been developed.[70] In this work the focus is on methylene skipped poly-ynes as the synthesis and hydrogenation of them pose unique challenges and are of specific relevance to this work, therefore semi-hydrogenations of isolated or conjugated alkynes are not discussed.

During this work, the hydrogenation of mono and di-ynes was easily completed with the Lindlar catalyst with excellent yields and very minor amounts of over-hydrogenated and

trans-isomer byproducts and this is well established in the literature. Multitudes of other methods have also been discovered and used in synthesis for the stereoselective semi-hydrogenation of a single alkyne to a *cis*-alkene and have been reviewed in great detail.[70] Skipped poly-ynes possessing three or more acetylenes are more unstable and give rise to higher amounts of semi-hydrogenation byproducts. In this section the methods developed for the semi-hydrogenation of skipped poly-ynes, mostly with three or more acetylenes are reviewed. Table 3.1 shows the methods that have successfully been utilized for this purpose in the literature.

Table 3.1 The methods successfully used in the stereoselective semi-hydrogenation of skipped poly-ynes to *cis*-poly-enes and their typical preparations.

Name	Type	Preparation
Lindlar[75]	Heterogeneous catalyst	Pd deposited on $CaCO_3$ poisoned with lead acetate and quinoline added under H_2 gas
Rosenmund[90]	Heterogeneous catalyst	Pd deposited on $BaSO_4$ with quinoline added under H_2 gas
Brown[91]	Heterogeneous catalyst	$Ni(OAc)_2$ reduced by $NaBH_4$ with ethylenediamine added under H_2 gas
Brown-Zweifel[92]	Non-catalytic	dialkyl borane made *in situ* forms an alkenyl-dialkyl borane with the alkyne, protonolyzed by acid
Alkoxytitanium(II)[93]	Non-catalytic	$Ti(OiPr)_4$ and iPrMgBr form an alkoxytitanium-acetylene complex, protonolyzed with water
Zinc catalyst[94, 95]	Heterogeneous catalyst	Zinc activated by 1,2-dibromoethane or as Zn(Cu), Zn(Cu/Ag) or Rieke zinc, protonolyzed by H_2O or alcohol
Diisobutylaluminium hydride[96]	Non-catalytic	DIBAL-H and alkyne form alkenylalanes, protonolyzed by methanol
Palladium (0)[97]	Homogeneous catalyst	Pd(0) and AcOH in presence of TMDSO

A rather limited number of these reactions has been carried out in the literature. Most of these examples are of di-ynes and tri-ynes and to a lesser extent tetra-ynes. Penta- and hexa-yne semi-hydrogenations are very rare in the literature and some of those examples are over half a century old. All of the methods listed above have drawbacks and advantages which center around the chemoselectivity of alkynes and alkenes which gives rise to the formation of over-hydrogenated and *trans*-isomer byproducts, which both lower the yields

as well as make the isolation of the desired *cis*-alkene more difficult. Other drawbacks can for example include reactivity towards other functional groups, formation of conjugation or reactivity only towards activated alkynes.

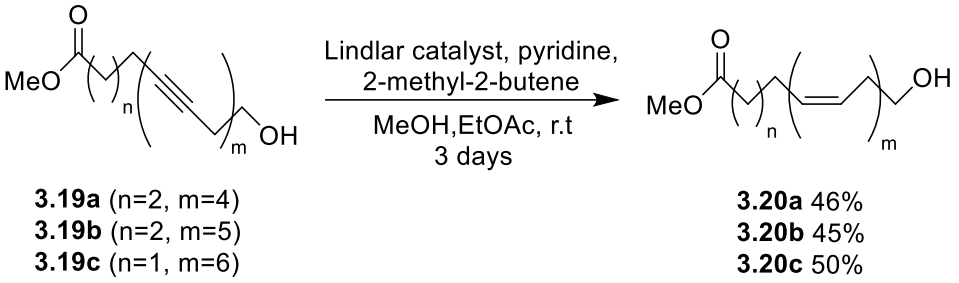
3.2.1 Pd-catalysts, Lindlar and Rosenmund

The so-called platinum metals have for decades been known for their properties and capabilities as hydrogenation catalysts. In the 1960s several studies established palladium as the most selective metal for conversion of alkynes to alkenes. The metals compared roughly as this: Pd>Pt>Ru>Rh>Ir. Similarly the selectivity for the formation of the *cis*-alkene was determined to be Pd>Rh>Pt>Ru≈Ir.[98] To further increase selectivity as well as reduce alkene reactivity the catalysts could be modified, both with the type of support material and additives called "catalyst poisons". Further influences such as temperature, agitation, amount of catalyst, amount of catalyst poisons, solvents and substrates would affect the reaction. In the 1950s modified catalysts started to emerge, beginning with the Lindlar catalyst which for the first-time allowed researchers to reliably stereoselectively semi-hydrogenate acetylenes to *cis*-alkenes with low amount of *trans*-isomer formation and over-hydrogenation.[75]

The Lindlar catalyst was the first catalyst and method deployed by researchers to stereoselectively semi-hydrogenate alkynes to *cis*-alkenes and has been featured in textbooks all around the globe for decades. The Lindlar catalyst is most generally described as palladium on calcium carbonate poisoned with lead. It is then employed under various conditions. Usually with quinoline but also pyridine or triethyl amine as added catalyst poison. These added catalyst poisons have been shown to rearrange the surface structure of the catalyst which then increases their chemoselectivity and stereoselectivity.[99] Lindlar reactions have been conducted in almost any common solvent, most commonly benzene, ethyl acetate and methanol; temperatures ranging from 0°C to room temperature; usually atmospheric pressure of H_2 and sometimes with extra additives such as cyclohexene or 2-methylbut-2-ene. Numerous functional groups are tolerated in the reaction, such as esters, carboxylic acids, hydroxyl groups, ketones, allylic halides, epoxides and many more protecting groups and the Lindlar catalyst has been successfully used in the hydrogenation of skipped di-, tri-, tetra-, penta- and hexa-ynes. These various conditions as well as different ratios of catalyst and catalyst poisons used have been reported by numerous authors and underline how no universal method exists in the semi-hydrogenation of skipped poly-ynes using the Lindlar catalyst. The substrates themselves seem to have a huge impact on the outcome of the reaction and researchers must seemingly tailor the conditions to them.

Reported poly-yne semi-hydrogenation yields using the Lindlar catalyst range from 62-90% for tri-ynes, 41-94% for tetra-ynes 40-87% for penta-ynes and 23-50% for hexa-ynes. Most authors do not make any mention of hydrogenation byproducts[100-111] or use methods to quantify or measure them that are probably not accurate enough to determine the amount of either over-hydrogenated or *trans*-isomer byproducts sufficiently. Therefore, they likely under-report them[77, 78, 81, 82, 112] and generally report in the higher range of yields. Other authors describe over-hydrogenated and *trans*-isomer byproducts that are formed and their separation which generally can only be achieved satisfyingly with argentation chromatography (see Chapter 6.2.2). These authors generally report lower yields (tri-enes 62-70% yield, tetra-enes 41%, 60% and 75% yield).[113-115]

Hwang et al. recently described a somewhat different conditions for the semi-hydrogenation of large poly-ynes. They submitted their substrates, methyl ester poly-yne precursors **3.19a-c** of ω-hydroxy PUFAs **3.20a-c** (depicted in Scheme 3.6) to these novel conditions, pointing out that the typical Lindlar catalysis conditions afforded products with a lot of over-hydrogenated byproducts.[116] They employed the Lindlar catalyst in 2-methylbut-2-ene:methanol:pyridine (4:4:1) solvent mixture for a tetra-yne and 2-methylbut-2-ene:methanol:ethyl acetate:pyridine (4:2:2:1) solvent mixture for penta- and hexa-ynes. These reactions lasted for 18 hours to 3 days and afforded products with low amount of over-hydrogenated byproducts according to ^{1}H-NMR in 45-50% yield but no mention was made of possible *trans*-isomers or their separation. Interestingly, the number of triple bonds in the structure did not affect the yields negatively, in fact they claimed the highest yield for the hexa-yne.



Scheme 3.6 Semi-hydrogenation of tetra-, penta- and hexa-yne methyl esters by Hwang et al. (The reaction for tetra-yne **3.19a** was completed in 18 hours and without EtOAc).[116]

Some authors describe purification on a reverse-phase HPLC or gel chromatography systems which separated the over-hydrogenated products. But, it remains unclear how effective it is, since most authors[107-109] did not specify it while others described high purity.[117] Yet others reported some over-hydrogenated byproducts still in the product after RP-LC purification or did an additional argentation chromatography.[115] Woollard et al. in 1978 did show successful separation of deuterated D_8-ARA and other less saturated C20 acids with Lipidex 5000 RP-gel partition chromatography.[117] One paper described the impurities separated on a RP-HPLC column as UV-absorbing, which indicates conjugated material not over-hydrogenated.[118] There is not much indication that *trans*-isomer byproducts of large poly-enes have been effectively separated by RP chromatography.

Authors sometimes describe certain conditions as being necessary for success for the catalytic hydrogenation of skipped poly-ynes but these claims were then not necessarily corroborated by others. Most authors executed the reactions at ambient temperature but the amount of quinoline used differed drastically (0.1 to 4.3 eq to the poly-yne, 0.025 to 1.07 eq to each acetylene or 0.1 to 0.9 mL quinoline per gram of catalyst). One paper described the use of triethylamine as the catalyst poison and a few others used pyridine. Kang and Britton described how stochiometric amount of quinoline was needed to prevent over-hydrogenation, as well as lower temperatures to prevent side-reactions.[119] Lellouche et al. described the need for exactly 0.01% pyridine in hexane for satisfying results.[120] The amount of catalyst can then vary a lot as well, 0.1-1.6 w/w ratio of the usually 5% palladium catalyst to the substrate (sometimes more) and some authors had to keep adding catalyst into the reaction over its duration for it to reach completion. Reaction times also varied a lot, from a few minutes up to 4 days, generally the slower reactions had more catalyst poison and/or additional additives but some substrates had certain groups in close

proximity to an acetylene which will make that acetylene then react much slower.[121] Some authors described the additional unsaturated additives as a crucial necessity to reduce or eliminate byproducts, these additives being mainly cyclohexene[122, 123] or 2-methyl-2-butene[116].

In addition to these greatly variable conditions some authors describe the Lindlar catalyst to be very lacking in terms of selectivity, yields or giving erratic results.[87, 124-126] These results might indicate that the semi-hydrogenation of each substrate is highly dependent on the exact reaction conditions and/or that the catalyst capabilities can be different depending on the manufacturer or the conditions. This can make any kind of optimization of these methods very difficult.

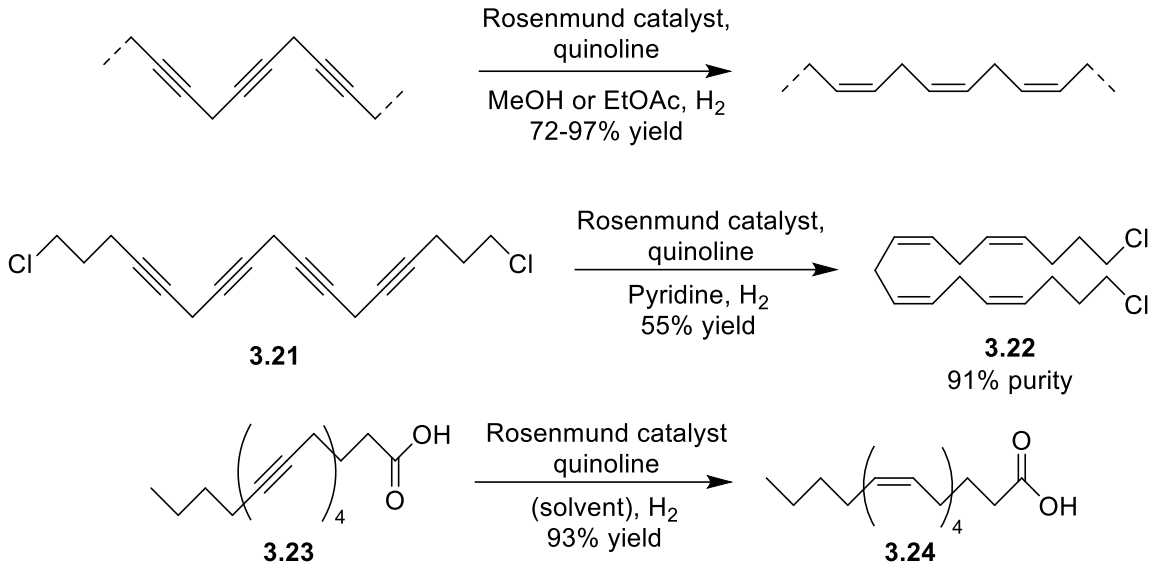
Lastly, some authors did not even fully describe their reaction conditions, for example the catalyst poison or the amount of it, temperature, catalyst manufacturer, reaction time or other factors.[101, 102, 105, 111, 127, 128] In the last two decades authors have generally been more detailed in their descriptions than earlier but reproducibility still remains uncertain and almost every synthetic group synthesizing skipped poly-enes uses their own unique method for the semi-hydrogenation.

Palladium on barium sulfate modified with various additives such as sodium acetate, sulfur containing molecules or nitrogenous bases is generally referred to as the Rosenmund catalyst, first published in 1918 as a catalyst for the transformation of acetyl chloride to an aldehyde.[90] After 1985 it can be seen used for the semi-hydrogenation of poly-alkynes to *cis*-alkenes and is then sometimes referred to as the Lindlar catalyst.[128-130] In this thesis it will be referred to as the Rosenmund catalyst and palladium on calcium carbonate with lead acetate as catalyst poison is referred to as the Lindlar catalyst. The Rosenmund catalyst has only been used successfully in the hydrogenation of a handful of methylene skipped polyunsaturated compounds, as the Rosenmund catalyst often shows too much reactivity. Therefore, the Lindlar catalyst is generally favored.

Six examples of skipped tri-yne hydrogenations with the Rosenmund catalyst can be found in the literature. Three authors report 72-76% yields while the other three report incredible 90-97% yields.[128-133] These authors did not mention over-hydrogenated or *trans*-isomer byproducts at all, except one paper which used argentation chromatography to remove "traces of overreduced or underreduced products" but the yields from the purification step were not reported.[129] Two skipped tetra-ynes hydrogenated by the Rosenmund catalyst have been reported in the literature. The dichloro skipped tetrayne **3.21** was hydrogenated in 55% yield after reverse-phase chromatography with only 91% purity according to GC analysis to produce the tetra-ene **3.22**, where the main impurity was the over-hydrogenated tri-ene byproduct. These were the only authors using the Rosenmund catalyst to semi-hydrogenate poly-ynes that seemed concerned with semi-hydrogenation byproducts.[125] These authors further described numerous trials with the Lindlar catalyst being too slow and affording low yield while the Rosenmund catalyst was much more active but afforded considerable over-hydrogenation.

The second example of a tetra-yne, the 19:4 n-6 tetra-yne acid **3.23** was hydrogenated to afford the tetra-ene acid **3.24** in dubious 93% yield. The authors did not describe the reaction conditions nor mention any byproducts but only that the ^{1}H and ^{13}C-NMR were satisfactorily compared to that of arachidonic acid (20:4 n-6).[134] The literature has therefore reported rather high yields for Rosenmund catalyzed tri- and tetra-yne semi-

hydrogenations. These reports have though not invoked much confidence in the catalyst and it has indeed not been used much by researchers since for this purpose. Scheme 3.7 illustrates the reported Rosenmund catalyzed semi-hydrogenations of poly-ynes.



Scheme 3.7 The reported Rosenmund catalyzed semi-hydrogenations of skipped tri- and tetra-ynes.

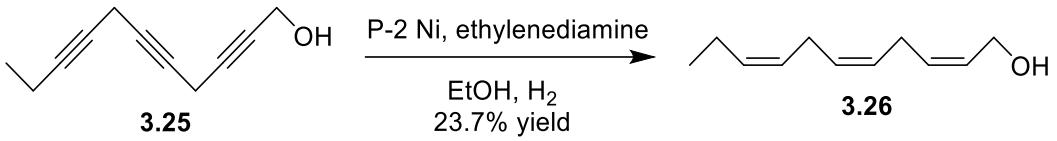
Despite these seemingly very successful reports, most authors that do try the Rosenmund catalyst describe immense over-hydrogenation or by-product formation[135] and in more recent years the catalyst has not seen much use in the semi-hydrogenation of skipped poly-ynes.

3.2.2 P2-Ni catalyst, the Brown catalyst

The use of a nickel catalyst to stereoselectively semi-hydrogenate alkynes was first described in 1972 by Brown and Ahuja.[136] Nickel acetate tetrahydrate was reduced with sodium borohydride in ethanol to produce the catalyst which they called P-2 nickel but has since generally been called the Brown catalyst. In the original paper they described multiple hydrogenations of alkenes to alkanes and then additionally the successful stereoselective semi-hydrogenation of 3-hexyne to *cis*-3-hexen with around 50:1 *cis*/*trans* ratio on a large scale. They then expanded on this method the following year with the addition of ethylenediamine as a catalyst poison to enhance selectivity for the *cis* product which was then achieved in 100:1 to 200:1 *cis*/*trans* ratios in excellent yields with around 2% over-hydrogenated product detected.[91]

Some regioselectivity of the Brown catalyst has been observed, where terminal TMS-protected alkynes or alkynes close to carbonyl or bulky protected alcohols react slowly or not at all. This can either be detrimental or useful for synthetic purposes. Additionally, the catalyst offers some benefits, it is prepared *in situ* from inexpensive materials in a very simple process, reaction times are usually short (0.5-3 hours), and the reactions are carried out at ambient temperature. For the semi-hydrogenation of skipped poly-ynes the Brown catalyst first appeared in 1981 when Mori and Ebata used it to semi-hydrogenate the skipped tri-ynol **3.25** to obtain the tri-ene **3.26** in low yield (24%) after "careful purification on a Merck Lobar column".[137] The authors did not in this short paper mention

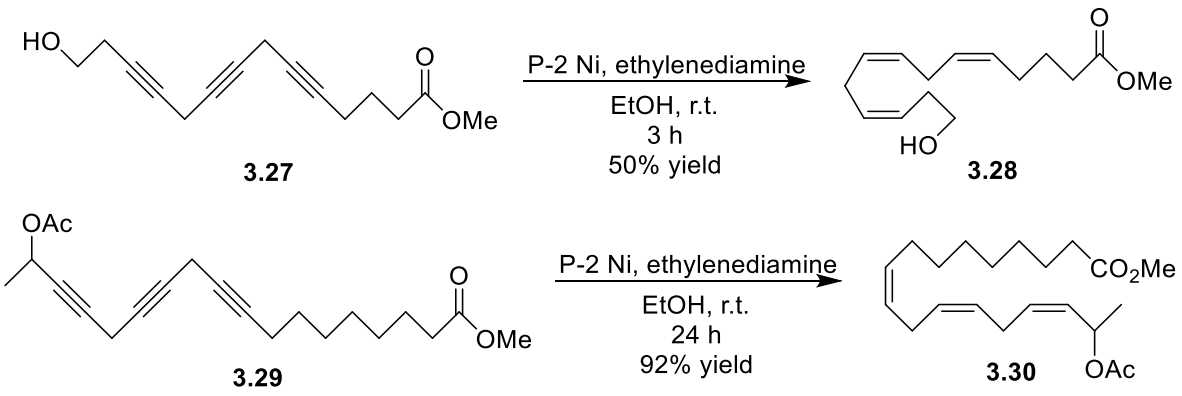
possible semi-hydrogenation byproducts except three small peaks in the ^{13}C-NMR which might indicate *trans* and which the authors estimated to be around 9%.



Scheme 3.8 The first published Brown catalyzed semi-hydrogenation of a skipped poly-yne, by Mori and Ebata.[137]

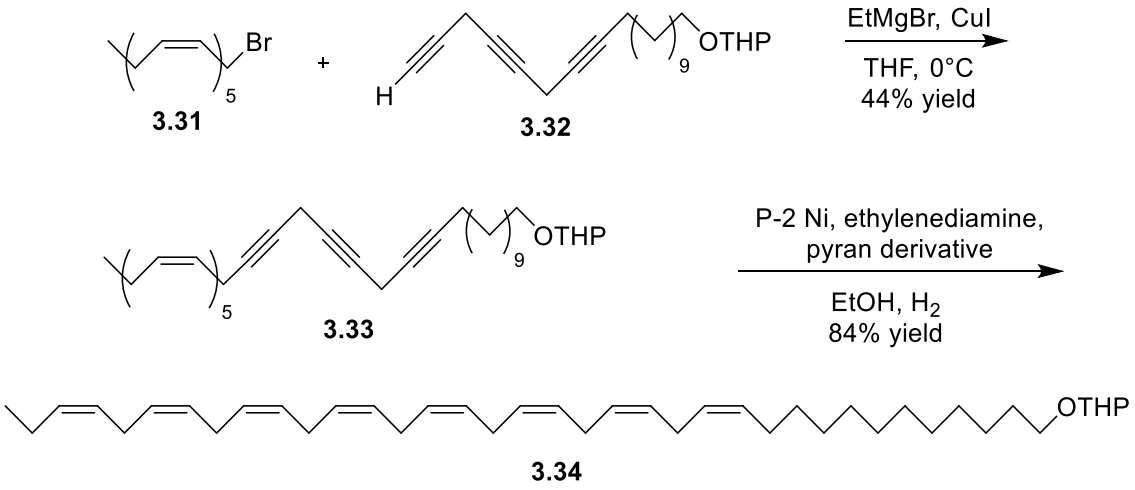
The Brown catalyst was sporadically used in the next two decades, mostly for di-ynes in 65-92% reported yields.[138-141] One paper described the hydrogenation of a di-yne and a subsequent production of the third double bond in a Wittig reaction. The authors did not mention the possibility of byproducts from the hydrogenation, which gives a pure product according to GC, but seem very concerned about the stereoselectivity of the Wittig reaction.[142] They then applied the final product to argentation chromatography and obtained a very pure sample, according to GC. The authors then mentioned the limitation of their methods to detect *trans*-isomers, something which most authors of the time did not bring to question. At the turn of the century many researchers started preferring the Brown catalyst for semi-hydrogenation of tri- and tetra-ynes over the then most commonly used Lindlar catalyst.[124] Several semi-hydrogenations of tri-ynes were reported in 50-93% yields, tetra-ynes in 43-78% yield and one example of a penta-yne[126] in 32% yield using the Brown catalyst. Like with the Lindlar catalyst, authors generally do not mention hydrogenation byproducts or claim the product to be completely pure without seemingly having investigated it.[85, 88, 143-147]

Han and Razdan in 1998, described the synthesis of the tri-ene synthon **3.28**. They used the Brown catalyst to semi-hydrogenate a di-yne and then a subsequent Wittig reaction to obtain the tri-ene.[116] The same group then two years later described an alternative method in which they obtained all three double bonds with the hydrogenation of the tri-yne **3.27**, obtaining the tri-ene in superior total yield (14% versus 33%). Both these papers made no mention of possible byproducts from the hydrogenation and Wittig reactions.[141] Hansen and Stenström in the same year described the semi-hydrogenation of a tri-yne **3.29**. By using palladium and platinum catalysts they obtained the tri-ene **3.30** with around 10% *trans* but using the Brown catalyst they claimed the product to be pure all-*cis* in 92% yield according to a 300 MHz NMR.[87] Qi et al. described the Brown catalytic semi-hydrogenation of multiple tetra-ynes without specifying the yields for the hydrogenation step. The purity of their products was claimed to be higher than 95% and only all-*cis* according to ^{1}H-NMR without specifying how they came to that conclusion.[148]



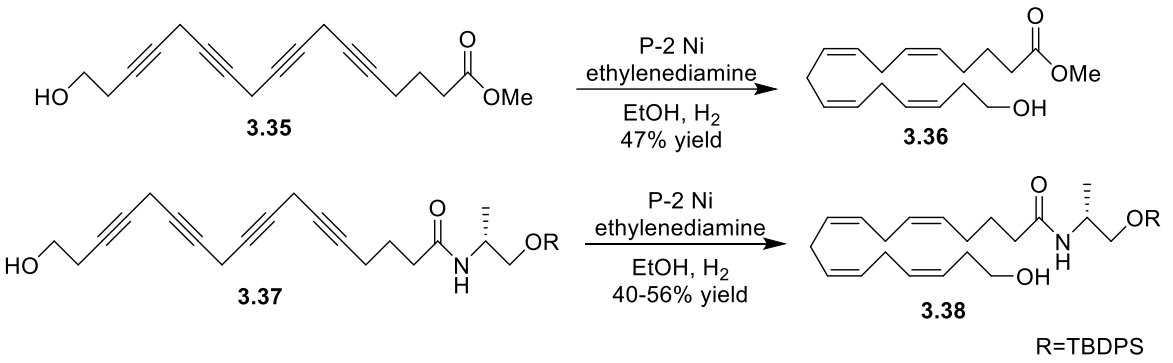
Scheme 3.9 The Brown catalyzed semi-hydrogenations of skipped tri-ynes by Razdan et al.[141] and Hansen and Stenström.[87]

Rezanka et al. described in 2008 the remarkable synthesis of skipped hepta- and octa-ene acids.[149] They coupled a penta-ene **3.31** derived from natural EPA to the tri-yne **3.32** to produce the penta-ene-tri-yne **3.33** in 44% yield. This large enyne was then partially semi-hydrogenated with the Brown catalyst to afford the octa-ene **3.34** in 84% yield purified on preparative TLC. This is depicted in Scheme 3.10. After deprotection of the alcohol and oxidation forming the acid, the authors claimed it to be 98% pure on GC and do not mention any possible hydrogenation byproducts.



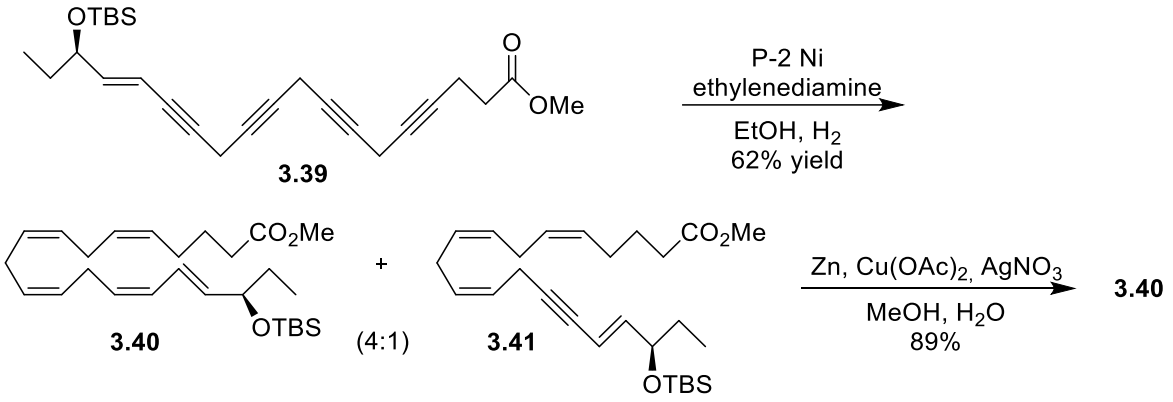
Scheme 3.10 Synthesis of a skipped octa-ene by Rezanka et al. semi-hydrogenating only three triple bonds with the Brown catalyst.[149]

Balas et al. described the Brown catalyzed semi-hydrogenation of a tetra-yne ester **3.35** and amide **3.37**, affording the tetra-ene products **3.36** and **3.38** in moderate yields 47% or 40-56%, respectively (see Scheme 3.11).[150] The authors remarked that the low yield was due to instability of the tetra-ynes. In the case of the amide, they also noted how sometimes a mixture of the tetra-ene product and a tri-ene-yne byproduct was obtained, where the triple bond closest to the amide was not reduced. By optimizing the reaction conditions, they did though manage to obtain the pure tetra-ene. They did not mention any further possible hydrogenation byproducts.



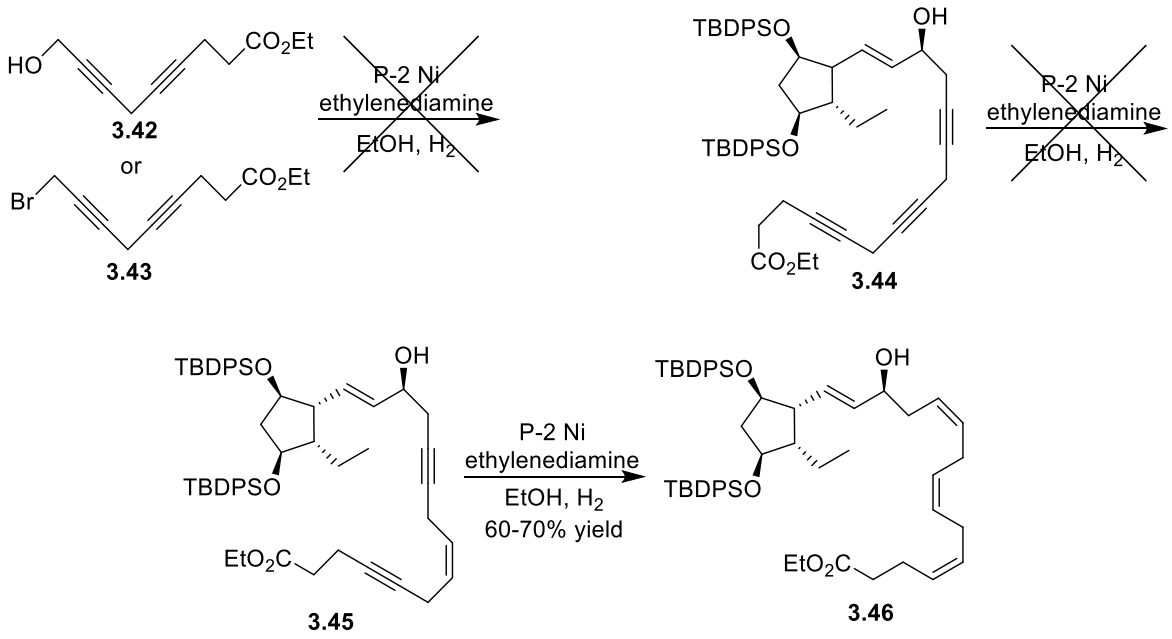
Scheme 3.11 The skipped tetra-yne semi-hydrogenations by Balas et al. using the Brown catalyst.[150]

Nanba et al. used the sequential Brown and (Zn(Cu/Ag)) semi-hydrogenations to manage to semi-hydrogenate the skipped tetra-yne **3.39**.[151] The Brown catalyst could only partly or very slowly reduce the triple bond conjugated to the *trans* double bond and, therefore, a 4:1 mixture of the penta-ene **3.40** and tetra-ene-yne **3.41** was obtained. The zinc amalgam catalyst finished the semi-hydrogenation obtaining **3.40** in 55% yield over these two steps but like many other authors, they did not mention any possible hydrogenation byproducts. This is illustrated in Scheme 3.12.



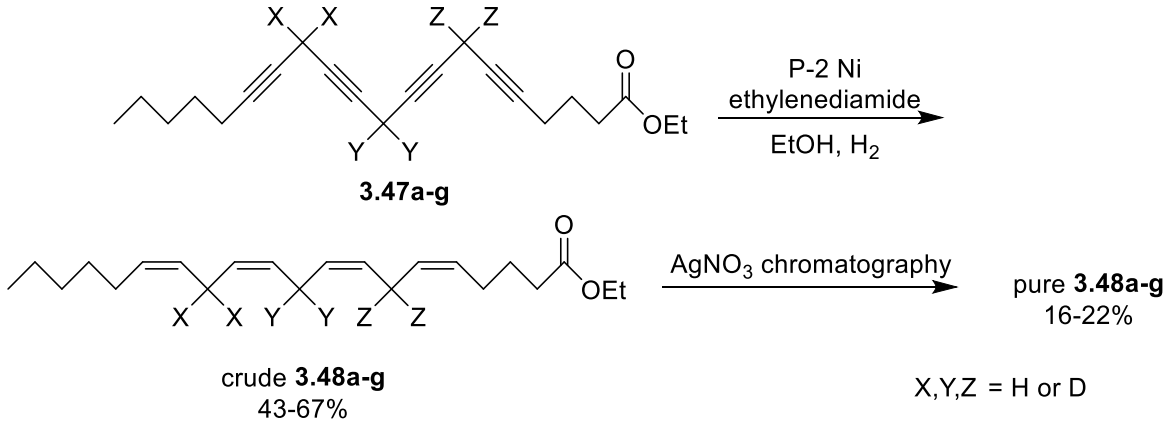
Scheme 3.12 The skipped tetra-yne semi-hydrogenation by Nanba et al. using the Brown catalyst producing a mixture which was semi-hydrogenated again to obtain the pure product.[151]

Taber et al. described the attempted semi-hydrogenation of skipped di-ynes **3.42**, **3.43** and tri-yne **3.44** with the Brown catalyst.[152] These trials surprisingly only afforded a complex mixture of products but the semi-hydrogenation of the yne-en-yne **3.45** afforded the desired product **3.46** in 60-70% yield depending on the stereoisomer. This is illustrated in Scheme 3.13. The authors claimed the product to contain only a trace amount of over-hydrogenated byproducts and the desired tetra-ene to be easily separated but did not elaborate further and did not mention the possibility of *trans*-isomers.



Scheme 3.13 The successful and unsuccessful Brown catalytic semi-hydrogenations by Taber et al.[152]

Only two papers mention argentation chromatography to purify the product from a Brown catalyzed semi-hydrogenation of poly-ynes. Fomich et al. reported the hydrogenation of a library of methylene-deuterated tetra-ynes **3.47a-g** ARA analogues semi-hydrogenated with the Brown catalyst. The authors mentioned how the products purified by silica chromatography were still a mixture of the desired tetra-ene as well as hydrogenation byproducts in 43-67% yield. The semi-hydrogenation products were then applied to argentation chromatography which afforded the pure tetra-enes **3.48a-g** in 16-22% final yield.[153]



Scheme 3.14 A library of seven different deuterated ARA analogues (where a-g represent different combinations of H or D) made by the Brown catalyzed semi-hydrogenation of corresponding tetra-ynes by Fomich et al.[153]

Finally, Firsov et al. described the use of the Brown catalyst poisoned with lead ($Pb(NO_3)_2$) to semi-hydrogenate an allyl deuterated EPA ethyl ester (EE).[126] The authors screened the Lindlar and Rosenmund catalysts and asserted that they produced more *trans*-isomers than the Brown catalyst. They then further asserted that the main impurities produced in the

Brown reactions supposedly according to GC-MS were only the over-hydrogenated byproducts which were more easily removed. They then produced their deuterated EPA EE penta-ene which is purified by argentation chromatography in 32% yield.

As with the Lindlar catalyst, the reaction conditions applied with the Brown catalyst vary. Mainly the amount of ethylenediamine which has been reported between 1-11 equivalents compared to the catalyst or 0.37-11.4 equivalents compared to the substrate. Increased amount of ethylenediamine supposedly reduces the amount of byproducts but the exact relationship is not entirely fleshed out. Most experiments were conducted at ambient temperature and reaction times usually between 3-5 hours but could vary between 30 minutes to 24 hours.

In very few cases authors have reported very low yields or a complex mixture of products using Brown catalyst but most authors who report about its use for poly-yne semi-hydrogenations claim moderate to excellent yields and usually excellent purity. The Lindlar and Brown catalysts have been by far the most common methods chosen by researchers doing poly-yne semi-hydrogenations, especially for poly-ynes bigger than tri-ynes. As for the Lindlar catalyst, the varying conditions for the Brown catalyst reactions make it hard to draw conclusions from the literature about the efficiency of the Brown catalyst versus the Lindlar catalyst. Possibly, the reaction efficiency is based heavily on the substrates themselves or the exact manufacturing of the catalysts. Many of the results reported in the literature must as well be considered with some hesitation since many authors do not seem to even consider the formation of possible byproducts and the products purity therefore put into question. Many authors cite GC chromatograms as definitive measurement of purity but *trans*-isomers are often notoriously difficult to separate. Additionally, when authors describe argentation chromatography performed on the compounds the yields drop down to 16-32% for tetra- and penta-enes which should reflect how important issue this is.

3.2.3 Brown-Zweifel hydroboration

Hydroboration, also called the Brown-Zweifel procedure after their description of the procedure in 1961, is a method that stereoselectively semi-hydrogenates alkynes to *cis*-alkenes.[92] This procedure generally involves the *in situ* production of dialkylboranes from the corresponding alkenes and either sodium borohydride/boron trifluoride or borane-dimethyl sulfide complex. The most common dialkylboranes used are dicyclohexyl-, catechol- and disiamyl-boranes. For skipped poly-ynes dicyclohexyl borane has been most used. After the addition of the alkyne to the dialkylborane the resulting vinyl dialkyl borane is protonolyzed, usually with acetic acid to produce the *cis*-alkene. Using sodium borohydride with boron trifluoride with large excess of the alkyne substrate will produce the trivinyl borane which is then similarly protonolyzed producing the *cis*-alkene. The reactions are generally carried out in dry THF or diglycol methyl ether (diglyme) at 0°C. These conditions tolerate esters, halides, acetals and protected alcohols, its main issue the reactivity towards aldehydes and ketons. These borane reagents can also readily reduce alkenes but impressive chemoselectivity for alkynes has been demonstrated. The increased addition of the borane reagent can also fully reduce an alkyne.

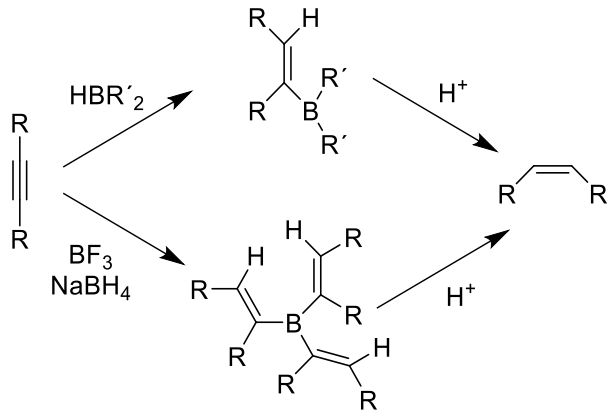
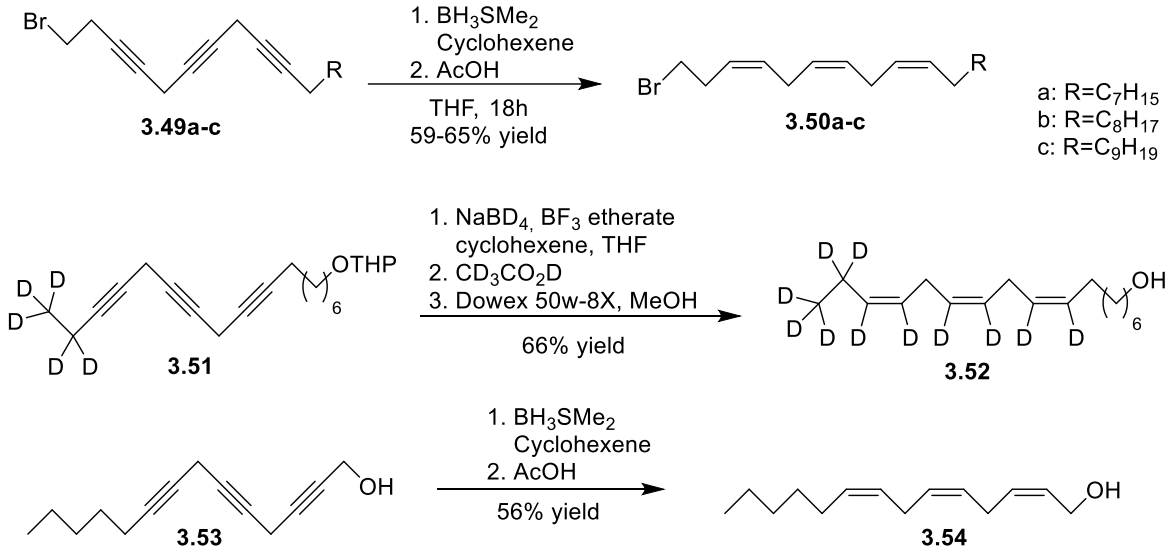


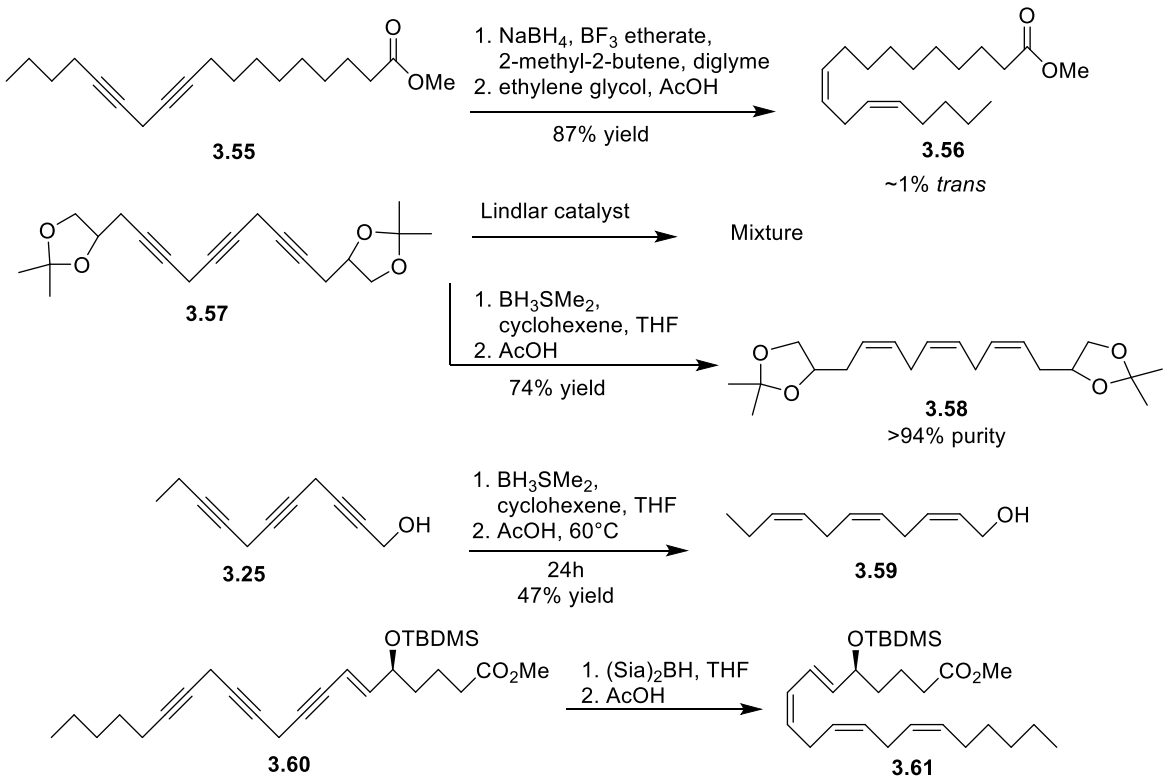
Figure 3.4 Formation of the vinyl dialkyl borane (top) and trivinyl borane (bottom) intermediates and their protonolysis during Brown-Zweifel hydroboration reactions.

This procedure has been successfully used in a handful of semi-hydrogenations of skipped di-ynes and tri-ynes. The yields are reported as 47-86%[135, 154-159] for tri-ynes, no larger skipped poly-ynes have been reported using this method. The reported stereochemistry is usually excellent. Millar and Underhill in 1986 mentioned the troublesome catalytic hydrogenations of methylene-interrupted poly-ynes which afforded both *trans*-isomers as well as over-hydrogenated products. The hydroboration of tri-ynes **3.49a-c** on the other hand afforded their tri-enes **3.50a-c** with no isomeric impurity detected according to capillary GC in 59-65% yield.[158] Svatos et al. in 1994 described the deuteration of a tri-yne **3.51** to afford tri-ene **3.52** in 66% yield, using $NaBD_4$ and CD_3CO_2D (d-acetic acid).[155] They remarked on this method having virtually no byproducts detectable according to GC while both the Lindlar and Brown catalysts produced products contaminated with *trans*-isomers as well as under and over-hydrogenated products. Blackburn et al. in 2003 did as well note the hydroboration of the tri-yneol **3.53** in 56% yield affording the pure stereoisomer of tri-enol **3.54** according to ^{13}C-NMR.[159] These hydroborations are depicted in Scheme 3.15.



Scheme 3.15 Skipped poly-yne hydroborations carried out by Millar and Underhill,[158] Svatos et al.[155] and Blackburn et al.[159] with all authors reporting complete stereocontrol of the resulting tri-enes.

Sgoutas et al. in 1969 on the other hand described the hydroboration of the di-yne **3.55** to produce the di-ene **3.56** in 87% yield which then measured ~1% *trans* according to IR.[156] Brudermüller and Musso successfully hydrogenated a symmetrical tri-yne diol with the Lindlar catalyst obtaining the tri-ene in 73% yield and 94% isomeric purity according to ^{1}H-NMR.[105] They did then not attain satisfying results using Lindlar catalyst on the very similar substrate, symmetric tri-yne diacetal **3.57**, obtaining obvious over-hydrogenation. However, employing the hydroboration method using dicyclohexylborane afforded the tri-ene **3.58** in 72% yield and in >94% isomeric purity according to ^{1}H-NMR.[157] Wong et al. did not mention any possible byproducts after their hydroboration of tri-yne **3.25** to tri-ene **3.59** which they obtained in 47% yield in 1985.[154] They did remark on how hydroboration was superior to the Brown catalyst which they deemed unsuitable for skipped tri-ynes, comparing their synthesis to Mori and Ebata[137] from 1981. Shimazaki et al. reported the semi-hydrogenation of a skipped tri-yne **3.60** in 1988.[135] They noted how the Rosenmund catalyst afforded too much of unidentified hydrogenation byproducts and hydroboration being a superior method affording their tetra-ene **3.61** in 95% purity by RP-HPLC after deprotection of t-butyldimethylsilyl ether and hydrolysis of the methyl ester. They did not describe further the reaction conditions nor mentioned the yields for the hydroboration reaction. These hydroboration reactions are illustrated in Scheme 3.16.



Scheme 3.16 The hydroborations described by Sgoutas et al.[156], Brudermüller and Musso[157], Wong et al.[154] and Shimazaki et al.[135]

Lastly, Crombie and Morgan alluded to a tri-yne hydroboration to obtain 14-dideuterated linolenic acid in their short communication from 1988 but did not describe conditions, purity nor yields.[160]

Benefits of this method include, no hydrogen gas needed and seemingly excellent stereoselectivity and little or no over-hydrogenation. The drawbacks to this method are the

sometimes moderate yields for tri-ynes, chemoselectivity, reactivity towards ketones, aldehydes, and alcohols (though there are examples of substrates with free hydroxyl groups) and cumbersome reactions. At least stochiometric ratio of borane reagents is needed for each alkyne in the substrate which might complicate reactions for the larger poly-ynes, notably no methylene-skipped poly-yne substrates greater than tri-ynes have been reported in the literature.

3.2.4 Miscellaneous other methods

Hungerford and Kitching in 1996 described the titanium(II) based semi-hydrogenation of alkynes to *cis* alkenes.[93] Using this method, they semi-hydrogenated conjugated di-ynes as well as skipped di-ynes and tri-ynes. The process involves using titanium isopropoxide ($Ti(OiPr)_4$) and isopropyl magnesium bromide (iPrMgBr) in large excess, 4-5 eq and 11-13 eq, respectively, for di-ynes and 8 eq and 20 eq for tri-ynes that form the alkoxytitanium-acetylene complexes. The reaction mixture is then quenched with H_2O or D_2O to obtain the *cis* alkene, see Figure 3.5.

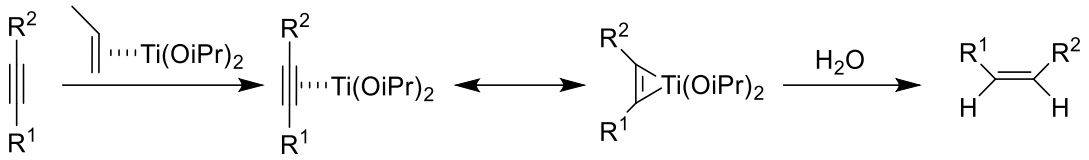
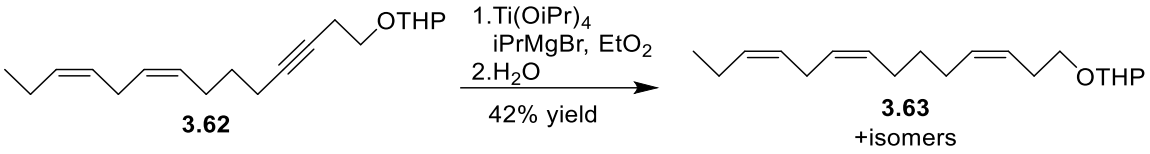


Figure 3.5 The semi-hydrogenation of an alkyne via the likely alkoxytitanium-acetylene complex.

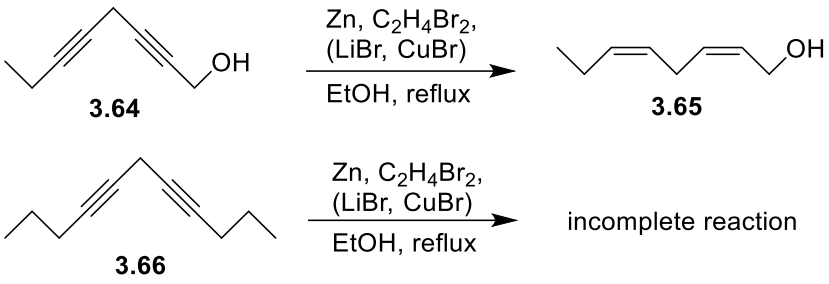
The authors described it as a simple procedure using cheap reagents affording negligible over-hydrogenation and without any detectable isomerization according to GC. The method offers additional advantages by using water as the proton supplier, especially for isomeric labeling, as well as chemoselectivity against terminal and *trans* alkenes. This method has though not gained any widespread usage and that is probably since it has some major drawbacks. The yields for di-enes were 42-53% and 26% for deuterated di-ene, for skipped tri-enes the yields were only reported for deuterating reactions, 25-33%. The di-ene-yne **3.62** was hydrogenated to tri-ene **3.63** in 42% yield, contaminated with isomers lacking the skipped di-ene system. The low yield, huge excess of reagents, reactivity towards most common active groups such as ketones, aldehydes, esters, carboxylic acids, alcohols, amines and thiols and possibly some rearrangements of double bonds have not made this method a desirable option to semi-hydrogenate skipped poly-ynes for chemists.[93, 161]



Scheme 3.17 The alkoxytitanium(II) mediated semi-hydrogenation of the di-ene-yne **3.62** where signs of isomerization of the skipped di-ene system was observed.[93]

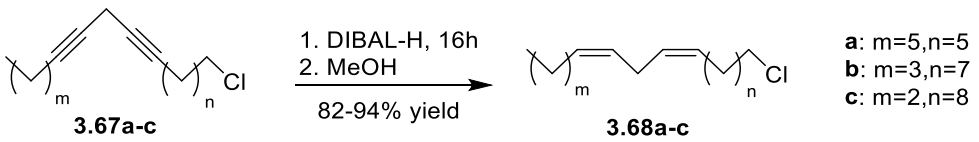
Many examples of activated zinc have been reported for the semi-hydrogenation of alkynes to *cis* alkenes. The zinc metal is activated with 1,2-dibromoethane or as a zinc copper couple Zn(Cu), other authors then report using zinc-copper-silver Zn(Cu/Ag) and in some cases Rieke zinc. This method is often highly stereoselective and regioselective, but some

authors note low (1-2%)[162] amounts of isomers for the hydrogenation of even single alkynes or incomplete reactions and in other cases over-hydrogenation or even high amount of *trans*-isomers.[163] Many successful examples of this reduction method have been reported, but mainly for mono-ynes and conjugated systems. Very few examples of skipped di-yne hydrogenations are found in the literature and the method then only proceeds if one of the triple bonds are activated by an electron donating group in close proximity, more reactive the closer the group is to the alkyne. Aerssens et al. demonstrated this method on an array of substrates, mainly mono-ynes and conjugated di-ynes, but also a few skipped di-ynes. The successful semi-hydrogenation of di-yne **3.64** to the di-ene **3.65** and the unsuccessful semi-hydrogenation of the di-yne **3.66**[163] are depicted in Scheme 3.18. The reaction was incomplete or unreactive for nonactivated or nonconjugated di-ynes.[70] No examples of skipped poly-ynes larger than di-ynes were found in the literature.



Scheme 3.18 One of the few examples of a zinc catalyzed skipped di-yne semi-hydrogenations. The authors did not report exact yields.[163]

Wilke and Müller pioneered the work on hydroalumination of alkynes beginning in 1956.[96] Then in 1963 Gensler and Bruno described the hydroalumination of skipped di-ynes to obtain some positional isomers of linoleic acid.[164] They used diisobutylaluminium hydride (DIBAL-H) to form the alkenylalanes with the di-ynes **3.67a-c** which were then protonolyzed with methanol. They obtained their chloro-di-enes **3.68a-c** in 82-94% yield, see Scheme 3.19. They noted very low *trans* and conjugated isomers (<1%), according to IR and UV spectra. Surprisingly, this method has since then not seen much use in poly-ene synthesis, even though chemoselectivity against alkenes has also been demonstrated. The main reason is most likely because of the DIBAL-H reactivity towards many functional groups, such as esters, epoxides, amides, tosylates and nitriles and large excess of DIBAL-H needed if any protic groups are present on the substrates. In addition the alkenylalanes are known to form dimers that produce butadiene units.[70]



Scheme 3.19 The hydroalumination reactions of di-ynes performed by Gensler and Bruno.[164]

Trost and Braslau demonstrated the homogeneous catalysis for the stereoselective semi-hydrogenation of alkynes using a palladium (0) and acetic acid catalytic system in the presence of tetramethyldisiloxane (TMDSO).[97] They only demonstrated the method on a single skipped di-yne, which was obtained in 59% yield in 13.4:1 ratio of the desired all-*cis* compound and another unidentified byproduct. This coupled with the semi-hydrogenation of a few other mono-yne substrates producing products with non-negligible

amount of *trans*-isomers has not made this method a sought-after option for the semi-hydrogenation of poly-ynes.

The methodologies discussed in this section are the methods that have currently been used to stereoselectively semi-hydrogenate skipped poly-ynes. To this authors knowledge no other methods have been successfully employed for such work. More methods have been demonstrated on mono-ynes or conjugated systems.[70] Methods that work perfectly well for mono-ynes then show considerable limitations for skipped poly-ynes, which underlines the distinctive properties of this system. In relevance to the work described in this thesis only semi-hydrogenation of skipped poly-ynes were reviewed, but this system proposes unique challenges.

As experienced during this work, as well as by many researchers mentioned above, the semi-hydrogenation byproducts are a universal problem. No matter the method a serious thought must be put into detecting them and then hopefully separate them from the desired compound. This makes one be extra suspicious for reports which claim very high or absolute purity and high yields of semi-hydrogenation of skipped poly-ynes. Many of these reports, especially older ones used technology which did not necessarily detect or separate these byproducts, but many, even recent ones, totally ignore them and don't demonstrate in a convincing manner the purity of the products. This makes it in some cases hard to conclude exactly what methods would be the best for the synthesis of skipped poly-enes. Chapter 6.2 details the semi-hydrogenation challenge undertaken during this project.

Lastly it is of worth to mention that this field is ever evolving, and new methods appear regularly, some of which might offer usability for the semi-hydrogenations of skipped poly-ynes.

3.3 The Wittig approach

The Wittig reaction is now a classical reaction first reported in 1954 by Wittig and Shöllkopf.[165] Much has been written on this reaction which exact mechanism has eluded chemists for decades.[166-168] In the initial paper the authors observed both *trans* and *cis* double bonds in equal amount but did not comment on it further and probably thought the reaction didn't offer any stereochemical properties. In the years that followed chemists quickly discovered how the reaction could favor the formation of *cis* or *trans* double bonds and by 1964 Bergelson and Shemyakin had systematically studied the stereoselectivity of the reaction and published a comprehensive paper on the stereoselective synthesis of unsaturated fatty acids using the Wittig reaction.[169] This paper opened up a completely new approach to poly-ene synthesis. In the wake of these discoveries numerous chemists started to report on multitudes of conditions and reagents that affected the *cis*/*trans* ratio.

Typically, the Wittig reaction involves an aldehyde or ketone and a triphenylphoshonium ylide which forms a carbon-carbon double bond exclusively at the sites of the former carbonyl and ylide. The reaction of an aldehyde and an ylide gives rise to a double bond which stereochemistry can be predicted by the properties of the ylide. If the ylide is non-stabilized the *cis*-alkene is formed in medium to high selectivity. Stabilized ylides form the *trans*-alkene in high selectivity. Other factors can determine further how strong selectivity

can be achieved, such as temperature, solvent or co-solvent, presence of lithium, dilution, and the bulkiness of the substrates.

Classically the mechanism has been assumed to be the nucleophilic attack of the ylide carbon on to the carbonyl compound to produce the zwitterionic betaine, where the bulkiness of the triphenylphosphine group as well as the two alkyl groups control the addition in such a way that the alkyl groups are anti to each other as well as the triphenylphosphine and oxide are anti to one another. This allows for the formation of the oxaphosphetane which then with the elimination of triphenylphosphine oxide affords the *cis* alkene. This is illustrated in Figure 3.6. Disassociation of the betaine which would then possibly re-assemble reversed would produce *trans*-alkene impurities. Substrates that would give rise to increased disassociation would then increase the *trans* ratio.

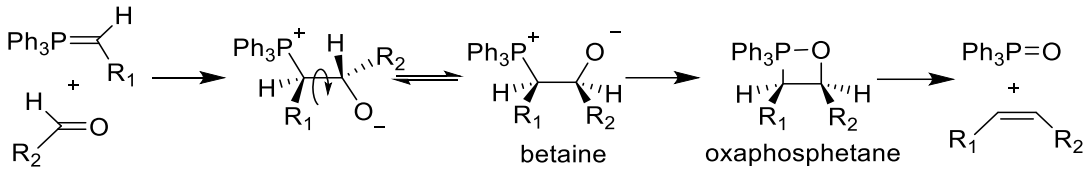


Figure 3.6 The classical mechanism proposed for the Wittig reaction of an ylide and an aldehyde, showing betaine and oxaphosphetane intermediates.

More recently it has been proposed and supported by computational calculations that the mechanism is more like a [2+2] cycloaddition where the direct and only intermediate is the irreversibly made oxaphosphetane as well as the lithium salt free reaction mechanism being fundamentally different from the lithium present reaction mechanism. The *cis*/*trans* selectivity of non-stabilized ylides is then based on the transition state of the oxaphosphetane ring forming, where both the phosphorus ligands (usually phenyl groups) and the alkyl groups connected to the ring carbons align in such a way to minimize steric interactions. In the case of non-stabilized ylides this "puckered" less hindered transition state leads to the *cis* alkene.[170, 171] This is illustrated in Figure 3.7.

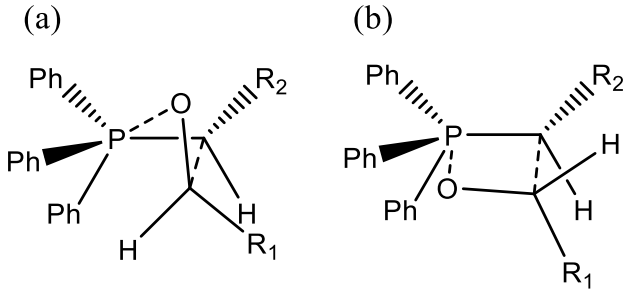
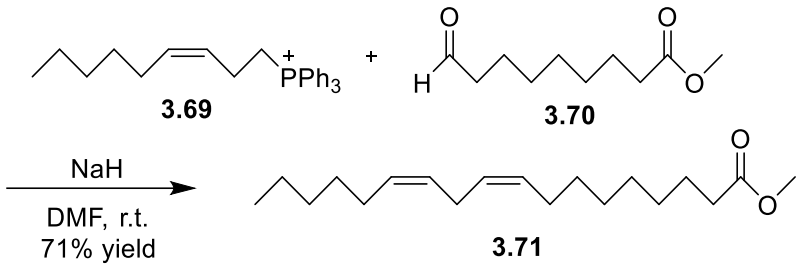


Figure 3.7 (a) The favored oxaphosphetane forming transition state leading to cis alkene for nonstabilized ylides. (b) The disfavored transition state leading to trans alkene for nonstabilized ylides.[170]

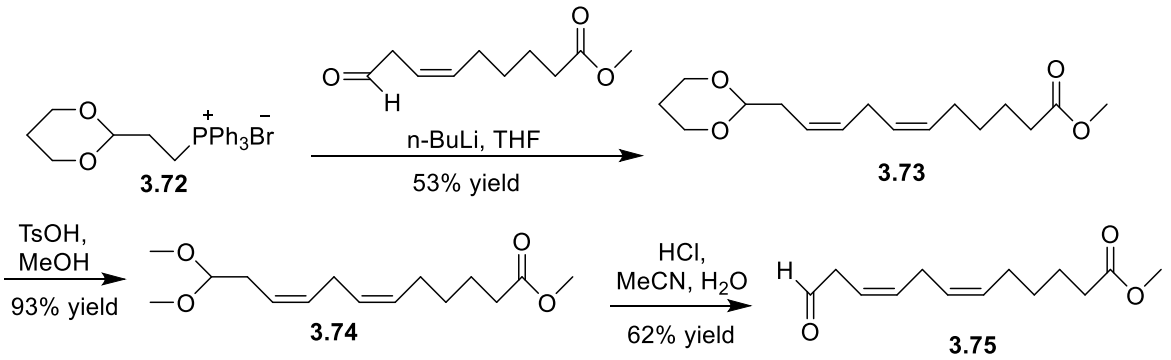
As mentioned earlier, Bergelson and Shemyakin were the first to demonstrate the usefulness of the Wittig reaction for the syntheses of polyunsaturated fatty acids in 1964.[169] They did not particularly delve into the syntheses of skipped polyunsaturated compounds with three or more double bonds except to mention the possibility. Their focus was the use of readily available starting materials that can be converted into either aldehydes or ylides. Their linoleic acid synthesis was then based on one of the Wittig reagents, the phosphonium salt **3.69**, having already one *cis*-double bond afforded from the catalytic hydrogenation of an acetylene. The Wittig reaction with the aldehyde **3.70** afforded the methyl ester linoleic acid **3.71** in 71% yield. This is illustrated in Scheme 3.20. They then

mentioned the possible formation of a *trans* double bond in the catalytic hydrogenation that can sometimes be conveniently removed by recrystallization of the phosphonium salt or by chromatography on neutral low activity grade alumina as the *trans,cis*-diene but in other cases to be difficult to remove. The Wittig reaction itself also produced *trans* double bonds which they estimated in up to 10% for the di-ene as well as the formation of conjugated double bonds either as the isomerization of the aldehyde to an α,β-unsaturated aldehyde or the ylide to the β-alkenylide. They did report the di-ene **3.71** as 97% pure according to GC after purification on neutral alumina. Their discussion did underline the main drawbacks of the Wittig reaction, namely the stereoselectivity of the double bond formed and then the conjugation of the unsaturated Wittig reagents in the presence of strong bases.



Scheme 3.20 The first reported synthesis of a natural fatty acid (LA) utilizing the Wittig reaction.

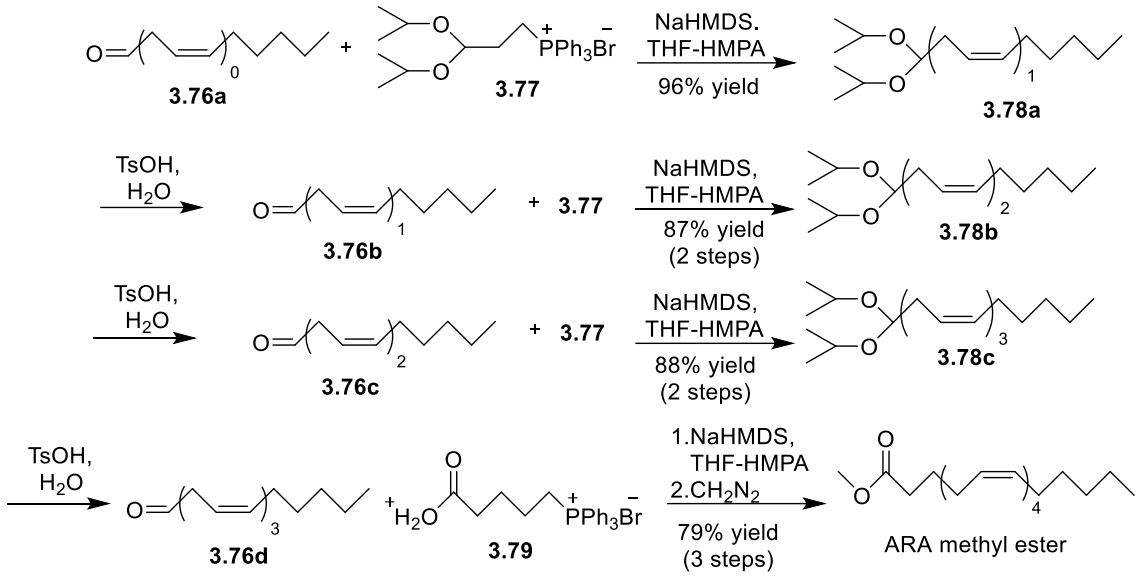
The poly-ene syntheses using Wittig reactions were established in more detail in the 1980s with the appearance of suitable homologation agents for the synthesis of 1,4-diene units. Beginning with Rakoff in 1984, the three-carbon acetal phosphonium bromide **3.72** was used to synthesize an 18:3 methyl ester in homologating Wittig reactions.[172] The main drawback of this synthesis was the extreme acidic conditions for the transacetalation of the 1,3-dioxane acetal **3.73** into the dimethoxy acetal **3.74** and finally into the aldehyde **3.75**, which promoted isomeric conjugation (up to 22%). Scheme 3.21 depicts one homologating cycle in the synthesis.



Scheme 3.21 The first Wittig homologation agent **3.72** demonstrated by Rakoff.[172]

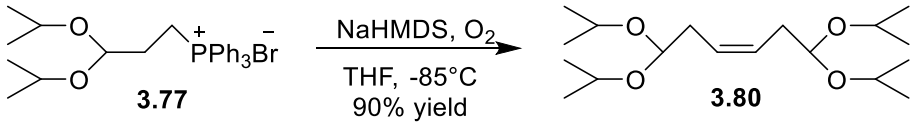
Viala and Santelli significantly improved the homologating cycle by utilizing the diisopropyl acetal **3.77** which they easily prepared from acrylaldehyde/prop-2-enal.[173] In 1988 they used **3.77** to synthesize arachidonic acid in three consecutive homologation Wittig reactions affording the final product in 58% total yield.[174] The aldehydes **3.76a-c** were reacted in a Wittig reaction with the homologating agent **3.77** to produce the unsaturated acetals **3.78a-c**. The final skipped tri-enal **3.76d** was then reacted to another Wittig agent **3.79** to produce the ARA methyl ester. The synthesis is depicted in Scheme

3.22. They cited high to excellent yields (87-96%) and excellent stereoselectivity for the homologating steps due to the absence of lithium salts, high dilution and low temperature conditions producing pure *cis* products. They did not mention conjugation and their only estimation on *trans* double bonds stems from the ^{13}C-NMR spectra of the products. They did mention 5% *trans* impurity if n-BuLi was used in the Wittig reaction.



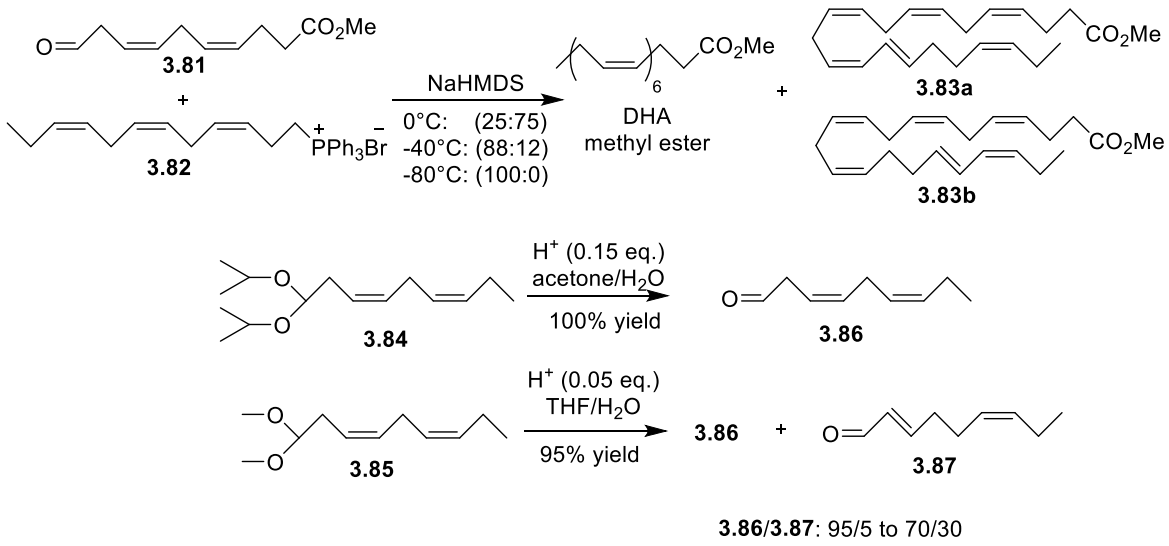
Scheme 3.22 The synthesis of the methyl ester of ARA by Viala and Santelli, using three cycles of the diisopropyl acetal homologating agent **3.77**.[174]

Viala and Sandri then described the use of the C6 homologation agent **3.80** in 1995 that already possesses a *cis* double bond produced by the oxidative dimerization of **3.77**, depicted in Scheme 3.23.[175] They first reported its use in the synthesis of α-linolenic acid, where 50% yield was afforded in the final Wittig reaction,[175] and then the same year, in a convergent synthesis of EPA and DHA where the Wittig reactions were achieved in 50-76% yields.[176]



Scheme 3.23 The first C6 homologating agent already possessing a *cis* double bond, produced by Viala and Sandri.[175]

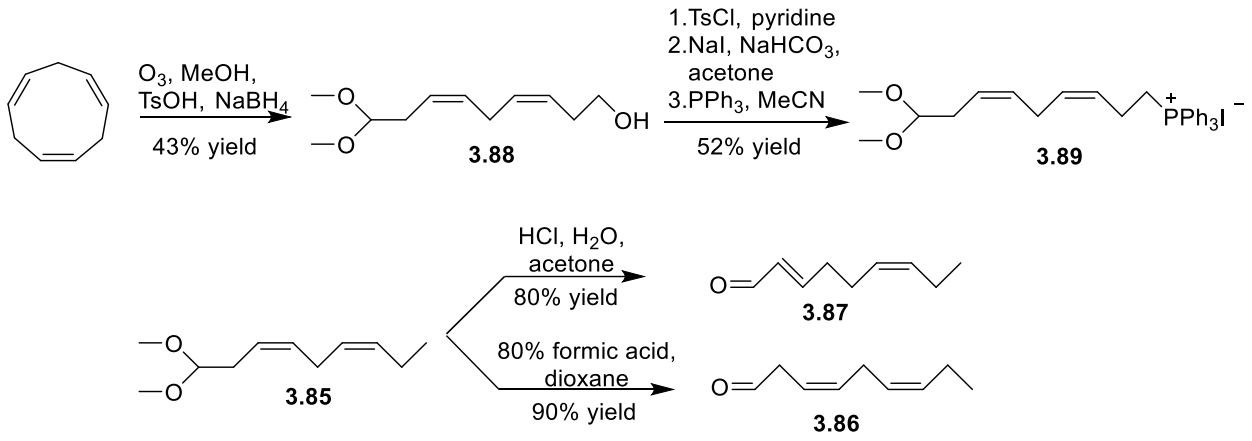
They only reported two instances of migration of double bonds. Firstly, in the Wittig reaction of the tri-ene phosphonium salt **3.82** and the di-ene aldehyde methyl ester **3.81** that afforded the DHA methyl ester, which interestingly produced the conjugated isomers **3.83a-b** in majority at 0°C but none at -80°C. Secondly, the deprotection of a di-ene acetal, where the dimethyl acetal **3.85** was deprotected with majority of the closest double bond migrated **3.87** while the corresponding diisopropyl acetal **3.84** was deprotected without migration **3.86**. The conjugation instances are depicted in Scheme 3.24. Otherwise only the pure *cis* products were assured by the authors but the only *cis/trans* estimation was made through ^{1}H and ^{13}C-NMR.



Scheme 3.24 The only double bond migration byproducts mentioned by Viala and Sandri in their synthesis of ALA, EPA and DHA. Their other Wittig reactions presumably affording pure compounds.[175, 176]

At this point, the Wittig homologating reactions seemed to emerge as a vastly superior alternative to the alkyne semi-hydrogenations for the synthesis of skipped poly-enes and this methodology was believed to afford absolutely stereoselective Wittig reactions. Santelli and coworkers in their previously cited 1998 paper[88] however revealed that this approach did, when minorly scaled up (up to 0.1 mole), exhibit all the numerous drawbacks of usual Wittig reactions, conjugation, *trans*-isomers and low yields. In 2002 Peng et al. reported the use of **3.77** to synthesize a di-ene aldehyde Wittig reagent in two consecutive Wittig reactions in 77% and 73% yields for each homologating step, mentioning that only pure *cis* products were produced but did not demonstrate that any further.[177]

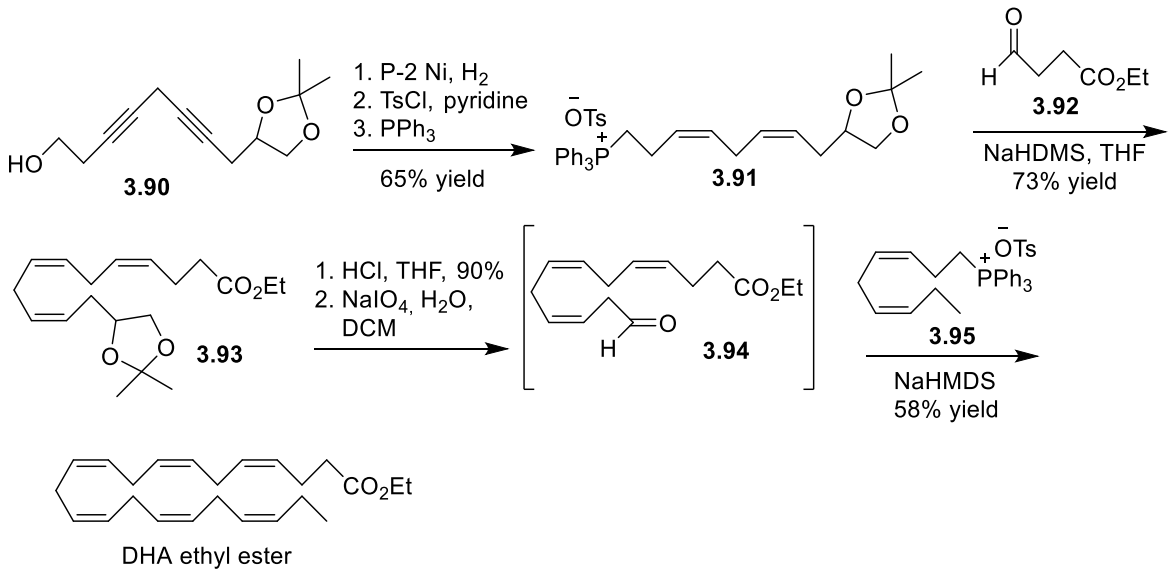
Lastly, in 2014 Mustafa et al. described the synthesis of a di-ene homologating agent **3.89** derived from the ozonolysis of cyclonona-1,4,7-triene via **3.88**, depicted in Scheme 3.25, from which they synthesized multiple skipped poly-ene systems such as pheromones and fatty acids, all the way up to a skipped octa-ene.[178] The authors also described an improved deprotection method where the dimethoxy acetal, e.g. **3.85**, was converted into the aldehyde **3.86** using formic acid in dioxane supposedly without any migration of double bonds. The Wittig reactions afforded the usual 62-76% yields and the authors mentioned the formation of possible *trans*-isomers but assured pure *cis* products after chromatography. Only normal silica gel chromatography was described, and no further estimation of *trans*-isomers was provided. The yields were generally lower with increased number of double bonds. Interestingly in one case a considerable amount of a penta-ene byproduct from the self-condensation of the phosphonium salt was isolated.



Scheme 3.25 The formation of the di-ene homologation agent **3.89** by Mustafa et al. and their dimethoxy acetal deprotection providing the pure di-ene aldehyde without any conjugated byproduct.[178]

This methodology of sequential Wittig reactions, interrupted by deprotection of the acetal group into the aldehyde or subsequent alteration into an alcohol via reduction, then a halide, usually bromide, and finally the phosphonium salt relies not only on the stereoselectivity of the Wittig reaction. Also, the outcome is dependent on the end-group modifications having limited or diminished effect on the migration of the double bonds that leads to conjugation in the aldehydes or phosphonium salts. The Wittig reactions in skipped poly-ene syntheses have reported yields generally around 50-75%, with most authors reporting *cis*/*trans* selectivity to be from 94:6[179] to pure *cis* products when at least one reactant is already possessing a double bond. Most of these authors did not definitively prove that no conjugation or *trans*-isomers formation took place in these reactions or that they could separate them with chromatography. This does not invoke much confidence in the Wittig approach and Santelli and coworkers, as mentioned earlier, already revealed how minor scale up showed how these seemingly perfectly stereoselective Wittig reactions started to exhibit all the usual drawbacks.[88] The uncertainty of stereoselectivity, conjugation problems for unsaturated reagents, sometimes instability or self-condensation of the substrates, low temperatures, strong bases, and moderate yields have made the Wittig reaction somewhat less appealing than the acetylenic approach. Consequently, in the last decade researchers synthesizing large, skipped poly-ene systems have mostly been using the acetylenic approach.

Finally, the combined use of the acetylenic and Wittig approaches can be employed to minimize the drawbacks and utilize the strengths of each method. Taber and You demonstrated this in 1995 in their convergent synthesis of DHA.[139] In accordance with Scheme 3.26 they used the two di-ene building blocks **3.91** and **3.95** afforded from the catalytic semi-hydrogenation of their corresponding di-ynes, e.g. **3.90**. **3.91** was attached to the ester aldehyde head **3.92** in a Wittig reaction, affording tri-ene **3.93** in 73% yield which was then coupled to the other di-ene **3.95** in a second Wittig reaction after a diol cleavage produced the unstable tri-ene aldehyde **3.94**. This afforded the hexa-ene structure of DHA, where two of the double bonds originated from the Wittig reactions and four of them from the catalytic semi-hydrogenations. The authors only relied on ^{13}C-NMR to establish the isomeric purity, which they deemed to be "quite clean" and no further attempts were made to establish *trans*-isomers or over-hydrogenated byproducts.



Scheme 3.26 Part of the total synthesis of the DHA ethyl ester by Taber and You, combining the acetylenic and Wittig approaches.[139]

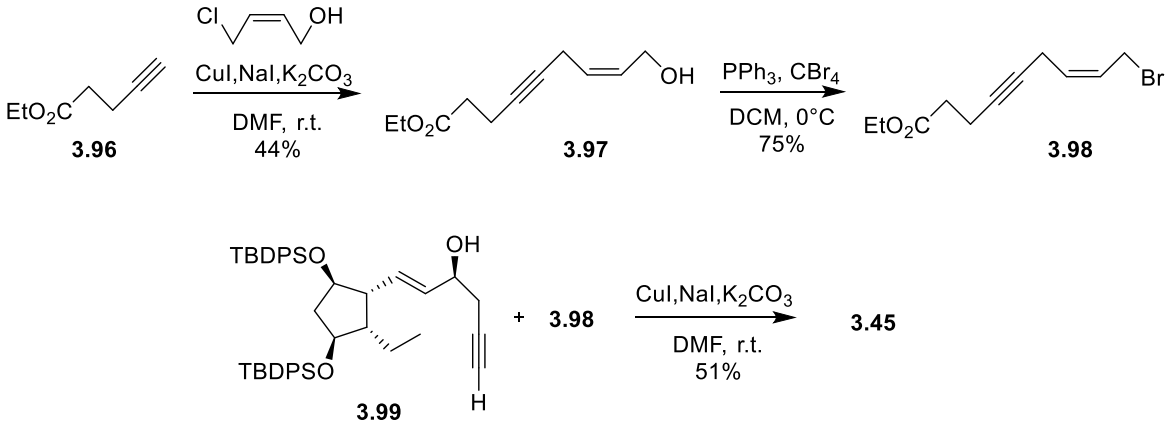
Many more authors have described syntheses using both methods to incorporate the poly-ene structure.[102, 113, 142, 147, 180, 181] The double bonds originated from Wittig reactions were generally assumed to be all-*cis* and no further efforts were made to confirm the *cis/trans* ratio.

3.4 Alternative strategies

The methods detailed in the previous sections have been shown to have certain downsides. Three main alternative strategies have been employed to produce skipped *cis* double bond systems and have been used in poly-ene total synthesis. Semi-hydrogenations of poly-ynes give rise to gradually more complex reaction mixtures based on number of acetylenes in the structure and the main goal for these alternative approaches can be summed up as, to avoid large skipped poly-yne substrates. The first method is simply using allyl halides as reactants instead of propargyl halides to produce 1,4-ene-ynes instead of the more unstable 1,4-di-ynes. The second approach is splitting up the semi-hydrogenations, so substrates with fewer acetylenes are semi-hydrogenated at a time. The third approach is using natural starting materials which already possess the desired poly-ene system.

The first alternative strategy that has been demonstrated is the allyl coupling. It involves the Jeffery or Lapitskaya copper coupling or the copper assisted Grignard coupling of a terminal acetylene to a *cis*-allyl halide, producing an 1,4-ene-yne system. The first matter to consider for this approach is that the allyl compound must already possess the *cis* configuration which has to be obtained in acceptable isomeric purity. The second matter is that the resulting substrate still must undergo a semi-hydrogenation reaction to convert the remaining acetylenes to *cis*-double bonds which will then likely proffer the same semi-hydrogenation byproducts as otherwise but possibly to a lesser extent.

Taber et al.[152] demonstrated this method successfully in 2008 for the synthesis of a tri-ene **3.46** after their semi-hydrogenation of the skipped tri-yne **3.44** that resulted in a complex mixture, as already depicted in Scheme 3.13. Scheme 3.27 depicts the synthesis of the ene-yne-ene compound **3.45**.



Scheme 3.27 The synthesis of the ene-yne-ene system by Taber et al. using the allyl coupling approach.[152]

They first coupled together the terminal acetylene **3.96** to the allyl 4-chloro-*cis*-buten-1-ol affording ene-yne **3.97** which they brominated to produce the allyl bromide **3.98.** That they coupled to the terminal acetylene **3.99** resulting in the yne-ene-yne **3.45**. The coupling yields were rather lower than usual yields (44% and 51%) for similar propargyl couplings (70-100%). The reason for that is mostly due to the S_N2 byproduct, where the nucleophilic attack took place on the olefin carbon, which then displaces the halide, instead of the halide carbon. This is illustrated in Figure 3.8. Taber et al. obtained the ene-yne-ene **3.45** in a 3:1 ratio against the byproduct which they could fortunately easily separate.

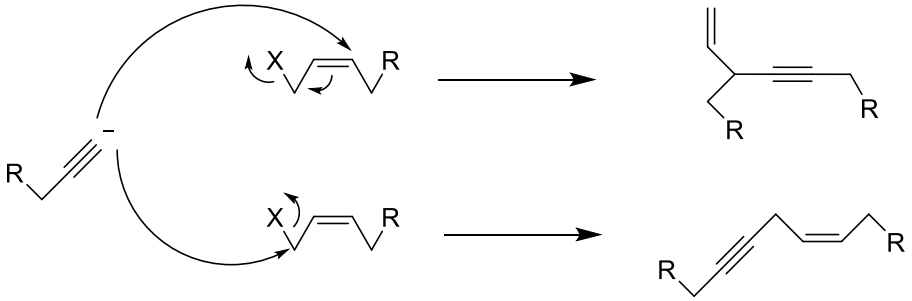
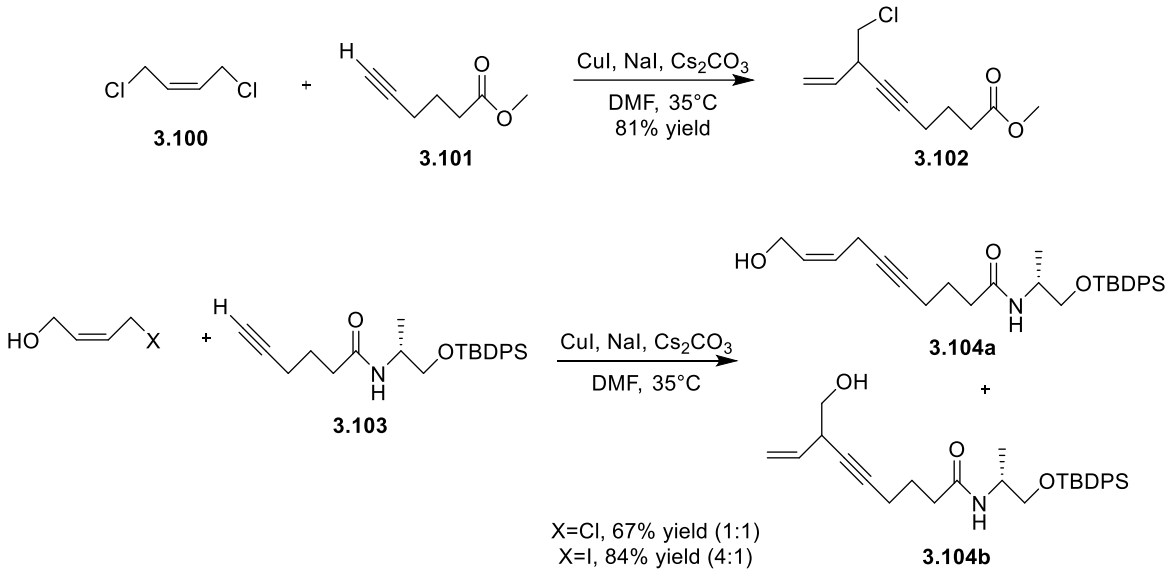


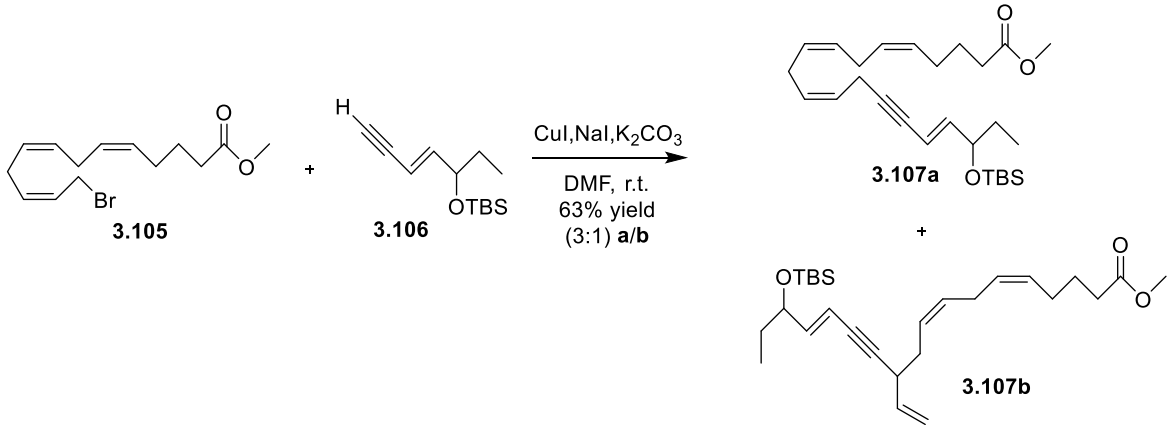
Figure 3.8 The two possible routes for the terminal acetylene copper coupling to an allyl halide, resulting in two regioisomers.

In the attempt to bypass the instability of their tetra-yne substrates **3.35** and **3.37**, Balas et al. ventured to produce ene-yne substrates to hopefully mitigate the instability issues.[150] Using the typical Lapitskaya conditions for the terminal alkyne **3.101** and the allyl **3.100**, they observed the unwanted regioselectivity since the reaction took place at the olefin carbon affording the chloride **3.102** as the only product in 81% yield. They did further experiments where they coupled 4-chloro-*cis*-buten-1-ol as the allyl compound to their acetylene amide **3.103** which afforded both regioisomers **3.104a-b** in a 1:1 ratio. They managed to improve the ratio in favor of the desired isomer to 4:1, by using 4-iodo-*cis*-buten-1-ol (see Scheme 3.28). Lastly, they tried the Grignard type reaction, where the amide **3.103** did not tolerate the conditions too well but was coupled to the di-chloro allyl **3.100** affording both regioisomers in a 1:1 ratio and 10% yield where the bis-substituted derivative was obtained as well (27%).



Scheme 3.28 The allyl coupling trials of Balas et al.[150]

Nanba et al.[151] described in their paper from 2018 the synthesis of the penta-ene **3.40**, see scheme 3.12. They also attempted the Lapitskaya copper coupling of the tri-ene **3.105** and terminal acetylene **3.106** resulting in a 3:1 ratio of the regioisomers **3.107a**/**3.107b** which they could then not separate by silica gel chromatography, see Scheme 3.29.



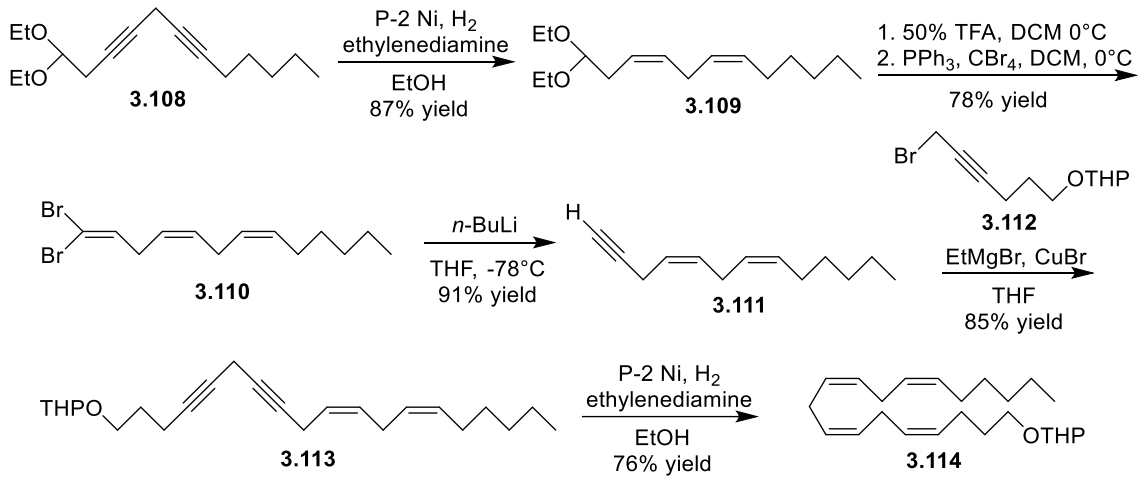
Scheme 3.29 The allyl coupling attempted by Nanba et al. which resulted in an inseparable regioisomer mixture.[151]

As a final example of an allyl coupling, Altundas et al.[143] semi-hydrogenated a tetra-yne but then added another acetylene unit to the structure through the copper coupling of the tetra-ene allyl bromide and three different terminal acetylenes. Three different tetra-ene-ynes were produced in rather poor or moderate yields (18%, 34% and 38-52%). The low yields are attributable to the allyl bromide coupling or the state of the tetra-ene which does not seem to have been purified in a meaningful way. These examples should demonstrate the main problem with this strategy, the regioselectivity, which results in low to moderate yields and the sometimes very difficult separation of isomers.

The second alternative method is based on splitting the semi-hydrogenations up. Instead of performing one semi-hydrogenation of a substrate with a large unstable skipped poly-yne framework, a few semi-hydrogenations would be performed on smaller substrates. This effectively partitions the semi-hydrogenation reaction into more reactions using smaller

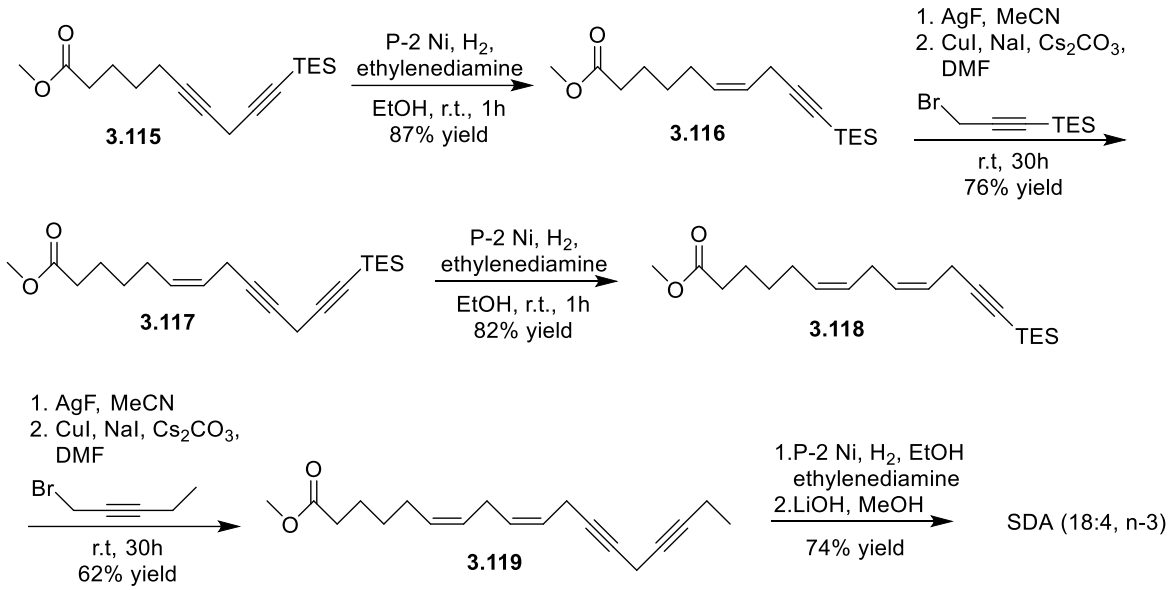
skipped poly-yne systems, where the instability of the substrates would be more manageable and lesser formation of the semi-hydrogenation byproducts are to be expected. On the other hand, this approach introduces extra steps, and the increased yields and purity of the poly-yne semi-hydrogenations must outweigh the material loss in the additional steps.

Heitz et al.[138] demonstrated this already in 1989, commenting on the instability of poly-ynes making them unfit for scale up. Their synthesis is depicted in Scheme 3.30. They coupled together two alkynes to produce the di-yne **3.108** which was then semi-hydrogenated by the Brown catalyst, affording **3.109**. They then manipulated the terminal acetal group to obtain the third alkyne using the Corey-Fuchs reaction[182] which afforded diene-yne **3.111** via the dibromo triene **3.110**, which they then coupled to the fourth alkyne **3.112** with the copper catalyzed Grignard reaction. The resulting di-ene-di-yne **3.113** was then semi-hydrogenated to produce the target tetra-ene **3.114**. The authors did still remark on the extreme care needed in the semi-hydrogenation steps to minimize the byproducts in the reactions.[138]



Scheme 3.30 The second alternative approach as demonstrated by Heitz et al. where they performed two semi-hydrogenations of skipped di-ynes instead of a single semi-hydrogenation of a tetra-yne.[138]

This method may be taken further by utilizing the inertness of silyl protected terminal alkynes under many semi-hydrogenation conditions. An alkyne is coupled to another silyl protected alkyne and then semi-hydrogenated using for example the Brown catalyst leaving the silyl-protected acetylene unreacted. The resulting ene-yne is then deprotected and ready to be coupled to a new alkyne which in turn can be semi-hydrogenated. This method was demonstrated in a Korean patent from 2010, where the researchers synthesized stearidonic acid (18:4, n-3) as depicted in Scheme 3.31.[183]

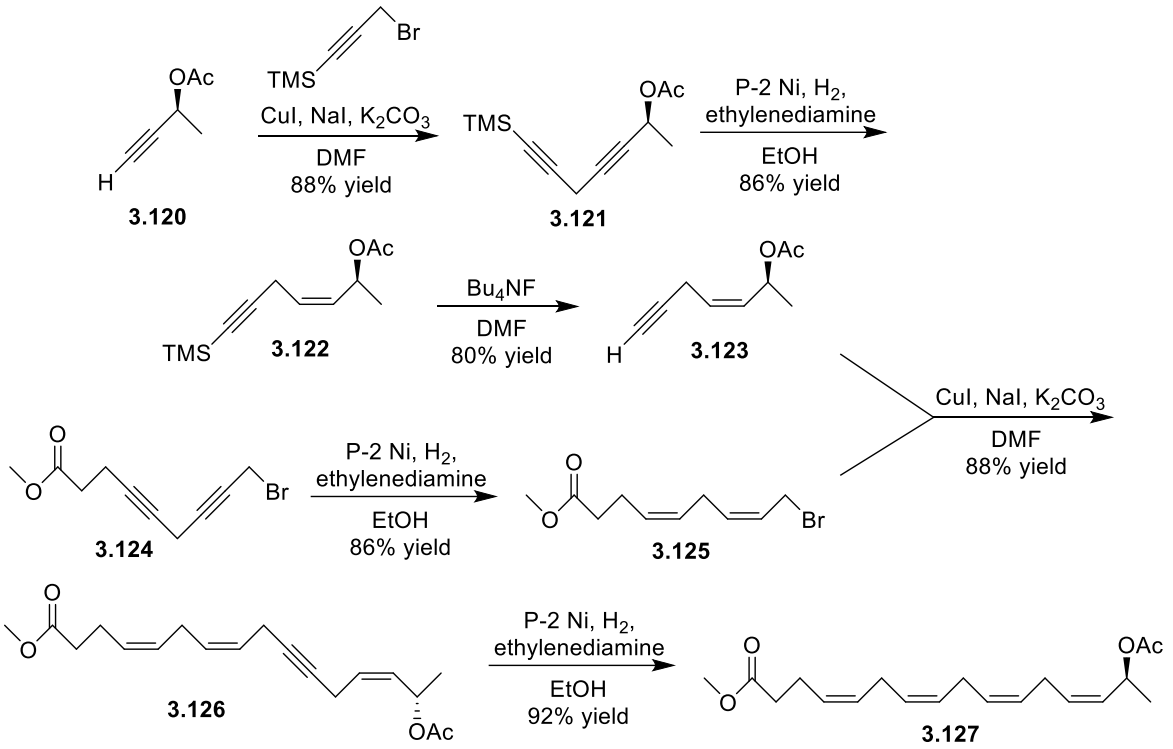


Scheme 3.31 Synthesis of stearidonic acid using the second alternative approach by repeated cycles of addition of TES protected acetylene, semi-hydrogenations and TES deprotection.[183]

The authors produced the TES protected di-yne **3.115** which was semi-hydrogenated with the Brown catalyst. The Brown catalyzed semi-hydrogenation left the TES protected terminal acetylene unaffected. The resulting en-yne **3.116** was then deprotected by AgF and copper coupled to a new TES protected propargyl bromide which afforded the ene-di-yne **3.117**. The ene-di-yne was then submitted to a Brown catalyzed semi-hydrogenation which once again left the TES protected acetylene unaffected, affording **3.118**. The deprotection and copper coupling was then repeated, except using 1-pent-2-yne as the propargyl substrate. This completed the desired framework and afforded the di-ene-di-yne **3.119**, which was then submitted to the final semi-hydrogenation resulting in SDA.

The semi-hydrogenation steps afforded their products in 87%, 82% and 74% yields. The researchers also semi-hydrogenated the corresponding skipped tetra-yne, all triple bonds at once, and obtained 71% yield. The patent did not address any specific impurities arising from the semi-hydrogenation of the tetra-yne and therefore it remains unclear why the multiple hydrogenations should be more beneficial, as the yields were much lower and the synthesis longer. A linear approach like that for the synthesis of stearidonic acid is unlikely to be more beneficial than a convergent synthesis with three fewer steps. The benefits of this linear synthesis of even larger poly-enes are unlikely to outweigh the benefit of a convergent synthesis through a poly-yne intermediate which should only be present for a very short time between its purification and semi-hydrogenation.

In 2001 Hansen and Stenstrøm described the first total synthesis of (-)-aplyolide, a cyclic molecule with four skipped *cis* double bonds.[184] A complex mixture of products was apparently afforded by the semi-hydrogenation of a tetra-yne substrate with the Brown catalyst, which did though contain up to 60% of the all-*cis* product according to GLC and ^{1}H-NMR analysis. This was surprising since the same reaction of a similar tri-yne had smoothly resulted in high yields. Instead, the authors decided to perform the semi-hydrogenations on smaller substrates, see Scheme 3.32.

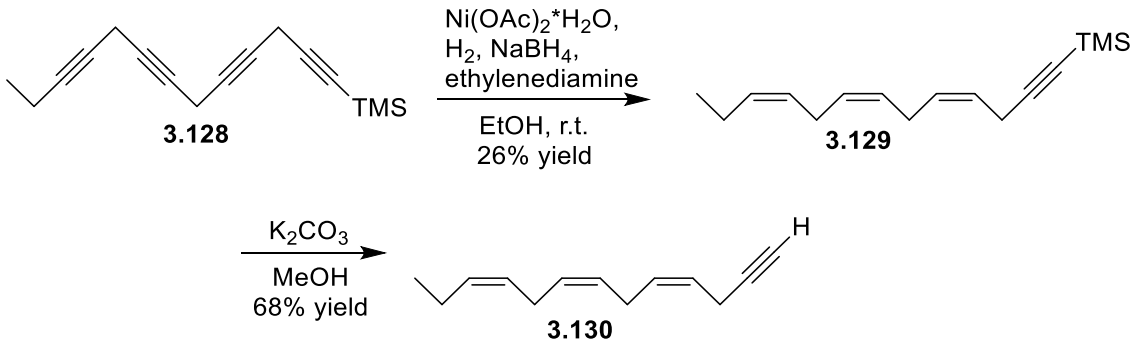


Scheme 3.32 The total synthesis of (-)-aplyolide by Hansen and Stenstrøm, utilizing a mix of the first and second alternative approaches.[184]

They began with coupling together TMS-protected propargyl bromide and the terminal alkyne **3.120**. The resulting di-yne **3.121** was then semi-hydrogenated, affording the ene-yne **3.122** since the TMS protected alkyne was unaffected. Another propargyl bromide di-yne **3.124** was produced and semi-hydrogenated, affording the di-ene propargyl bromide **3.125**. After TMS-deprotection of the ene-yne **3.122**, the di-ene **3.125** and ene-yne **3.123** were then coupled in a very successful allyl coupling reaction producing the tri-ene-yne **3.126** and it subsequently semi-hydrogenated to produce the tetra-ene **3.127**. The semi-hydrogenations were achieved in 86%, 86% and 92% yields and afforded the desired tetra-ene with acceptable minor *trans* impurities according to ^{13}C-NMR.

A patent granted in 2013 describes the synthesis of a ^{13}C-labeled DHA.[104] In this patent a ^{13}C-labeled penta-yne was produced which was then semi-hydrogenated affording the corresponding penta-ene. This penta-ene was then brominated and coupled as the allyl bromide to a terminal acetylene unit in 49-59% yield and semi-hydrogenated affording the hexa-ene in 75-85% yield. Adding a single triple bond to a poly-ene structure via allyl coupling for the purpose of semi-hydrogenating it and then adding another is most likely not an efficient method for the synthesis of large skipped poly-enes.

Another very recent example of this approach was demonstrated by Zhang et al. in 2020.[185] The authors described the synthesis of a 7-(*S*)-hydroxy-DHA, a DHA derivative. Part of the synthesis is depicted in Scheme 3.33.



Scheme 3.33 The partial semi-hydrogenation on a TMS-protected tetra-yne by Zhang et al.[185]

The TMS protected tetra-yne **3.128** was synthesized by the usual copper coupling procedures and then submitted to a semi-hydrogenation via the Brown catalyst. Interestingly, the authors found the compound to be unstable on silica and thus used it crude. The resulting product with three of the four alkynes semi-hydrogenated, leaving the TMS protected terminal alkyne intact, afforded the tri-ene-yne **3.129** in 26% yield. The TMS group was then cleaved under basic conditions producing the tri-ene-yne **3.130** in 68% yield. The authors then used this substrate in a Sonogashira coupling with a vinyl iodide to produce the *trans* bond in their target material. But this compound could in theory also be used in another copper coupling reaction with a propargyl halide, producing a larger skipped poly-ene-yne system which would then be semi-hydrogenated. The supplied ^{1}H-NMR spectra of the compounds did not indicate any over-hydrogenation which might make further purification (removal of semi-hydrogenation byproducts) unnecessary for this kind of approach, but the rather low yields, about 18% over the two steps, does not bode well.

Modification and manipulation of pure fatty acids, specifically PUFAs can be another very powerful tool in the synthesis of other poly-ene products, making use of the already established skipped poly-ene frameworks as well as chain length and position of double bonds. The drawback being the expensive, sometimes hard to obtain pure starting materials. Pure EPA and DHA concentrates are though now commercially available as well as many other natural PUFAs and they have been used for such purposes. Rezanka et al. demonstrated the synthesis of large poly-enes containing seven or eight skipped double bonds as mentioned before in Section 3.2.2.[149] For this synthesis, they utilized the Grignard coupling of a synthesized tri-yne to a tetra- or penta-ene allyl bromide derived from natural EPA and ARA as already shown in Scheme 3.10.

The coupling was achieved in 44% yield and the following semi-hydrogenation of the three alkynes in 84% yield. Methods like this could possibly be the only plausible way to produce skipped all-*cis* alkenes with seven or more double bonds using alkynes since the instability of the skipped poly-ynes must become unmanageable. A 2018 review by Vik and Hansen delves into many such applications in the literature, where researchers have demonstrated how the large PUFAs, EPA, DHA, ARA and DPA have been chemically manipulated to produce some intermediate substrates which have then been used to obtain natural or unnatural fatty acids, eicosanoids and other fatty acid derivatives as well as myriads of other lipid molecules found in nature.[186] Chapter 5.6 puts forth a proposition of an alternative synthetic pathway towards DHA-like MEL **5** utilizing this method.

To summarize, the first approach has considerable downsides. The allyl coupling generally takes place in poor or moderate yields and the regioisomer mixture is sometimes very difficult to separate. Some authors have though managed to find success using this method even with large, skipped poly-ene allyl halides. The second approach is little bit more undefined, but it is quite clear that semi-hydrogenation of smaller skipped poly-ynes, such as di-ynes or tri-ynes is easier to handle, offers less formation of byproducts and higher yields. The problematic part is the coupling of the smaller fragments together to form the desired skipped poly-ene framework. This involves manipulation of the terminal ends and then coupling reactions, for example allyl coupling, and subsequent semi-hydrogenations on those substrates These extra steps can reduce yields considerably but can nonetheless outweigh the difficulties involved with the larger skipped poly-ynes. The third approach utilizes what nature has to offer and is a very desirable option. The biggest downside is that the desired poly-ene framework must be approachable from the natural compounds. The natural PUFAs are manipulated from the acid end which means that the derived compounds are usually limited to having the most common skipped poly-ene framework omega ends (n-3 and n-6).

Powerful olefin metathesis reactions have been developed to produce *cis* alkenes with high stereoselectivity.[187, 188] These methods have been employed in many total syntheses but their use in the synthesis of skipped poly-ene frameworks are rather limited. With many double bonds present in the structure the regioselectivity can be a problem as well as undesired homodimerizations and ring-closures.[189] Some new catalysts have demonstrated excellent stereoselectivity for many substrates, but usually when these metathesis catalysts are used on more complex substrates for cross-metathesis reactions (two different substrates), the stereoselectivity is usually reduced.[190] So, in addition to the sometimes less than excellent stereoselectivity, the selective synthesis of skipped or conjugated di-enes has remained difficult, not to mention skipped poly-enes using olefin metathesis methods.

Many other examples of *cis* double bond generating methods have been reported but they usually have niche applications and therefore not applicable or relevant to the synthesis of large all-*cis* skipped poly-enes because of severe drawbacks for that kind of synthesis. These drawbacks include cumbersome handling, toxicity, expensive materials, low chemo- or stereoselectivity or the need of very specific substrates. Many methods have also never been reported on or demonstrated for skipped poly-ene substrates and not within this project's scope to test all the myriad of potential alkene producing methods.

4 Synthesis of the DHA-like MEL 5

This chapter outlines and discusses the design, execution, and main synthetic challenges for the synthesis of the DHA-like methoxylated ether lipid MEL **5**.[3] Section 4.1 briefly covers the initial stereoselective synthesis of 16:0 MEL **2** by Ställberg, the subsequent first stereoselective synthesis of an unsaturated MEL, the 16:1 MEL **1** by Magnússon and Haraldsson and then the initial attempts of Magnússon, Lúthersson and Haraldsson at the synthesis of the MEL **5**. Section 4.2 outlines the final design and execution of the synthesis of MEL **5**.[3]

4.1 Prior syntheses

4.1.1 First stereoselective syntheses of saturated and mono-unsaturated MELs 1 and 2

In 1990 Ställberg introduced the first synthesis of stereoisomerically pure methoxylated ether lipids. The syntheses of the four stereoisomers of the 16:0 MEL **2** for the first time confirmed the natural isomer to be (2'*R*,2*S*)-**2**.[50] To do that he used the chiral building blocks (*R*)- and (*S*)-solketals and (*R*)- and (*S*)-glycidyl benzyl ethers (depicted in Figure 4.1), available from the chiral pool, obtained from D-mannitol and L-ascorbic acid.

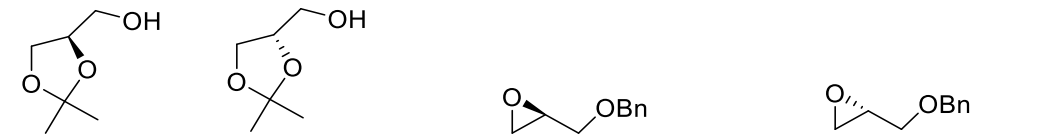
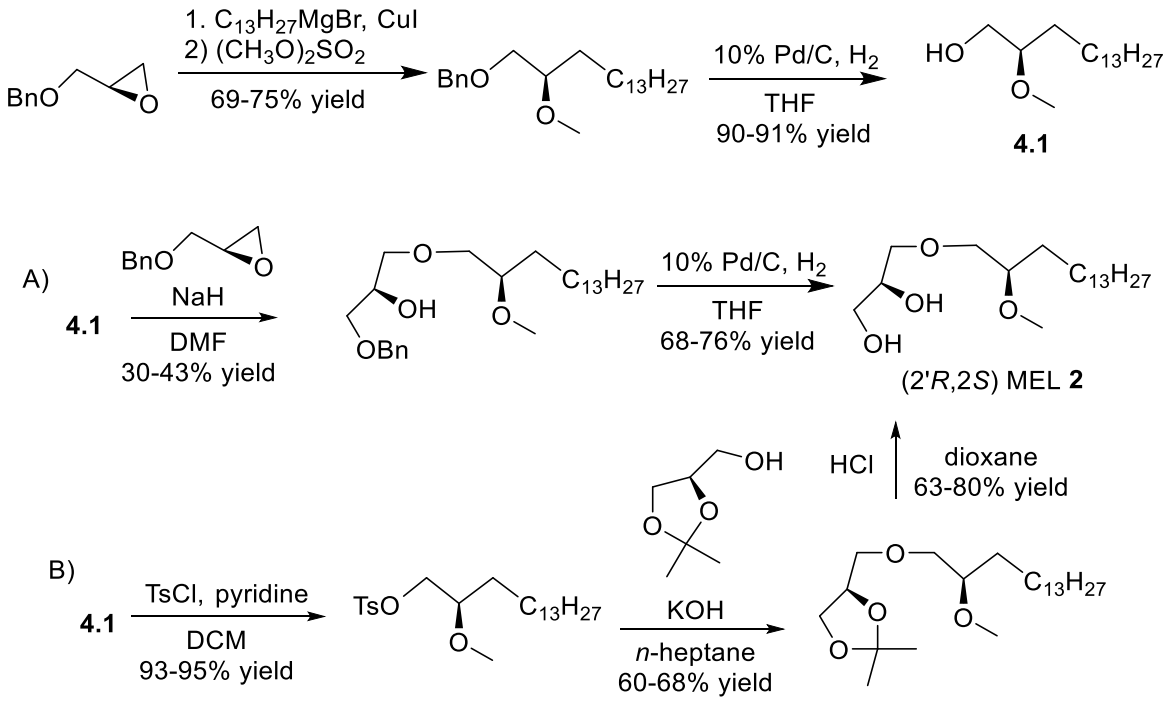


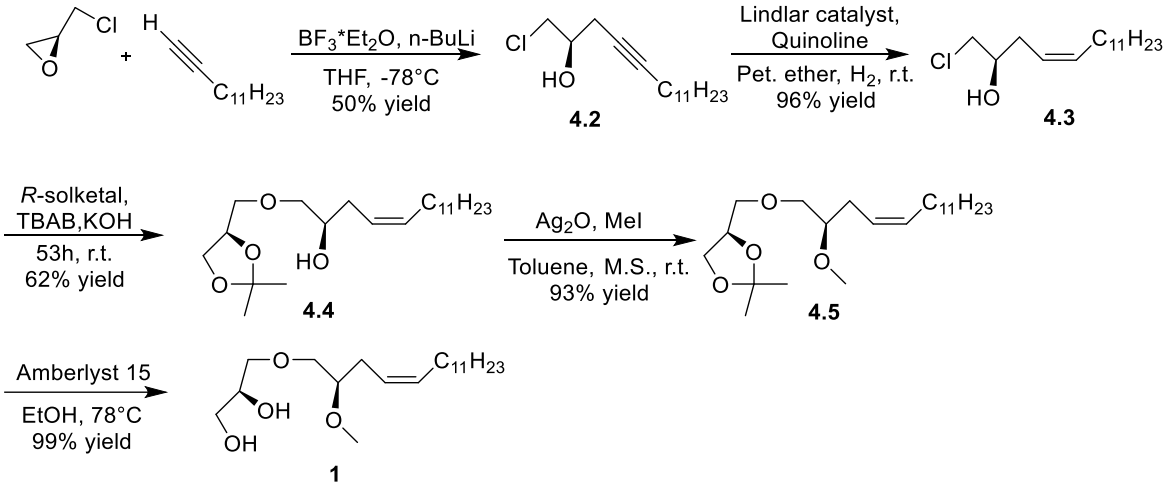
Figure 4.1 The chiral building blocks used by Ställberg[50] for the syntheses of the four stereoisomers of the 16:0 MEL **2**.

By starting with the appropriate chiral building blocks, Ställberg synthesized all four possible stereoisomers of MEL **2**. Each isomer could be synthesized in two ways by the use of the methoxylated alcohol intermediate **4.1** in accordance with Scheme 4.1, where the natural (2'*R*,2*S*)-**2** enantiomer is depicted. First the epoxide on a glycidyl benzyl ether was opened with tridecyl magnesium bromide and the resulting oxide subsequently methylated in the same flask. The benzyl group was then deprotected by catalytic hydrogenolysis over palladium. In one approach the resulting alcohol **4.1** was used to attack a second glycidyl benzyl ether which was then benzyl deprotected, resulting in the MEL **2** (see A in Scheme 4.1). Alternatively, in the second approach the alcohol **4.1** was tosylated and then coupled to the appropriate solketal enantiomer using powdered KOH, and then finally deprotected under acidic conditions (see B in Scheme 4.1).



Scheme 4.1. First total synthesis of an enantiopure MEL, the MEL **2** by Ställberg.[50]

Magnússon and Haraldsson then in 2010 were the first to report an enantioselective synthesis of a monounsaturated MEL, the 16:1 MEL **1** using solketal and epichlorohydrin as the chiral building blocks.[64] This is illustrated in Scheme 4.2. They coupled together the *S*-epichlorohydrin and the tridec-1-yne lithium adduct, in the presence of BF_3, utilizing a method developed by Yamaguchi and Hirao.[191] Here the BF_3 seems to stabilize the oxide which then does not form another epoxide in an intra-molecular substitution reaction. The resulting chlorohydrin **4.2** could only be coupled to the solketal in very low yield with the formation of an unwanted conjugated enynol as the major product. The chain of events seems to be an intramolecular formation of an epoxide intermediate, which was then opened up by an elimination reaction involving the methylene group located in between the epoxide and the alkyne to form a primary allyl alcohol.



Scheme 4.2 The first synthesis of the enantiopure 16:1 (2'*R*,2*S*)-MEL **1** by Magnússon and Haraldsson.[64]

When, on the other hand, chlorohydrin **4.2** was semi-hydrogenated via Lindlar catalyst prior to the coupling to yield the *cis* configuration in chlorohydrin **4.3**, and then coupled to the solketal the reaction proceeded without the enynol byproduct formation and furnished the glyceryl ether alcohol **4.4**. In this coupling reaction no solvent was used but TBAB as a phase-transfer agent as well as a catalyst. This together with an NMR investigation showed that the reaction must go through an epoxy-intermediate which forms *in situ.* After the coupling, the methylation of the alcohol was brought about via Ag_2O and MeI in toluene to accomplish the acetonide methyl ether **4.5**. Deprotection of the acetonide was accomplished with wet Amberlyst 15 in 95% ethanol under reflux yielding the final product MEL **1** in 27% overall yield over 5 steps which gave the same optical activity and ^{1}H-NMR spectra as had been established for the natural compound. After the successful enantioselective synthesis of the MEL **1**, the focus of the group shifted towards synthesis of the more complex MELs, incorporating polyunsaturated structures.

4.1.2 Initial MEL DHA 5 synthesis attempts

Using the same strategy as for the MEL **1** synthesis described above, the retrosynthetic analysis shown in Figure 4.2 was designed for the synthesis of the polyunsaturated MEL **5** that was then carried out by Magnússon and Lúthersson.[192] The target product MEL **5** was disconnected to the hydroxy hexa-ene precursor **4.6** that in turn was further disconnected to *R*-solketal and hexa-yne chlorohydrin intermediate **4.7**. That was further disconnected to the di-yne chlorohydrin **4.8** and tetra-yne bromide **4.9** fragments, with the latter being further disconnected as described in Figure 4.3.

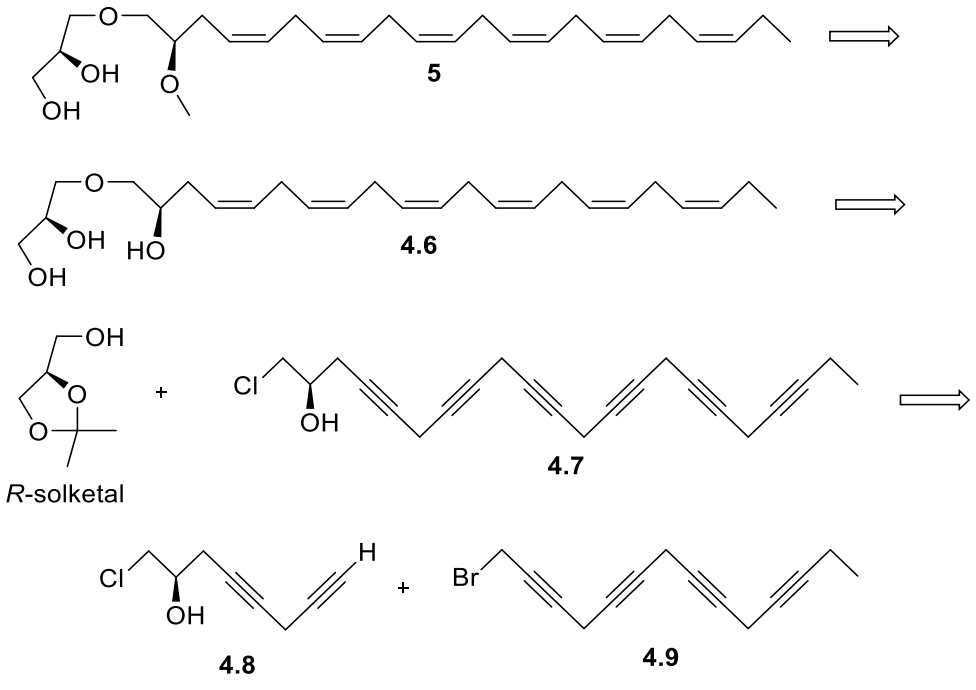


Figure 4.2 Initial retrosynthetic approach for the synthesis of MEL **5**.

The main features of this synthesis are the two chiral centers and then the polyunsaturated methylene-interrupted (skipped) hexa-ene hydrocarbon chain. The chiral configurations would be achieved using the same chiral building blocks as for the MEL **1** and the polyunsaturated tail would be accomplished by the acetylenic approach using the copper

mediated coupling reaction developed by Lapitskaya and coworkers.[86] A series of these reactions interrupted by TMS-deprotections or bromination reactions would generate a methylene-interrupted tetra-yne chain **4.9** (see retrosynthesis in Figure 4.3). Similarly, further coupling of tetra-yne **4.9** and di-yne chlorohydrin **4.8** results in the methylene interrupted hexa-yne chlorohydrin **4.7** (see retrosynthesis in Figure 4.2). Hexa-yne chlorohydrin **4.7** would then be stereoselectively semi-hydrogenated prior to attaching it to the *R*-solketal head moiety. And, as is implied by Figure 4.2, both the ether moieties are introduced at later stages in the synthesis.

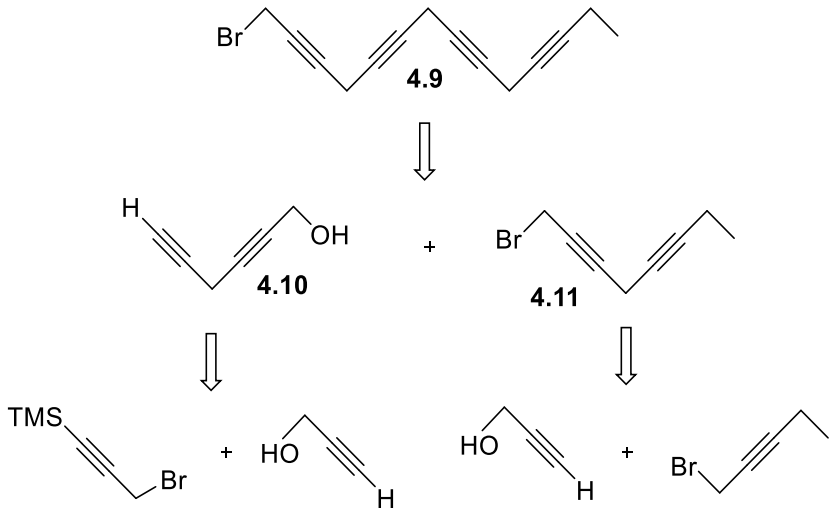


Figure 4.3 The retrosynthetic approach for the tetra-yne tail fragment **4.9**.

The problems with this strategy were the instability of the poly-ynes, semi-hydrogenation of the poly-yne tail **4.7** and then the coupling of the resulting poly-ene tail to the solketal. The semi-hydrogenation of the hexa-yne **4.7** showed considerable amount of over-hydrogenated byproducts in the ^{1}H-NMR spectrum which was something that had to be addressed. In addition to that it became clear early on that the poly-yne compounds were very unstable. This strategy then had another serious drawback in subjecting the either labile hexa-yne or the hexa-ene framework to harsh reaction conditions. The key step, coupling together the hexa-ene and the solketal, at most managed to produce a crude product in 37% yield (true yield much lower), but the hexa-ene or the hexa-yne frameworks did not handle the solvent-free strong alkaline condition of the reaction. In fact, the labile hexa-yne structure underwent carbonation in an exothermic reaction under these conditions. This strategy managed to produce a small crude sample which did include the target molecule, the MEL **5**, according to HRMS, but no other satisfactory spectra could be obtained. Further details are provided in a master thesis by Einar Lúthersson.[192] To make the synthesis feasible and bypass these problems another synthetic approach had to be designed. The second and more successful strategy is outlined in the following section.

4.2 Successful synthesis of DHA-like MEL 5

To synthesize the skipped poly-ene structure for the MEL **5**, the acetylenic approach seemed most straight forward, despite the literature as well as the experience from the

initial MEL **5** strategy exposing certain problems. As revealed in Section 4.1.2, the skipped poly-yne structures proved to be very sensitive to oxidation and polymerization. In addition, the stereoselective semi-hydrogenation of six triple bonds at once proved to be anything but simple and the desired poly-ene products were always contaminated with considerable amounts of over-hydrogenated and *trans*-isomer byproducts. Other strategic options, such as the Wittig approach, meant complete redesign of the synthesis and there was also huge uncertainty about its efficiency and stereoselectivity. Considerable effort then went into optimizing reactions to produce the poly-ynes in good yields and exploring different semi-hydrogenation catalysts and conditions in an attempt to find ideal conditions to semi-hydrogenate the hexa-yne substrate.

Figure 4.4 reveals the retrosynthetic analysis of the modified synthetic approach. It may be divided into three main retrosynthetic steps and the corresponding synthetic operations: (1) The functional group interconversion (FGI) to the hexa-yne **11** refers to the final steps of the synthesis that includes the stereoselective semi-hydrogenation, one of the main challenges of the synthesis; (2) Hexa-yne **11** is then disconnected to the tetra-yne tail fragment **12** and the di-yne head fragment **13**; (3) The tetra-yne **12** is further disconnected as was depicted in Figure 4.3 and the di-yne **13** is in turn further disconnected to the isopropylidene protected glyceryl glycidyl ether **14** corresponding to the establishment of the di-yne moiety along with the methyl ether. The head piece synthon **14** possessing both chiral centres is disconnected to the commercially available chiral precursors *R*-solketal and *S*-epichlorohydrin. The last one also involves the diastereomeric control of the two chiral centres of the molecule. The synthesis is to a large extent based on the acetylenic approach with the stepwise copper promoted coupling of propargyl bromides with terminal alkynes dominating in the synthesis. The synthesis of tetra-yne **12** is based on three such steps, the synthesis of di-yne head part **13** one and the coupling of **12** with **13** to accomplish hexa-yne **11** on one, the total of five such steps in the overall synthesis.

The total synthesis is dealt with in the three corresponding main synthetic tasks that are fully described in the corresponding three sub-sections of this section. Besides that, the major challenges related to sorting out the stereochemistry and the diastereomeric control in the preparation of the head piece synthon **14** are described and discussed in further detail in Chapter 6.1.

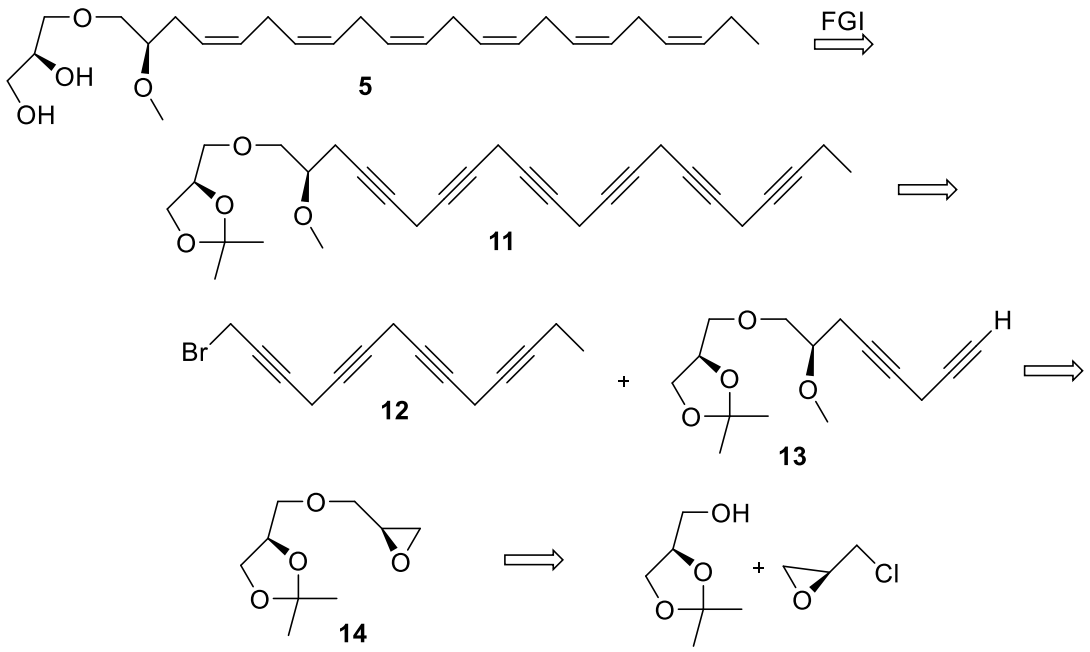


Figure 4.4 Second retrosynthetic approach for the synthesis of the MEL **5**.

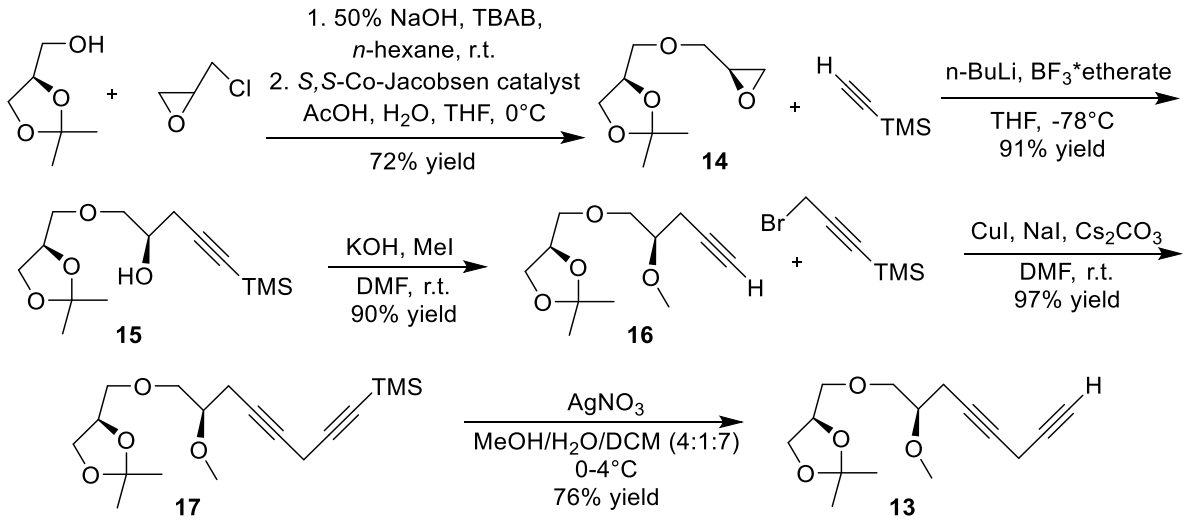
The main goal of the second retrosynthesis was to prevent the need to subject the poly-enes or poly-ynes to harsh reaction conditions. This was addressed by introducing both chiral carbons simultaneously and right in the beginning of the synthesis through the crucial isopropylidene protected glyceryl glycidyl ether **14**. The key head piece **14** could then also be used as a common starting point for the syntheses of all the MELs **1**-**10**. Other important goals included attempts to find better semi-hydrogenation conditions for the hexa-yne and the use of argentation (silver ion) chromatography to remove the semi-hydrogenation byproducts.

As has been discussed, the instability of the skipped poly-ynes is well-known and widely reported in the literature and was obvious during this work. Skipped di-ynes can be stored pure and concentrated for about 1-2 weeks in a freezer without too much deterioration. Tri-ynes are more unstable and deteriorate considerably over a few days in a freezer. Tetra-ynes were produced as white crystals that went yellow in minutes, even under vacuum, and deteriorated considerably overnight in a freezer. Some authors have in the past described procedures where tri- and tetra-ynes could be stored dissolved in solvents in a freezer for some time without much deterioration.[193] One paper does report the successful storage of a skipped tetra-yne acid at -20°C for up to months without describing the exact conditions any further.[115] But any storage of these compounds should not be advised. Penta- and hexa-ynes are very unstable and proved to decompose or polymerize very rapidly after their purification and any attempts at storage afforded huge losses with subsequent "messy" and low yielding reactions. This general instability made it very important to make the synthesis as convergent as possible and be performed as quickly as possible. Over the course of this work, it was discovered that limiting the time it takes to get from the poly-yne compounds to the semi-hydrogenation step was vital to increase the yields and made work-up and purification also smoother. Streamlining this process, was a huge factor in making the synthesis possible and greatly increased yields were obtained as a result.

Another point that might be worth making regards the obtainment of the hexa-yne **11**. One could couple together different kinds of poly-ynes. 1+5, 2+4 and 3+3, where the numbers represent the number of triple bonds in each substrate for the final copper coupling reaction. Increased number of triple bonds in a substrate, as in higher the number, increases the instability of the substrate. By synthesizing two tri-ynes (3+3) which would then combine into the hexa-yne could be thought to be the best option but as the tri-ynes were both semi-unstable and the head piece containing the expensive chiral centers should be subjected to as few reactions as possible it was decided against. The 1+5 strategy would not be as convergent and producing a penta-yne which is then submitted to at least two other reactions to turn into a hexa-yne not viable at all, as well as former synthesis and experience within the group in coupling together a mono-yne headpiece to a tri- or tetra-yne tail already showed characteristically poor yields. Kunau et al. as mentioned in Chapter 3.1 also achieved much better yields changing their di-Grignard substrates from mono-yne acids to di-yne.[80] The 2+4 strategy was therefore the best option as di-ynes are relatively stable and therefore only one unstable intermediate, the tetra-yne.

4.2.1 Synthesis of the di-yne head piece fragment 13

The synthesis of the di-yne head fragment **13** is depicted in Scheme 4.3. It was started by coupling the two chiral synthons *R*-solketal and *S*-epichlorohydrin together, introducing both the chiral carbons into the structure. The solvent-free conditions with KOH and catalytic amount of TBAB were originally used and afforded the isopropylidene protected glyceryl glycidyl ether **14** in 50% yield when using equimolar amount of the reactants. By increasing the *S*-epichlorohydrin to 1.5 equivalents, 67% yield was afforded based on the solketal. Later during this work, it was discovered that using 50% NaOH in water as the base, adding *n*-hexane as a solvent and heating to reflux the yields were improved to 86%. The product **14** was then only obtained with diastereomeric purity of 93-95%, which is 86-90% diastereomeric excess (*de*), much greater diastereomeric impurity than was to be expected based on the purity of the starting materials, which should have been around 95% de. To obtain **14** in satisfying purity, a hydrolytic kinetic resolution (HKR) reaction was employed using the Cobalt-Jacobsen catalyst which then also removed the need to use stereopure epichlorohydrin as a starting material and afforded the product **14** in 72% yield based on the solketal and in >98% *de*. This reaction and its diastereomeric purity control are discussed in further details in Chapter 6.1.



Scheme 4.3 Synthesis of the di-yne head part **13**.[3]

The next step involved the addition of the first acetylene unit to the glyceryl glycidyl ether **14**. The epoxide was opened with a TMS protected acetylene with the help of *n*-BuLi in dry THF at -78°C. The reaction only proceeded in the presence of boron trifluoride (BF_3) by the method described by Hirao and Yamaguchi.[191] The *n*-BuLi was added to the cooled alkyne, 30 minutes later the epoxide **14** was added and then the BF_3 slowly over a few minutes and the reaction stirred further for 2 hours at -78°C. The reaction was then abruptly quenched by a saturated NH_4Cl solution. This procedure afforded the TMS-protected mono-yne alcohol **15** in 91% yields at best.

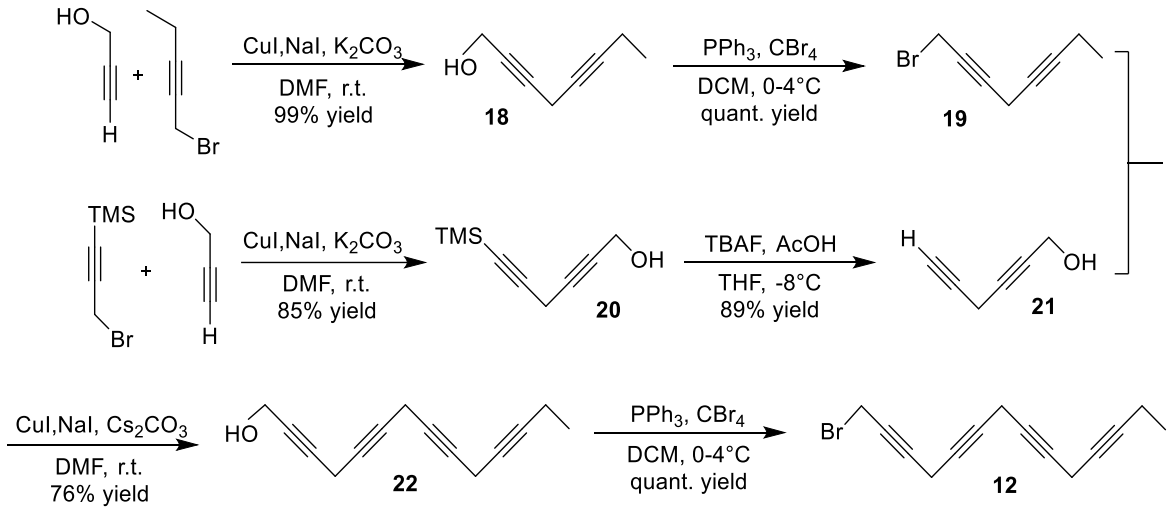
Next the TMS group was removed, and the alcohol methylated simultaneously using KOH and MeI in DMF at r.t. This reaction would often not run to completion, affording around 50% yield of the mono-yne methyl ether **16** and the rest would be the TMS deprotected alcohol. The yield for this reaction could be improved to 65-75% by carefully guaranteed water-free conditions, using fresh DMF, freshly ground KOH and nitrogen flow. If the reaction mixture turned orange or even brown after addition of the base considerably lower yields were obtained (<50%). By adding molecular sieves, the yields could be further pushed up to 86-90% and around 4-7% of the TMS deprotected alcohol were recovered as well. A small amount of the allene byproduct (<1%) was visible in the ^{1}H-NMR spectra of the product **16** after silica purification.

The second acetylene unit was then added to the structure by the use of the copper coupling method developed by Lapitskaya et al.[86] The mono-yne **16** and a TMS-protected propargyl bromide were coupled together to achieve the TMS protected di-yne **17** in 97% yield. As stated before, the copper coupling conditions developed by Lapitskaya et al. were used for all the ensuing copper couplings for this project. The method uses CuI, NaI and Cs_2CO_3 in DMF. K_2CO_3 was used instead of Cs_2CO_3 for couplings involving two simple mono-yne substrates since the two bases gave similar yields in such cases. Di-yne **17** was obtained in >80% yield when using 2 equivalents of the TMS protected propargyl bromide and one equivalent of all the other components. Using less than 2 equivalents of the TMS protected propargyl bromide generally left some of the mono-yne **16** unreacted. By increasing the amount of the salts CuI, NaI and Cs_sCO_3 to two, five and four equivalents, respectively, the above stated excellent yields were achieved.

To complete the di-yne head piece fragment **13** synthesis, the second TMS group was then removed using silver nitrate in the solvent mixture MeOH/H_2O/DCM (4:1:7), affording the di-yne **13** in 76% yield following a procedure described by Orsini and coworkers.[194] The silver deprotection method was necessary in the case of the di-yne **17**, since usual TMS deprotection methods, such as alkali bases or fluoride ions, produced large amounts of allene byproduct. The silver nitrate method promoted the lowest amount of allene formation and therefore the highest yield. The yield from this reaction fluctuated initially between 40-70%, sometimes for no apparent reason. Cooling the reaction to 0°C increased the reaction time but did not affect the yields. The reaction would usually not reach completion entirely according to TLC, even after additional silver nitrate was added. The biggest effect on yields was the substrate freshness and if not stored for longer than overnight in a freezer afforded yields were steadily 70-76%. The small amounts of allene produced could be easily removed by silica gel chromatography. The head fragment **13** was obtained in 43% total yield based on the solketal.

4.2.2 Synthesis of the tetra-yne tail fragment 12

The next task was to attach the head piece fragment **13** to the bromide tetra-yne tail fragment **12**. The synthesis of the tetra-yne tail fragment **12** is depicted in Scheme 4.4.



Scheme 4.4 Synthesis of the tetra-yne tail fragment **12**.[3]

Initially, the poly-yne substrates were sometimes stored in a freezer, up to few days before use. The latter steps in the synthesis then generally afforded only moderate yields. When the synthesis had been streamlined, reactions optimized, specific care taken to do the synthesis as fast as possible and handling of the substrates during work-up, purification and preparation done carefully under argon blanket and in as short a time as possible, drastic improvements in yields were obtained.

The tetra-yne **12** synthesis is highly convergent, using three propargyl sub-units as the starting materials, two times propargyl alcohol, one TMS-protected propargyl bromide and the 1-bromopent-2-yne synthon which incorporates the omega-3 framework. The omega-3 di-yne **18** was made by the copper coupling of propargyl alcohol and 1-bromopent-2-yne. Initially 1.5 equivalents of the bromide substrate were used in accordance with former protocols as well as the salts CuI, NaI and K_2CO_3 in 1 eq, 2 eq and 2 eq, respectively. This

afforded **18** in around 70-75% yield after 48 hours as based on the propargyl alcohol. Using one equivalent of all reagents afforded even better yields after only 24 hours, at best around 91% after purification by silica gel chromatography. But, by skipping the purification and using the crude **18**, 99% yield was obtained for **19** in the following Appel brominating reaction[195] based on the propargyl starting materials. Some amount of the desired product was unescapably removed on the silica gel column with the small amount of allene byproduct. Removing this purification step and easily isolate the next reaction product afforded the optimal yield.

The following reaction, the brominating Appel reaction was accomplished using triphenylphosphine and tetrabromomethane in DCM. This reaction was performed at around 0°C and was usually completed in 30-60 minutes. The main difficulty with this reaction was the large amount of triphenylphosphine oxide along with unreacted triphenylphosphine that had to be removed from the reaction mixture. Initially, silica was added to the reaction mixture after completion until a thick slurry was formed. The solvent was then removed under vacuum and the resulting brownish silica was placed on a fritted disc and the product eluted with DCM and petroleum ether (1:9). This procedure afforded the propargyl bromide di-yne **19** in 70-75% yield, usually contaminated with a small amount of triphenylphosphine that was not purified further. This procedure was improved drastically if the reaction mixture was diluted with diethyl ether to the point of the phosphines starting to precipitate from the solution and the solution then put directly on a short silica gel column eluting with diethyl ether but also dichloromethane if the column started to become blocked. This enabled the removal of most of the phosphines and then a second normal silica gel column using 2% ethyl acetate in petroleum ether afforded the propargyl bromide di-yne **19** in quantitative yields only contaminated with a small amount of bromoform.

The synthesis of di-yne **20** was accomplished with the copper coupling of a second propargyl alcohol unit to a TMS protected propargyl bromide to produce the TMS protected di-yne intermediate **20**. This reaction went through similar development as the copper coupling for **18** described above. The yields were though quite different as **20** was usually afforded in around 68-75% yield after purification. No consistent beneficial effect was produced through tweaking the reaction conditions, and the best yields obtained were 85% when using 1 eq of all substrates and stirring the reaction for 48 hours at room temperature.

The subsequent TMS deprotection of **20** to furnish the di-yne **21** was then initially performed in the same way as described earlier for the di-yne headpiece precursor **17**, with silver nitrate. In the aqueous work up, a considerable amount of the product remained dissolved in the water phase and general yield was therefore only around 60-66%. Interestingly, the same reaction conditions using silver trifluoromethanesulfonate (AgOTf) while also limiting the water in the aqueous work up afforded 79% yield. Negligible allene formation was observed in these reactions that was easily removed by silica gel chromatography. This reaction was then improved further by using TBAF in dry THF with acetic acid to reach neutrality[150] and no aqueous work up, only silica gel filtration and chromatography. A small amount of the allene byproduct was formed but easily removed by the silica gel chromatography along with a small amount of the desired product. This procedure afforded the product **21** in 82-89% yield after purification.

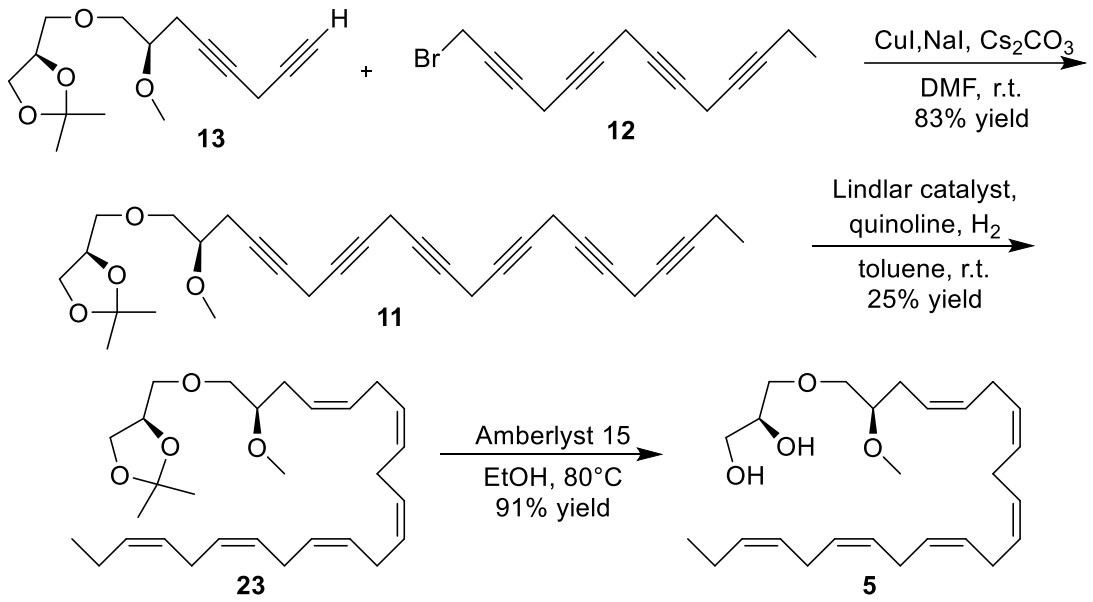
The two resulting di-ynes **19** and **21** were then copper coupled together to obtain the tetra-yne alcohol **22**, now using cesium carbonate instead of potassium carbonate. This reaction

gave initially very poor results with less than 50% yield and was very messy, with the formation of a lot of brown oil or sludge which was usually only soluble in acetone or DCM. By increasing the mol equivalents of the bromide **19** and the salts some improvement in terms of yield was observed (57-61%). Then as discussed before, with greater care taken with the substrates and speeding up the process, 68-76% yields were obtained. The tetra-yne substrate **22** now showing high instability, was obtained as fine white crystals after evaporation of the solvent after silica gel purification. These crystals quickly turned the shade of yellow after exposure to air and after storage under vacuum had turned dark yellow with parts morphed into an orange oil. To maximize the yields for the upcoming reactions, the best course of action was to immediately after purification and evaporation of most of the solvent to start the next reaction.

The next reaction was an Appel brominating reaction that went through similar development as discussed earlier for the di-yne **19**. The bromination of **22** thus afforded initially the corresponding tetra-yne bromide **12** in very poor yields but after said development, **12** was afforded as white crystals (which quickly turned yellow), contaminated with small amount of bromoform and other solvents, in practically quantitative yields (calculated from the ^{1}H-NMR spectrum). By submitting the product to a lengthy evaporation under vacuum to remove all solvent residues only allowed it to deteriorate further and therefore potentially reduce the yields for the next reaction. The total yield for the tetra-yne tail fragment **12** synthesis was thus achieved in 57% yield as based on the most expensive substrate, the TMS protected propargyl bromide.

4.2.3 The final steps of the synthesis of MEL 5

The tetra-yne bromide **12** was after purification immediately employed in the fifth and last copper coupling with the di-yne head piece **13**. The final reactions, the head and tail copper coupling, the penultimate and all-important stereoselective semi-hydrogenation, and the final acetal deprotection are depicted in Scheme 4.5.



Scheme 4.5 The final steps in the DHA-like MEL **5** synthesis.[3]

The very labile hexa-yne **11** was afforded in around 66% yield initially after 48-hour reaction time under the normal copper coupling reaction conditions. After streamlining the process, using just under 2 equivalents of the tetra-yne bromide **12** and quenching the reaction after 18 hours, 76-83% yields were achieved. The excess of the bromide tail **12** was needed to make sure that all of the di-yne **13** was reacted, since it had very similar chromatographic properties as the hexa-yne **11** and was thus difficult to remove by flash chromatography. As the hexa-yne **11** was so unstable, and in order to guarantee the best possible yields in the next reaction, it was preferably only roughly concentrated under vacuum after a silica gel column treatment and immediately employed in the subsequent reaction.

That reaction involved the simultaneous stereoselective semi-hydrogenation of the six acetylenes present in the hexa-yne **11**. This reaction proved to produce large amounts of byproducts, namely *trans*-isomers and over-hydrogenated products. And as was discussed in Chapter 3.2, no obvious solutions to overcome that existed in the literature. This key step of the acetylenic approach went therefore under further investigation and multiple conditions and catalysts were explored both for substrate **11** as well as other similar poly-yne substrates of various sizes in the attempt to find the best possible conditions. Different commercial preparations of the Lindlar catalyst were also explored and are denoted by roman numerals. This is discussed further in Section 6.2. Table 4.1 lists a few different semi-hydrogenation conditions that were tried on the hexa-yne **11**.

Table 4.1 Semi-hydrogenations performed on the hexa-yne **11** to produce the DHA-like MEL **5**.

Catalyst	Catalyst poison	Solvent	Reaction time	Vinyl integration	Yield
Lindlar **I**	quinoline	benzene	50 min	11.38 (94%)	31%
Lindlar **II**	quinoline	Et_2O	32 min	10.23 (85%)	31%
Lindlar **III**	quinoline	toluene	52 min	10.28 (86%)	60%
Lindlar **III**	pyridine	MeOH[a]	incomplete	-	-
Brown	eda	EtOH	80 min	10.47 (87%)	44%

[a] 2-methyl-2-butene also added.

The vinyl proton integration from the ^{1}H-NMR spectra gives an estimation of the amount of over-hydrogenated product. There are twelve vinyl protons on the compound and an integration of about ten (83%) might indicate that every single molecule had lost one double bond and therefore be 100% over-hydrogenated product. But since many of the molecules are over-hydrogenated by two, three, four or possibly even five or six double bonds, the vinyl proton integration gives only a relative estimation of the over-hydrogenation and the desired hexa-ene product needs to be isolated to obtain the actual yield. The yield in Table 4.1. is the yield after normal silica gel chromatography, which affords the product along with all the over-hydrogenated and *trans*-isomer byproducts and does therefore not reflect the true yield of the desired product. Different Lindlar catalysts were used during this work, different batches of the same catalyst as well as from different manufacturers. Each individual bottle of catalyst is referred to by a roman numeral.

The highest integration of the vinyl protons (94%) was achieved with the Lindlar **I** catalyst in benzene with quinoline. What was then discovered was that the same catalyst obtained from other bottles, different batches, (Lindlar **II** and **III**) could not repeat these results to the same success. This suggests a major reproducibility problem with using the Lindlar catalyst, which is something that has been mentioned in the literature. The low yield with the Lindlar **I** catalyst could be attributed to the condition of the starting material, which

was by then not properly handled. Integration of 85% was achieved with similar yield but in diethyl ether with the Lindlar **II** catalyst and very similar integration was afforded in toluene (86%) but in considerably higher yield (60%) with the Lindlar **III** catalyst. Unfortunately, the conditions described by Hwang and coworkers,[116] which gave promising results for other substrates **24** (MEL **10**), **69** (EPA) and **74** (DHA) reacted the hexa-yne **11** very slowly or stopped completely very early on, since no change was seen on TLC after the first minutes, even though more catalyst was added and the reaction allowed to run for 48 hours. Lastly, the Brown catalyst afforded similar vinyl integration as the Lindlar **II** and **III** catalysts but with somewhat lesser yield (44%) than the Lindlar **III** catalyst.

Two runs were also submitted to argentation chromatography, the run using Lindlar **III** in toluene and the one using the Brown catalyst. The Lindlar run was purified by 10% $AgNO_3$ impregnated silica gel and eluted through a column, while the Brown run was purified on a preparative 10% $AgNO_3$ impregnated TLC plate. Table 4.2 depicts the yields for each run.

Table 4.2 The yield obtained for the semi-hydrogenation and subsequent argentation chromatography.

Catalyst	Normal silica gel purification yield	Argentation chromatography yield	Total yield	Vinyl integration
Lindlar **III**	60%	42%	25%	11.87 (99%)
Brown	44%	50%	22%	11.84 (99%)

The highest yield for the semi-hydrogenation step was achieved by the Lindlar **III** catalyst in toluene which afforded a very pure sample of compound **23** in about 25% yield after argentation chromatography. Very similar results were though achieved by the Brown catalyst which afforded the pure compound **23** in 22% yield. The Brown catalyst might possibly be the better candidate here for further experiments since this reaction was probably not optimized fully and as has been alluded at earlier, the Lindlar catalyst might have reproducibility problems.

Argentation chromatography can remove over-hydrogenated product as is clearly visible by the ^{1}H-NMR before and after spectra, which in the case of the compound **23** removed all or nearly all over-hydrogenated products. The other issue is the *trans*-isomers that are produced in the reaction and if they have all been removed as well. Argentation chromatography does separate *cis*- and *trans*-isomers, but how efficient these purifications were in the case of compound **23** is not clear from the NMR spectrum. The IR spectra do show how the argentation chromatography reduced the *trans*-isomer content but did not offer accurate quantification, this is discussed further in Chapter 6.3.

The pure hexa-ene **23** was then finally deprotected using wet Amberlyst 15 refluxed in 96% ethanol affording the hexa-ene MEL **5** in 91% yield.

To obtain the optimal yields the acetylene synthesis was performed over 6 days with the mono-yne head **16** priorly made. The di-yne substrates were stored only overnight in a freezer to reduce the workload, and other substrates always immediately reacted or kept in a copper coupling reaction overnight. Just over 8% total yield for the DHA-like MEL **5** were thus achieved, as based on the solketal starting material. Based on this experience and results in the literature, some alternative synthetic strategies are postulated in Chapter 5.6.

5 Synthesis of other MELs and PUFAs

The original shark and dogfish liver oil sample examined by the group contained seven different types of MELs as detected by the HPLC/MS/MS system.[1] The number of carbons and unsaturation level of the carbon chain of each compound can be deduced from the accurate mass. The MELs detected were thus, two saturated ones, 16:0 and 18:0, three monounsaturated types, 16:1 and two types of 18:1 and then two polyunsaturated, 18:3 and 22:6. The 16:1 and one of the 18:1 were probably the most abundant MELs **1** and **3**, whose structure has already been established, where the unsaturation is positioned on the fourth carbon (Δ4) in the fatty chain. The other 18:1 type does not have its structure established but was hypothesized to be the omega-9 type, the MEL **6**, a structural analogue of oleic acid which is common in shark liver oil as well as the abundant selachyl alcohol having the same omega-9 structure. The 22:6 would most likely be the remarkable DHA-like MEL **5** and the 18:3 one, which has been detected before,[53] has never had its structure determined.

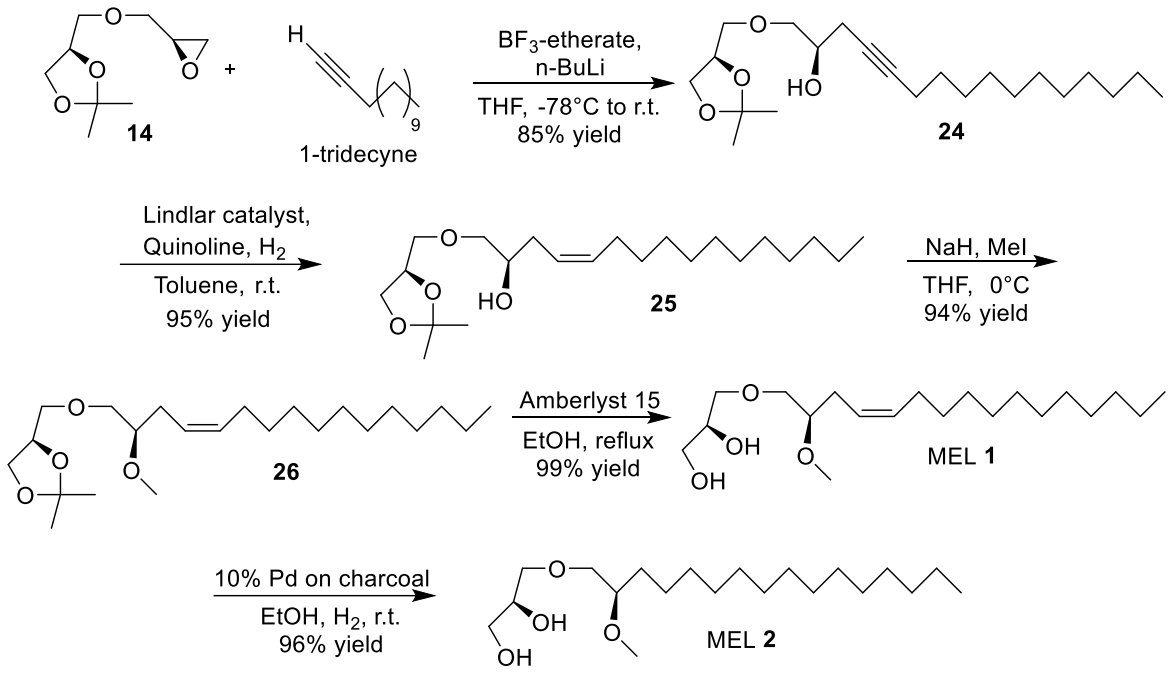
The goal was to use the new head piece **14** and synthesize all these compounds.[2] Then it would be possible to look at the degradation breakdown of their MS/MS spectra, compare them to the natural samples and confirm their exact structure. As for the unknown 18:3 MEL, a rational guess of an omega-3 (MEL **8**) or omega-8 (MEL **7**) structure was made.[5] The MEL **7** having the first double bond be in the Δ4 position, just as the most common types. Another omega-6 type (MEL **9**) was then also synthesized, but the MS/MS spectra of all three synthesized 18:3 MELs did not fit the one obtained from the natural sample. This is discussed further in Chapter 5.7.

An unprecedented 16:4 type was also synthesized, the MEL **10**, which was used to test semi-hydrogenation conditions before the cumbersome synthesis of the DHA-like MEL **5** was undertaken, but also for curiosity reasons. In addition to these MELs a range of PUFA ethyl esters were synthesized as well for testing reasons, such as a 16:4 type, SDA, EPA and DHA, using the same acetylenic methodology for the poly-yne synthesis.

Section 5.1 covers the two in one synthesis of 16:1 MEL **1** and 16:0 MEL **2**. Section 5.2 covers the syntheses of the two types of 18:1 MELs, MEL **3** and **6** as well as the synthesis of the 18:0 MEL **4**.[4] Section 5.3 covers the syntheses of the three different 18:3 MELs, the MELs **7**, **8** and **9**. Section 5.4 covers the synthesis of the non-natural 16:4 MEL **10**. Section 5.5 will then cover a modified approach to MEL **8** using a combined Wittig and acetylenic approach.[5] Section 5.6 puts forth theorized alternative approaches to the synthesis of MEL **5** based on the manipulation of pure natural DHA or using the combined Wittig and acetylenic approaches. Section 5.7 then covers the MS/MS fragmentation comparisons and finally Section 5.8 covers the syntheses of the PUFA ethyl esters.

5.1 Syntheses of the MELs 1 and 2

Magnússon and Haraldsson synthesized MELs **1** and **2** in 2010[64], applying their synthetic strategy outlined in Scheme 4.2. As discussed earlier, this strategy was limited in how it was not applicable to polyunsaturated MEL substrates. The new approach, using the key head piece **14** would be a convenient method to synthesize all possible MELs and was first demonstrated for MELs **1** and **2** as depicted in Scheme 5.1.[2]



Scheme 5.1 The in tandem synthesis of 16:1 and 16:0 MELs **1** and **2**.[2]

The epoxide on the head piece **14** was opened by the nucleophilic attack of the lithium adduct of 1-tridecyne in the presence of BF_3 in dry THF. The reaction went smoothly and afforded after flash chromatography the mono-yne alcohol **24** in 85% yield. The alcohol **24** was then submitted to a Lindlar catalyzed semi-hydrogenation in toluene which afforded the mono-ene alcohol **25** after flash chromatography as a colourless oil in 95% yield. The alcohol was then methylated by MeI in the presence of NaH as the base. This afforded the mono-ene **26** in 94% yield. The isopropylidene protecting group was then removed by refluxing the mono-ene **26** in the presence of Amberlyst 15 in 96% ethanol which afforded MEL **1** in 99% yield after simple filtration. The MEL **1** was then applied to further hydrogenation by 10% palladium on charcoal to achieve the MEL **2**, which was afforded in 96% yield after filtering over celite. The total yield for MEL **1** was 54% and MEL **2** 52%, as based on the solketal. This approach was a huge improvement from Magnússon's and Haraldsson's previous synthesis which afforded MEL **2** in 27% total yield and used two chiral starting materials.

The synthesis of MEL **1** and **2** was then used to confirm the stereochemistry of the new synthetic approach based on the head piece **14** by comparison of ^{1}H-NMR, ^{13}C-NMR and the optical activity of the products MEL **1** and **2** to the available values in the literature. The specific rotation values are listed in Table 5.1.

Table 5.1 Specific rotation values for MELs 1 and 2 by the new synthesis compared with available values.

Compound	Isolated from shark oil	Ställberg	Magnússon and Haraldsson	The new synthesis
MEL **1**	-3.0[50]	-3.1[50]	-3.0[64]	-2.9[a]
MEL **2**	-12.0[196]		-12.5[64]	-11.1[b]

[a] *c* 2.27, $CHCl_3$, 20°C, [b] *c* 1.78, $CHCl_3$, 20°C.

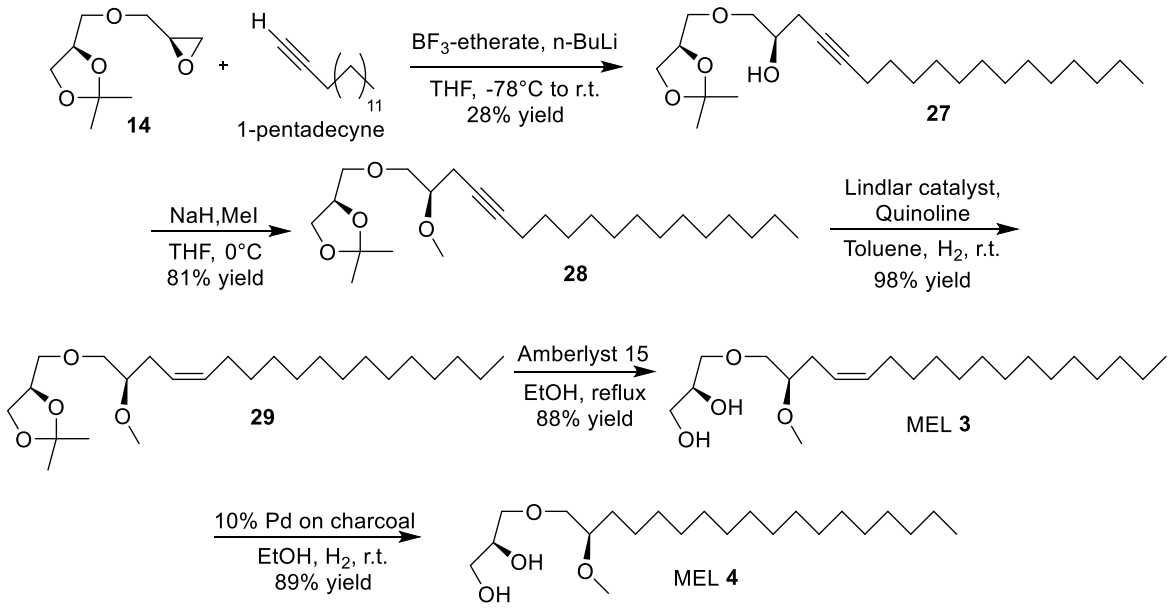
The NMR spectra in tandem with the specific rotation data firmly establishes the absolute configuration of the MELs **1** and **2** and then also the important key head piece **14** which was then further used to synthesize all the other MELs.

5.2 The 18:1 and 18:0 MEL syntheses

Two MELs with the 18:1 configuration were detected in the initial shark and dogfish oil sample. MEL **3** (18:1 n-14) is one of the well-known and most abundant MELs and would with no doubt represent one of the 18:1 MELs detected. The other 18:1 MEL was yet to be structurally established. The 18:1 n-9 ether lipid (selachyl alcohol) is one of the most common types of the unsubstituted ether lipids as well as oleic acid (n-9) being abundant in the liver oil of sharks. This encouraged the synthesis of MEL **6** as the possible candidate for the second 18:1 MEL in the sample. The MELs **3** and **6** were then synthesized and their MS/MS degradation spectra compared to that of the 18:1 MELs detected in the original oil sample to confirm their structure. The 18:0 MEL **4** was also synthesized as the fully hydrogenated product of MEL **3**.[4]

5.2.1 Syntheses of the MELs 3 and 4

The initial synthetic strategy for 18:1 n-14 MEL **3** was to use the key head piece synthon **14** and mirror the MEL **1** synthesis, except using the 1-pentadecyne instead of the 1-tridecyne. The resulting alcohol was then to be methylated with MeI in the presence of a base, then hydrogenated with the Lindlar catalyst and finally deprotected with Amberlyst 15 in ethanol. Scheme 5.2 depicts the total synthesis.



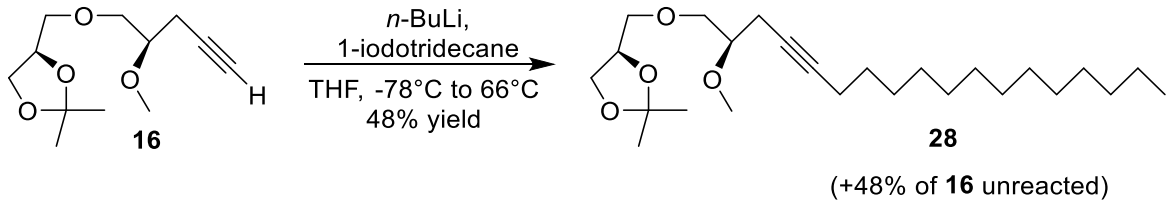
Scheme 5.2 The in tandem first synthesis of the 18:1 n-14 MEL **3** and 18:0 MEL **4**.[4]

The first step for this synthesis went quite poorly. The mono-yne alcohol **27** was only isolated in around 20-28% yield, except one attempt which afforded 40% as the best yield but could not be repeated. Contrary to the similar 16:1 MEL **1** synthesis, where the reaction of the head piece **14** and 1-tridecyne went smoothly, the 1-pentadecyne would not

operate in the same manner. The 1-pentadecyne seemed to not dissolve properly under the cold reaction condition as well as only partly dissolving in the eluting solvents on the silica column, making the purification very cumbersome. Attempts were made with other solvents, bases and increased temperatures but these attempts only afforded no reaction or very low yields (10-20%).

The mono-yne alcohol **27** was then methylated with MeI using NaH as the base in dry THF affording the mono-yne **28** in 81% yield. Stereoselective semi-hydrogenation with the Lindlar catalyst in toluene with quinoline gave the *cis*-mono-ene **29** in 98% yield. The deprotection via reflux in the presence of Amberlyst 15 in 96% ethanol gave the MEL **3** in 88% yield. Finally, the MEL **3** was fully hydrogenated by 10% palladium on activated charcoal to produce the MEL **4**, which was afforded in 89% yield after filtering over celite. The MEL **3** was thus afforded in 14% total yield based on the solketal, 20% if the one-time 40% yield is to be considered in the coupling reaction and the MEL **4** was achieved in 12.5% total yield (which would be obtainable without the extra step of semi-hydrogenating, presumably in slightly better yield) .

Unsatisfied with the yield in the first step of the MEL **3** synthesis, a different approach was explored. The second approach was to remove the apparent troublemaker, the 1-pentadecyne from the equation and instead employ the mono-yne head **16**. The terminal acetylene would be used as the nucleophile and attack a 1-halotridecane to obtain the desired mono-yne **28**. The key differing step for the second approach for the MEL **3** synthesis is depicted in Scheme 5.3.



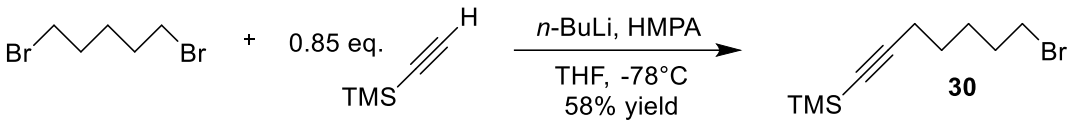
Scheme 5.3 The key reaction for the second synthesis of the MEL **3**.[4]

The mono-yne **16** was synthesized as depicted in Scheme 4.3. The yields for this reaction were at most only 48%, but another 48% of the unreacted **16** was recovered, 96% total material recovered. The maximum yield was achieved by heating the reaction to a gentle reflux after the addition of the 1-iodotridecane. Using HMPA did not increase the yield and only lowered the recovery of the starting material. The mono-yne **28** was then used to obtain the desired MEL **3** as before, depicted in Scheme 5.2. The strict total yield for this approach would be 24% for MEL **3** and 22% for MEL **4**, but repeated reactions of the unreacted **16** from the key reaction depicted above (since almost no material was lost) would push the total yield to around 48% for MEL **3** and 43% for MEL **4** as based on the solketal.

5.2.2 Synthesis of the MEL 6

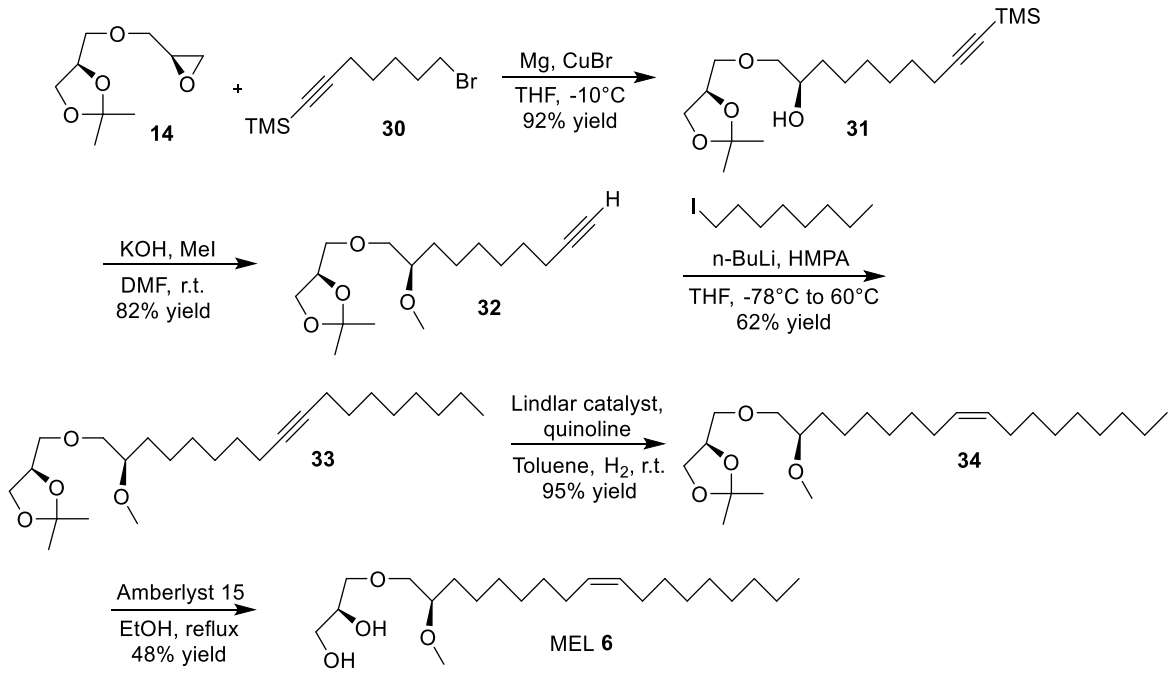
The 18:1 n-9 MEL **6** was the first MEL to be synthesized that did not possess the Δ4-double bond, but rather the first double bond located on the ninth carbon on the *O*-alkyl chain. Using the lithium adduct of the acetylene to open up the epoxide on the head piece **14** was then not an option so another method was developed. A new “linker” was

synthesized which would "link" together the head piece **14** and the rest of the carbon chain. Scheme 5.4. depicts the synthesis of the TMS-protected alkyne bromide linker **30**.[197]



Scheme 5.4 The synthesis of the TMS-protected alkyne bromide linker **30**.

The commercially available substrates 1,5-dibromopentane and TMS-protected acetylene were reacted in 1 to 0.85 molar ratios in the presence of *n*-BuLi and HMPA in dry THF. This afforded the linker **30** in 50-58% yield after some tedious chromatography to separate the desired mono-yne compound from the unreacted 1,5-dibromopentane and the bis-acetylenated byproduct. The subsequent synthesis of the MEL **6** is depicted in Scheme 5.5.[4]



Scheme 5.5 The synthesis of the 18:1 n-9 MEL **6**.[4]

The first reaction followed a procedure developed by Alam and coworkers.[198] They developed a robust method for the ring-opening of epoxides with Grignard reagents catalyzed by copper. The head piece **14** and the Grignard reagent of **30** in the presence of CuBr in dry THF at -10°C afforded the mono-yne alcohol **31** in 92% yield. No reaction took place without the copper salt, and if only cooled to 0°C the main product was the halohydrin byproduct. The mono-yne alcohol **31** was then submitted to the simultaneous TMS-deprotection and alcohol methylation using MeI and KOH as the base. Like the similar reaction of **15** to **16**, only about 50% of the substrate was methylated after an hour and then remained as such. By adding water and extracting the reaction material into ether, concentrate on vacuum and then repeat the reaction, about 78% yield of mono-yne **32** was afforded. This reaction was optimized further with molecular sieves which pushed the yields to 81-82%. The lithium adduct of **32** was then reacted with 1-iodooctane to afford the mono-yne **33** in 30-40% yield initially, without HMPA and letting the reaction reach

room temperature. But with the addition of HMPA and gradually allowing the temperature rise to reflux the afforded yields reached 62%. 1-bromooctane gave no product even with HMPA.

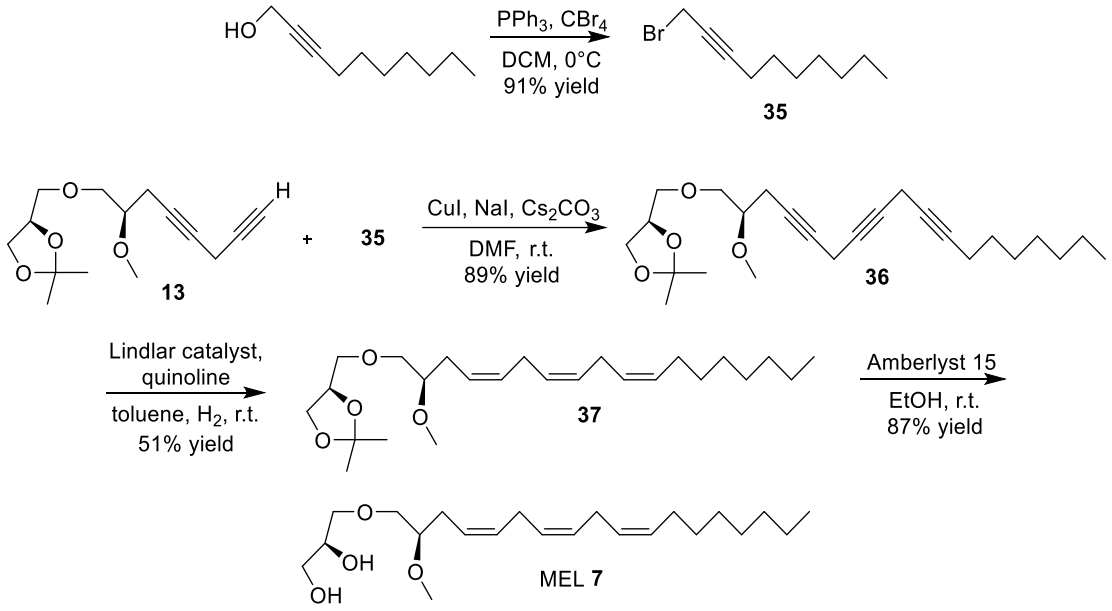
The mono-yne **33** was then submitted to the Lindlar catalyzed semi-hydrogenation with quinoline in toluene which afforded the *cis*-mono-ene **34** in 95% yield. Lastly, the isopropylidene protection group was removed by reflux in 96% ethanol with Amberlyst 15 which afforded the MEL **6** in 48% yield. The surprisingly low yield for this last step was undoubtedly because of deteriorated material being used and does not represent the possible yield for this reaction, which on all comparable substrates is around 85-100%. The MEL **6** was thus afforded in 15% total yield but ignoring the last step should be obtainable in about 30% total yield as based on the solketal.

5.3 The 18:3 syntheses

In addition to the six MELs detected and already described in the shark and dogfish oil, there was an 18:3 MEL which had been detected before[53] but never had its exact structure confirmed. The 18:3 MEL was the least abundant in the sample and, unlike the others, had a very simple MS/MS spectra (low fragmentation). The initial thought was that it possibly possessed the Δ4-position for the first double bond and then two additional methylene interrupted double bonds adjacent to it, making it an n-8 derivative (MEL **7**). The MEL **7** would be simple to synthesize from the di-yne head piece **13** using the already established strategy.[5] When the MS/MS degradation spectrum of the synthesized MEL **7** did not align with the spectrum obtained from the natural sample other alternative structures had to be considered. Other likely candidates were thought to be the n-3 MEL **8**, an α-linolenic acid derivative or the n-6 MEL **9**, a γ-linolenic acid derivative.

5.3.1 Synthesis of the MEL 7

The synthesis of the MEL **7** starting from the di-yne **13** is depicted in Scheme 5.6.[5]



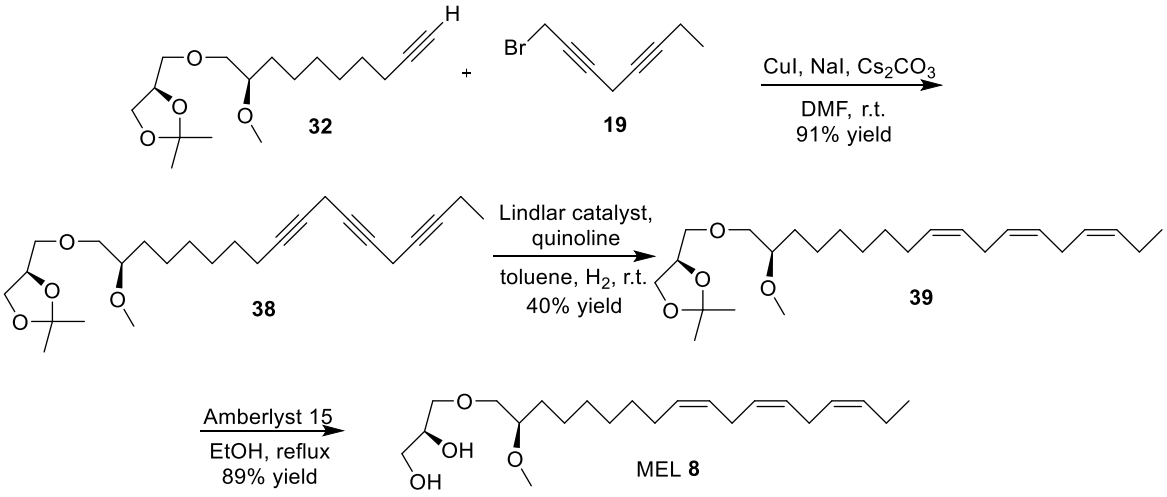
Scheme 5.6 The synthesis of 18:3 n-8 MEL **7**.[5]

The synthesis of the di-yne **13** is depicted in Scheme 4.3. The n-8 mono-yne propargyl bromide **35** was made from commercially available dec-2-yn-1-ol in an Appel reaction, which afforded the mono-yne **35** in 91% yield. The di-yne **13** and mono-yne **35** were reacted in a copper coupling reaction which afforded the tri-yne **36** in 89% yield. The tri-yne **36** was then submitted to the Lindlar catalyzed semi-hydrogenation with quinoline in toluene which afforded the tri-ene **37** in 98% yield after flash chromatography. The product still contaminated with the semi-hydrogenation byproducts was then purified further by argentation chromatography affording the pure tri-ene **37** in 52% yield. Overall, the semi-hydrogenation step afforded the tri-ene **37** in 51% yield. The isopropylidene deprotection was then accomplished as before by reflux in 96% ethanol with Amberlyst 15, affording the MEL **7** in 87% yield. The synthesis of MEL **7** was completed in 17% total yield as based on the solketal.

As the solketal, the expensive chiral starting material is on the head part of the molecule, a more efficient way could be to modify the synthesis such that the head part would be the mono-yne **16** and the tail part would be a di-yne. That approach would submit the head part to fewer reactions and therefore retain the solketal better. The experience with these copper couplings on the other hand has indicated, as has been stated before, generally lower yields when mono-yne head parts are being copper coupled to long tails. Since all the copper couplings gave very good to excellent yields, no further explorations were done in this case.

5.3.2 Synthesis of the MEL 8

The second candidate for the 18:3 MEL was the n-3 MEL **8**. As the first double bond on the chain was located on the ninth carbon the same intermediate as for the synthesis of 18:1 n-9 MEL **6**, the mono-yne **32** was used. The synthesis of MEL **8** is depicted in Scheme 5.7, starting from the mono-yne **32**.



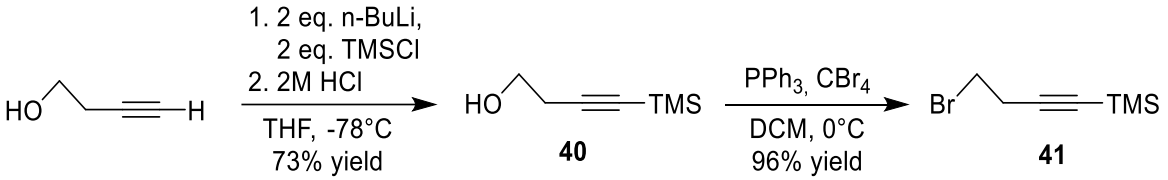
Scheme 5.7 The synthesis of the 18:3 n-3 MEL **8**.

The mono-yne **32** coupled to the di-yne **19** would establish the desired tri-yne structure. This was accomplished yet again via the same copper coupling as before, which afforded the tri-yne **38** in 91% yield. The tri-yne **38** was semi-hydrogenated in the same manner as for the MEL **7** and was afforded in 78% yield after flash chromatography and then in 51%

yield after argentation chromatography, 40% yield in all for the semi-hydrogenation step. Also same as before, the tri-ene **39** refluxed in 96% ethanol in the presence of Amberlyst 15 removed the isopropylidene group affording the MEL **8** in 89% yield. The MEL **8** was thus obtained in just under 18% overall yield as based on the solketal.

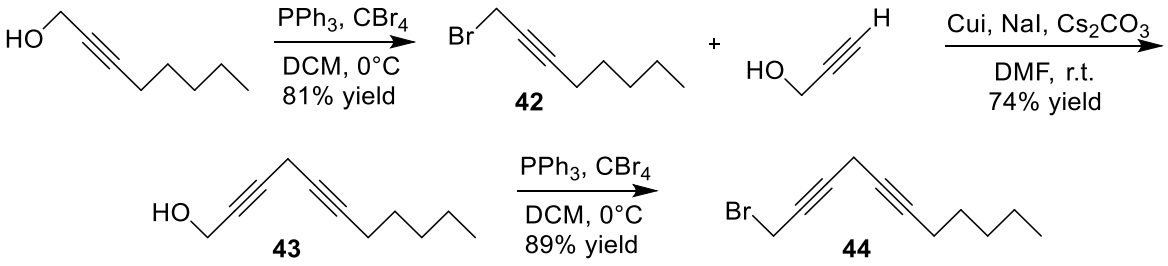
5.3.3 Synthesis of the MEL 9

Neither the n-8 nor n-3 18:3 MELs did align with the MS/MS spectra of the natural compound. A third and final 18:3 MEL synthesis was then performed. An unusual n-6 variant was tested. To accomplish this structure another and shorter linker had to be synthesized as the first double bond in the chain would be located on the sixth carbon. The synthesis of the new mono-yne linker **41** is depicted in Scheme 5.8.



Scheme 5.8 The synthesis of the TMS-protected alkyne linker **41**.

Commercially available but-3-yn-1-ol was allowed to react with two equivalents of TMS-chloride and *n*-BuLi in dry THF to afford the mono-yne alcohol **40** in 73% yield after addition of 2M HCl and vigorous stirring. The alcohol was then brominated in an Appel reaction affording the mono-yne linker **41** in 96% yield. The linker **41** attached to the head piece **14** would position the first double bond on the chain on the sixth carbon. To finish the desired n-6 framework an n-6 di-yne propargyl bromide **44** was required, see Scheme 5.9.

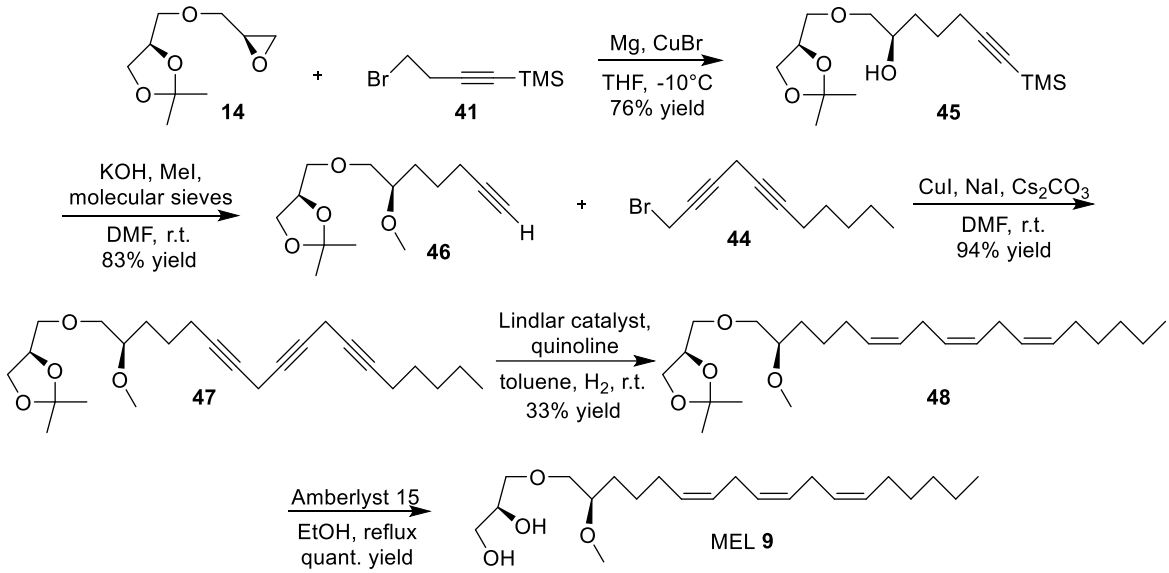


Scheme 5.9 Synthesis of the n-6 di-yne tail part **44**.

Its synthesis was accomplished by the Appel bromination of oct-2-yn-1-ol, which afforded the mono-yne **42** in 81% yield after flash chromatography. Copper coupling of the resulting mono-yne **42** and propargyl alcohol gave the di-yne alcohol **43** in 74% yield. The di-yne **44** was then afforded in another Appel bromination reaction in 89% yield after flash chromatography.

As for the synthesis of MEL **8**, the linker **41** was reacted with magnesium metal to obtain the corresponding Grignard reactant. The Grignard reactant was then reacted with the head piece **14** in the presence of CuBr to obtain the mono-yne **45** (see Scheme 5.10). Initially, this reaction gave very poor yields. This was because some amount of bromoform eluted with the linker **41** off the silica column. To resolve that, some further flash

chromatography was applied until no bromoform residue was left and the reaction then went smoothly, affording the mono-yne **45** in 76% yield. The mono-yne **45** was then similarly as for **15** and **31** deprotected and methylated in the same operation using MeI and KOH. And as before, the substrate would only methylate in part but with the addition of molecular sieves the mono-yne **46** was afforded in 83% yield. The mono-yne **46** and di-yne **44** were then copper coupled together, affording the tri-yne **47** in 94% yield.



Scheme 5.10 The synthesis of the 18:3 n-6 MEL **9**.

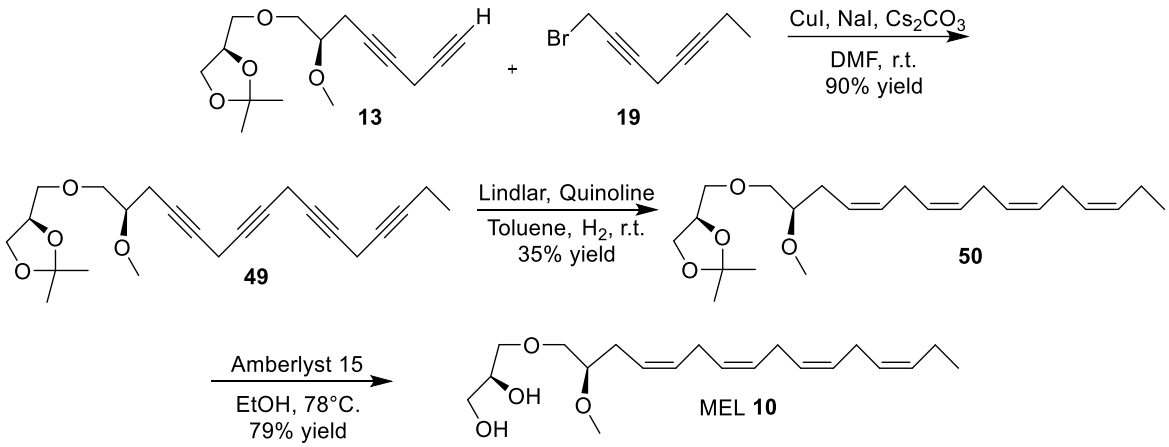
The key semi-hydrogenation step was conducted in the same manner as for the other 18:3 MELs and afforded the tri-ene **48** in 78% yield after flash chromatography and then the argentation chromatography afforded 43% yield, in all 33% yield for the semi-hydrogenation. The semi-hydrogenation yields were somewhat lower for this product compared to the other 18:3 MELs and is most likely explained by the condition of the tri-yne **47** as it was submitted to the semi-hydrogenation and the reaction was not optimized further. The final isopropylidene deprotection afforded the MEL **9** in quantitative yields. Thus, the MEL **9** was obtained in 14% total yield as based on the solketal.

Surprisingly, none of the above 18:3 MEL structures seemed to correspond to the one found in the natural shark and dogfish liver oil sample, since none of their MS/MS spectra matched. The true structure of the polyunsaturated 18:3 MEL therefore remains unknown.

5.4 Synthesis of the MEL 10

The MEL **10** is not one of the natural compounds found in nature. It was synthetically easily accessible from the di-ynes **13** and **19**, intermediates in the MEL **5** synthesis. MEL **10** is highly unsaturated, like MEL **5**, but the simpler synthesis and lesser instability of the tetra-yne precursor allowed for easier access to semi-hydrogenation trials of poly-yne MEL substrates. The MEL **10** fits very well in between the hexa-ene MEL **5** and the tri-ene MELs **7**, **8** and **9** under investigation in the semi-hydrogenation studies. It also offers a direct comparison with SDA when it comes to the trans contaminant evaluation as will be

discussed later. Scheme 5.11 depicts the MEL **10** synthesis from the di-yne intermediates **13** and **19**.



Scheme 5.11 Synthesis of the non-natural MEL **10**.

The di-ynes **13** and **19** were synthesized as covered before and submitted to the same copper coupling conditions as for **13** and **12**. The reaction was allowed to run for about 40 hours at r.t. (which was probably more than was needed) and after aqueous work-up purified by flash chromatography, which afforded the tetra-yne **49** as a yellow liquid in 90% yield. The unstable tetra-yne **49** was then immediately employed in a stereoselective semi-hydrogenation reaction. Multiple trials were made to semi-hydrogenate the tetra-yne **49** and some of them are listed in Table 5.2.

Table 5.2 Semi-hydrogenation trials of compound **49** to produce the tetra-ene MEL **10**.

Catalyst	Catalyst poison	Solvent	Reaction time	Vinyl integration	Yield
Lindlar **II**[a]	none	benzene	57 min	3.03 (38%)	Low
Lindlar **II**[a]	quinoline	benzene	46 min	6.44 (81%)	Low
Lindlar **II**[a,b]	quinoline	Et_2O	60 min	5.86 (73%)	Low
Lindlar **II**	quinoline	Et_2O	21 min	6.85 (86%)	36%
Lindlar **III**	quinoline	toluene	1h 34 min	6.98 (87%)	77%
Lindlar **V**	pyridine	MeOH[c]	6h 44 min	6.98 (87%)	51%
Brown	eda	EtOH	32 min	6.50 (81%)	54%

[a] small scale (~20mg). [b] 0°C. [c] 2-methyl-2-butene also added.

The reaction without any catalyst poison gave overwhelmingly over-hydrogenated product. The reactions performed in benzene and diethyl ether gave low yields and similar integration, except when performing the reaction at 0°C in diethyl ether, the reaction time tripled, and much lower integration was obtained. The best semi-hydrogenation outcome that was achieved for the semi-hydrogenation of the tetra-yne **49** was in toluene with quinoline as additive. The reaction was monitored by TLC and ran for about 90 minutes at r.t. and on completion was filtered through celite, concentrated, and purified on a silica column. This afforded the crude tetra-ene **50** as a faintly yellow oil in 77% yield, by far the highest that was achieved for this compound. This crude product was then purified further

on a 10% $AgNO_3$ impregnated preparative TLC plate which afforded a band of the pure tetra-ene **50** as an almost colourless liquid in 46% yield, 35% yield in total for the semi-hydrogenation. Surprisingly, the conditions tributed to Hwang et al. did not offer higher integration as for other substrates but only the characteristically fair yield and greatly increased reaction time. Similarly, the Brown catalyst afforded fair yield but even lower integration.

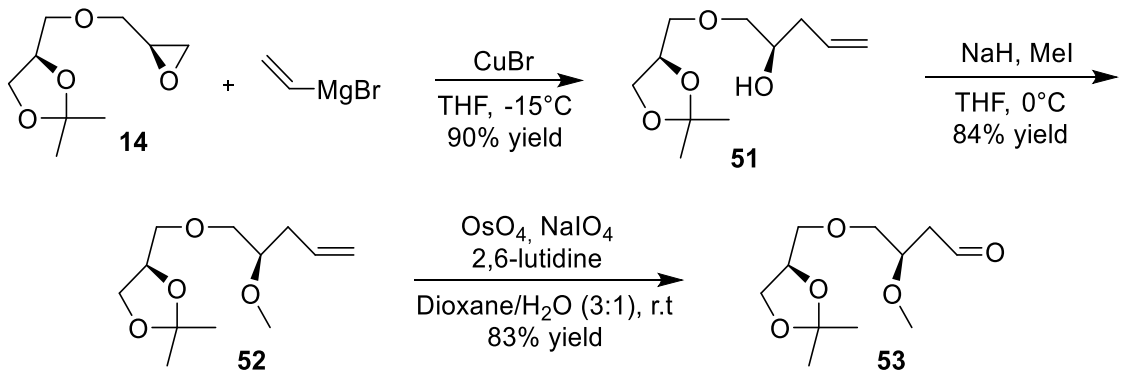
Lastly, the isopropylidene group was removed. By refluxing the tetra-ene **50** in 96% ethanol in the presence of Amberlyst 15 and then purifying the product on a short silica column the tetra-ene diol, MEL **10** was afforded in 79% yield. This lower yield than for other similar isopropylidene deprotection reactions performed was most likely due to the substrate **50** that was used in the reaction had been stored for some time, and this reaction then not optimized further. The synthesis of the MEL **10** was thus achieved in just under 11% overall yield over 8 steps based on the solketal starting material.

5.5 Wittig and acetylenic approaches combined

As has been described, the semi-hydrogenation of skipped poly-yne compounds has some drawbacks, mainly the unstable skipped poly-yne substrates and formation of large amounts of byproducts in that reaction. With every additional triple bond in the skipped poly-yne chain, a seemingly larger ratio of byproducts is afforded. A noticeable drop in quality of the semi-hydrogenation product of a tri-yne is seen when compared to a di-yne. The alternative approach of combining the Wittig reaction with the acetylenic approach to limit the need for large poly-yne systems has been discussed.

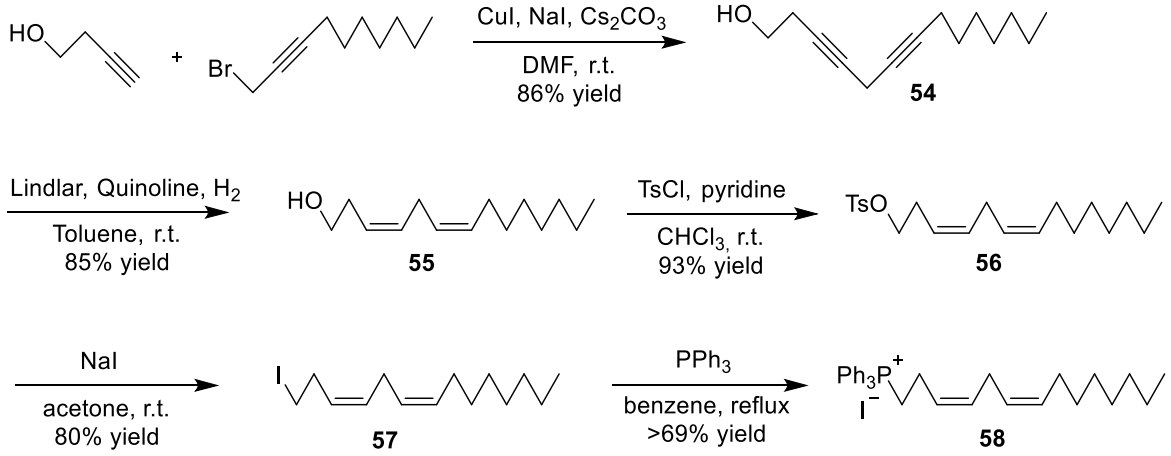
To compare these methods an alternative synthetic approach was developed for MEL **7**. This new approach should significantly increase the yield for the semi-hydrogenation but as well introduce many new reactions and additional steps to the synthesis. The stereoselectivity of the Wittig reaction was also under question, but as was covered in Chapter 3.3, the stereoselectivity is usually very good or excellent, but not guaranteed to be so. The new approach is based around the formation of the Δ4-double bond with a Wittig reaction, using the di-ene phosphonium iodide tail part **58** and the modified aldehyde head piece **53**. The preparations of the two Wittig reactants, **53** and **58**, are depicted in Schemes 5.12 and 5.13, respectively.

The synthesis of the aldehyde **53** (as all the other MEL syntheses described in this thesis) made use of the universal head piece **14**, this time to react with vinyl magnesium bromide in the presence of CuBr at -15°C in dry THF (see Scheme 5.12). This afforded the mono-ene alcohol **51** in 90% yield. **51** was then methylated with MeI in the presence of NaH as a base affording the methoxy mono-ene **52** in 85% yield. **52** was then converted into an aldehyde in an oxidizing cleavage reaction using osmium tetraoxide and sodium periodate in the presence of 2,6-lutidine which has been shown to reduce the formation of an α-hydroxy ketone byproduct.[199]



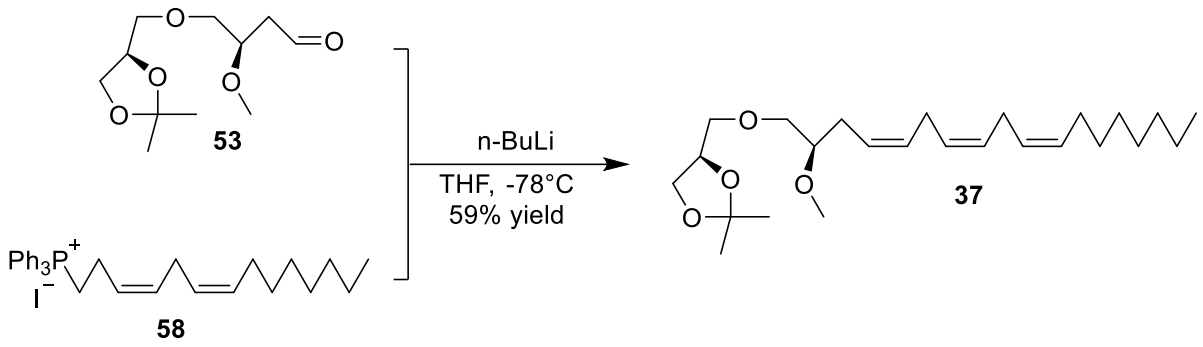
Scheme 5.12 The synthesis of the modified head group **53**.

The phosphonium iodide tail part **58** synthesis (see Scheme 5.13) started with the copper coupling of homo-propargyl alcohol and 1-bromo-2-decyne, the same way as before, affording the di-yne **54** in 86% yield. The di-yne **54** was then semi-hydrogenated using the Lindlar catalyst with quinoline in toluene, affording the di-ene **55** in 85% yield with very low *trans* and over-hydrogenated byproducts as observed by IR and NMR spectroscopy. The di-ene alcohol **55** was not purified further by argentation chromatography, but tosylated with TsCl using pyridine as a base affording the tosylate **56** in 93% yield. The tosylate **56** was then converted to the iodide **57** in a Finkelstein reaction in 80% yield and finally to the targeted phosphonium salt **58** by refluxing in benzene in the presence of triphenylphospine. This afforded **58** as a sticky solid in 69% yield at least (not all material was recovered) after washing with diethyl ether to remove the unreacted triphenyl phosphine and iodide.



Scheme 5.13 Synthesis of the phosphonium iodide di-ene **58** for the Wittig reaction.

The phosphonium salt **58** as obtained was then used in a Wittig reaction with the aldehyde **53** using n-BuLi as a base (which should be exchanged with a non-lithium base according to the literature) in dry THF. This afforded the tri-ene **37** in 59% yield as depicted in Scheme 5.14. This reaction was not optimized any further.



Scheme 5.14 The Wittig reaction affording the isopropylidene protected 18:3 n-8 MEL **7**.

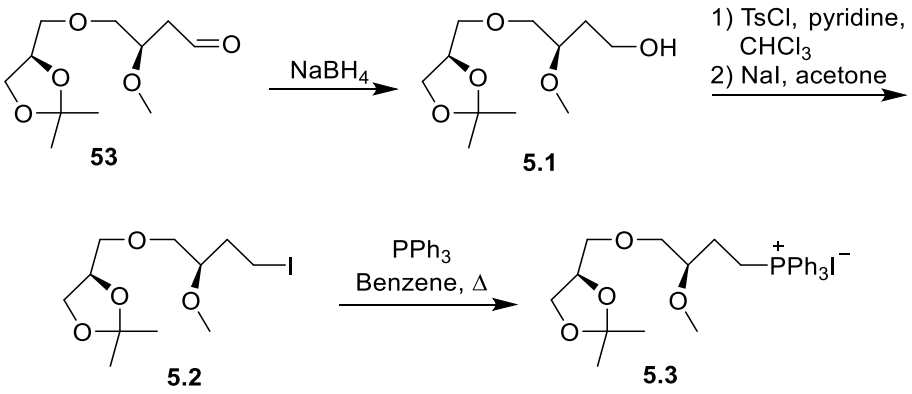
IR spectra comparison on the 18:3 n-8 MEL **37** products derived from the acetylenic approach on one hand and the combined approach on the other was made. It is hard to draw conclusions about the exact *trans*-isomer content from the IR spectra of the MELs. The IR spectroscopy is not a very accurate tool to measure low *trans* contents, besides the *trans* band overlapping with another band in the MEL spectra. A noticeable difference was though seen in the spectra after argentation chromatography on the acetylenic derived product which indicates much lower *trans* content after the argentation chromatography and almost identical IR spectra to the Wittig product was obtained, with the Wittig product only showing a marginally higher peak. This supports the assumption that the approach involving the Wittig reaction and the di-yne semi-hydrogenation does not produce much *trans* content but the tri-yne semi-hydrogenation approach does.

The ^{1}H-NMR comparison revealed almost no over-hydrogenated byproducts in the Wittig based product, with the vinyl protons integrating for 5.71 (95%). This means there was some over-hydrogenation resulting from the Lindlar semi-hydrogenation reaction of the di-yne **54** which was not purified by silver ion chromatography. By comparison, the product afforded from the semi-hydrogenation of the tri-yne **36**, showed more over-hydrogenated byproducts according to the ^{1}H-NMR, where the vinyl protons integrated for 5.41 (90%). This comparison underlines how the increased number of acetylenes in the poly-ynes submitted to semi-hydrogenations increases the amounts of byproducts which supports the hypothesis that a combined approach could be the most optimal.

Thus, the Wittig reaction product **37** was achieved in 27% total yield as based on the solketal without any argentation chromatography and with small amount of over-hydrogenated byproduct and negligible amount of *trans*-isomers. The same compound **37** afforded from the semi-hydrogenation of the tri-yne **36** was afforded in 38% yield, but with significantly more impurities, both over-hydrogenated and *trans*. The argentation chromatography then afforded a very pure sample of **37** with proton integration of 6.06 (101%) but in lower total yield of 18%. If the Wittig reaction affords a mostly stereopure product, which in this case it seemingly did even though a lithium base was used, the argentation purification could be applied to the di-ene tail if needed. This would then lower the yield only for the less expensive tail part and then possibly remove any need for argentation chromatography of the compound that includes the chiral carbons. These possibilities might cement the combined approach as the most efficient, but this has still to be demonstrated further.

5.6 Alternative strategies for the MEL 5 synthesis

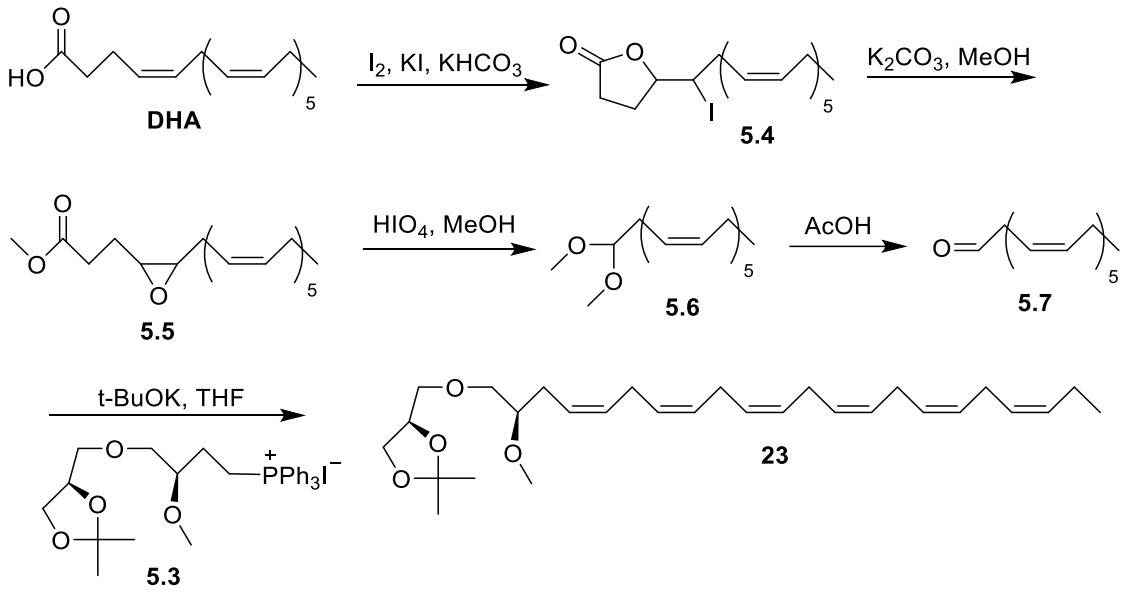
An alternative method for the MEL **5** synthesis that circumvents the difficult poly-yne instability and semi-hydrogenation issues could offer considerable synthetic advantages. As mentioned briefly before, the chemical manipulation of pure PUFAs could be a worthwhile strategy for the total synthesis of highly unsaturated MELs. Already containing the all-*cis* skipped poly-ene structure, pure natural DHA could be used as the starting material for the synthesis of the DHA-like MEL **5**. A proposal for such a venture is presented in Schemes 5.15 and 5.16 where the phosphonium iodide substrate **5.3** is derived from the head part **53** and pure natural DHA is converted to the penta-ene aldehyde substrate **5.7**, respectively. These two substrates would then be attached in a Wittig reaction.



Scheme 5.15 Proposed synthesis of head piece **5.3** for the alternative strategy for MEL **5**.

The aldehyde head part **53** was derived from the original head piece **14** and used in the Wittig synthesis described earlier. This aldehyde could be reduced to produce the alcohol **5.1** via sodium borohydride for example and then converted to the tosylate which in turn could be exchanged with iodide affording **5.2** or be used further as the tosylate. The Wittig reagent phosphonium iodide **5.3** would then be produced by reflux in benzene in the presence of triphenylphosphine. The phosphonium iodide head part **5.3** with both chiral centers established could now attempt a Wittig reaction with the penta-ene aldehyde **5.7** derived from natural DHA to produce the desired MEL **5**.

Corey et al.[200] first reported the iodolactonization of ARA where the carboxylic function forms an iodolactone from the closest double bond. When the iodolactone was treated with a base in methanol an epoxide was formed, selectively on the double bond closest to the carboxylic acid. This method has since been demonstrated on EPA and DHA. These epoxides are very important since they can then give access by oxidative cleavage to large skipped poly-ene aldehydes. Scheme 5.16 depicts an example of a strategy which would convert natural DHA to a skipped penta-ene aldehyde **5.7** which could be used in a Wittig reaction with the ylide of **5.3** to produce the hexa-ene **23**, the isopropylidene protected MEL **5**.



Scheme 5.16 Proposed conversion of natural DHA to the penta-ene aldehyde reagent **5.7** and the following Wittig reaction producing the acetal protected MEL **5**.

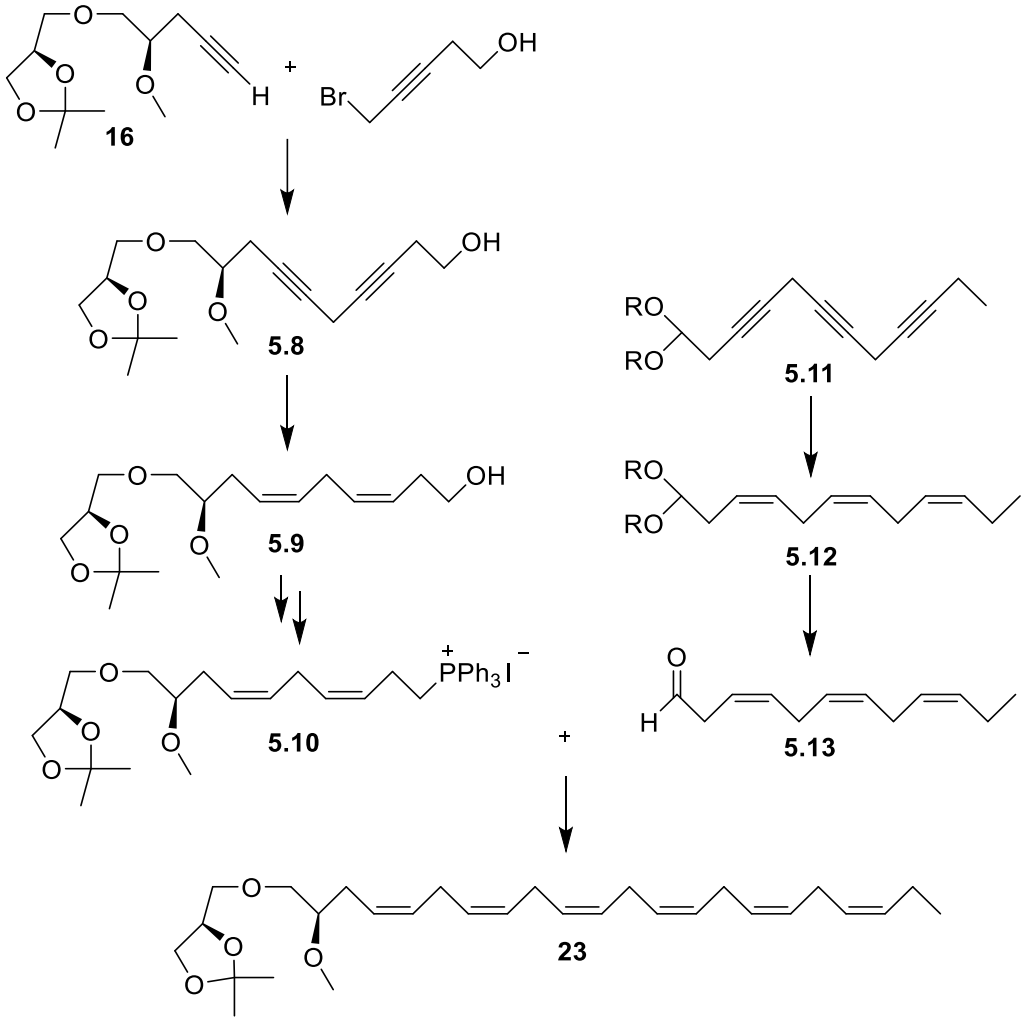
Different methods have been developed for the synthesis of **5.7** and Scheme 5.16 puts forth an example of a strategy which could be attempted. The carboxylic group in DHA can be made to form the γ-lactone with the closest double bond on the chain in the presence of I_2, KI and $KHCO_3$ producing **5.4**. A mild base in methanol will open up the lactone forming the methyl ester epoxide **5.5** via intramolecular elimination of the iodide. Periodic acid in methanol can then cleave the epoxide and afford the dimethyl acetal **5.6** which should easily convert to the corresponding aldehyde **5.7** under acidic conditions. This methodology has been demonstrated on similar compounds as well as DHA and the penta-ene aldehyde **5.7** has been produced before in similar fashion.[201, 202] The aldehyde **5.7** would then be the second reagent in the Wittig reaction with the phosphonium iodide head group **5.3** affording the desired acetal protected DHA-like MEL **5**, compound **23**.

An alternative approach to this method would be to use the aldehyde **53** as the first Wittig reagent and transmute the penta-ene aldehyde **5.7** further to a phosphonium halide as the second reagent. The efficiency of this approach would depend on the over-all yield for the transmutation of the labile penta-ene substrate to the phosphonium halide reagent.

A synthetic pathway like this would circumvent certain difficulties as mentioned before, but possibly create its own. Downsides to this strategy is the use of possibly expensive pure DHA as starting material and possible low yields or stereoselectivity in the all-important Wittig reaction. Pure DHA is now commercially available, but Corey and coworkers did in 1987 also publish a very convenient method to isolate DHA from inexpensive fish oil by converting it to the iodolactone which was then isolated and converted back to the acid (14g of DHA from 200g of oil).[203]

If for whatever reason the approaches described above were to be unfeasible or impossible one could still theorize some other strategies to circumvent the imposing hexa-yne semi-hydrogenation. Some combination of smaller substrates for semi-hydrogenation and Wittig reactions as have been demonstrated in the literature for some poly-ene syntheses could be more efficient than the methodology described in Chapter 4.2. Supporting this is the synthesis of MEL **7** by the combined approaches (Section 5.5) which demonstrated how

very low amounts of byproducts were present in the final product. To obtain excellent purity some further argentation chromatography could though be needed, but as mentioned in Section 5.5 it could be performed on the less expensive di-ene semi-hydrogenation product instead of the expensive final product. Finally, Scheme 5.17 puts forth an example of a possible strategy for the total synthesis of MEL **5** by the combined Wittig and acetylenic approaches.



Scheme 5.17 An example of a possible strategy for the synthesis of MEL **5** by the combined Wittig and acetylenic approach. R=alkyl.

The mono-yne head part **16** could be copper coupled to 5-bromo-pent-3-yn-1-ol, which if not commercially available is accessible through homopropargyl alcohol and formaldehyde. This would produce the di-yne **5.8** which would then be submitted to a semi-hydrogenation reaction, affording the di-ene **5.9** which by well-established methods would be converted to the Wittig reagent **5.10**. The tri-yne **5.11** could then also be semi-hydrogenated to produce the tri-ene **5.12** and since it would be a semi-hydrogenation of a tri-yne it would probably warrant argentation purification for optimal purity. Deprotection would then produce the second Wittig reagent, the aldehyde **5.13**. The Wittig reaction of **5.10** and **5.13** could then possibly produce the desired hexa-ene **23** in better yield than the hexa-yne semi-hydrogenation described in Chapter 4.2.

5.7 MS/MS fragmentation comparisons

A HPLC/MS analytical method to investigate the MEL fragment isolated from the original shark and dogfish liver oil sample was developed by Albertsdóttir as an M. Sc. project.[1] The results of this project identified seven different MELs and accurate mass and MS/MS degradation spectra were acquired for all of them. From the accurate mass the molecular formula and level of unsaturation can be deduced. The position of the double bonds and therefore the absolute structure can then be approximately deduced by the MS/MS fragmentation pattern. To prove the absolute structure, the fragmentation pattern of a synthesized MEL must accurately display the same fragments and in similar relative intensity if fragmented under the same conditions.

The MS/MS fragmentation spectra of both the 18:1 MELs identified in the sample were compared to the spectra of the synthesized MEL **3** and **6**. The one eluting first from the column (18:1A) showed the same fragmentation as MEL **6**, see Figure 5.1. The one eluting next (18:1B) was also a perfect fit for MEL **3**, see Figure 5.2. The 22:6 MEL was compared to the synthesized DHA-like MEL **5** which was also, as expected, a perfect fit, see Figure 5.3. Finally, the 18:3 MEL was compared to the synthesized MELs **7**, **8** and **9**, which all displayed significantly different patterns, but none fit the natural sample, see Figure 5.4. Albertsdóttir had already compared the 16:1 MEL to the then synthesized MEL **1** and observed identical spectra.[1]

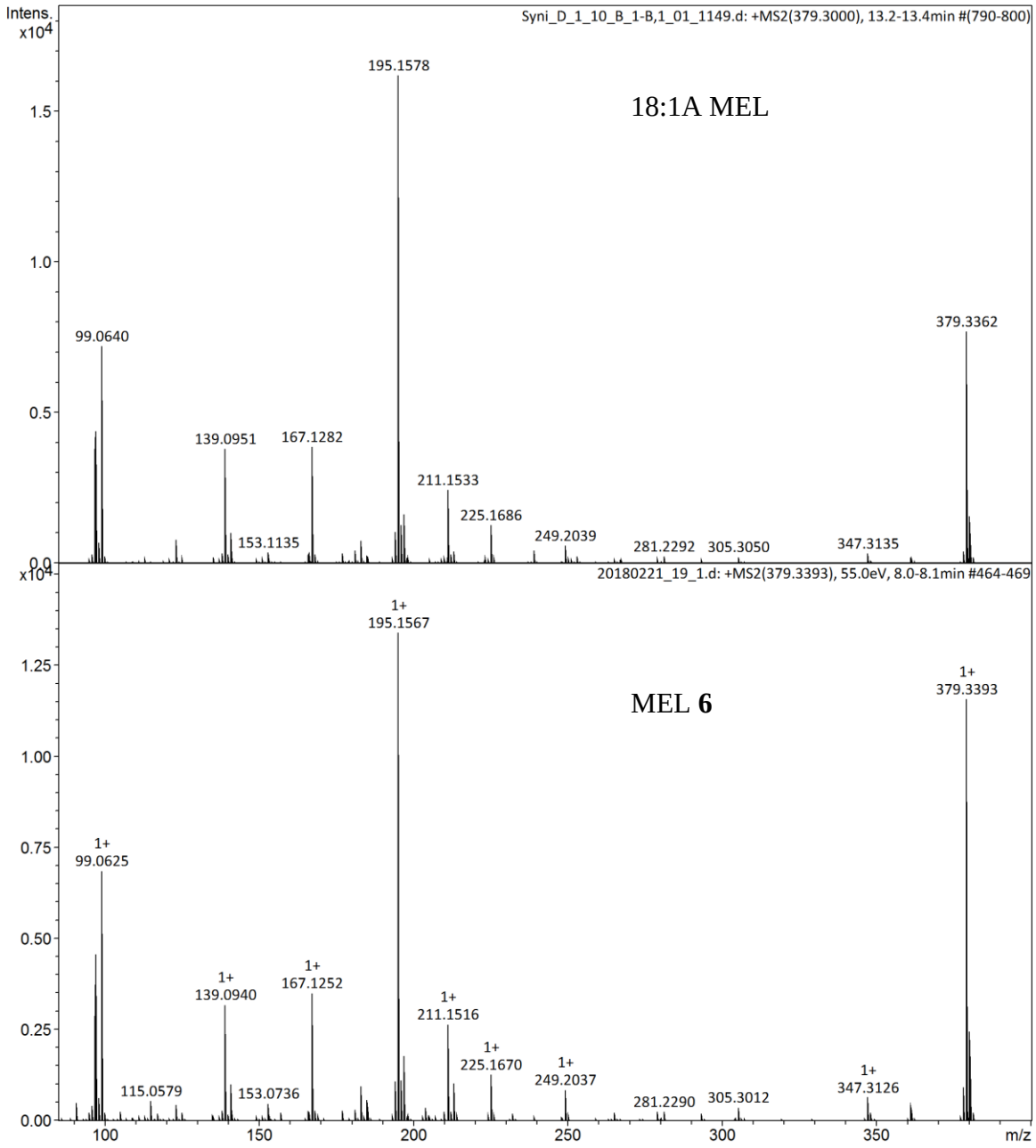


Figure 5.1 The MS/MS fragmentation of the synthesized MEL **6** and the 18:1A MEL found in the shark liver oil sample.

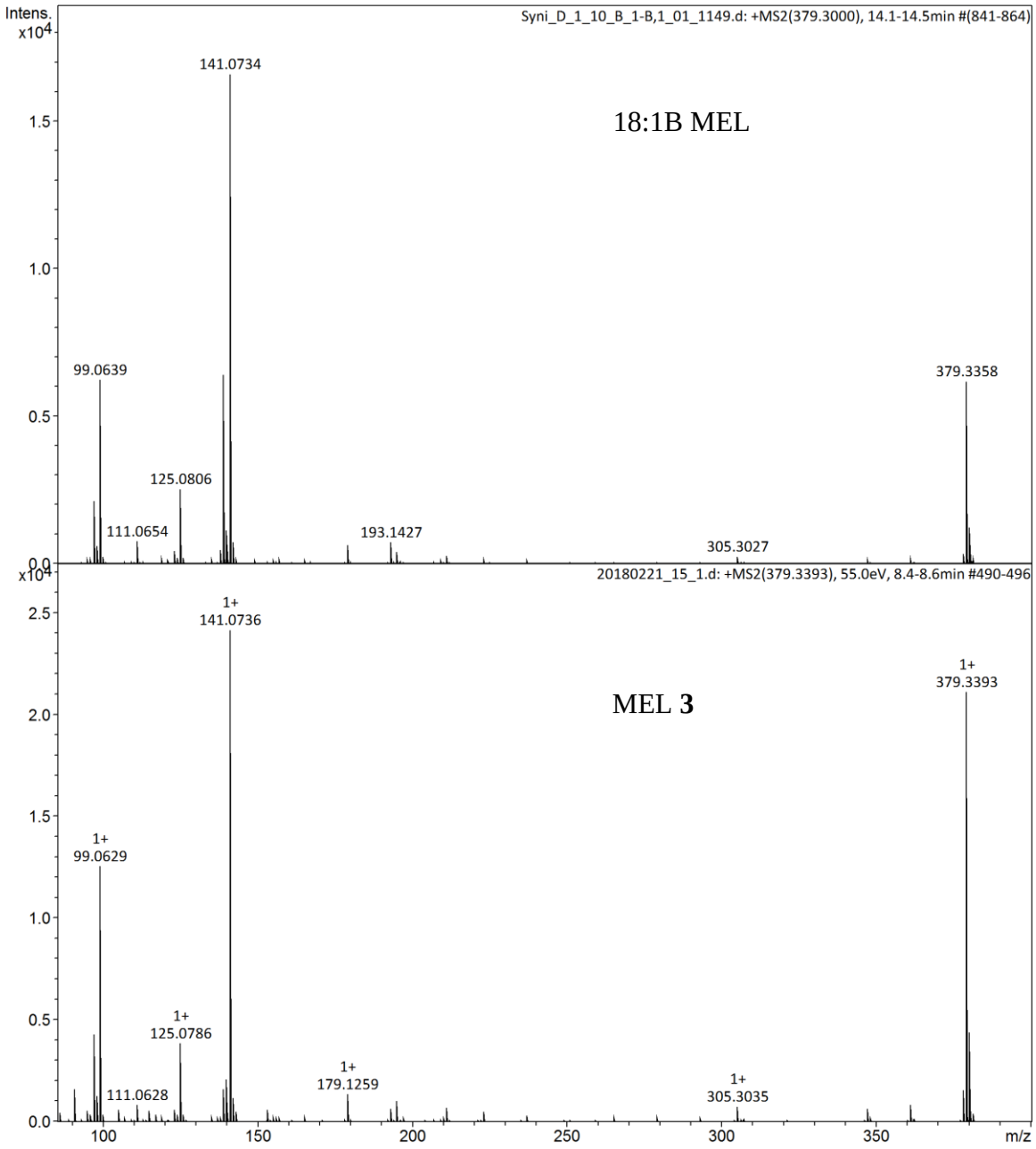


Figure 5.2 The MS/MS fragmentation of the synthesized MEL **3** and the 18:1B MEL found in the shark liver oil sample.

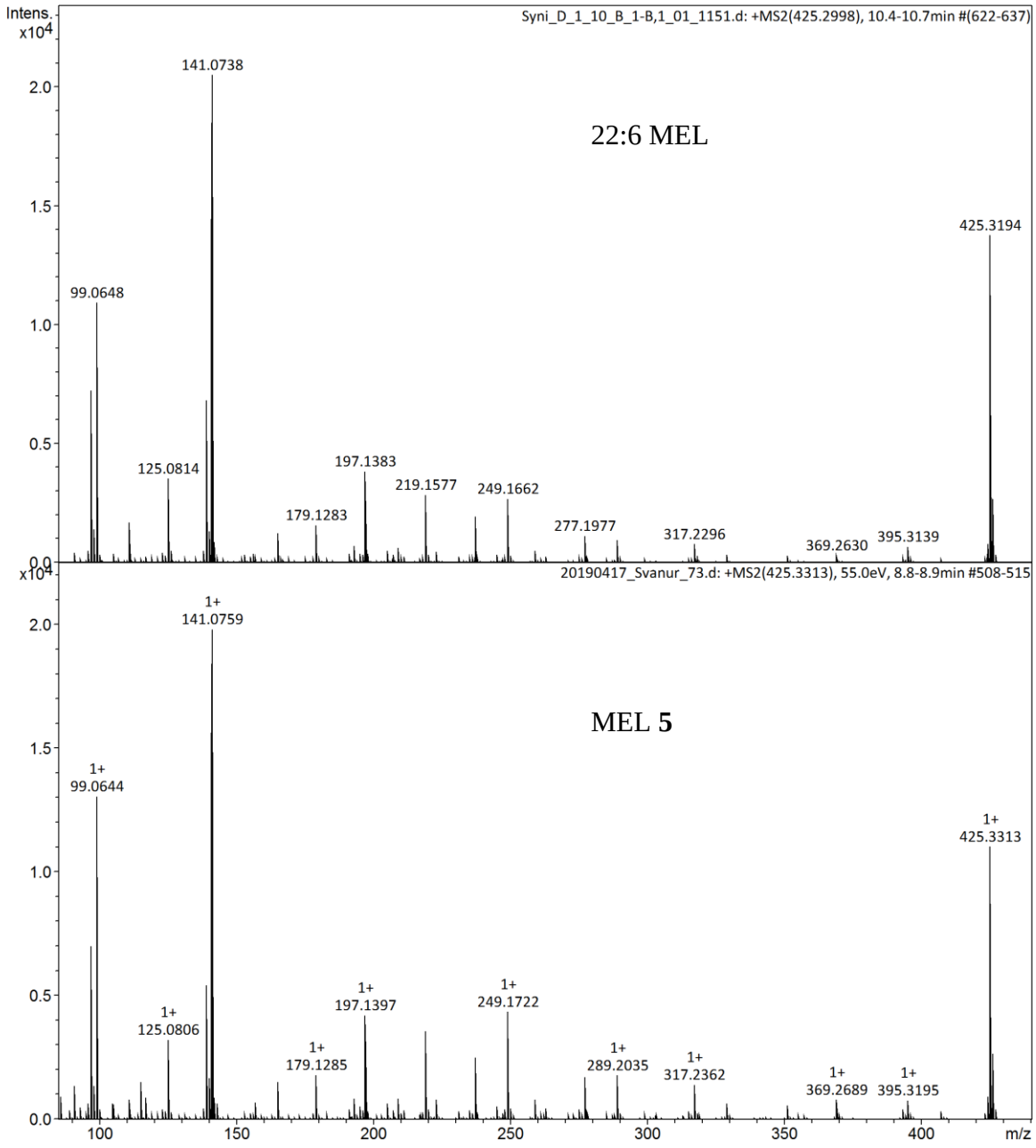


Figure 5.3 The MS/MS fragmentation of the synthesized MEL **5** and the 22:6 MEL found in the shark liver oil sample.

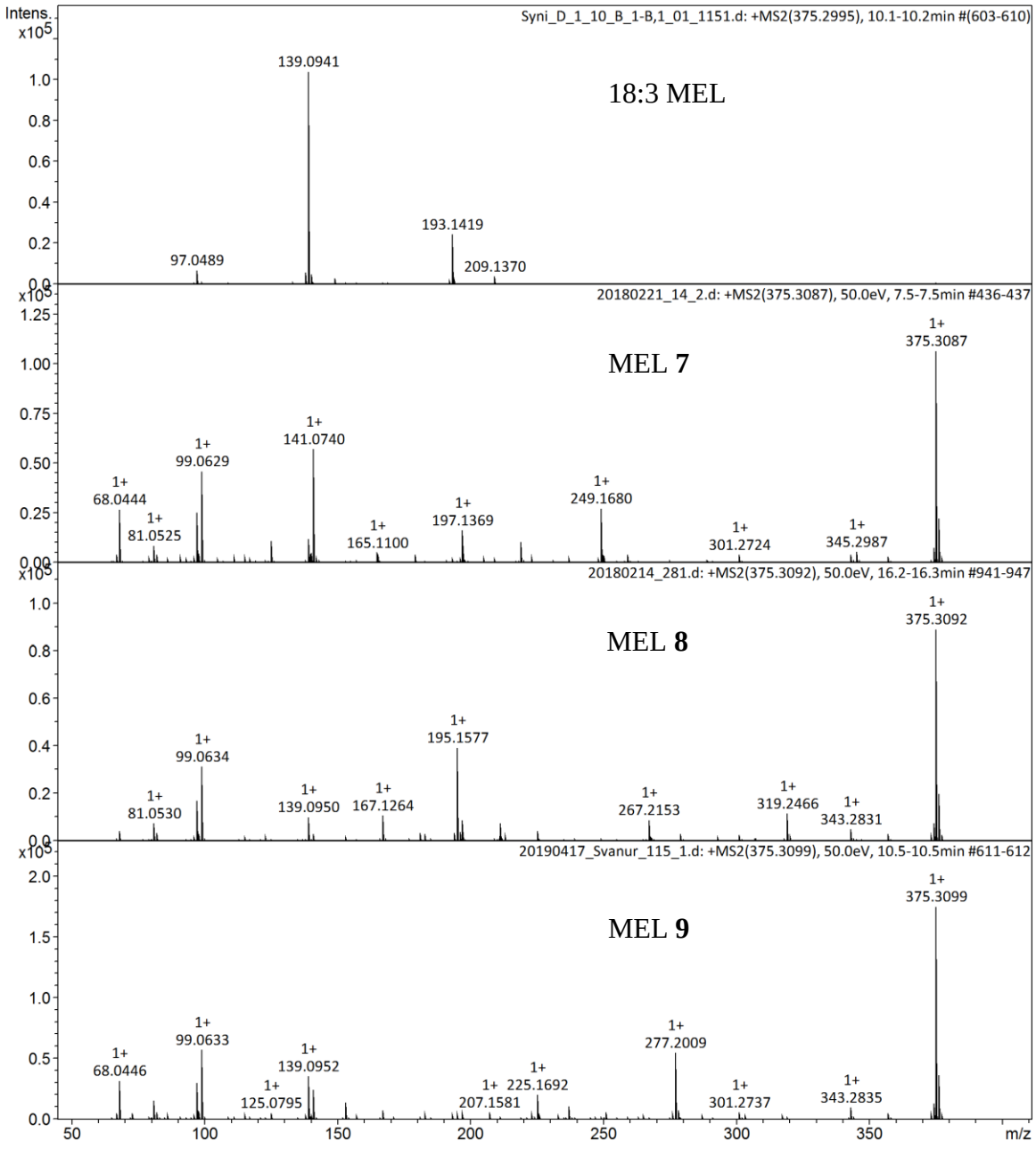


Figure 5.4 The MS/MS fragmentation of the synthesized MELs **7**, **8**, **9** and the 18:3 MEL found in the shark liver oil sample.

The 18:3 MEL which was originally discovered in 1982[53] could unfortunately not have its absolute double bond configuration established by these efforts. Three different types of MELs, an n-3, n-6 and n-8 were synthesized and their fragmentation spectra compared to it. It is possible that the true configuration could be very unusual and not follow the skipped configuration, but the fragmentation spectrum obtained does raise some questions. First of all, only very low fragmentation was observed over a range of different collision energies as well as that the 99 m/z fragment is missing but present in the spectra for all the other MELs.

What was expected and already established were the absolute structures of the 22:6 and one of the 18:1 MELs which corresponded nicely to the spectra of MEL **5** and MEL **3**,

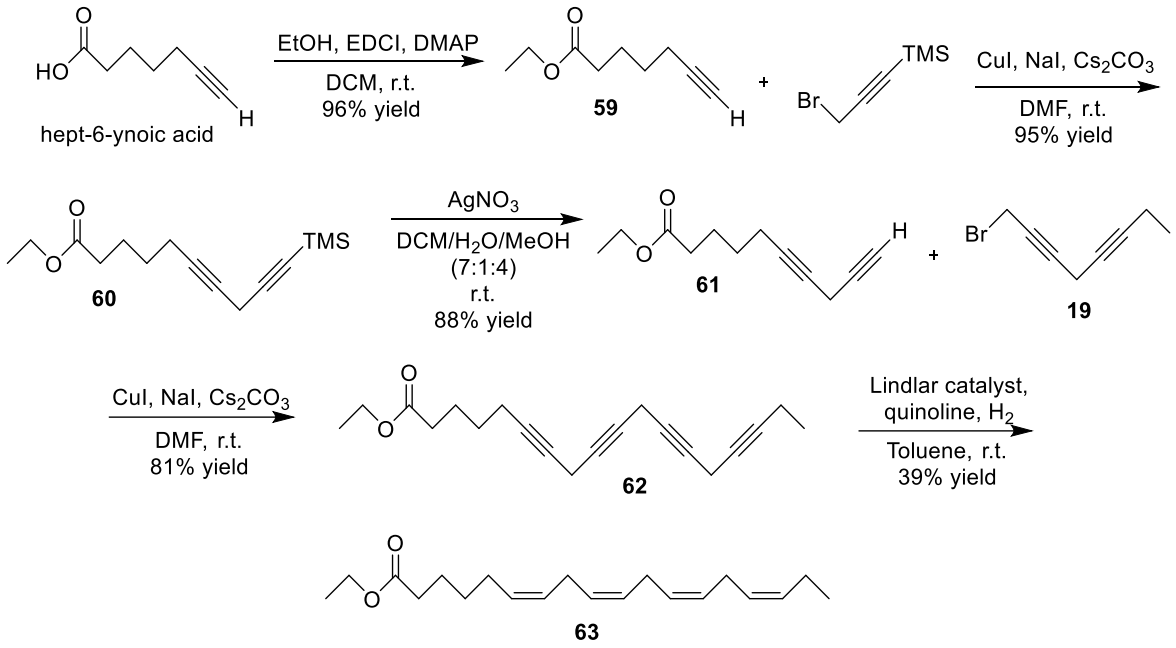
respectively. The n-9 structure of MEL **6** has never been confirmed before, but its fragmentation fit perfectly to the 18:1A MEL from the sample. These results confirm the MELs in the original sample to be, 16:0, 16:1 n-12,[1] 18:0, 18:1 n-14, 18:1 n-9 and 22:6 n-3 (DHA-like). The last type, the 18:3 is still unestablished, but its structure does not correspond to a normal skipped configuration of a n-3, n-6 or n-8 carbon chain.

5.8 Synthesis of PUFA EEs

As a part of this work, the syntheses of large poly-yne substrates and submitting them to semi-hydrogenation reactions, the natural polyunsaturated fatty acids SDA, EPA and DHA were synthesized. Their syntheses were from a curiosity standpoint very interesting, as these molecules are of high biological importance and similar syntheses in the past have been somewhat lacking in describing exact conditions of the final products. These products are as well very accessible from the intermediates and methods described for the DHA-like MEL **5** synthesis. These syntheses were also of particular interest in one aspect, as these compounds could be submitted to *trans* analysis under specific GC methods and compared to established mono-*trans* standards, which would very accurately confirm their *trans*-isomer contents. As will be discussed in detail in Chapter 6.3, the exact *trans* content of the MEL **5** can be difficult to measure. As the poly-yne intermediates of the PUFAs would be semi-hydrogenated and purified under the same conditions as the poly-yne **11** they could give clues into the actual *trans* content of the MEL **5**, specifically the synthesized DHA adorning all six double bonds.

The syntheses of the PUFAs are, as for the MEL **5**, based on the acetylenic approach and rely on the copper coupling reactions as described before. The only real difference is the head-group that now is a simple ethyl ester derived from commercially available mono-yne carboxylic acids. The total synthesis of SDA from hept-6-ynoic acid is depicted in Scheme 5.18, the synthesis of the tri-yne tail piece **65** is depicted in Scheme 5.19, the total synthesis of EPA from hex-5-ynoic acid in Scheme 5.20 and the total synthesis of DHA from pent-4-ynoic acid is depicted in Scheme 5.21. Finally, Scheme 5.22 reveals the synthesis of a 16:4 EE, easily acquired from the di-ynes **73** and **19**.

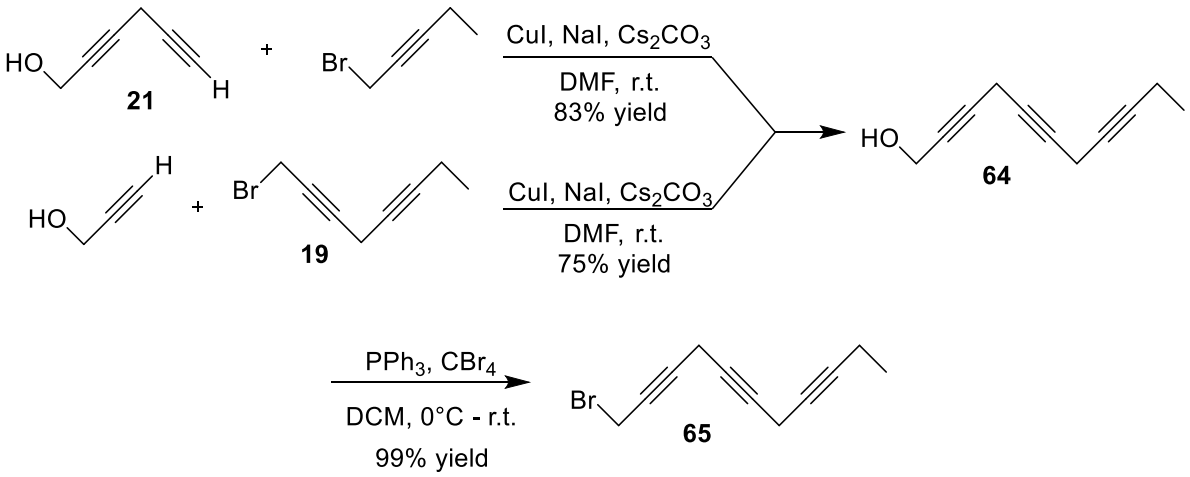
The starting mono-yne acid was chosen so that the number of carbons between the acid group and the triple bond corresponded to the natural target fatty acid. For SDA, the starting point was hept-6-ynoic acid which was esterified by ethanol via EDCI as a coupling agent and DMAP as a catalytic base, which afforded the mono-yne EE **59** in 96% yield. Coupling this product to a TMS protected propargyl bromide with the familiar copper coupling conditions afforded the TMS-protected di-yne **60** in 95% yield when isolated. The subsequent deprotection was accomplished with $AgNO_3$, affording the di-yne **61** in 88% yield over two steps, when using the crude di-yne **60**. One attempt was made to deprotect the TMS group under the same conditions as were successful for the di-yne intermediate **20** using TBAF and AcOH in dry THF. This reaction only afforded some unidentified byproduct and none of the desired compound **61**.



Scheme 5.18 Synthesis of the SDA EE **63**.

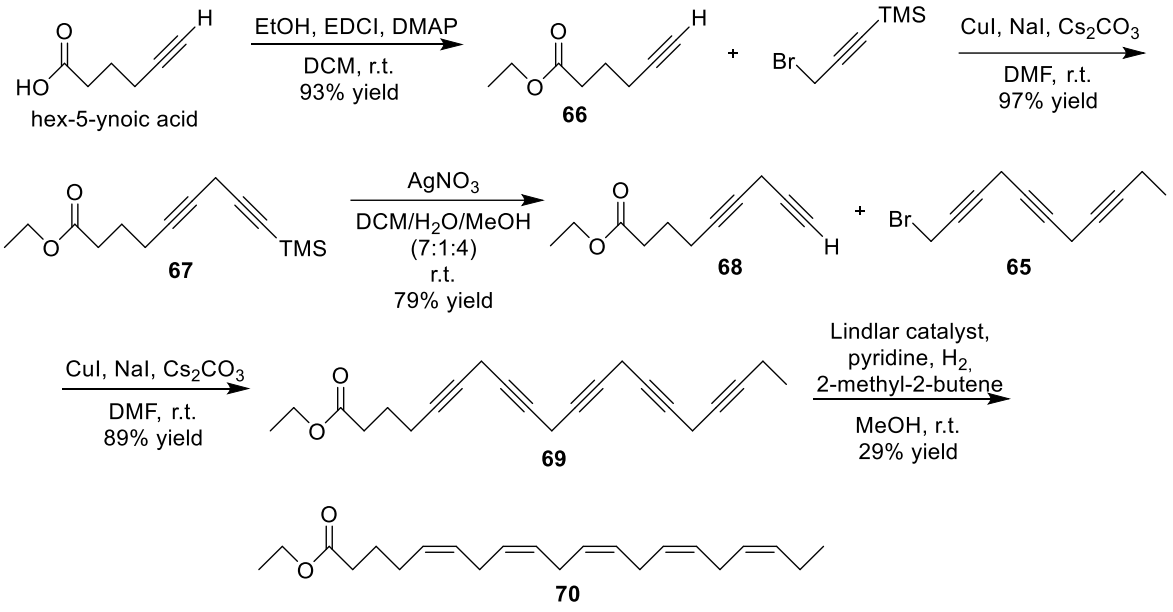
The deprotected di-yne **61** was then coupled to the appropriate tail part, which in this case was the already described di-yne **19**. This copper coupling afforded the tetra-yne **62** in 81% yield at best. Both the Lindlar and the Rosenmund catalyst afforded considerable byproducts when semi-hydrogenating the tetra-yne. The most favorable conditions were achieved using the Lindlar catalyst with 0.5 eq. quinoline in toluene. The temperature, either 0°C or r.t. did not seem to affect the outcome much. Thus, the crude product was afforded in 70% yield as a mixture with the semi-hydrogenation byproducts. This mixture was then applied to a 5% $AgNO_3$ impregnated preparative TLC and the pure SDA EE **63** was afforded in 55% yield off the plate, in all, 39% yield for this reaction. Accordingly, the SDA EE **63** was accomplished in 27% total yield over five steps as based on the hept-6-ynoic acid.

To accomplish the EPA EE **70** synthesis, a tri-yne tail part had to be synthesized. The synthetic route is depicted in Scheme 5.19. Using the already established di-ynes **19** and **21** the tri-yne **64** could be arrived at by two different routes. Either the coupling of **21** and bromopent-2-yne or the coupling of propargyl alcohol and **19**. The former coupling was achieved in 83% yield but the di-yne **21** had been achieved in 76% yield. The latter route was achieved in 75% yield (never optimized further) where the di-yne **19** had been afforded in quantitative yield. Total yields for the tri-yne **64** are thus 63% and 75%, respectively, making the latter route more optimal. The bromination of **64** was completed in an Appel reaction, affording the tri-yne **65** in 99% yield.



Scheme 5.19 Synthesis of the tri-yne **65** for the EPA EE synthesis.

The synthesis of the EPA EE **70** was completed essentially in the same way as for the SDA EE **63** as depicted in Scheme 5.20. The starting material, hex-5-ynoic acid was esterified, affording the mono-yne **66** in 93% yield. The following copper coupling to the TMS-protected propargyl bromide afforded the di-yne **67** in 97% yield and the subsequent deprotection afforded the di-yne **68** in 79% yield. The di-yne **68** was then coupled with the tri-yne **65**, affording the penta-yne **69** in 89% yield.

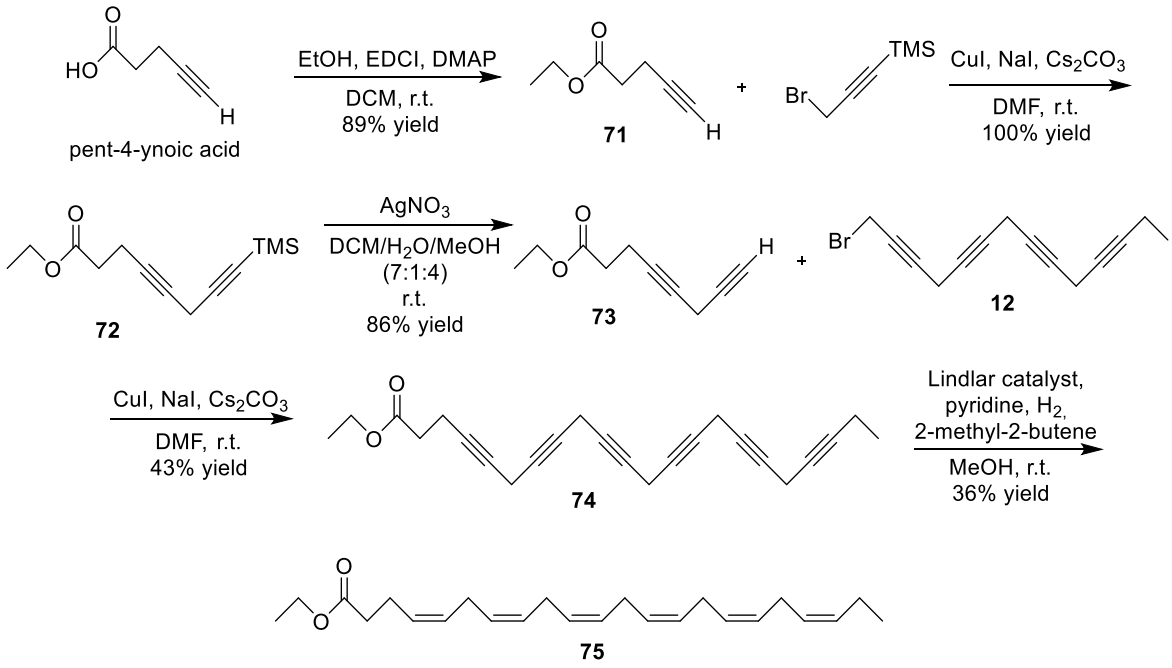


Scheme 5.20 Synthesis of the EPA EE **70**.

The final semi-hydrogenation step was attempted with the Lindlar, Rosenmund and Brown catalysts which all afforded considerable amounts of byproducts. The conditions which afforded the best outcome were the conditions developed by Hwang et al.[116] using the Lindlar catalyst in methanol and pyridine and 2-methyl-2-butene as additives. This reaction afforded the product in lower yield than the quinoline and toluene method and still had obvious signs of byproducts in their NMR and IR spectra but considerably lesser. The best

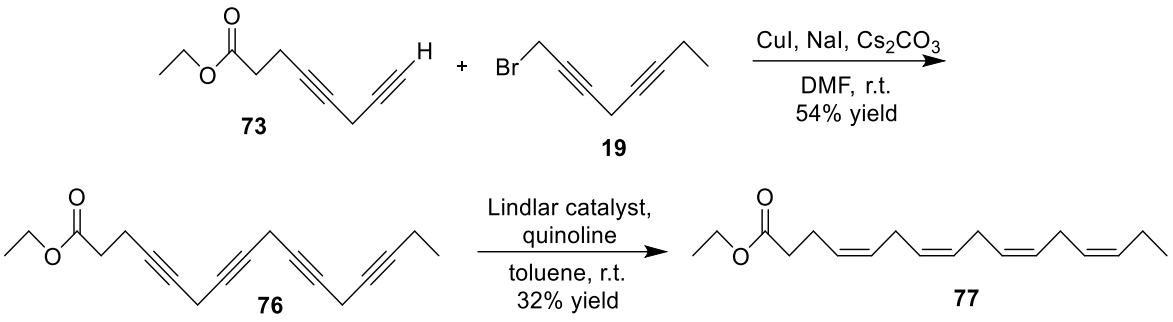
attempt afforded the crude product in 54% yield after normal silica gel chromatography and then the pure product in 54% yield off the 10% $AgNO_3$ impregnated preparative TLC plate. In all, 29% yield for this reaction. This synthesis was not attempted further but there was a considerable room for improvement as the semi-hydrogenation was very slow and was stopped before completion. Accordingly, the EPA EE **70** was afforded in 18% total yield over five steps as based on the hex-5-ynoic acid.

The DHA EE **75** was also essentially made in the same way as the others, as depicted in Scheme 5.21. The pent-4-ynoic acid was esterified, affording the mono-yne **71** which was rather volatile and could only be roughly concentrated. The coupling of **71** and TMS-protected propargyl bromide afforded the di-yne **72** in quantitative yields and the subsequent deprotection afforded the di-yne **73** in 86% yield. As for the DHA-like MEL **5** the tetra-yne **12** was needed to complete the poly-yne framework, and the copper coupling of **73** and **12** afforded the hexa-yne **74** in 43% yield. This reaction was not optimized further and could most likely be improved to the level of what was demonstrated for the hexa-yne **11**. The same conditions as for the EPA EE **70** proved to afford the best outcome for the semi-hydrogenation of **74** which afforded the crude product in 66% yield and then the pure DHA EE **75** was recovered in 54% yield off the 10% $AgNO_3$ impregnated preparative TLC plate. In all, 36% yield was obtained from the reaction. The DHA EE **75** was afforded in 12% total yield over five steps as based on the pent-4-ynoic acid.



Scheme 5.21 Synthesis of the DHA EE **75**.

Lastly the 16:4 EE **77** was synthesized in the same manner as before, using the di-ynes **19** and **73** to produce the tetra-yne **76** which was then semi-hydrogenated as can be seen in Scheme 5.22.



Scheme 5.22 Last two steps in the synthesis of the 16:4 EE **77**.

The tetra-yne **76** was made from left-over material from the DHA EE **75** synthesis, which could explain the average yield for the coupling reaction which was not optimized further. The 16:4 EE **77** was obtained in 79% yield from the semi-hydrogenation and the pure compound then recovered in 41% yield by argentation chromatography, in total 32%. This was also not optimized any further.

These synthesized PUFAs were obtained in excellent purity after argentation chromatography, except for some *trans* content in the case of **75**. The post semi-hydrogenation purity challenge is discussed in Chapter 6.2 and the *trans*-isomer evaluation and measurements are discussed in Chapter 6.3.

6 The main challenges

The main obstacles experienced during this project could be summarized into three main challenges. Firstly, the diastereomeric purity of the compounds; secondly, the application and optimization of the semi-hydrogenation reactions; finally, the measuring or estimation of *trans*-isomers in the final product.

Section 6.1 discusses the diastereomeric purity of compound **14** and the steps taken to address it.[2] Section 6.2 discusses the challenges of the stereoselective semi-hydrogenation of poly-ynes and details the trials and optimizations of the semi-hydrogenations of some of the di-, tri-, tetra-, penta-, and hexa-ynes performed for this project. Finally, Section 6.3 discusses the measurements of *trans*-isomer impurities in poly-enes, the difficulties involved, and the results achieved during this project.

6.1 The diastereomeric challenge

In their previously described synthesis of the MEL **1** Magnússon and Haraldsson had shown that the chiral configuration of the epichlorohydrin starting material was preserved completely as was confirmed by Mosher ester analysis.[64] The new synthetic strategy which was based around the diastereomeric head piece synthon **14** made directly from (*R*)-solketal (98% ee) and (*S*)-epichlorohydrin (97% ee) needed a similar approval of the stereochemistry being preserved. The mechanism for its creation was assumed to involve the nucleophilic attack of the deprotonated alcohol of the solketal exclusively on the epoxide methylene carbon. The resulting alkoxide then displaces the chloride intramolecularly, producing a new epoxide. The diastereomeric impurities in the product **14** were then expected, based on enantiopurity of the starting materials, to be around 2.5% (95% de) provided that the reaction took place exclusively on the epoxide methylene site rather than the chloride methylene group. Figure 6.1 depicts all four possible stereoisomers of **14** and their expected percentage in the product of the reaction of (*R*)-solketal and (*S*)-epichlorohydrin.

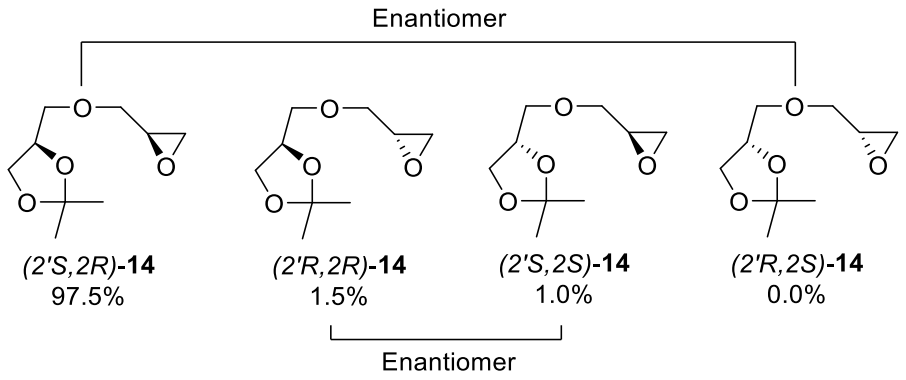


Figure 6.1 All possible stereoisomers of **14** and their respective percentage in the reaction product of (*R*)-solketal and (*S*)-epichlorohydrin.

Each set of enantiomers displayed identical ^{1}H-NMR spectra but the spectra between the diastereomers were slightly different. The peaks in the spectra of the diastereomers are mostly the same and overlap, except in the case of the methylene group protons belonging to the *sn*-1 position of the solketal, i.e. the one forming the ether linkage. They resonated as two separated doublets of doublets signals for one set of enantiomers (desired) and as a single doublet for the other set (undesired) and, importantly, did not overlap. This is illustrated in Figure 6.2. This difference was used to accurately integrate and determine the diastereomeric impurity.

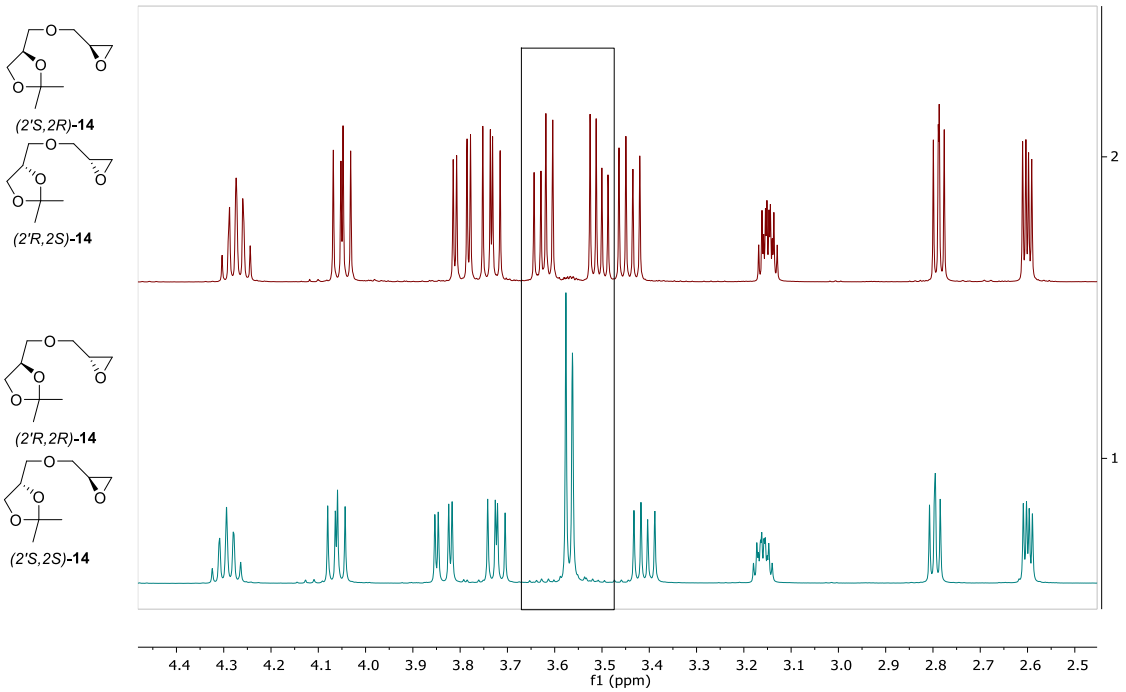


Figure 6.2 ^{1}H-NMR spectra of each set of enantiomers of the head piece synthon **14**, showing the almost identical spectra of the glycerol region, except for the part within the square. Small amount of diastereomeric impurity is visible in each spectrum.

The ratio of the diastereomers in each sample was calculated by using the integration of the diastereomeric peak versus the two flanking doublet of doublets peaks of the desired isomer. This way the amount of unwanted diastereomers (2'*R*,2*R*)-**14** and (2'*S*,2*S*)-**14** could be established in good accuracy comparable to the Mosher ester methods stated earlier. The unwanted (2'*R*,2*S*)-**14** enantiomer does remain invisible however, but it should only exist in trace amounts. The error in calculating the ratio increased slightly for very low amounts of diastereomers (<2%) and was complicated by the interfering signals in the baseline such as satellites of the peaks belonging to the desired isomer.

Unfortunately, the ^{1}H-NMR analysis revealed that the formation of **14** in the aforementioned reaction did not preserve the intended configuration completely. According to the ^{1}H-NMR there seemed to be at best around 4.5-6.5% of diastereomers present (sometimes more), which would be an increase of diastereomeric impurities of about 2-4% relevant to the expected value of 2.5%. This was not ideal for the integrity of the synthesis and kicked off considerable effort to identify the problem and reduce the diastereomeric impurities.

6.1.1 Identifying the problem

For the reaction of (*R*)-solketal (98% ee) and (*S*)-epichlorohydrin (97% ee) to form the key compound (2'*S*,2*R*)-**14**, the amounts of diastereomers (2'*R*,2*R*)-**14** and (2'*S*,2*S*)-**14** were expected to be 1.5% and 1.0% of the product, respectively (Figure 6.1). The enantiomer (2'*R*,2*S*)-**14** would only form when the small amount of impurities, the (*S*)-solketal and (*R*)-epichlorohydrin would react and therefore only in trace amounts. This only holds with the assumption that the alkoxide nucleophilic attack on the epichlorohydrin takes place exclusively on the epoxide methylene carbon. Attack on the chlorocarbon would afford the unwanted (2'*R*,2*R*)-**14** diastereomer. Another possibility would be the chemical racemization of the epichlorohydrin during the reaction, where bromide from the catalyst or chloride formed in the reaction would attack the epoxide. This would in turn intramolecularly displace the other chloride which would result in the opposite stereochemistry. This is illustrated in Figure 6.3.

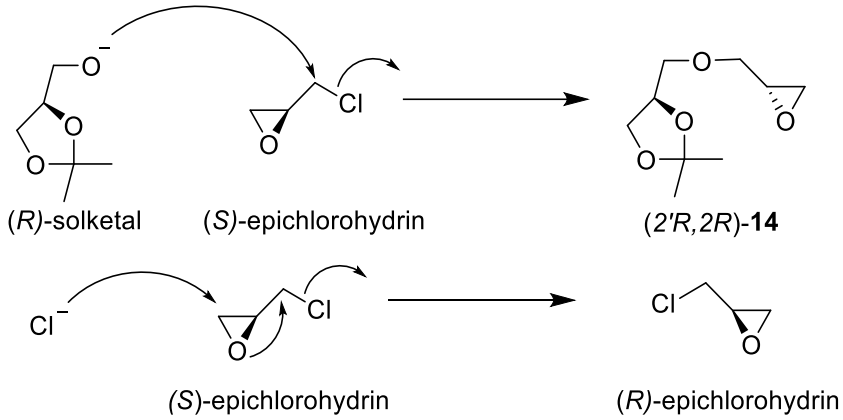


Figure 6.3 Possible pathways for the racemization of the head piece synthon **14**, affording the unwanted diastereomer (2'*R*,2*R*)-**14** or the unwanted substrate (*R*)-epichlorohydrin.

A few reaction conditions, listed in Table 6.1, were screened for this reaction in the attempt to both increase yields and identify factors that could influence the additional formation of diastereomers.

Table 6.1 Comparison of different reaction conditions affecting yield and diastereomeric ratio.

Base	Solvent	Epichloro-hydrin (eq)	Catalyst	Temp	Yield (%)	Diastereomer (%)
KOH	-	1	0.2eq TBAB	r.t.	49-53	4.5-6.5[a]
KOH	-	1	0.2eq TBAB	0°C	50	5
KOH	DMSO	1	0.2eq TBAB	r.t.	13	28
KOH	H_2O/DCM	1	0.2eq TBAB	r.t.	0	-
NaH	THF	1	DMAP	0°C	16	19
KOH	-	1	0.2eq TBAB BF_3*Et_2O	0°C	18	63
NaOH	H_2O	1	0.04eq TBAB	r.t.	34	3.5
NaOH	-	1	0.04eq TBAB	r.t.	33	4
NaOH	H_2O	1	0.4eq TBAB	r.t.	38	7
KOH	-	1.2	0.2eq TBAB	r.t.	60	5.6
KOH	-	1.5	0.2eq TBAB	r.t.	64	5.0
KOH	-	2	0.2eq TBAB	r.t.	69	4.8
KOH	-	1.5	0.04eq TBAB	0°C	61-67	3.6-4.3[a]
NaOH	H_2O/hexane	2	0.1eq TBAB	60°C	86	5.9
KOH	-	1.3	0.2eq TBAB	r.t.	56	3.3[b]
KOH	-	1.6	0.04eq TBAB	0°C	61	3.6[c]
KOH	-	1.6	0.04eq TBAB	0°C	63	3.8[d]

[a] Range over multiple reactions, [b] synthesis of the (2'*S*,2*R*)-**14** isomer, [c] (2'*R*,2*R*)-**14** isomer, [d] (2'*R*,2*R*)-**14** isomer.

Initially a few conditions were screened in the attempt to increase the yields. The use of DMSO and DCM as solvents and NaH as a base in THF gave very poor results. NaOH gave around 20% lower yield than KOH, both with and without water as solvent. Increasing the amount of TBAB, increased the yield inconsequentially (38%) but also increased the diastereomeric impurity. By then the highest yields were achieved with 1 eq of freshly ground KOH and 0.2 eq of TBAB mixed with the substrates under solvent-free conditions. These conditions afforded around 50% yield and diastereomeric impurity around 4.5-6.5%. As the pure (*R*)-solketal was more expensive, increasing the amount of (*S*)-epichlorohydrin to 1.5 eq in the reaction increased the yields, based on the solketal, to 64%. Increasing it further to 2 eq only marginally improved the yields (69%).

By lowering the temperature to 0°C the diastereomeric impurity seemed to be generally slightly lower than at room temperature but, more importantly, decreasing the amount of TBAB in the reaction to 0.04 eq reduced the impurity considerably, to around 3.6-4.3%. Without the phase-transfer/catalyst TBAB there was no reaction or very low yield. Using TBACl afforded similar results but a bit lower yield and $TBAHSO_4$ afforded much lower yield (46%). Another approach was then discovered which afforded higher yields. Adding *n*-hexane to the reaction, using 50% NaOH in water as the base and bringing the reaction to a reflux afforded **14** in typically 81-82% yield but the best results reached 86%. This came with the downside of increased diastereomeric impurity, around 6%.

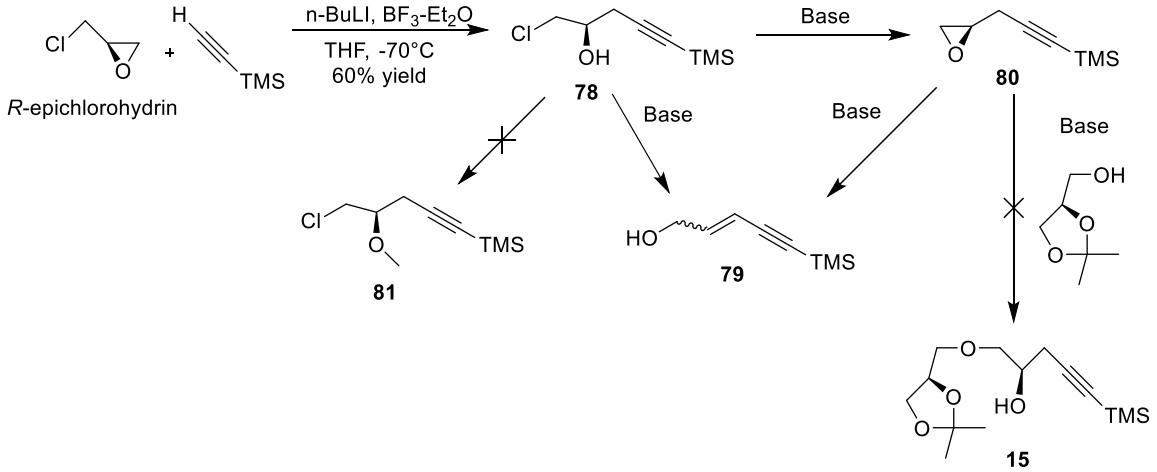
An attempt to estimate the amount of each isomer in the product was made using a chiral GC column and a synthesized standard of each stereoisomer. Only a partial separation was achieved. The diastereomers (2'*R*,2*R*)-**14** and (2'*S*,2*R*)-**14** had the same retention time and (2'*S*,2*S*)-**14** and (2'*R*,2*S*)-**14** as well. The method could only separate the isomers based on

the chirality of the solketal part of the molecule. The method could then not determine the amount of (2'*R*,2*R*)-**14** in the desired product, but it did indeed confirm that the combined amount of (2'*S*,2*S*)-**14** and (2'*R*,2*S*)-**14** proved to be around 0.8% when the expected maximum would be 1%. This confirmed the increased diastereomeric impurity in the product to be caused by increased amount of the (2'*R*,2*R*)-**14** isomer only as had been anticipated.

Lastly, limited attempts to separate the diastereomers of **14** or the alcohol and methoxy derivatives **15** and **16** on HPLC and chiral-HPLC were unsuccessful. A more exhaustive screening of HPLC-columns could reveal a preparative method which would isolate the desired isomer.

6.1.2 Attempts at alternative synthesis

To tackle the diastereomer problem an alternative synthesis was attempted where the dissatisfying head piece **14** reaction was bypassed. The various attempts are shown in Scheme 6.1. Opening the epoxide on epichlorohydrin with a TMS-protected lithium acetylide with BF_3 in dry THF gave the chlorohydrin **78** in 60% yield which is a structural analogue of the mono-yne **4.2** formed in the old MEL **1** synthesis. The coupling of the solketal to that structure proved very problematic, just as Magnússon and Haraldsson had observed before.[64] When attempting to couple chlorohydrin mono-yne **78** to the solketal, only small amount of the coupled product was formed in a mixture with the enynol **79**.



Scheme 6.1 The intermediates and byproducts in the attempted alternative head piece synthesis.

The basic conditions needed to couple the units together would produce the enynol **79** in high yield and virtually none of the desired product was detected. The solution of Magnússon and Haraldsson of hydrogenating the triple bond was not an option in this instance since that would destroy the prerequisites for the copper coupling reaction. Converting the chlorohydrin to the epoxide **80** which would then be reacted with the potassium salt of solketal to accomplish the solketal alcohol ether **15** was attempted. Various basic conditions were screened. Potassium hydroxide in diethyl ether would only afford 30% of the epoxide **80**, but stochiometric amount of t-BuOK afforded the epoxide **80** without formation of **79**. n-BuLi, EtMgBr, NaH and DBU all yielded either small fraction of the epoxide mixed with byproducts or left most of the chlorohydrin unreacted. Unfortunately, all attempts aiming at coupling the chlorohydrin **78** or epoxide **80** to the

solketal or the potassium salt of the solketal were unsuccessful. Using titanium isopropoxide as an epoxide activator also gave no reaction with potassium solketal salt.[204]

Attempts to methylate the hydroxyl group on **78** to form the methyl ether **81** which would then be unable to form the epoxide **80** and therefore the side-product **79** were unsuccessful as well. Basic conditions only gave the side-product **79**, BF_3 added gave no reaction as did Ag_2O with MeI in toluene. The methylated compound **81** would also most likely not be reactive towards the solketal as was the case for the structural analogue for the MEL **1** demonstrated by Magnússon and Haraldsson. No further attempts at an alternative head synthesis were made but the focus instead put on kinetically resolving the diastereomers.

6.1.3 Hydrolytic kinetic resolution (HKR)

Another approach to address this problem was to use kinetic resolution to separate the diastereomers. The term kinetic resolution usually applies when a chiral catalyst or reagent reacts with enantiomers at different reaction rates which then results in an enantiomerically enriched product. The compounds at issue are diastereomeric so only one diastereomer was affected using the methods described below, which were based on a lipase biocatalyst and a chemical catalyst. Since the observed flaw in the diastereomeric purity was only because of the losses in enantiocontrol on the epoxide site of **14**, it was anticipated that by targeting the epoxide or its alcohol derivative a kinetic resolution of the two diastereomers (2'*R*,2*R*)-**14** and (2'*S*,2*R*)-**14** or their corresponding alcohol derivatives should be possible. That would leave only the desired product (2'*S*,2*R*)-**14** and the (2'*S*,2*S*)-**14** diastereomer in an amount corresponding to the enantiomeric purity of the solketal starting material, under 1%.

The first attempt at kinetic resolution was to use enzymes. Lipases have been used to kinetically resolve enantiomers of a whole variety of substrates.[205] The lipase acylates alcohol stereoisomers with an acylating agent of choice with different reaction rates. The unreacted alcohol isomer and the acylated one should then be easily separated by chromatography yielding enantiomerically enriched products. This is illustrated in Figure 6.4 for the TMS-protected mono-yne alcohol **15**.

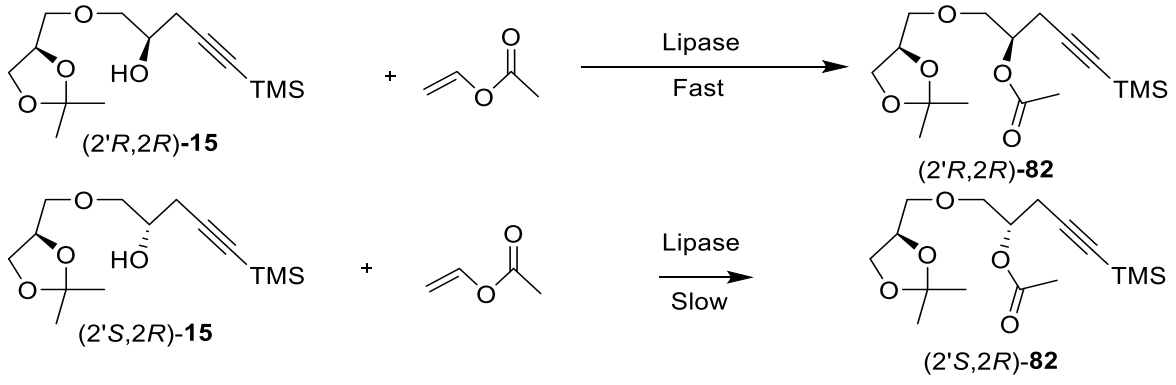


Figure 6.4 Kinetic resolution of **15** using lipase.

The TMS-acetylene alcohol diastereomers (2'*R*,2*R*)- and (2'*S*,2*R*)-**15** were used as substrates and screened against six different lipases using vinyl acetate as the acylating

agent. Two of the lipases (*Pseudomonas sp.* and *Pseudomonas fluorescens* from Amano) showed activity for the substrates as well as reacting the (2'*R*,2*R*)-**15** at a faster rate. One of the lipases (*Pseudomonas fluorescens*) was used on a substrate with 5.2% diastereomeric impurity and after 6 hours and about 50% conversion the product **82** showed 3.5% diastereomeric impurity. These preliminary results were promising but not pursued any further since the focus shifted towards a chemical catalyst which was very efficient.

The second attempt on kinetic resolution, this time focusing on the key head synthon **14**, was to attempt hydrolytic kinetic resolution (HKR) catalyzed by the chiral cobalt(III) salen complex (Co-Jacobsen catalyst) (see Figure 6.5). This complex discovered in the late 1990s has risen to prominence in the last 15-20 years as a highly selective hydrolytic kinetic resolving catalyst for terminal epoxides.[206] The catalyst, under very mild conditions stereoselectively catalyzes terminal epoxides to react with water to form the corresponding 1,2-diols. The (*S,S*)-Co-Jacobsen catalyst would then react only the problematic (2'*R*,2*R*)-**14** (as well as the enantiomer (2'*R*,2*S*)-**14** which should only exist in trace amounts) producing the diol **83**. The desired (2'*S*,2*R*)-**14** isomer would be unaffected as well as the other unwanted (2'*S*,2*S*)-**14** diastereomer. This is illustrated in Figure 6.5.

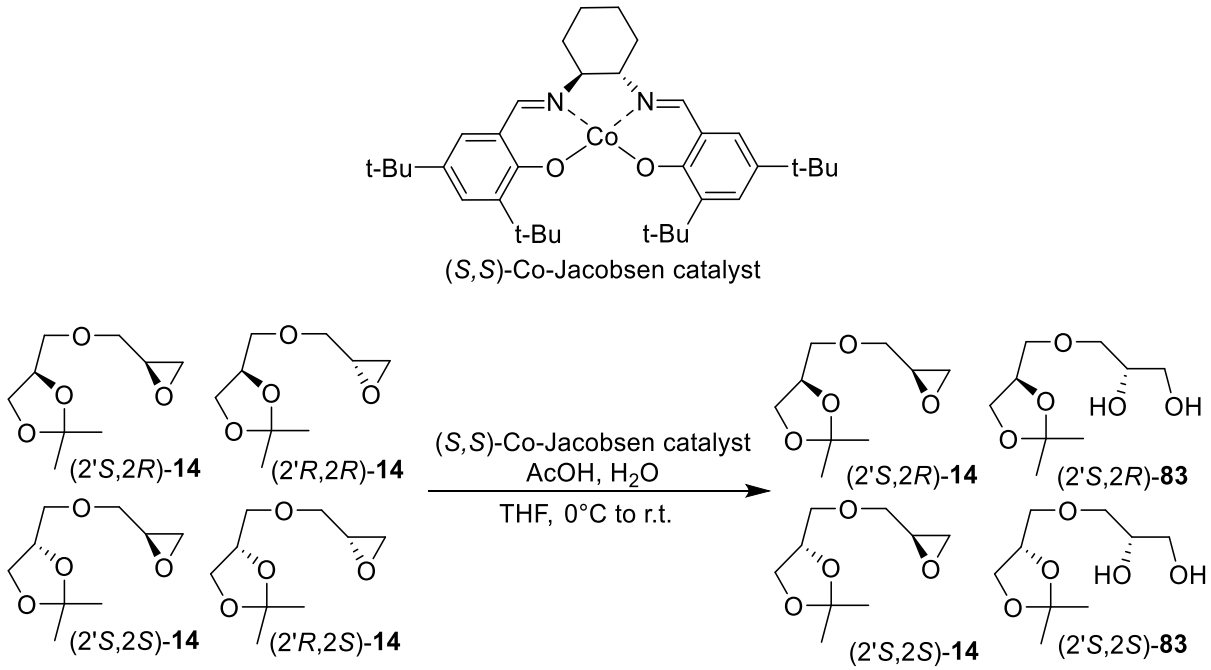
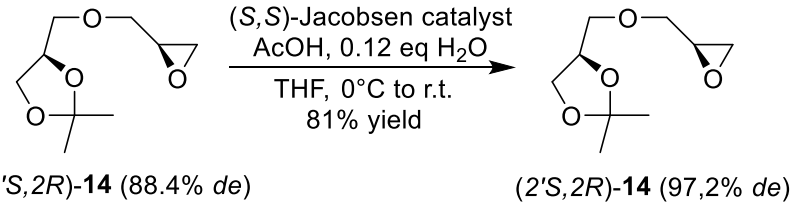


Figure 6.5 The cobalt (*S,S*)-Jacobsen catalyzed kinetic resolution of the key head piece synthon **14**, depicting all four isomers.

This method proved successful in removing the unwanted (2'*R*,2*R*) diastereomer. A typical procedure went as follows: The red catalyst was first dissolved in toluene and activated by the addition of acetic acid. The solution was stirred for a few hours exposed to the air until dark brown. The solvent was then removed and the brown solid re-dissolved in dry THF and cooled to around 0°C. Then the key head piece synthon **14** with diastereomeric impurity of 5.8% was added along with 0.12 equivalents of water and the resulting solution then stirred overnight. This afforded after chromatography the (2'*S*,2*R*)-**14** in 81% yield

with diastereomeric impurity that had dropped to 1.4%. This reaction is depicted in Scheme 6.2.

The catalyst did not affect the (2'*S*,2*S*) diastereomer, which amounted to around 1% and the maximum diastereomeric excess achievable then around 98%. These results showed that the key head piece synthon **14** could be achieved in at least 97.2% *de*, which is better than what was originally expected from the synthesis which was 95% *de*.

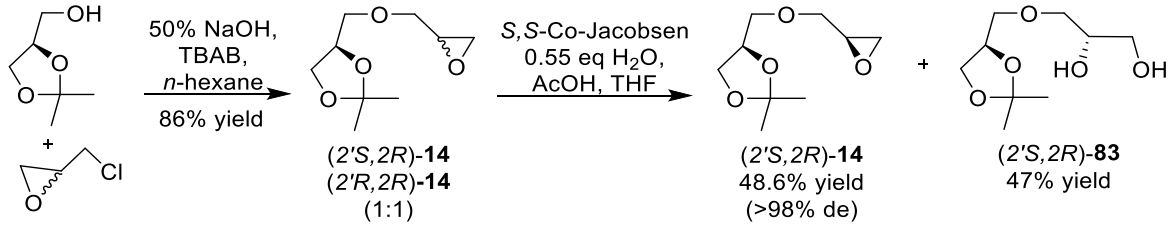


Scheme 6.2 Hydrolytic kinetic resolution of the head piece (2'*S*,2*R*)-**14** synthesized from two chiral starting materials, using the Co-Jacobsen catalyst.

The head piece synthesis at this point offered **14** in 97.2% *de* using the two expensive chiral starting materials (*R*)-solketal and (*S*)-epichlorohydrin. The coupling reaction and the subsequent HKR were afforded in 86% and 81% yields and two equivalents of the (*S*)-epichlorohydrin were used. This resulted in around 70% of the (*R*)-solketal and only 35% of the (*S*)-epichlorohydrin were being utilized.

6.1.4 HKR on diastereomers from racemic epichlorohydrin

As the key head synthon **14** was being kinetically resolved so efficiently by the catalyst the question arouse if it could not be done in a more economical fashion by using racemic epichlorohydrin. (*R*)-solketal and *rac*-epichlorohydrin were then submitted to the same coupling reaction as for the enantiopure compounds which then afforded a 50/50 mixture of (2'*S*,2*R*)-**14** and (2'*R*,2*R*)-**14**. This reaction and the following HKR are depicted in Scheme 6.3.

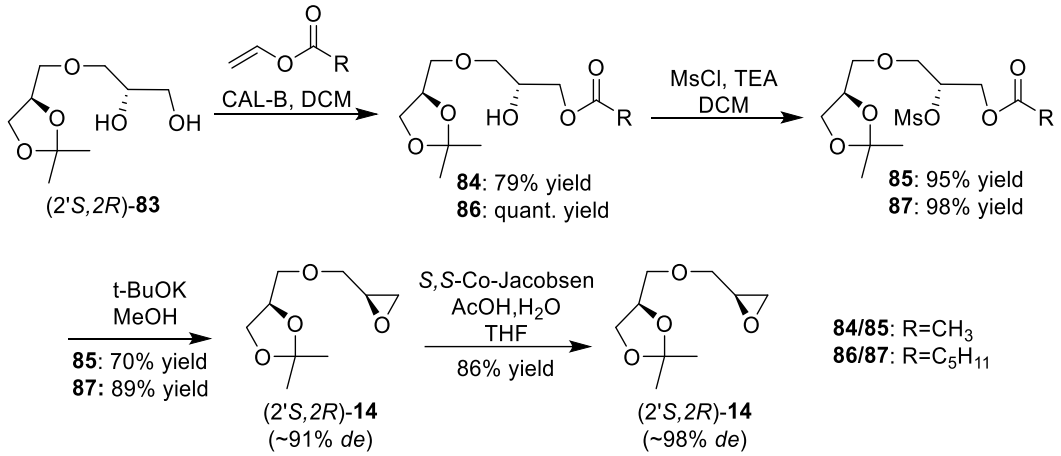


Scheme 6.3 The 50/50 mixture of the (2'*S*,2*R*) and (2'*R*,2*R*)-isomers of **14** kinetically resolved by the Co-Jacobsen catalyst affording the almost pure (2'*S*,2*R*)-isomer and the diol co-product.[2]

The Co-Jacobsen kinetic resolution of the (2'*S*,2*R*)-**14** and (2'*R*,2*R*)-**14** (1:1) mixture afforded the unreacted (2'*S*,2*R*)-**14** isomer in excellent yield and diastereomeric purity, less than 1% of diastereomers as estimated from the ^{1}H-NMR spectrum which was the theoretical minimum. The diol byproduct (2'*S*,2*R*)-**83** was also isolated in excellent yield. In this manner, 42% of the *R*-solketal would end up in the product while another 40% of the expensive chiral starting material ended up as the diol byproduct **83**. A strategy was then formulated to reclaim the solketal fraction that ended up in the diol.

6.1.5 Inversion of configuration of co-product by use of HKR

Pehma et al.[65] demonstrated in 2012 how they could take a diol co-product from a Co-Jacobsen kinetic resolution reaction and have it converted back to an epoxide while also inverting its configuration. A similar strategy was then developed and carried out for the diol **83** and those results are detailed in Scheme 6.4.[2]



Scheme 6.4 The epoxidation of the diol byproduct (2'S,2*R*)-**83** and the simultaneous inversion of configuration, resulting in the desired (2'S,2*R*)-**14**.[2]

The diol **83** was acylated with both vinyl acetate and hexanoate using the *Candida antarctica* lipase B (CAL-B) affording the alcohols **84** and **86** in 79% and quantitative yields, respectively. That lipase is known to offer superb regioselectivity towards the primary alcohol groups in various glycerol derivatives.[207, 208] The alcohols **84** and **86** were both mesylated, using mesyl chloride and triethylamine as a base in DCM. This afforded the acyl mesylates **85** and **87** in 95% and 98% yield after purification on a silica gel column. The next step, the important closure of the epoxide and the resulting inversion of configuration was accomplished by using potassium tert-butoxide as the base in methanol. This reaction initially afforded the desired (2'*S*,2*R*)-**14** in only 70% yield for both acyl mesylates **85** and **87**, but by using the base freshly isolated, 89% yields were afforded using the hexanoate mesylate **87** (not optimized further for **85**). At this point the product had around 4.5% diastereomeric impurities which should reflect the purity of the starting diol **83**. The product was therefore submitted to a second HKR reaction and this afforded the (2'*S*,2*R*)-**14** in 86% yield with around 1% diastereomeric impurity.

This simple addition to the synthesis therefore afforded the (2'*S*,2*R*)-**14** in 72% yield as based on the use of the (*R*)-solketal as the sole chiral precursor. This significantly improved the efficient use of the chiral starting materials of the original approach, which was based on the use of two enantiopure starting materials of which 70% of (*R*)-solketal and 35% of (*S*)-epichlorohydrin accrued in the afforded head piece **14**.

6.1.6 Use of HKR on racemic starting materials

A final approach using no enantiopure starting materials was then theorized. Noticing a certain symmetry in the head piece **14** a novel approach using diglycidyl ether **88** was

developed. The diglycidyl ether mixture should be composed of three stereoisomers as shown in Figure 6.6.

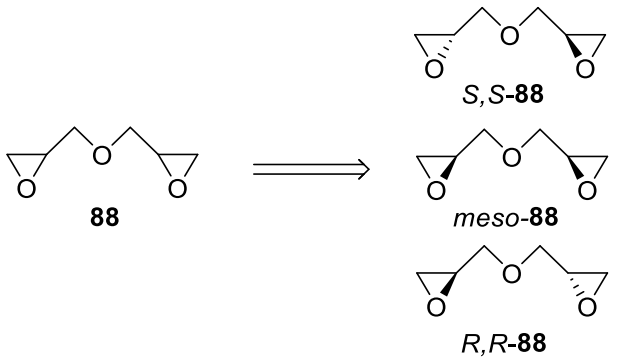


Figure 6.6 The diglycidyl ether **88** mixture split into its three stereoisomeric components.

By subjecting the diglycidyl ether **88** stereoisomer mixture to a Co-Jacobsen catalyzed reaction a mixture of products, depicted in Figure 6.7, should be afforded. By using the *S,S*-Co-Jacobsen catalyst the product corresponding to the *meso*-compound would be the desired deprotected derivative of (2'*S*,2*R*)-**14**, the diol *S,S*-**89**. It should be easily separated from its co-products, the unreacted *S,S*-**88** and the tetraol *S,S*-**90** obtained from the *R,R*-**88** enantiomer. This method, if successful, would establish both stereocenters in the key head piece synthon **14** without the use of expensive enantiopure starting materials.

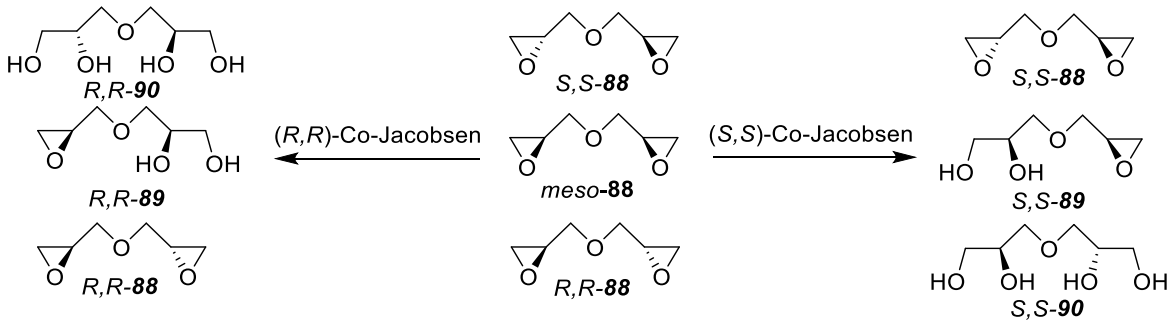
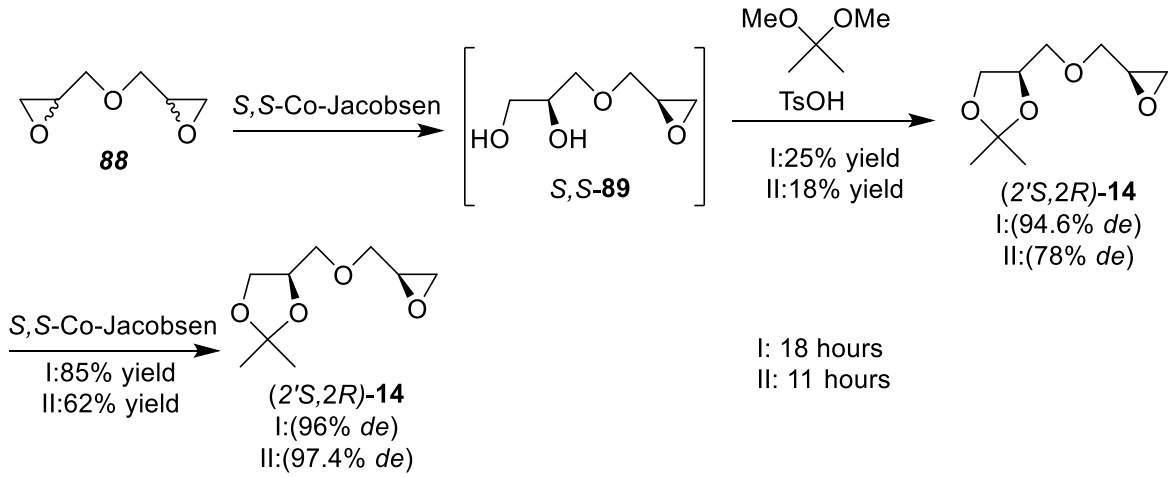


Figure 6.7 All stereoisomers of diglycidyl ether **88** and their expected products from a hydrolytic kinetic resolution reaction by the Co-Jacobsen catalyst.

The diglycidyl ether can be obtained by commercial means or for example by coupling of epichlorohydrin and allyl alcohol which product is then epoxidized via m-CPBA.

The diglycidyl ether **88** mixture was reacted with 0.9-0.95 equivalents of water in the presence of *S,S*-Jacobsen catalyst. The solvent was evaporated, and the crude reaction mixture applied to a silica column chromatography where the three products were easily separated. The middle compound, second off the column was the desired diol *S,S*-**89** which was then directly acetal protected in the presence of 2,2-dimethoxypropan, catalyzed by p-toluenesulfonic acid to afford, after short work-up and a silica column treatment the desired (2'*S*,2*R*)-**14**. This is depicted in Scheme 6.5.



Scheme 6.5 The synthesis of enantiopure (2'*S*,2*R*)-**14** from non-enantiopure starting material, the diglycidyl ether **88**.

Somewhat different results were achieved based on the reaction time of the original HKR reaction. When allowed to react for 18 hours with the catalyst, the (2'*S*,2*R*)-**14** was afforded in 25% yield, after acetal protection, with around 2.7% diastereomer present (94.6% *de*), similar to what was expected of the original synthesis. This product was then subjected to a second Co-Jacobsen reaction, which afforded (2'*S*,2*R*)-**14** in 85% yield and had 2.0% diastereomeric impurity (96% *de*). This is marginally better than the original synthesis expected and could not be improved further by subjecting it to another HKR reaction with the catalyst. When the diglycidyl ether **88** was reacted for 11 hours with the catalyst, (2'*S*,2*R*)-**14** was afforded in 18% yield with 11% diastereomeric impurity present (78% *de*). This product was then subjected to a second Co-Jacobsen reaction, yielding (2'*S*,2*R*)-**14** in 62% yield with diastereomeric impurity of 1.3% (97.4% *de*) which was similar to the purity achieved when using both starting materials enantiopure.

These results could probably be improved by further optimization, but some balance might have to be reached in the first Co-Jacobsen reaction if increased amount of the (2'*S*,2*S*)-**14** isomer is being formed, since it can then not be removed by further Co-Jacobsen reactions. This alternative method based on no enantiopure starting materials afforded (2'*S*,*2R*)-**14** in 11% yield at 97.4% de and in 21% yield at 96% de as based on the diglycidyl ether starting material **88**.

6.2 The Poly-yne semi-hydrogenation challenge

A lot of work went into optimization of the semi-hydrogenations of skipped poly-ynes for this project. One of the major issues was dealing with the instability of the skipped poly-ynes and large effort was put into streamlining the synthesis, work-up and purification of these compounds to minimize the time until they were submitted to the semi-hydrogenation. The other major issue concerned the reproducibility of the semi-hydrogenations. Finding the right catalyst and then the right reaction conditions could be a daunting task, especially since the large poly-yne substrates were so time-sensitive and needed considerable effort to make.

The most obvious candidate for the semi-hydrogenations of skipped poly-ynes was the Lindlar catalyst. The Lindlar catalyst is universally most known and is the only catalyst that has been used on penta- and hexa-ynes in the literature. The prominence of the Brown catalyst in recent years and many authors stating its superiority to the Lindlar catalyst made it also an attractive choice for these experiments. The Rosenmund catalyst has not seen as much success but has been used in a few cases. The sometimes wildly different results reported in the literature as well as the non-reporting of purification steps or even reaction conditions as well as uncertainty regarding the final purity of the reported semi-hydrogenation products made it very difficult to strategically review the semi-hydrogenations of large, skipped poly-ynes to make conclusions on the best conditions. Therefore, during this work many experiments were done to try to hone in on the optimal conditions for the poly-yne substrates involved in this project. It is not at all obvious what conditions or catalyst should be superior, and in the effort of pursuing the best conditions one should bear in mind Paul Rylander's words back in 1967 in his book on catalytic hydrogenation.[209]

> "The successful outcome of catalytic hydrogenation depends upon the propitious choice of a number of factors. Foremost among these is the proper choice of a catalyst, a choice most easily made by uncovering a suitable precedent. Other factors that include temperature, pressure, agitation, amount of catalyst, mode of addition, and solvent may also have a decisive influence. Fortunately, acceptable results can often be obtained throughout such a wide range of conditions that, unless there is a need to optimize a process, little attention need be given to these factors. But when the most appropriate catalyst is already in use and the reduction still fails in some respect, it is only through a change in process conditions that a satisfactory result can be obtained. An unavoidable complication in an examination of the effect of process variables on the outcome of a reduction is the fact that "the most appropriate catalyst" is not necessarily invariable but may itself change with a change in process conditions."

Rylander's words succinctly describe one of the main issues with this project. The desired skipped poly-ene products could be produced by different catalysts and conditions but in almost all cases, considerable amounts of byproducts were produced and in some cases only a small amount of the actual desired compound was produced. The challenge was then to find the most optimized conditions, a pursuit which was quickly made difficult by the number of parameters to consider and reproducibility problems. This chapter will give brief overview of the process which cumulated in the final products described earlier in Chapters 4 and 5.

The main semi-hydrogenation byproducts are the molecules where one or more of the carbon-carbon double bonds in the desired skipped all-*cis* poly-ene framework have either undergone further hydrogenation to an alkane or isomerized into the *trans*-configuration, affording either the over-hydrogenated or *trans*-isomer byproduct. The occurrence of these byproducts is usually very low or not observed at all for mono-yne and sometimes di-yne substrates for both the Lindlar and Brown catalysts. The problem arises when semi-hydrogenating larger skipped poly-yne structures. Generally, the triple bond adsorption rate to the catalysts is much higher than that for the double bond, which makes these catalysts successful at semi-hydrogenation. When double bonds are adsorbed to the catalyst, they can undergo further hydrogenation or isomerization leading to the byproducts.

When a structure of a compound has multiple triple bonds, one can imagine that the substrate itself stays adsorbed to the catalyst more often or for a longer period of time. This must mean increased proximity of other double bonds present in the structure to the catalyst which could increase their adsorption to the catalyst which would in turn increase the prospect of their isomerization or full hydrogenation. Thus, the more triple bonds present in a compound, the more efficient the catalysts could become at hydrogenating double bonds and the amounts of byproducts would rise in relation with the number of triple bonds in the substrate. This relation was noticed during this work, where all poly-yne semi-hydrogenation reactions involving substrates larger than di-ynes produced noticeable amounts of byproducts that also increased with increasing number of triple bonds. The poly-yne compounds under investigation during this work were all skipped and maybe that has a bearing on these results as compounds with multiple isolated triple bonds could behave differently.

The byproducts produced in poly-yne semi-hydrogenations have very similar chromatographic properties as the corresponding desired skipped poly-ene compounds and are difficult to separate except with argentation chromatography. The over-hydrogenated byproducts could be estimated from the ^{1}H-NMR spectrum of the semi-hydrogenation reaction product. The signs of over-hydrogenation are the decreased integration of the vinyl protons around 5.2-5.5 ppm and of the methylene protons around 2.8 ppm. The spectrum will also show an increase in the integration of the areas around 1.3 ppm and 0.9 ppm indicating a saturated chain. In the case of the omega-3 substrates a triplet signal indicating an omega-6 framework would appear just up-field of the omega-3 signal as well. This is illustrated in Figure 6.8.

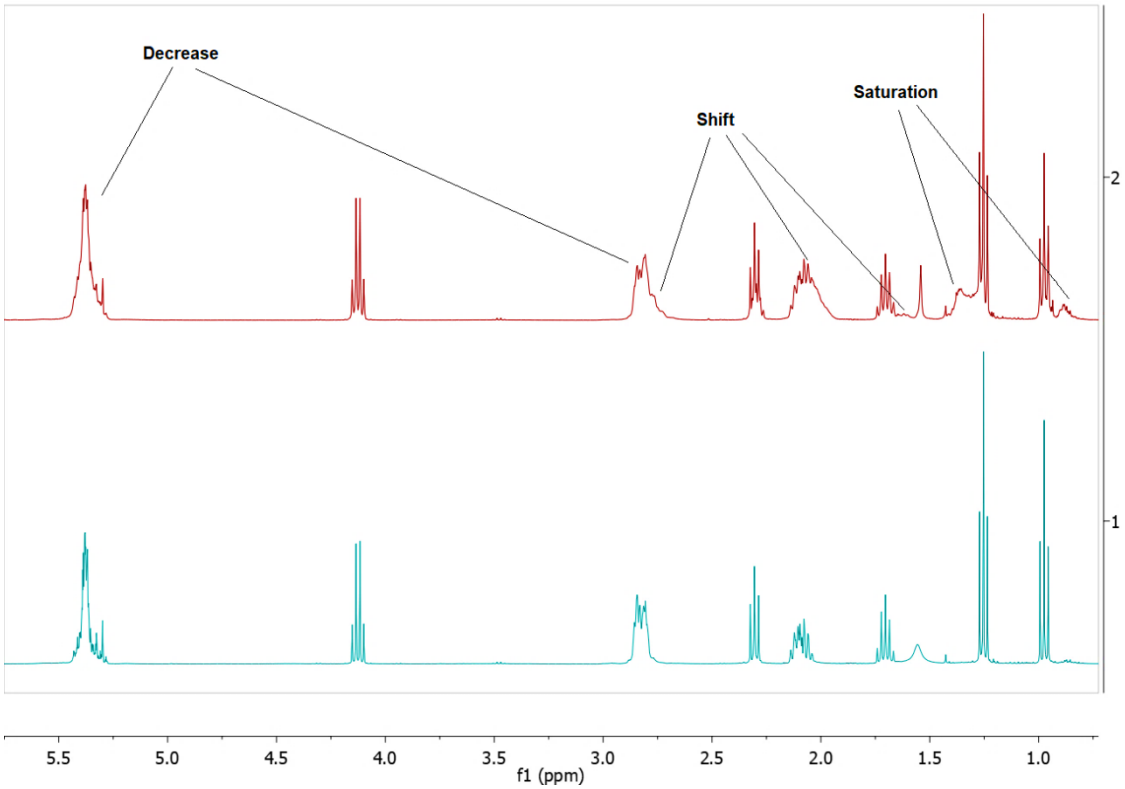


Figure 6.8 ^{1}H-NMR spectra of synthesized EPA EE **70** after semi-hydrogenation via the Lindlar catalyst before (top) and after (bottom) argentation chromatography. The top spectrum shows all the obvious signs of over-hydrogenation.

The amount of *trans*-isomer impurities was harder to measure, but the distinct C-H out of plane bend for *trans*-double bonds at 966-969 cm^{-1} could be used to roughly estimate the amounts of *trans*-isomers and compare products before and after argentation chromatography.

6.2.1 Catalyst explorations – general conditions

When pursuing the right catalyst and conditions for this work, it became apparent that the instability of the poly-ynes was affecting the results. When hydrogenating tri-, tetra-, penta- or hexa-ynes the substrates had to be as fresh as possible. This introduced a considerable inconvenience, since storing them was quite difficult or impossible. The substrates had to be synthesized and then immediately hydrogenated. This made the exploration of conditions and catalysts quite cumbersome. The preparation of tetra-ynes was considerably easier than penta- or hexa-ynes as well as the substrates being more stable. Much of the optimization was therefore performed on the tetra-yne **62** precursor to the SDA EE **63** initially, and then on the tetra-yne **49** precursor to the MEL **10** and those optimized procedures then carried out on the more unsaturated compounds.

It was revealed early on that when switching to a new bottle/batch of the Lindlar catalyst, worse results were obtained under the same conditions and therefore immediately clear that reproducibility could be a major issue. So, in addition to the different reaction conditions, a few different batches and manufacturers of the Lindlar catalyst were tested. The success of

a particular reaction was assessed mainly on the measured yields and the calculated amount of over-hydrogenation based on the ^{1}H-NMR spectrum.

Concerning agitation, good results were obtained from semi-hydrogenations conducted in a PARR hydrogenation reactor, but no discernable difference was noticed between The PARR reactor and magnetic stirring in round-bottom flasks sealed with septa mounted with hydrogen filled balloons. The PARR reactor was quickly replaced by the much more easily managed magnetic stirring. The reactions could then also be easily monitored closely by TLC, which made the reaction completion easy to detect and eliminated troublesome estimations of reaction times in the PARR reactor.

Large number of semi-hydrogenation trials were performed to explore the best reaction conditions for the poly-ynes. The tetra-yne **62** was used initially in these trials and the samples being fresh and not allowed to deteriorate in storage was imperative to obtain reliable results. The Lindlar catalyst was the main catalyst candidate, and many different parameters were explored, such as solvent, temperature, catalyst loading, catalyst poisons and catalyst poison ratio. It was also clear that the catalyst itself sometimes behaved differently, when compared between manufacturers or even between different batches.

Using no quinoline gave overwhelmingly over-hydrogenated products. The apparent benefit of quinoline was maximized around 0.5 equivalents, less of it only increased over-hydrogenation and more did not generally give any added benefit. Using more than 1 equivalents of quinoline increased reaction times or in a few cases completely stopped the reactions. A reaction performed in methanol with quinoline afforded an immensely over-saturated product. Benzene, THF, toluene and diethyl ether all gave satisfying results. Cooling the reactions to 0°C did not seem to affect the reactions much on small scale, except to increase the reaction times and sometimes offered lowered vinyl integration.

Using additional additives, such as cyclohexene and 2-methyl-2-butene gave mixed results based on which catalyst was used. Using cyclohexene with the Lindlar **III** catalyst in toluene did not have any effect. Cyclohexene with the Lindlar **I** catalyst in toluene retarded the reaction but in THF it had no discernible effect and the reaction completed smoothly with marginally lesser over-hydrogenation. This combined with the noticeable drop in product quality after switching between batches from Lindlar **I** to Lindlar **II** and the wildly different reaction times between some catalysts (15 minutes to 5 hours for the same substrate and conditions) prompted some further inquiries to establish the best catalyst for the semi-hydrogenations of the larger poly-ynes. Other authors have reported similar observations with the Lindlar catalyst, bad reproducibility both in house as well as between groups.

In organic synthesis of poly-ene compounds, many authors have ignored the semi-hydrogenation byproducts while others have used insufficient methods to measure them or separate from their desired product. The products of a skipped poly-yne semi-hydrogenation have very similar chromatographic properties and can be very difficult to separate, especially *trans*-isomers. Some success has been described using RP-HPLC to remove over-hydrogenated products, but the efficiency of such methods is still unclear. No data has been put forward about RP-HPLC capability to separate *trans*-isomers of large poly-enes. One method has proved to be very effective in separating all-*cis* compounds from their over-hydrogenated and *trans*-isomer byproducts and that is argentation or silver ion (Ag^{+}) chromatography.

6.2.2 Argentation chromatography

Argentation chromatography started to appear in the literature in 1961[210]-1962[211, 212] to separate molecules based on their level of unsaturation and stereochemistry. The ability of silver ions to form weak charge-transfer π-complexes with carbon-carbon double bonds makes it possible to separate compounds according to their degree of unsaturation. They also bond more strongly to *cis*-double bonds than *trans*, making the separation of stereoisomers possible as well. Already in the 1960's, chemists were using countercurrent distribution methods[213], silica gel thin-layer chromatography[212] and silica gel column chromatography[211] with silver ions to separate multitudes of compounds, with an explosion of publications detailing its use. Later, HPLC methods using silver ion impregnated stationary phases have become widely reported within lipid research.[214]

Seemingly this technique saw less and less use with time except within dedicated lipid research groups. Many comprehensive reviews have been written, such as Morris in 1966,[215] oldest but still relevant and others more recent.[216, 217] One review[218] explicitly with the aim to revive awareness for the technique, which might be warranted as has been discussed in Chapter 3 and will be detailed further below.

In fat and oil industry as well as lipid research argentation chromatography has been used successfully for numerous applications such as isolation of both EPA and DHA from oil in high purity, high yield recovery of high purity EPA esters from algae and fish oil[219] and separating trans derivatives of EPA and DHA after isomerization reactions with p-toluenesulfinic acid to name a few.[220]

Over-hydrogenated products and *trans*-isomers are prevalent byproducts in semi-hydrogenation reactions of alkynes, specifically enhanced in the hydrogenation of poly-ynes. Argentation chromatography has been used to effectively separate desired all-*cis* poly-ene compounds from their semi-hydrogenation byproducts. But the reported organic syntheses of all-*cis* skipped poly-ene systems by semi-hydrogenation of the corresponding poly-ynes in the literature, has mostly seen this technique absent. Only a handful of authors have described its use for the purification of their semi-hydrogenation products.

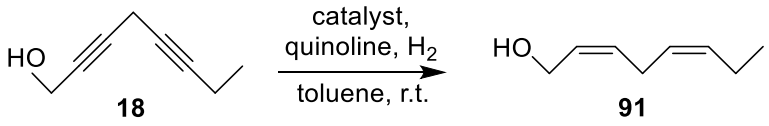
Kerdesky et al.[127] in 1985 described the semi-hydrogenation of a skipped tri-yne in 94% yield and then stated that argentation chromatography can readily remove over and under hydrogenated byproducts. Demin et al.[221] in 1994 reported the hydrogenation of a tri-yne using Lindlar catalyst, where one triple bond was isolated, and the other two skipped. They described a large amount of over-hydrogenated byproducts, which eluted with the main product on TLC and HPLC, but on 10% Ag^+ impregnated TLC the separation was very good. They concluded that the argentation purification was necessary to achieve pure product. Khrimian et al.[123] in 2004 described the semi-hydrogenation of a di-yne with the Lindlar catalyst obtaining 95% pure product which was then afforded in 99% purity after argentation chromatography. Fomich et al.[153] then in 2016 described the synthesis of multiple deuterated arachidonates which they stated to be generally afforded as crude samples after semi-hydrogenation. Only after argentation chromatography and obtaining considerably lower yield, pure samples were obtained.

Not many other examples of silver ion chromatography are mentioned in the literature in the context of skipped poly-ene synthesis and no authors describe its use after semi-

hydrogenation of skipped penta- or hexa-ynes. Authors who do not use or mention argentation chromatography do then mostly ignore the semi-hydrogenation byproducts.

6.2.3 Di-yne experiments

Hydrogenations of a short chain di-yne alcohol **18** to produce the di-ene alcohol **91** were performed, see Scheme 6.6. Five different Lindlar catalysts and one Rosenmund catalyst were tried, and very diverse outcomes were obtained. All the reactions were performed in toluene at room temperature, using 0.25 eq. of quinoline and 0.8 wt/wt ratio of catalyst to substrate. Less quinoline was used than normally to try to exaggerate the production of the byproducts to better see differences between the catalysts. The results are presented in Table 6.2. Lindlar **I-IV** were obtained from Sigma-Aldrich as palladium on calcium carbonate poisoned with lead, Lindlar **V** was obtained from TCI (Belgium) and Lindlar **VI-VII** were obtained from Sigma Aldrich as "Lindlar catalyst".



Scheme 6.6 Semi-hydrogenation trials on the di-yne **18**.

Table 6.2 Semi-hydrogenation trials performed on the di-yne **18**, using different catalysts in toluene.

Catalyst	Reaction time	Vinyl proton int.	
		Measured	%
Lindlar **III**	36 min	2.97	74
Lindlar **IV**	59 min	2.83	71
Lindlar **V**	57 min	3.44	86
Lindlar **VI**	Inactive	-	-
Lindlar **VII**	Inactive	-	-
Rosenmund	Few min	-	-

The substrate **18** was not ideal since the hydrogenation products evaporated easily under vacuum which made the yield calculations inconclusive. The ^{1}H-NMR spectra did though give the vinyl proton percentage which was used to compare the catalysts.

The Lindlar **V** catalyst showed obvious superiority over the other catalysts. Much less over-hydrogenation was observed in the ^{1}H-NMR spectra and the indicative *trans* double bond peak in the IR spectra was less than half of what was seen in the spectra produced by the product of Lindlar **III** and **IV**. Lindlar **VI** and **VII** were different batches of the same catalyst and were both completely inactive under these conditions. The Rosenmund catalyst was way too active and yielded almost only over-hydrogenated product.

Another trial using the di-yne alcohol **54** with different catalysts to afford the di-ene **55** was performed (see the Wittig approach). Three different catalyst manufacturers with two batches of one of them (Lindlar **III** and **IV**), in total 4 catalysts. The reactions were performed in toluene with 0.4 equivalents of quinoline as catalyst poison, except in the case of Lindlar **VII**, where no quinoline was added. The results can be seen in Table 6.3.

Table 6.3 The results for the semi-hydrogenations of substrate **54** to compare Lindlar catalysts.

Catalyst	Reaction time	Vinyl proton int.		Yield
		Measured	%	
Lindlar **III**	36 min	3.90	98	78%
Lindlar **IV**	36 min	3.92	98	80%
Lindlar **V**	51 min	3.88	97	76%
Lindlar **VII**[a,b]	2h 49 min	3.74	94	87%

[a] Three times the amount of catalyst used. [b] No quinoline added.

For these reactions, the catalysts **III, IV** and **V** gave almost identical results, except for the reaction time which was different for each manufacturer. Very low over-hydrogenation was seen in the ^{1}H-NMR spectra and almost no visible side-products were observed on 10% $AgNO_3$ impregnated TLC. The Lindlar **VII** behaved radically different, it was much slower, and more catalyst was added into the reaction to aid it to completion. No quinoline was used and it is not clear if the catalyst was treated in some way by the manufacturer, but no such explanations were present on the labeling. The Lindlar **VII** did afford higher yields than the others but there was noticeably more over-hydrogenation taking place instead. The Rosenmund catalyst was also tried, both in toluene and methanol with 0.2, 0.5 and 2 equivalents of quinoline, but in all cases gave almost only over-hydrogenated products.

6.2.4 Tri- and tetra-yne experiments

As mentioned before, multiple semi-hydrogenation trials were conducted on the tetra-yne **62** (the SDA precursor), to try to optimize the reaction conditions. The Lindlar **I** catalyst in benzene had given very promising results for the semi-hydrogenation of the MEL **5** precursor the hexa-yne **11**. The vinyl proton integration was over 90% but the yield was very low (30%) as proper handling of the hexa-yne had not been implemented at that point. Subsequent use of another batch of the same catalyst (Lindlar **II**) would always show increased over-hydrogenation and could never approach the same results. The following trials on the tetra-yne **62** narrowed down the optimal conditions for newly purchased catalysts (Lindlar **II**, **III**, **IV**, **V**, and **VI**).

The best results were conducted with Lindlar **III** (**IV** very similar) in toluene at room temperature using 0.5 equivalents of quinoline and about 1:1 weight ratio of catalyst to substrate. The Lindlar **III** proved to be superior and was used under these conditions to semi-hydrogenate all the tri-yne MEL substrates **36**, **38** and **47** as well as the tetra-yne MEL substrate **49**. The best results achieved for the Lindlar **II** for the substrate **62** as well as all the Lindlar **III** results for the above-mentioned substrates are listed in Table 6.4.

Table 6.4 Semi-hydrogenations of tri- and tetra-ynes using the Lindlar catalyst in toluene with quinoline.

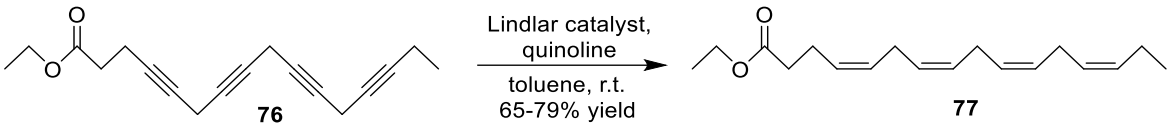
Target Molecule	Lindlar Catalyst	Reaction time	Vinyl proton int.		Yield
			Measured	%	
SDA EE[a]	**II**	43 min	6.85	86	50%
SDA EE	**III**	1h 48 min	7.04	88	70%
MEL **7**	**III**	45 min	5.19	86	73%
MEL **7**	**III**	41 min	4.99	83	68%
MEL **7**[b]	**III**	1h 37min	5.39	90	98%
MEL **8**	**III**	33 min	4.83	81	53%
MEL **8**	**III**	1h 20 min	5.17	86	78%
MEL **9**	**III**	47 min	5.38	90	78%
MEL **10**	**III**	1h 34 min	7.18	90	77%

[a] 0°C,[b] larger scale (400mg)

These conditions gave satisfying results for the tri- and tetra-yne substrates and were subsequently used on the penta- and hexa-yne substrates (see Section 6.2.5). The integration of 88-90% was achieved for all substrates (except only 86% for MEL **8**) using this method and yields around 70-78%. Lastly, when semi-hydrogenating MEL **7** on a larger scale (400 mg) than normally (60-100 mg) where the sample was only roughly concentrated, a surprising 98% yield was achieved with good vinyl integration. This seems to indicate that a larger scale or not concentrating the poly-yne samples could play a crucial role in increasing yields significantly.

It has to be realized at this point that the vinyl proton integration results show clearly that these products are not pure as some over-hydrogenation has occurred and therefore these yields are not the final yields. The subsequent argentation chromatography to obtain the pure compounds is discussed in Section 6.2.7.

More catalyst trials were conducted. Firstly, the performance of the Lindlar **III** and **V** were compared on the 16:4 tetra-yne **76**. Scheme 6.7 depicts the semi-hydrogenation reaction.



Scheme 6.7 Semi-hydrogenation trials producing the 16:4 EE **91**.

The tetra-yne **76** was semi-hydrogenated three times. Once with the Lindlar **V** and two times with the Lindlar **III** catalyst, with and without 2-methyl-2-butene as an additive. The results are shown in Table 6.5.

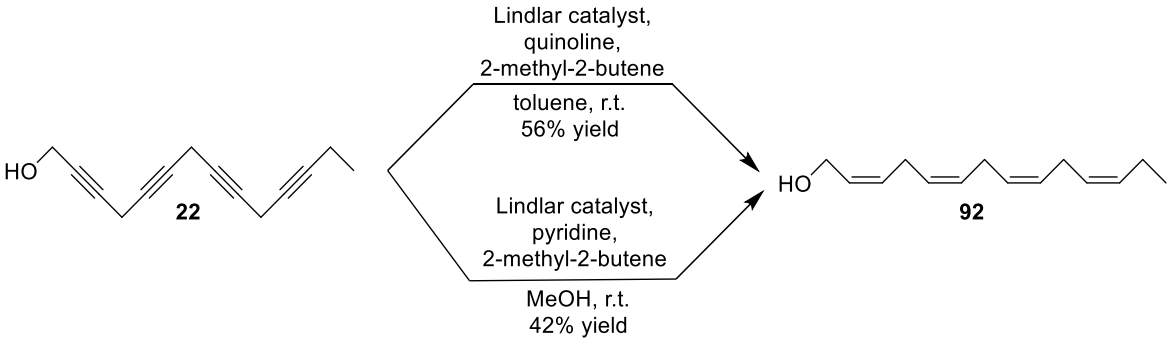
Table 6.5 Semi-hydrogenations of **76** in toluene with quinoline.

Catalyst	Reaction time	Vinyl proton int.	Yield
Lindlar **III**	1h 51m	6.76 (85%)	77%
Lindlar **III**[a]	1h 11m	7.13 (89%)	65%[b]
Lindlar **V**	1h 49m	7.20 (90%)	79%

[a] 12.6 eq. of 2-methyl-2-butene was added. [b] Some material was lost during preparation which impacted the yield slightly.

For the Lindlar **III** reaction, the vinyl proton integration indicates more over-hydrogenation than for the Lindlar **V** reaction. The Lindlar **III** reaction could then be improved to a similar level as for the Lindlar **V** reaction by the addition of 2-methyl-2-butene.

Finally, comparison of the two conditions, Lindlar catalyst in toluene with quinoline and 2-methyl-2-butene and the conditions described by Hwang et al.[116] who used Lindlar catalyst in methanol with pyridine and 2-methyl-2-butene was made. The Lindlar **V** catalyst was used. Scheme 6.8 depicts the reaction and Table 6.6 details the results.



Scheme 6.8 The semi-hydrogenations of the tetra-yne 22 producing the tetra-ene **92**.

Table 6.6 Semi-hydrogenation results for the tetra-yne **22**.

Catalyst	Additive	Solvent	Vinyl proton int. Measured	%	Yield
Lindlar **V**	quinoline 2-methyl-2-butene	Toluene	7.29	91	56%
Lindlar **V**	pyridine, 2-methyl-2-butene	MeOH	7.78	97	42%

The products **92** exhibited very clean spectra, though the toluene/quinoline route had noticeably higher over-hydrogenation. The MeOH/pyridine route had close to perfect integration and therefore very low over-hydrogenation, but the yields were somewhat lower.

These results indicated that the Lindlar **V** catalyst was slightly better than **III**. And the Hwang et al. conditions gave incredibly good vinyl integration but somewhat lower yields, at least for an alcohol substrate.

6.2.5 Penta- and hexa-yne experiments

The main challenge was to semi-hydrogenate the penta- and hexa-yne substrates and few methods were employed for that purpose. Firstly, the results for the Lindlar catalyst in toluene are listed in Table 6.7.

Table 6.7 Semi-hydrogenations of skipped poly-ynes using the Lindlar catalyst in toluene with quinoline.

Target Molecule	Lindlar Catalyst	Reaction time	Vinyl proton int. Measured	%	Yield
EPA EE	**III**	28 min	8.39	84	65%
EPA EE[a]	**III**	42 min	8.61	86	54%
EPA EE	**V**	4h 16 min	8.81	88	69%
DHA EE	**III**	1h 6 min	10.22	85	78%
MEL **10**	**III**	1h 34 min	7.18	90	77%
MEL **5**	**III**	52 min	10.28	86	60%

[a] 80% of normal catalyst loading

Similar vinyl proton integration was achieved for both PUFA EE and MEL substrates (85-88%). The MEL **5** was achieved in somewhat lower yields than the PUFA EEs. Noticeably, the Lindlar **V** catalysts, when tried on the EPA EE substrate gave better results than the Lindlar **III** catalyst, even though the reaction time increased more than six-fold.

The promising conditions described by Hwang et al. were also tested on these substrates and the results are shown in Table 6.8.

Table 6.8 Semi-hydrogenations using Lindlar in MeOH with pyridine and 2-methyl-2-butene.

Target Molecule	Lindlar catalyst	Reaction Time	Vinyl proton int. Measured	%	Yield
EPA EE	**V**	3h 13 min	9.42	94	54%
EPA EE[a]	**V**	5h 40 min	9.10	91	56%
DHA EE	**V**	2h 43 min	11.56	96	67%
MEL **10**	**V**	6h 44 min	7.01	88	51%
MEL **5**	**V**	Incomplete	-	-	-

[a] 0°C

The semi-hydrogenations using these conditions gave very good results on the PUFA EE substrates, the cleanness of the spectra was initially obvious when compared to the other methods and the vinyl proton integration (94% and 96%) proved to be superior. The only drawback was the noticeably lower yields 54-67% and longer reaction times. Cooling the reaction to 0°C gave lower integration in the case of the EPA EE. The semi-hydrogenation of **74**, the precursor to DHA EE, gave 67% yield and excellent integration, very promising results for the DHA-like MEL **5**.

It was therefore unfortunate that the semi-hydrogenation on the MEL substrates did not produce similar results. The semi-hydrogenation of the MEL **10** substrate gave worse results than for the Lindlar in toluene route and the semi-hydrogenation of the MEL **5** substrate stopped showing any sign of continuation after a few minutes. The reaction started off showing all seven spots on TLC, indicating that the starting material, product and all semi-hydrogenated intermediates were present in the reaction, but did then not change, for more than 24 hours. Further experiments, tweaking the ratios of solvent versus pyridine and 2-methyl-2-butene might be warranted to try to produce more desirable outcome.

6.2.6 Brown catalyst experiments

Finally, the Brown catalyst was also tried on the hexa-yne substrates. Those results are listed in Table 6.9.

Table 6.9 Results of the semi-hydrogenation of skipped poly-ynes using the Brown catalyst.

Target Molecule	Ethylene-diamide	Reaction time	Vinyl proton int. Measured	%	Yield
DHA EE	0.55 eq	22 min	7.88	66	99%[a]
DHA EE	0.5 eq	14 min	9.04	75	84%[a]
DHA EE	0.9 eq	15 min	8.73	72	96%[a]
DHA EE	2.7 eq	12 min	9.31	78	83%[a]
MEL **10**	2.2 eq	32 min	6.56	82	54%
MEL **5**	2.2 eq	80 min	10.47	87	44%

[a] Only filtered

The DHA EE precursor was semi-hydrogenated a few times with different amounts of eda. This substrate was semi-hydrogenated very quickly, and the end of the reaction seemingly had to be monitored very closely to prevent over-hydrogenation as the best integration was

achieved with the shortest reaction time. The very high yields relate to that no chromatography was performed, only filtering of the catalyst. The Brown catalyst did not perform nearly as well as the Lindlar catalyst for the DHA EE hexa-yne substrate. The MEL **10** substrate did not perform particularly well under these conditions either, but the MEL **5** substrate did achieve marginally better integration but lower yield. Some further optimization could improve these results, and might be warranted for the MEL **5** substrate, since the Lindlar catalyst might be more prone to reproducibility issues.

6.2.7 Purity issues of poly-ynes after semi-hydrogenation

When hydrogenating these poly-ynes the complexity and amounts of side-products increased with every additional acetylene group on the molecule. The hydrogenation of skipped poly-yne systems, which because of their labile acetylene-locked methylene groups have increased stability issues, a suitable catalyst system for their semi-hydrogenation was one of the goals in this work. A handful of methods have been used successfully in the semi-hydrogenation of skipped poly-ynes in the work presented herein, but results from other authors have varied dramatically throughout the decades. The number of alkynes, the solvent, catalyst, catalyst manufacturer, other active groups, amount and type of catalyst poison and temperature have all been showed to significantly affect the reaction outcome, and between authors sometimes in contradictory ways. For these reasons, no universal method has been developed and it is very hard to predict exactly what conditions and catalyst will be successful for a certain substrate. The results achieved here show that the proper argentation purification after the semi-hydrogenation is a very powerful tool to obtain pure products from semi-hydrogenation reactions and therefore mitigating the downsides any particular method/catalyst possesses.

Many authors have had great success in the semi-hydrogenation of di-, tri- and tetra-ynes using the Lindlar or Brown catalysts. Many authors do though not properly address the possibility of hydrogenation impurities in their products or declare high purity and refer to methods that do not inspire great confidence in those estimations. Only a handful of penta- and hexa-yne semi-hydrogenations have been reported, in all cases using the Lindlar catalyst. Osbond and Wickens[78] as well as Pabon[82] demonstrated it all the way back in the 1960s but with questionable purity. Two patents have been given for the synthesis of DHA[103] and a ^{13}C-labeled DHA[104] in 2013. Neither patent describes any purification beyond a normal silica gel chromatography and over-hydrogenation is visible in their ^{1}H-NMR spectra and *trans*-isomers unaddressed. Hwang et al.[116] did then in 2016 describe the semi-hydrogenation of penta- and hexa-ynes into ω-hydroxy EPA and DHA methyl esters in fair yields (45-50%). They mentioned the RP-HPLC purification of the acids on scale up (>100mg) but otherwise normal silica gel chromatography. Where the ^{1}H-NMR spectra are accessible, they without exception show the typical signs of over-hydrogenation for these penta- and hexa-enes and *trans*-isomers were not addressed.

During this work, no matter the catalyst or conditions, the semi-hydrogenation of skipped poly-yne compounds did always show over-hydrogenation in ^{1}H-NMR as well as *trans* band in IR. Argentation chromatography was then used to isolate the desired skipped all-*cis* poly-ene products with excellent purity. The use of preparative TLC plates for the isolation of the product was not ideal, especially for the penta- and hexa-ynes, since the method has increased risk of exposing these labile compounds to light and air as well as no successful method was employed in visualizing the bands very clearly. When purifying the larger poly-enes the degree of separation between the product and one of the byproduct

bands was always low, which resulted in either very low yield or lower purity when harvested from the plate. This could most likely be improved upon by more efficient methods such as impregnated preparative-LC.

As has been described, argentation chromatography was considered essential during this work to obtain pure products of the poly-enes. All main poly-ene products were submitted to argentation chromatography, either on 10% $AgNO_3$ impregnated preparative TLC plates or impregnated silica used in a flash column chromatography. Table 6.10 describes successful argentation purifications for the poly-ene products, the vinyl proton integration before and after, as well as the yields.

Table 6.10 Successful argentation purifications of skipped poly-yne substrates.

Target Molecule	Catalyst	Catalyst Poison	Vinyl proton int.		Yields [%]		
			Before	After	Crude	Ag^+	Total
SDA EE	**III**	quinoline	7.04	7.97/8	70	55	39
EPA EE	**III**	quinoline	8.61	9.89/10	54	11	6
EPA EE	**V**	quinoline	8.81	9.84/10	69	38	26
EPA EE	**V**	pyridine	9.42	9.93/10	54	54	29
DHA EE	**III**	quinoline	10.22	12.01/12	78	18	14
DHA EE	**V**	pyridine	11.56	11.98/10	67	54	36
MEL **7**	**III**	quinoline	5.39	6.06/6	98	54	51
MEL **8**	**III**	quinoline	5.17	5.80/6	78	51	39
MEL **9**	**III**	quinoline	5.38	5.97/6	78	43	33
MEL **10**	**III**	quinoline	7.18	7.97/8	77	46	35
MEL **5**	**III**	quinoline	10.28	11.70/12	60	42	25
MEL **5**	**Brown**	eda	10.47	11.84/12	45	50	23

Great success was clearly achieved in purifying these compounds using argentation chromatography. Only the MEL **5** was a little bit lacking, as one of the byproduct bands did not completely separate from the product band on the preparative TLC.

The main drawbacks for the semi-hydrogenation of these skipped poly-ynes are, as has been covered, the instability of the skipped poly-yne substrates, the production of over-hydrogenated and *trans*-isomer byproducts and the sometimes-low reproducibility, especially with the Lindlar catalyst. The instability issue can be endured by careful handling and organization of the synthesis or bypassed by semi-hydrogenating smaller substrates and then combine them. The other two drawbacks have to do with the catalyst that is used and requires careful selection of the catalyst or luck.

Chapter 5.6 described alternative strategies which might be better than the pure acetylenic methodology used in this project. New catalysts are always emerging, and these drawbacks could be minimized in the future with new technologies which would then elevate this methodology further. This is an active research scene and many examples from the last few years show innovative methods and catalysts, such as palladium nanoparticles, silica supported copper nanoparticles,[222] monodispersed nickel nanoparticles,[223] first-row transition-metalated porous organic polymers[224] and many more.[225] These examples and any others could lead to even more efficient skipped poly-yne semi-hydrogenations in the future.

6.3 *Trans* isomer measurements

Trans and *cis* carbon-carbon double bonds have different physical and chemical properties. Several methods can be used to distinguish between the *cis*- and *trans*-isomers of a certain molecule, but to measure the exact proportions can be difficult especially in cases with more than one double bond present in the molecule. During this work, the synthesis of methylene interrupted all-*cis* poly-ene hydrocarbon chains, there was interest to accurately assess the amount of the unwanted *trans*-isomers present in the products. This chapter shortly discusses the methods available for distinguishing or separating *cis*- and *trans*-isomers and explains why the assessment of the unwanted *trans*-isomer percentage in poly-ene compounds can be difficult.

6.3.1 *Trans*-evaluation by NMR spectroscopy

In ^{1}H-NMR spectra the vicinal coupling signal for the alkene protons generally appears between 5.2 to 5.5 ppm for non-conjugated double bonds. In a *cis* double bond, the signal is more upfield but a *trans* double bond is generally further downfield, though the difference is only about 0.04 ppm so the signals still overlap. The coupling constants are also different, around 6-14 Hz for a *cis* double bond but 11-18 Hz for a *trans*. These differences can be used to distinguish between two monounsaturated isomers, but to accurately assess the amount of isomer proportions in a sample can be very difficult or impossible using these differences since the signals generally overlap even with only one double bond present in the structure. If there is more than one double bond present any accurate measurements of isomer proportions are practically impossible using this method.[226]

Different chemical shifts can be observed for double bonds in a ^{13}C-NMR spectrum based on their configuration. This has been studied in detail for unsaturated fatty acids and esters.[227-229] The difference in the chemical shift of the double bond carbons is usually less than 0.5 ppm where the *trans* carbons appear slightly higher upfield than *cis* but this difference is tiny and is skewed by neighboring double-bonds or proximity to a carboxyl-end. A bigger difference is observed in the chemical shift of the methylene groups situated on each side of the double bond. Allylic methylene group beside a *cis* double bond is observed around 27 ppm but at around 32.5 ppm if it is *trans* except for methylene groups very close to either end of the fatty acid which can be more flexible.

Similar trend is seen in polyunsaturated compounds where a methylene group between two *cis* bonds resonates around 25.5 ppm, a methylene group between one *trans* and one *cis* bond around 30.5 ppm and a methylene group between two *trans* bonds around 35.5 ppm.[227] Figure 6.9 depicts two spectra of the synthesized DHA EE before and after argentation chromatography. The before spectrum shows the two expected *trans* peaks at 30.5 and 32.5. The spectrum of the purified sample shows no or very weak signal in the *trans* region but was measured by GC to have 4.5% of mono-*trans*-isomers. Many other peaks are visible in the before spectra resulting from other over-hydrogenated and *trans* impurities.

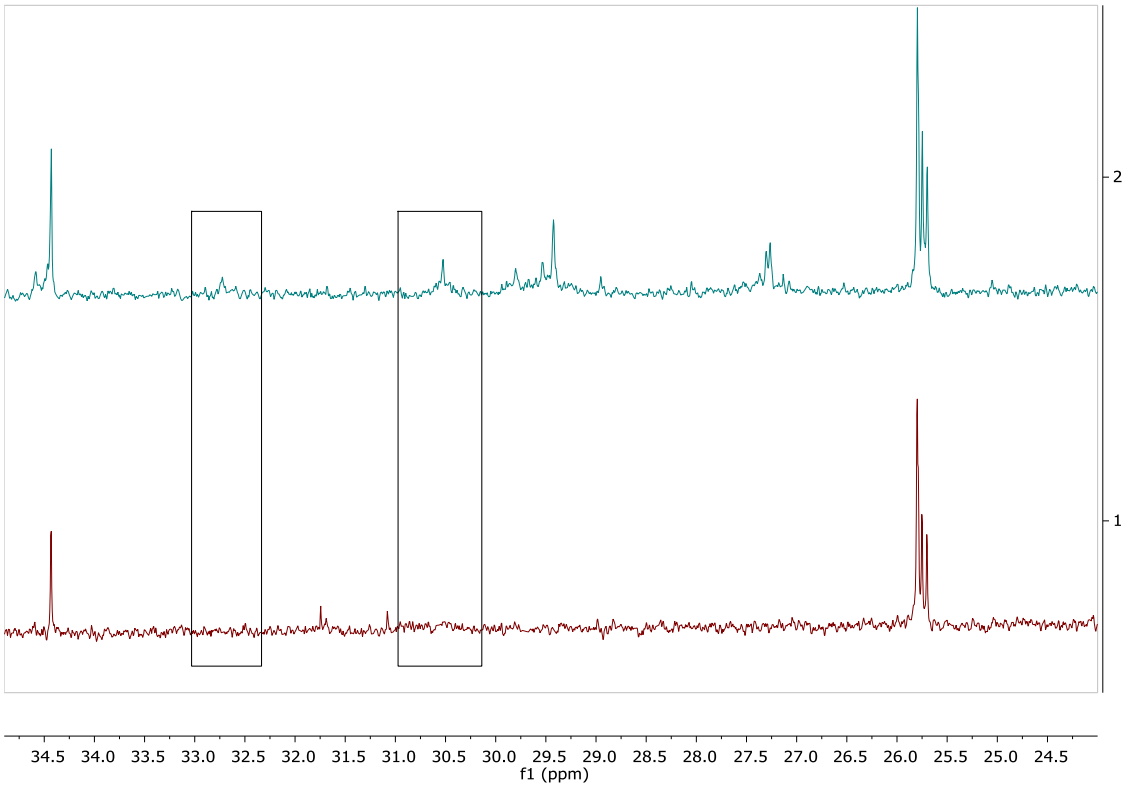


Figure 6.9 A section of the ^{13}C-NMR spectra of the synthesized DHA EE **75** before (top) and after (bottom) argentation chromatography.

^{13}C-NMR can and has been used to differentiate between isomers and detect *trans*-isomers in a sample, but to accurately measure the low percentage of *trans* in a poly-ene compound can be quite difficult using this method. A strong sample is required and a large signal to noise ratio. The methylene *trans* peaks which would be very small do then not necessarily congregate exactly in one spot making the uncertainty of the measurement very high. This becomes even more complex if the double bonds are close to other moieties or if there are also over-hydrogenated byproducts present in the sample. This makes NMR not an ideal method to estimate *trans*-isomer ratio in a semi-hydrogenated product. Figure 6.10 depicts the spectra of compound **23** and the MEL **5**, after argentation chromatography, where a visible peak is seen at exactly 30.5 ppm, the expected *trans* region. Integration of the region shows it to be around 4-5% compared to the *cis* region for both compounds.

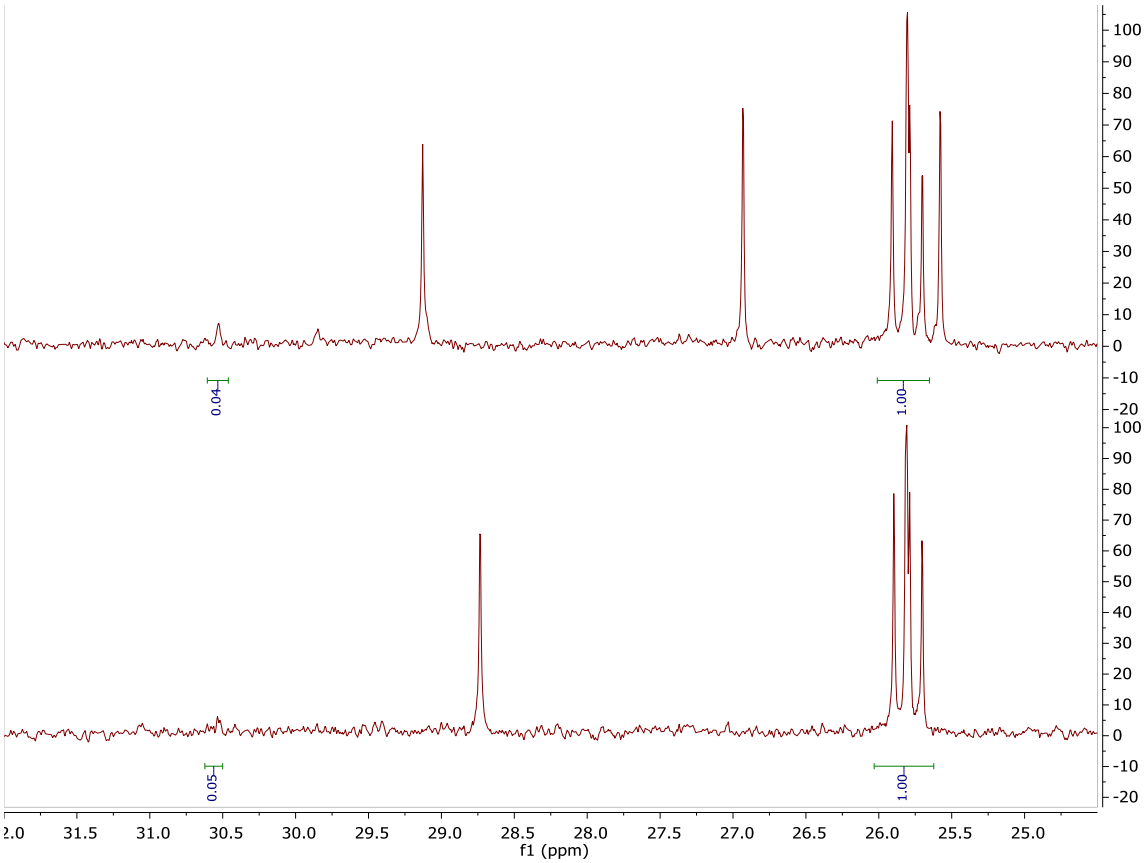


Figure 6.10 Section of the ^{13}C-NMR of the compounds **23** (top) and MEL **5** (bottom) after argentation chromatography.

6.3.2 *Trans*-evaluation by IR spectroscopy

Significant difference of *cis* and *trans* double bonds is then observed with Fourier transform infrared spectroscopy (FT-IR). A few different bands have been uniquely identified for both *cis* and *trans* double bonds, but most of them are of weak intensity and in complex molecules overlap with each other as well as bands belonging to other functional groups. Table 6.11 lists the relevant bands belonging to *cis* and *trans* double bonds.[230-232]

Table 6.11 IR bands identified for *cis* and *trans* configured double bonds.

Functional group	Band position (cm^{-1})	Assignment
Trans *Cis*	3025-3028 3008-3018	ν_{sym}=C-H stretch
Trans *Cis*	1666 1654	C=C stretch
Trans *Cis*	966-969 690-750	=C-H out-of-plane bending

Most of the peaks listed in Table 6.11 are not of much practical use in the accurate *cis*/*trans* measurements being discussed, due to low intensity or overlapping and interferences with other bands, except the *trans* C-H out-of-plane bending at around 967 cm^{-1}. This highly characteristic band has been used extensively in the fat and oil industries

to measure *trans* content easily and rapidly in a variety of fat and oil samples. *Trans*-free reference samples have to be used which adequately mirror the composition in the sample to be measured as well as a standard curve based on, usually, methyl elaidate standards. But the method is still subjected to interferences from other bands that are inherently part of the samples which reduces the accuracy and sensitivity, especially at low *trans* levels (<5%).

In 1969 Allen[233] published a method for *trans* measurements of fats using the absorbance bands for *trans* at 967 cm^{-1} and another belonging to the acid or ester group which were then compared to known samples containing methyl elaidate and oleate. This method worked fine to identify pure stereo-isomers but was in no way accurate enough to measure low *trans*% in samples, as well as showing small differences in values between regioisomers, different values based on the position of the *trans* double bond in the chain.[227] Researchers have continually been improving these FT-IR methods throughout the decades for oil samples, but they have still today not reached the accuracy needed when compared to GC methods for samples with very low *trans* levels.[234]

As of 2010 an attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy method has been validated based on a procedure measuring the height of the negative second derivative of the trans absorption band at 966 cm^{-1} to measure *trans* content in a fat or oil sample. This is the best method available to date for IR measurements of *trans* content in oil but is still only recommended for samples containing >1.8% trans content as a percentage of total fat content.[235] This method has only been validated for mono-*trans* fatty esters using methyl elaidate or trielaidin as standards for a calibration curve. Due to these reasons, it is almost impossible to accurately measure trans percentage for novel polyunsaturated compounds with IR methods. IR methods can therefore not be used to precisely measure *trans* content in a MEL sample but can nonetheless sometimes be used to detect the presence of *trans* and observe a change in the amount of *trans* due to purification performed on the sample.

It was important in this work to try to as accurately as possible to measure or estimate the *trans* ratio in the final products. As explained above, that proved to be a rather difficult task. Using infrared spectra, it was clear that after the initial semi-hydrogenation considerable amount of *trans* double bonds were present in the poly-ene products as the 967 cm^{-1} peak was clearly visible. Using a calibration curve made with oleic and elaidic methyl esters mixed in different proportions ranging from 1% to 50% an attempt to estimate the *trans* ratio was made. Comparing the peak proportions of the IR spectra of the synthetic SDA put the trans amount between 4-10%. After purification on 10% $AgNO_3$ silica preparative TLC plate the same method showed the trans percentage to be around 0-2%. Figures 6.11 and 6.12 show the transmittance IR spectra of the synthesized SDA EE **63** before and after argentation chromatography, respectively. The spectrum measured after the argentation chromatography shows that the band at 969 cm^{-1} is almost completely gone.

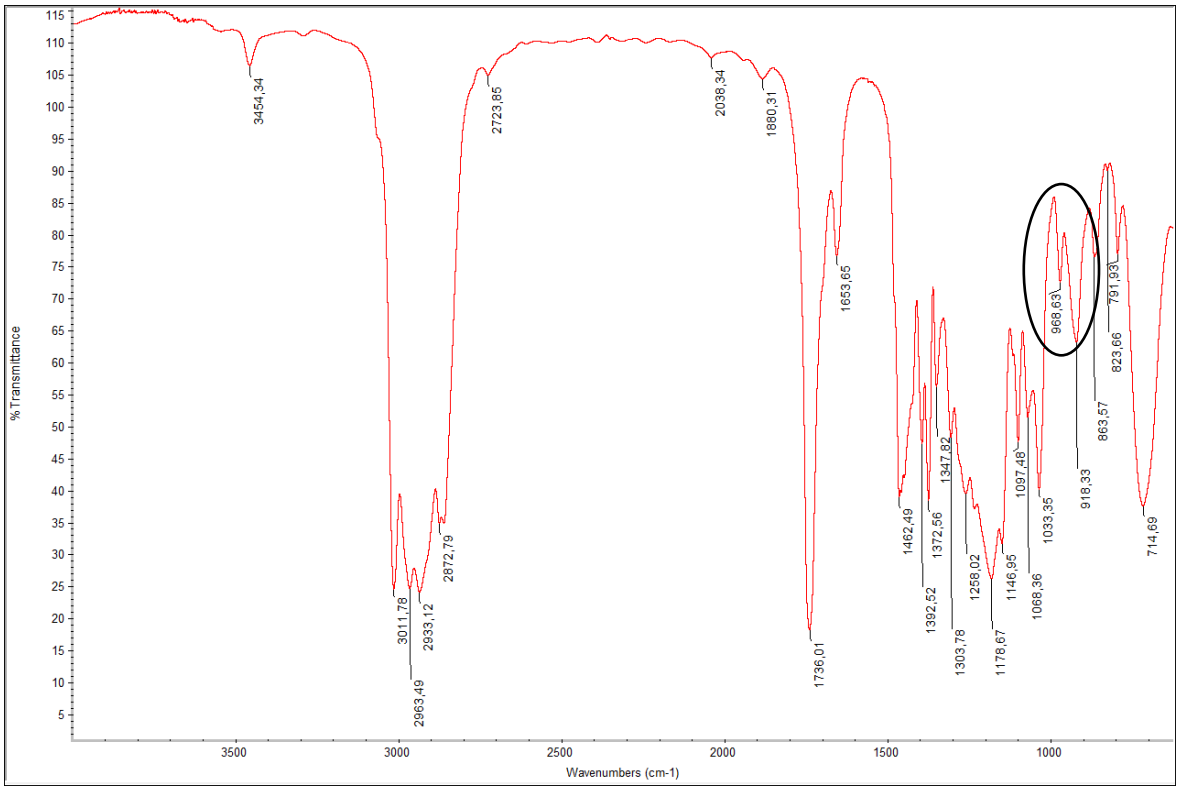


Figure 6.11 The transmittance IR spectrum of the synthesized SDA EE **63** before argentation chromatography. The *trans* band at 969 cm^{-1} is visible inside the black circle.

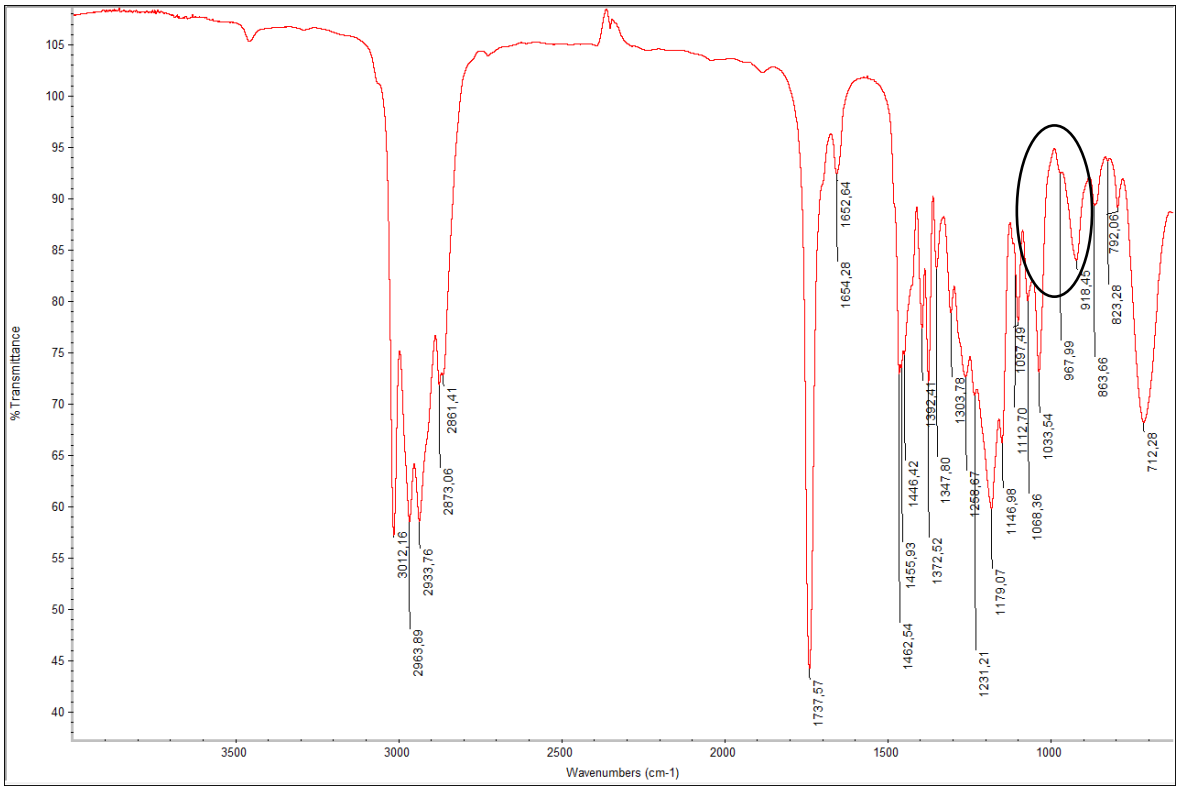


Figure 6.12 The transmittance IR spectrum of the synthesized SDA EE **63** after argentation chromatography. The *trans* band at 969 cm^{-1} within the black circle is almost completely gone.

This method was not accurate enough to exactly measure the *trans* content but did nonetheless clearly show the effect of the silver ion chromatography in removing the *trans*-isomers. Unfortunately, in the MEL samples another band at around 975 cm^{-1} interfered greatly with the *trans* band, which made any accurate estimation impossible. Nonetheless, difference in the band height and shift was observed before and after silver ion purification, see Figure 6.13.

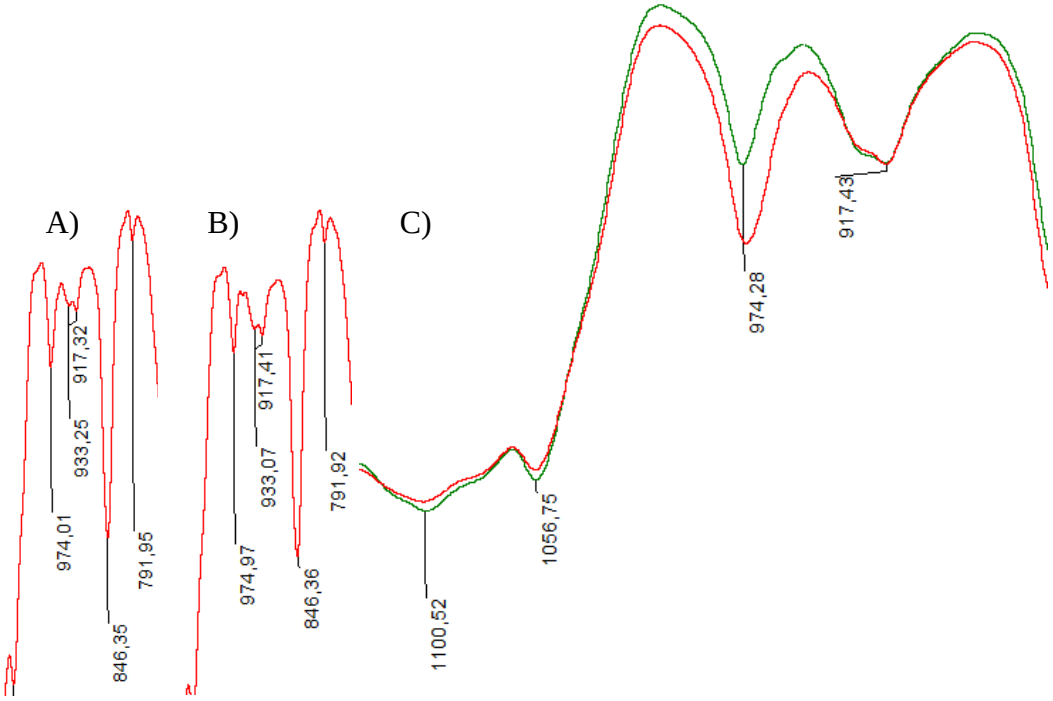


Figure 6.13 The relative reduction in the IR transmittance spectra of the band at 974 before purification A) and shift to 975 after purification B) for the n-6 MEL **9** substrate **48**. C) shows the IR transmittance of the compound **23** before (red) and after (green) silver ion purification

6.3.3 *Trans*-evaluation by GC analysis

The most accurate method and the one used in the oil industry to measure exact *trans* percentage in a sample is with gas chromatography. Methods and standards have been established and are in wide use that can separate stereoisomers based on a single double bond being *cis* or *trans* in a polyunsaturated fatty acid. The problem here is that to be sure of the *trans* proportions in a certain compound it is necessary to verify the *trans* peaks in the chromatogram. This involves obtaining *trans*-isomer standards and then establish a method that can separate them which is not necessarily an easy task but has been done for example for EPA and DHA which are of great interest to the fish oil industry.

By the late 1970s the separation of *cis* and *trans*-isomers of 18:2 had been achieved on GC.[236] By 2005 the mono-*trans* and di-*trans*-isomers of EPA and DHA were made in an isomerization reaction of natural EPA and DHA and then separated with silver ion chromatography into groups based on the number of *trans* double bonds. These groups were then separated into almost individual peaks on GC which all elute separately from the all-*cis* EPA and DHA. This formed the foundation of all accurate *trans*-measurements in fish oil in the industry today.[220]

As discussed above, no definitive way to measure the *trans* content in the synthetic polyunsaturated MEL compounds exists using NMR or IR. Furthermore, no verified GC method exists for these compounds and therefore practically impossible to accurately measure the *trans* percentage in the final MEL compounds without significant syntheses and GC method development. The second-best choice was to synthesize the comparable PUFAs, SDA, EPA and DHA using similar methods and most importantly the same hydrogenation conditions. These PUFAs are of huge interest in the marine and algae oil industry and have well defined GC *trans* percentage measurement methods and standards. SDA has a similar poly-ene tail structure to MEL **10** and DHA to MEL **5**. After semi-hydrogenation and purification via silver ion chromatography the PUFAs were measured by established GC methods at EPAX in Ålesund, Norway and BASF in Sandefjord, Norway. Table 6.11 shows the results of these measurements.

Table 6.12 IR estimations and GC measurements of *trans*-isomers in the PUFA products.

Substrate	Method	*Trans* content
SDA EE **63**	Lindlar in toluene	<0.4%[a]
EPA EE **70**	Lindlar in toluene	8.0%[b]
EPA EE **70**	Lindlar in toluene	1.05%
EPA EE **70**	Lindlar in MeOH	0.99%
DHA EE **75**	Lindlar in MeOH	4.25%
DHA EE **75**	Lindlar in toluene	4.4%[c]
DHA EE **75**	Lindlar in toluene	6.8%[c]

[a] No actual *trans*-isomer standard, [b] Before argentation chromatography, [c] same semi-hydrogenation reaction but two separate preparative TLC plates

The SDA EE sample showed three tiny peaks in proximity to the main peak. No *trans*-isomer standard for SDA EE was available but the positions of the peaks, one in front of the all-*cis* peak and the rest behind mirrored the EPA and DHA samples. If assumed to be the *trans*-isomer peaks, the sample contained less than 0.4% *trans*-isomers. One EPA sample that had not been purified by silver ion chromatography was measured and showed over 30% impurities, of which about 8% were the mono *trans*-isomers. The same EPA hydrogenation product, after silver ion purification, showed 1.05% *trans*-isomer impurities. Another EPA EE sample which was semi-hydrogenated under the conditions described by Hwang et al.[116] showed *trans*-isomers just below 1%. The DHA EE samples showed over 4% *trans*-isomer content for both Lindlar catalyzed semi-hydrogenation methods.

One of the DHA EE **75** semi-hydrogenation products was isolated on two individual 10% $AgNO_3$ preparative TLC plates where the collected fragment from the first plate was measured to contain 4.4% *trans*-isomers and the second plate 6.8%. This underlines how proper isolation on the preparative TLC plates was difficult for the hexa-enes regardless of semi-hydrogenation method. The band belonging to the desired product overlapped somewhat with the supposed mono-*trans* band on the prep-TLC plates. More efficient argentation chromatography methods employed should enable the obtainment of more desirable results for the larger poly-ynes.

7 Conclusions

The head piece **14** proved to be a powerful synthon for the synthesis of the MEL compounds and its development made possible the greatly improved synthesis of MEL **1**, by increasing the yields from 27% to 54% as well as open up the possibility to synthesize polyunsaturated MELs. The unfortunate diastereomeric impurity in the headpiece **14** that was realized was then dealt with by the employment of the Co-Jacobsen catalyst which then also allowed the significant improvement in the economy of the synthesis by reducing the chiral starting materials to one and then possibly zero. (*Paper I*)

Good success was achieved in synthesizing compounds possessing large, skipped poly-ene frameworks by catalytic semi-hydrogenation with the acetylene approach despite high instability of the skipped poly-yne compounds. The acetylene methodology proved to be capable of synthesizing, at least, compounds possessing six skipped double bonds such as the DHA EE **75** and MEL **5** in around 10% total yield. Great success was achieved in increasing yields and efficiency of the iterative copper coupling reactions by taking great care in handling the labile compounds during work-up and purification as well as limit the time of their existence as much as possible. Argentation chromatography showed great efficiency in removing the undesired semi-hydrogenation byproducts as visible in the NMR spectra of the final products. (*Paper II*)

By adding a carbon chain linker of different lengths to the synthesis, the unsaturation can be positioned anywhere on the fatty chain, and this allowed the synthesis of the 18:1 n-9 MEL **6**. The MS/MS comparison to the original shark and dogfish liver oil sample proved the existence of MEL **6** for the first time. (*Paper III*)

The syntheses of three 18:3 MEL derivatives were performed to try to pinpoint the exact structure of the 18:3 MEL detected in the liver oil sample. Surprisingly, none of the synthesized MELs compared to the one detected. So, the only conclusion drawn was that it had neither the typical Δ4 unsaturation, n-3 or n-6 structure. Furthermore, a Wittig and acetylenic combined approach was tested on the MEL **7**. This proved to be very successful, even though not the most favorable reaction conditions were used. A combined approach might be the most favorable strategy moving forward, utilizing both their strength, and minimizing weaknesses. (*Paper IV, accepted by ChemistrySelect* & *Paper V, manuscript*)

Finally, a few notable PUFA EEs were synthesized (SDA, EPA, DHA), which was a valuable experience in streamlining the experimental procedures and allowed for comparisons to the MEL synthesis as well as the literature. GC results compared to *trans*-isomer standards gave insight into the true purity concerning *trans* content which could be extrapolated onto the MEL synthesis. (*Paper VI, manuscript*)

Hopefully these new synthetic strategies can now allow access to pure MELs to be used for biological screenings and tests which might give us further glimpses into their function and purpose in animals and deepen further our understanding of the complexity of life.

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List of publications

- Paper I: Asymmetric synthesis of methoxylated ether lipids. A Glyceryl Glycidyl ether key building block design, preparation and synthetic application

- Paper II: Asymmetric synthesis of methoxylated ether lipids. Total synthesis of n-3 polyunsaturated Docosahexaenoic acid-like methoxylated ether lipid

- Paper III: Asymmetric synthesis of methoxylated ether lipids. Total synthesis of two monounsaturated C18:1 and a saturated C18:0 methoxylated ether lipid derivative

- Paper IV: Asymmetric synthesis of methoxylated ether lipids: Total synthesis of a triene C18:3 omega-8 MEL derivative (Manuscript accepted by ChemistrySelect)

Paper I

Paper II

Paper III

Paper IV