Norwegian University
of Life Sciences

Master's Thesis 2018 60 ECTS
Faculty of Chemistry, Biotechnology and Food Science

A New Method for the Synthesis of Fatty Acid Amides

Christiane Færestrand Ellefsen
Teacher Education in Natural Sciences

Acknowledgements

The work described in this thesis was done at the department of Chemistry, the Faculty of Chemistry, Biotechnology and Food Science, at the Norwegian University of Life Sciences.

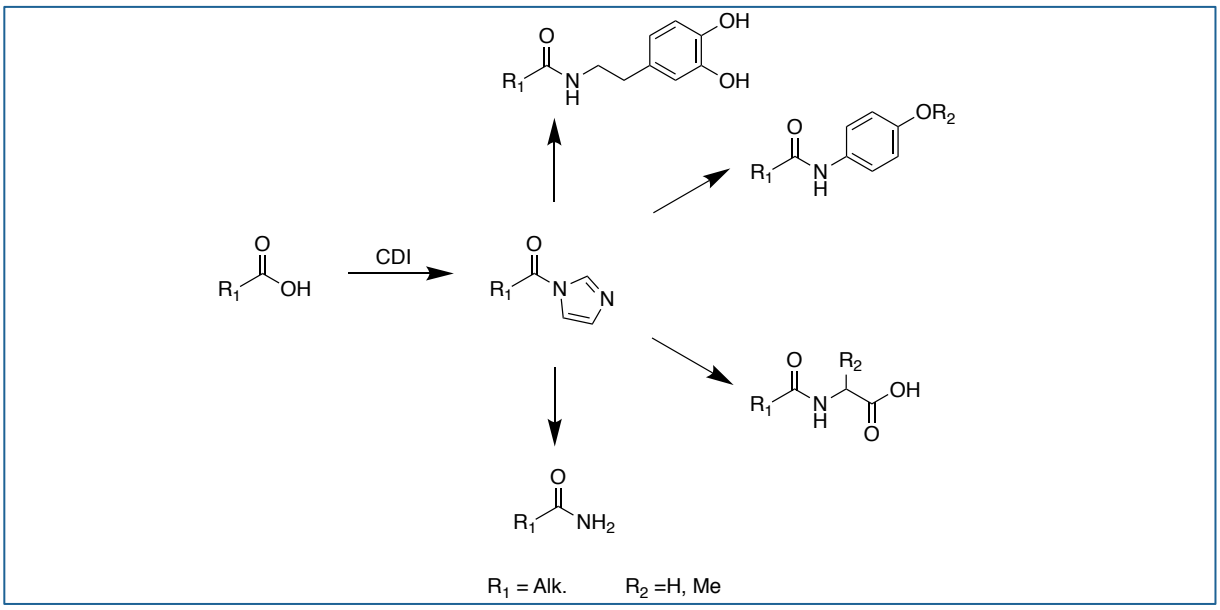
I wish to express my gratitude towards my main supervisor, Professor Yngve H. Stentrøm, and my supervisors Dr. Simen Antonsen and Dr. Jens M. J. Nolsøe, for allowing me this experience, and for your continuous aid and encouragement. You have all inspired me tremendously!

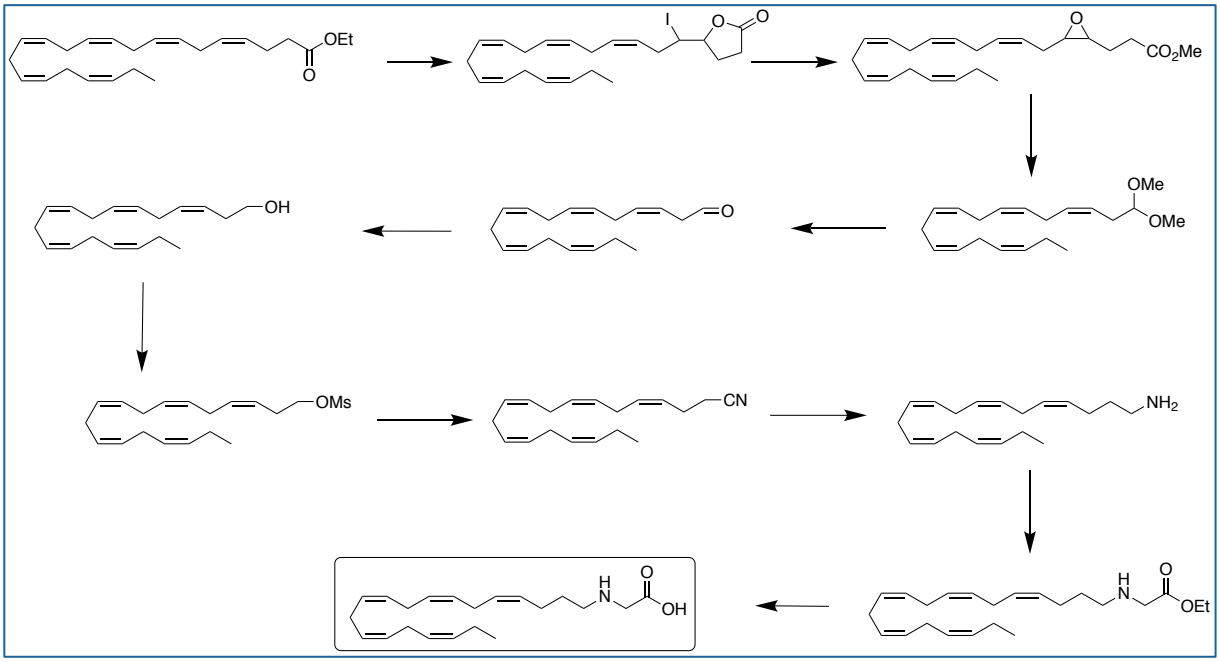
I also wish to thank Professor Dag Ekeberg and Senior Engineer Hanne Devle for performing HR-MS analyses, and Senior Engineer Anne Gravdal for ordering chemicals. To Dr. Ida Aarum for helpful advice now and again, and to the rest of the chemistry department, both students and staff, for participating in making me feel right at home.

I particularly wish to thank Mali Ristun, with whom I worked together on part of this project, for great cooperation and for being a terrific companion. To Malene Gaulin and Erik Tylleskär, for making each day at the lab a real joy; it would not have been the same without you.

Last but not least, I wish to thank my family and friends for putting up with me these past few months, and for your ever-present love and support. You're the best!

Graphical abstract





Abstract

The amide bond is an essential part of many natural products and is also found in a lot of today's pharmaceuticals. The fatty acid amides are a group of natural products that has shown biological activity towards several receptors in both the peripheral and the central nervous system. The traditional methods for the formation of amide bonds tend to give low to moderate yields when utilized on long chain fatty acids, and so, an effective and reliable method for the synthesis of these interesting compounds seems to be in demand.

In this thesis, a method using *N,N'*-carbonyldiimidazole (CDI) as coupling reagent in the preparation of fatty acid amides is tested.

A total of 20 fatty acid amides have been prepared, within the groups *N*-acyldopamines, *N*-acylamino phenols, *N*-acylamino acids, and primary fatty acid amides. Among these, the *N*-acylamino acids gave the best results, with yields over 80 %. The primary fatty acid amides were made in low yields with saturated fatty acids, but quantitative yields with the polyunsaturated fatty acids. Both the *N*-acyldopamines and the *N*-acylamino phenols were prepared in moderate yields.

A synthesis path towards an analogue of ω-3 DPA using DHA as starting material, was also followed. The first 8 steps were conducted; in a total yield of 7 %. The final two steps remain to be done. Several of the reactions were not optimized, and improvements of the yields should be possible.

Sammendrag

Amidbindingen er en essensiell del av mange naturstoffer, og er også å finne i mange av de legemidler som benyttes i dag. Fettsyreamider er en gruppe naturstoffer som har vist biologisk aktivitet mot flere reseptorer i både det perifere og det sentrale nervesystemet. Tradisjonelle metoder for dannelse av amidbindinger gir ofte lavt til moderat utbytte når de benyttes med lange fettsyrekjeder. Dermed virker det å være et behov for effektive og pålitelige metoder for syntese av disse interessante forbindelsene.

I denne oppgaven blir en metode der *N,N'*-karbonyldiimidazol (CDI) anvendes som koblingsreagens i tillagingen av fettsyreamider utprøvd.

Totalt 20 fettsyreamider er blitt laget, innenfor gruppene *N*-acyldopaminer, *N*-acylamino-fenoler, *N*-acylaminosyrer og primære fettsyreamider. Blant disse var det *N*-acylaminosyrene som ga best resultater, med utbytter over 80 %. De primære fettsyreamidene ble laget i lave utbytter med mettede fettsyrer, men i kvantitative utbytter med flerumettede fettsyrer. Både *N*-acyldopaminene og *N*-acylaminofenolene ble tillaget i moderate utbytter.

En syntesevei mot en analog av ω-3 DPA med DHA som utgangsmateriale, ble også fulgt. De første 8 stegene ble utført, med et samlet utbytte på 7 %. De siste to trinnene gjenstår. Flere av reaksjonene ble ikke optimalisert, og forbedringer av utbyttene bør være mulig.

Abbreviations and trivial names

2-AG	2-arachidonoylglycerol
AA	(*5Z, 8Z, 11Z, 14Z*)-eicosa-5,8,11,14-tetraenoic acid
Acetaminophen	*N*-(4-hydroxyphenyl)acetamide
AEA	*N*-(*5Z, 8Z, 11Z, 14Z*)-(2-hydroxyethyl) eicosa-5,8,11,14-tetraenoic amide
AM404	*N*-4-hydroxyphenyl-(*5Z,8Z,11Z,14Z*)-eicosa-5,8,11,14-tetraenoic amide
BOP	Benzotriazol-1-yloxytris (dimethylamino) phosphonium hexafluorophosphate
Capsaicin	*N*-(*6E*)-(3-methoxy-4-hydroxy-benzyl)-8-methyl-6-nonenamide
CB1	Cannabinoid receptor 1
CB2	Cannabinoid receptor 2
CDI	*N,N*′-carbonyldiimidazole
Chloramphenicol	2,2-dichloro-*N*-[1,3-dihydroxy-1-(4-nitrophenyl) propan-2-yl] acetamide
COX	Cyclooxygenase
DCC	*N,N*-dicyclohexylcarbodiimide
DCM	Dichloromethane
DHA	(*4Z, 7Z, 10Z, 13Z, 16Z, 19Z*)-docosa-4,7,10,13,16-hexaenoic acid
DHA-EE	Ethyl ester of docosahexaenoic acid
DPA	(*7Z, 10Z, 13Z, 16Z, 19Z*)-docosa-7,10,13,16,19-pentaenoic acid
EDC	*N*-ethyl-*N'*-dimethylaminopropylcarbodiimide
EPA	(*5Z, 8Z,11Z, 14Z, 17Z*)-eicosa-5,8,11,14,17-pentanoic acid
EPA-EE	Ethyl ester of eicosapentaenoic acid
EtOAc	Ethyl acetate
FAA	Fatty acid amide
GPR18	G protein-coupled receptor 18
HMDS	Hexamethyldisilazane
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole
LOX	Lipoxygenase
N-palmitoylethanol-amine	*N*-(2-hydroxyethyl) hexadecanoic amide
NAA	*N*-acylamino acids
NAE	*N*-acylethanolamines
NADA	*N*-arachidonoyldopamine
NAGly	*N*-*a*rachidonoylglycine
NAP	*N*-acylamino phenols
NDA	*N*-acyldopamines

N-docosatetraenoyl-ethanolamine	*N*-(*7Z, 10Z, 13Z, 16Z*)-(2-hydroxyethyl) docosa-7,10,13,16-tetraenoic amide
OA	Octadecanamide
Octadecapentaenoic acid	(*3Z, 6Z, 9Z, 12Z, 15Z*)-octadeca-3,6,9,12,15-pentaenoic acid
OLDA	*N*-(3,4-dihydroxy-benzylmethyl)-(*9Z*)-octadeca-9-enoic amide
PFAM	Primary fatty acid amides
PGE2	*(5Z, 11α, 13E, 15S)*-11,15-dihydroxy-9-oxo-prosta-5,13-dien-1-oic acid
PUFA	Polyunsaturated fatty acid
Sphingosine	(*4E*)-1,3(*R*)-dihydroxy-(*2S*)-amino-octadec-4-ene
Taurine	2-aminoethanesulfonic acid
THC	Δ^9-tetrahydrocannabinol
THF	Tetrahydrofuran
TRPV1	Transient receptor potential vanilloid-type 1 channel
TRPV4	Transient receptor potential vanilloid-type 4 channel

General remarks

IUPAC nomenclature is used in the naming of compounds. Figures and schemes are drawn using ChemDraw Professional 17.0.

Table of Contents

Acknowledgements ii
Graphical abstract iii
Abstract iv
Sammendrag v
Abbreviations and trivial names vi
General remarks viii
1 Introduction 1
1.1 Natural Products 1
1.1.1 Biological activity of fatty acid amides 3
1.1.2 *N*-acylethanolamines 4
1.1.3 *N*-acyldopamines 5
1.1.4 *N*-acylaminophenols 6
1.1.5 *N*-acylamino acids 7
1.1.6 Primary fatty acid amides 8
1.2 Chemical background 9
1.2.1 Synthesis of PUFA metabolites, derivatives and analogues 9
1.2.2 Amide bond formation 11
1.3 Aim and background 15
2 Results and discussion 16
2.1 Fatty acid amide-syntheses 16
2.1.1 Synthesis of *N*-acyldopamides 16
2.1.2 Synthesis of *N*-acylamino phenols 17
2.1.3 Synthesis of *N*-acylamino acids 17
2.1.4 Synthesis of primary fatty acid amides 18
2.1.5 Summary 22
2.2 Synthesis of 3-aza ω-3 DPA 24
3 Conclusion 27
4 Experimental 28
4.1 General hydrolyzing method 28
4.2 General amidation method 28
Synthesis of *N-3,4*-dihydroxybenzylmethyl-(*5Z, 8Z, 11Z, 14Z*)-eicosa-5,8,11,14-tetraenoic amide 29
Synthesis of *N*-3,4-dihydroxybenzylmethyl-*(9Z)*-octadeca-9-enoic amide 30
Synthesis of *N-3,4*-dihydroxybenzylmethyl-*(9Z, 12Z)*-octadeca-9,12-dienoic amide 31

Synthesis of *N*-4-hydroxyphenyl-*(5Z,8Z,11Z,14Z)*-eicosa-5,8,11,14-tetraenoic amide .. 32
Synthesis of *N*-4-hydroxyphenyl-(*9Z*)-octadeca-9-enoic amide 33
Synthesis of *N-4*-methoxyphenyl-(*9Z*)-octadeca-9-enoic amide 34
Synthesis of *N-4*-methoxyphenyl hexadecanoic amide 35
Synthesis of amino carbomethoxy methane hydrochloride 36
Synthesis of 1(*S*)-amino-1-carbomethoxy ethane hydrochloride 37
Synthesis of 1*(R)*-amino-1-carbomethoxy ethane hydrochloride 37
Synthesis of *N*-carbomethoxy methyl-*(5Z, 8Z, 11Z, 14Z)*-eicosa-5,8,11,14-tetraenoic amide 38
Synthesis of *N*-carboxymethyl-*(5Z, 8Z, 11Z, 14Z*)-eicosa-5,8,11,14-tetraenoic amide... 39
Synthesis of *N*-carbomethoxy methyl butanoic amide 40
Synthesis of *N*-carboxymethyl butanoic amide 41
Synthesis of *N*-carbomethoxy methyl hexanoic amide 42
Synthesis of *N*-carboxymethyl hexanoic amide 43
Synthesis of *N*-carbomethoxy methyl decanoic amide 44
Synthesis of *N*-carboxymethyl decanoic amide 45
Synthesis of *N*-carbomethoxy methyl propanoic amide 46
Synthesis of *N*-carboxymethyl propanoic amide 47
Synthesis of *N*-1*(S)*-carbomethoxyethyl decanoic amide 48
Synthesis of *N*-1*(S)*-carboxyethyl decanoic amide 49
Synthesis of *N*-1*(R)*-carbomethoxyethyl decanoic amide 50
Synthesis of *N*-1*(R)*-carboxyethyl decanoic amide 51
Synthesis of *(5Z, 8Z, 11Z, 14Z, 17Z)*-eicosa-5,8,11,14,17-pentaenoic acid 52
Synthesis of *(4Z, 7Z, 10Z, 13Z, 16Z, 19Z)*-docosa-4,7,10,13,16,19-hexaenoic acid 53
Synthesis of *(9Z*)-octadeca-9-enoic amide 54
Synthesis of hexadecanoic amide 55
Synthesis of *(9Z, 12Z)*-octadeca-9,12-dienoic amide 56
Synthesis of octadecanoic amide 57
Synthesis of *(4Z, 7Z, 10Z, 13Z, 16Z, 19Z)*-docosa-4,7,10,13,16,19-hexaenoic amide 58
Synthesis of *(5Z, 8Z, 11Z, 14Z, 17Z)*-eicosa-5,8,11,14,17-pentaenoic amide 59
Synthesis of (*7Z, 10Z, 13Z, 16Z, 19Z*)-*5*-iodo-docosa-7,10,13,16,19-pentaenoic-4-lactone 60
Synthesis of (*7Z, 10Z, 13Z, 16Z, 19Z*)-methyl 4,5-epoxy-docosa-7,10,13,16,19-pentaenoate 61
Synthesis of (*3Z, 6Z, 9Z, 12Z, 15Z*)-1,1-dimethoxyoctadeca-3,6,9,12,15-pentaene 62

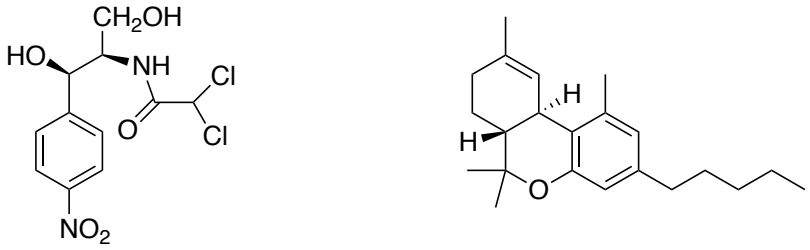
Synthesis of (*3Z, 6Z, 9Z, 12Z, 15Z*)-octadeca-3,6,9,12,15-pentaenal 63
Synthesis of (*3Z, 6Z, 9Z, 12Z, 15Z*)-octadeca-3,6,9,12,15-pentaen-1-ol 64
Synthesis of (*3Z, 6Z, 9Z, 12Z, 15Z*)-octadeca-3,6,9,12,15-pentaenyl methanesulfonate. 65
Synthesis of (*4Z, 7Z, 10Z, 13Z, 16Z*)-nonadeca-4,7,10,13,16-pentaenenitrile..... 66
Synthesis of (*4Z, 7Z, 10Z, 13Z, 16Z*)-nonadeca-4,7,10,13,16-pentaenal 67
Synthesis of (*4Z, 7Z, 10Z, 13Z, 16Z*)-nonadeca-4,7,10,13,16-pentaen-1-amine 68
5 References 69
6 Appendix i
6.1 Additional reactions i
Synthesis of hexanoic acid i
Synthesis of (2-acetoxy)-propanoic acid ii
6.2 Spectra iii

1 Introduction

1.1 Natural Products

All living organisms require a range of organic compounds to complete their life cycles. Such compounds are referred to as natural products. The natural products are divided into primary and secondary metabolites. Primary metabolites are found in nearly all living beings, and within this group we find carbohydrates, amino acids, fats and nucleic acids; compounds that are vital for the survival of the organisms. The secondary metabolites are specific to certain organisms, and unlike the primary metabolites, their presence is not vital to the organism. Instead, they tend to have special properties that are beneficial to the organism, such as partaking in various defense mechanisms.[1]

The synthesis of natural products has become a significant focus of the pharmaceutical industry as a lot of today's drugs are either natural products themselves, or analogues of such. For example, when it comes to small-molecule anticancer drugs, only about 17 % of the approved drugs are completely synthetic compounds, leaving the remaining 83 % as either isolated natural products, or derivatives, modifications, or mimics of such.[2] One example is chloramphenicol, a natural product isolated from the bacteria *Streptomyces venezuelae*, which was later synthesized and used as a broad-spectrum antibiotic drug.[3] Another example is Δ^9-tetrahydrocannabinol (THC), a constituent of the plant *Cannabis sativa,* and source of the effects associated with the narcotic drugs known as marijuana, hashish or cannabis which is derived from this plant.[4] These drugs are also used for medicinal purposes in parts of the world.



Chloramphenicol THC

Figure 1-1 Molecular structures of the natural products chloramphenicol and THC.

One major class of natural products is the lipids; a structurally diverse group carrying out a variety of important tasks in nature. IUPAC defines them as "(…) substances of biological origin that are soluble in nonpolar solvents".[5] One property the lipids are responsible for is the selective permeability of all eukaryotic cell membranes; a property that is crucial for the cell's

ability to carry out its operations. A big part of the cell membrane consists of lipids, and mainly three groups; namely phospholipids, glycolipids and sterols. Phospholipids are, as the name suggests, lipids containing phosphate, whereas the glycolipids contain a carbohydrate moiety. Either of these groups can be bound to a fatty acid, which provides these otherwise non-lipid molecules with lipid properties.[6]

Fatty acids (FA) are defined as aliphatic monocarboxylic acids with a chain of 4-28 carbons, a chain that can be either saturated or unsaturated.[5] Polyunsaturated fatty acids (PUFA) and especially the ω-3 PUFAs such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are of particular interest, as ingestion of these is viewed to be beneficial to human health.[7-8] Another important PUFA is the ω-6 fatty acid arachidonic acid (AA). Together with EPA, AA gives rise to a family of important C-20 compounds called eicosanoids. The PUFAs' metabolism thus leads to a range of compounds with different functions and effects in nature and in the human body. The prostaglandins for instance, are eicosanoids known to inhibit gastric acid secretion, control blood pressure and act as mediators of inflammation.[9]

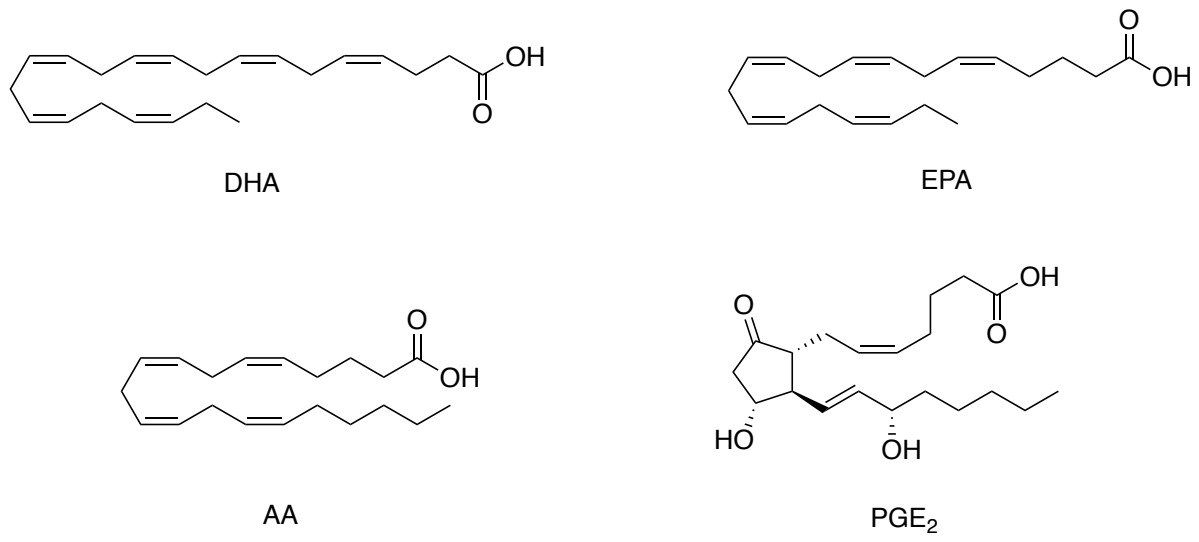


Figure 1-2 Molecular structures of the ω-3 PUFAs DHA and EPA, the ω-6 PUFA AA, and the prostaglandin PGE2, a metabolite of AA.

Besides the prostaglandins and other prostanoids which are metabolized from either the ω-3 or ω-6 FAs by an enzyme class called cyclooxygenases (COX), PUFAs also give rise to many other compounds. AA is converted into leukotrienes and lipoxins by another enzyme class; the lipoxides (LOX), whereas EPA and DHA are converted into protectins, maresins, and resolvins by these same enzymes. These compounds are also involved in inflammation processes. Some act as pro-inflammatory mediators, such as PGE_2 and other AA-metabolites, whilst the EPA

and DHA-metabolites are believed to either inhibit or resolve the inflammation process.[10-12] The ω-6 metabolites are also generally vasoconstrictors, whereas the ω-3 metabolites are vasodilators.[13]

The PUFA metabolites are synthesized on demand and are swiftly degraded thereafter through β-oxidation, a process that leads to a chain shortening of two carbons by each cycle. This degradation happens in the mitochondria and the peroxisomes in the cells.[14]

1.1.1 Biological activity of fatty acid amides

The fatty acid amides (FAA) have a simple general structure: $R_1CONR_2R_3$. They are often divided into subgroups according to what amine they are made up of, which gives us the *N*-acylethanolamines (NAE), *N*-acyldopamines (NAD), *N*-acylamino phenols (NAP), *N*-acylamino acids (NAA), primary fatty acid amides (PFAM), and a large number of other *N*-acylamides, among which we find the ceramides and sphingomyelins i.e.[15]

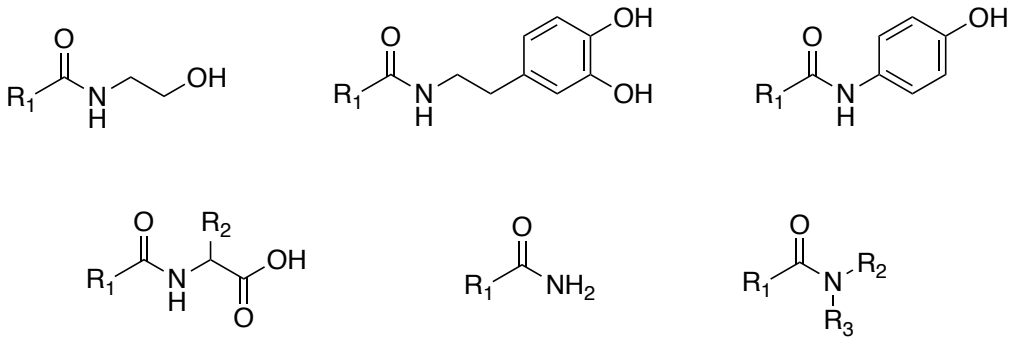
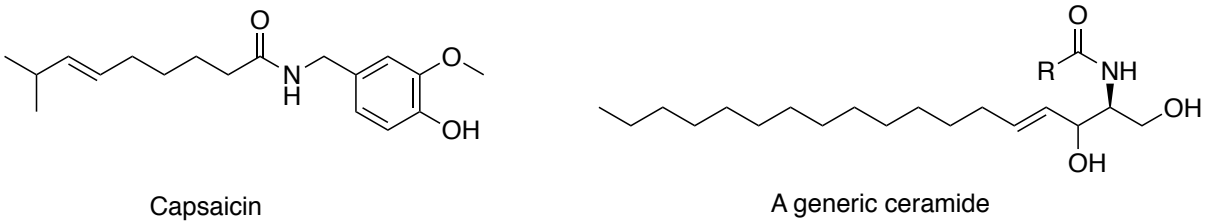


Figure 1-3 General structures of NAEs, NADs, NAPs, NAAs, PFAMs and other *N*-acylamides. R_1 = alkyl, R_2, R_3 =H, alkyl, acyl, aryl etc.

One *N*-acylamide that has long been known is capsaicin; the compound responsible for the burning orosensation that is experienced upon eating chili peppers. Capsaicin binds to Transient Receptor Potential Vanilloid-type 1 ($TRPV_1$), a heat-activated receptor which also responds to chemical toxins.[16]

Other FAAs that have been recognized for a long time are the ceramides; a sub-group to the sphingolipids. Ceramides consist of the amino-alcohol sphingosine bound to a fatty acid residue through an amide bond. They are known to induce apoptosis, and has due to this property been suggested as anticancer agents.[17] Ceramide metabolism is also highly active during lung development, and it plays an important role in maintaining a healthy lung, both structurally and functionally.[18]

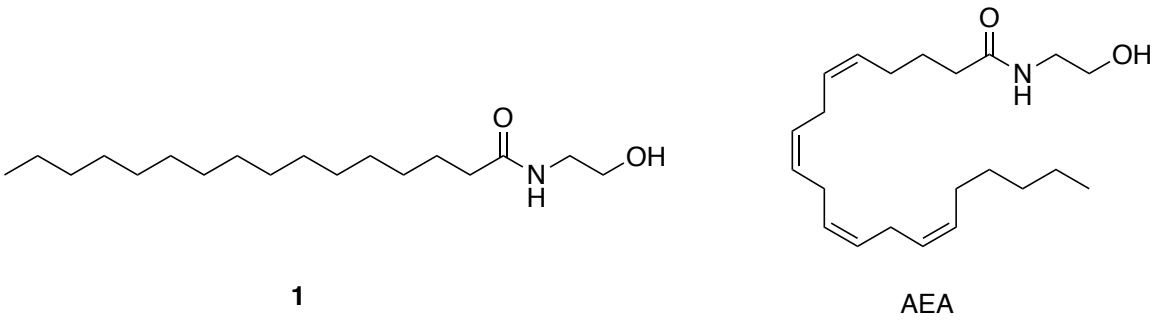


Capsaicin A generic ceramide

Figure 1-4 Molecular structures of the *N*-acyl amide capsaicin, and the general structure of a ceramide.

1.1.2 *N*-acylethanolamines

In 1957 *N*-palmitoylethanolamine (**1**) was discovered as an anti-inflammatory agent present in egg yolk.[19] Since then, several other NAEs have been discovered. The first to receive extensive interest was anandamide (AEA), which was identified as an endogenous ligand to the cannabinoid receptors CB_1 and CB_2 in the mammalian brain in 1992.[20] These receptors were previously known to be a binding site to THC, and anandamide was thus assigned as the natural ligand for CB_1, and as an important member of the endocannabinoid system, which in addition consists of the receptors CB_1 and CB_2, the ligand of CB_2 2-arachidonoylglycerol (2-AG), the enzyme fatty acid amide hydrolase (FAAH) and the membrane protein AEA transporter. Research has later shown that AEA is involved in pain-suppressing, it can induce over-eating, and it is involved in the regulation of emotions, in addition to several other properties, all of which makes it an interesting objective for further research.[21-23] AEA also binds to the capsaicin-receptor $TRPV_1$.[24-25] AEA is readily hydrolyzed into AA and 2-aminoethanol by FAAH in the cells.[26-27] One approach towards prolonging and enhancing its effects is therefore to find potent inhibitors of FAAH to prevent this degradation.



1 AEA

Figure 1-5 Molecular structures of the NAEs palmitoylethanolamine (**1**) and anandamide (AEA).

Several other NAEs have been isolated from the mammalian brain and peripheral tissues. *N*-oleoyl- (**2**) and *N*-stearoylethanolamine (**3**) are, together with **1** the most abundant in brain tissues, but AEA, *N*-linoleoyl- (**4**), *N*-linolenoyl- (**5**), *N*-dihomo-γ-linolenoyl- (**6**), and *N*-docosatetraenoylethanolamine (**7**) are also present, in lower concentrations.[28-29]

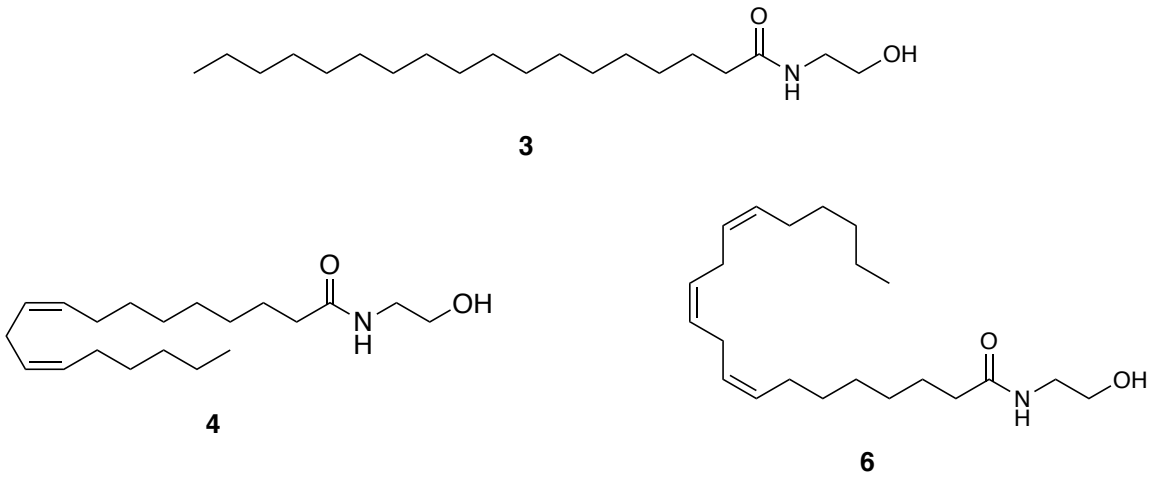


Figure 1-6 Molecular structures of the NAEs stearoylethanolamine (**3**), linoleoylethanolamine (**4**) and dihomo-γ-linolenoylethanolamine (**6**).

1.1.3 *N*-acyldopamines

Members of the NAD family are also active towards the endocannabinoid system. Both *N*-arachidonoyldopamine (NADA) and *N*-oleoyldopamine (OLDA) are key compounds in this group. The NADs target both $TRPV_1$ and CB_1 receptors.[30-31] NADA has been shown to bind to $TRPV_1$ with a potency similar to that of capsaicin.[30] It does not, however, bind to dopamine-receptors.[31] The long alkyl tail as well as the vanillyl-like head makes NADA and capsaicin structurally similar, which might explain the similarity in affinity towards $TRPV_1$. OLDA also binds strongly to $TRPV_1$, producing hyperalgesia, but it exhibits only weak interactions at CB_1.[32] Docosapentaenoyl- (**8**), stearoyl- (**9**) and palmitoyldopamine (**10**) have also been isolated from the mammalian brain. Both **9** and **10** bind to CB_1 and $TRPV_1$ with low affinities. Their roles are not fully known, but they have been shown to strengthen the effects of NADA and AEA.[33]

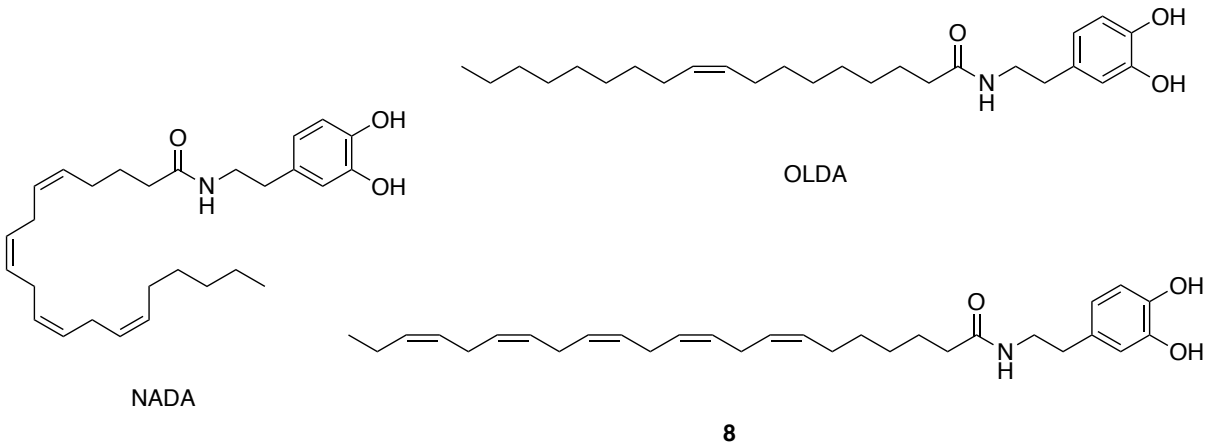


Figure 1-7 Molecular structures of the NADs NADA, OLDA and ω-3 DPA dopamine (**8**).

A handful synthetic NADs have been tested towards the endocannabinoid system, namely stearidonoyl- (**11**), pinolenoyl- (**12**), α-linolenoyl-dopamine (**13**), as well as **8** and the NAD of EPA (**14**).[31] The ω-6 NADs are the strongest inhibitors of FAAH. Several of the NADs also inhibit the AEA transporter, and all bind to CB_1.[31]

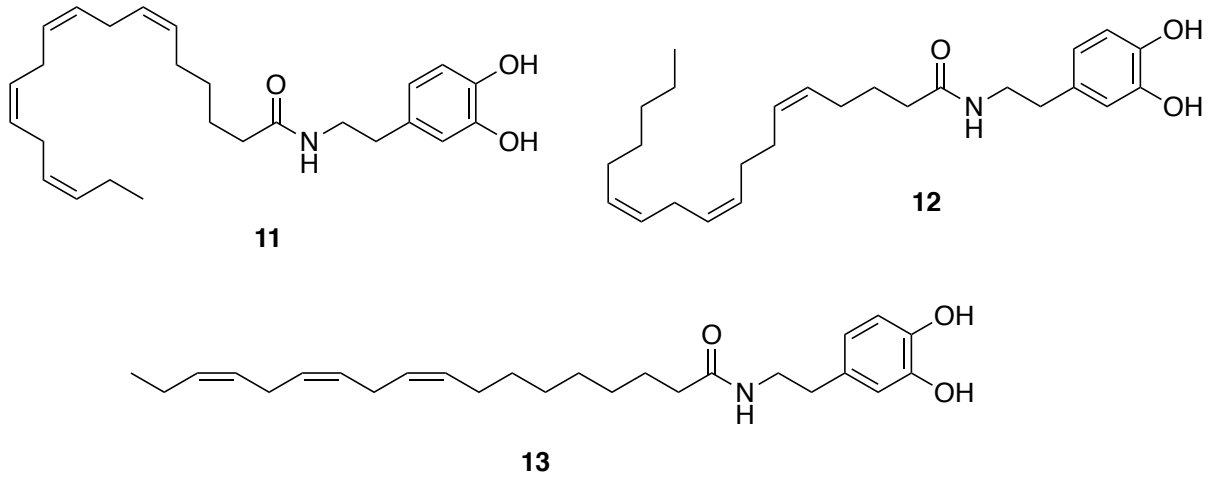
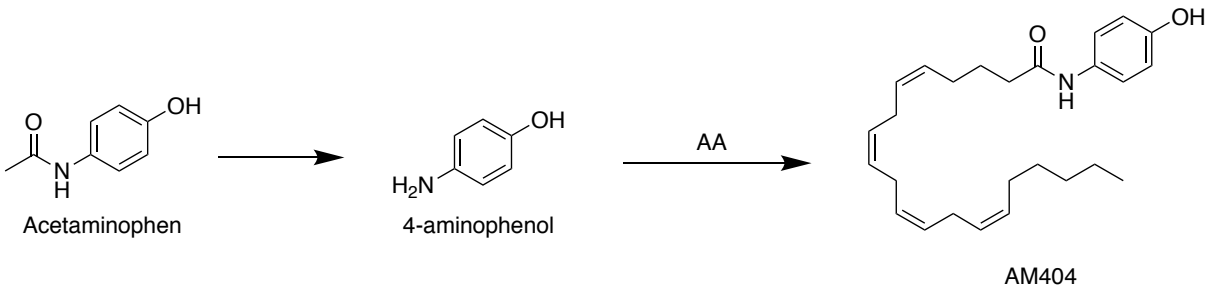


Figure 1-8 Molecular structures of the NADs stearidonoyldopamine (**11**), pinolenoyldopamine (**12**) and α-linolenoyldopamine (**13**).

1.1.4 *N*-acylaminophenols

Another FAA that is known to bind to the cannabinoid receptors is AM404, a metabolite of the commonly used analgesic and antipyretic drug paracetamol or acetaminophen, and AA presumably (Scheme 1-1). AM404 is an activator of $TRPV_1$ and inhibits uptake of AEA into the cells.[34] Additionally, AM404 inhibits members of the COX-family. Due to these properties, AM404 is believed to be the active metabolite behind the effects of the popular drug.[35]



Scheme 1-1 Supposed metabolism of acetaminophen to AM404.

Synthetic palmitoyl-aminophenols have been tested for inhibitory action against FAAH, and for affinity towards CB_1, however there seems to be only weak interactions.[36] Synthetic NAPs with shorter alkyl chains (C-12 and C-10) have also been prepared and tested for anticancer and antioxidant activity, but were found to be weakly active at best.[37]

1.1.5 *N*-acylamino acids

Amongst the NAAs, the *N*-arachidonoylamino acids are the most prominent and well known, especially *N*-arachidonoyl glycine (NAGly), which suppresses tonic inflammatory pain.[38-39] NAGly is structurally very similar to AEA, and it has been suggested to be a metabolite of AEA, and that its biosynthesis happens through oxidation of AEA.[40] Despite this similarity, NAGly shows no signs of activity towards neither CB_1 nor CB_2.[41]

The *N*-arachidonoylamino acids have been tested for inhibitory action against FAAH, and the different compounds' efficacy in this respect seems species-dependent. *N*-arachidonoyl-isoleucine (**15**) for instance, is capable of inhibiting only human FAAH, whereas NAGly is known to inhibit FAAH in rodent tissues much more efficiently than in human tissues.[42] It is generally supposed that the inhibition of FAAH allows the concentration of AEA to remain elevated for longer, and that the source of the pain suppressing and anti-inflammatory effects of NAGly and its fellow NAAs lies here.[43]

In addition to inhibitory action, NAGly has been identified as the endogenous ligand of GPR18, a G-protein coupled receptor present in the immune system, which has lately been nominated as another member of the endocannabinoid system.[44]

Several other glycine NAAs have been discovered, such as *N*-butyrylglycine (**16**), *N*-hexanoyl-glycine (**17**), *N*-decanoylglycine (**18**), and *N*-benzoylglycine (**19**).[15]

Other amino acids that are present in this group are alanine, *γ*-aminobutyric acid, serine, phenylalanine, taurine and valine.[15] *N*-Arachidonoyltaurine (**20**) is another FAA that binds to $TRPV_1$, and it is also active towards another receptor of this family; $TRPV_4$.[45]

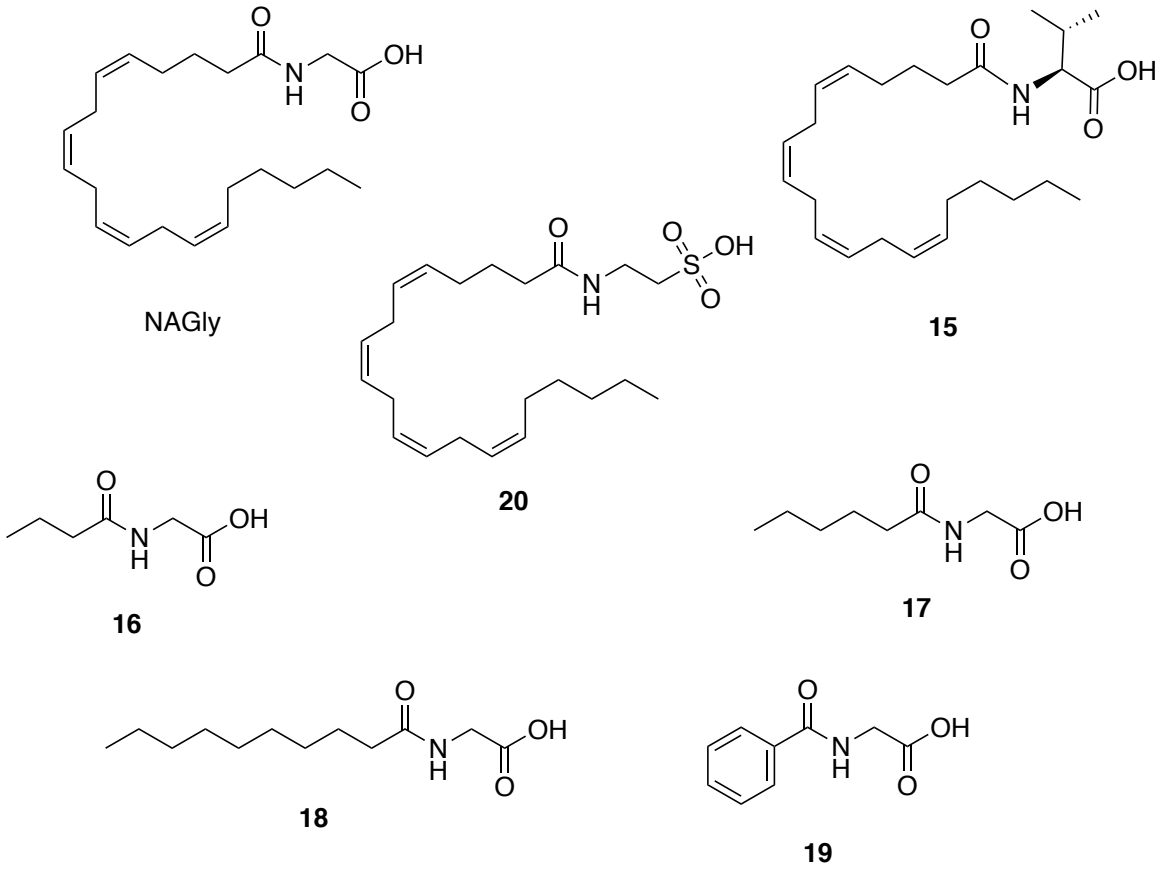


Figure 1-9 Molecular structures of the NAAs NAGly, arachidonoylisoleucine (**15**), arachidonoyltaurine (**20**), butyrylglycine (**16**), hexanoylglycine (**18**), decanoylglycine (**17**) and benzoylglycine (**19**).

1.1.6 Primary fatty acid amides

In 1989, Arafat *et al.*[46] reported the isolation of the five PFAMs oleamide (OA), palmitamide (**21**), palmitoleamide (**22**), elaidamide (**23**) and linoleamide (**24**), from human plasma. OA was later isolated from the cerebrospinal fluid of cat, rat and human, and it was discovered that it can induce physiological sleep in rats.[47] Other effects that have been connected to OA is the regulation of memory processes, hypothermia, activation of serotonin-receptor subtypes and more.[15] It is believed to interact either directly or indirectly with CB_1 and $TRPV_1$, as well as with several other receptors usually not associated with the endocannabinoid system.[48] Other PFAMs have also been found to possess biological properties; **24** increases Ca^{2+} flux,[49] whereas erucamide (**25**) is the main angiogenic lipid in bovine mesentery tissue.[50]

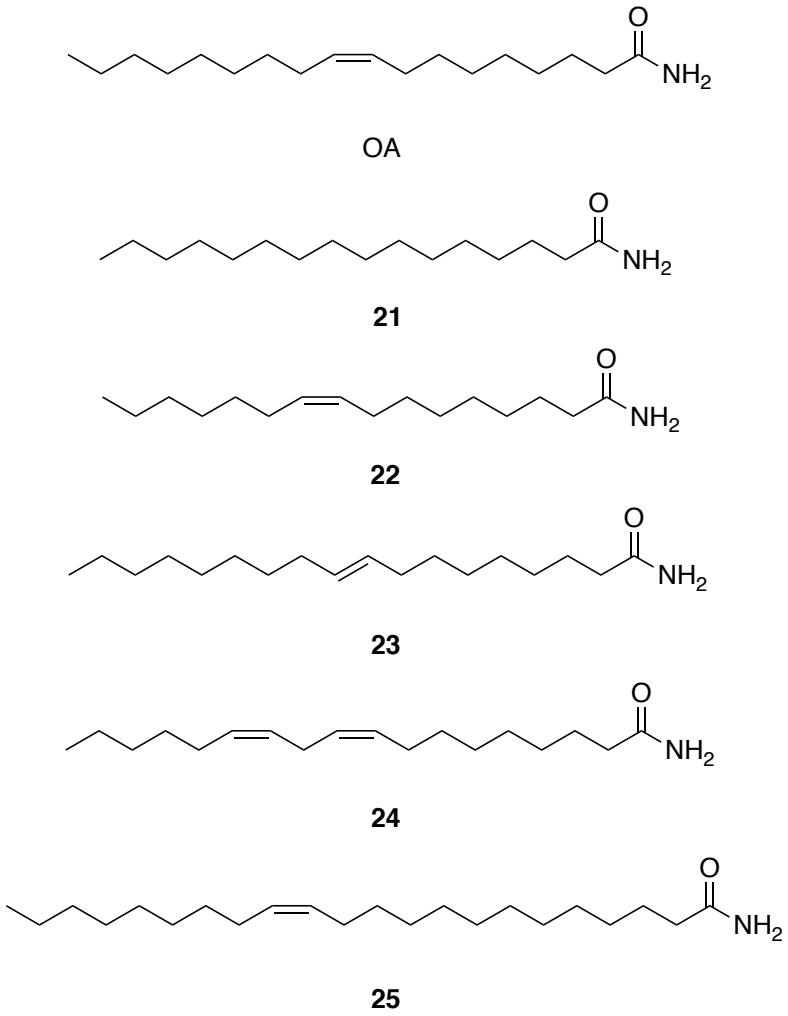


Figure 1-10 Molecular structures of the PFAMs oleamide (OA), palmitamide (**21**), palmitoleamide (**22**) elaidamide (**23**), linoleamide (**24**) and erucamide (**25**).

1.2 Chemical background

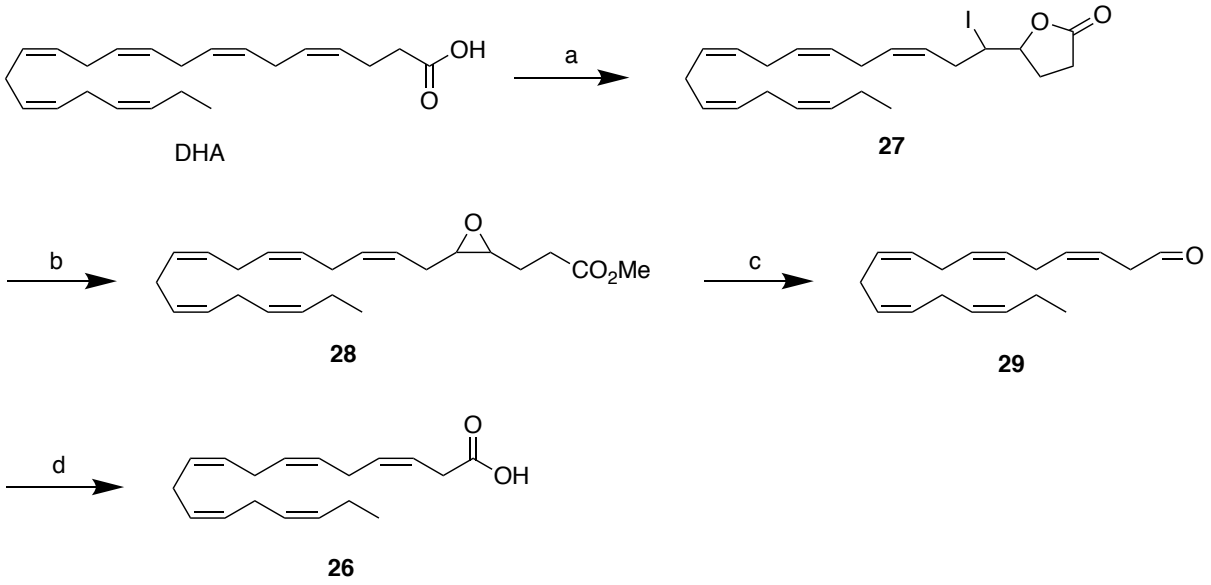
Analogues of natural products sometimes exhibit enhanced or more specific pharmacological effects than the naturally occurring compounds. For this reason, it is of interest to synthesize natural product analogues. These compounds can also be tailored to be absorbed, distributed, metabolised and excreted according to ones needs. This makes the analogues more suitable for medicinal purposes than the natural products themselves.

1.2.1 Synthesis of PUFA metabolites, derivatives and analogues

As discussed earlier, PUFA metabolism leads to a variety of compounds with important and interesting abilities. Syntheses of such metabolites, derivatives and analogues with PUFAs as starting material can also be done in the lab, an approach discussed by Vik and Hansen[51] in a recently published review. This method was first used by Corey *et al.*[52] who synthesized

epoxides from AA using regioselective internal epoxidation in 1979. These epoxides were later used to synthesize oxidation products towards leukotrienes by the same group.[53-54]

Corey *et al.*[55] later used iodo-lactonization to separate DHA and EPA from a mixture, exploiting that they would end up as γ- and δ-iodo-lactones respectively. Kuklev *et al.*[56] later used this method in the synthesis of octadecapentaenoic acid (**26**) from DHA, a synthesis that further entailed epoxidation of iodo-lactone **27** into epoxide **28**, which was converted into the aldehyde **29**. The final step was oxidation of **29**, yielding fatty acid **26**.



Scheme 1-2 The synthesis of **26** as described by Kuklev *et al.*[56] Reagents: (a) K_2CO_3, I_2, EtOH; (b) Et_3N, MeOH; (c) H_5IO_6, Et_2O; (d) CrO_3, H_2SO_4, H_2O, acetone.

Over the last few decades, Skattebøl and associates have reported a series of semi-syntheses with PUFAs as starting materials, using the above-mentioned methods and others.[57-62]

Some of the compounds that have been reported are a group 3-hetero PUFA analogues, where the β-carbon is substituted with oxygen or sulfur.[63-65] Compounds **30**-**32** which are shown in figure 1.11 are examples of such analogues. The goal of these studies is to retain the biological properties of the PUFAs whilst making them resilient against β-oxidation.

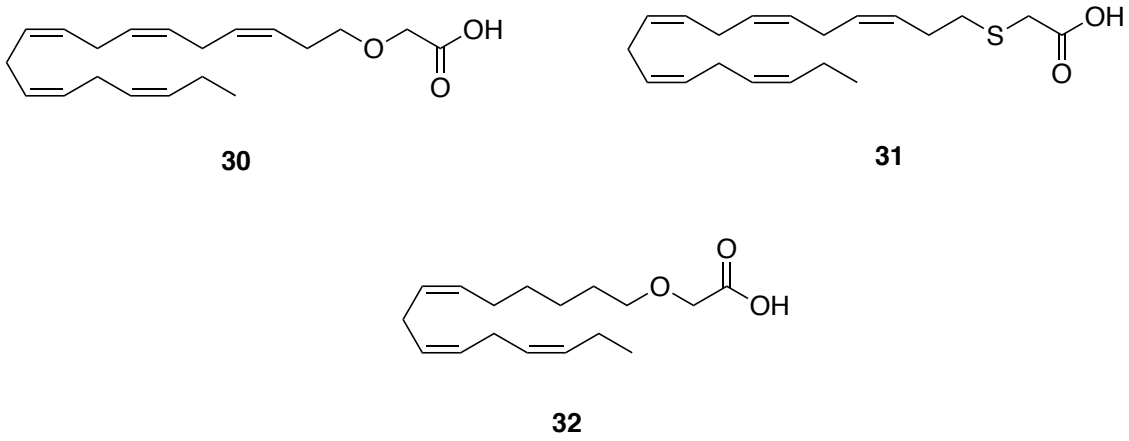
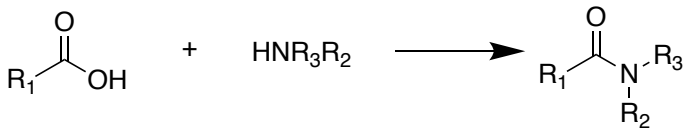


Figure 1-11 3-oxa and 3-thia PUFA analogues.

1.2.2 Amide bond formation

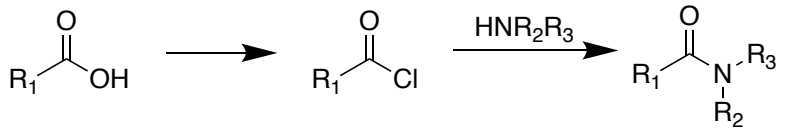
The acylation of nitrogen to form an amide bond is the most common type of acylation in the preparation of small molecule drug candidates.[66] The amide bond is also an essential part of all peptides and proteins; natural products that are indispensable in basically all biological processes, and the formation of amide bonds is therefore also of significant importance in peptide synthesis.

Several methods have been reported as useful for the formation of amide bonds. The simplest method involves having a carboxylic acid, or the corresponding ester, react with an amine (Scheme 1-3).[67] This method usually gives low yields, and requires high temperatures. Because of this, different methods to activate the carboxylic acid before it is reacted with the amine, have been developed.



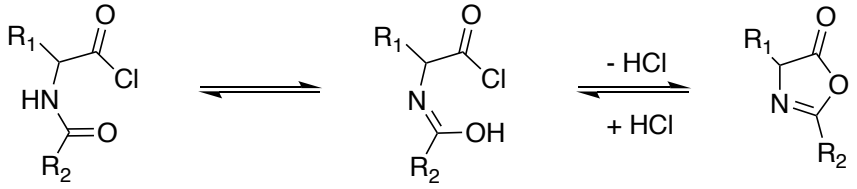
Scheme 1-3 Simple amidation from a carboxylic acid and an amine.

The use of acyl halides is most common in this respect (Scheme 1-4). Acyl halides are highly reactive and easy to prepare and are thus a great alternative. Acyl chlorides in particular have proved to be useful, and they are the most common group of reagents in *N*-acylation.[66]



Scheme 1-4 Acyl chloride amidation reaction.

The downside to this method is that the formation of the acyl halide might also produce harmful by-products such as HCl(g) and/or CO(g). Additionally, it may lead to hydrolysis, racemization, and a number of side-reactions, because the acyl halides are so strongly activated.[68] Simple amino acid chlorides for instance, cyclize rapidly into oxazolones (Scheme 1-5), which leaves the method impractical in peptide synthesis, as this will lead to racemization.[69]



Scheme 1-5 Cyclization of an *N*-acylamino acid into an oxazolone.[69]

Coupling reagents that have been developed specifically for the formation of amide bonds are numerous. One of the first that was reported is the diimide *N,N'*-dicyclohexylcarbodiimide (DCC),[70] an example of what has later become a large group of diimide coupling reagents. The success of the reaction depends strongly on the solvent in use, and different diimides that work well with different solvents have been developed. Another challenge with this method is that the by-products are ureas, and they can be difficult to remove from the mixture. Diimides with ureas that are easier to remove have thus been developed. One such example is EDC.[68]

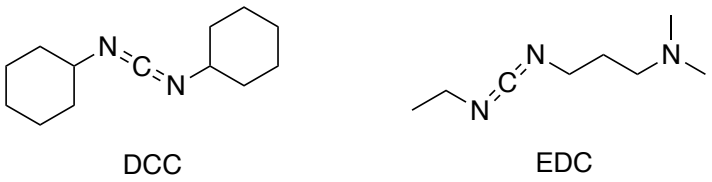
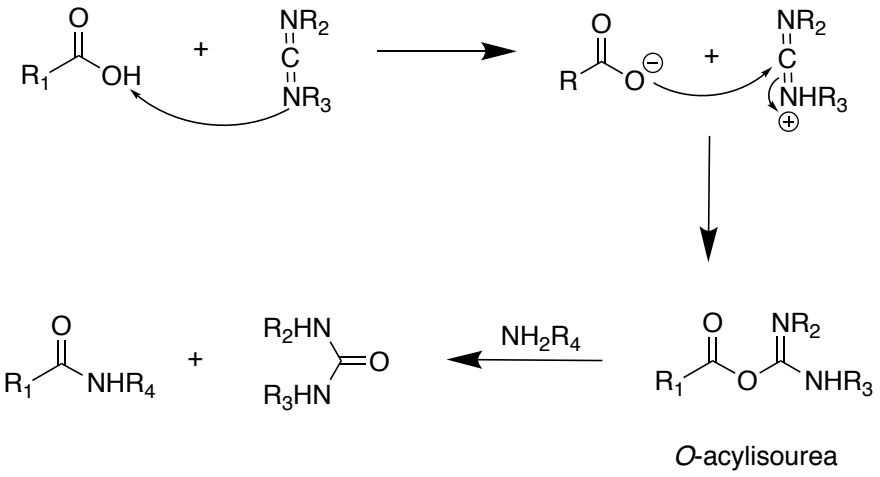


Figure 1-12 The diimide coupling reagents DCC and EDC.

The principle behind the diimide-mechanism is simple (Scheme 1-6). The active acylating agent, "*O*-acylisourea", is first created. This reacts with the amine to form the amide. The amine also needs to be added before long to avoid side-reactions.[69]



Scheme 1-6 Diimide coupling mechanism.[68]

It is common to use additives in the form of 1-hydroxybenzotriazoles with the diimide-method. This reduces racemization and formation of by-products and leads to a higher degree of purity. HOBt and HOAt are commonly used in this respect, and several derivatives of these are also available.[71] The 1-hydroxybenzotriazole reacts with the *O*-acylisourea, and thus creates another activated intermediate; a benzotriazole-ester (**Figure 1-14**).[69]

Figure 1-13 Molecular structures of the benzotriazole additives HOBt and HOAt.

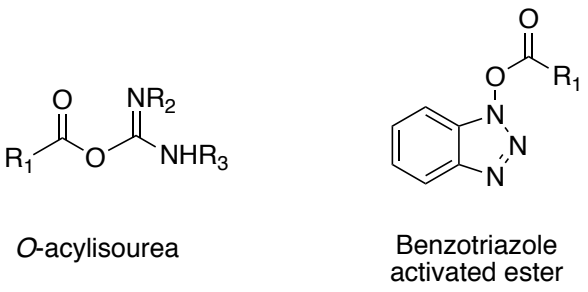


Figure 1-14 The activated intermediate *O*-acylisourea, and the activated benzotriazole ester that is acquired when a benzotriazole additive is used.

Benzotriazole coupling reagents are also available. These are usually in the form of either a phosphonium-, ammonium- or uronium salt. One member of this group that has been used successfully is BOP (Figure 1-15).[72-73] The advantages with these reagents is that the by-

products are easily removed, the method gives good yields, and only a few possible side-reactions.[69]

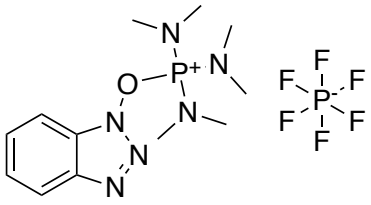
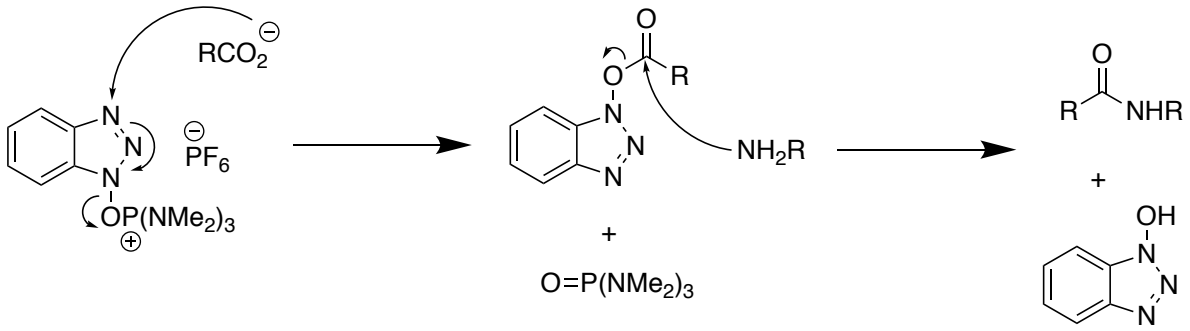


Figure 1-15 Molecular structure of the coupling reagent BOP.

The carboxylic acid is activated as a benzotriazole-intermediate which is reacted with the amine (Scheme 1-7). The disadvantage with BOP is that one by-product, hexamethylphosphoric-triamide, is highly carcinogenic.[74-75] This leaves the use of this reagent problematic, especially for industrial purposes. Variations of BOP have been tested and found potent, however they all have certain limitations when it comes to sensitivity to either moisture or heat. High prices as well as safety issues also opposes the use of these reagents.[69]



Scheme 1-7 BOP coupling mechanism.[68]

The possibility of using enzymes to create amide bonds has also been explored. However, the isolation process makes this method both costly and inefficient, and thus not practical for large scale, nor industrial use.[76-77]

The carboxylic acid can also be activated as an acylimidazole. The coupling reagent *N,N'*-carbonyldiimidazole, CDI (Figure 1-16), is used in this respect.[78] The acylimidazole reacts with the amine to give the amide (Scheme 1-8).[68]

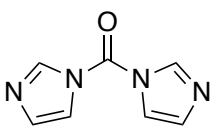
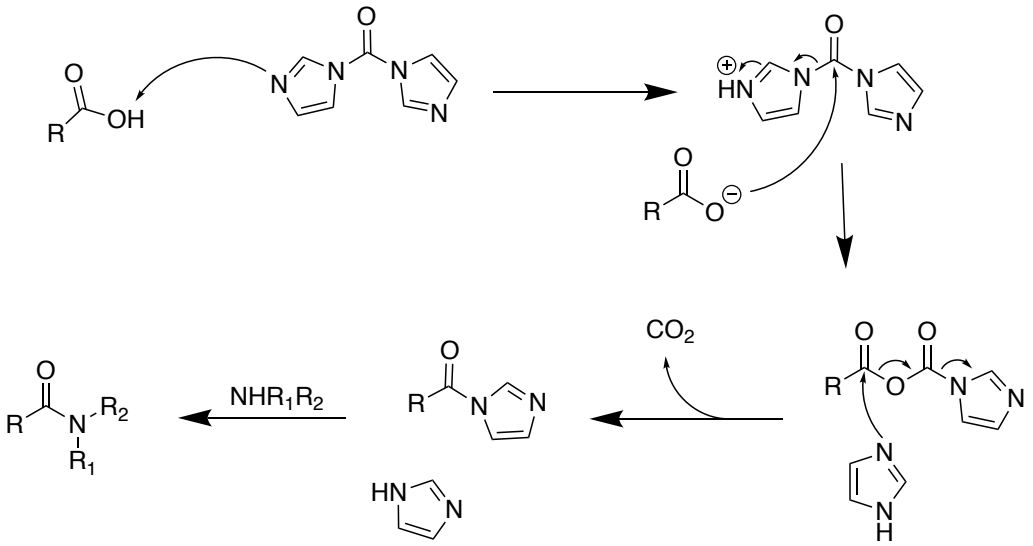


Figure 1-16 Molecular structure of the coupling reagent CDI.



Scheme 1-8 CDI coupling mechanism.[68]

The main advantage of this method is that the reaction is irreversible; with the formation of CO_2 (g). The initial reaction happens quite fast, and the imidazole by-products are easily removed in an acidic work-up. A crucial detail regarding this method is keeping the reaction environment dry, as CDI is readily hydrolyzed to imidazole when subjected to H_2O. Despite this, CDI has in fact been used in amidation reactions in water.[79]

CDI has been used to synthesize both NAEs and several new FAAs of both PUFAs and saturated fatty acids mostly in high yields.[80]

1.3 Aim and background

As discussed earlier, the amide bond is an essential part of a large number of natural products. Many methods are available for the formation of amide bonds; however, especially when working with long chain fatty acids, the most common methods seems to provide only low to moderate yields, and several of the methods have limitations because of the harsh conditions that are required for the reactions to take place. Taken into consideration that these reagents often are expensive and lead to toxic by-products, there seems to be room for a method that works well under mild conditions, and with a range of both fatty acids and amines.

This thesis attempts to assess the scopes and limitations of a method using CDI to synthesize NADs, NAPs, NAAs and PFAMs. The work described herein can be seen as complementary to the works of a few other master students.[81-83]

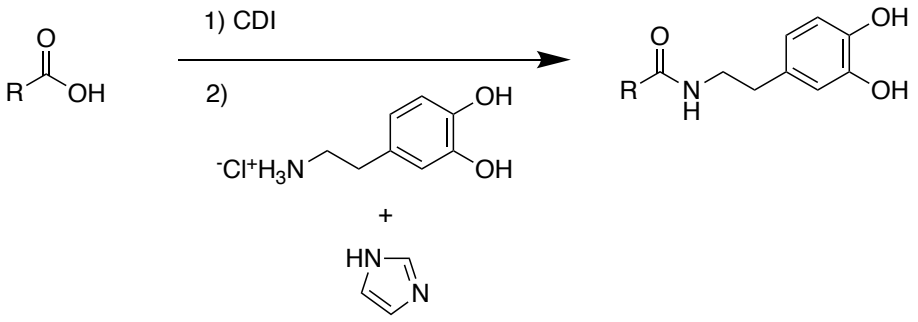
Secondly, a synthetic path towards 3-aza ω-3 DPA (**33**) is tested, using some of the methods described in section 1.2.1.

2 Results and discussion

2.1 Fatty acid amide-syntheses

2.1.1 Synthesis of *N*-acyldopamides

Linoleoyldopamine (**34**) was at first attempted synthesized from linoleic acid and dopamine hydrochloride, in THF. This reaction yielded only 9 % of the desired product. A new approach was therefore needed. One theory of what caused the poor yield was that the two hydroxyl-groups on the amine would hold on to moisture. Another possible source of the problem was the low solubility of the HCl salt. The amine salt was therefore azeotroped in anhydrous THF, and free-based with imidazole in anhydrous DCM before it was used in the amidation reaction. This method worked well, and it was used to synthesize NADA and OLDA in addition to **34**, in yields of 77 %, 58 % and 77 % respectively.



R: $(CH_2)_3(CH{=}CHCH_2)_4(CH_2)_3CH_3$ $(CH_2)_7CH{=}CH(CH_2)_7CH_3$ $(CH_2)_7(CH{=}CHCH_2)_2(CH_2)_3CH_3$

NADA OLDA **34**

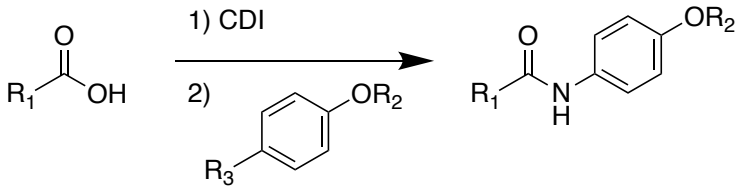
Scheme 2-1 Reaction scheme for the synthesis of NADs.

Czarnocki *et al.*[72] describes the successful synthesis of a few NADs, and among them NADA, OLDA and **34**, with BOP as coupling reagent, in yields ranging from 87 – 98 %. OLDA has also been synthesized with the acyl chloride method, in 97 % yield.[84] Several NADs have also been made using mixed anhydrides, in yields between 55 and 60 %.[31]

2.1.2 Synthesis of *N*-acylamino phenols

The natural product AM404 was prepared from 4-aminophenol hydrochloride and AA. The achieved yield was 61 %. The method was then repeated with oleic acid, giving compound **35** in a yield of 86%.

The amine 4-methoxyphenylamine was used to synthesize amides with structures analogous to AM404. This was done with oleic acid and palmitic acid, yielding compounds **36** in 42 % yield and **37** in 61 % yield.



a: R_1= $(CH_2)_3(CH{=}CHCH_2)_4(CH_2)_3CH_3$, $(CH_2)_7CH{=}CH(CH_2)_7CH_3$ R_2 = H R_3 = $NH_3^+Cl^-$

AM404 **35**

b: R_1= $(CH_2)_7CH{=}CH(CH_2)_7CH_3$, $(CH_2)_{14}CH_3$ R_2 = Me R_3 = NH_2

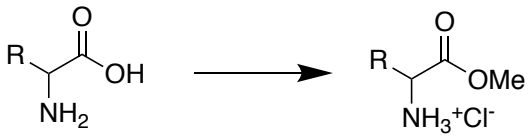
36 **37**

Scheme 2-2 Reaction scheme for the synthesis of NAPs.

The natural product AM404 has previously been synthesized in 48 % yield by Sinning *et al.*[85] with the acyl chloride method, whereas **37** has been synthesized by Di Marzo *et al.*[86] with EDC and HOBt in a yield of 64 %.

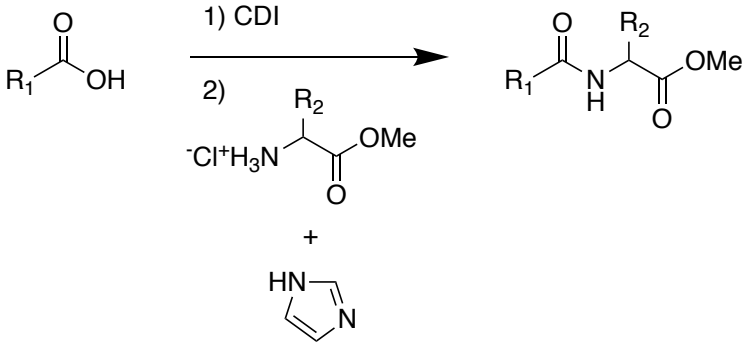
2.1.3 Synthesis of *N*-acylamino acids

Amino acids, with the general structure R-C(NH_2)CO_2H have zwitterionic abilities and relatively high polarities and are therefore poorly soluble in organic solvents. Moreover, the carboxylic acid moiety could interfere with the reaction, making protection of this group essential. This was done by converting it into a methyl ester (Scheme 2-3).



Scheme 2-3 Amino acid protection. Reagents and conditions: $SOCl_2$, MeOH, 60°C, 4h. R=H, Me.

The direct use of this hydrochloride salt in the amidation reaction was attempted at first, however as the salt was only partly soluble in DCM and THF, this reaction only gave low yields. The hydrochloride salt was free-based with imidazole before it was added to the reaction. This method gave high yields and clean products, and simple hydrolysis yielded the final products.



a: $R_1 = (CH_2)_nCH_3$, $(CH_2)_4(CH=CHCH_2)_4CH_4CH_3$ $R_2 = H$

16: n = 2,
17: n = 4,
18: n = 8,
38: n = 1.

NAGly

b: $R_1 = (CH_2)_8CH_3$ $R_2 = Me$

39 + **40**

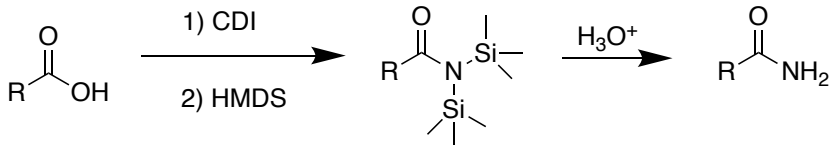
Scheme 2-4 Reaction scheme for the synthesis of NAAs.

The natural products NAGly, **16**, **17**, and **18**, and additionally propanoylglycine (**38**) were synthesized from their corresponding FAs. These reactions all yielded from 81 to >95 %. Alanine amides were made from decanoic acid with both L- (**39**) and D-alanine (**40**) in quantitative yields.

NAGly has previously been synthesized in 34 % yield with the acyl chloride method.[41] The NAA **16** has been synthesized with the acyl chloride method in a yield of 78 %,[87] while **38** has been synthesized with the acyl chloride method in a yield of 25 %,[88] and via the corresponding anhydride.[89]

2.1.4 Synthesis of primary fatty acid amides

The primary fatty acid amides were made using hexamethyldisilazane (HMDS). The amine replaces the imidazole unit, and the trimethylsilyl-units are cleaved in the work-up, leaving behind a primary amide. The primary amides OA, **21**, **24**, stearamide (**41**), DHA-amide (**42**), and EPA-amide (**43**) were prepared using this method.



R: $(CH_2)_7CH=CH(CH_2)_7CH_3$ OA | $(CH_2)_{14}CH_3$ **21** | $(CH_2)_7(CH=CHCH_2)_2(CH_2)_3CH_3$ **24** | $(CH_2)_{12}CH_3$ **41**

$(CH_2)_2(CH=CHCH_2)_6CH_3$ **42** | $(CH_2)_3(CH=CHCH_2)_5CH_3$ **43**

Scheme 2-5 Reaction scheme for the synthesis of primary fatty acid amides.

The polyunsaturated fatty acids (PUFAs) were made in quantitative yields; however, the more saturated FAs gave low yields from 10-35 %. Several attempts were made to improve these discouraging results, but without success. THF was used rather than DCM, the relative amount of CDI was increased, the relative amount of HMDS was increased, and the reaction time was increased; all without improving the yields of the reaction.

HMDS is a sterically hindered amine, and a poor nucleophile,[90] which might be the cause for the low yields in some of the reactions. This theory was supported by observations of the intermediate *N*-acylimidazole in the crude NMR-spectra.

In figures **Figure 2-1** and **Figure 2-2** are shown the crude NMR-spectra from an attempted amidation of palmitic acid with HMDS. In the ^{1}H NMR spectrum, the peaks from 7 – 8.5 ppm represent the protons on the imidazole moiety. In the ^{13}C NMR spectrum, the peak at 169.62 ppm represents the carbonyl. The chemical shift is lower than the shift from the primary amide carbonyl (~176 ppm). Additionally, a couple of peaks are found between 115 – 135 ppm. These represent the carbons on the imidazole moiety.

The *N*-acylimidazole was expected to be unstable and to hydrolyze if present in the work-up. However, previous studies on the stability of *N*-acylimidazoles might support the observations, as other *N*-acylimidazoles have shown some stability towards both acidic, basic and aqueous environments in the past.[91-93]

When the crude product was attempted purified by flash chromatography, around 70 % of the fatty acid was obtained despite that the spectra of the crude compounds revealed little or no sign of them. A small amount of the desired amide was also obtained. This might indicate that if the *N*-acylimidazole really was present in the crude sample, it was hydrolyzed on the column, probably due to the acidic character of the silica gel.

OA and **24** have been synthesized via their acyl chlorides in yields of 76 % and >95 % respectively.[94] The PFAMs **21** and **41** have also been synthesized via their acyl chlorides.[95] The natural product **25** has been synthesized using urea and a catalyst, in 92 % yield.[96] Primary amides have also been synthesized directly from palm oil and urea with an enzyme catalyst.[77]

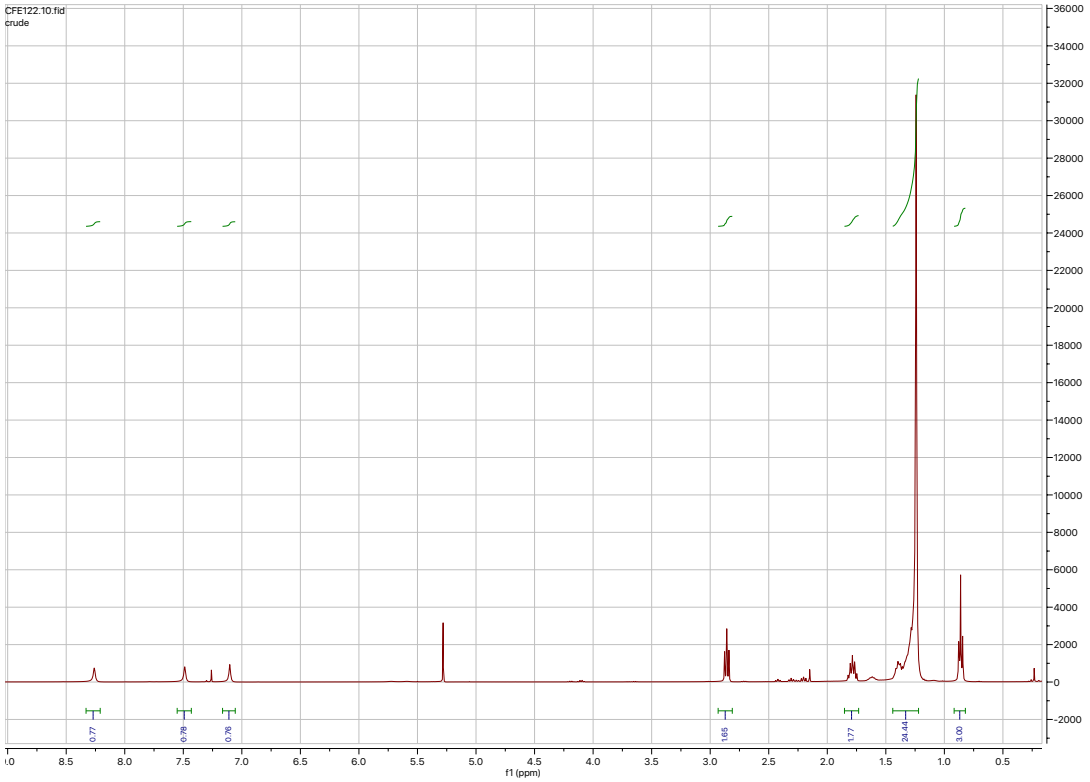


Figure 2-1 ^{1}H NMR spectrum of the crude product of an attempted amidation reaction with palmitic acid.

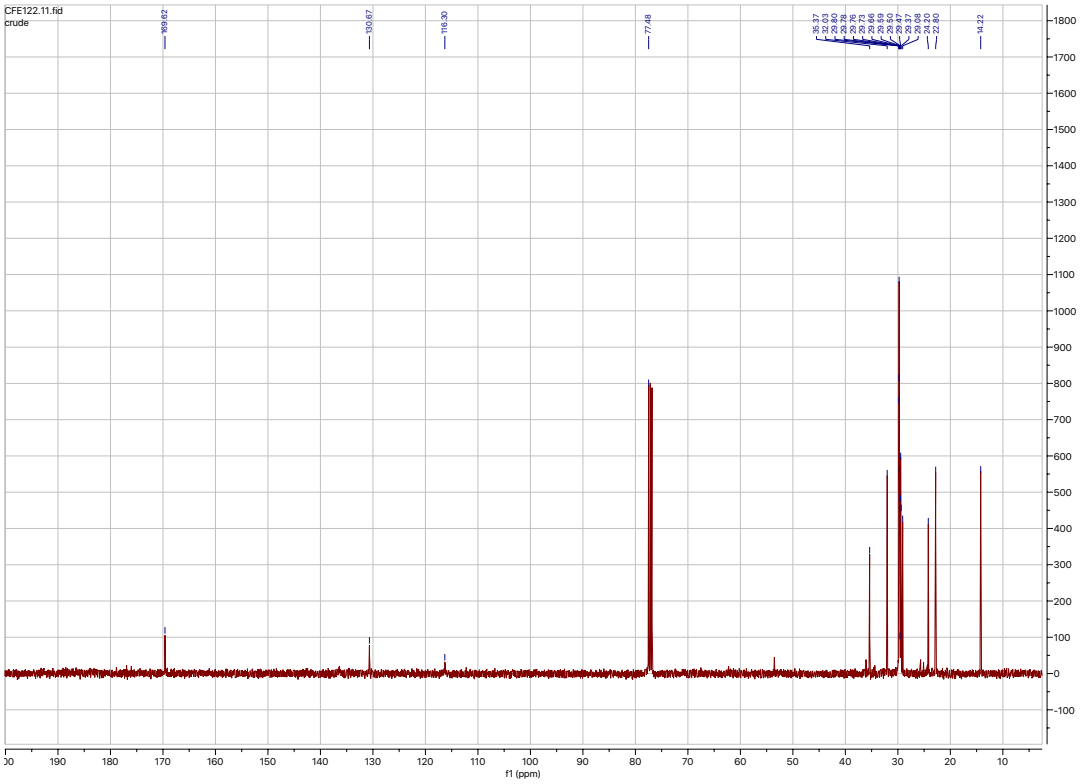


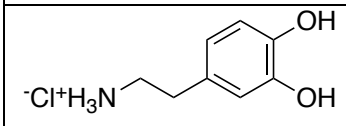
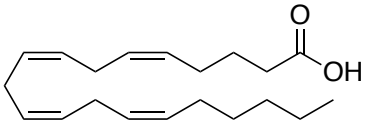
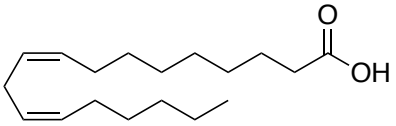
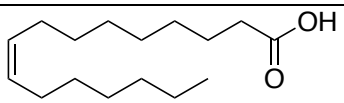
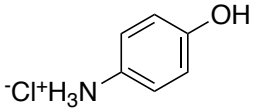
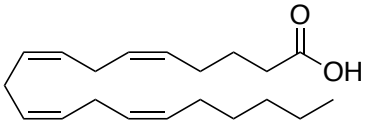
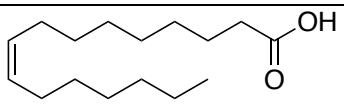
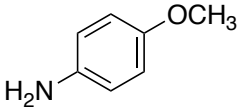
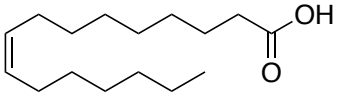
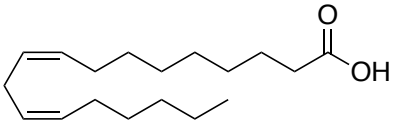
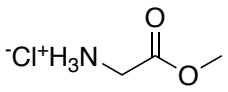
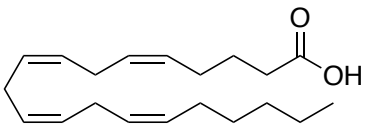
Figure 2-2 ^{13}C NMR spectrum of the crude product of an attempted amidation reaction with palmitic acid.

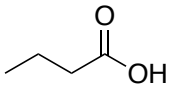
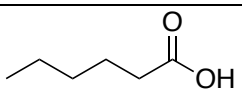
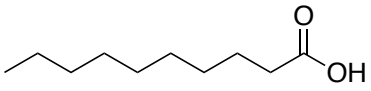
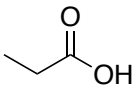
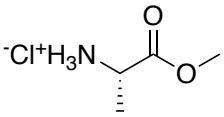
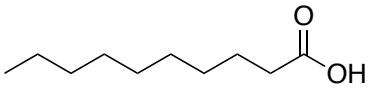
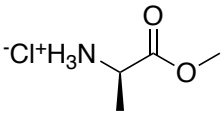
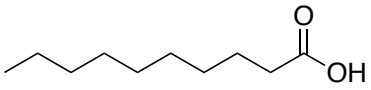
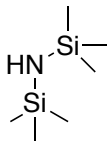
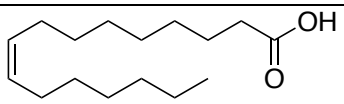
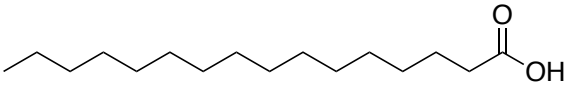
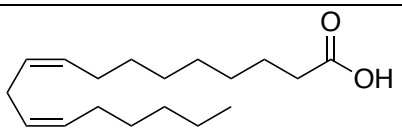
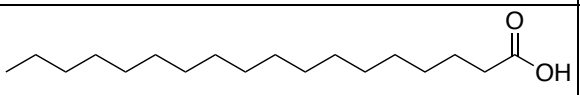
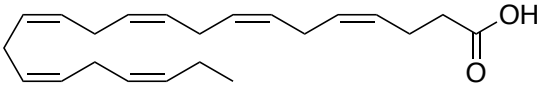
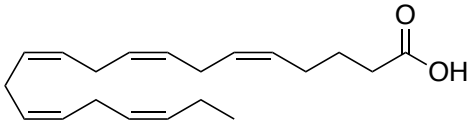
2.1.5 Summary

As shown by Johansson[52] and Johannessen[81] the amidation method has several advantages, and works well on a wide range of both fatty acids and amines. A total number of 20 FAAs has been synthesized here, in yields ranging from 10 to >95 %. The results of all amidation reactions are summarized in

Table 2-1.

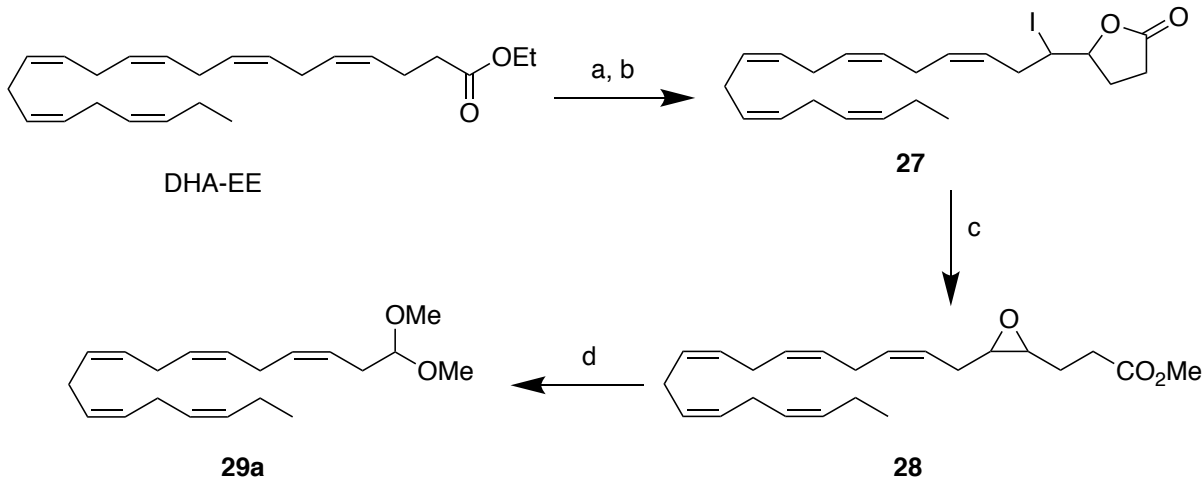
Table 2-1 Results from all amidation reactions.

Amine	Fatty acid	Yield:	#
		77 %	NADA
		77 %	OLDA
		58 %	**34**
		61 %	AM404
		86 %	**35**
		44 %	**36**
		62 %	**37**
		95 %	NAGly

		>95 %	**16**
		>95 %	**17**
		81 %	**18**
		93 %	**38**
		>95 %	**39**
		>95 %	**40**
		10 %	OA
		24 %	**21**
		32 %	**24**
		35 %	**41**
		>95 %	**42**
		>95 %	**43**

2.2 Synthesis of 3-aza ω-3 DPA

The iodo-lactone **27** was synthesized from DHA-EE following the protocol described by Langseter[58] *et al*. The crude yield of the reaction was 88 %, which is lower than the previously reported quantitative yields. The product was converted into epoxide **28** without further purification, following the protocol described by Flock[63] *et al*. in a crude yield of 78 %, which is lower than the previously reported 89 %. The acetal **29a** was then synthesized from crude **28** following the same protocol. After purification using column chromatography the product was obtained in a yield of 40 %, which is comparable to the reported 46 %.



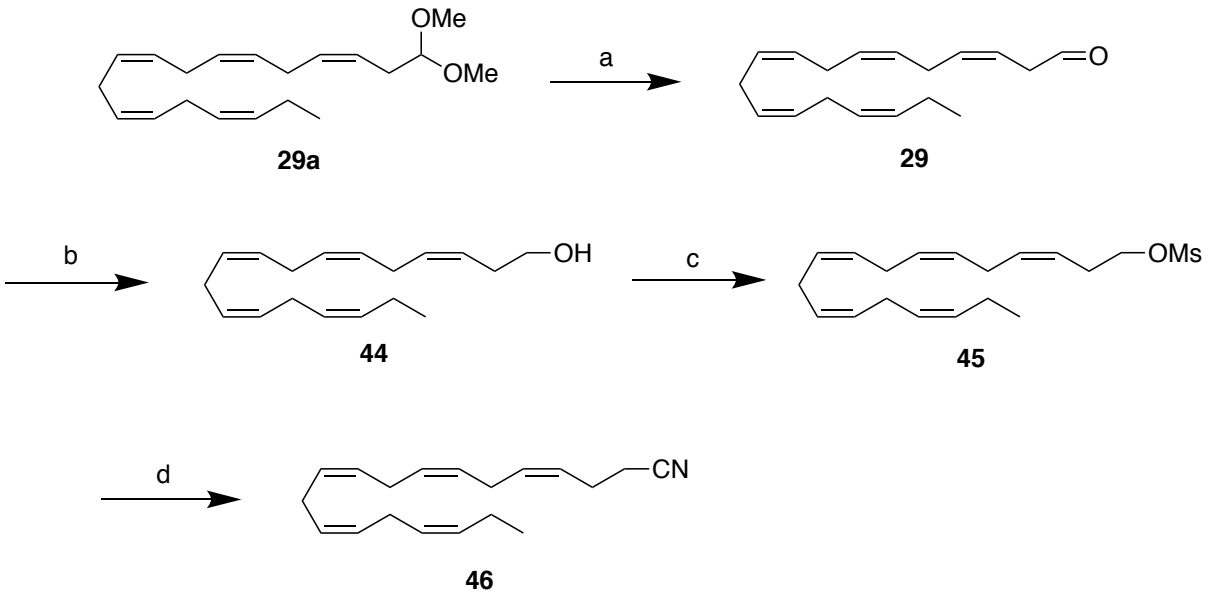
Scheme 2-6 Synthesis of acetal **29a**. Reagents and conditions: (a) $LiOHxH_2O$ in $MeOH/H_2O$ (1:1), rt, 4h; (b) HI, $KHCO_3$, I_2 in THF, 0°C, 16h; (c) MeOH, K_2CO_3, rt, 3h; (d) Periodic acid, MeOH, rt, 6h.

Acetal **29a** was hydrolyzed to form aldehyde **29** and then reduced to alcohol **44** in yields of 88 % and 87 %, following the protocol described by Flock *et al.*[63] The previously reported yields of these reactions are 95-97 % and 80 % respectively.

The alcohol was converted into mesylate **45** which was converted into nitrile **46**, following the protocol described by Fillipova *et al.*[97] (EPA) and Pangopolous[98] (DHA). The yields were of the reactions were 99 % and 59 %. The previously reported yields of these reactions are 94 % and 68 %.

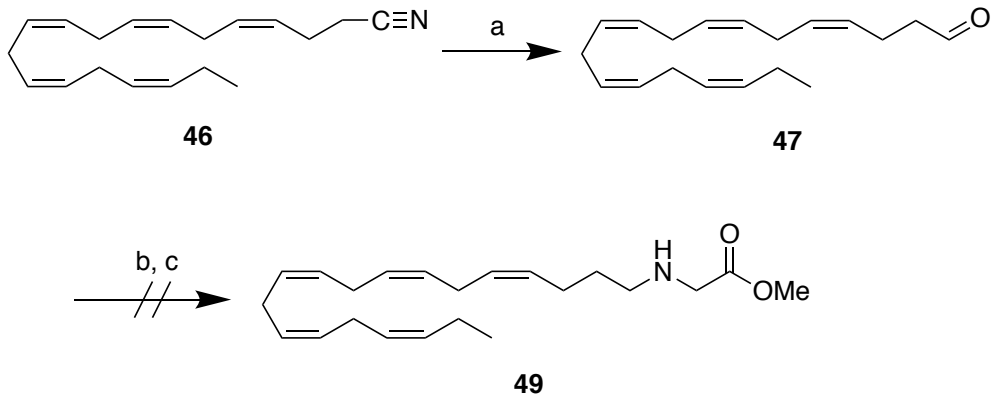
The suspected reason for the low yield in the last reaction is that the DMSO which was used as solvent could contain some amount of water. To avoid this, other conditions to synthesize **46** was attempted, using acetonitrile, 18-crown-6 and KCN. This method yielded 39 % of the nitrile on the first attempt. The method was not optimized, and it would be expected that the yield could be increased. One suggestion for improvement is warming the reaction up to 70°C right

from the start rather than leaving it at rt for 24h first. The work-up should also be possible to optimize, preferably so that chromatographic purification would be superfluous.



Scheme 2-7 Synthesis of **45**. Reagents and conditions: (a) Formic acid in dioxane, rt, 1.5h; (b) $NaBH_4$ in MeOH, 0°C, 30min; (c) MsCl and TEA in DCM, 0°C to rt, 2.5h; (d) KCN, 18:cr:6 and acetonitrile, rt, 24h, then 70°C, 3.5h.

Nitrile **46** was reduced to aldehyde **47** following the protocol described by Antonsen *et al.*[99] The aldehyde was not purified but used directly in reductive amination with methyl glycinate hydrochloride and $NaBH_3CN$. This reaction was not successful, as none of the desired product **49** was obtained.

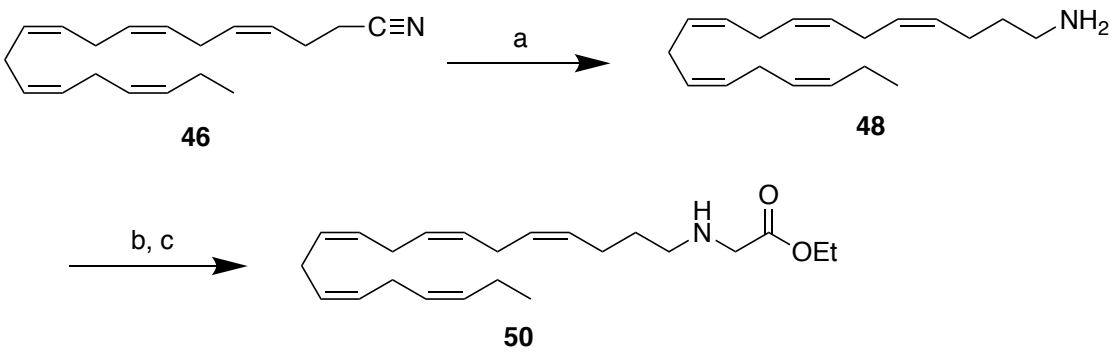


Scheme 2-8 Synthesis of **49**. Reagents and conditions: (a) DIBAL-H in hexane, -78°C, 2h; (b) methyl glycinate hydrochloride, KOH in MeOH, rt, 45min; (c) $NaBH_3CN$ in MeOH, rt, 12h.

A new approach towards **33** was devised; reduction of nitrile **46** into amine **48,** rather than aldehyde **47**, followed by reductive amination with ethyl glyoxylate.

Reduction of nitrile **46** into amine **48** was done with $LiAlH_4$ in THF. The work-up was done with saturated Rochelle salt-solution, however getting rid of the aluminum-species still turned out to be challenging. The crude yield of the reaction was 60 %. This should be possible to improve, by optimizing the work-up conditions.

The amine was to be used in reductive amination with ethyl glyoxylate and $NaBH_3CN$. This was not done due to time limitations. The final step would be hydrolysis of the ester **50** giving **33**.



Scheme 2-9 Synthesis of **48**. Reagents and conditions: (a) $LiAlH_4$ in THF, 0°C, 36h. (b) Ethyl glyoxylate in toluene. (c) $NaBH_3CN$ in MeOH.

3 Conclusion

A total number of 20 fatty acid amides were synthesized in varying yields. NADs were made in satisfactory yields starting with dopamine hydrochloride. NAPs were made in satisfactory yields using both 4-aminophenol and 4-methoxyphenylamine. NAAs were made in mostly quantitative yields from the methyl esters of the amino acids. The yields of the PFAMs varied according to the degree of unsaturation; the PUFA-amides were made in highest yields.

The amidation method can, given these as well as the previously described results, be considered an easy and reliable method that works well with a variety of amines in combination with PUFAs. The method has some limitations when used with saturated fatty acids, and this becomes even more evident when primary amides are to be made. However, as the saturated fatty acids are not as sensitive as the PUFAs, their requirement for a mild amidation method is not as immediate, and other available methods, such as the acyl halide method, should be adequate.

The coupling reagent CDI is a versatile and cheap reagent that works well at ambient temperatures and with a variety of fatty acids and amines. Amine hydrochloride salts are also compatible with the method, thanks to the basic by-product of the activation step: imidazole. When the imidazole that is formed in the reaction is not sufficient, the amine hydrochloride salt can be free-based in imidazole, and then added directly to the reaction.

The first 8 steps in a 10-step synthetic route towards 3-aza n-3 DPA (**33**) was followed, yielding a total of 7 %. Several of the reactions were only attempted once due to time limitations, and improvement of most yields should be possible. The conversion from mesylate **45** to nitrile **46**, and the following reduction to amine **48** are natural candidates for optimization.

4 Experimental

All reactions were done in a N_2-atmosphere. All reagents were used as purchased, unless otherwise mentioned.

Thin layer chromatography (TLC) was performed on TLC silica gel F_{254} sheets, and $KMnO_4$-solution was used for development. Silica gel 60 (40-63µm) from Merck was used for column chromatography. Optical rotation was measured on a PerkinElmer instrument, Model 341.

NMR spectra were recorded on a Bruker Ascend 400 instrument at 25°C, at 400 MHZ for ^{1}H NMR and 100 MHz for ^{13}C NMR. $CDCl_3$ was used as solvent, and the reference peaks were calibrated to 7.26ppm (^{1}H NMR) and 77.16ppm (^{13}C NMR). When DMSO-d_6 was used as solvent, the peaks were calibrated to 2.50 (^{1}H NMR) and 39.52 (^{13}C NMR).

HR-MS spectra were measured on an Autospec Ultima (EI/70eV) instrument from Micromass Ltd, and IR spectra on an Agilent 5500 Series FT-IR instrument with an ATR diamond cell.

4.1 General hydrolyzing method

The ester (1 equiv.) was dissolved in ethanol (5mL) and H_2O (5mL). $LiOHxH_2O$ (5 equiv.) was added, and the mixture was set to stirring in a N_2-atmosphere at rt for 12h.

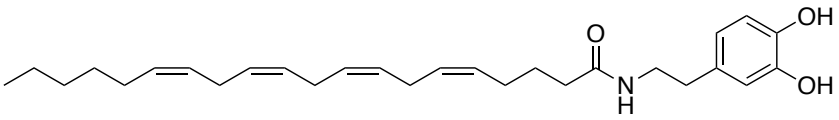
H_2O (15mL) was added, and the sample was acidified with HCl to pH <5, before it was saturated with NaCl. Hexane was added (10mL), the phases separated, and the water phase was extracted with hexane (3x10mL). The organic phases were finally dried with Na_2SO_4, and the solvent was evaporated.

4.2 General amidation method

The fatty acid (1 equiv.) was dissolved in DCM (5mL), and CDI (1.1 equiv.) was added. The solution was left with stirring in a N_2-atmosphere at rt for 30 minutes before the amine (2 equiv.) was added. The mixture was set to stirring in a N_2-atmosphere at rt for 12h.

DCM (25mL) and saturated NH_4Cl-solution (20mL) was added and the mixture was acidified with HCl to pH 2. The mixture was transferred to a separatory funnel, the phases separated, and the inorganic phase was extracted with DCM (3x10mL). The organic phases were dried with Na_2SO_4 for 30min, and the solvent was evaporated.

Synthesis of *N-3,4*-dihydroxybenzylmethyl-(*5Z, 8Z, 11Z, 14Z*)-eicosa-5,8,11,14-tetraenoic amide



NADA

Yield: 77 %.

Method:

General method for amidation was used, with AA (79mg, 0.26mmol), CDI (76mg, 0.47mmol), and dopamine hydrochloride (108mg, 0.570mmol), which was azeotroped in THF, and free-based with imidazole (220mg, 3.23mmol) in 5mL anhydrous DCM for 30 minutes prior to addition to the reaction.

The crude sample was purified by column chromatography, where the product was eluted with 50 % EtOAc in hexane.

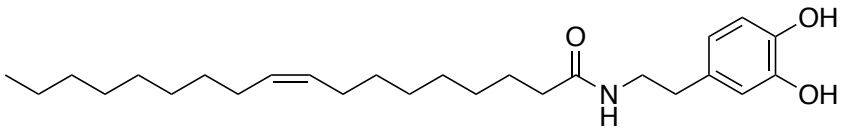
Data:

***R_f*:** (50 % EtOAc in hexane).

^{1}H NMR (400MHz, $CDCl_3$): δ 7.81 (s, 1H, OH), 6.80 (d, *J* = 8.0 Hz, 1H), 6.77 – 6.72 (m, 1H), 6.70 (s, 1H, OH), 6.57 – 6.49 (m, 1H), 5.79 (t, *J* = 5.1 Hz, 1H, NH), 5.36 (qt, *J* = 17.2, 6.9 Hz, 8H), 3.45 (q, *J* = 6.8 Hz, 2H), 2.80 (dq, *J* = 12.2, 6.3 Hz, 6H), 2.67 (t, *J* = 7.1 Hz, 2H), 2.21 – 2.13 (m, 2H), 2.05 (h, *J* = 7.4 Hz, 4H), 1.67 (p, *J* = 7.4 Hz, 2H), 1.42 – 1.14 (m, 8H), 0.88 (t, *J* = 6.7 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 174.30 (C), 144.42 (C), 143.21 (C), 130.54 (CH), 130.39 (CH), 128.98 (CH), 128.81 (CH), 128.64 (CH), 128.31 (CH), 128.07 (CH), 127.83 (CH), 127.51 (CH), 120.40 (C), 115.44 (CH), 115.23 (CH), 41.09 (CH_2), 36.13 (CH_2), 34.88 (CH_2), 31.52 (CH_2), 29.32 (CH_2), 27.23 (CH_2), 26.57 (CH_2), 25.65 (CH_2), 25.63 (CH_2), 25.53 (CH_2), 22.58 (CH_2), 14.09 (CH_3).

Synthesis of *N*-3,4-dihydroxybenzylmethyl-*(9Z)*-octadeca-9-enoic amide



OLDA

Yield: 58 %.

Method:

General method for amidation was used, with oleic acid (146mg, 0.517mmol), CDI (126mg, 0.777mmol), and dopamine hydrochloride (190mg, 1.00mmol), which was azeotroped in anhydrous THF and free-based with imidazole (344mg, 5.05mmol) in 5mL anhydrous DCM for 30 minutes prior to addition to the reaction.

The sample was purified further using column chromatography, where the product was eluted with 50 % EtOAc in hexane.

Data:

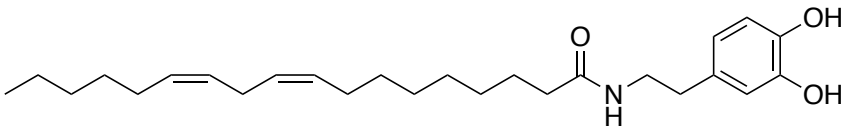
***R_f*:** (50 % EtOAc in hexane).

^{1}H NMR (400MHz, $CDCl_3$): δ 7.86 (s, 1H, OH), 7.05 (s, 1H, OH), 6.79 (d, *J* = 8.0 Hz, 1H), 6.73 (d, *J* = 1.9 Hz, 1H), 6.52 (dd, *J* = 8.0, 1.9 Hz, 1H), 5.94 (t, *J* = 5.7 Hz, 1H, NH), 5.33 (tq, *J* = 9.4, 5.2 Hz, 2H), 3.44 (q, *J* = 6.8 Hz, 2H), 2.65 (t, *J* = 7.0 Hz, 2H), 2.22 – 2.09 (m, 2H), 2.02 – 1.90 (m, 4H), 1.67 – 1.49 (m, 2H), 1.34 - 1.23 (m, 22H), 0.87 (t, *J* = 6.8 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 174.75 (C), 144.49 (C), 143.22 (C), 130.44 (CH), 130.02 (CH), 129.73 (CH), 120.43 (C), 115.56 (CH), 115.35 (CH), 41.07 (CH_2), 36.77 (CH_2), 34.84 (CH_2), 31.92 (CH_2), 29.78 (CH_2), 29.73 (CH_2), 29.55 (CH_2), 29.34 (CH_2), 29.24 (CH_2), 29.20 (CH_2), 29.15 (CH_2), 27.24 (CH_2), 27.20 (CH_2), 25.79 (CH_2), 22.70 (CH_2), 14.13 (CH_3).

IR: 3321, 3008, 2930, 2852, 1633, 1521 cm^{-1}.

Synthesis of *N-3,4*-dihydroxybenzylmethyl-*(9Z, 12Z)*-octadeca-9,12-dienoic amide



34

Yield: 77 %.

Method:

General method for amidation was used, with linoleic acid (144mg, 0.513mmol), CDI (147mg, 0.907mmol), and dopamine hydrochloride (200mg, 1.05mmol), which was azeotroped in THF, and free-based with imidazole (393mg, 5.77mmol) in 5mL anhydrous DCM for 30 minutes prior to addition to the reaction.

The crude sample was purified by column chromatography, where the product was eluted with 50 % EtOAc in hexane.

Data:

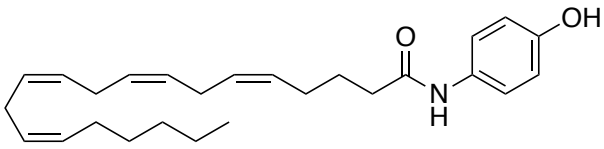
***R_f*:** (50 % EtOAc in hexane).

^{1}H NMR (400MHz, $CDCl_3$): δ 7.89 (s, 1H, OH), 6.80 (d, *J* = 8.0 Hz, 1H), 6.74 (d, *J* = 2.0 Hz, 1H), 6.54 (dd, *J* = 8.0, 2.0 Hz, 1H), 5.80 (t, *J* = 5.7 Hz, 1H, NH), 5.44 – 5.25 (m, 4H), 3.46 (q, *J* = 6.9 Hz, 2H), 2.76 (t, *J* = 6.4 Hz, 2H), 2.67 (t, *J* = 7.1 Hz, 2H), 2.19 – 2.11 (m, 2H), 2.04 (q, *J* = 6.9, 5.7 Hz, 4H), 1.66 – 1.50 (m, 2H), 1.41 – 1.19 (m, 14H), 0.88 (t, *J* = 6.9 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 174.76 (C), 144.54 (C), 143.34 (C), 130.52 (CH), 130.38 (CH), 130.16 (CH), 128.19 (CH), 128.02 (CH), 120.52 (C), 115.57 (CH), 115.33 (CH), 41.14 (CH_2), 36.91 (CH_2), 34.99 (CH_2), 31.64 (CH_2), 29.73 (CH_2), 29.46 (CH_2), 29.33 (CH_2), 29.28 (CH_2), 29.24 (CH_2), 27.32 (CH_2), 25.87 (CH_2), 25.75 (CH_2), 22.70 (CH_2), 14.20 (CH_3).

IR: 3148, 3008, 2930, 2857, 1711, 1638 cm^{-1}.

Synthesis of *N*-4-hydroxyphenyl-*(5Z,8Z,11Z,14Z)*-eicosa-5,8,11,14-tetraenoic amide



AM404

Yield: 61 %.

Method:

General method for amidation was used, with AA (215mg, 0.706mmol), CDI (182mg, 1.12mmol), and 4-aminophenol hydrochloride (161mg, 1.11mmol).

The sample was purified further using column chromatography, where the product was eluted with EtOAc.

Data:

***R_f*:** 0.68 (50 % EtOAc in hexane).

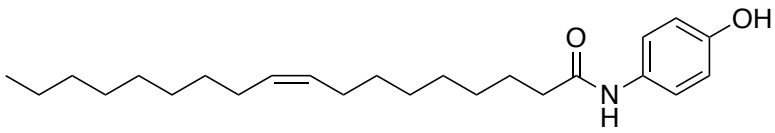
^{1}H NMR (400MHz, $CDCl_3$): δ 7.24 (d, *J*=8.8Hz, 2H), 7.17 (s, 1H), 6.73 (d, *J*=8.8Hz, 2H), 6.29 (s, 1H), 5.37 (m, 8H), 2.81 (m, 6H), 2.34 (t, *J*=15.1Hz, 2H), 2.16 (q, *J*=6.4Hz, 2H), 2.05 (q, *J*=6.9Hz, 2H), 1.81 (p, *J*=14.9Hz, 2H), 1.39 – 1.23 (m, 6H), 0.89 (t, *J*=13.7Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 171.73 (C), 153.33 (C), 130.55 (C), 129.97 (CH), 128.99 (CH), 128.97 (CH), 128.62 (CH), 128.29 (CH), 128.12 (CH), 127.84 (CH), 127.52 (CH), 122.76 (2xCH), 115.81 (2xCH), 36.77 (CH_2), 31.51 (CH_2), 29.32 (CH_2), 27.22 (CH_2), 26.60 (CH_2), 25.64 (CH_2), 25.42 (CH_2), 22.57 (CH_2), 14.08 (CH_3).

IR: 3310, 3008, 2857, 1655, 1515 cm^{-1}.

HR-MS: Found: 395.2818, calculated: 395.2824.

Synthesis of *N*-4-hydroxyphenyl-(*9Z*)-octadeca-9-enoic amide



35

Yield: 86 %.

Method:

General method for amidation was used, with oleic acid (285mg, 1.01mmol), CDI (245mg, 1.51mmol), and 4-aminophenol hydrochloride (300mg, 2.06mmol).

The sample was purified further using column chromatography, where the product was eluted with 50 % EtOAc in hexane.

Data:

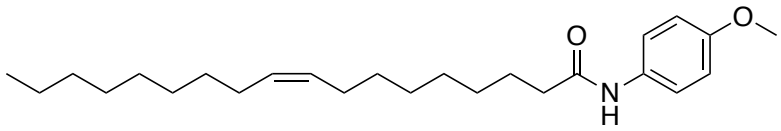
***R_f*:** 0.57 (50 % EtOAc in hexane).

^{1}H NMR (400MHz, $CDCl_3$): δ 7.25 (d, *J* = 8.9 Hz, 2H), 6.74 (d, *J* = 8.8 Hz, 2H), 5.42 – 5.27 (m, 2H), 2.41 – 2.26 (m, 2H), 2.02 (q, *J* = 6.7 Hz, 4H), 1.72 (p, *J* = 7.4 Hz, 2H), 1.40 – 1.21 (m, 20H), 0.89 (t, *J* = 6.8 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 172.74 (C), 153.78 (C), 130.10 (2xCH), 129.86 (2xCH), 123.26 (C), 115.94 (2xCH), 37.49 (CH_2), 32.01 (CH_2), 29.88 (CH_2), 29.85 (CH_2), 29.64 (CH_2), 29.44 (CH_2), 29.43 (CH_2), 29.43 (CH_2), 29.39 (CH_2), 29.29 (CH_2), 27.34 (CH_2), 27.31 (CH_2), 25.90 (CH_2), 22.79 (CH_2), 14.22 (CH_3).

IR: 3316, 2919, 2852, 1750, 1610 cm^{-1}.

Synthesis of *N-4*-methoxyphenyl-(*9Z*)-octadeca-9-enoic amide



36

Yield: 44 %.

Method:

General method for amidation was used, with oleic acid (303mg, 1.07mmol), CDI (211mg, 1.30mmol), and 4-methoxyphenylamine (181mg, 1.47mmol).

The resulting sample, which was a dark, brown solid, was purified further with column chromatography, and the product was eluted with 10 % EtOAc in hexane as a white solid.

Data:

***R_f*:** 0.93 (50 % EtOAc in hexane).

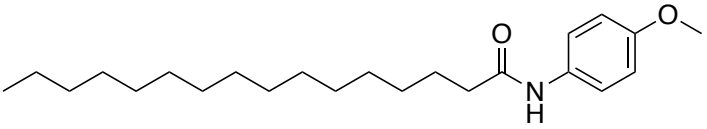
^{1}H NMR (400MHz, $CDCl_3$): δ 7.40 (d, *J*=9.0Hz, 2H), 7.10 (s, 1H), 6.84 (d, *J*=9.0Hz, 2H), 5.34 (m, 2H), 3.78 (s, 3H), 2.32 (t, *J*=15.2Hz, 2H), 2.00 (m, 4H), 1.72 (t, *J*=14.4Hz, 2H), 1.63 (s, 1H), 1.34 – 1.25 (m, 20H), 0.87 (t, *J*=6.8Hz, 1H).

^{13}C NMR (100MHz, $CDCl_3$): δ 207.08 (C), 129.87 (2xCH), 121.78 (2xCH), 114.25 (2xCH), 55.61 (CH_3), 37.79 (CH_2), 32.03 (CH_2), 31.06 (CH_2), 29.90 (CH_2), 29.84 (CH_2), 29.65 (CH_2), 29.45 (CH_2), 29.40 (CH_2), 29.26 (CH_2), 27.35 (CH_2), 27.30 (CH_2), 25.81 (CH_2), 14.24 (CH_3).

IR: 3310, 3014, 2930, 2857, 1661, 1605, 1510 cm^{-1}.

HR-MS: Found: 387.3138, calculated: 387.3137.

Synthesis of *N-4*-methoxyphenyl hexadecanoic amide



37

Yield: 62 %.

Method:

General method for amidation was used, with palmitic acid (367mg, 1.43mmol), CDI (256mg, 1.58mmol), and 4-methoxyphenylamine (194mg, 1.58mmol). A mixture of 10mL hexane and 5mL DCM was used as solvent for the reaction, and later 10mL THF was added.

The resulting sample, which was a purplish solid, was purified using flash chromatography, where the product was eluted with 20 % EtOAc in hexane as a white solid.

Data:

***R_f*:** 0.61 (50 % EtOAc in hexane).

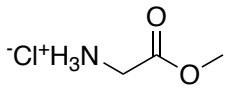
^{1}H NMR (400MHz, $CDCl_3$): δ 7.41 (dd, *J*= 9.0 & 5.0 Hz, 2H), 6.85 (dd, *J*= 9.0 & 4.8Hz, 2H), 3.78 (s, 3H), 2.32 (t, *J*=15.1Hz, 2H), 1.72 (p, *J*=14.6Hz, 2H), 1.25 (m, 24H), 0.88 (t, *J*=13.7Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 171.30 (C), 156.50 (C), 131.20 (C), 121.83 (2xCH), 114.30 (2xCH), 55.63 (CH_3), 37.86 (CH_2), 32.07 (CH_2), 29.84 (CH_2), 29.82 (CH_2), 29.80 (CH_2), 29.76 (CH_2), 29.63 (CH_2), 29.53 (CH_2), 29.50 (CH_2), 29.44 (CH_2), 25.86 (CH_2), 22.84 (CH_2), 14.26 (CH_3).

IR: 3299, 2919, 2852, 1650, 1549 cm^{-1}.

HR-MS: Found: 361.2997, calculated: 361.2981.

Synthesis of amino carbomethoxy methane hydrochloride



51

Yield: >95 %.

Method:

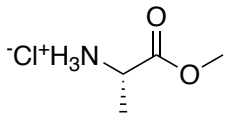
To a solution of glycine (996mg, 13.3mmol) in 20 mL of MeOH was added thionyl chloride (4.75g, 40.0mmol) dropwise at 0°C. The mixture was then refluxed at 60°C for 4h, before the solvent was removed under reduced pressure; leaving behind a white, cotton-like substance.

Data:

^{1}H NMR (400MHz, DMSO-*d*$_6$): δ 8.49 (s, 3H, $N\underline{H_3}^+$), 3.76 (d, *J*=21.0Hz, 3H), 3.33 (d, *J*=4.2Hz, 2H)

^{13}C NMR (100MHz, DMSO-*d*$_6$): δ 167.83 (C), 52.30 (CH_3), 39.27 (CH_2)

Synthesis of 1(*S*)-amino-1-carbomethoxy ethane hydrochloride



52

Yield: >95 %.

Method:

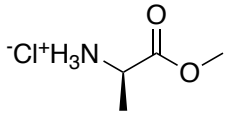
To a solution of L-alanine (995mg, 11.2mmol) in 20 mL of methanol was added thionyl chloride (4.00g, 33.6mmol) dropwise at 0°C. The mixture was refluxed at 60°C for 4h, before the solvent was removed under reduced pressure; leaving behind a white, cotton-like substance.

Data:

^{1}H NMR (400MHz, DMSO-*d*$_6$): δ 8.63 (s, 3H, NH_3^+), 4.05 (q, *J*=6.2Hz, 1H), 3.72 (s, 3H), 3.35 (s, 3H).

^{13}C NMR (100MHz, DMSO-*d*$_6$): δ 170.20 (C), 52.56 (CH_3, 47.56 (CH_2), 15.46 (CH_3).

Synthesis of 1*(R)*-amino-1-carbomethoxy ethane hydrochloride



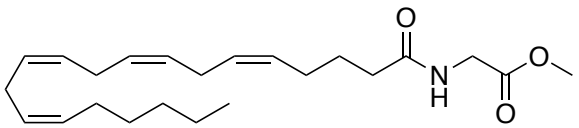
53

Yield: >95 %.

Method:

To a solution of D-alanine (372mg, 4.18mmol) in 20 mL of methanol was added thionyl chloride (1.490g, 12.5mmol) dropwise at 0°C. The mixture was refluxed at 60°C for 4h, before the solvent was removed under removed pressure; leaving behind a white, cotton-like substance.

Synthesis of *N*-carbomethoxy methyl-*(5Z, 8Z, 11Z, 14Z)*-eicosa-5,8,11,14-tetraenoic amide



NAGly-ME

Yield: 95 %.

Method:

General method of amidation was used, with AA (114mg, 0.374mmol), CDI (103mg, 0.635mmol), and **51** (142mg, 1.13mmol), which was free-based with imidazole (256mg, 3.76mmol) in 5mL anhydrous DCM for 30 minutes prior to addition to the reaction.

Data:

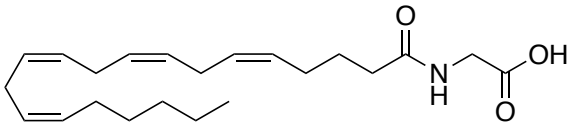
***R_f*:** 0.47 (50 % EtOAc in hexane).

^{1}H NMR (400MHz, $CDCl_3$): δ 6.01 (s, 1H, NH), 5.42-5.29 (m, 8H), 4.03 (d, J = 5.2 Hz, 2H), 3.75 (s, 3H), 2.84-2.78 (m, 6H), 2.34 – 2.21 (m, 2H), 2.12 (q, J = 6.9 Hz, 2H), 2.04 (q, J = 6.9 Hz, 2H), 1.72 (p, J = 7.5 Hz, 2H), 1.42 – 1.16 (m, 6H), 0.88 (t, J = 6.8 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 173.14 (C), 170.67 (C), 130.62 (CH), 129.13 (CH), 128.97 (CH), 128.71 (CH), 128.34 (CH), 128.29 (CH), 127.97 (CH), 127.64 (CH), 52.48 (CH_3), 41.30 (CH_2), 35.76 (CH_2), 31.62 (CH_2), 29.43 (CH_2), 27.33 (CH_2), 26.71 (CH_2), 25.74 (CH_2), 25.44 (CH_2), 25.68 (CH_2), 14.18 (CH_3).

IR: 3294, 3008, 2930, 2857, 1756, 1655 cm^{-1}.

Synthesis of *N*-carboxymethyl-*(5Z, 8Z, 11Z, 14Z)*-eicosa-5,8,11,14-tetraenoic amide



NAGly

Yield: 90 %.

Method:

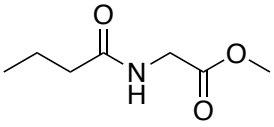
General method of hydrolyzation was used, with NAGly-ME (107mg, 0.285mmol) and $LiOHxH_2O$ (62mg, 1.5mmol) in 3 mL of EtOH and 3mL H_2O.

Data:

^{1}H NMR: (400MHz, $CDCl_3$): δ 8.23 (s, 1H, OH), 6.41 (s, 1H, NH), 5.43-5.29 (m, 8H), 4.03 (d, *J* = 5.0 Hz, 2H), 2.84-2.78 (m, 6H), 2.32 – 2.21 (m, 2H), 2.11 (q, *J* = 7.0 Hz, 2H), 2.05 (q, *J* = 6.9 Hz, 2H), 1.71 (p, *J* = 7.4 Hz, 2H), 1.45 – 1.19 (m, 6H), 0.88 (t, *J* = 6.8 Hz, 3H).

^{13}C NMR: (100MHz, $CDCl_3$): δ 174.54 (C), 172.94 (C), 130.65 (CH), 129.10 (CH), 128.95 (CH), 128.75 (CH), 128.42 (CH), 128.22 (CH), 127.95 (CH), 127.64 (CH), 41.66 (CH_2), 35.70 (CH_2), 31.63 (CH_2), 29.44 (CH_2), 27.34 (CH_2), 26.68 (CH_2), 25.74 (CH_2), 25.46 (CH_2), 22.69 (CH_2), 14.19 (CH_3).

Synthesis of *N*-carbomethoxy methyl butanoic amide



16-ME

Yield: >95 %.

Method:

General method for amidation was used, with butyric acid (0.185mL, 2.00mmol), CDI (490mg, 3.02mg) and **51** (757mg, 6.03mmol), which was free-based with imidazole (2.08g, 30.6mmol) in 5mL anhydrous DCM prior to addition to the reaction.

The sample was purified using flash chromatography, where the product was eluted with 75 % EtOAc in hexane.

Data:

***R*$_f$:** 0.34 (75 % EtOAc and hexane).

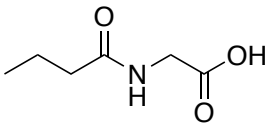
^{1}H NMR (400MHz, $CDCl_3$): δ 6.93 (s, 1H, NH), 3.82 (d, *J*=5.6Hz, 2H), 3.55 (s, 3H), 2.07 (t, *J*=14.9Hz, 2H), 1.49 (m, 2H), 0.78 (t, *J*=7.4Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 173.66 (C), 170.34 (C), 51.81 (CH_3), 40.80 (CH_2), 37.67 (CH_2), 18.76 (CH_2), 13.35 (CH_3).

IR: 3282, 2964, 1745, 1655, 1532 cm^{-1}.

HR-MS: Calculated: 159.0859, found: 159.0903.

Synthesis of *N*-carboxymethyl butanoic amide



16

Yield: 52 %.

Method:

General method for hydrolyzation was used, with **16-ME** (100mg, 0.685mmol) and $LiOHxH_2O$ (150mg, 3.57mmol) in EtOH (4mL) and H_2O (4mL).

Data:

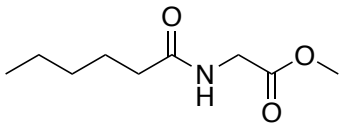
^{1}H NMR (400MHz, $CDCl_3$): δ 10.27 (s, 1H, OH), 6.64 (t, *J*=5.3Hz, 1H, NH), 4.03 (d, *J*=5.2Hz, 2H), 2.24 (t, *J*=7.5Hz, 2H), 1.65 (p, *J*=7.4Hz, 2H), 0.93 (t, *J*=7.3Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 175.04 (C), 172.74 (C), 41.59 (CH_2), 38.12 (CH_2), 19.13 (CH_2), 13.71 (CH_3).

IR: 3321, 3087, 2964, 1733, 1627, 1549 cm^{-1}.

HR-MS: Calculated: 145.0739, found: 145.0737.

Synthesis of *N*-carbomethoxy methyl hexanoic amide



17-ME

Yield: >95 %.

Method:

Method: General method for amidation was used, with hexanoic acid (85mg, 0.73mmol), CDI (190mg, 1.17mmol), and **51** (265mg, 2.11mmol), which was free-based with imidazole (478mg, 7.02mmol) in anhydrous DCM for 30 minutes prior to addition to the reaction.

Data:

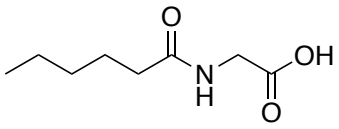
***R*$_f$:** 0.25 (50 % EtOAc in hexane).

^{1}H NMR (400MHz, $CDCl_3$): δ 6.35 (s, 1H, NH), 3.96 (d, *J*=5.2Hz, 2H), 3.68 (s, 3H), 2.18 (t, *J*=15.2Hz, 2H), 1.58 (p, *J*=14.9Hz, 2H), 1.25 (m, 4H), 0.83 (t, *J*=6.6Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 173.56 (C), 170.59 (C), 52.21 (CH_3), 41.10 (CH_2), 36.21 (CH_2), 31.35 (CH_2), 25.23 (CH_2), 22.34 (CH_2), 13.86 (CH_3).

IR: 3294, 2958, 2863, 1756, 1655, 1543 cm^{-1}.

Synthesis of *N*-carboxymethyl hexanoic amide



17

Yield: 94 %.

Method:

General method for hydrolyzation was used, with **17-ME** (101mg, 0.543mmol) and $LiOHxH_2O$ (115mg, 2.74mmol) in 2mL EtOH and 2mL H_2O.

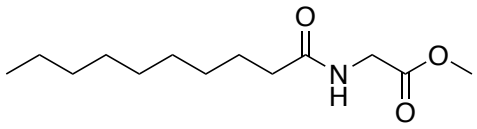
Data:

^{1}H NMR (400MHz, $CDCl_3$): δ 10.67 (s, 1H, OH), 6.51 (t, J = 5.1 Hz, 1H, NH), 4.05 (d, J = 5.2 Hz, 2H), 2.28 (t, J = 7.1 Hz, 2H), 1.63 (p, *J*=7.4, 2H), 1.42 – 1.17 (m, 4H), 0.77 (t, J = 7.1 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 175.03 (C), 172.77 (C), 41.62 (CH_2), 36.30 (CH_2), 31.40 (CH_2), 25.35 (CH_2), 22.42 (CH_2), 13.98 (CH_3).

IR: 3305, 3070, 2930, 2869, 2639, 1728, 1638, 1543 cm^{-1}.

Synthesis of *N*-carbomethoxy methyl decanoic amide



18-ME

Yield: 81 % (per amine).

Method:

General method for amidation was used, with decanoic acid (188mg, 1.09mmol), CDI (195mg, 1.20mmol) and methyl glycinate (62mg, 0.70mmol).

The sample was purified with column chromatography, where the product was eluted with 50 % EtOAc in hexane.

Data:

***R_f*:** 0.40 (50 % EtOAc in hexane).

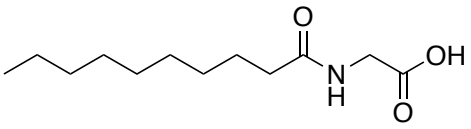
^{1}H NMR (400MHz, $CDCl_3$): δ 6.51 (s, 1H, NH), 3.94 (d, *J*=5.4Hz, 2H), 3.66 (s, 3H), 2.17 (t, *J*=7.6Hz, 2H), 1.55 (s, 2H), 1.18 (m/s, 12H), 0.80 (t, *J*=6.7Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 173.75 (C), 170.57 (C), 52.15 (CH_3), 41.08 (CH_2), 36.20 (CH_2), 31.80 (CH_2), 29.40 (CH_2), 29.32 (CH_2), 29.21 (CH_2), 25.57 (CH_2), 22.59 (CH_2), 14.01 (CH_3).

IR: 3321, 3070, 2919, 2852, 1750, 1644, 1543 cm^{-1}.

HR-MS: Found: 243.1832, calculated: 243.1834.

Synthesis of *N*-carboxymethyl decanoic amide



18

Yield: 51 %.

Method:

General method for hydrolyzation was used, with **18-ME** (100mg, 0.411mmol) and $LiOHxH_2O$ (84mg, 2.00mmol), in 4mL EtOH and 4mL H_2O.

Data:

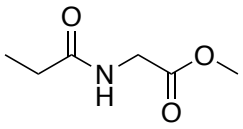
^{1}H NMR (400MHz, $CDCl_3$): δ 6.02 (s, 1H, OH), 4.09 (d, *J*=5.2Hz, 2H), 2.26 (t, *J*=15.3Hz, 2H), 1.65 (p, *J*=14.4Hz, 2H), 1.26 (m, 12H), 0.88 (t, *J*=6.8Hz, 3H)

^{13}C NMR (100MHz, $CDCl_3$): δ 179.62 (C), 174.05 (C), 41.62 (CH_2), 36.55 (CH_2), 36.55 (CH_2), 34.14 (CH_2), 32.05 (CH_2), 29.44 (CH_2), 29.25 (CH_2), 25.73 (CH_2), 24.88 (CH_2), 22.85 (CH_2), 14.29 (CH_3).

IR: 3020, 2924, 2857, 1711, 1650, 1560 cm^{-1}.

HR-MS: Found: 229.1693, calculated: 229.1678.

Synthesis of *N*-carbomethoxy methyl propanoic amide



38-ME

Yield: 93 %.

Method:

General method for amidation was used, with propanoic acid (155mg, 2.09mmol), CDI (486mg, 3.00mmol), and **51** (654mg, 5.21mmol), which was free-based with imidazole (1.44g, 21.2mmol) in 5mL anhydrous DCM for 30 minutes prior to addition to the reaction.

The product was purified further using flash chromatography, where it was eluted with 75 % EtOAc in hexane.

Data:

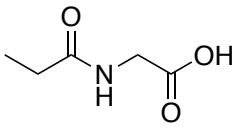
***R_f*:** 0.14 (50 % EtOAc in hexane).

^{1}H NMR (400MHz, $CDCl_3$): δ 6.25 (s, 1H, NH), 4.00 (d, *J*=5.3Hz, 2H), 3.70 (s, 3H), 2.24 (q, *J*=7.6Hz, 2H), 1.12 (t, *J*=7.6Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 174.13 (C),170.68 (C), 52.33 (CH_3), 41.19 (CH_2), 29.31 (CH_2), 9.65 (CH_3).

IR: 3310, 2986, 1745, 1655, 1538 cm^{-1}.

Synthesis of *N*-carboxymethyl propanoic amide



38

Yield: 78 %.

Method:

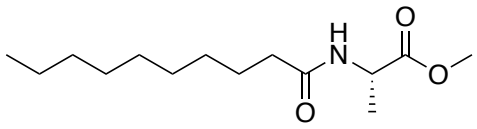
General method for hydrolyzation was used, with **38-ME** (151mg, 1.03mmol) and $LiOHxH_2O$ (218mg, 5.20mmol) in EtOH (2mL) and H_2O (2mL).

Data:

^{1}H NMR (400MHz, DMSO-*d*$_6$): δ 12.48 (s, 1H, OH), 8.06 (d, *J* = 6.1 Hz, 1H, NH), 3.71 (d, *J* = 5.9, 2H), 2.12 (q, *J* = 7.6 Hz, 2H), 0.99 (t, *J* = 7.5 Hz, 3H).

^{13}C NMR (100MHz, DMSO-*d*$_6$): δ 173.70 (C), 171.91 (C), 40.99 (CH_2) 28.63 (CH_2), 10.22 (CH_3).

Synthesis of *N*-1*(S)*-carbomethoxyethyl decanoic amide



39-ME

Yield: >95 %.

Method:

General method for amidation was used, with decanoic acid (181mg, 1.05mmol), CDI (256mg, 1.58mmol), and **52** (441mg, 3.15mmol), which was free-based with imidazole (716mg, 10.5mmol) in 5mL anhydrous DCM for 30 minutes prior to addition to the reaction.

Data:

R_f:

$[\alpha]^{20}_{589}$ = +4.5° (c=0.076, $CHCl_3$).

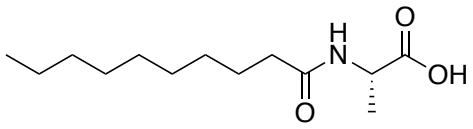
^{1}H NMR (400MHz, $CDCl_3$): δ 6.62 (d, *J* = 7.3 Hz, 1H, NH), 4.43 (p, *J* = 7.3 Hz, 1H), 3.58 (s, 3H), 2.19 – 1.97 (m, 2H), 1.48 (p, *J* = 7.4 Hz, 2H), 1.24 (d, *J* = 7.2 Hz, 3H), 1.21 – 1.07 (m, 12H), 0.72 (t, *J* = 6.8 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 173.48 (C), 172.86 (C), 52.02 (CH_3), 47.68 (CH), 36.10 (CH_2), 31.66 (CH_2), 29.27 (CH_2), 29.20 (CH_2), 29.08 (CH_2), 29.07 (CH_2), 25.47 (CH_2), 22.45 (CH_2), 17.89 (CH_3), 13.85 (CH_3).

IR: 3294, 2924, 2852, 1745, 1644, 1538, 1454 cm^{-1}.

HR-MS: Calculated mass: 257.1991 Found mass: 257.2007.

Synthesis of *N*-1*(S)*-carboxyethyl decanoic amide



39

Yield: >95 %.

Method:

General method of hydrolyzation was used, with **39-ME** (203mg, 0.790mmol) and $LiOHxH_2O$ (166mg, 3.96mmol), in 2 mL EtOH and 2 mL H_2O.

Data:

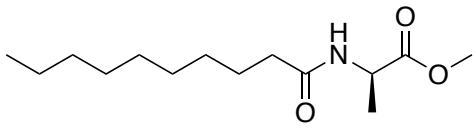
$[\alpha]^{20}_{589}$ = -16.2° (c=0.154, $CHCl_3$).

^{1}H NMR (400MHz, $CDCl_3$): δ 11.94 (s, 1H, OH), 6.72 (d, J = 7.2 Hz, 1H, NH), 4.53 (p, J = 7.2 Hz, 1H), 2.21 (t, J = 7.7 Hz, 2H), 1.56 (p, J = 7.2 Hz, 2H), 1.39 (d, J = 7.1 Hz, 3H), 0.82 (t, J = 6.7 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 175.70 (C), 174.43 (C), 48.25 (CH), 36.34 (CH_2), 31.84 (CH_2), 29.42 (CH_2), 29.30 (CH_2), 29.25 (CH_2), 29.16 (CH_2), 25.69 (CH_2), 22.64 (CH_2), 18.15 (CH_3), 14.07 (CH_3).

IR: 3310, 3070, 2924, 2852, 2617, 1722, 1622, 1543 cm^{-1}.

Synthesis of *N*-1*(R)*-carbomethoxyethyl decanoic amide



40-ME

Yield: >95 %.

Method:

General method for amidation was used, with decanoic acid (173mg, 1.00mmol), CDI (270mg, 1.67mmol), and **53** (483mg, 3.45mmol), which was free-based with imidazole (735mg, 10.8mmol) in 5mL anhydrous DCM for 30 minutes prior to addition to the reaction.

Data:

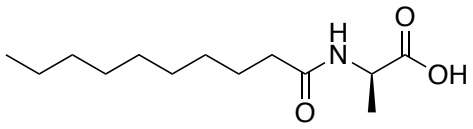
$[\alpha]^{20}_{589}$ = -5.8° (c=0.076, $CHCl_3$).

^{1}H NMR (400MHz, $CDCl_3$): δ 6.60 (d, *J* = 7.3 Hz, 1H, NH), 4.44 (p, *J* = 7.3 Hz, 1H), 3.59 (s, 3H), 2.17 – 2.00 (m, 2H), 1.49 (p, *J* = 7.5 Hz, 3H), 1.25 (d, *J* = 7.2 Hz, 3H), 1.19 – 1.06 (m, 20H), 0.73 (t, *J* = 6.9 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 173.50 (C), 172.86 (C), 52.04 (CH_3), 47.69 (CH), 36.12 (CH_2), 31.67 (CH_2), 29.28 (CH_2), 29.21 (CH_2), 29.09 (CH_2), 29.07 (CH_2), 25.47 (CH_2), 22.46 (CH_2), 17.92 (CH_3), 13.87 (CH_3).

IR: 3294, 2924, 2852, 1745, 1644, 1538, 1454 cm^{-1}.

Synthesis of *N*-1*(R)*-carboxyethyl decanoic amide



40

Yield: 76 %.

Method:

General method of hydrolyzation was used, with **40-ME** (151mg, 0.588mmol) and $LiOHxH_2O$ (123mg, 2.93mmol), in 2 mL EtOH and 2 mL H_2O.

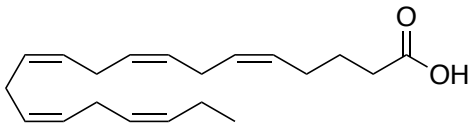
Data:

$[\alpha]^{20}_{589}$ = +4.5° (c=0.154, $CHCl_3$).

^{1}H NMR (400MHz, $CDCl_3$): δ 11.31 (s, 1H, OH), 6.63 (d, *J* = 7.1 Hz, 1H, NH), 4.55 (p, *J* = 7.0 Hz, 1H), 2.11 (t, *J* = 7.7 Hz, 2H), 1.58 (p, *J* = 7.1 Hz, 2H), 1.41 (d, J = 7.1 Hz, 3H), 1.35 – 1.11 (m, 12H), 0.83 (t, *J* = 6.7 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 175.81 (C), 174.41 (C), 48.29 (CH), 36.41 (CH_2), 31.88 (CH_2), 29.46 (CH_2), 29.33 (CH_2), 29.29 (CH_2), 29.21 (CH_2), 25.71 (CH_2), 22.68 (CH_2), 18.20 (CH_3), 14.12 (CH_3).

Synthesis of *(5Z, 8Z, 11Z, 14Z, 17Z)*-eicosa-5,8,11,14,17-pentaenoic acid



EPA

Yield: >95 %.

Method:

General method for hydrolyzation was used, with the EPA-EE (1.03g, 3.12mmol), LiOHxH_2O (630mg, 15.0mmol), in 5mL EtOH and 5mL H_2O.

Data:

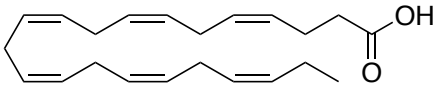
^{1}H NMR (400MHz, $CDCl_3$): δ 5.38 (m, 10H), 2.83 (m, 8H), 2.37 (t, *J*=7.5Hz, 2H), 2.10 (dq, *J*= 14.6 & 19.8Hz, 4H), 1.72 (p, *J*=14.8Hz, 2H), 0.97 (t, *J*=7.5Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 180.40 (C), 132.12 (CH), 129.15 (CH), 128.85 (CH), 128.67 (CH), 128.37 (CH), 128.28 (CH), 128.26 (CH), 128.18 (CH), 127.98 (CH), 127.14 (CH), 33.55 (CH_2), 26.56 (CH_2), 25.74 (2xCH_2) 25.65 (2xCH_2), 24.59 (CH_2), 20.67 (CH_2), 14.37 (CH_3).

IR: 3014, 2964, 1705 cm^{-1}.

HR-MS: Calculated mass: 302.2246 Found mass: 302.2230.

Synthesis of *(4Z, 7Z, 10Z, 13Z, 16Z, 19Z)*-docosa-4,7,10,13,16,19-hexaenoic acid



DHA

Yield: 33 %.

Method:

General method for hydrolyzation was used, with DHA-EE (527mg, 1.48mmol), $LiOHxH_2O$ (295mg, 7.03mmol) in 5mL EtOH and 5mL H_2O.

Data:

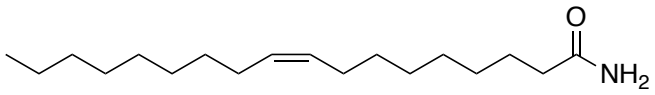
***R_f*:** 0.69 (25 % EtOAc in hexane).

^{1}H NMR (400MHz, $CDCl_3$): δ 11.25 (s, 1H, O<u>H</u>), 5.38 (m, 12H), 2.82 (m, 10H), 2.41 (m, 4H), 2.08 (p, *J*=14.8Hz, 2H), 0.98 (t, *J*=7.5Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 179.64 (C), 131.90 (CH), 129.47 (CH), 128.45 (CH), 128.20 (CH), 128.16 (CH), 128.13 (CH), 127.99 (CH), 127.97 (CH), 127.88 (CH), 127.78 (CH), 127.44 (CH), 126.93 (CH), 33.94 (CH_2), 25.54 (CH_2), 25.53 (2xCH_2), 25.49 (CH_2) 25.45 (CH_2), 22.38 (CH_2), 20.47 (CH_2), 14.18 (CH_3).

IR: 3008, 2964, 2930, 2678, 1711 cm^{-1}.

Synthesis of *(9Z)*-octadeca-9-enoic amide



OA

Yield: 10 %.

Method:

General method for amidation was used, with oleic acid (285mg, 1.01mmol), CDI (186mg, 1.15mmol), and HMDS (0.46mL, 2.0mmol).

The sample was dissolved in 15 mL DCM, and HCl was added to a pH of 2. Then the sample was set in a N_2-atmosphere at rt with stirring for 12h. After this, the sample was washed with 10 % HCl (3x10mL) and 10 % NaOH (3x10mL). The organic phases were dried with Na_2SO_4 for 30 minutes, and the solvent was removed under reduced pressure.

The sample was purified with flash chromatography, and the product was eluted with 50 % EtOAc in hexane.

Data:

***R_f*:** 0.41 (50 % EtOAc in hexane).

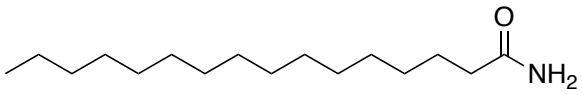
^{1}H NMR (400MHz, $CDCl_3$): δ 5.99 (s, 1H), 5.48 (s, 1H), 5.33 (m, 2H), 2.21 (t, *J*=15.3Hz, 2H), 2.00 (p, *J*=18.7 & 5.9Hz, 3H), 1.62 (t, *J*=7.0Hz, 2H), 1.28 (d, *J*=17.1Hz, 19H), 0.87 (t, *J*=6.8Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 176.31 (C), 130.14 (CH), 129.85 (CH), 36.10 (CH_2), 32.03 (CH_2), 29.89 (CH_2), 29.82 (CH_2), 29.73 (CH_2), 29.65 (CH_2), 29.44 (CH_2), 29.36 (CH_2), 29.33 (CH_2), 29.24 (CH_2), 27.35 (CH_2), 27.29 (CH_2), 25.64 (CH_2), 22.81 (CH_2), 14.24 (CH_3).

IR: 3355, 3187, 2924, 2852, 1711, 1633 cm^{-1}.

HR-MS: Found: 281.2718, calculated: 281.2719.

Synthesis of hexadecanoic amide



21

Yield: 24 %.

Method:

General method for amidation was used, with palmitic acid (332mg, 1.29mmol), CDI (202mg, 1.25mmol), and HMDS (0.46mL, 2.0mmol), in 5mL DCM and 10mL hexane.

The sample was dissolved in 15mL DCM, and HCl was added to a pH of 2. Then the sample was set in a N_2-atmosphere at rt with stirring for 12h. After this, the sample was washed with 10 % HCl (3x10mL) and 10 % NaOH (3x10mL). The organic phases were dried with Na_2SO_4 for 30 minutes, and the solvent was removed under reduced pressure.

The sample was purified with flash chromatography, where the product was eluted with 20 % EtOAc in hexane.

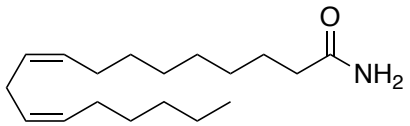
Data:

***R_f*:** 0.34 (50 % EtOAc in hexane).

^{1}H NMR (400MHz, $CDCl_3$ δ 5.37 (s, 2H, NH_2), 2.22 (t, *J*=15.3Hz, 2H), 1.63 (p, *J* = 7.5 Hz, 2H), 1.36 – 1.21 (m, 24H), 0.88 (t, *J* = 6.8 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 35.95 (CH_2), 31.93 (CH_2), 29.70 (CH_2), 29.66 (CH_2), 29.61 (CH_2), 29.49 (CH_2), 29.37 (CH_2), 29.35 (CH_2), 29.25 (CH_2), 25.55 (CH_2), 22.70 (CH_2), 14.13 (CH_3).

Synthesis of *(9Z, 12Z)*-octadeca-9,12-dienoic amide



24

Yield: 32 %.

Method:

General method for amidation was used, with linoleic acid (281mg, 1.00mmol), CDI (356mg, 2.20mmol), and HMDS (0.46mL, 2.0mmol).

The sample was dissolved in 15 mL DCM, and HCl was added to a pH of 2. Then the sample was set in a N_2-atmosphere at rt with stirring for 12h. After this, the sample was washed with 10 % HCl (3x10mL) and 10 % NaOH (3x10mL). The organic phases were dried with Na_2SO_4 for 30 minutes, and the solvent was removed under reduced pressure.

The sample was purified with flash chromatography, and the product was eluted with 50 % EtOAc in hexane.

Data:

***R_f*:** 0.32 (50 % EtOAc in hexane).

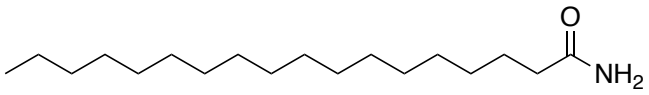
^{1}H NMR (400MHz, $CDCl_3$): δ 5.05 (m, 5H), 2.77 (t, *J*=6.5Hz, 2H), 2.22 (t, *J*=15.3Hz, 2H), 2.05 (q, *J*=6.8Hz, 3H), 1.64 (m, 2H), 1.32 (m, 13H), 0.89 (t, *J*=6.8Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 175.55 (C), 130.37 (CH), 130.17 (CH), 128.20 (CH), 128.04 (CH), 36.05 (CH_2), 31.66 (CH_2), 29.73 (CH_2), 29.48 (CH_2), 29.37 (CH_2), 29.34 (CH_2), 29.25 (CH_2), 27.34 (CH_2), 27.33 (CH_2), 25.77 (CH_2), 25.64 (CH_2), 22.71 (CH_2), 14.20 (CH_3).

IR: 3361, 3210, 2930, 2857, 2499, 1912, 1728, 1655, 1599 cm^{-1}.

HR-MS: Found: 279.2574, calculated: 279.2562.

Synthesis of octadecanoic amide



41

Yield: 35 %.

Method:

General method for amidation was used, with stearic acid (290mg, 1.02mmol), CDI (365mg, 2.25mmol), and HMDS (0.46mL, 2.0mmol), in 5mL DCM and 10mL hexane.

The sample was dissolved in 15mL DCM, and HCl was added to a pH of 2. Then the sample was set in a N_2-atmosphere at rt with stirring for 12h. After this, the sample was washed with 10 % HCl (3x10mL) and 10 % NaOH (3x10mL). The organic phases were dried with Na_2SO_4 for 30 minutes, and the solvent was removed under reduced pressure.

The sample was purified with flash chromatography, and the product was eluted with 20 % EtOAc in hexane.

Data:

***R_f*:** 0.36 (50 % EtOAc in hexane).

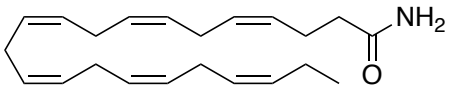
^{1}H NMR (400MHz, $CDCl_3$): δ 5.44 (s, 2H, NH_2), 2.22 (t, *J*=15.2Hz, 2H), 1.63 (p, *J*=14.4Hz, 2H), 1.25 (s, 24H), 0.88 (t, *J*=6.8Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 175.71 (C), 36.05 (CH_2), 32.04 (CH_2), 29.81 (CH_2), 29.79 (CH_2), 29.77 (CH_2), 29.73 (CH_2), 29.60 (CH_2), 29.48 (CH_2), 29.46 (CH_2), 29.36 (CH_2), 25.66 (CH_2), 22.81 (CH_2), 14.23 (CH_3).

IR: 3389, 3187, 2919, 2852, 1644, 1471, 1420 cm^{-1}.

HR-MS: Found: 283.2877, calculated: 283.2875.

Synthesis of *(4Z, 7Z, 10Z, 13Z, 16Z, 19Z)*-docosa-4,7,10,13,16,19-hexaenoic amide



42

Yield: >95 %.

Method:

General method for amidation was used, with **DHA** (162mg, 0.493mmol), CDI (169mg, 1.04mmol), and HMDS (0.21mL, 1.0mmol).

The sample was dissolved in 15 mL DCM, and HCl was added to a pH of 2. Then the sample was set in a N_2-atmosphere at rt with stirring for 12h. After this, the sample was washed with 10 % HCl (3x10mL) and 10 % NaOH (3x10mL). The organic phases were dried with Na_2SO_4 for 30 minutes, and the solvent was removed under reduced pressure.

The sample was purified with flash chromatography, and the product was eluted with 50 % EtOAc in hexane.

Data:

***R_f*:** 0.00 (20 % EtOAc in hexane).

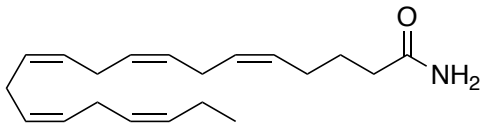
^{1}H NMR (400MHz, $CDCl_3$): δ 10.96 (s, 1H), 5.39 (m, 12H), 2.85 (s, 10H), 2.42 (s, 4H), 2.07 (p, *J*=14.7Hz, 2H), 0.98 (t, *J*=7.5Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 174.86 (C), 132.20 (CH), 129.67 (CH), 128.73 (CH), 128.48 (CH), 128.44 (CH), 128.42 (CH), 128.22 (CH), 128.20 (CH), 128.14 (CH), 128.02 (CH), 127.16 (CH), 35.78 (CH_2), 25.79 (CH_2), 25.77 (CH_2), 25.69 (CH_2), 23.34 (CH_2), 20.70 (CH_2), 14.41 (CH_3).

IR: 3014, 2904, 1677 cm^{-1}.

HR-MS: Found: 327.2545, calculated: 327.2562.

Synthesis of *(5Z, 8Z, 11Z, 14Z, 17Z)*-eicosa-5,8,11,14,17-pentaenoic amide



43

Yield: >95 %.

Method:

General method for amidation was used, with **EPA** (298mg, 0.985mmol), CDI (360mg, 2.22mmol), and HMDS (0.46mL, 2.0mmol).

The sample was purified using flash chromatography, and the product was eluted with 50 % EtOAc in hexane.

Data:

***R_f*:** 0.00 (20 % EtOAc in hexane).

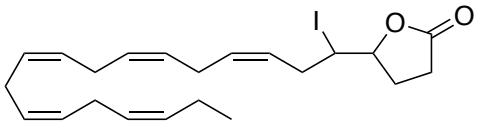
^{1}H NMR (400MHz, $CDCl_3$): δ 5.38 (m, 12H), 2.83 (m, 8H), 2.22 (t, *J*=15.2Hz, 2H), 2.10 (dq, *J*=19.6 & 14.7Hz, 4H), 1.72 (p, *J*=14.9Hz, 2H), 0.97 (t, *J*=7.5Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 175.28 (C), 132.21 (CH), 129.16 (CH), 129.00 (CH), 128.74 (CH), 128.42 (CH), 128.35 (CH), 128.31 (CH), 128.22 (CH), 128.01 (CH), 127.14 (CH), 35.26 (CH_2), 26.71, (CH_2), 25.79 (CH_2), 25.68 (CH_2), 25.38 (CH_2), 20.69 (CH_2), 14.40 (CH_3).

IR: 3014, 2964, 1655 cm^{-1}.

HR-MS: Found: 301.2414, calculated: 301.2406.

Synthesis of (*7Z, 10Z, 13Z, 16Z, 19Z*)-*5*-iodo-docosa-7,10,13,16,19-pentaenoic-4-lactone



27

Yield: 88 %.

Method:

DHA-EE (10.0g, 28.0mmol) was dissolved in a mixture of EtOH and H_2O (30mL each), and $LiOHxH_2O$ (5.67g, 135mmol) was added. The mixture was stirred for 4h whilst being monitored by use of TLC (20 % EtOAc in hexane).

To the mixture was added 90mL H_2O, and the solution was cooled to 0°C. Then 20 mL of a 57 % HI-solution, 10mL $KHCO_3$-solution and finally 2 spatula tips of $LiOHxH_2O$ was added, to a pH of 8. I_2 (21.4g, 84.2mmol) in 70mL THF was added dropwise, and the solution was left at 0-4°C in the dark for 18h.

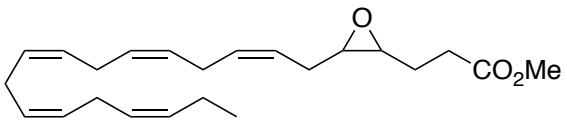
NaS_2O_3 in THF was added, and the solution was saturated with NaCl. The product was extracted with hexane (4x50mL), and the extract was washed with brine (2x50mL). $Na_2S_2O_3$ was then added to the solution until it went from dark brown to light yellow. The organic extract was dried with Na_2SO_4 for 30 minutes, before the solvent was removed under reduced pressure.

Data:

^{1}H NMR (400MHz, $CDCl_3$): δ 5.48 (s, 2H), 5.44 – 5.25 (m, 9H), 4.39 (t, *J* = 5.8 Hz, 1H), 3.33 (s, 6H), 2.88 – 2.74 (m, 9H), 2.41 (ddd, *J* = 7.1, 5.8, 1.4 Hz, 2H), 2.07 (td, *J* = 7.5, 1.3 Hz, 2H), 0.97 (t, *J* = 7.5 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 176.29 (C), 132.03 (CH), 131.56 (CH), 128.76 (CH), 128.60 (CH), 128.41 (CH), 127.93 (CH), 127.85 (CH), 127.38 (CH), 127.03 (CH), 126.76 (CH), 80.77 (CH), 67.94 (CH), 34.66 (CH_2), 28.54 (CH_2), 27.36 (CH_2), 25.91 (CH_2), 25.70 (CH_2), 25.67 (CH_2), 25.57 (CH_2), 20.58 (CH_2), 14.33 (CH_3).

Synthesis of (*7Z, 10Z, 13Z, 16Z, 19Z*)-methyl 4,5-epoxy-docosa-7,10,13,16,19-pentaenoate



28

Yield: 78 %.

Method:

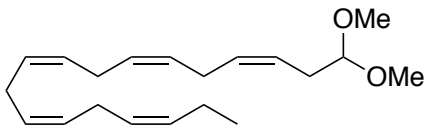
To a stirring solution of crude **27** (11.2g, 24.6mmol) in 150mL of MeOH was added K_2CO_3 (5.06g, 36.7mmol). The solution was stirred at rt for 3h before 50 mL H_2O was added, and the product was extracted with hexane (6x25mL). The extract was washed with brine (2x25mL), and dried with Na_2SO_4 (30min), before the solvent was removed under reduced pressure.

Data:

^{1}H NMR (400MHz, $CDCl_3$): δ 5.58 – 5.41 (m, 2H), 5.41 – 5.14 (m, 8H), 3.67 (s, 3H), 2.99 – 2.92 (m, 2H), 2.86 – 2.77 (m, 8H), 2.53 – 2.46 (m, 2H), 2.44 – 2.33 (m, 1H), 2.26 – 2.19 (m, 1H), 2.05 (p, *J* = 7.4 Hz, 2H), 1.97 – 1.86 (m, 1H), 1.85 – 1.71 (m, 1H), 0.95 (t, *J* = 7.5 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 173.14 (C), 132.00 (CH), 130.64 (CH), 128.57 (CH), 128.48 (CH), 128.33 (CH), 127.94 (CH), 127.81 (CH), 127.70 (CH), 126.99 (CH), 124.20 (CH), 56.58 (CH), 55.91 (CH), 51.69 (CH_3), 30.97 (CH_2), 26.20 (CH_2), 25.80 (CH_2), 25.63 (CH_2), 25.61 (CH_2), 25.52 (CH_2), 23.33 (CH_2), 20.54 (CH_2), 14.26 (CH_3).

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



29a

Yield: 40 %.

Method:

Crude **28** (6.71g, 18.7mmol) was dissolved in anhydrous MeOH (150mL) before periodic acid was added (4.70g, 20.5mmol). The mixture was set to stirring at rt for 6h before 50mL H_2O was added, and the product was extracted with hexane (6x25mL). The extract was then washed with H_2O (3x25mL) and brine (2x25mL). The product was dried with $MgSO_4$ (15min), and the solvent was removed under reduced pressure.

The crude sample was purified by flash chromatography, where the product was eluted as a colourless oil, with 5 % EtOAc in hexane.

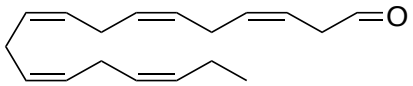
Data:

***R_f*:** 0.37 (5 % EtOAc in hexane).

^{1}H NMR (400MHz, $CDCl_3$): δ 5.53 – 5.45 (m, 2H), 5.45 – 5.19 (m, 8H), 4.39 (t, *J* = 5.8 Hz, 1H), 3.33 (s, 6H), 2.92 – 2.68 (m, 8H), 2.41 (t, *J* = 6.3 Hz, 2H), (p, *J* = 7.3 Hz, 2H), 0.97 (t, *J* = 7.5 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 132.17 (CH), 130.43 (CH), 128.71 (CH), 128.43 (2xCH), 128.19 (CH), 128.10 (CH), 128.00 (CH), 127.15 (CH), 124.09 (CH), 104.22 (CH), 53.08 (2xCH_3), 31.16 (CH_2), 25.98 (CH_2), 25.77 (CH_2), 25.76 (CH_2), 25.67 (CH_2), 20.69 (CH_2), 14.40 (CH_3).

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



29

Yield: 88 %.

Method:

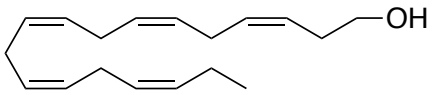
To a stirring solution of acetal **29a** (927mg, 3.05mmol) in dioxane (10mL) was added 12mL aqueous formic acid (80 %). After 1.5h at rt, 25mL H_2O was added, the product was extracted with hexane (3x25mL), and the extract was washed with aq. sat. $NaHCO_3$-solution (25mL). Then, the extract was washed with brine, dried with $MgSO_4$ (15min), and the solvent removed under reduced pressure, leaving behind a colourless oil.

Data:

^{1}H NMR (400MHz, $CDCl_3$): δ 9.66 (s, 1H, C<u>H</u>O), 5.78 - 5.51 (m, 2H), 5.49 – 5.23 (m, 8H), 3.21 (d, *J* = 7.3 Hz, 2H), 2.82 (m, 8H), 2.07 (p, *J* = 7.2 Hz, 2H), 0.97 (t, *J* = 7.5 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 199.36 (CHO), 133.22 (CH), 132.17 (CH), 128.96 (CH), 128.74 (CH), 128.55 (CH), 127.92 (CH), 127.89 (CH), 127.23 (CH), 127.09 (CH), 118.81 (CH), 42.60 (CH_2), 26.09 (CH_2), 25.77 (CH_2), 25.75 (CH_2), 25.66 (CH_2), 20.67 (CH_2), 14.38 (CH_3).

Synthesis of (*3Z, 6Z, 9Z, 12Z, 15Z*)-octadeca-3,6,9,12,15-pentaen-1-ol



44

Yield: 87 %.

Method:

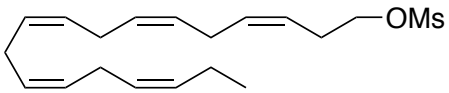
To a stirring solution of aldehyde **29** (546mg, 2.12mmol) in ice-cooled MeOH (6.4mL) was added $NaBH_4$ (207mg, 5.47mmol) in 8.2mL MeOH. The mixture was stirred at 0°C for 30min before 1.4M HCl was added (8.2mL). The product was then extracted with hexane-ether (2:1) (1x20mL+2x10mL), washed with brine (2x10mL), and dried with $MgSO_4$ (15min). The solvent was removed under reduced pressure, leaving behind a colourless oil.

Data:

^{1}H NMR (400MHz, $CDCl_3$): δ 5.64 – 5.50 (m, 1H), 5.45 – 5.10 (m, 9H), 3.66 (t, *J* = 6.5 Hz, 2H), 2.93 – 2.75 (m, 8H), 2.36 (q, *J* = 6.6 Hz, 2H), 2.08 (p, *J* = 7.1 Hz, 2H), 1.46 (s, 1H), 0.97 (t, *J* = 7.5 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 132.20 (CH), 131.24 (CH), 128.72 (CH), 128.47 (CH), 128.44 (CH), 128.17 (CH), 128.12 (CH), 128.00 (CH), 127.15 (CH), 125.80 (CH), 62.35 (CH_2), 30.96 (CH_2), 25.90 (CH_2), 25.79 (CH_2), 25.77 (CH_2), 25.68 (CH_2), 20.70 (CH_2), 14.41 (CH_3).

Synthesis of (*3Z, 6Z, 9Z, 12Z, 15Z*)-octadeca-3,6,9,12,15-pentaenyl methanesulfonate



45

Yield: >95 %.

Method:

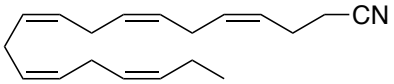
To a stirring, ice-cooled solution of alcohol **44** (476mg, 1.83mmol) in anhydrous DCM (5mL) and triethylamine (0.51mL, 3.7mmol) was added methanesulfonyl chloride (0.28mL, 3.7mmol). The reaction mixture was allowed to reach rt. After 2.5h brine (10mL) was added, and volatile substances removed in vacuo. The product was then extracted with EtOAc (3x10mL), washed with sat. $NaHCO_3$-solution (2x10mL) and brine (2x10mL) and dried over Na_2SO_4 (30min). The solvent was removed under reduced pressure, leaving behind a brown oil.

Data:

^{1}H NMR (400MHz, $CDCl_3$): δ 5.63 – 5.49 (m, 1H), 5.51 – 5.17 (m, 9H), 4.22 (t, *J* = 6.8 Hz, 2H), 3.00 (s, 3H), 2.83 (m, 8H), 2.54 (q, *J* = 6.8 Hz, 2H), 2.07 (p, *J* = 7.2 Hz, 2H), 0.97 (t, *J* = 7.5 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 132.20 (CH), 132.05 (CH), 128.80 (CH), 128.76 (CH), 128.55 (CH), 128.02 (CH), 127.94 (CH), 127.62 (CH), 127.12 (CH), 123.46 (CH), 69.16 (CH_2), 37.64 (CH_3), 27.50 (CH_2), 25.87 (CH_2), 25.78 (CH_2), 25.76 (CH_2), 25.68 (CH_2), 20.69 (CH_2), 14.41 (CH_3).

Synthesis of (*4Z, 7Z, 10Z, 13Z, 16Z*)-nonadeca-4,7,10,13,16-pentaenenitrile



46

Yield: 59 % (method I), 39 % (method II).

Method I:

To a solution of mesylate **45** (619mg, 1.83mmol) in 5mL DMSO was added KCN (182mg, 2.79mmol). The solution was stirred at 70°C for 2.5h before H_2O (25mL) was added. The product was extracted with EtOAc and hexane. The extract was washed thoroughly with H_2O, before it was dried over Na_2SO_4 (30min) before the solvent was removed under reduced pressure.

The product was purified with flash chromatography, where it was eluted with EtOAc in hexane (1:15), as a yellow oil.

Method II:

Mesylate **45** (129mg, 0.38mmol) was dissolved in 2mL acetonitrile, and 18-crown-6 (65mg, 0.25mmol) and KCN (42mg, 0.64mmol) was added. The mixture was stirred overnight at rt. After 24h, the mixture was warmed to 70°C, and stirred for another 3.5h. Then, H_2O (15mL) was added, and the product was extracted with hexane (3x10mL) and EtOAc (2x10mL), washed with H_2O (2x10mL) and dried over Na_2SO_4 (30min).

The product was purified with flash chromatography, where it was eluted with 10 % EtOAc in hexane.

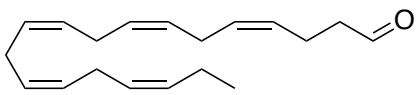
Data:

***R_f*:** 0.43 (10 % EtOAc in hexane).

^{1}H NMR (400MHz, $CDCl_3$): δ 5.62 – 5.49 (m, 2H), 5.45 – 5.16 (m, 8H), 2.83 (m, 8H), 2.44 – 2.33 (m, 4H), 2.08 (p, J = 7.2 Hz, 2H), 0.98 (t, J = 7.5 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 132.19 (CH), 131.58 (CH), 128.85 (CH), 128.75 (CH), 128.55 (CH), 127.96 (CH), 127.91 (CH), 127.54 (CH), 127.10 (CH), 125.62 (CH), 119.40 (C), 25.77 (2xCH_2), 25.67 (CH_2), 23.41 (CH_2), 20.68 (CH_2), 17.62 (CH_2), 14.40 (CH_3).

Synthesis of (*4Z, 7Z, 10Z, 13Z, 16Z*)-nonadeca-4,7,10,13,16-pentaenal



47

Yield: 93 %.

Method:

To a solution of nitrile **46** (101mg, 0.375mmol) in 3mL hexane at -78°C was added 1M DIBAL-H in hexane (0.56mL, 0.56mmol) dropwise. The mixture was stirred for 2.5h. Then, sat. aq. Rochelle salt (2.5mL) was added dropwise to the reaction mixture at 0°C, and it was stirred at 0°C to rt overnight. Sat. aq. Rochelle salt (15mL) and 10mL EtOAc was added, and the product was extracted with EtOAc (4x10mL). The extract was washed with brine (2x10mL) and dried over $MgSO_4$ (15min). The solvent was removed under reduced pressure, leaving behind a yellow oil.

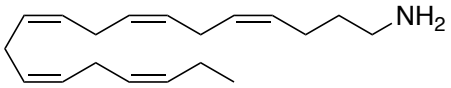
Data:

***R_f*:** 0.36 in EtOAc/hexane (1:15).

^{1}H NMR (400MHz, $CDCl_3$): δ 9.78 (s, 1H, C<u>H</u>O), 5.50 – 5.25 (m, 10H), 2.95 – 2.74 (m, 8H), 2.57 – 2.47 (m, 0.5H), 2.41 (t, *J* = 6.7 Hz, 0.5H), 2.39 – 2.29 (m, 1H), 2.22 (q, *J* = 6.6 Hz, 1H), 2.07 (p, *J* = 7.3 Hz, 2H), 0.97 (t, *J* = 7.5 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 201.90 (CHO), 132.05 (CH), 129.43 (CH), 128.59 (CH), 128.39 (CH), 128.34 (CH), 128.30 (CH), 128.28 (CH), 128.14 (CH), 128.08 (CH), 128.00 (CH), 127.88 (CH), 127.85 (CH), 127.69 (CH), 127.02 (CH), 43.68 (CH_2), 25.65 (CH_2), 25.64 (CH_2), 25.55 (CH_2), 20.57 (CH_2), 14.28 (CH_3).

Synthesis of (*4Z, 7Z, 10Z, 13Z, 16Z*)-nonadeca-4,7,10,13,16-pentaen-1-amine



48

Yield: 60 %.

Method:

To a solution of nitrile **46** (47mg, 0.17mmol) in 1mL anhydrous THF at 0°C was added 1M $LiAlH_4$ in THF (0.50mL, 0.50mmol). The mixture was warmed to rt and stirred overnight. Another 2mL THF was added, and the mixture was stirred for another 24h before sat. aq. Rochelle salt (15mL) was added dropwise, and it was let to stir for another 5h. The phases were separated, and the inorganic phase was extracted with EtOAc (3x10mL). The combined organic phases were washed with sat. aq. Rochelle salt (10mL) and brine (10mL) and dried over Na_2SO_4 (30min) before the solvent was removed under reduced pressure.

Data:

***R_f*:** 0.36 (20 % EtOAc in hexane).

^{1}H NMR (400MHz, $CDCl_3$): δ 5.51 – 5.23 (m, 9H), 2.92 – 2.77 (m, 7H), 2.75 – 2.67 (m, 1H), 2.56 – 2.23 (m, 2H), 2.16 – 2.00 (m, 3H), 1.53 (q, *J* = 7.3 Hz, 1H), 0.97 (t, *J* = 7.5 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 132.17 (CH), 129.71 (CH), 128.69 (CH), 128.43 (CH), 128.37 (CH), 128.32 (CH), 128.23 (CH), 128.20 (CH), 128.00 (CH), 127.13 (CH), 41.84 (CH_2), 33.49 (CH_2), 25.76 (CH_2), 25.67 (CH_2), 24.75 (CH_2), 20.68 (CH_2), 14.40 (CH_3).

5 References

1. Dewick, P. M., **2009;** pp 7-8. ISBN: 978-0-470-74168-9
2. Newman, D. J.; Cragg, G. M., *J. Nat. Prod.* **2016,** *79* (3), 629-61.
3. Dewick, P. M., **2009;** pp 147-48. ISBN: 978-0-470-74168-9
4. Mechoulam, R. a. G., Y., *J. Am. Chem. Soc.* **1964,** *86*, 1646-47.
5. Moss, G. P., Smith, P. A. S., and Tavernier D., *Pure Appl. Chem.* **1995,** *67* (8/9), 1307-75.
6. Mathews, C. K., van Holde, K. E., Ahern, K. G., 3 ed.; Pearson: **2000;** pp 315-23. ISBN: 978-0-8053-3066-3
7. Simopoulos, A. P., *Am. J. Clin. Nutr.* **1991,** *54* (3), 438-63.
8. Palmquist, D. L., *Prof. Anim. Scient.* **2009,** *25*, 207-49.
9. Dewick, P. M., **2009;** pp 58-64. ISBN: 978-0-470-74168-9
10. Wiktorowska-Oxczarek, A., Berezinska M and Nowak, JZ., *Adv. Clin. Exp. Med.* **2015,** *24* (6), 931-41.
11. Michalak, A.; Mosińska, P.; Fichna, J., *Front. Pharmacol.* **2016,** *7*, 459-59.
12. Serhan, C. N.; Petasis, N. A., *Chem. Rev.* **2011,** *111* (10), 5922-43.
13. Gabbs, M.; Leng, S.; Devassy, J. G.; Monirujjaman, M.; Aukema, H. M., *Adv. Nutr.* **2015,** *6* (5), 513-40.
14. Prost, I.; Dhondt, S.; Rothe, G.; Vicente, J.; Rodriguez, M. J.; Kift, N.; Carbonne, F.; Griffiths, G.; Esquerré-Tugayé, M.-T.; Rosahl, S.; Castresana, C.; Hamberg, M.; Fournier, J., *Plant Physiol.* **2005,** *139* (4), 1902-13.
15. Farrell, E. K.; Merkler, D. J., *Drug Discov. Today* **2008,** *13* (13), 558-68.
16. Caterina, M. J.; Schumacher, M. A.; Tominaga, M.; Rosen, T. A.; Levine, J. D.; Julius, D., *Nature* **1997,** *389*, 816.
17. Morad, S. A. F., and Cabot, M. C. , *Nat. Rev. Cancer* **2012,** *13* (1), 51-65.
18. Petrache, I. a. B., E. V., *Annu. Rev. Physiol.* **2016,** *78* (1), 463-80.
19. Kuehl, F. A. J., Jacob, T. A., Ganleym O. H., Ormond, R. E., and Mesinger, M. A. P., *J. Am. Chem. Soc.* **1957,** *79*, 5577-78.
20. Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A. and Mechoulam R., *Science* **1992,** *258*, 1946-49.
21. Walker, J. M., Huang, S. M., Strangman, N. M., Tsou, K., and Sañudo-Peña M. C., *Proc. Natl. Acad. Sci. USA* **1999,** *96*, 12198-203.
22. Williams, C. M., and Kirkham, T. C., *Psychopharmacology* **1999,** *143*, 315-17.

23. Kathuria, S., Gaetani, S., Fegley, D., Valiño, F., Duranti, A., Tontini, A., Mor, M., Tarzia, G., La Rana, G., Calignano, A., Guistino, A., Tattoli, M., Palmery, M., Cuomo, V., and Piomelli, D., *Nat. Med.* **2003,** *9*, 76-81.
24. Zygmunt, P. M.; Petersson, J.; Andersson, D. A.; Chuang, H.-h.; Sørgård, M.; Di Marzo, V.; Julius, D.; Högestätt, E. D., *Nature* **1999,** *400*, 452.
25. Smart, D.; Gunthorpe, M. J.; Jerman, J. C.; Nasir, S.; Gray, J.; Muir, A. I.; Chambers, J. K.; Randall, A. D.; Davis, J. B., *Br. J. Pharmacol.* **2000,** *129* (2), 227-30.
26. Deutsch, D. G. a. C., S. A., *Biochem. Pharmacol.* **1993,** *46* (5), 791-96.
27. Deutsch, D. G., Ueda, N. and Yamamoto, S., *Prostag. Leukotr. Ess.* **2002,** *66*, 201-10.
28. Koga, D., Santa, T., Fukushima, T., Homma, H., and Imai, K., *J. Chromatogr. B* **1996,** *690*, 7-13.
29. Mechoulam, R., Fride, E. and Di Marzo, V., *Eur. J. Pharmacol.* **1998,** *359*, 1-18.
30. Huang, S. M.; Bisogno, T.; Trevisani, M.; Al-Hayani, A.; De Petrocellis, L.; Fezza, F.; Tognetto, M.; Petros, T. J.; Krey, J. F.; Chu, C. J.; Miller, J. D.; Davies, S. N.; Geppetti, P.; Walker, J. M.; Di Marzo, V., *Proc. Natl. Acad. Sci. USA* **2002,** *99* (12), 8400.
31. Bisogno, T.; Melck, D.; Bobrov, M.; Gretskaya, N.; Bezuglov, V.; De petrocellis, L.; Di Marzo, V., *Biochem. J.* **2000,** *351 Pt 3*, 817-24.
32. Chu, C. J. H., S. M.; De Petrocellis, L.; Bisogno, T.; Ewing, S. A.; Miller, J. D.; Zipkin, R. E.; Daddario, N.; Appendino, G.; Di Marzo, V.; and Walker, J. M., *J. Biol. Chem.* **2003,** *278* (16), 13633-39.
33. De Petrocellis, L.; Chu, C. J.; Moriello, A. S.; Kellner, J. C.; Walker, J. M.; Di Marzo, V., *Br. J. Pharmacol.* **2004,** *143* (2), 251-56.
34. De Petrocellis, L.; Bisogno, T.; Davis, J. B.; Pertwee, R. G.; Di Marzo, V., *FEBS Lett.* **2000,** *483* (1), 52-56.
35. Högestätt, E. D., Jönsson, B. A. G., Ermund, A., Andersson, D. A., Björk, H., Alexander, J. P., Cravatt, B. F., Basbaum, A. I., and Zygmunt, P. M., *J. Biol. Chem.* **2005,** *280*, 31405-12.
36. Vandevoorde, S.; Jonsson, K.-O.; Fowler, C. J.; Lambert, D. M., *J. Med. Chem.* **2003,** *46* (8), 1440-48.
37. Takahashi, N., Ohba, T., Yamauchi, T., and Higashiyama, K., *Bioorg. Med. Chem.* **2006,** *14*, 6089-96.
38. Huang, S. M., Bisogno, T., Petros, T. J., Chang, S. Y., Zavitsanos, P. A., Zipkin, R. E., Sivakumar, R., Coop, A., Maeda, D. Y., De Petrocellis, L., Burstein, S., Di Marzo, V., and Walker, J. M., *J. Biol. Chem.* **2001,** *276*, 42639-44.

39. Burstein, S. H.; McQuain, C. A.; Ross, A. H.; Salmonsen, R. A.; Zurier, R. E., *J. Cell. Biochem.* **2011,** *112* (11), 3227-33.
40. Burstein, S. H.; Rossetti, R. G.; Yagen, B.; Zurier, R. B., *Prostag. Oth. Lipid M.* **2000,** *61* (1-2), 29-41.
41. Sheskin, T.; Hanuš, L.; Slager, J.; Vogel, Z.; Mechoulam, R., *J. Med. Chem.* **1997,** *40* (5), 659-67.
42. Cascio, M. G. M., A.; Ligresti, A.; Appendino, G.; Burstein, S.; Di Marzo, V., *Biochem. Biophys. Res. Commun.* **2004,** *314*, 192-96.
43. Burstein, S. H., *Mol. Pharmacol.* **2018,** *93* (3), 228.
44. Kohno, M.; Hasegawa, H.; Inoue, A.; Muraoka, M.; Miyazaki, T.; Oka, K.; Yasukawa, M., *Biochem. Biophys. Res. Commun.* **2006,** *347* (3), 827-32.
45. Saghatelian, A., McKinney, M. K., Bandell, M., Patapoutian, A., and Cravatt, B. F., *Biochem.* **2006,** *45* (30), 9007-15.
46. Arafat, E. S.; Trimble, J. W.; Andersen, R. N.; Dass, C.; Desiderio, D. M., *Life Sci.* **1989,** *45* (18), 1679-87.
47. Cravatt, B. F., Prospero-Garcia, O., Siuzdak, G., Gilula, N. B., Henriksen, S. J., Boger, D. L., and Lerner, R. A., *Science* **1995,** *268*.
48. Hiley, C. R.; Hoi, P. M., *Cardiovasc. Drug Rev.* **2007,** *25* (1), 46-60.
49. Lo, Y.-K.; Tang, K.-Y.; Chang, W.-N.; Lu, C.-H.; Cheng, J.-S.; Lee, K.-C.; Chou, K.-J.; Liu, C.-P.; Chen, W.-C.; Su, W.; Law, Y.-P.; Jan, C.-R., *Biochem.Pharmacol.* **2001,** *62* (10), 1363-69.
50. Wakamatsu, K., Masaki, T., Itoh, F., Kondo, K. and Sudo, K., *Biochem. Biophys. Res. Commun.* **1990,** *168* (2), 423-29.
51. Vik, A.; Hansen, T. V., *Org. Biomol. Chem.* **2018**.
52. Corey, E. J., Niwa, H., and Falck, J. R., *J. Am. Chem. Soc.* **1979,** *101* (6), 1586-87.
53. Corey, E. J.; Marfat, A.; Falck, J. R.; Albright, J. O., *J. Am. Chem. Soc.* **1980,** *102* (4), 1433-35.
54. Corey, E. J.; Albright, J. O.; Barton, A. E.; Hashimoto, S., *J. Am. Chem. Soc.* **1980,** *102* (4), 1435-36.
55. Wright, S. W.; Kuo, E. Y.; Corey, E. J., *J. Org. Chem.* **1987,** *52* (19), 4399-401.
56. Kuklev, D. V.; Aizdaicher, N. A.; Imbs, A. B.; Bezuglov, V. V.; Latyshev, N. A., *Phytochemistry* **1992,** *31* (7), 2401-03.
57. Flock, S.; Antonsen, S.; Gallantree-Smith, H.; Langseter, A. M.; Skattebøl, L.; Stenstrøm, Y., *Tetrahedron* **2016,** *72* (30), 4518-22.
58. Langseter, A. M., Stenstrøm, Y. and Skattebøl, L., *Molecules* **2014,** *19*, 3804-12.

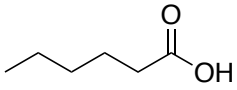
59. Holmeide, A. K.; Skattebøl, L.; Sydnes, M., *J. Chem. Soc. Perkin Trans. 1* **2001,** (16), 1942-46.
60. Hansen, T. V.; Skattebøl, L., *Tetrahedron Lett.* **2004,** *45* (13), 2809-11.
61. Vik, A.; Hansen, T. V.; Holmeide, A. K.; Skattebøl, L., *Tetrahedron Lett.* **2010,** *51* (21), 2852-54.
62. Flock, S.; Kristin Holmeide, A.; Skattebøl, L., *Synth. Commun.* **2007,** *37* (22), 4005-15.
63. Flock, S., Lundquist, M. and Skattebøl, L., *Acta Chem. Scand.* **1999,** *53*, 436-45.
64. Hamberg, M.; Chechetkin, I. R.; Grechkin, A. N.; León, I. P.; Castresana, C.; Bannenberg, G., *Lipids* **2006,** *41* (5), 499-506.
65. Pitt, M. J.; Easton, C. J.; Ferrante, A.; Poulos, A.; Rathjen, D. A., *Chem. Phys. Lipids* **1998,** *92* (1), 63-69.
66. Carey, J. S., Laffan, D., Thomson, C., and Williams, M. T., *Org. Biomol. Chem.* **2006,** *4*, 2337-47.
67. Dunlap, F. K., *J. Am. Chem. Soc.* **1902,** *24*, 758-63.
68. Montalbetti, C. A. G. N.; Falque, V., *Tetrahedron* **2005,** *61* (46), 10827-52.
69. Joullié, M. M., and Lassen, K. M., *ARKIVOC* **2010,** *viii*, 189-250.
70. Sheehan, J. C. a. H., G. P., *J. Am. Chem. Soc.* **1955,** *77*, 1067-68.
71. König, W.; Geiger, R., *Chem. Ber.* **1970,** *103* (3), 788-98.
72. Czarnocki, Z., Matuszewska, M. P. and Matuszewska I., *Org. Prep. Proced. Int.* **1998,** *30* (6), 699-702.
73. Castro, B. D., J. R.; Evin, G; Selve, C., *Tetrahedron Lett.* **1975,** *14*, 1219-22.
74. Zapp, J. A., *Am. Ind. Hyg. Assoc. J.* **1975,** *36* (12), 916-19.
75. Lee, K. P. a. T. H. J., *J. Natl. Cancer Inst.* **1982,** *36*, 157-71.
76. Tremblay, H.; St-Georges, C.; Legault, M.-A.; Morin, C.; Fortin, S.; Marsault, E., *Bioorg. Med. Chem. Lett.* **2014,** *24* (24), 5635-38.
77. Al-Mulla, E. A. J.; Yunus, W. M. Z. W.; Ibrahim, N. A. B.; Rahman, M. Z. A., *J. Oleo Sci.* **2010,** *59* (2), 59-64.
78. Paul, R.; Anderson, G. W., *J. Am. Chem. Soc.* **1960,** *82* (17), 4596-600.
79. Sharma, R. K. a. J., Rahul., *Synlett* **2007,** *4*, 603-06.
80. Johansson, S. J. R.; Johannessen, T.; Ristun, M. S.; Ellefsen, C. F.; Antonsen, S.; Nolsøe, J. M. J.; Hansen, T. V.; Stenstrøm, Y. H., *Synlett* **2018,** *DOI: 10.1055/s-0037-1611939*.
81. Johannessen, T. Norwegian University of Life Sciences, Ås, **2018**.
82. Johansson, S. J. R. Norwegian University of Life Sciences, Ås, **2017**.
83. Ristun, M. S. Norwegian University of Life Sciences, Ås, **2018**.

84. Takao, K.; Noguchi, K.; Hashimoto, Y.; Shirahata, A.; Sugita, Y., *Chem. Pharm. Bull.* **2015,** *63* (4), 278-85.
85. Sinning, C.; Watzer, B.; Coste, O.; Nüsing, R. M.; Ott, I.; Ligresti, A.; Marzo, V. D.; Imming, P., *J. Med. Chem.* **2008,** *51* (24), 7800-05.
86. Di Marzo, V., Ligresti, A., Morera, E., Nalli, M., and Ortar, G., *Bioorg. Med. Chem.* **2004,** *12* (5161-5169).
87. Bondi, S.; Eissler, F., *Biochem. Z.* **1910,** *23*, 499-513.
88. Hell, Z.; Cwik, A.; Finta, Z.; Horváth, Z., *J. Mol. Catal. A - Chemical* **2002,** *184* (1), 191-95.
89. Abbott, L. D., *J. Biol. Chem.* **1942,** *145* (1), 241-45.
90. Pearson, A. J., and Roush, W. R., John Wiley & Sons: **1999**. ISBN: 978-0471979272
91. Fee, J. A.; Fife, T. H., *J. Org. Chem.* **1966,** *31* (7), 2343-46.
92. Fife, T. H.; Natarajan, R.; Werner, M. H., *J. Org. Chem.* **1987,** *52* (5), 740-46.
93. Zaramella, S.; Strömberg, R.; Yeheskiely, E., *Eur. J. Org. Chem.* **2002,** *2002* (15), 2633-39.
94. Fong, C.; Wells, D.; Krodkiewska, I.; Hartley, P. G.; Drummond, C. J., *Chem. Mater.* **2006,** *18* (3), 594-97.
95. Guy, J. B.; Smith, J. C., *J. Chem. Soc.* **1939,** (0), 615-18.
96. Awasthi, N. P.; Upadhayay, S. K.; Singh, R. P., *J. Oleo Sci.* **2008,** *57* (9), 471-75.
97. Filippova, L.; Aarum, I.; Ringdal, M.; Dahl, M. K.; Hansen, T. V.; Stenstrøm, Y., *Org. Biomol. Chem.* **2015,** *13* (16), 4680-85.
98. Pangopoulos, M. K. Norwegian University of Life Sciences, Ås, **2016**.
99. Antonsen, G. S.; Gallantree-Smith, H.; Görbitz, H. C.; Hansen, V. T.; Stenstrøm, H. Y.; Nolsøe, M. J., *Molecules* **2017,** *22* (10).

6 Appendix

6.1 Additional reactions

Synthesis of hexanoic acid



54

Yield: 54 %.

Method:

To 40mL H_2O at 0°C was carefully added H_2SO_4 (5mL) under stirring. The solution was cooled to rt, and 1-hexanol (4.9mL, 40mmol) was added. Then, $KMnO_4$ (14g, 80mmol) was added in small portions in minute-laps. The solution was refluxed at 40°C, overnight.

A 39 % $NaHSO_4$-solution (approx. 50mL) was added, to a colorless solution. The phases were separated, and the water phase was extracted with 50mL diethyl ether. The organic phases were extracted with 5M NaOH-solution (20mL), and the extract was washed with diethyl ether (30mL). The extract was then acidified with HCl to pH 1, and the two phases were separated. The water phase was extracted with diethyl ether (30mL), and the extract, together with the organic phase, was dried with Na_2SO_4 (30min), before the solvent was removed under reduced pressure.

The product was finally purified with column chromatography, where it was eluted with 25 % EtOAc in hexane.

Data:

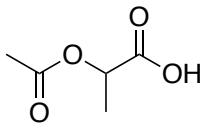
***R_f*:** 0.71 (25 % EtOAc in hexane).

^{1}H NMR (400MHz, $CDCl_3$): δ 11.99 (s, 1H, O<u>H</u>), 2.36 – 2.31 (m, 2H), 1.62 (p, *J* = 7.6 Hz, 2H), 1.39 – 1.24 (m, 4H), 0.89 (t, *J* = 7.2, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 180.91 (C), 34.23 (CH_2), 31.33 (CH_2), 24.47 (CH_2), 22.41 (CH_2), 13.93 (CH_3).

IR: 2931, 2875, 1712 cm^{-1}.

Synthesis of (2-acetoxy)-propanoic acid



55

Yield: 29 %

Method:

To a stirred solution of lactic acid (1.27g, 14.1mmol) in 2mL THF was added acetyl chloride (4mL, 56.3mmol). The solution was stirred at rt for 2h while being monitored by TLC. The solvent was removed under reduced pressure, and the product was distilled at 117°C and 12 mmHg and then purified with column chromatography, where the product was eluted with 1:50 MeOH in $CHCl_3$.

Data:

R_f: 0.20 (MeOH in $CHCl_3$, 1:50).

^{1}H NMR (400MHz, $CDCl_3$): δ 11.88 – 11.57 (m, 1H, O<u>H</u>), 5.05 – 4.98 (m, 1H), 2.17 – 1.99 (m, 3H), 1.48 – 1.41 (m, 5.4 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 175.90 (C), 170.88 (C), 68.26 (CH), 20.40 (CH_3), 16.61 (CH_3).

IR: 3473, 2998, 2925, 2634, 1723 cm^{-1}.

6.2 Spectra

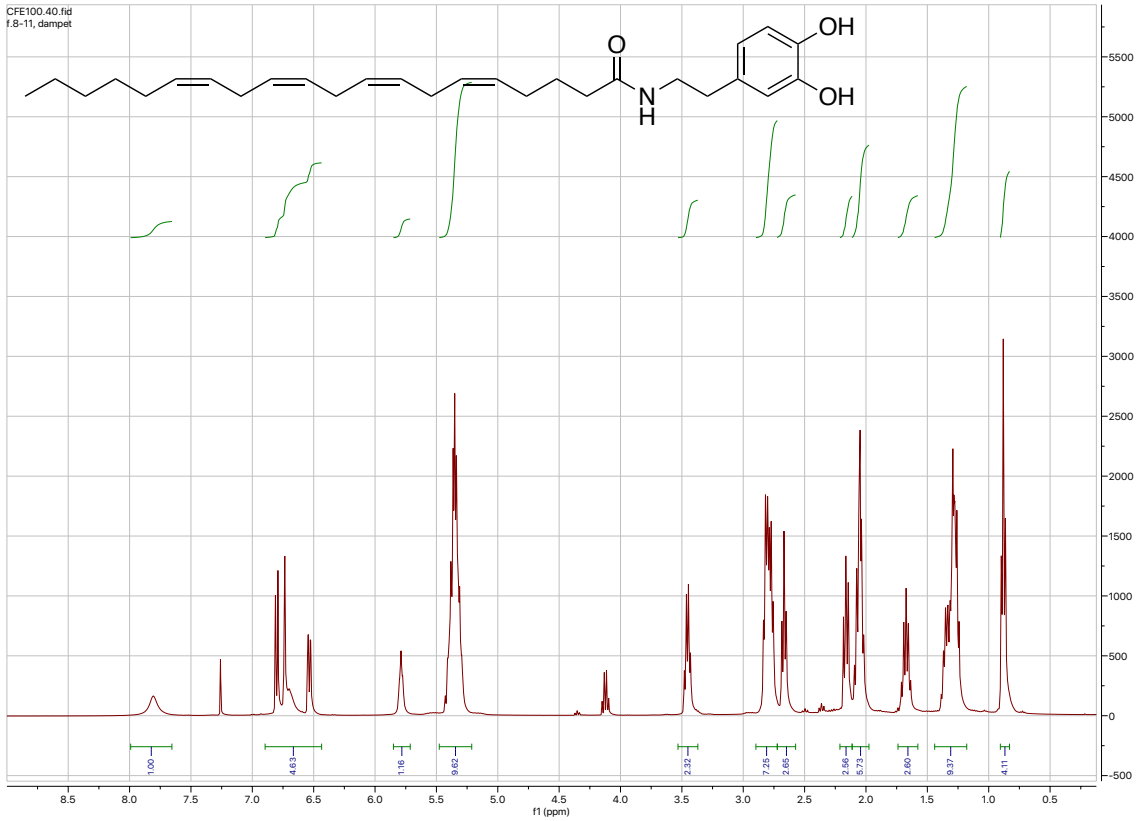


Figure 6-1 ^{1}H NMR spectrum of NADA.

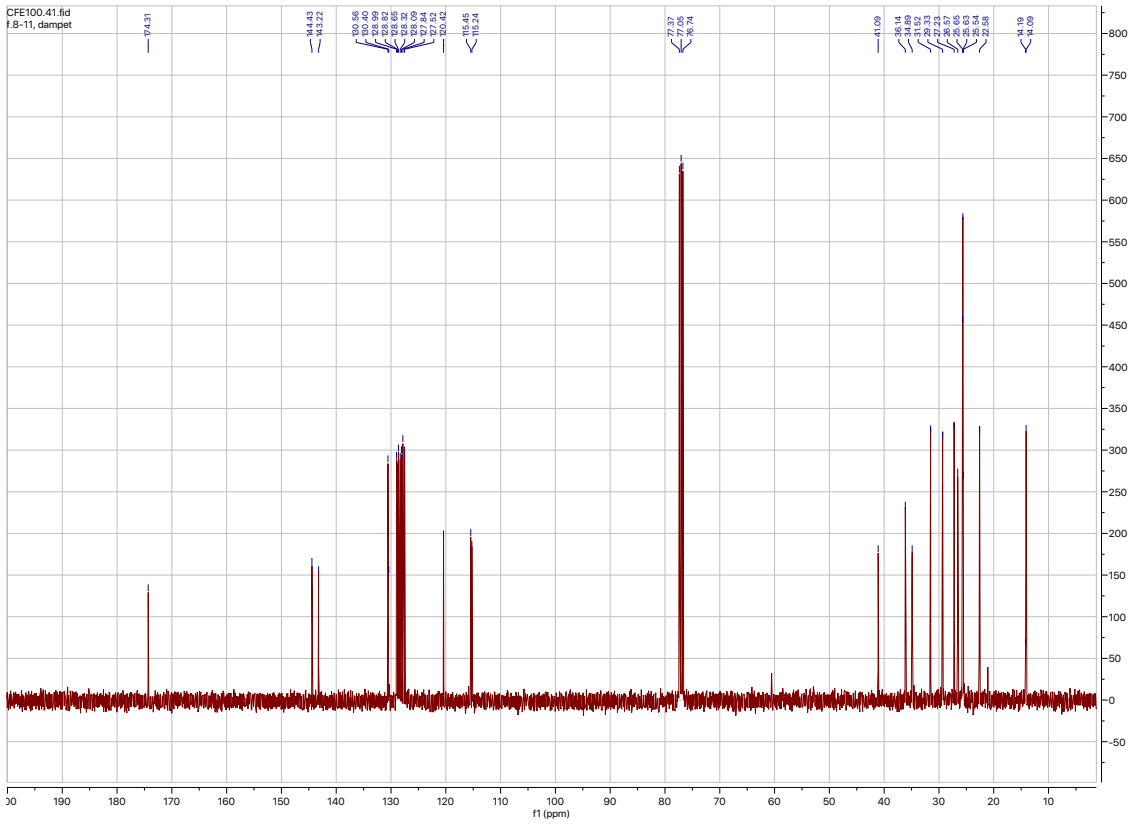


Figure 6-2 ^{13}C NMR spectrum of NADA.

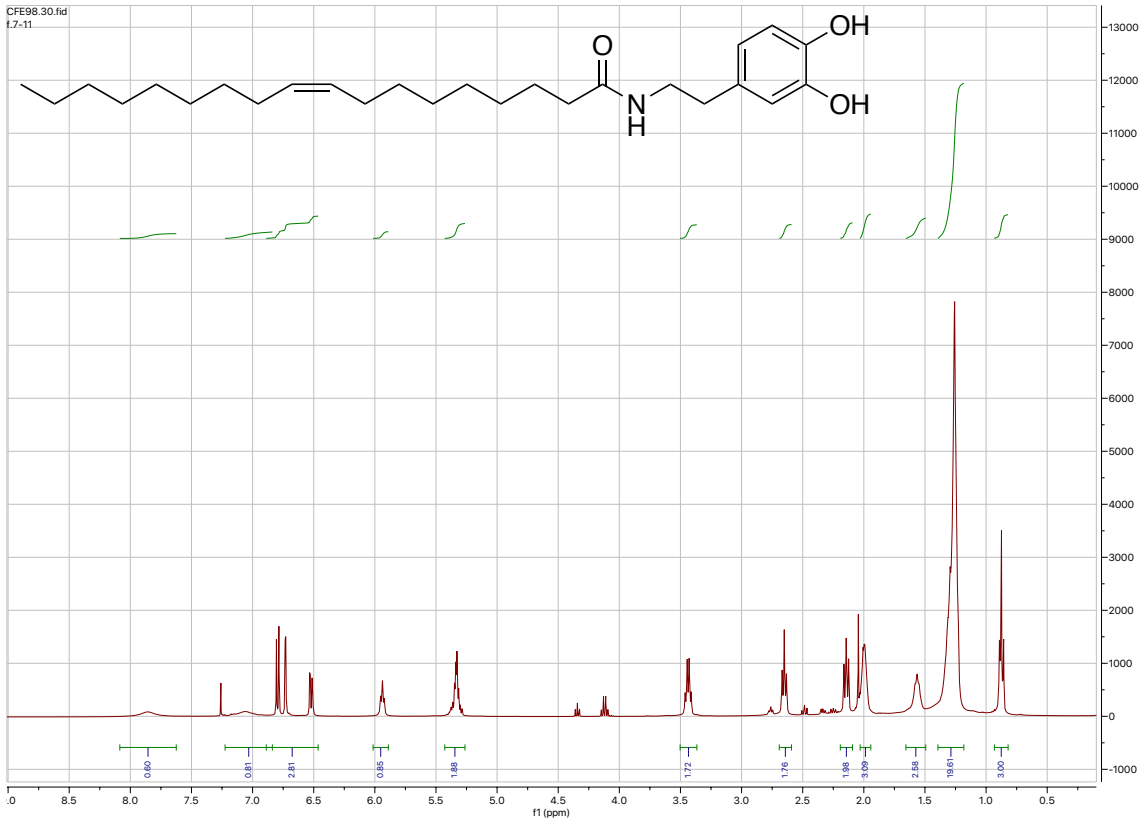


Figure 6-3 ^{1}H NMR spectrum of OLDA.

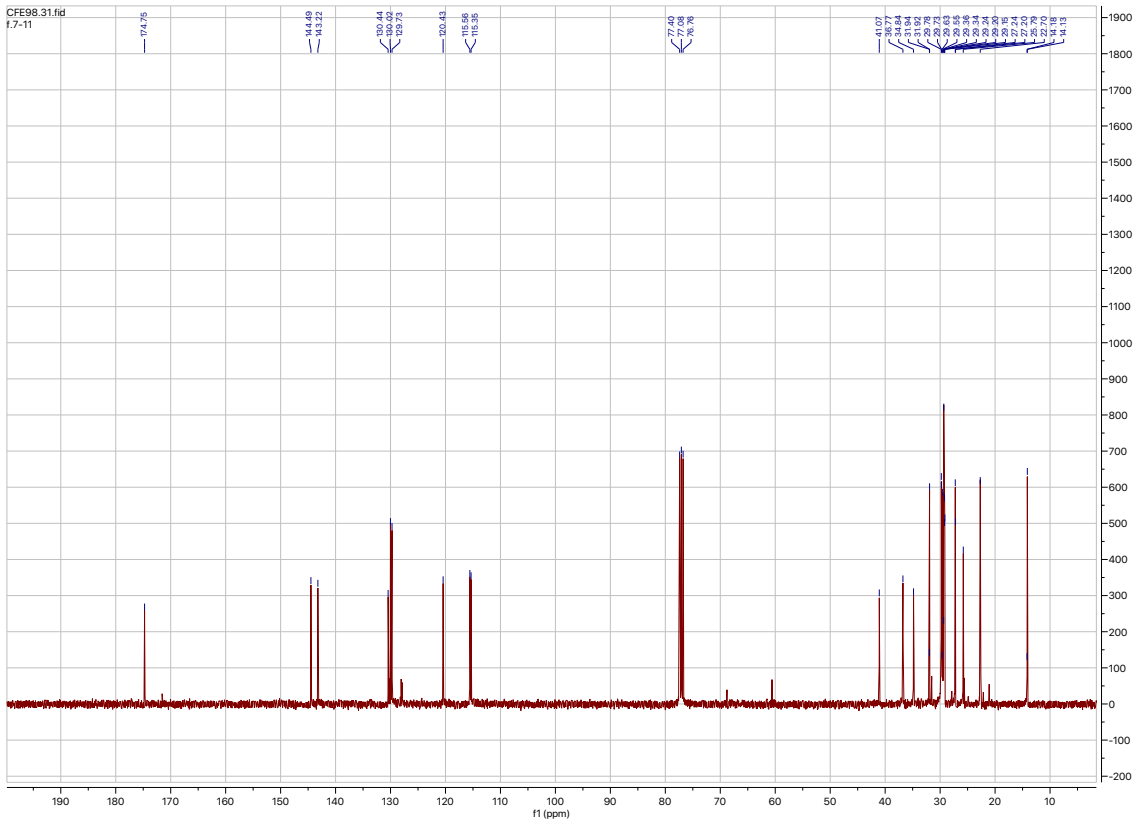


Figure 6-4 ^{13}C NMR spectrum of OLDA.

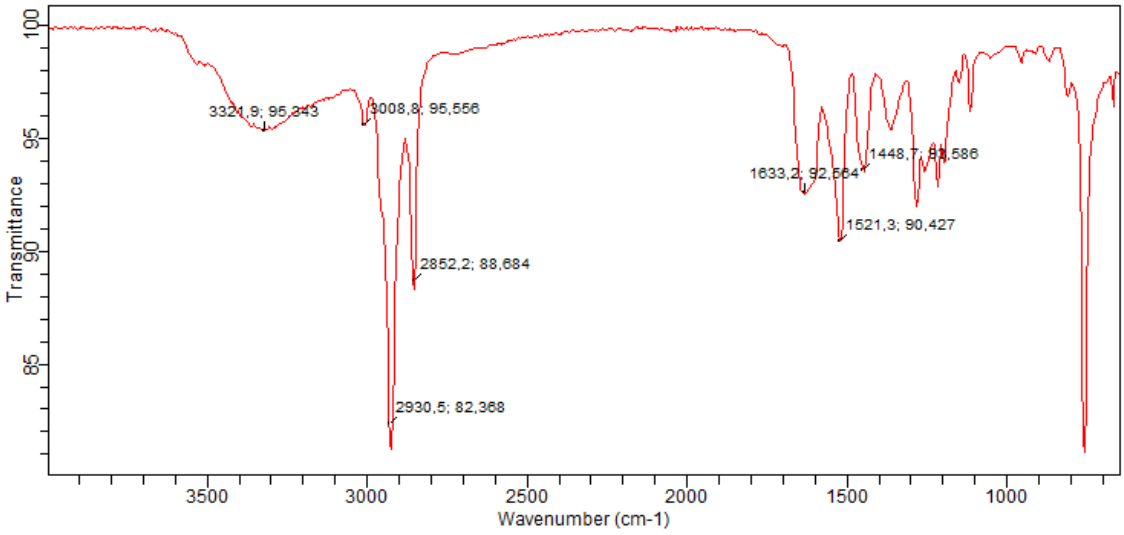


Figure 6-5 IR-spectrum of OLDA.

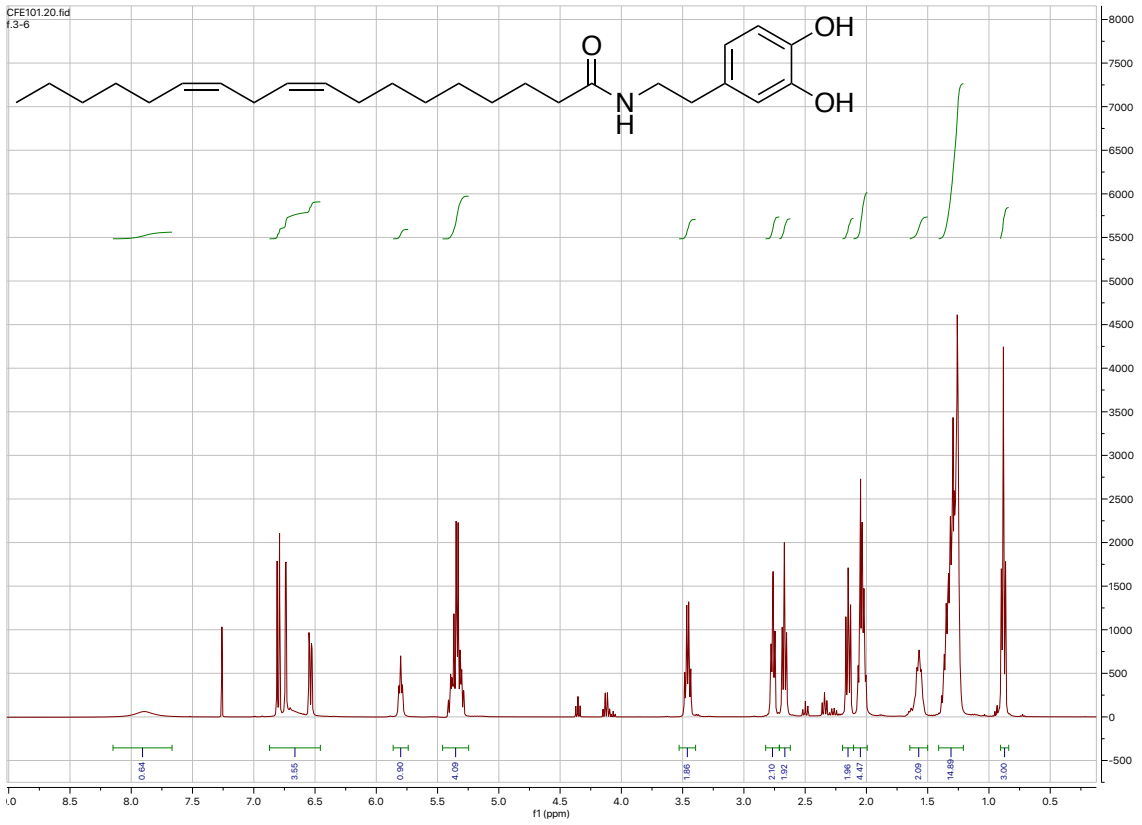


Figure 6-6 ^{1}H NMR spectrum of **34**.

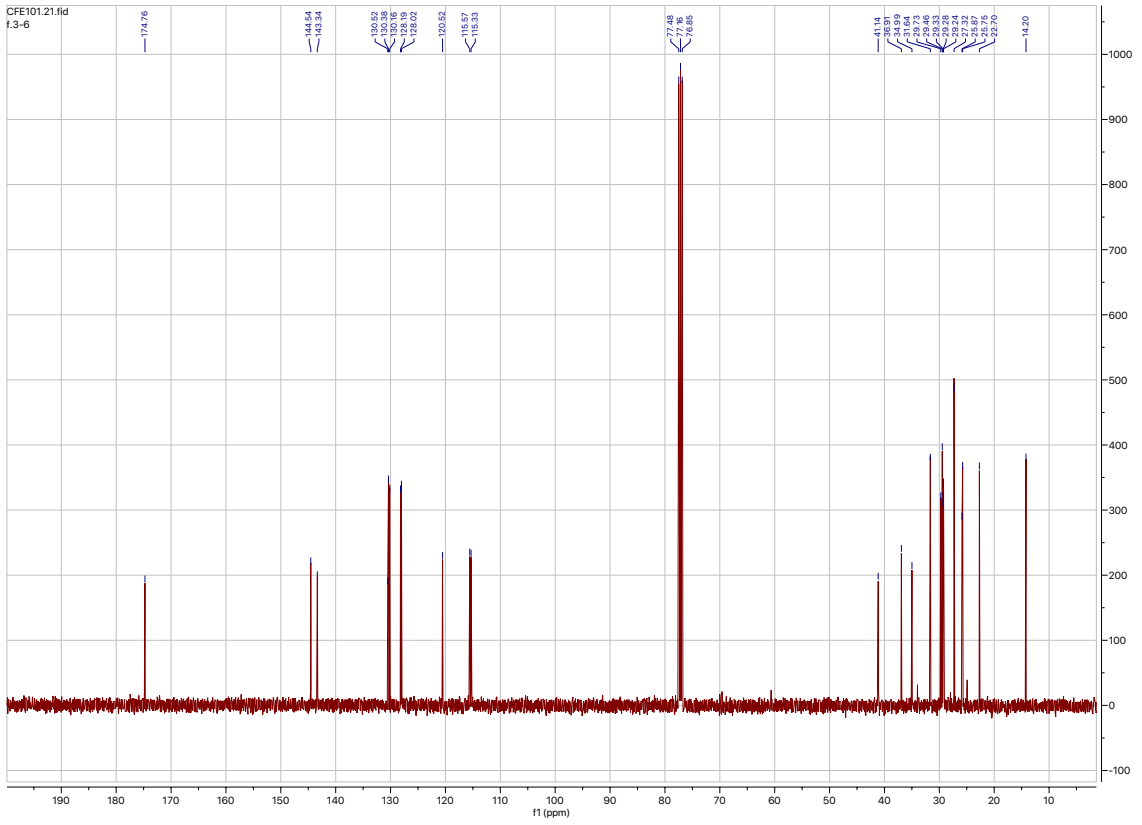


Figure 6-7 ^{13}C NMR spectrum of **34**.

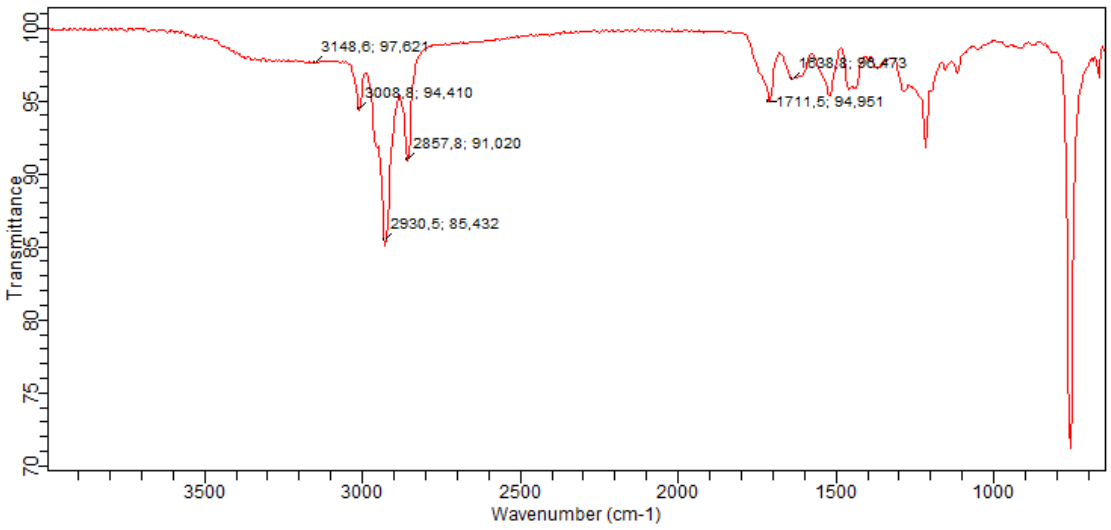


Figure 6-8 IR-spectrum of **34**.

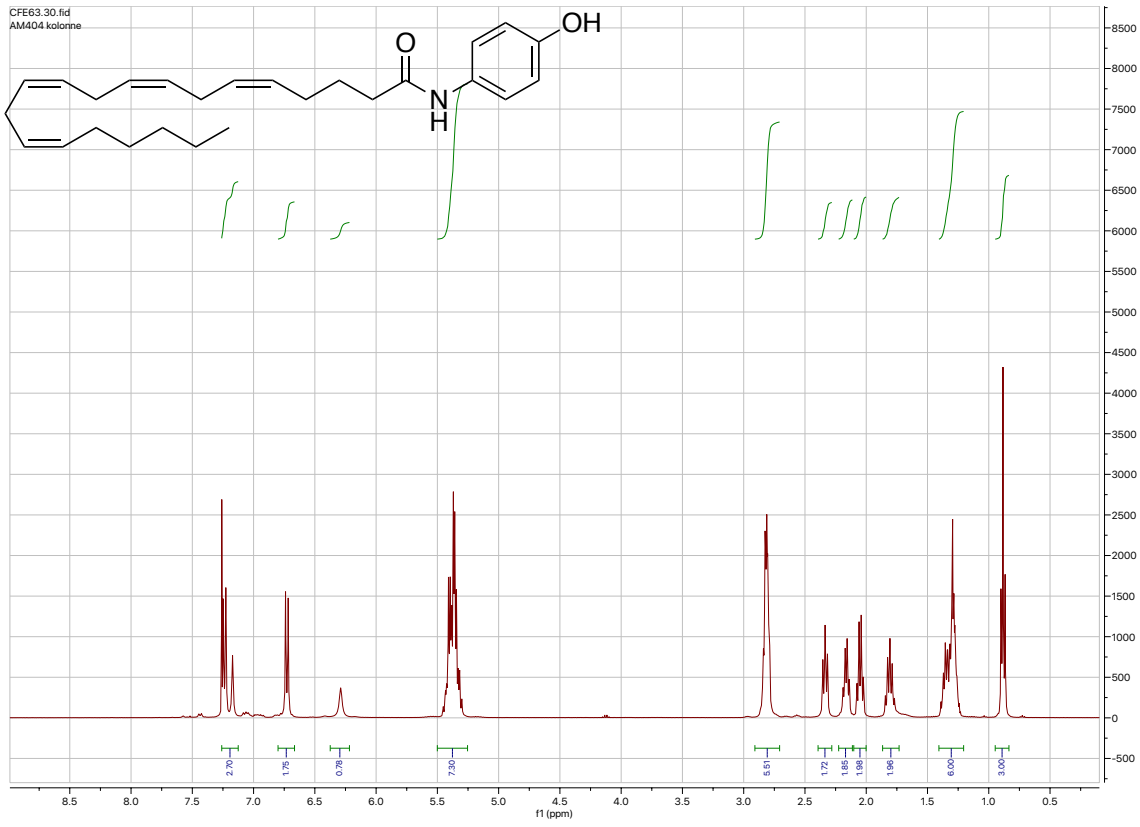


Figure 6-9 ^{1}H NMR spectrum of AM404.

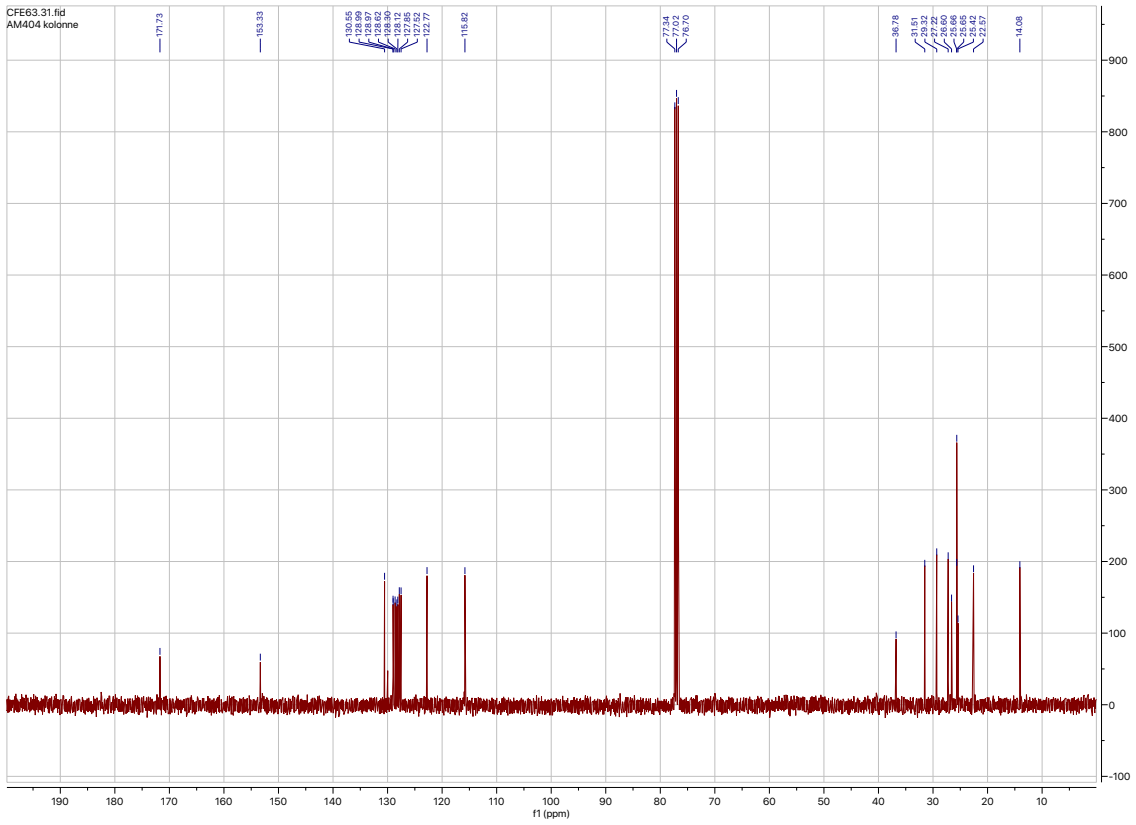


Figure 6-10 ^{13}C NMR spectrum of AM404.

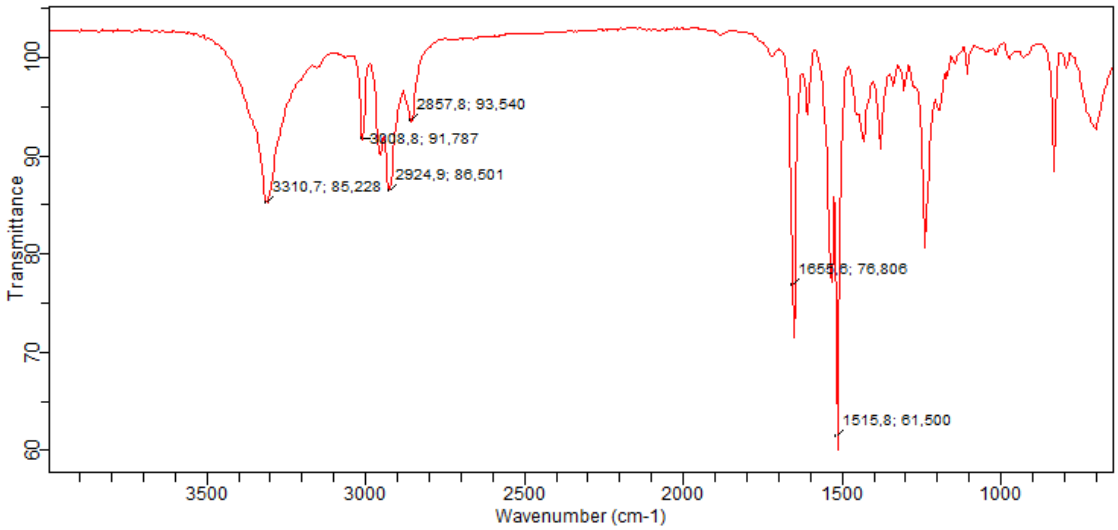
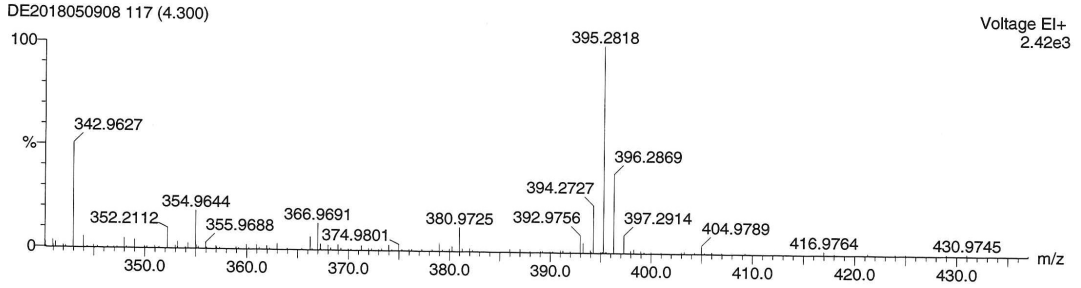


Figure 6-11 IR-spectrum of AM404.

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

Monoisotopic Mass, Odd and Even Electron Ions
175 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)

CFE53
DE2018050908 117 (4.300)



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
395.2818	395.2824	-0.6	-1.6	9.0	1	C26 H37 N O2
	395.2811	0.7	1.8	9.5	2	C24 H35 N4 O
	395.2797	2.1	5.2	4.5	3	C23 H39 O5
	395.2784	3.4	8.6	5.0	4	C21 H37 N3 O4

Figure 6-12 HR-MS spectrum of AM404.

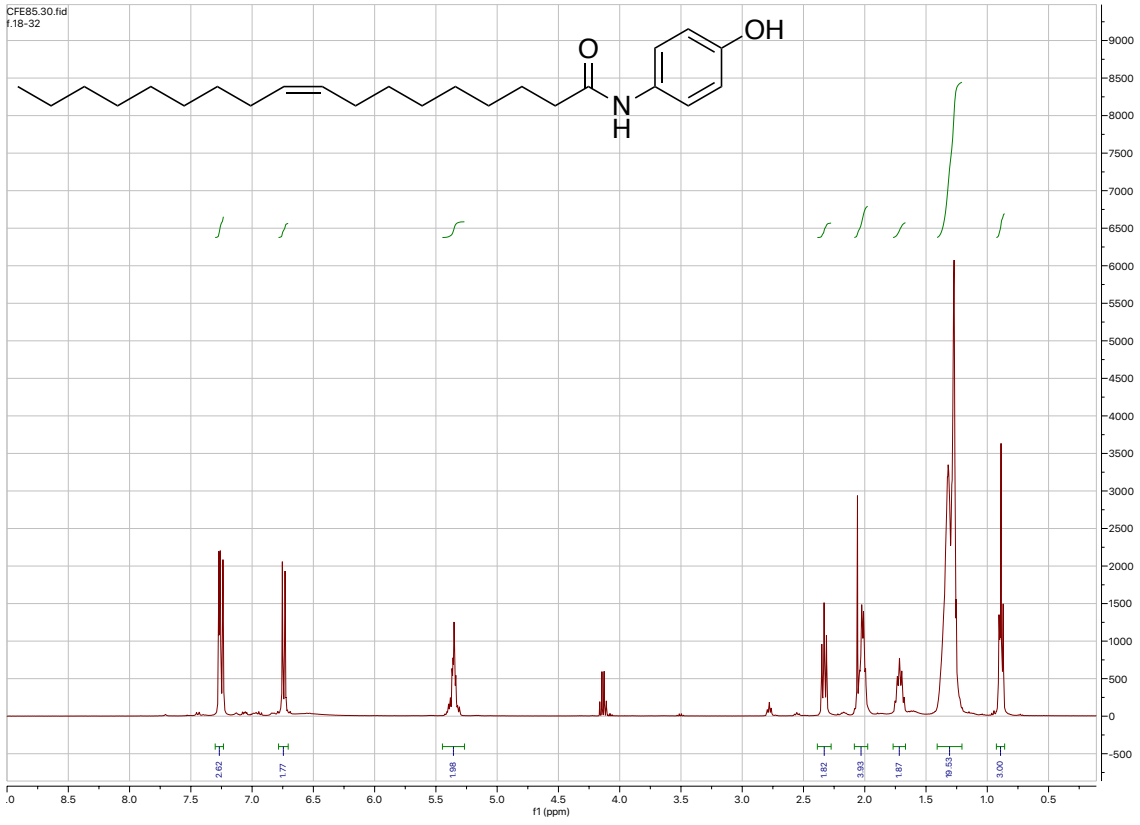


Figure 6-13 ^{1}H NMR spectrum of **35**.

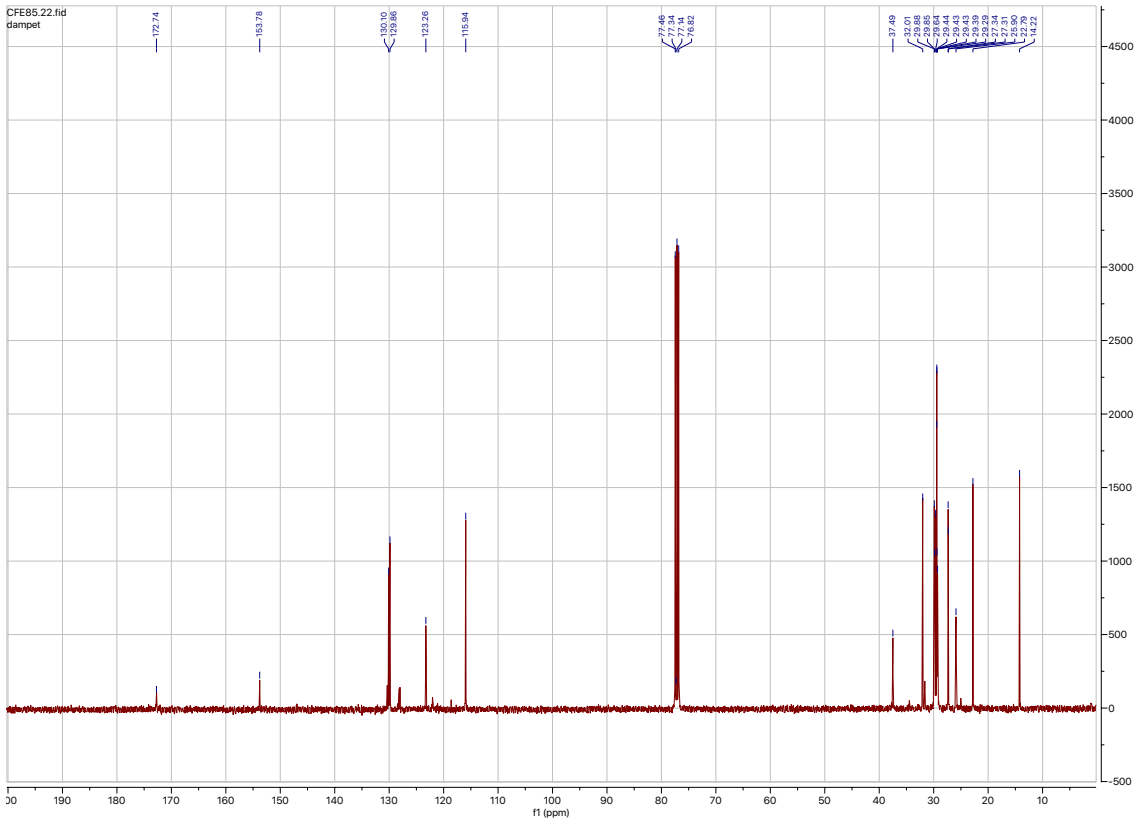


Figure 6-14 ^{13}C NMR spectrum of **35**.

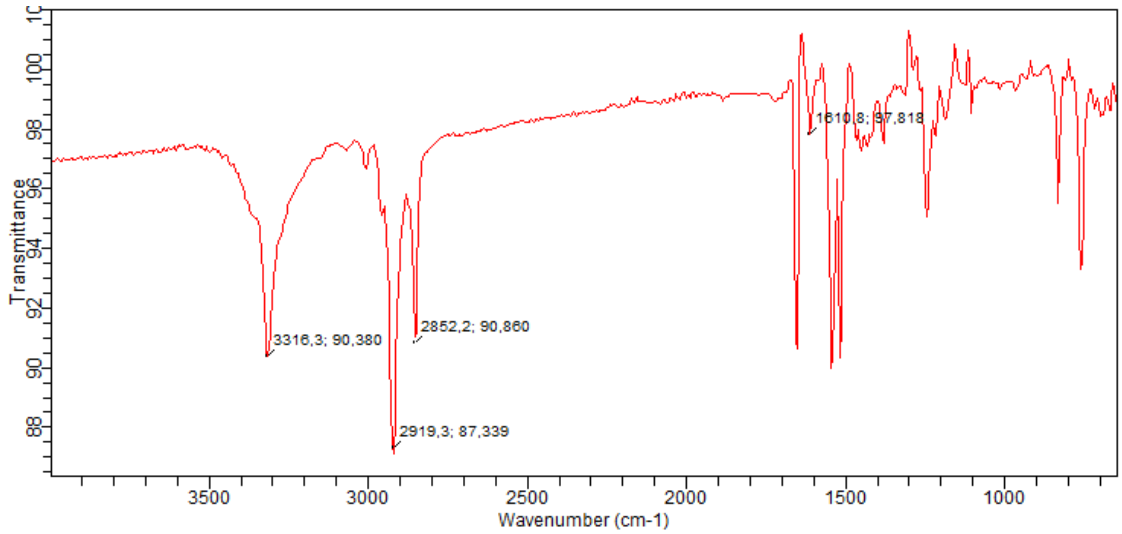


Figure 6-15 IR-spectrum of **35**.

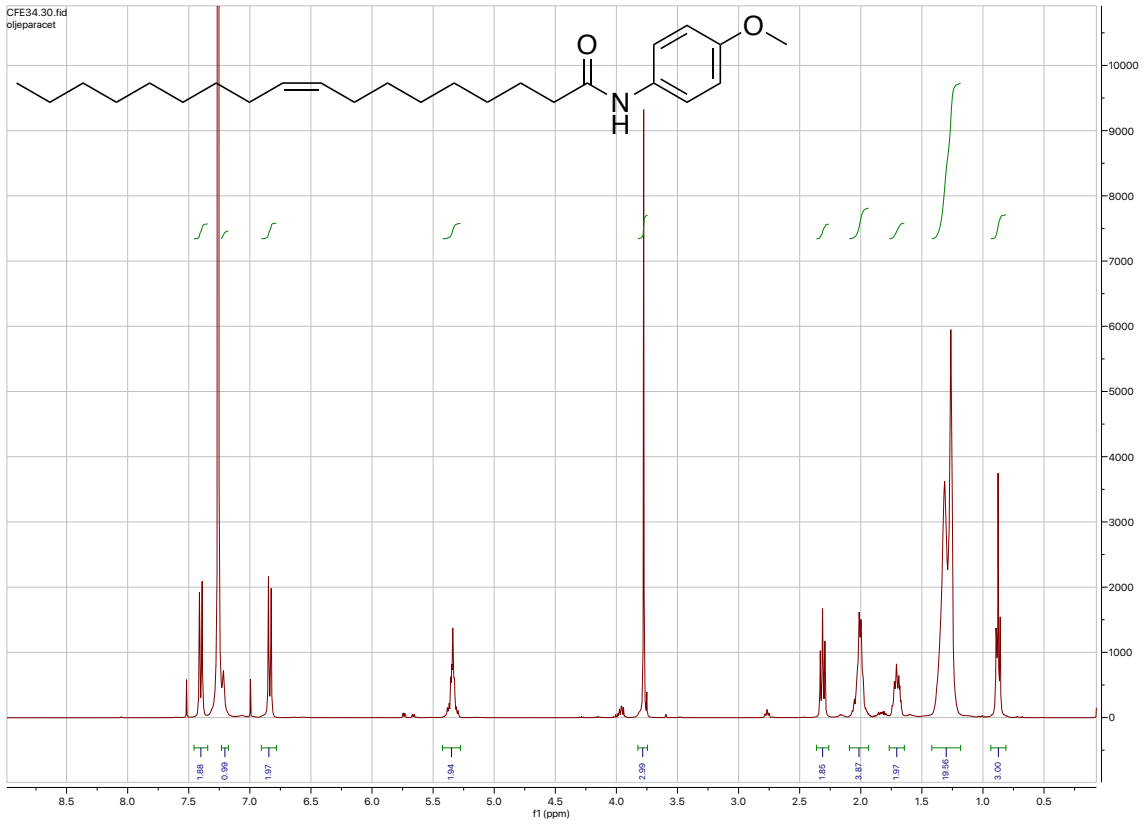


Figure 6-16 ^{1}H NMR spectrum of **36**.

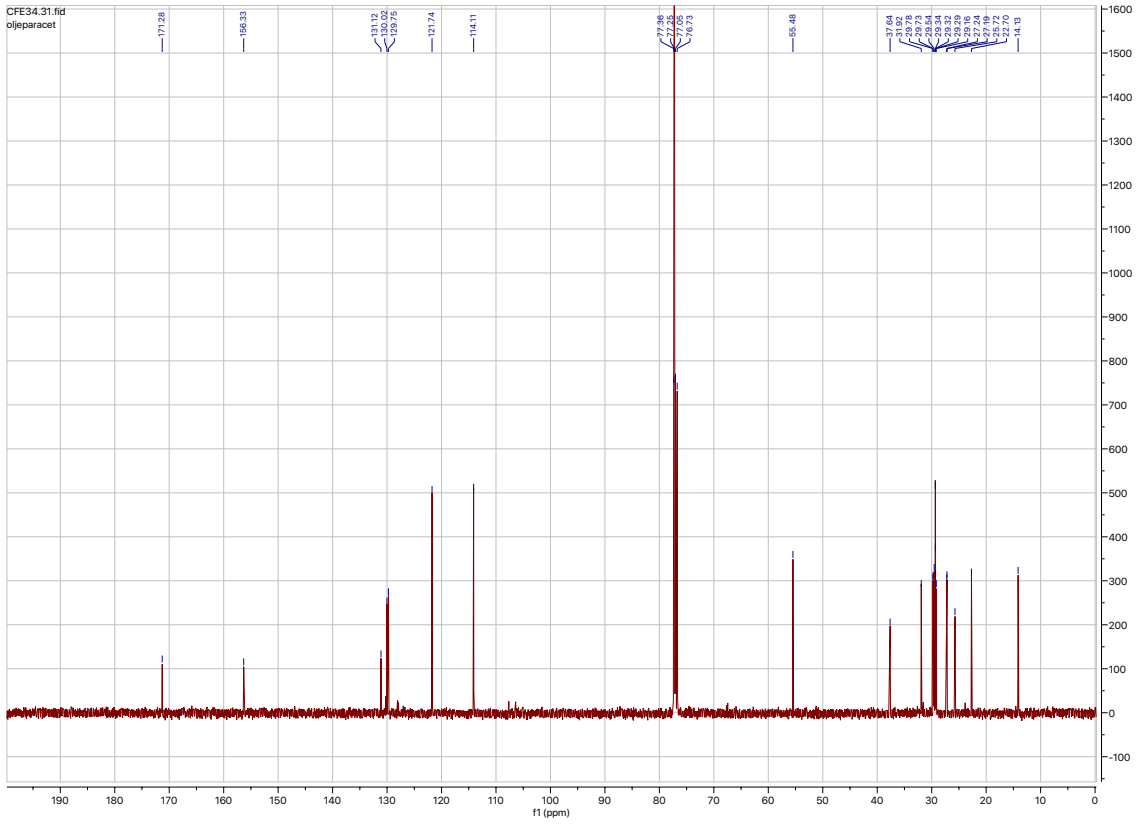


Figure 6-17 ^{13}C NMR spectrum of **36**.

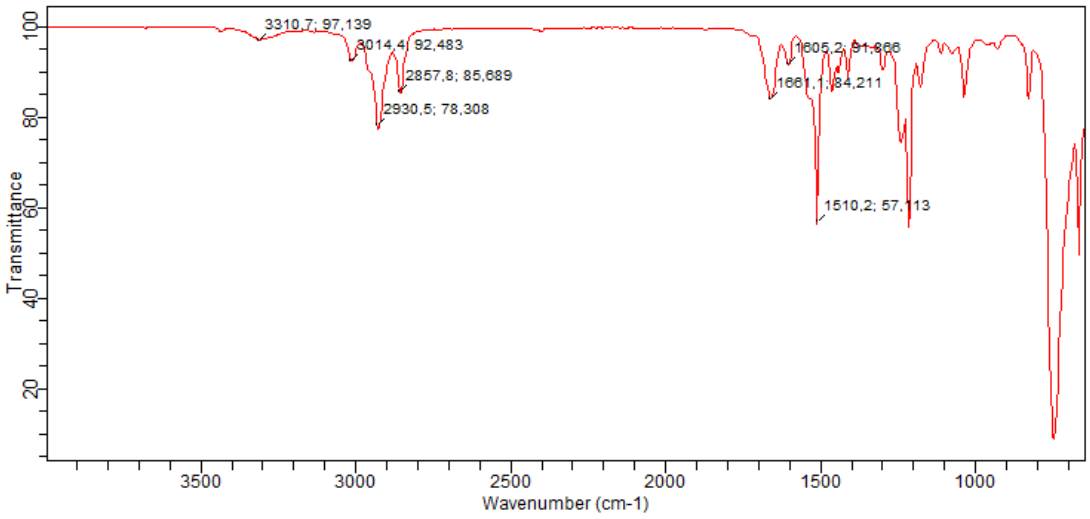
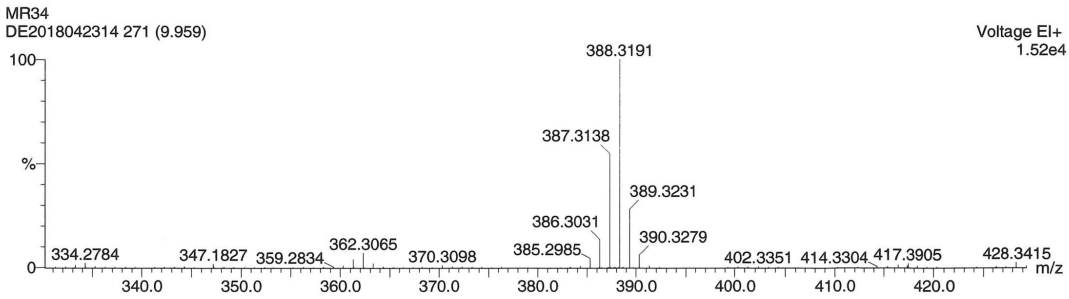


Figure 6-18 IR-spectrum of **36**.

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

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



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
387.3138	387.3137	0.1	0.2	6.0	1	C25 H41 N O2
	387.3110	2.8	7.1	1.5	2	C22 H43 O5

Figure 6-19 HR-MS spectrum of **36**.

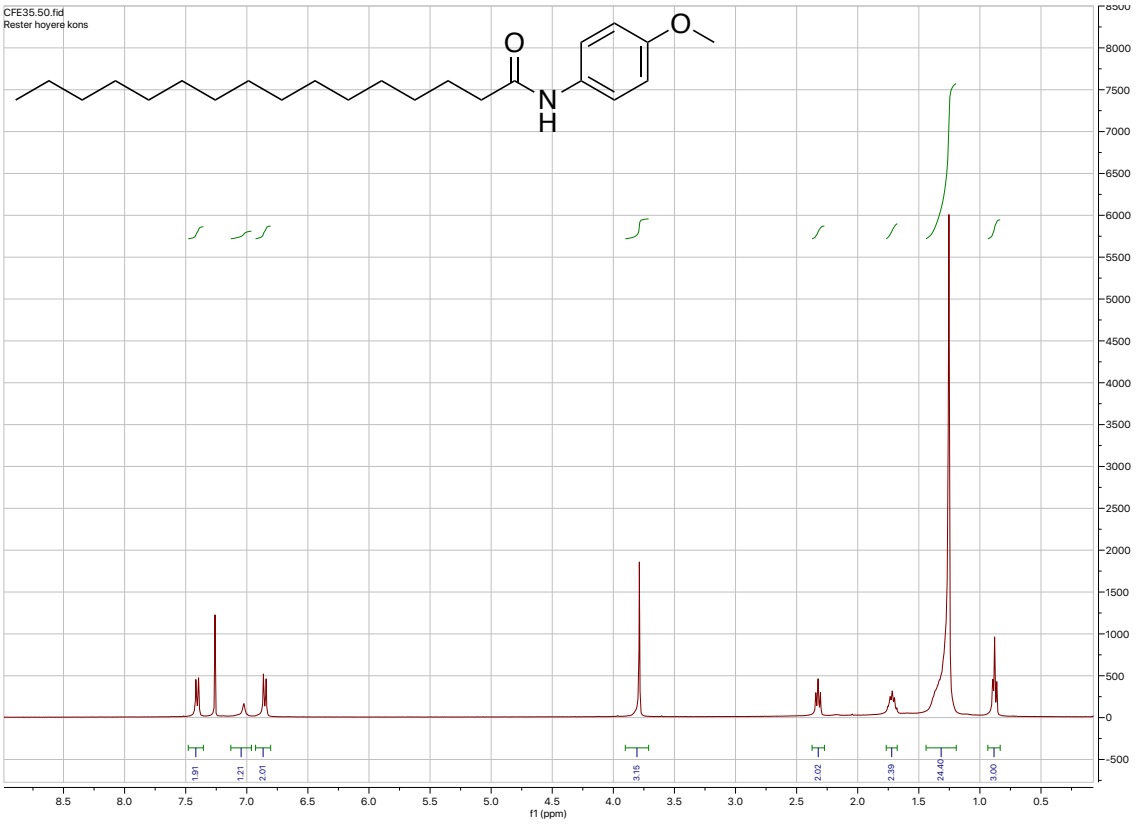


Figure 6-20 ^{1}H NMR spectrum of **37**.

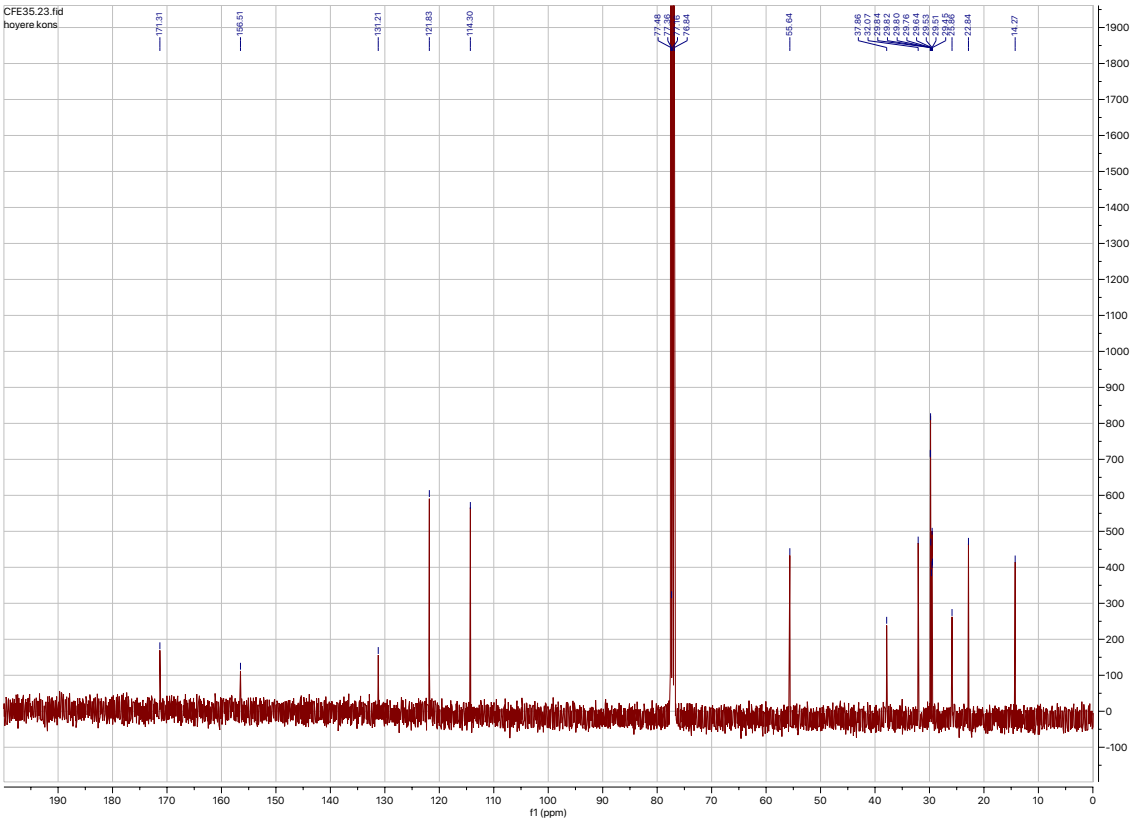


Figure 6-21 ^{13}C NMR spectrum of **37**.

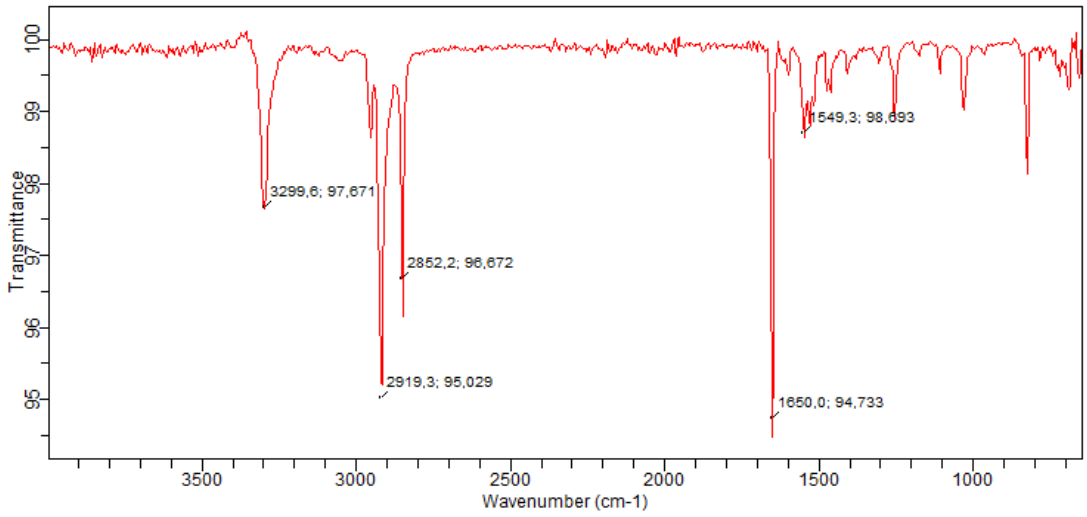
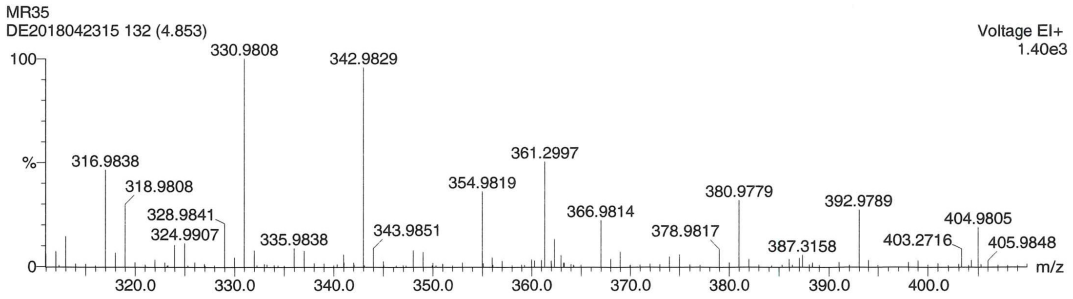


Figure 6-22 IR-spectrum of **37**.

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

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



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
361.2997	361.2981	1.6	4.5	5.0	1	C23 H39 N O2

Figure 6-23 HR-MS spectrum of **37**.

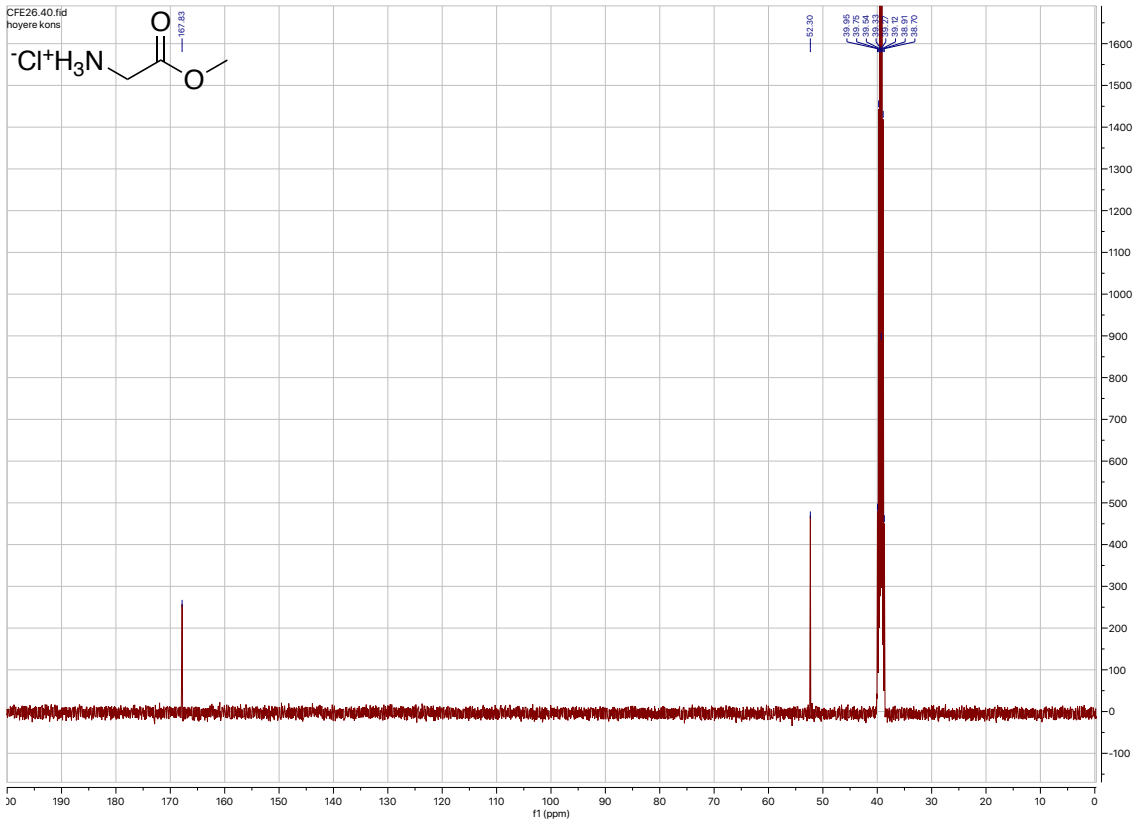


Figure 6-24 ^{13}C NMR spectrum of **51**.

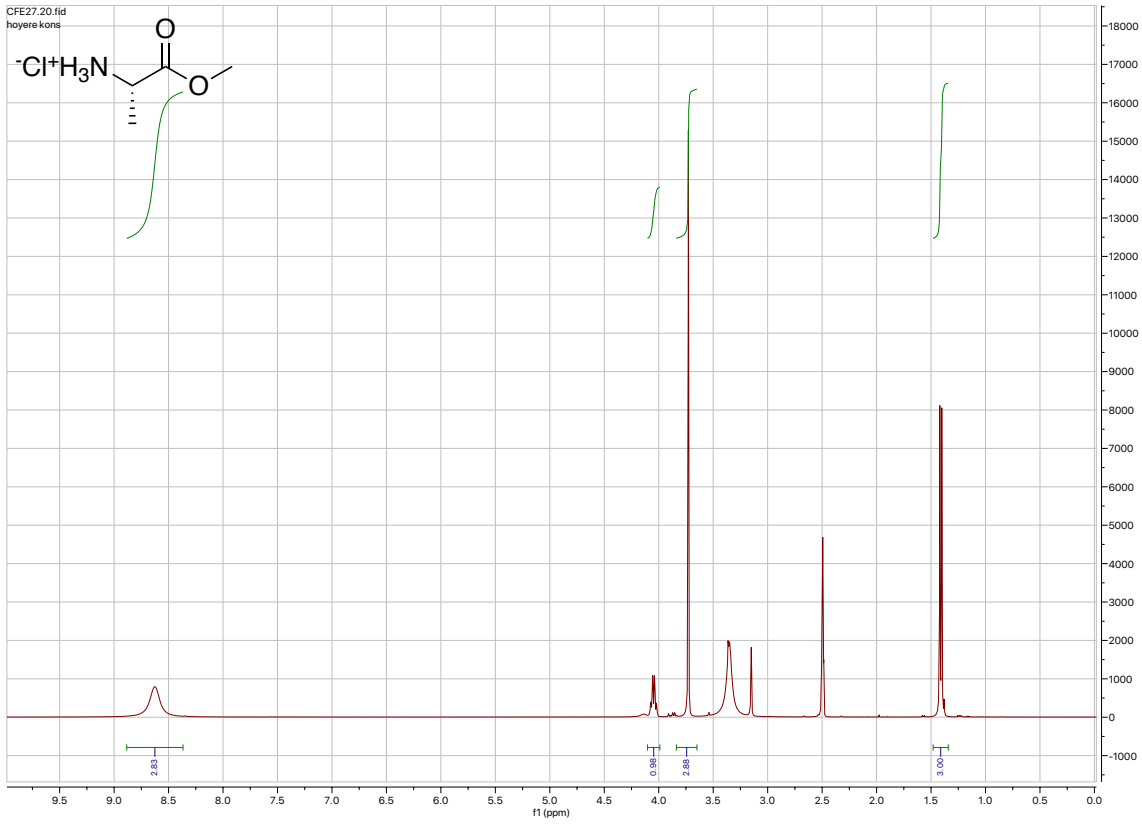


Figure 6-25 ^{1}H NMR spectrum of **52**.

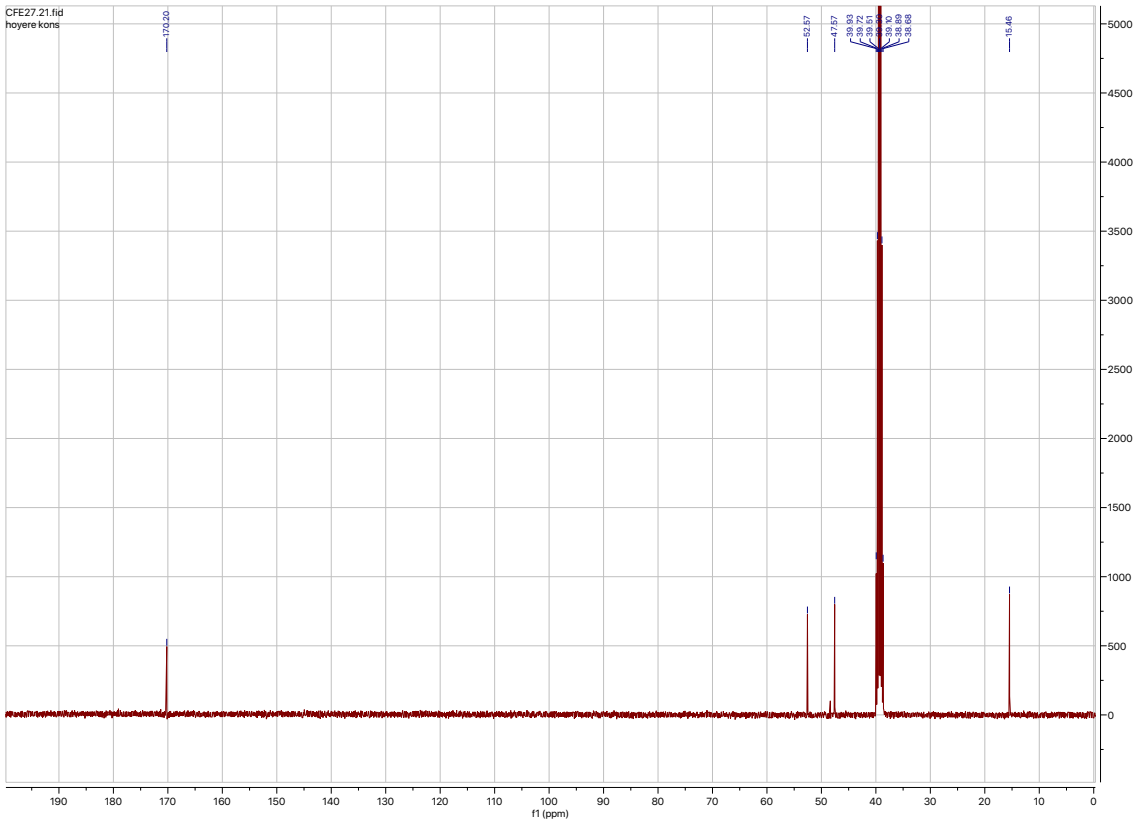


Figure 6-26 ^{13}C NMR spectrum of **52**.

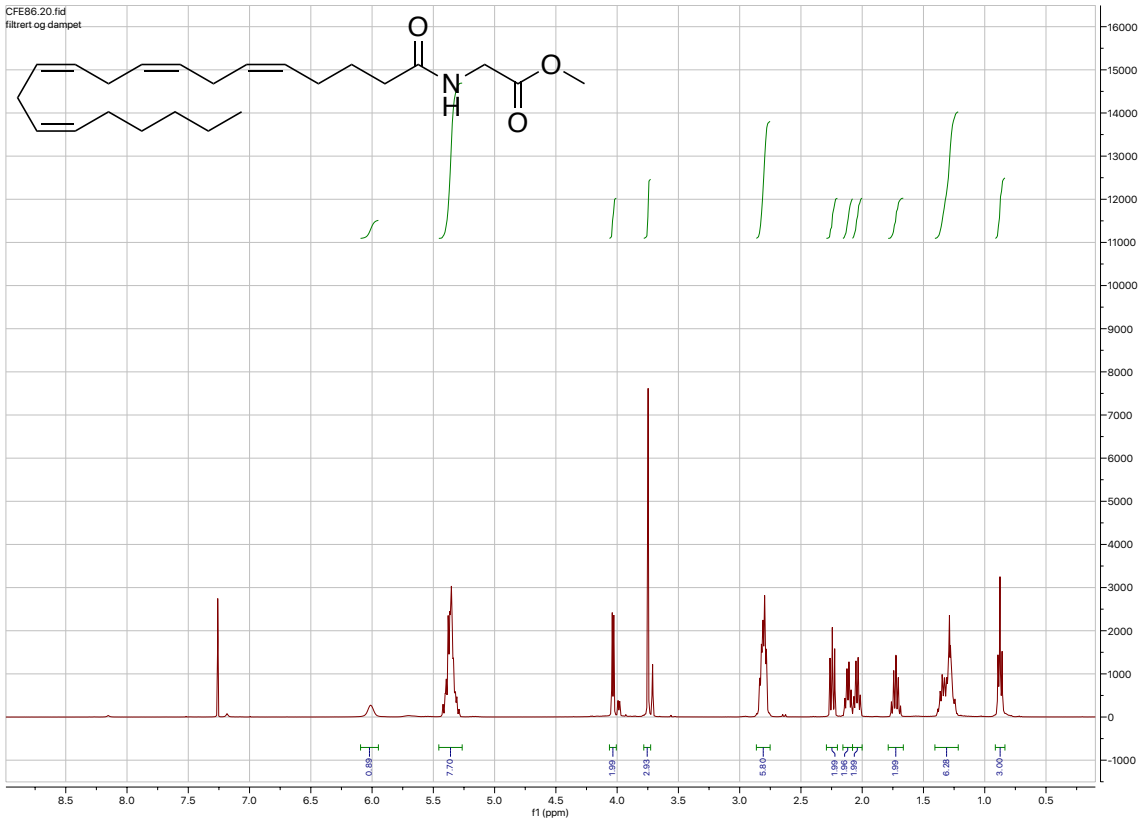


Figure 6-27 ^{1}H NMR spectrum of NAGly-ME.

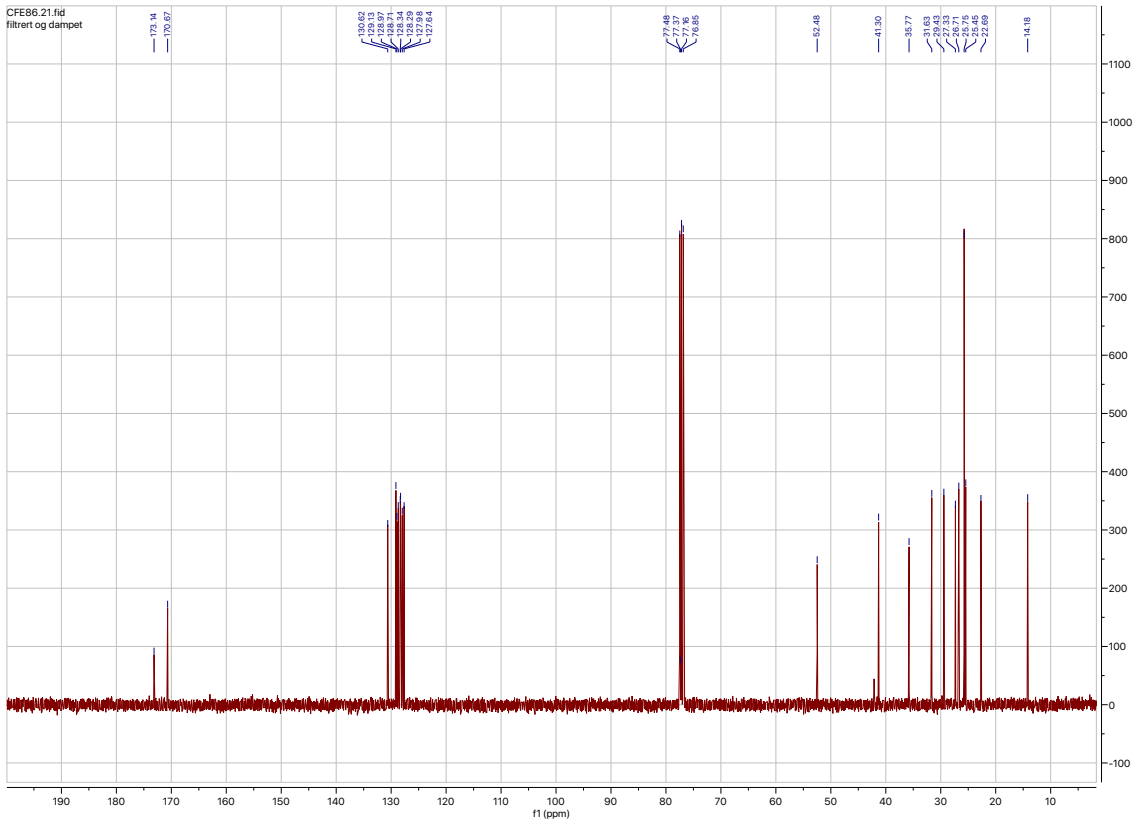


Figure 6-28 ^{13}C NMR spectrum of NAGly-ME.

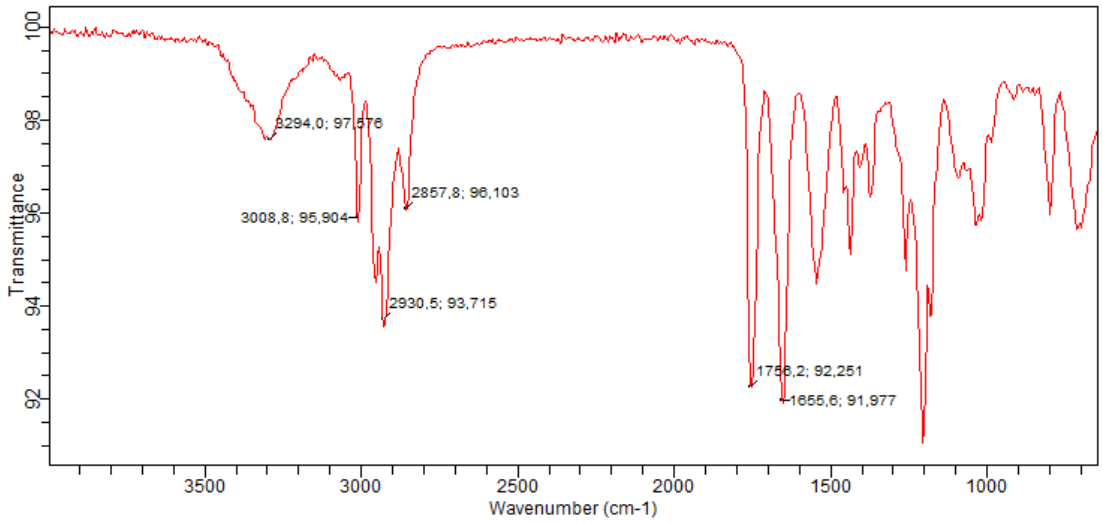


Figure 6-29 IR-spectrum of NAGly-ME.

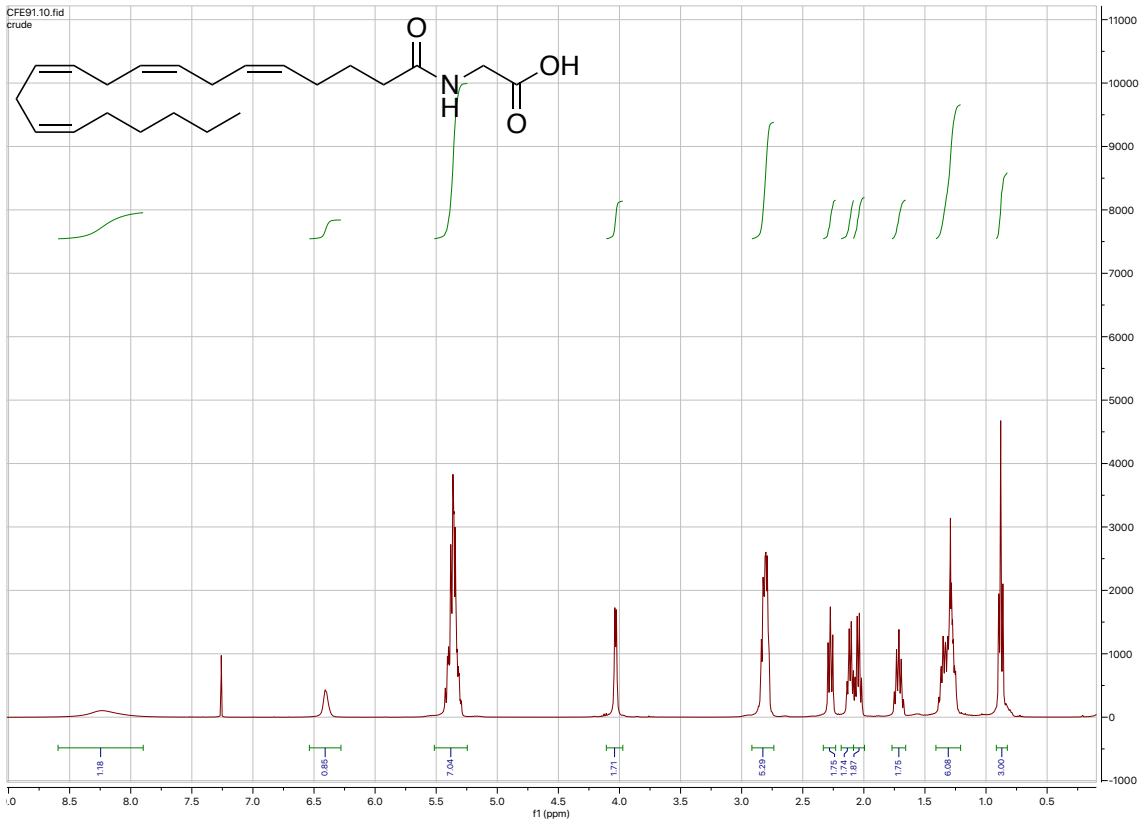


Figure 6-30 ^{1}H NMR spectrum of NAGly.

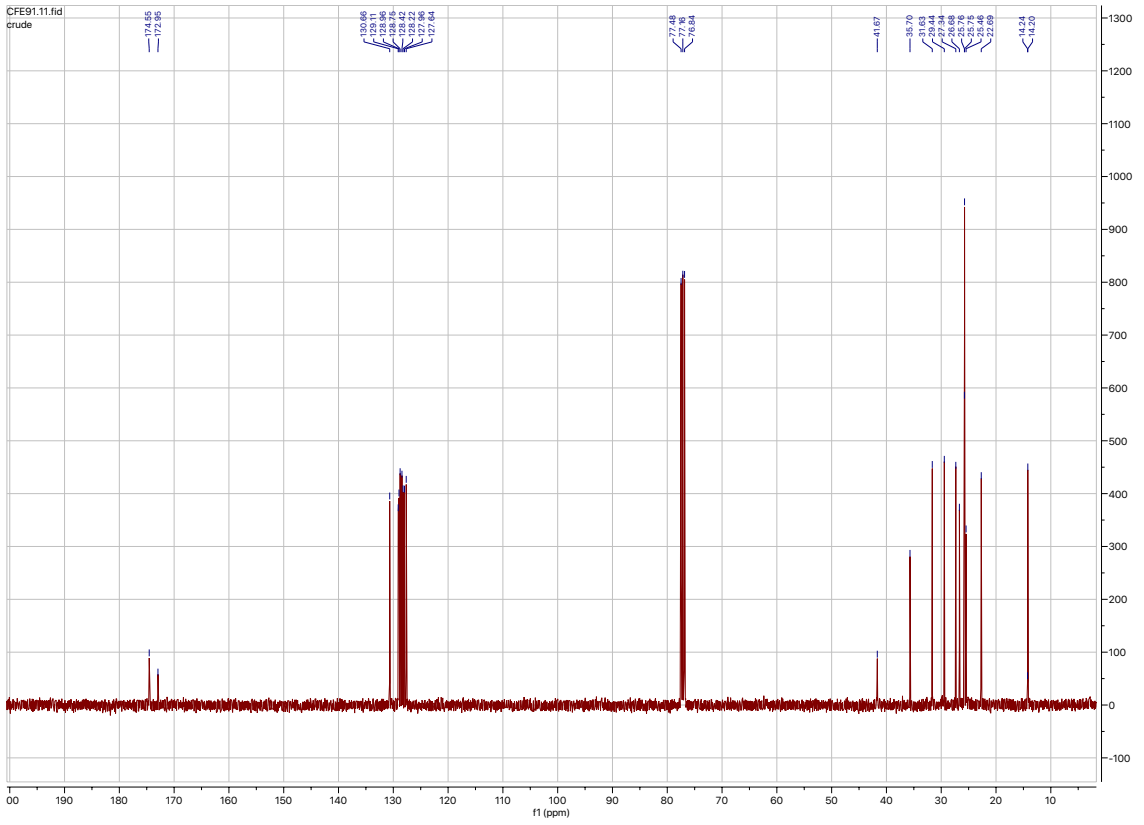


Figure 6-31 ^{13}C NMR spectrum of NAGly.

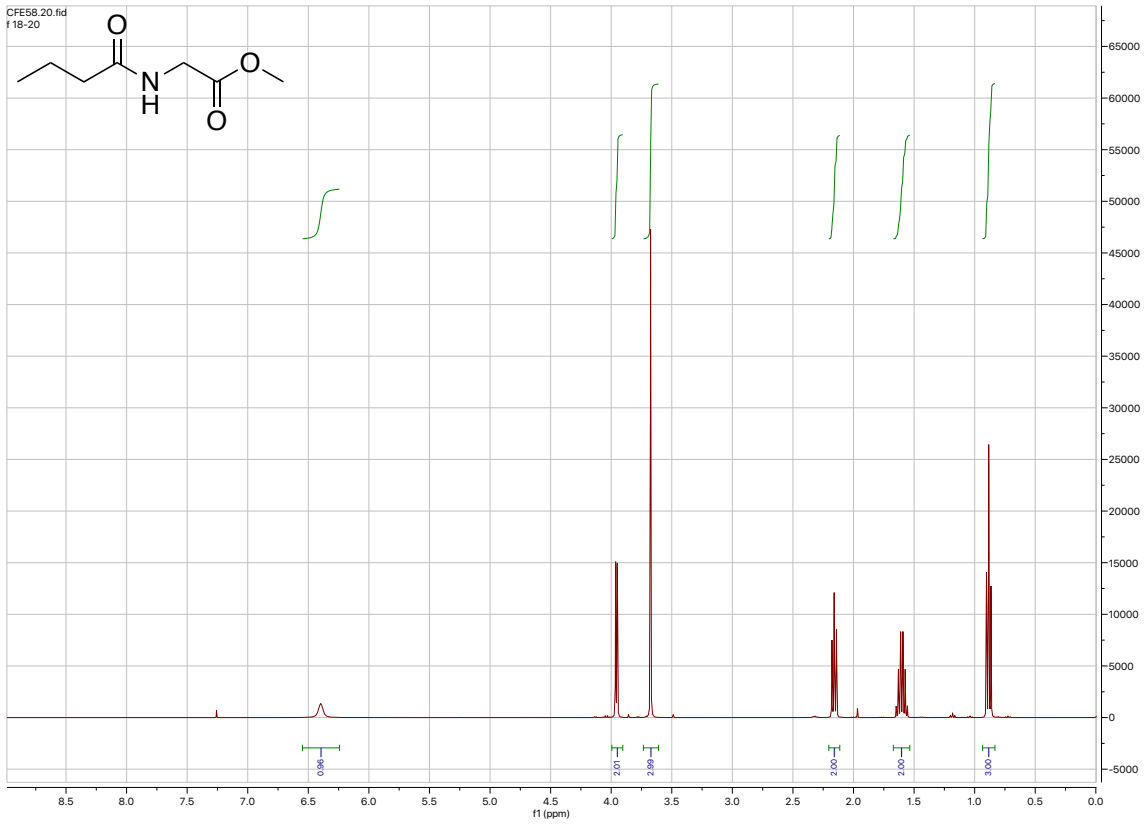


Figure 6-32 ^{1}H NMR spectrum of **16-ME**.

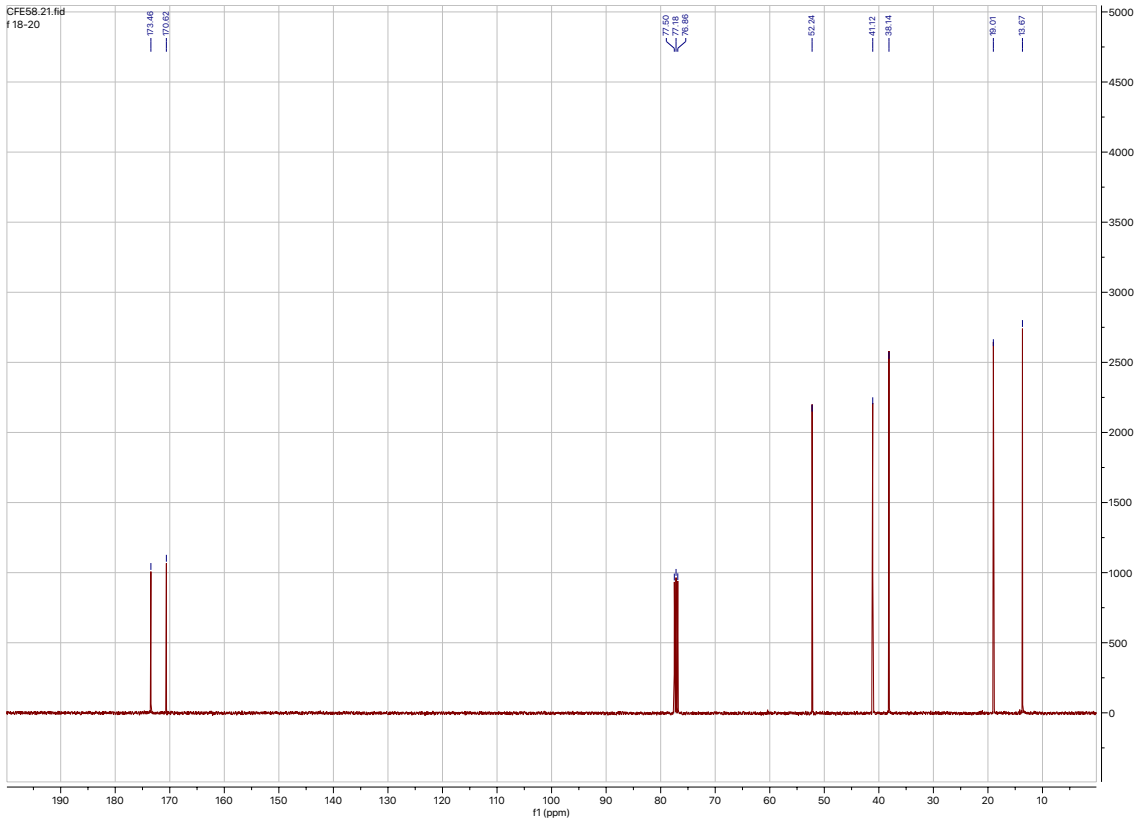


Figure 6-33 ^{13}C NMR spectrum of **16-ME**.

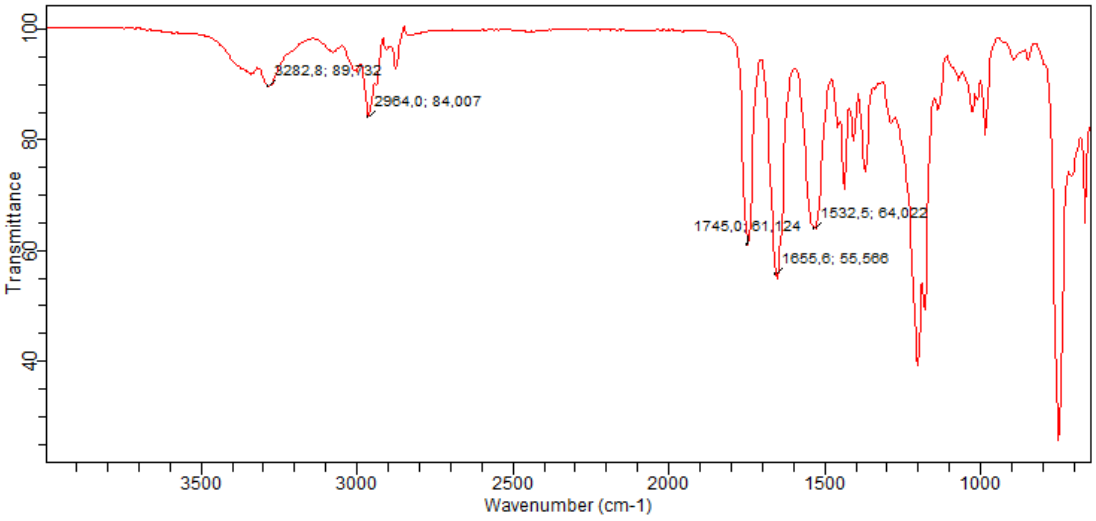
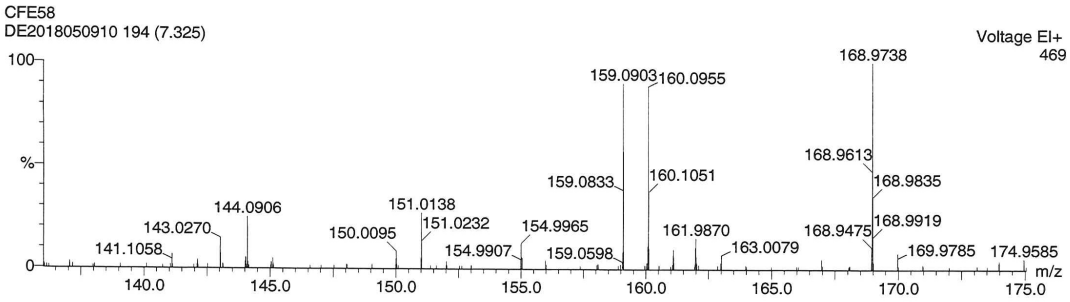


Figure 6-34 IR-spectrum of **16-ME**.

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

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



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
159.0903	159.0895	0.8	4.8	2.0	1	C7 H13 N O3

Figure 6-35 HR-MS spectrum of **16-ME**.

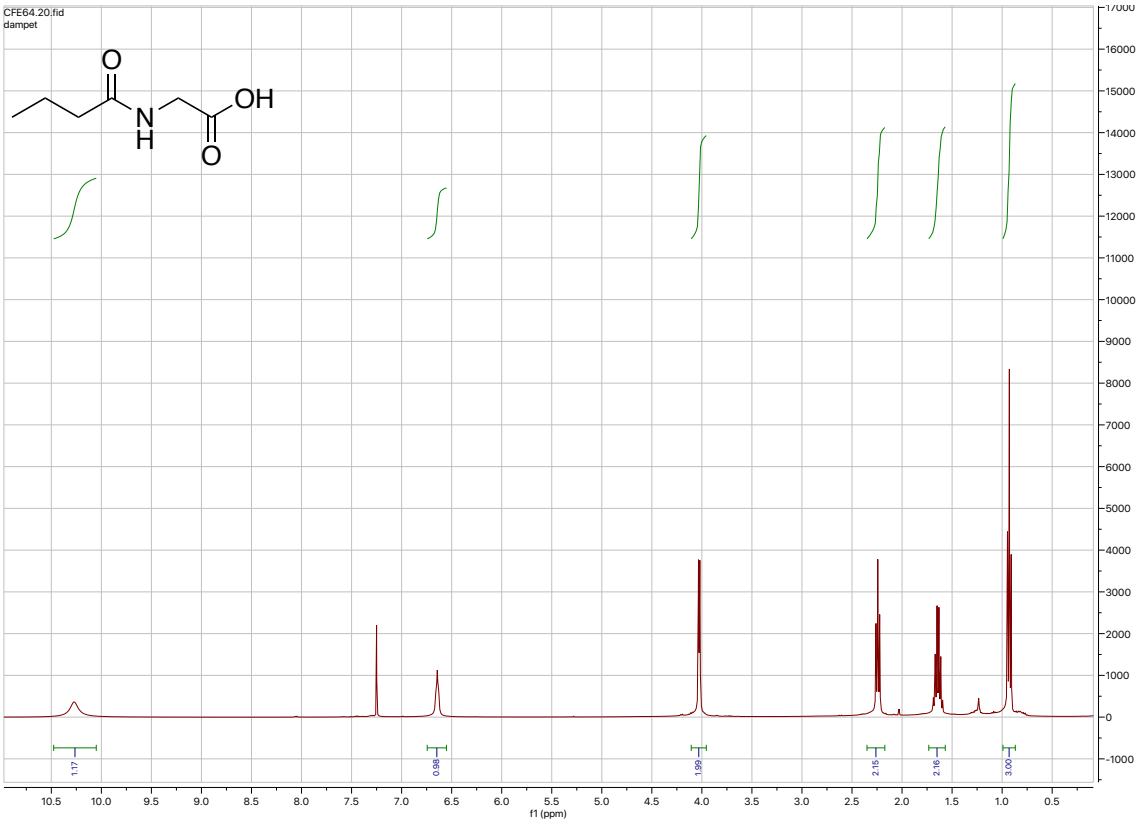


Figure 6-36 ^{1}H NMR-spectrum of **16**.

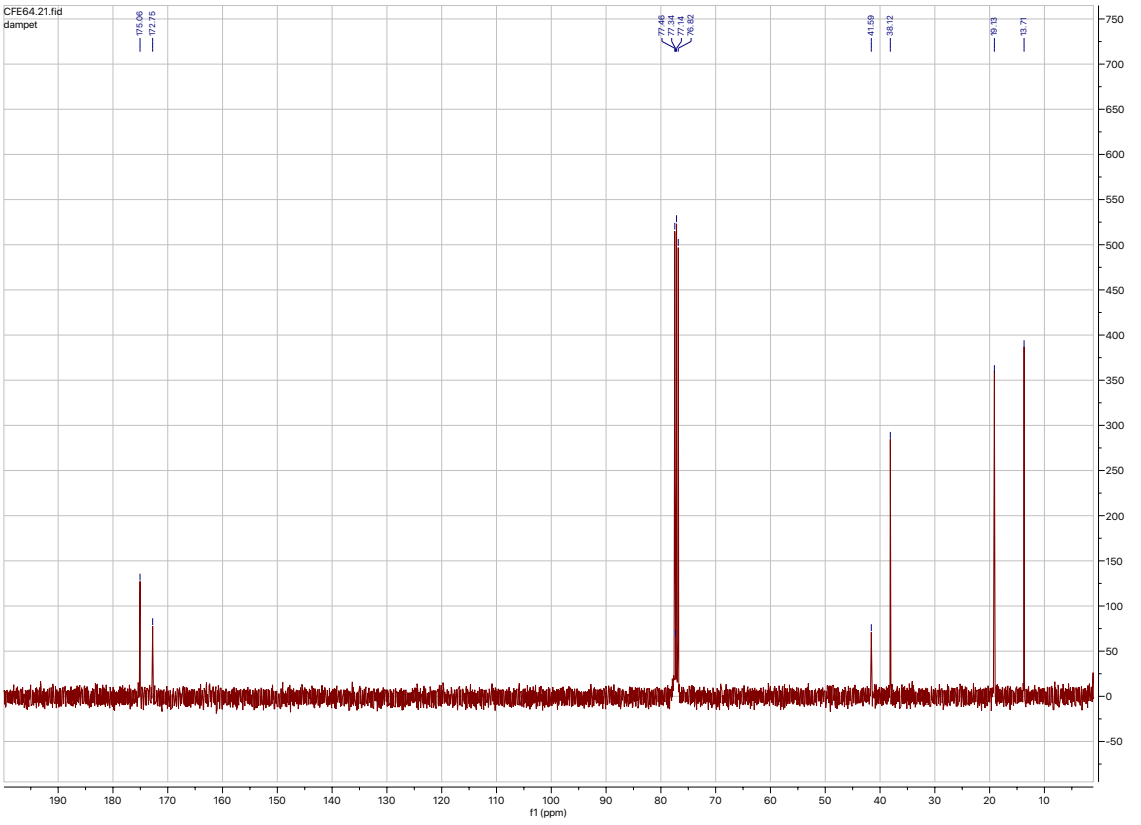


Figure 6-37 ^{13}C NMR spectrum of **16**.

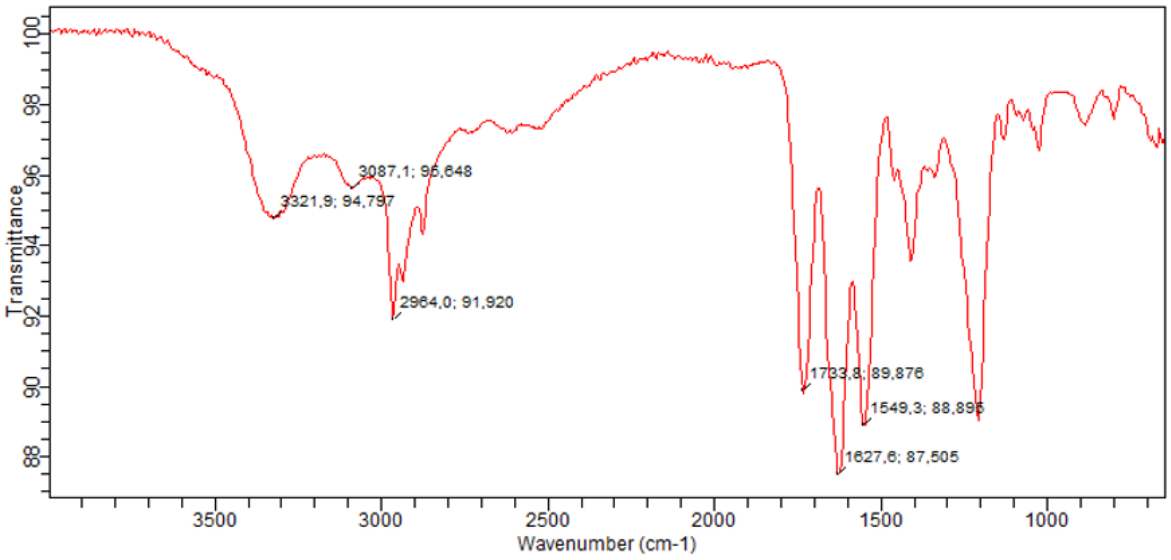
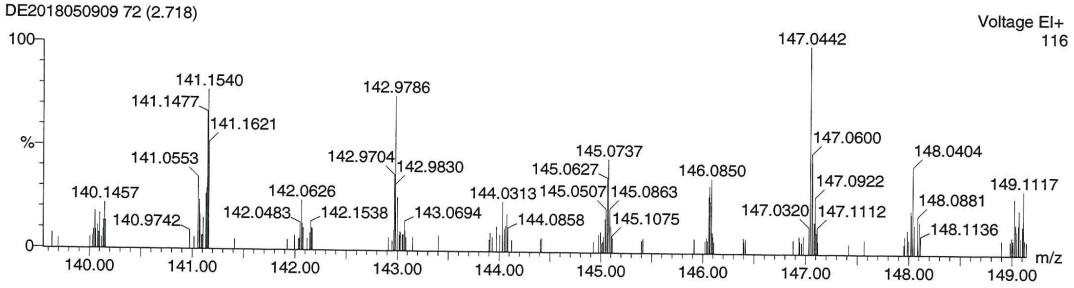


Figure 6-38 IR-spectrum of **16**.

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

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

CFE64
DE2018050909 72 (2.718)



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
145.0737	145.0739	-0.2	-1.3	2.0	1	C6 H11 N O3
	145.0726	1.1	7.9	2.5	2	C4 H9 N4 O2

Figure 6-39 HR-MS spectrum of **16**.

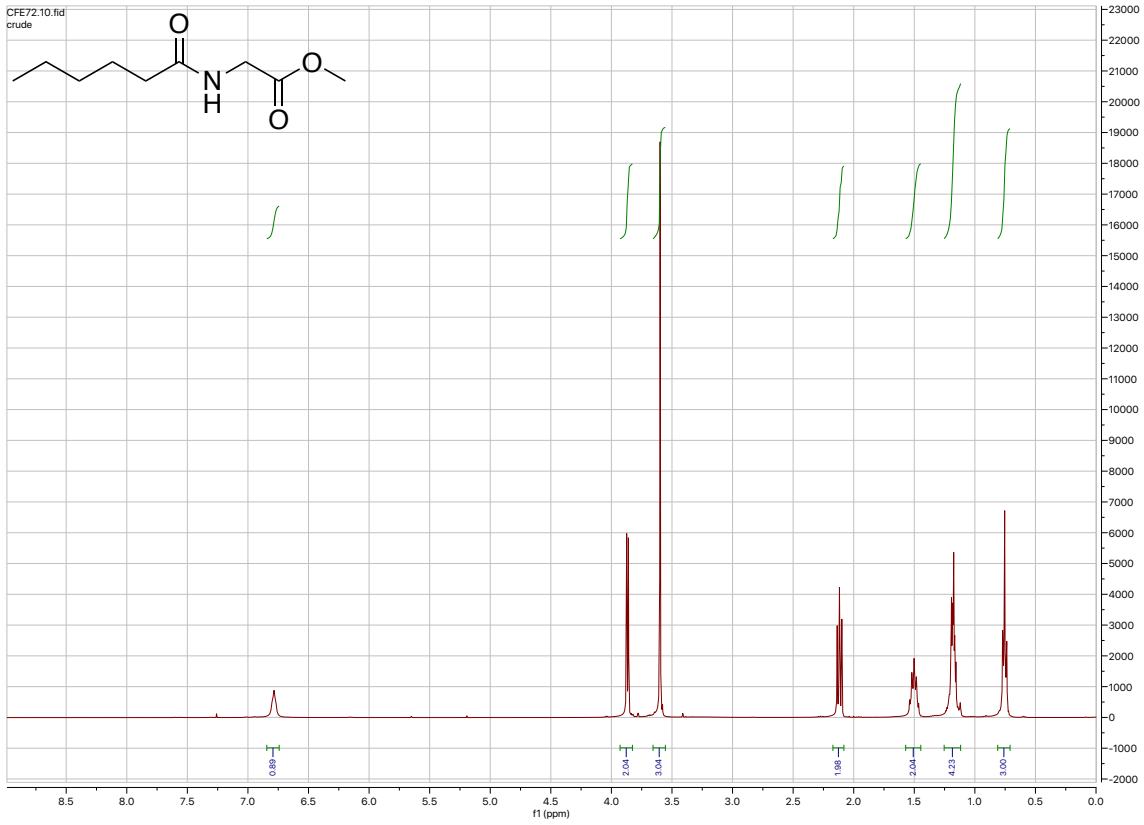


Figure 6-40 ^{1}H NMR spectrum of **17-ME**.

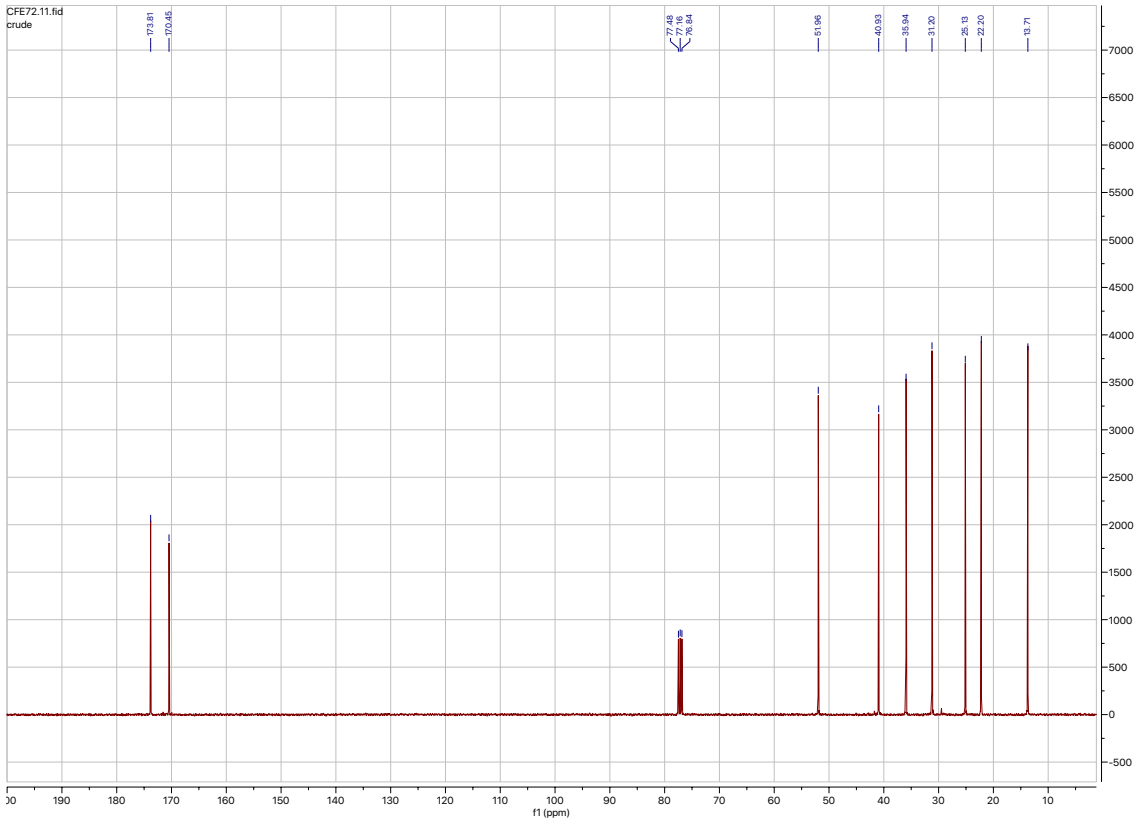


Figure 6-41 ^{13}C NMR-spectrum of **17-ME**.

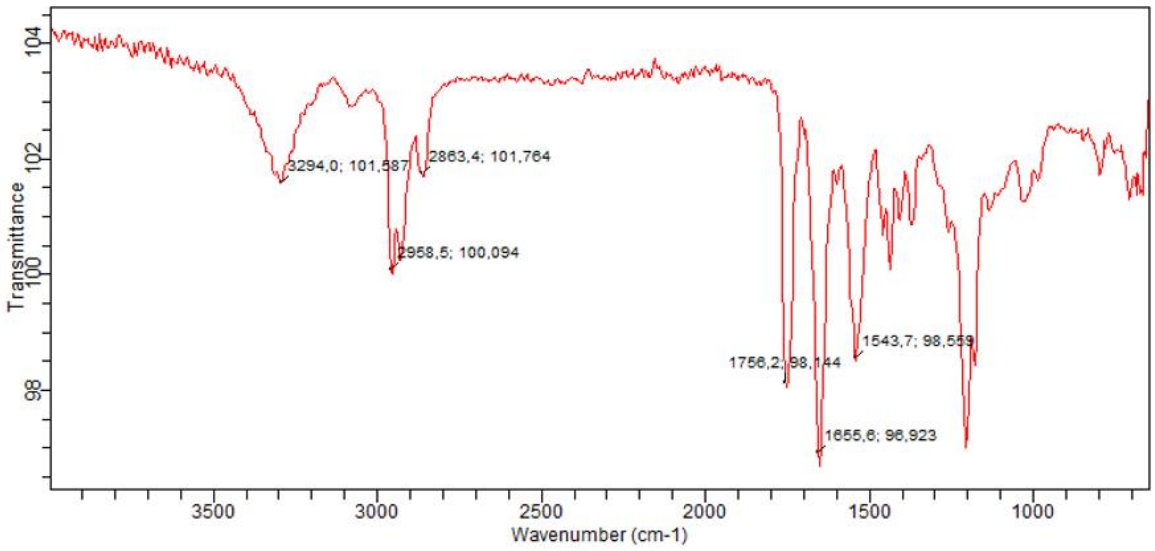


Figure 6-42 IR-spectrum of **17-ME**.

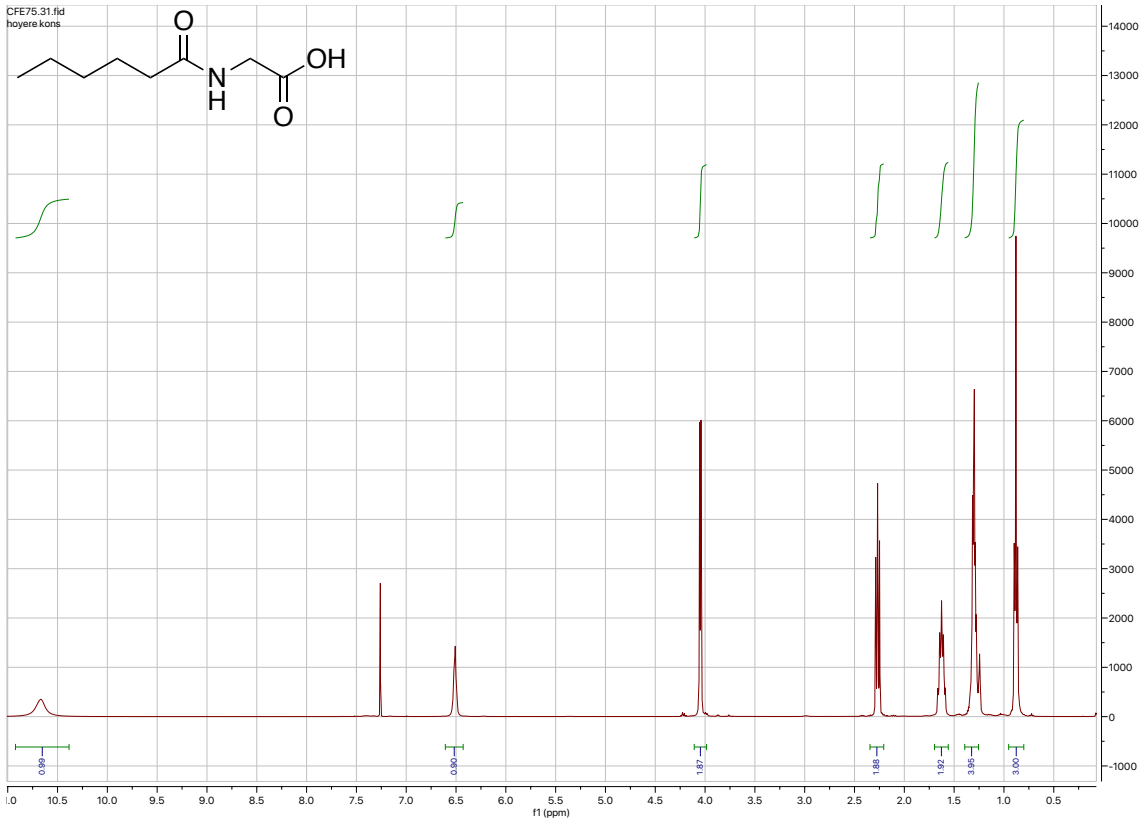


Figure 6-43 ^{1}H NMR spectrum of **17**.

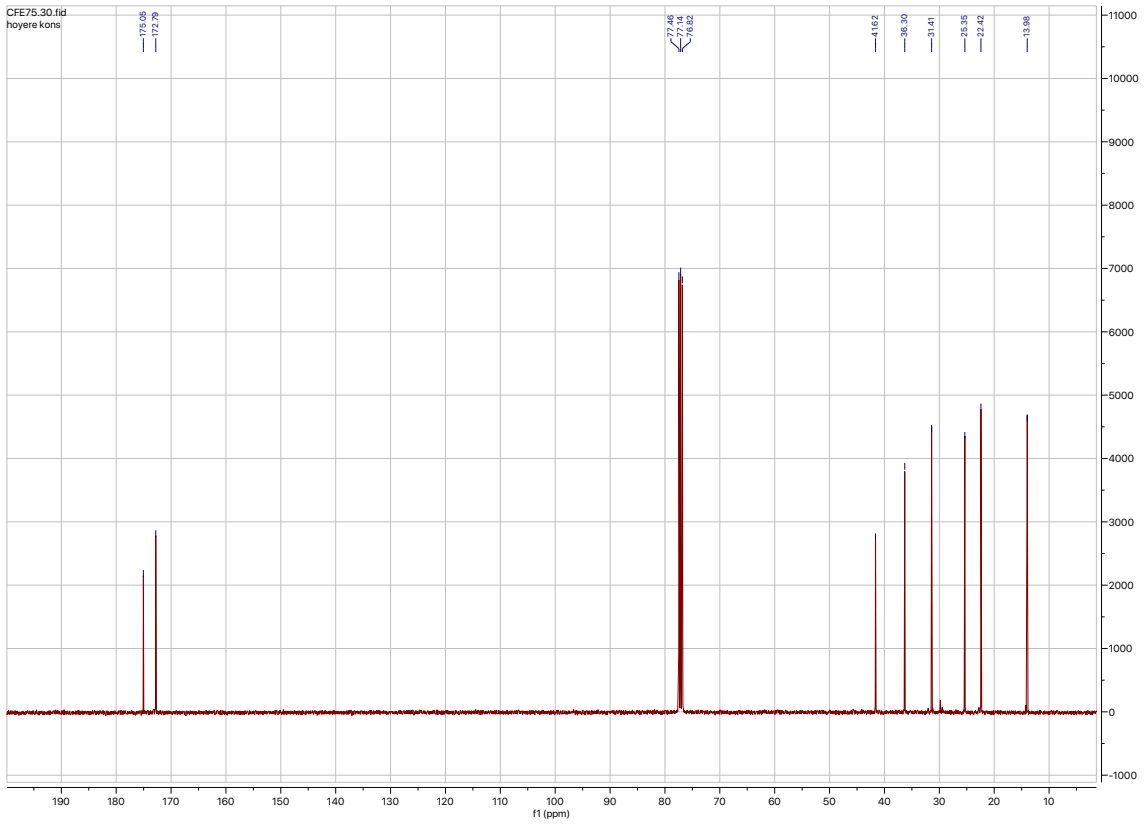


Figure 6-44 ^{13}C NMR spectrum of **17**.

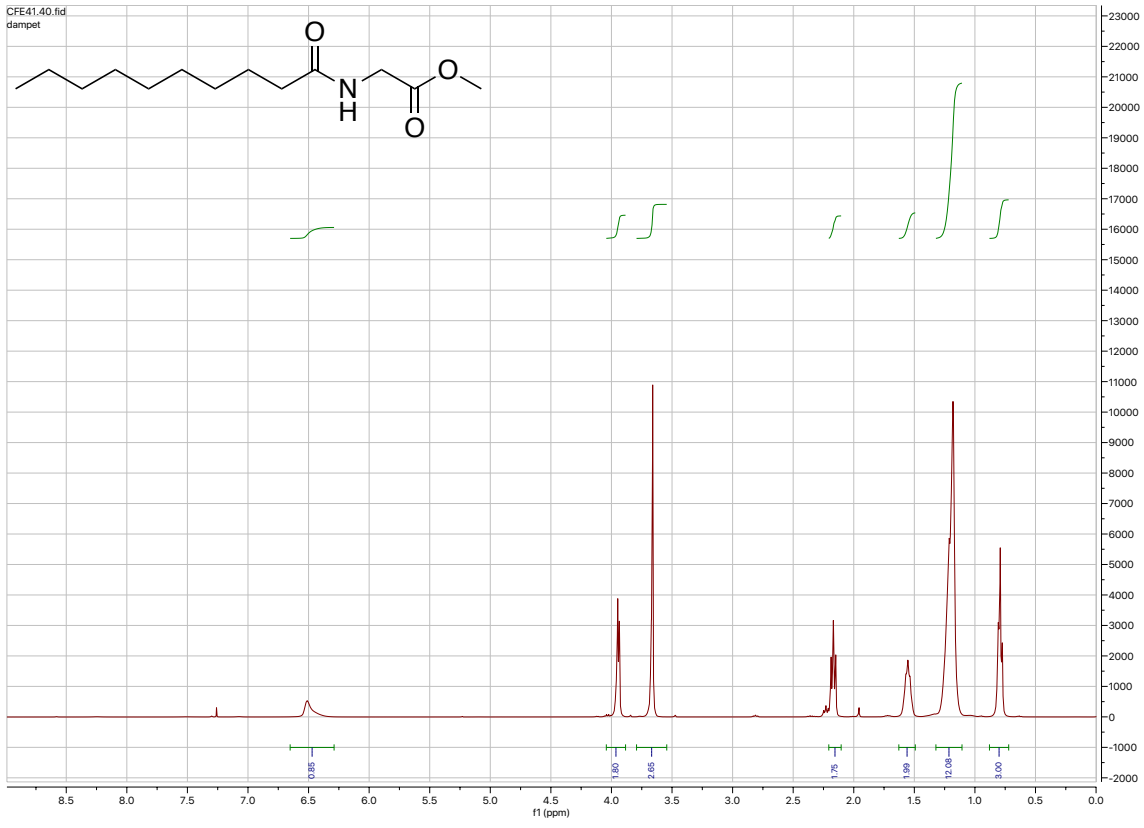


Figure 6-45 ^{1}H NMR spectrum of **18-ME**.

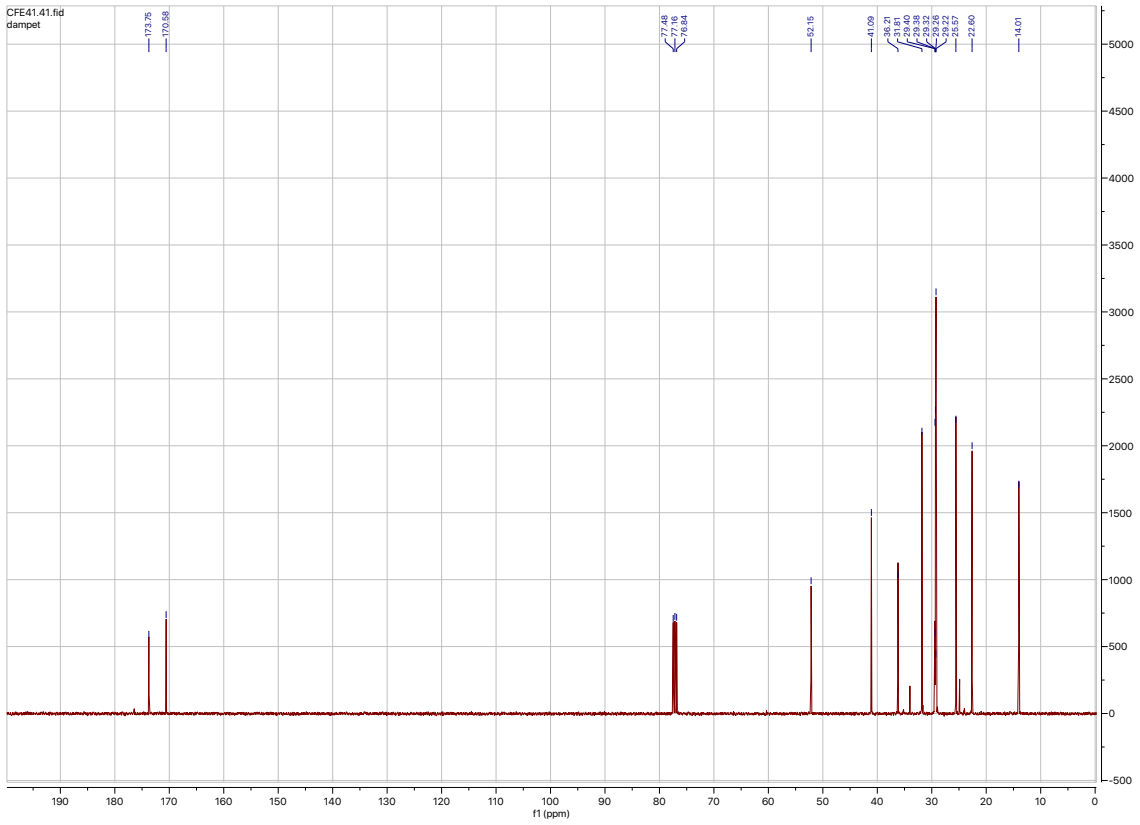


Figure 6-46 ^{13}C NMR spectrum of **18-ME**.

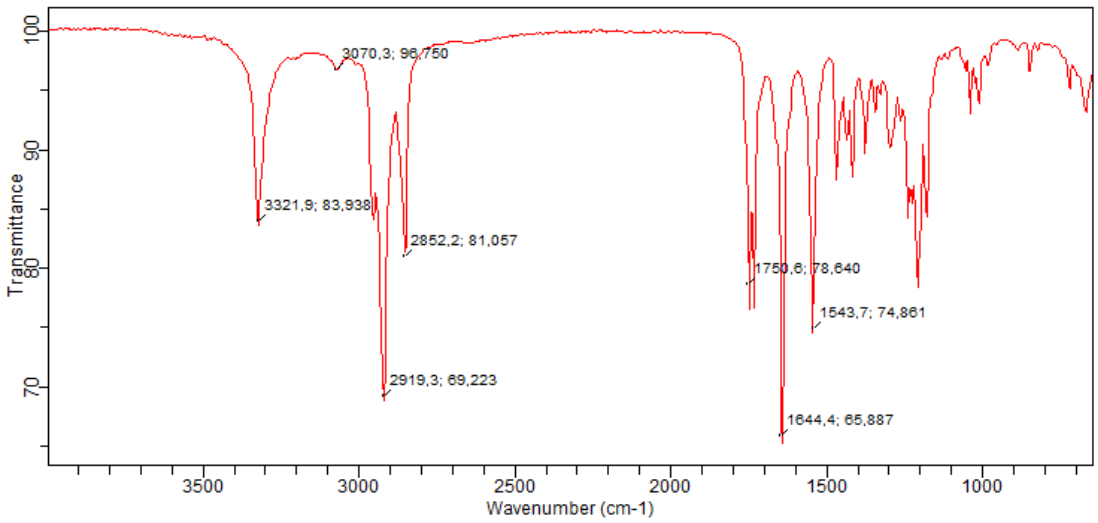
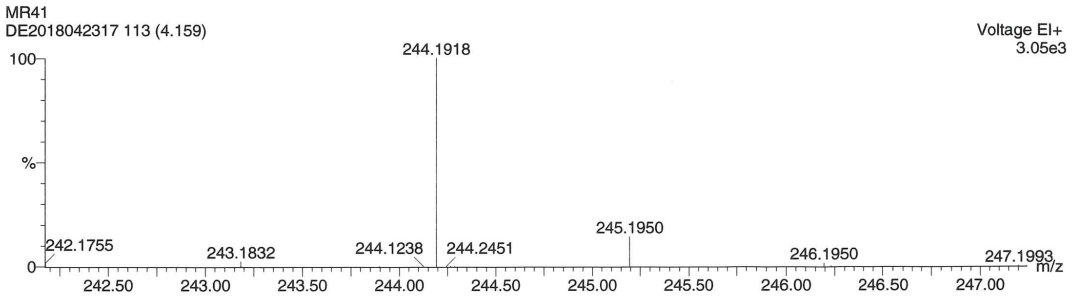


Figure 6-47 IR-spectrum of **18-ME**.

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

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



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
243.1832	243.1834	-0.2	-1.0	2.0	1	C13 H25 N O3

Figure 6-48 HR-MS spectrum of **18-ME**.

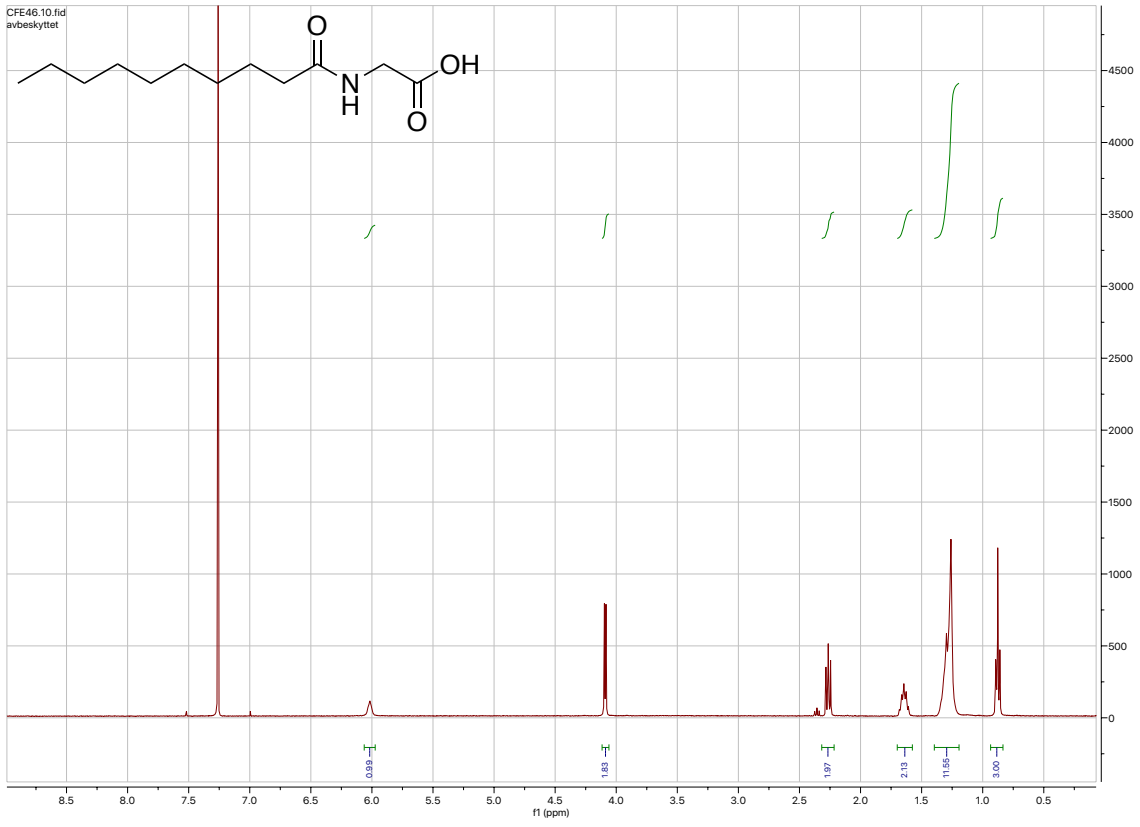


Figure 6-49 ^{1}H NMR spectrum of **18**.

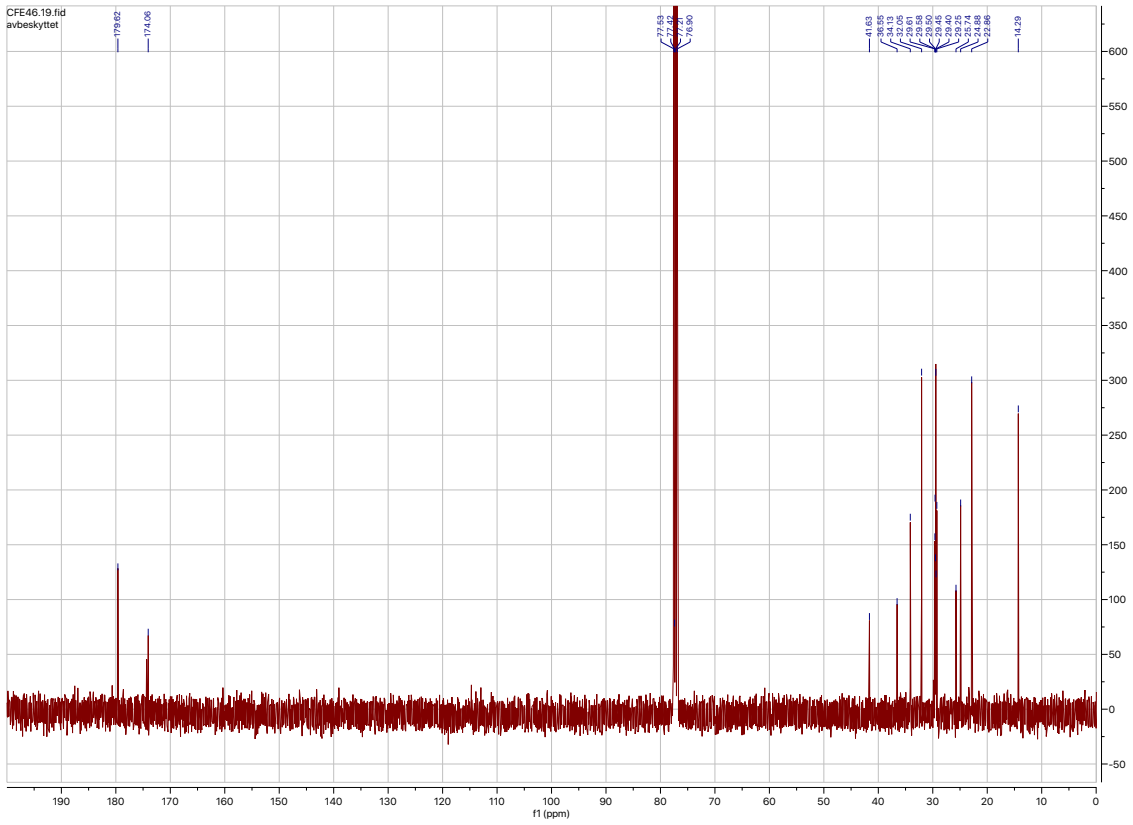


Figure 6-50 ^{13}C NMR spectrum of **18**.

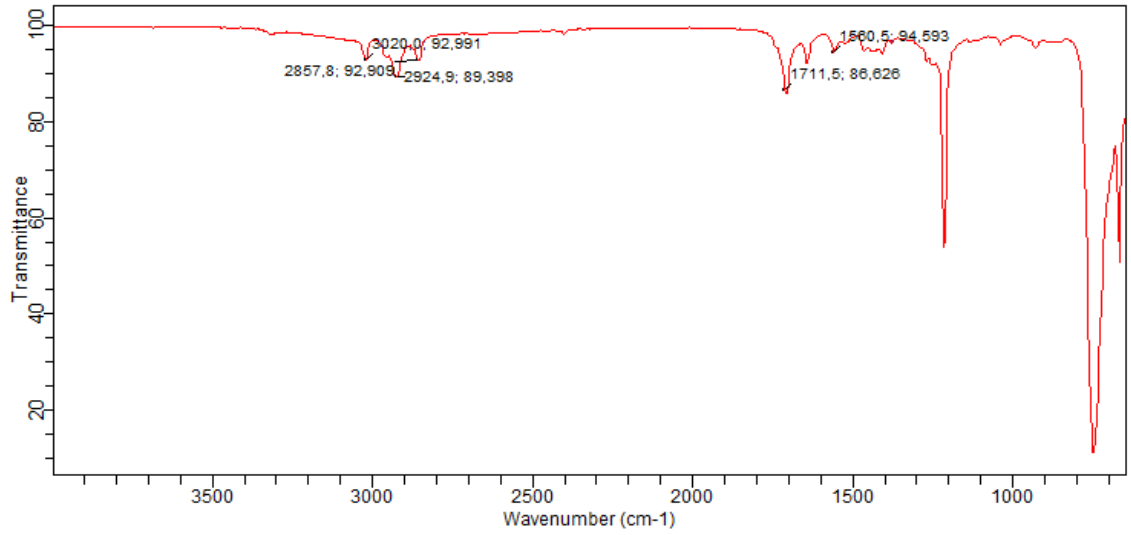
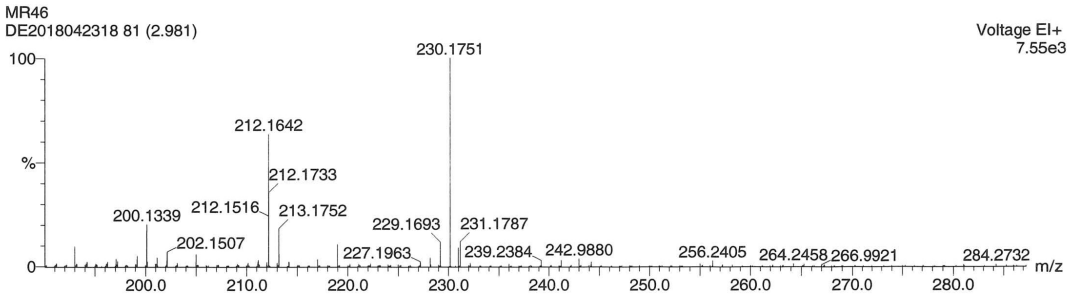


Figure 6-51 IR-spectrum of **18**.

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

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



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
229.1693	229.1678	1.5	6.6	2.0	1	C12 H23 N O3

Figure 6-52 HR-MS spectrum of **18**.

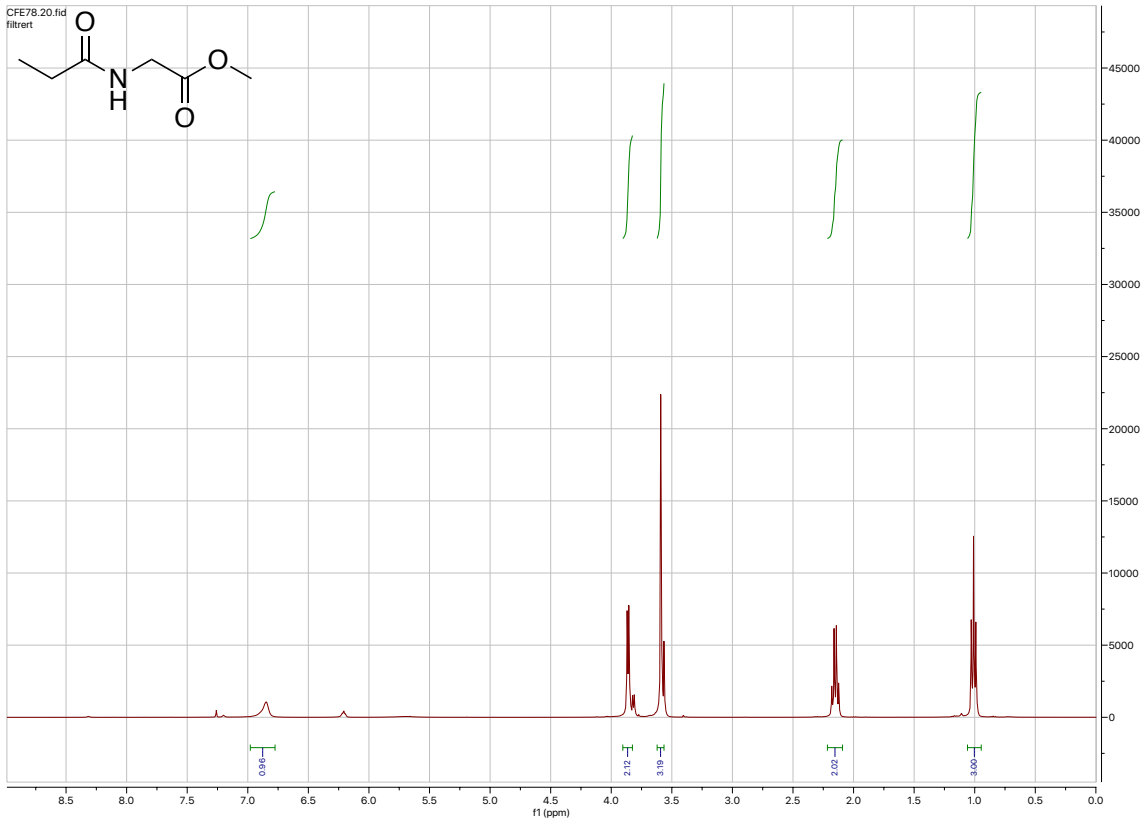


Figure 6-53 ^{1}H NMR spectrum of **38-ME**.

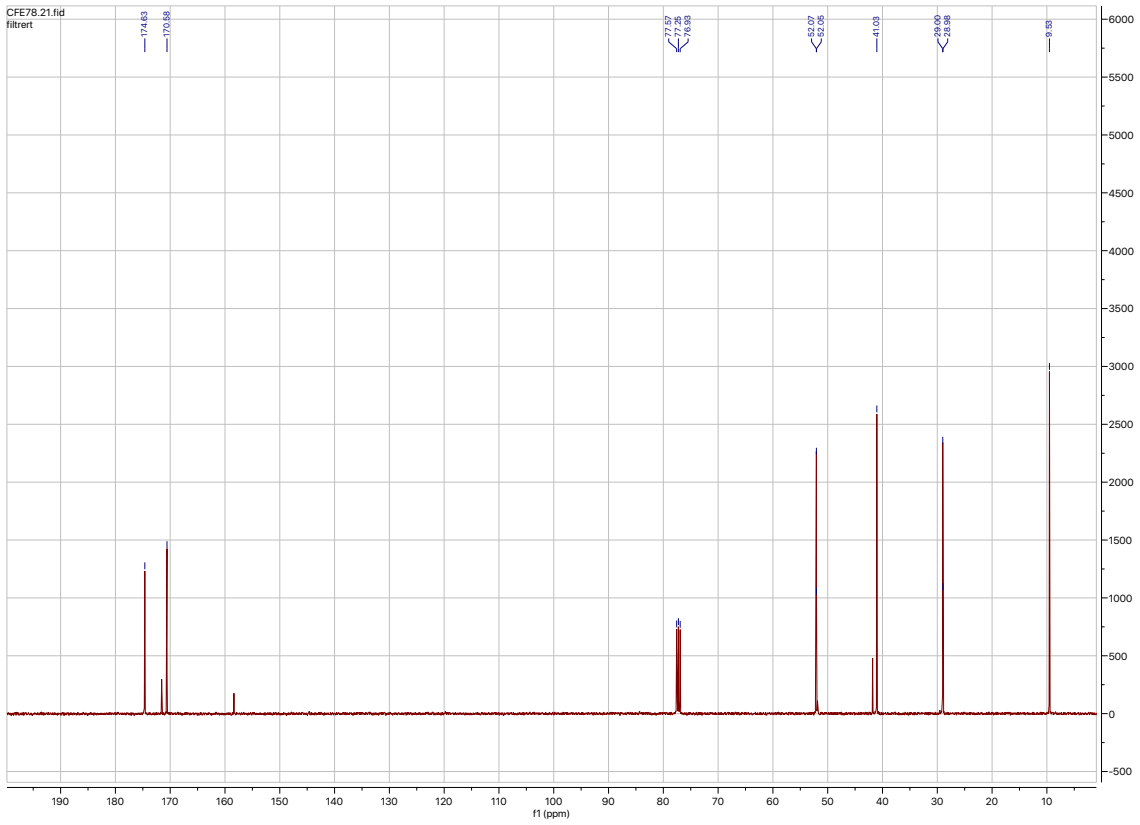


Figure 6-54 ^{13}C NMR spectrum of **38-ME**.

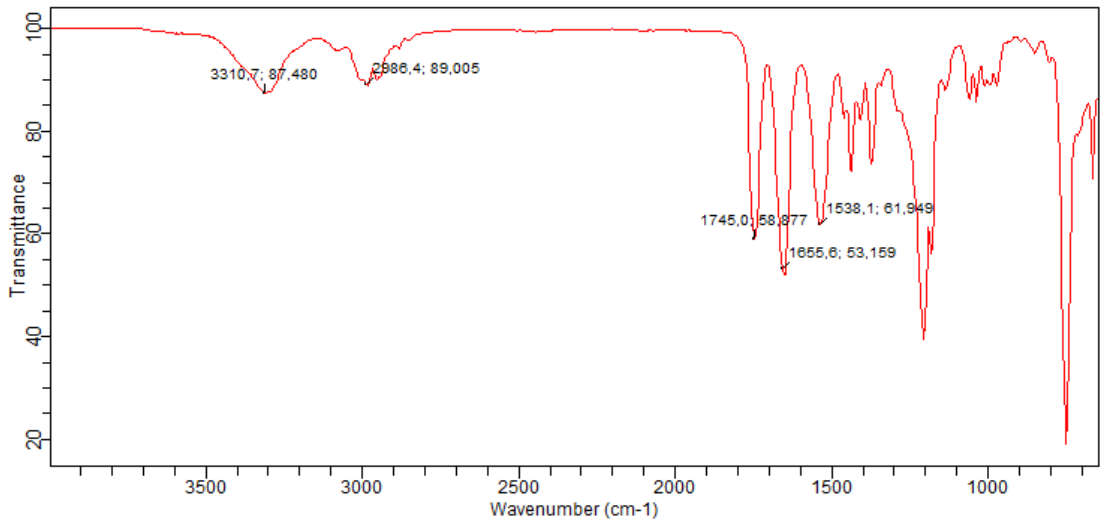


Figure 6-55 IR-spectrum of **38-ME**.

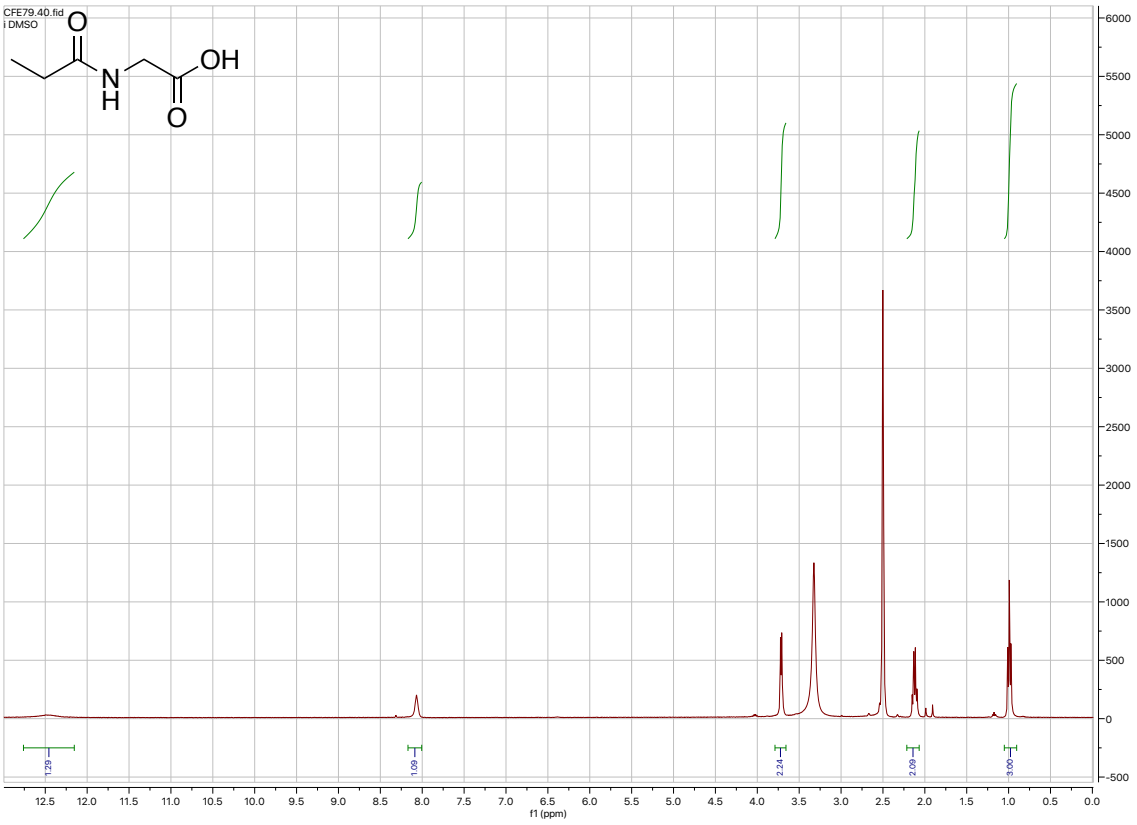


Figure 6-56 ^{1}H NMR spectrum of **38**.

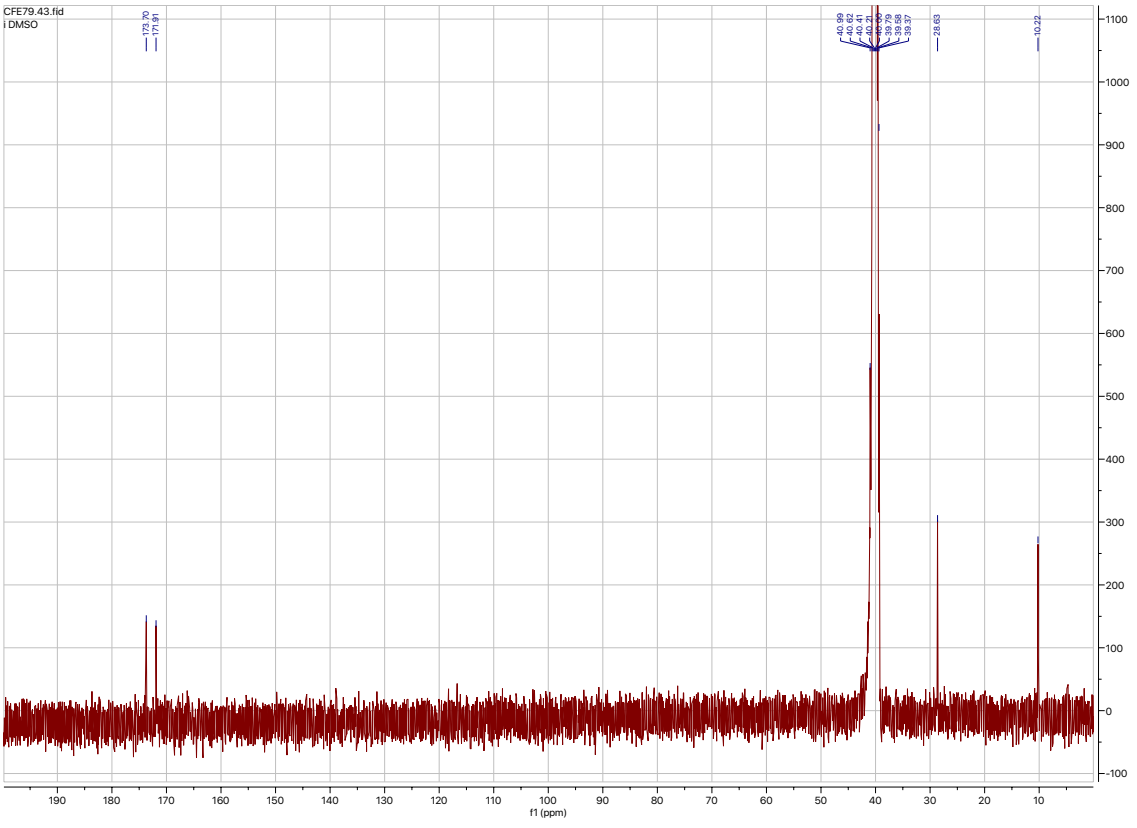


Figure 6-57 ^{13}C NMR spectrum of **38**.

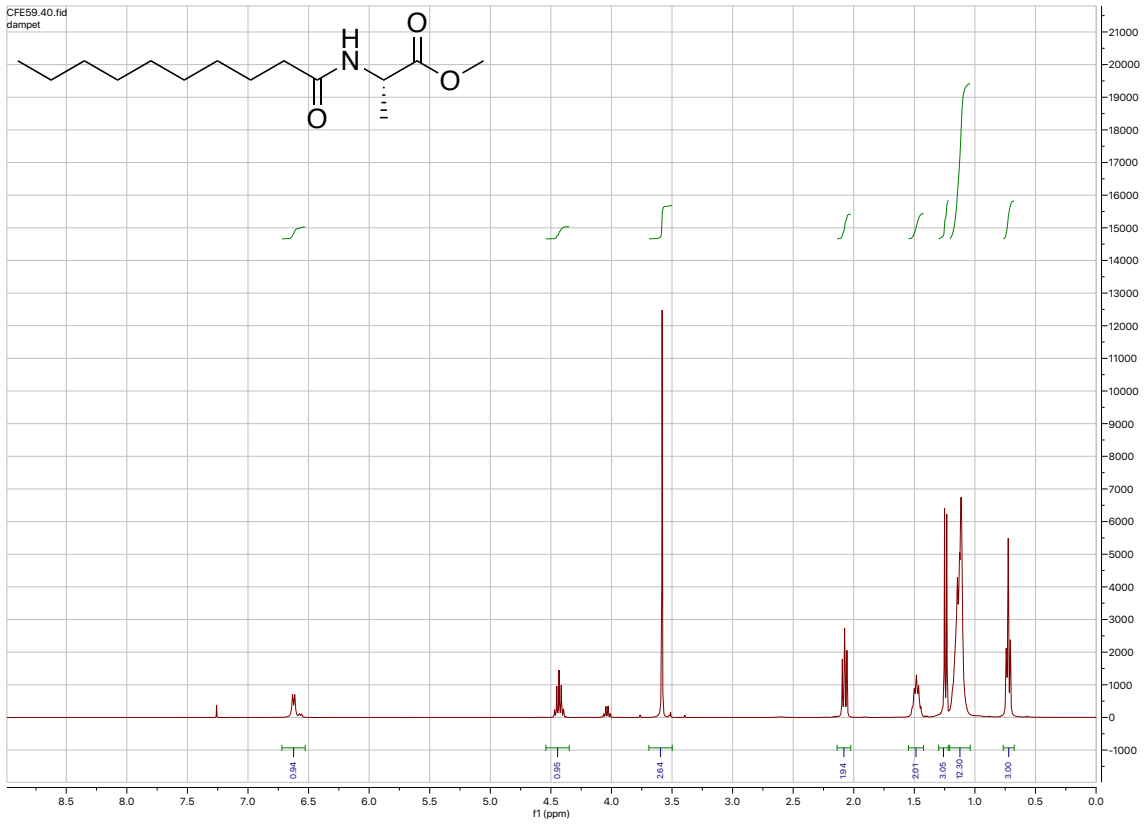


Figure 6-58 ^{1}H NMR spectrum of **39-ME**.

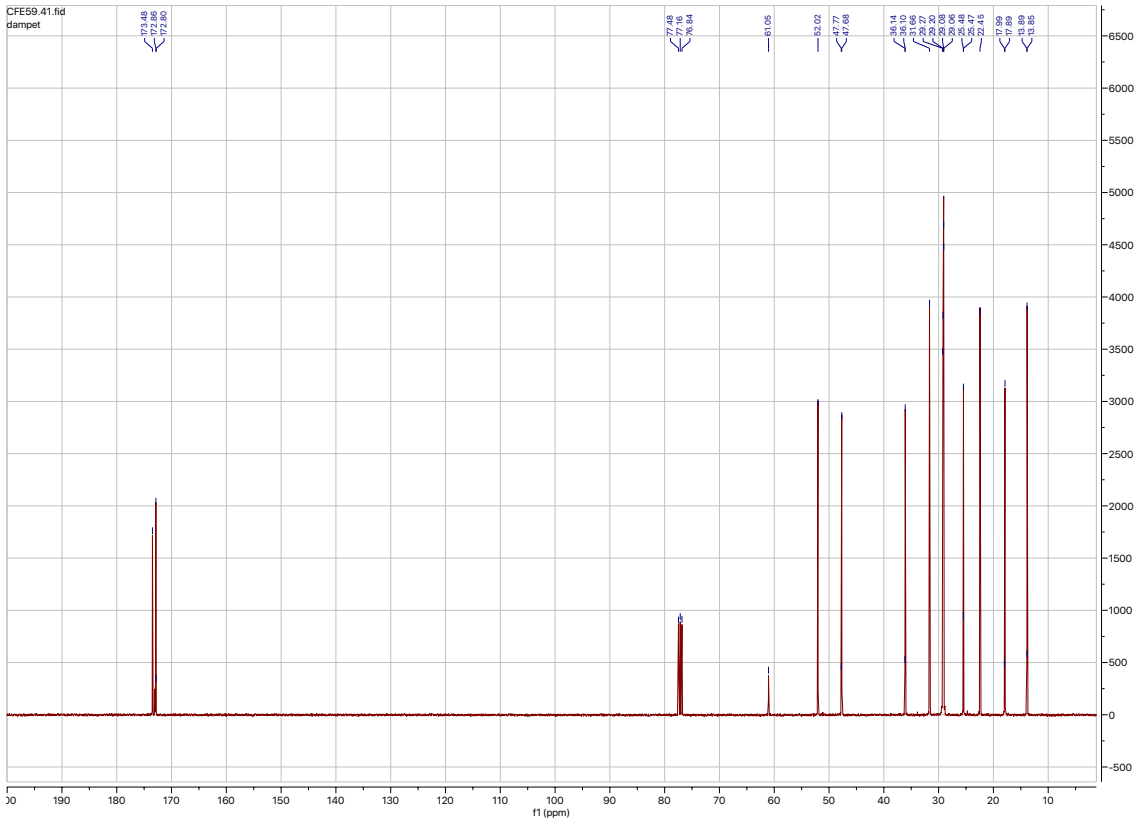


Figure 6-59 ^{13}C NMR spectrum of **39-ME**.

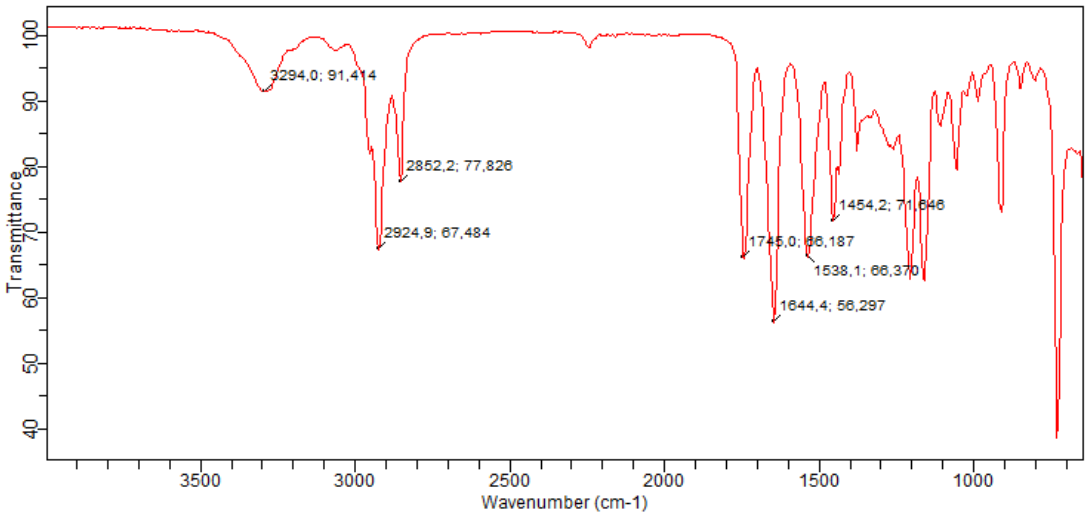
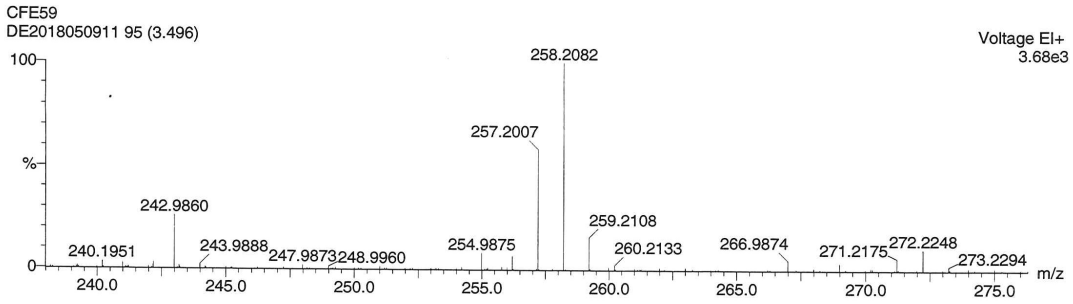


Figure 6-60 IR-spectrum of **39-ME**.

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

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



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
257.2007	257.2018	-1.1	-4.2	6.5	2	C17 H25 N2
	257.1991	1.6	6.2	2.0	1	C14 H27 N O3

Figure 6-61 HR-MS spectrum of **39-ME**.

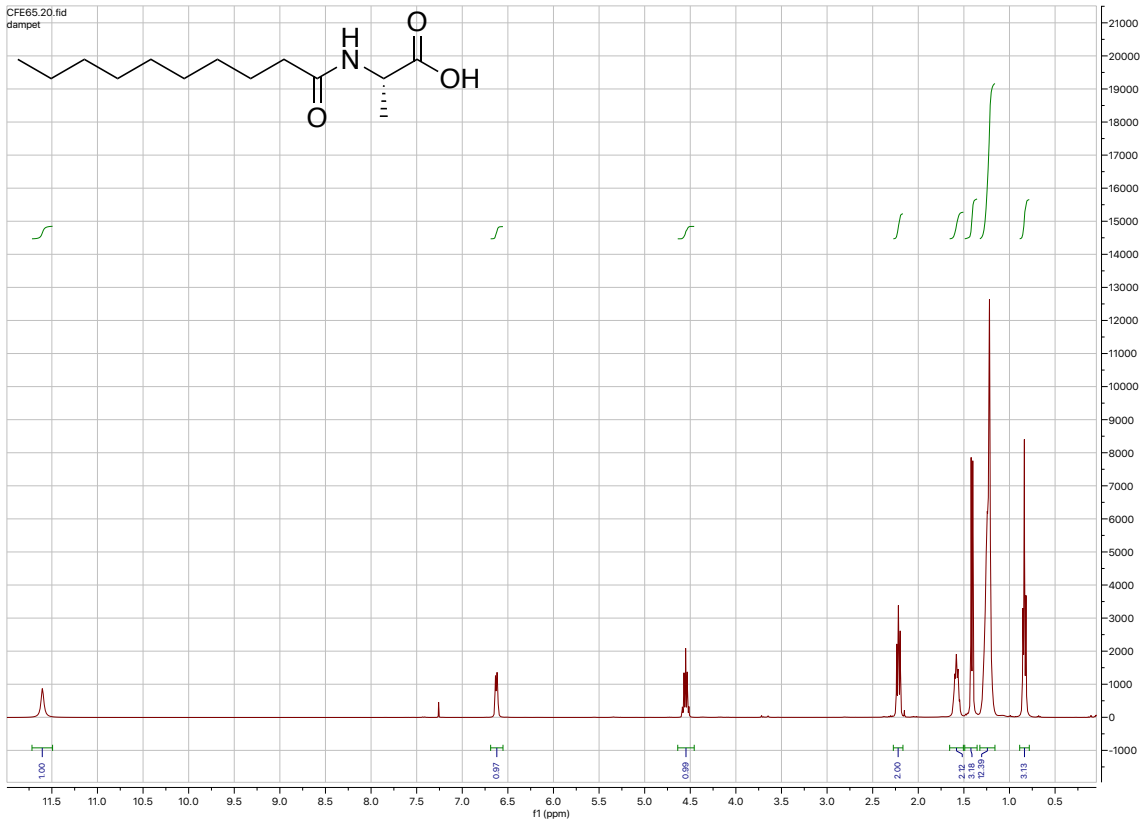


Figure 6-62 ^{1}H NMR spectrum of **39**.

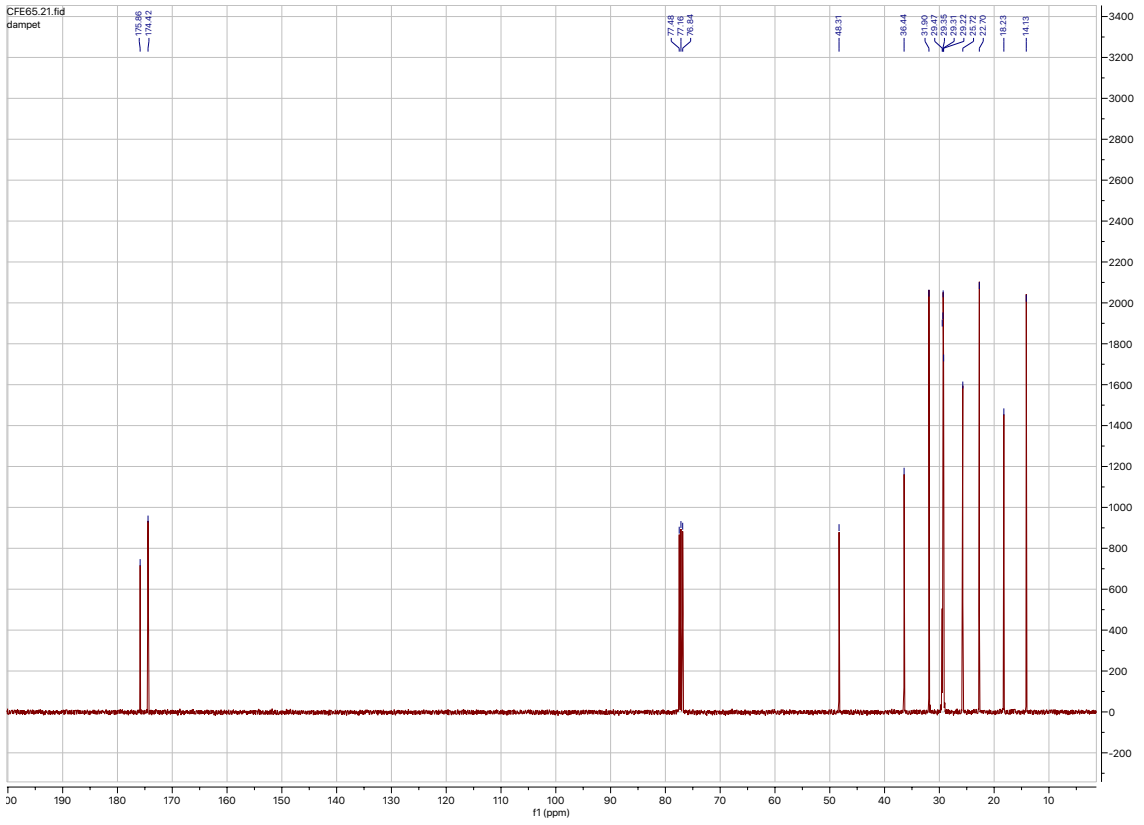


Figure 6-63 ^{13}C NMR spectrum of **39**.

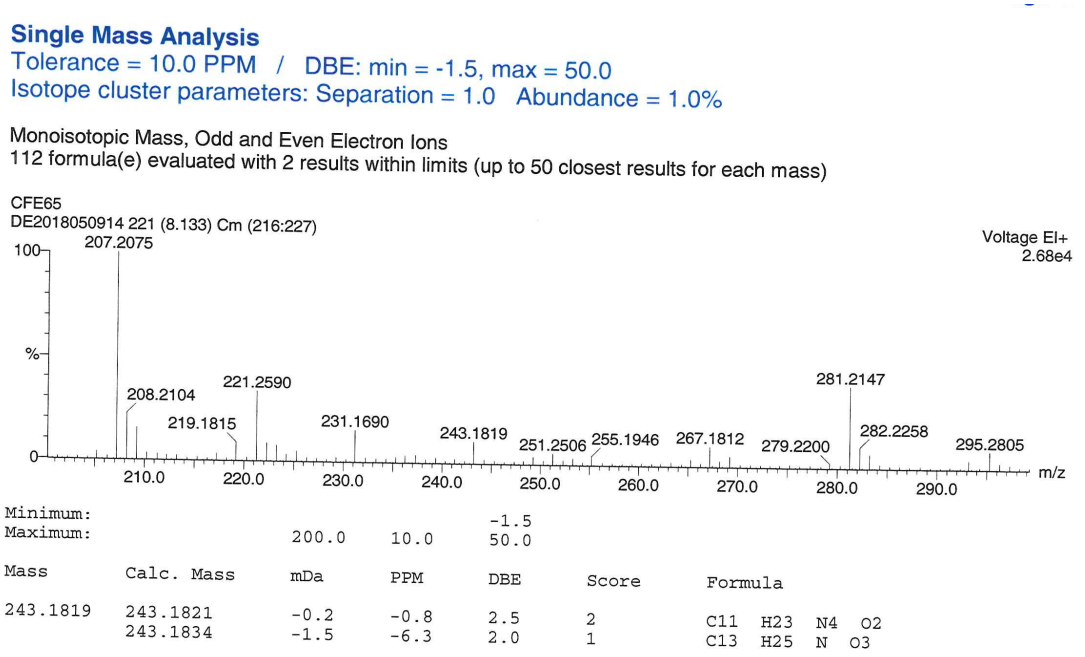


Figure 6-64 HR-MS spectrum of **39**.

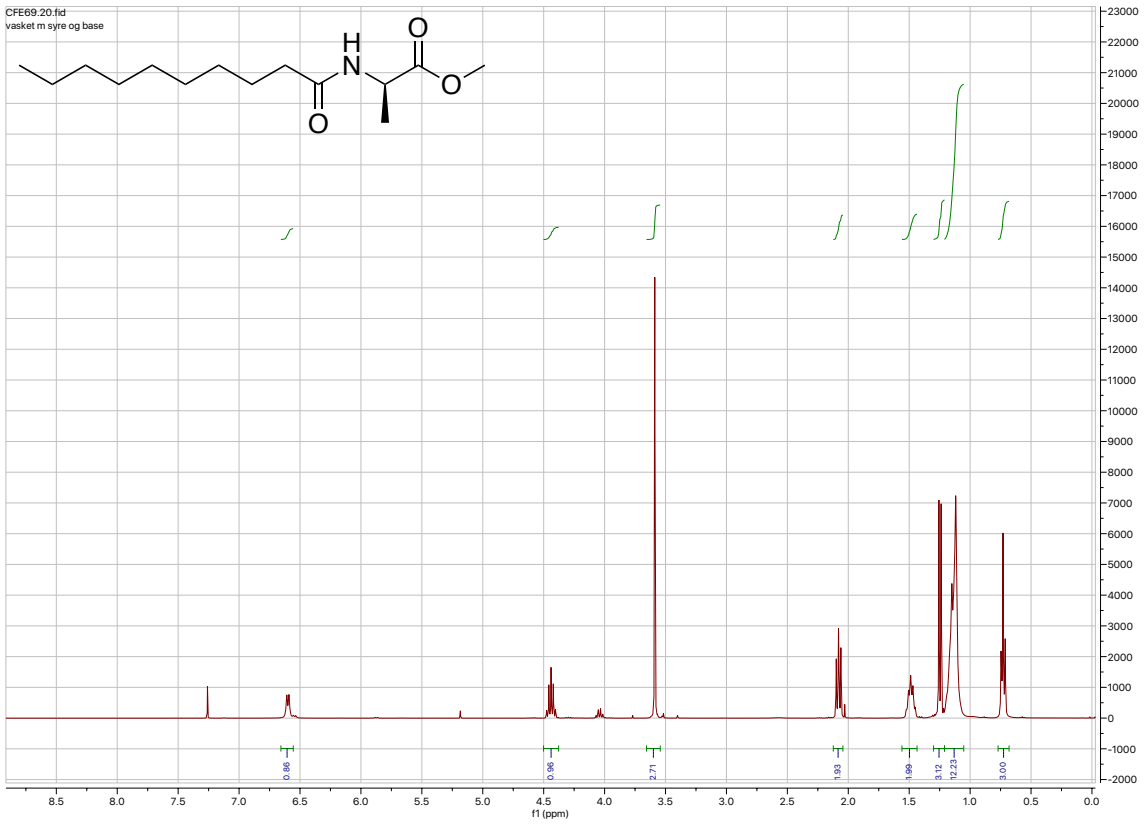


Figure 6-65 ^{1}H NMR spectrum of **40-ME**.

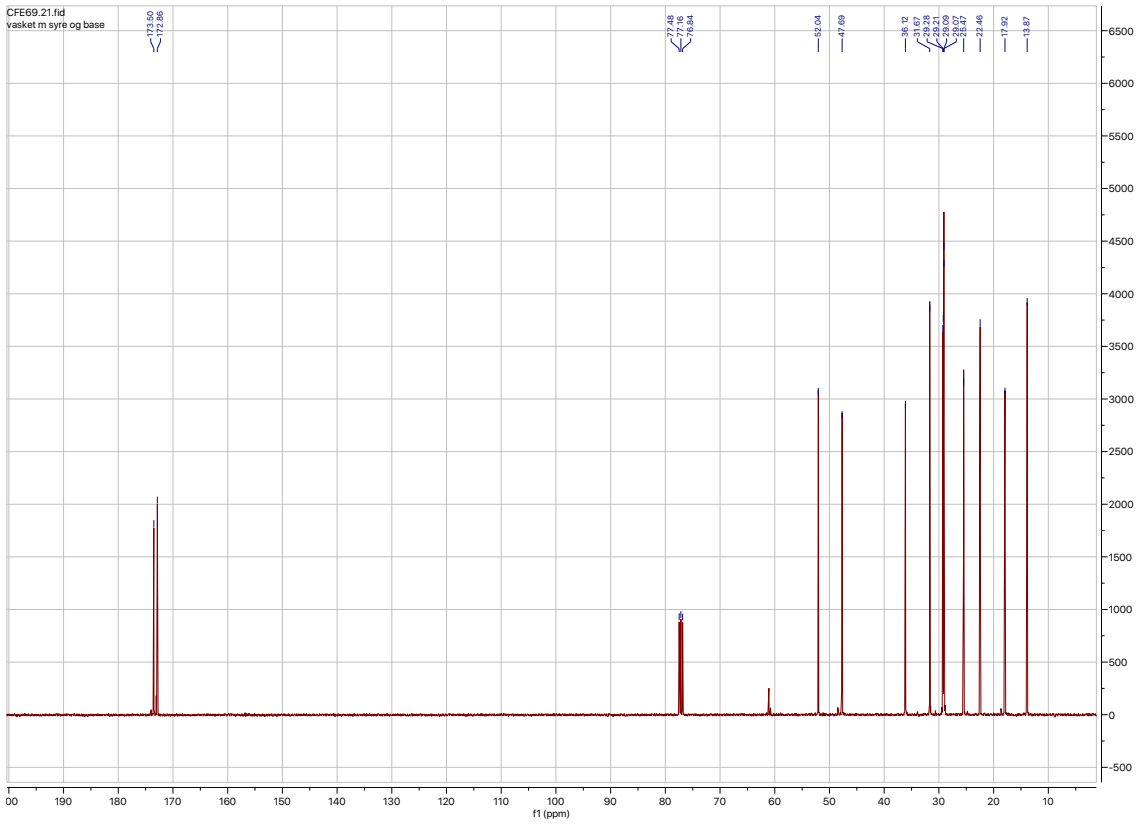


Figure 6-66 ^{13}C NMR spectrum of **40-ME**.

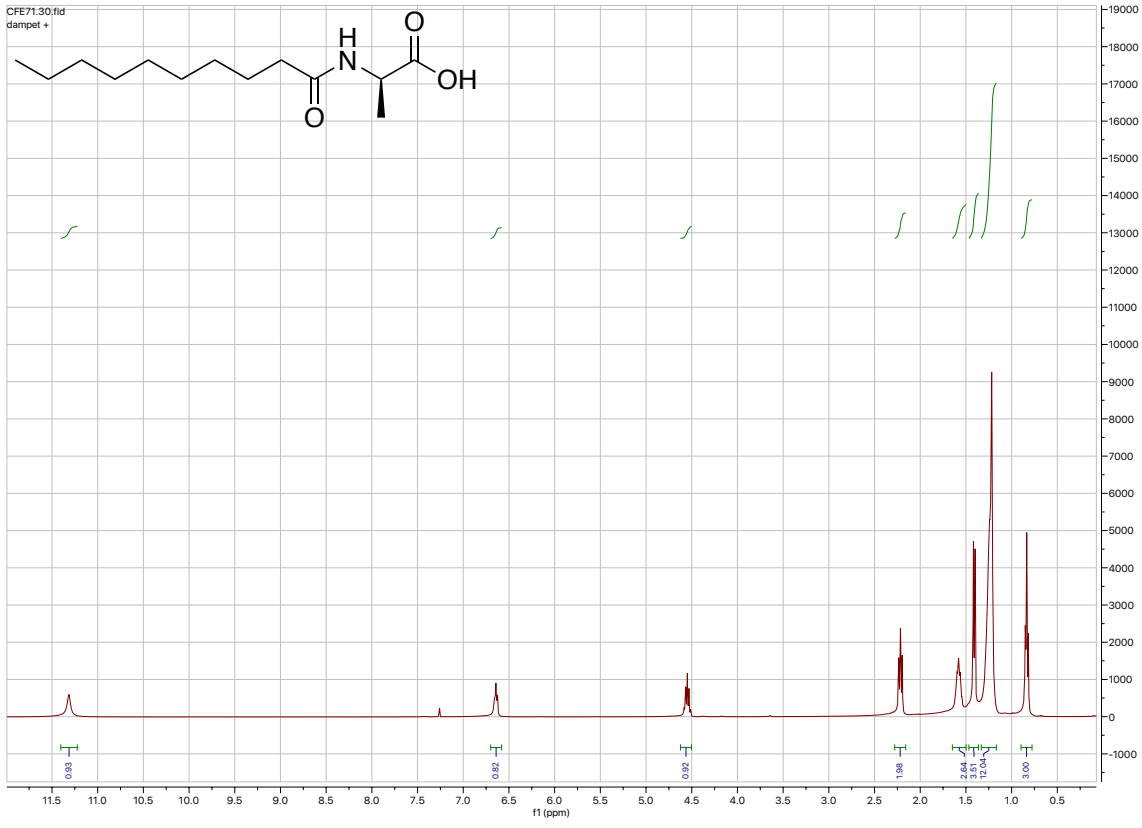


Figure 6-67 ^{1}H NMR spectrum of **40**.

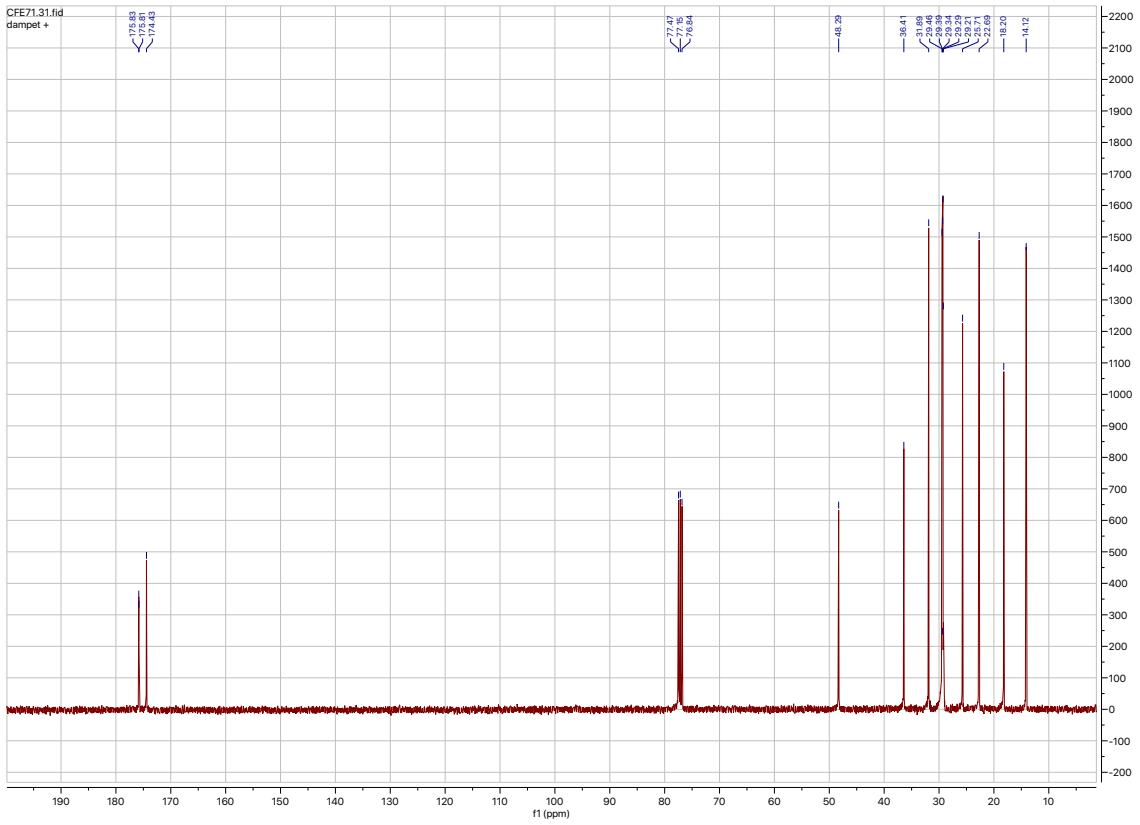


Figure 6-68 ^{13}C NMR spectrum of **40**.

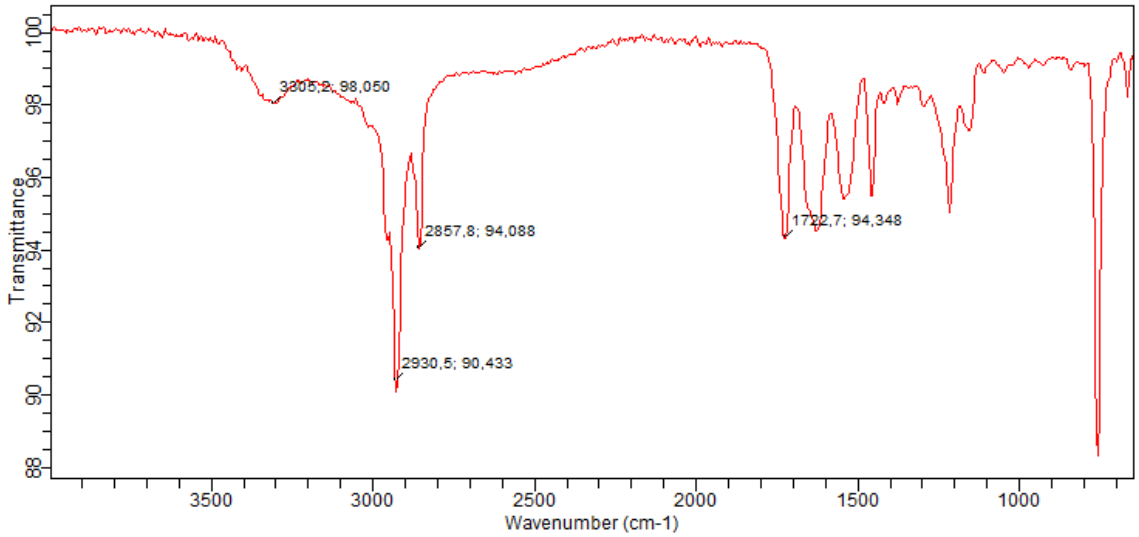


Figure 6-69 IR-spectrum of **40**.

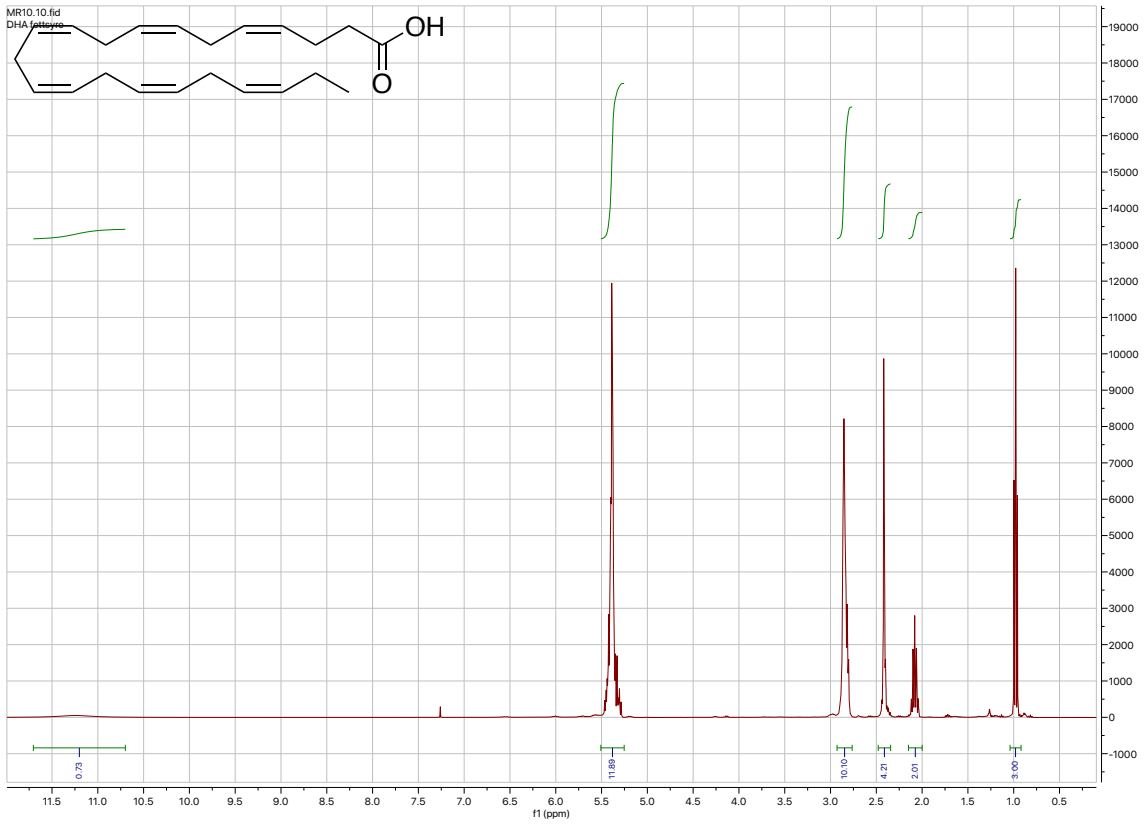


Figure 6-70 ^{1}H NMR spectrum of DHA.

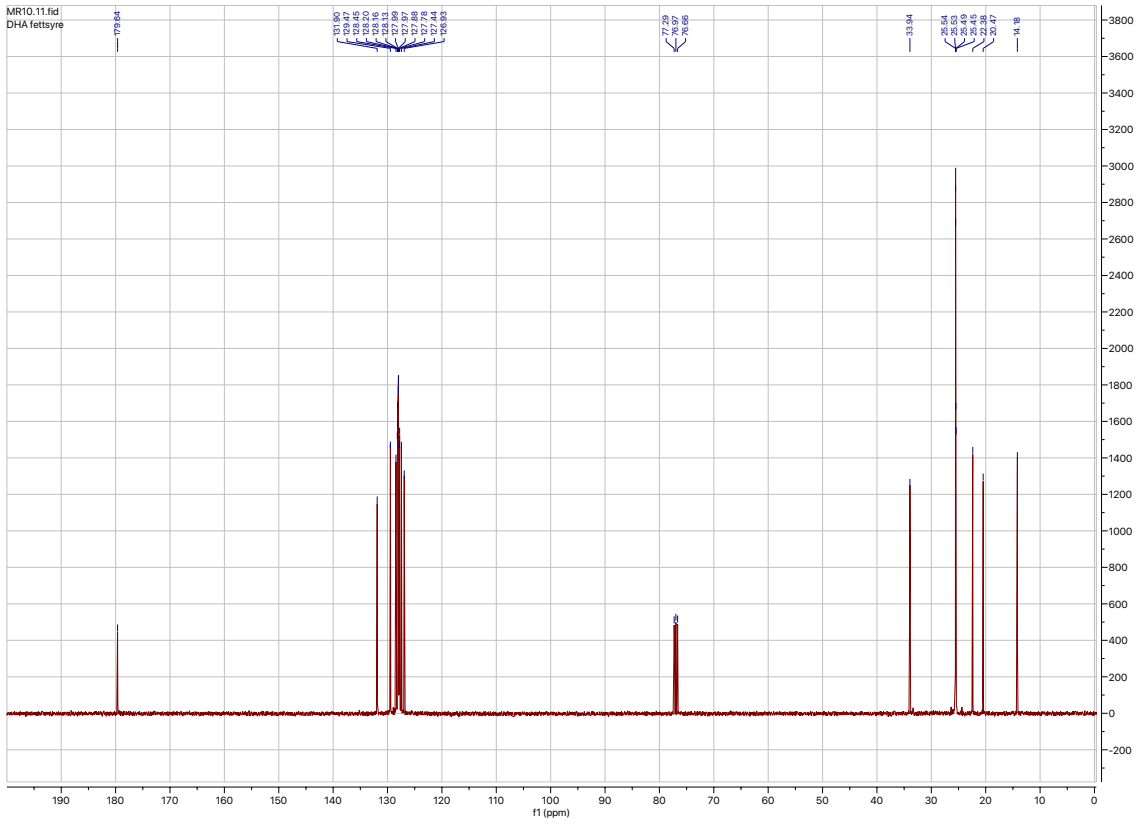


Figure 6-71 ^{13}C NMR spectrum of DHA.

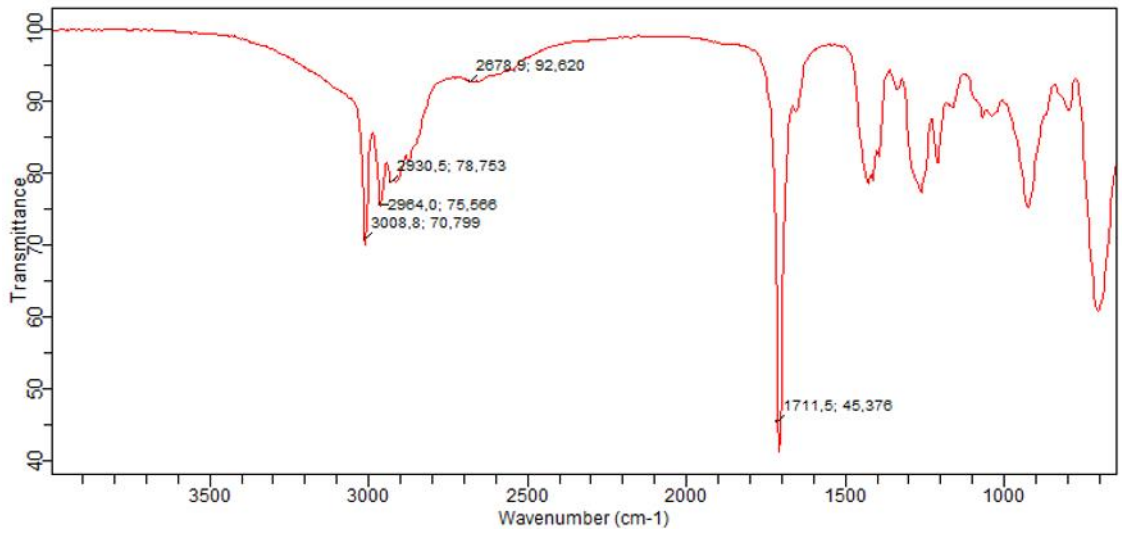


Figure 6-72 IR-spectrum of DHA.

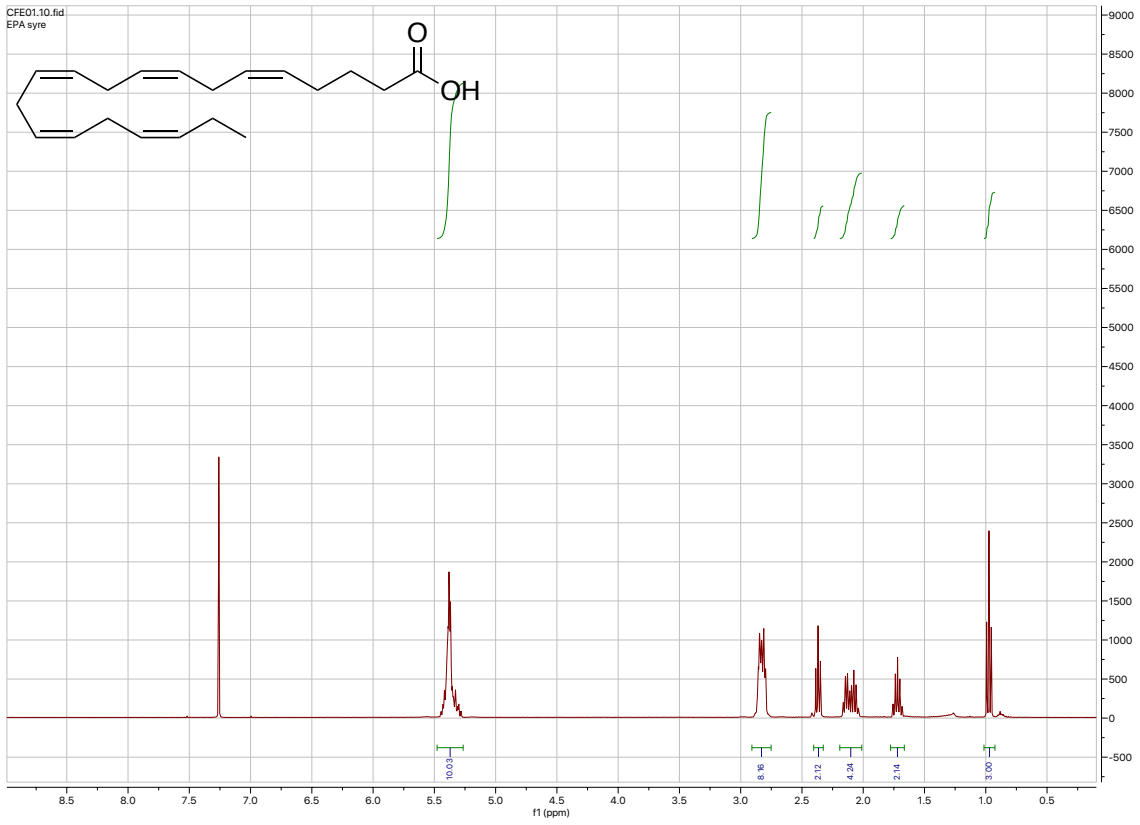


Figure 6-73 ^{1}H NMR spectrum of EPA.

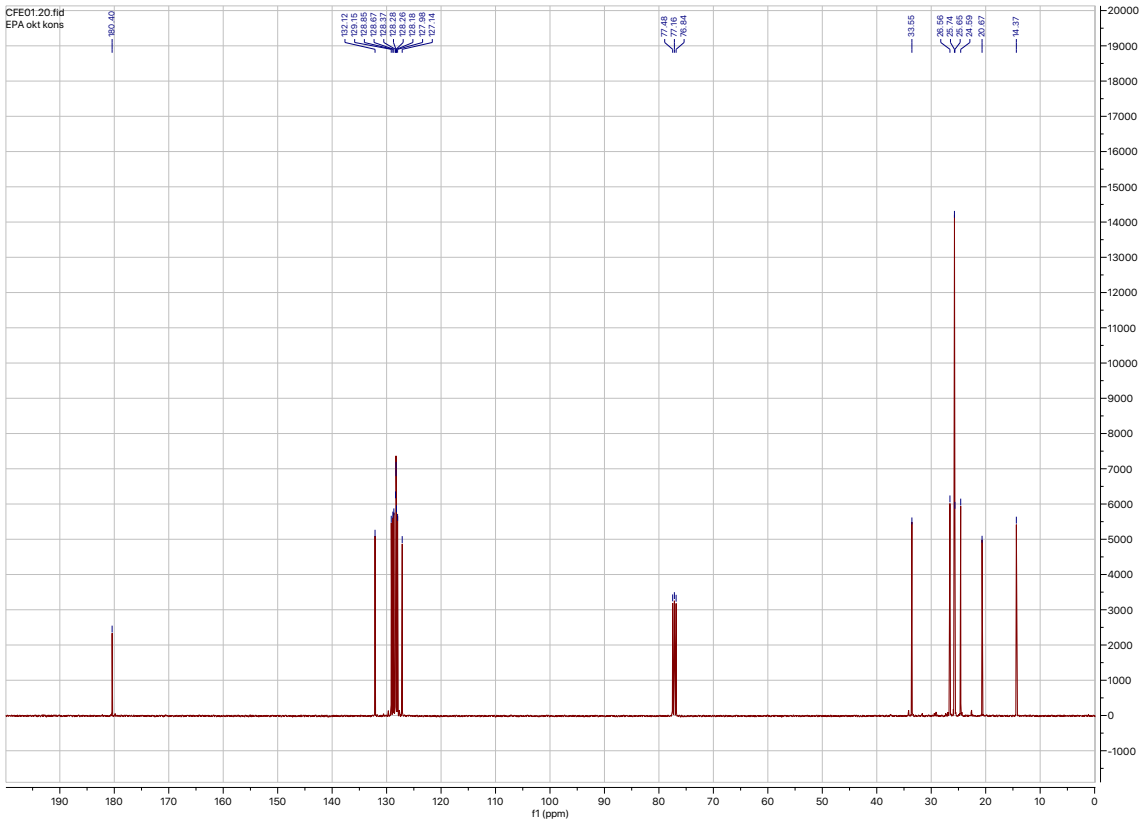


Figure 6-74 ^{13}C NMR spectrum of EPA.

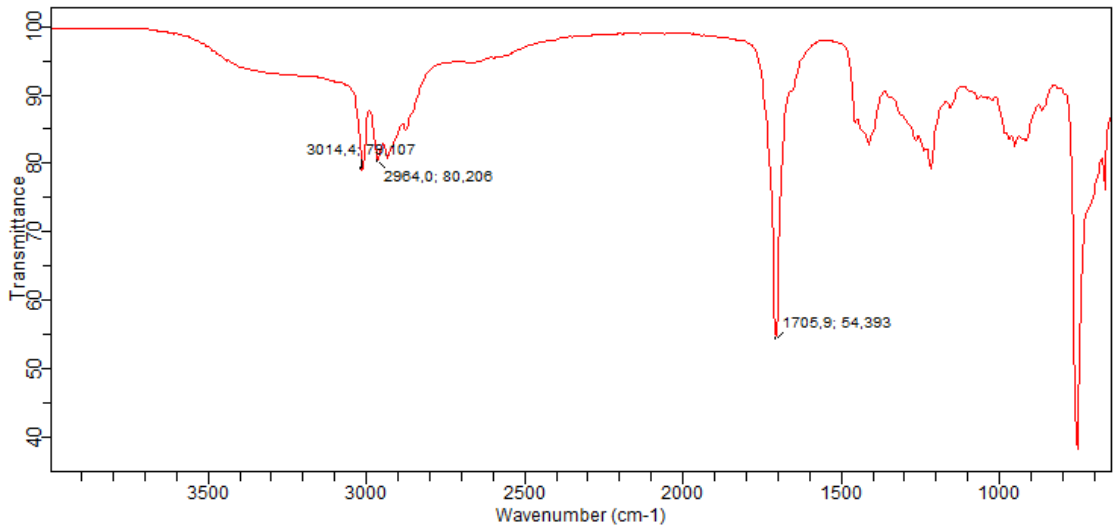
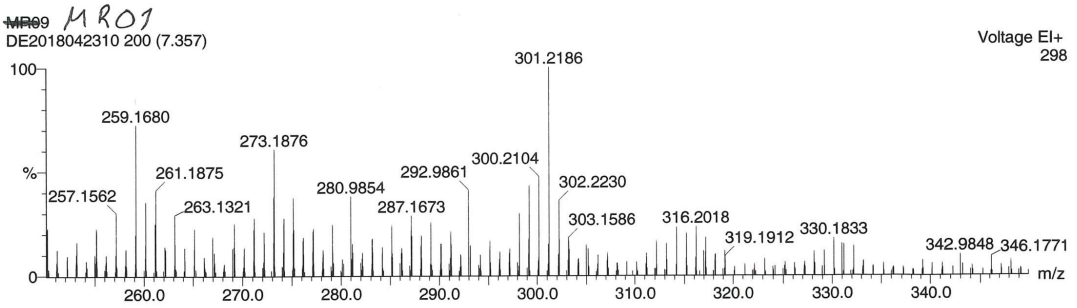


Figure 6-75 IR-spectrum of EPA.

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

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

~~MR09~~ MR01
DE2018042310 200 (7.357)



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
302.2230	302.2246	-1.6	-5.2	6.0	1	C20 H30 O2

Figure 6-76 HR-MS spectrum of EPA.

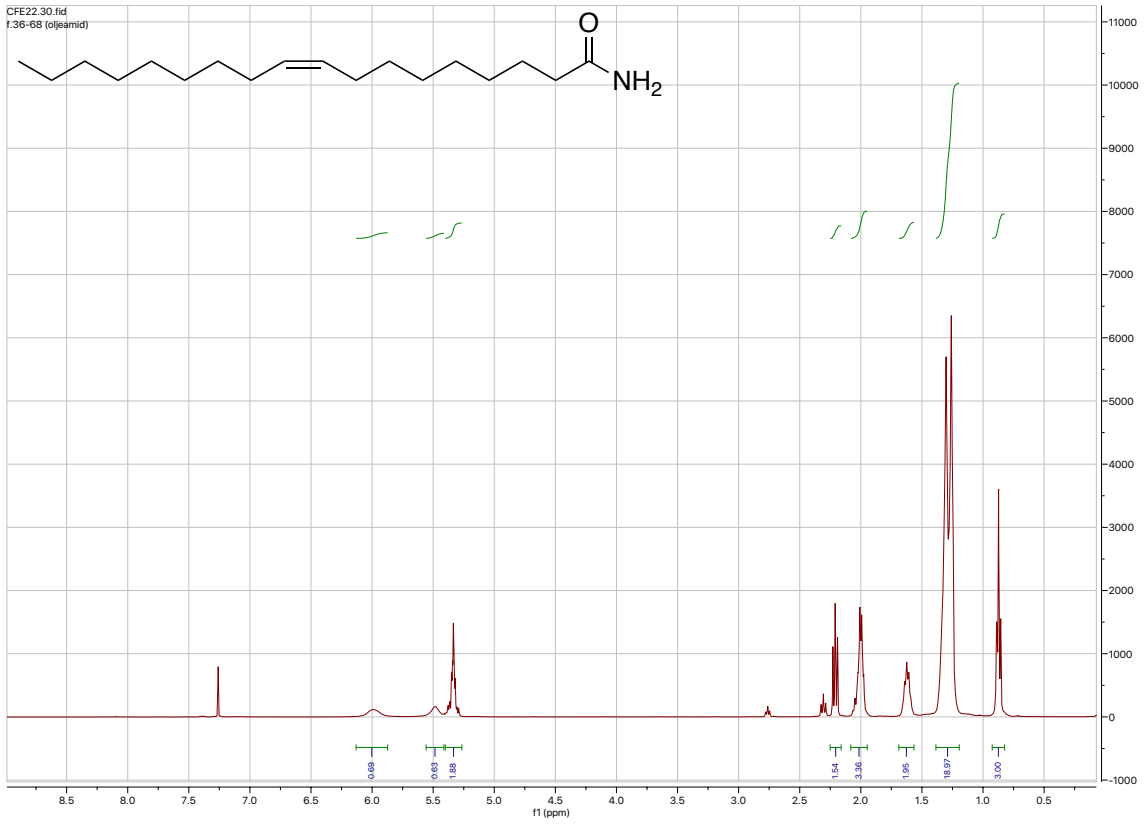


Figure 6-77 ^{1}H NMR spectrum of OA.

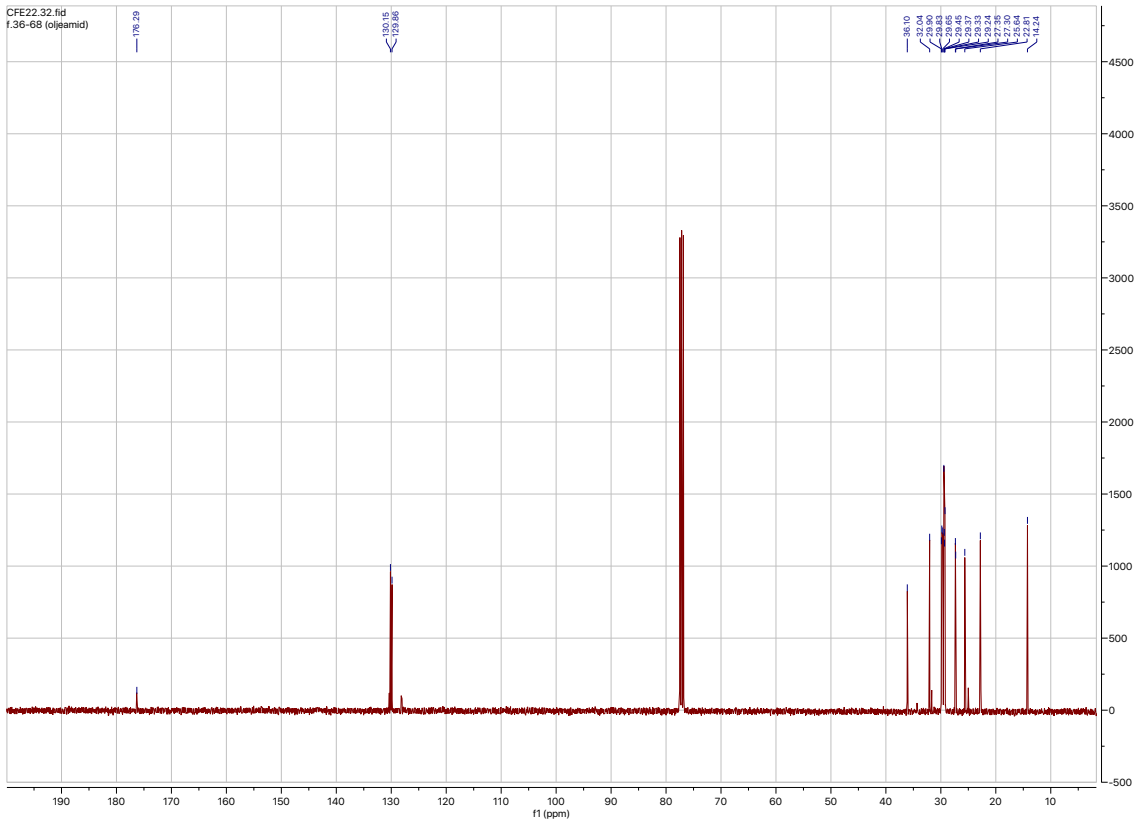


Figure 6-78 ^{13}C NMR spectrum of OA.

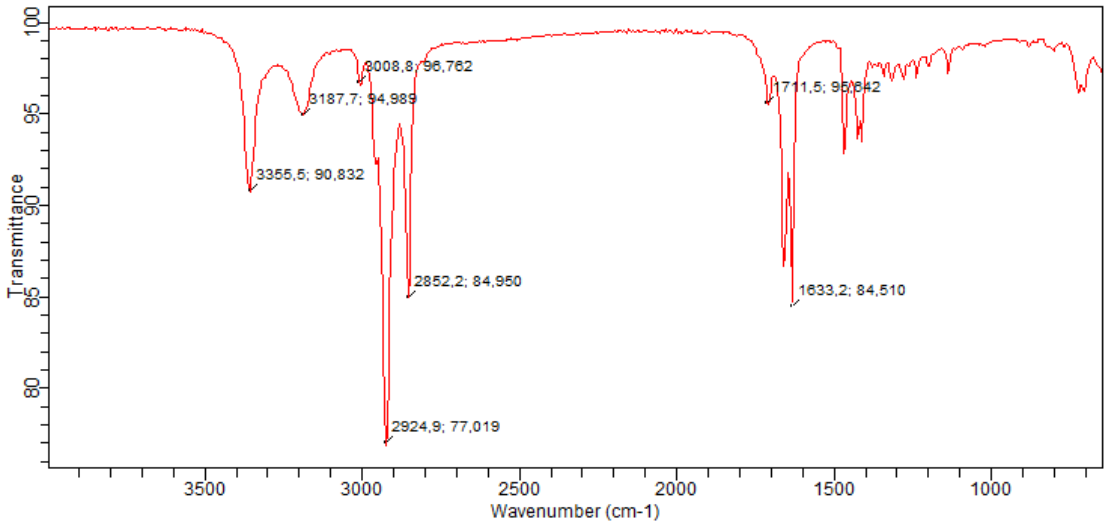
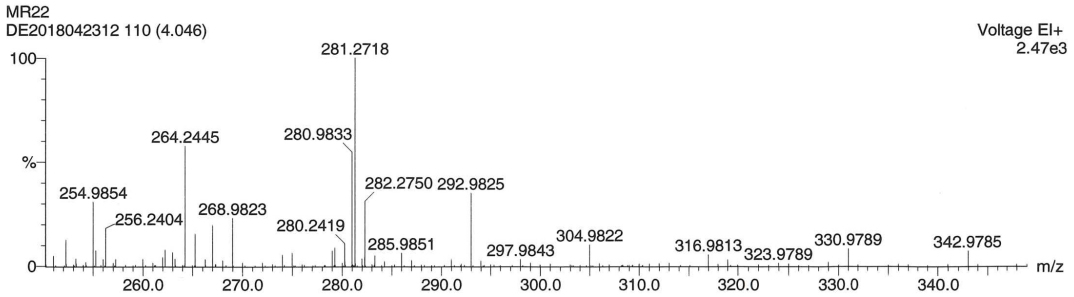


Figure 6-79 IR-spectrum of OA.

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

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



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
281.2718	281.2719	-0.1	-0.2	2.0	1	C18 H35 N O

Figure 6-80 HR-MS spectrum of OA.

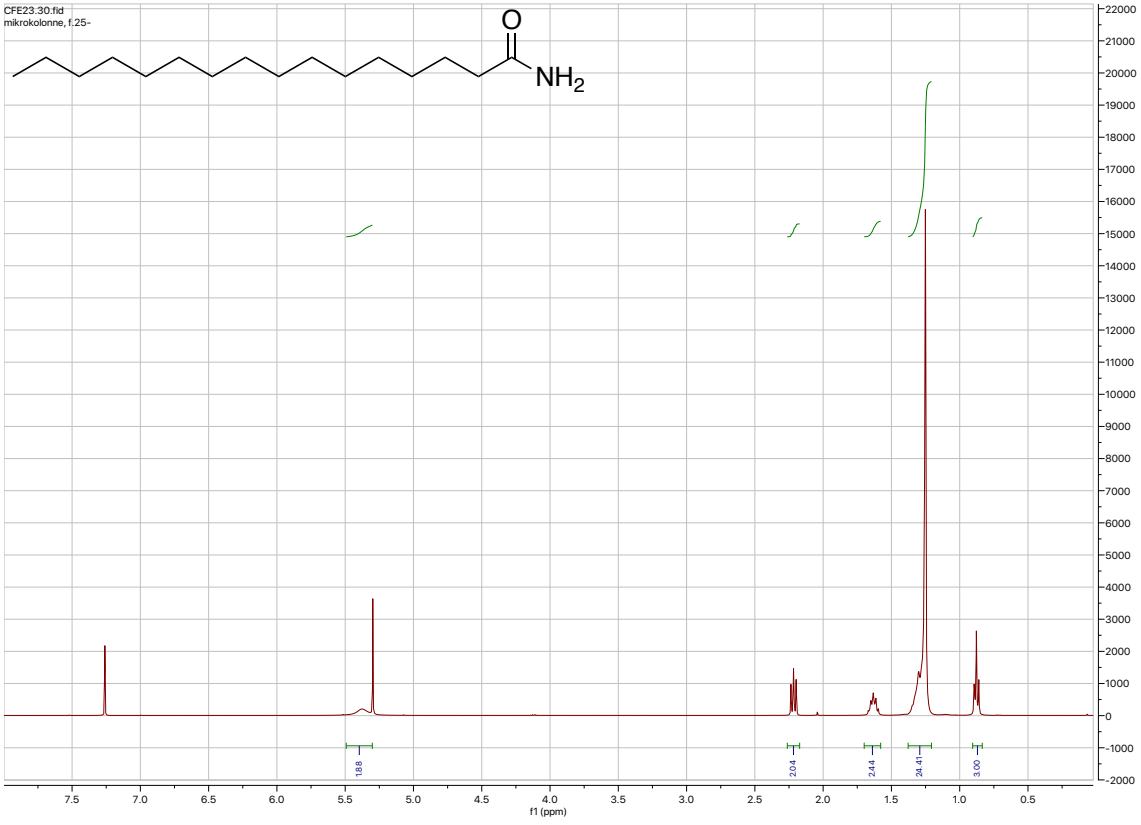


Figure 6-81 ^{1}H NMR spectrum of **21**.

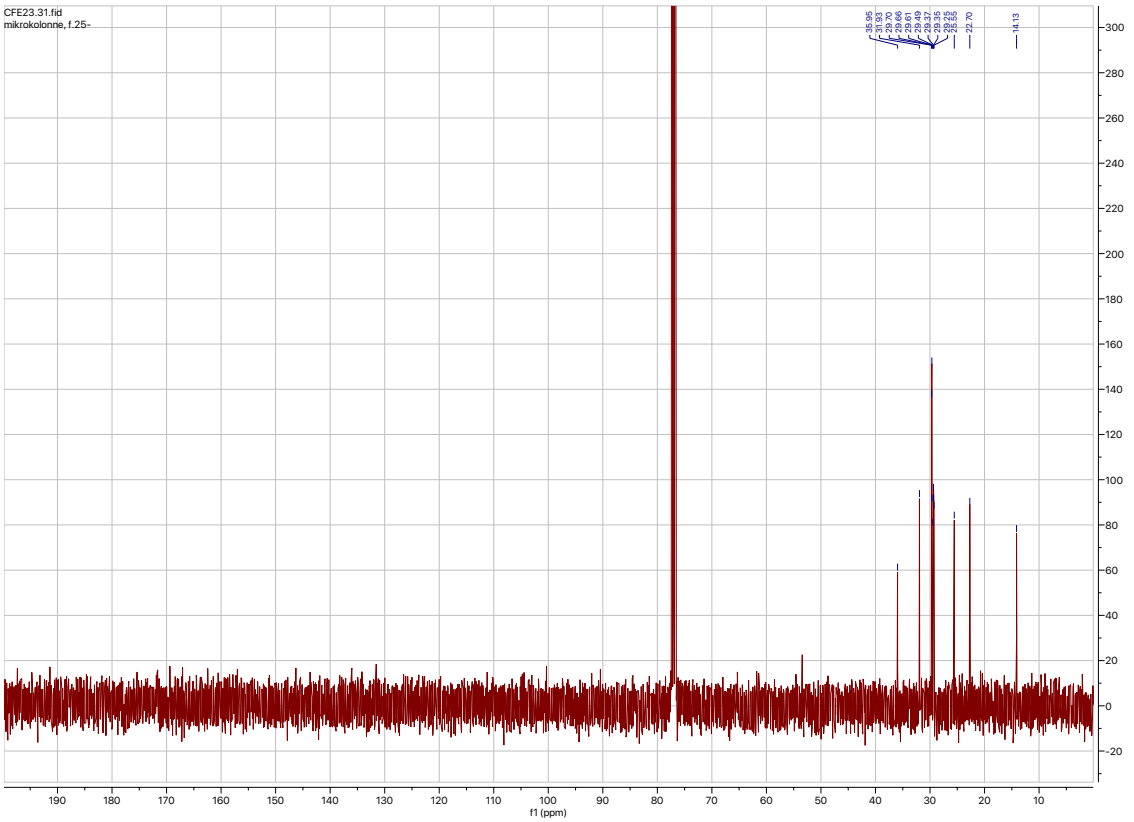


Figure 6-82 ^{13}C NMR spectrum of **21**.

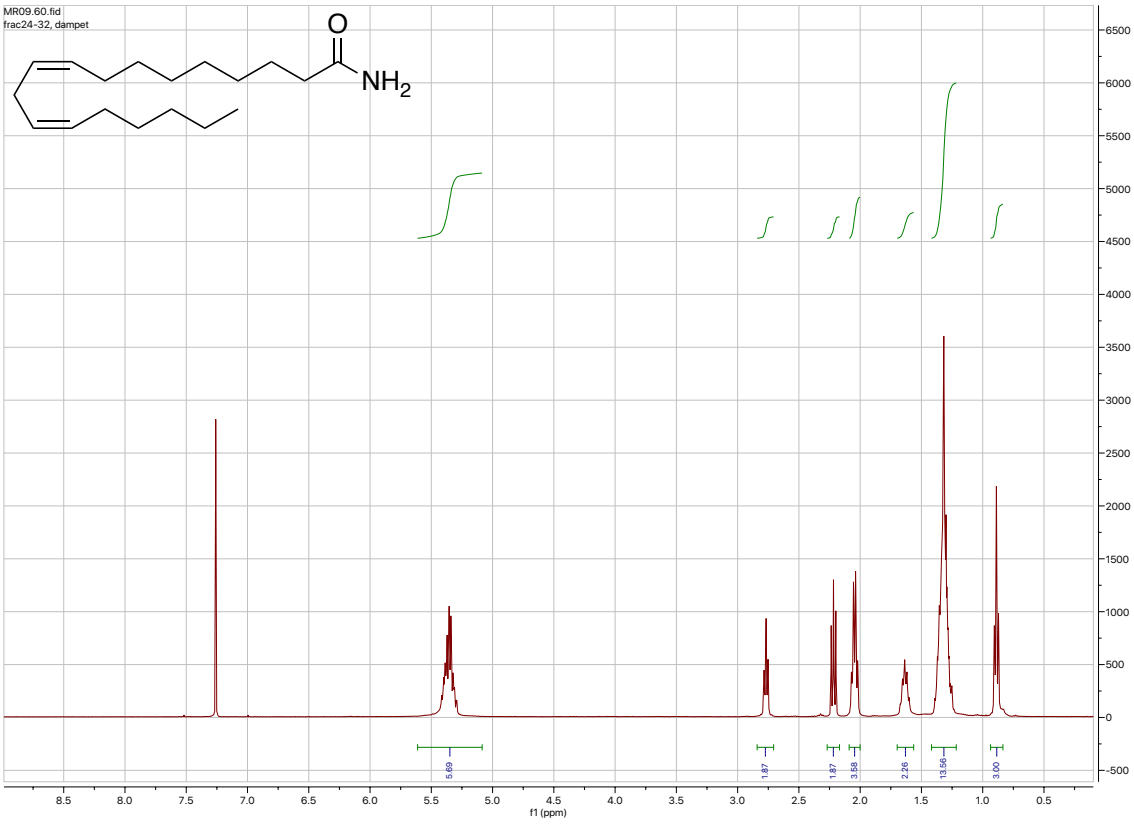


Figure 6-83 ^{1}H NMR spectrum of **24**.

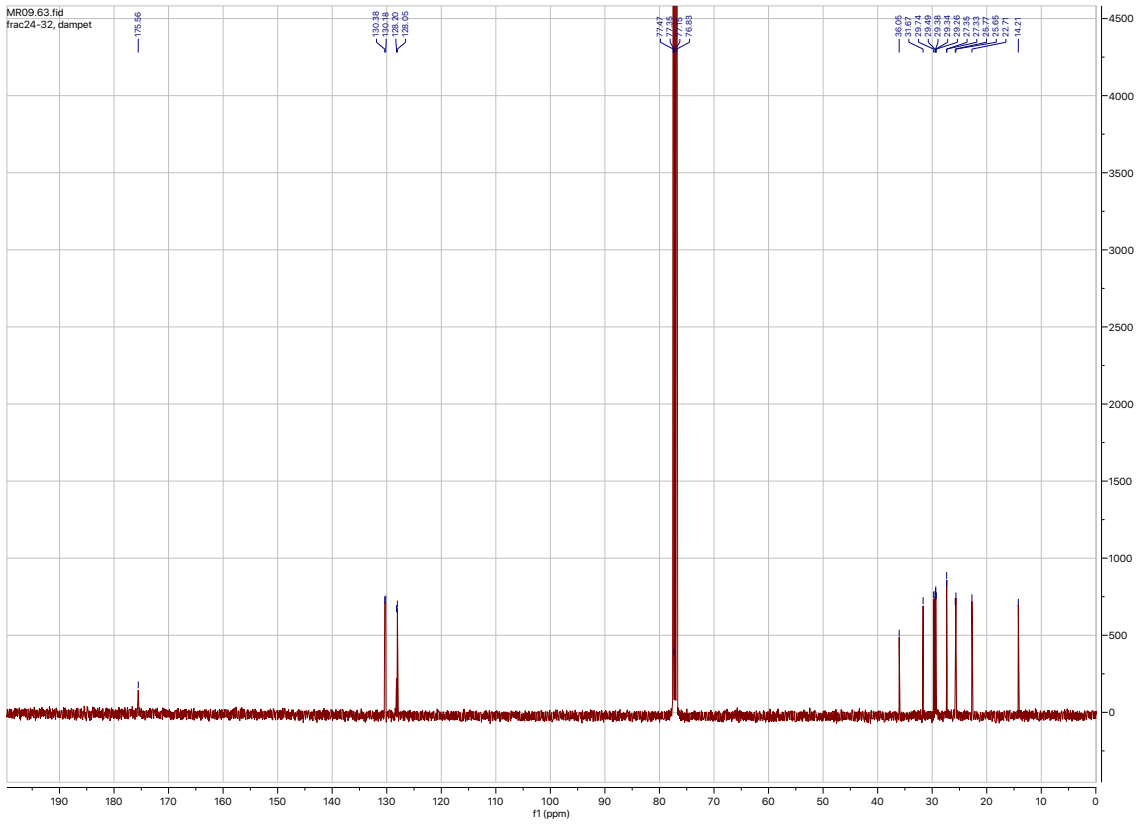


Figure 6-84 13C NMR spectrum of **24**.

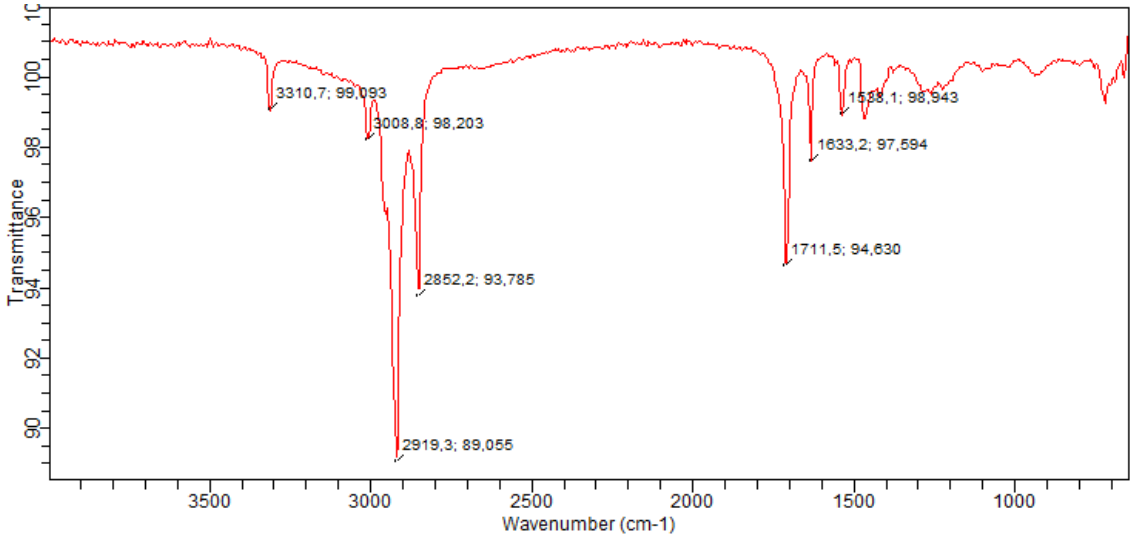
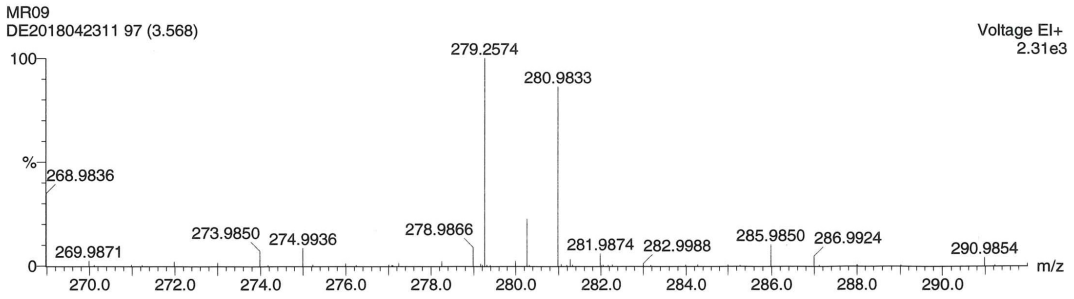


Figure 6-85 IR-spectrum of **24**.

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

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



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
279.2574	279.2562	1.2	4.2	3.0	1	C18 H33 N O

Figure 6-86 HR-MS spectrum of **24**.

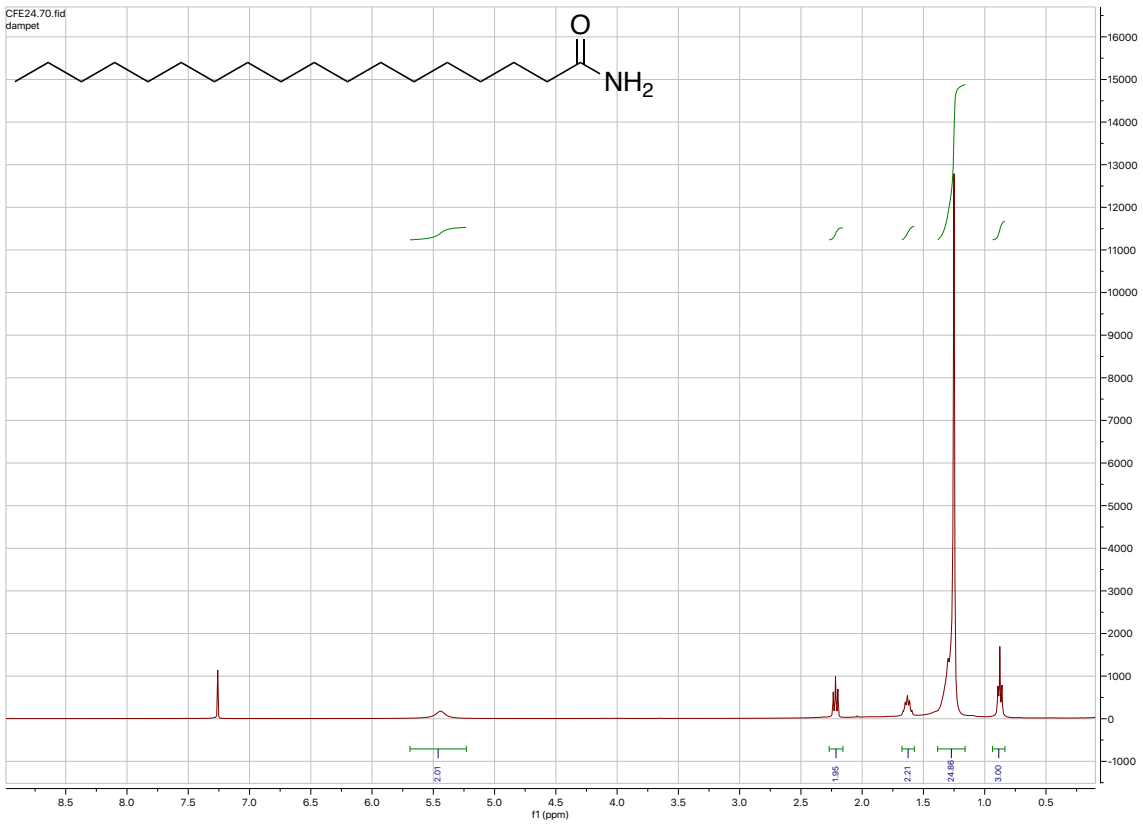


Figure 6-87 ^{1}H NMR spectrum of **41**.

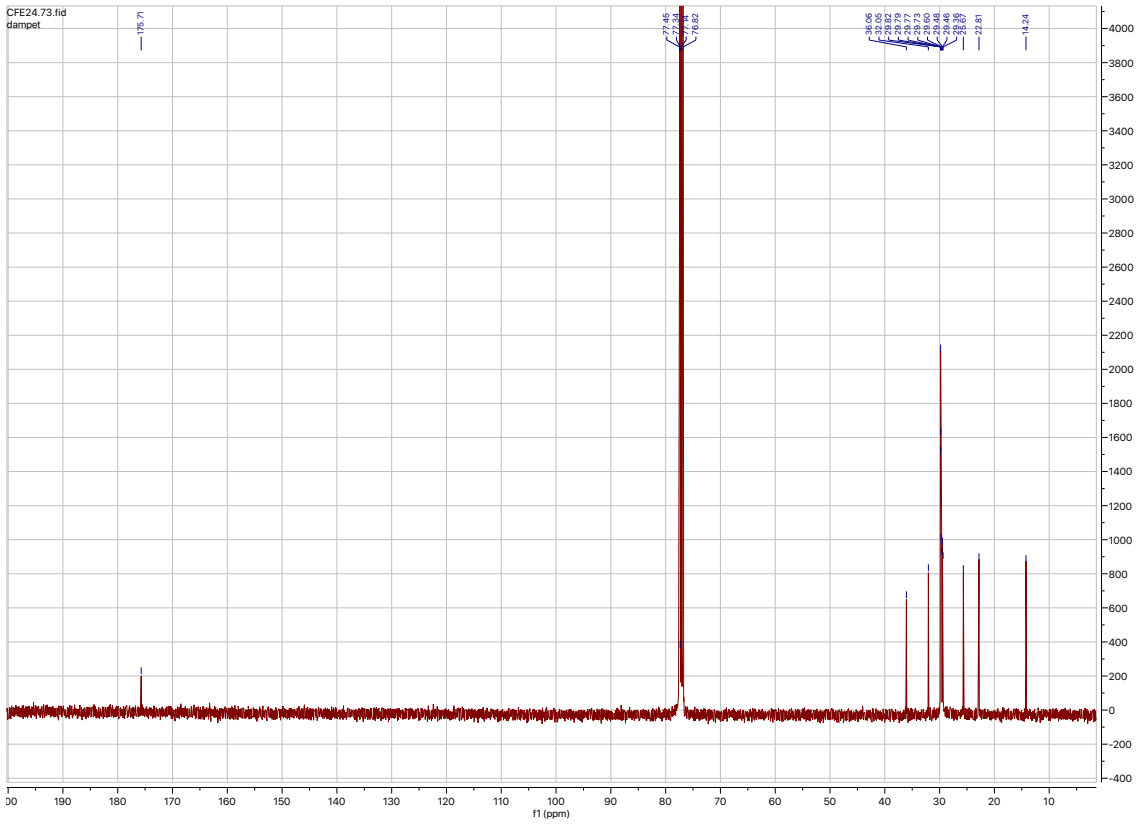


Figure 6-88 ^{13}C NMR spectrum of **41**.

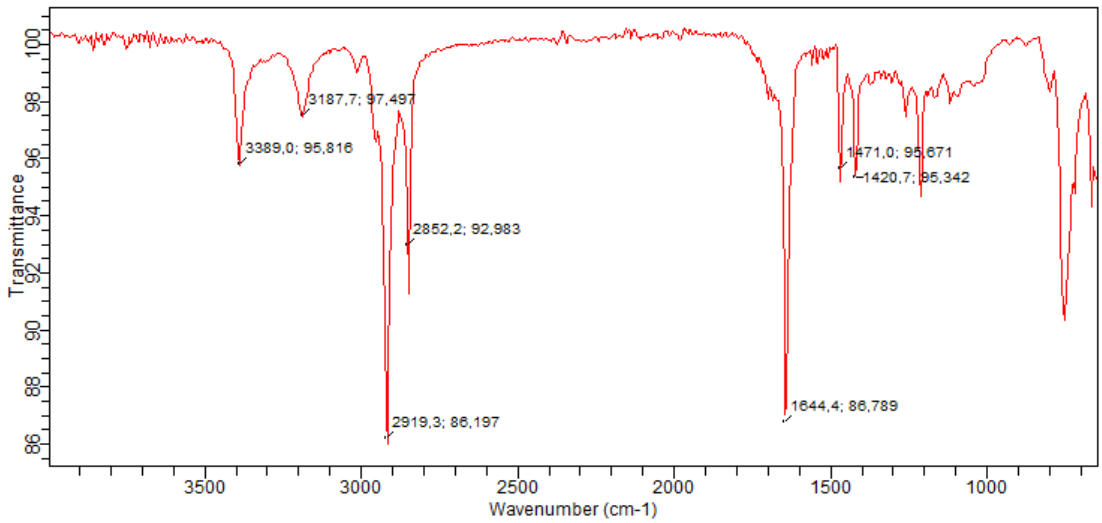
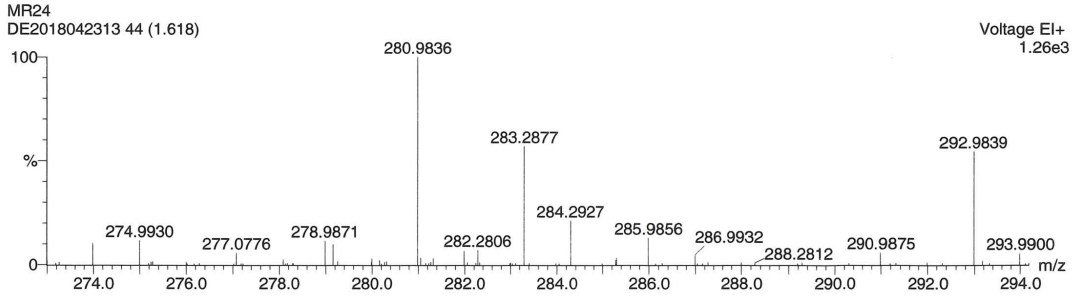


Figure 6-89 IR-spectrum of **41**.

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

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



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
283.2877	283.2875	0.2	0.7	1.0	1	C18 H37 N O

Figure 6-90 HR-MS spectrum of **41**.

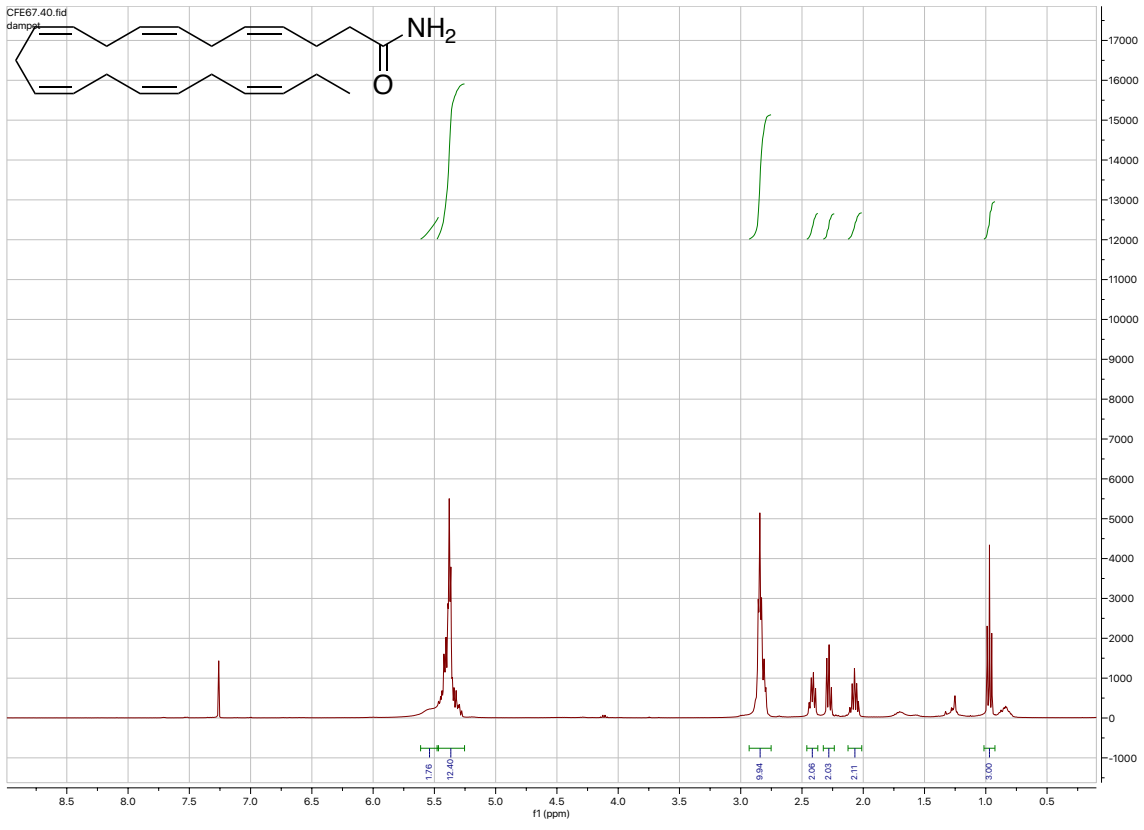


Figure 6-91 1H NMR spectrum of **42**.

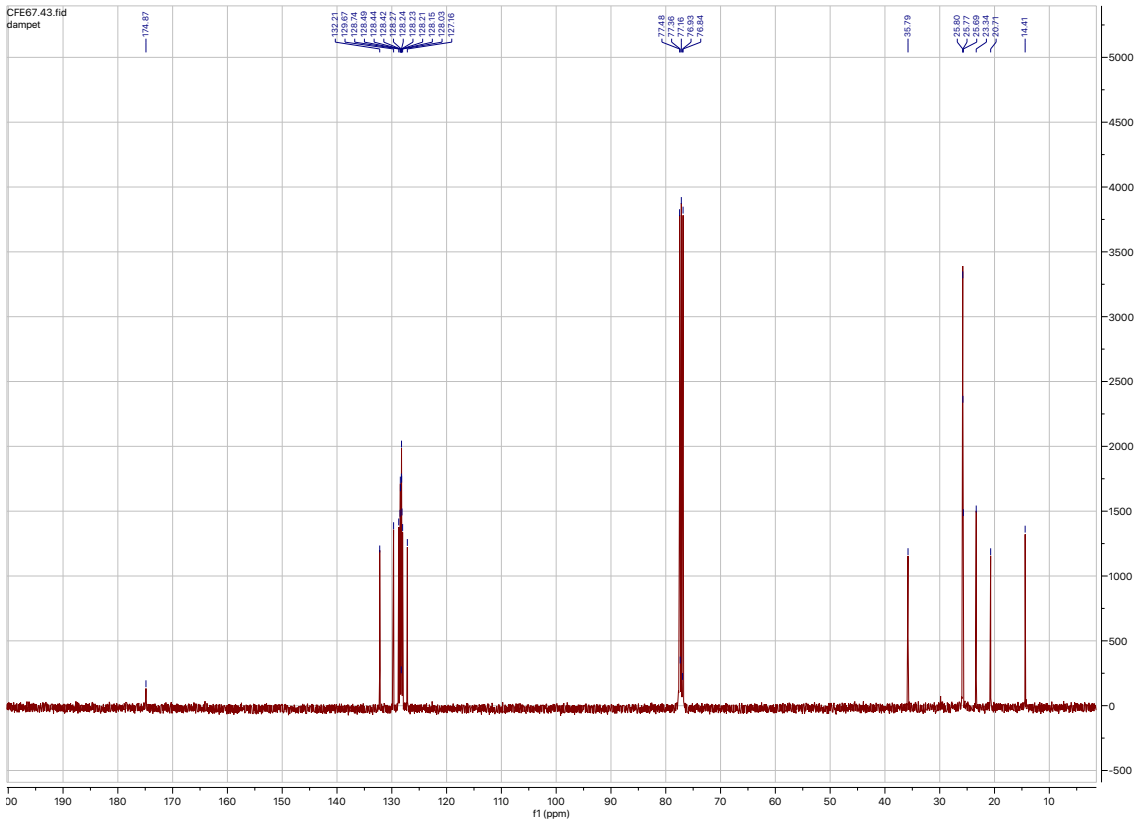


Figure 6-92 13C NMR spectrum of **42**.

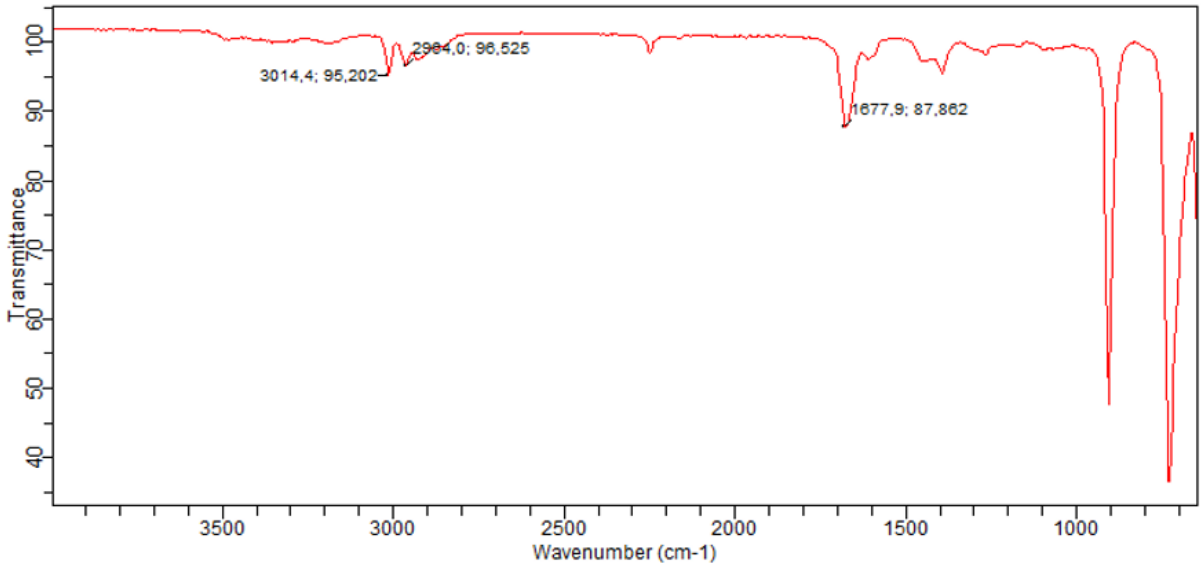
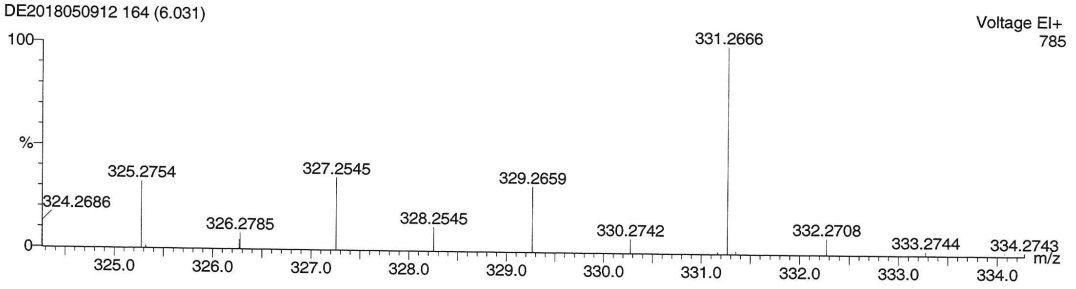


Figure 6-93 IR-spectrum of **42**.

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

Monoisotopic Mass, Odd and Even Electron Ions
147 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)

CFE12
DE2018050912 164 (6.031)



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
327.2545	327.2549	-0.4	-1.1	7.5	3	C20 H31 N4
	327.2535	1.0	2.9	2.5	2	C19 H35 O4
	327.2562	-1.7	-5.2	7.0	1	C22 H33 N O
	327.2522	2.3	7.1	3.0	4	C17 H33 N3 O3

Figure 6-94 HR-MS spectrum of **42**.

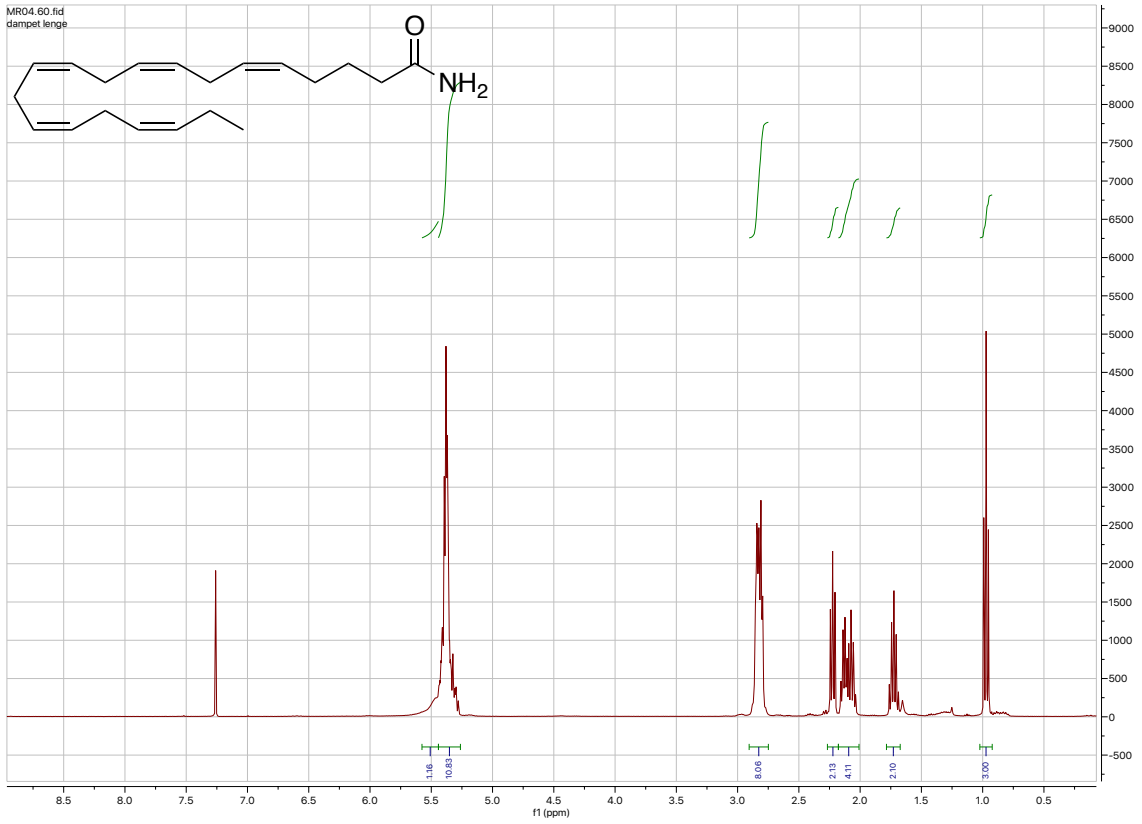


Figure 6-95 ^{1}H NMR spectrum of **43**.

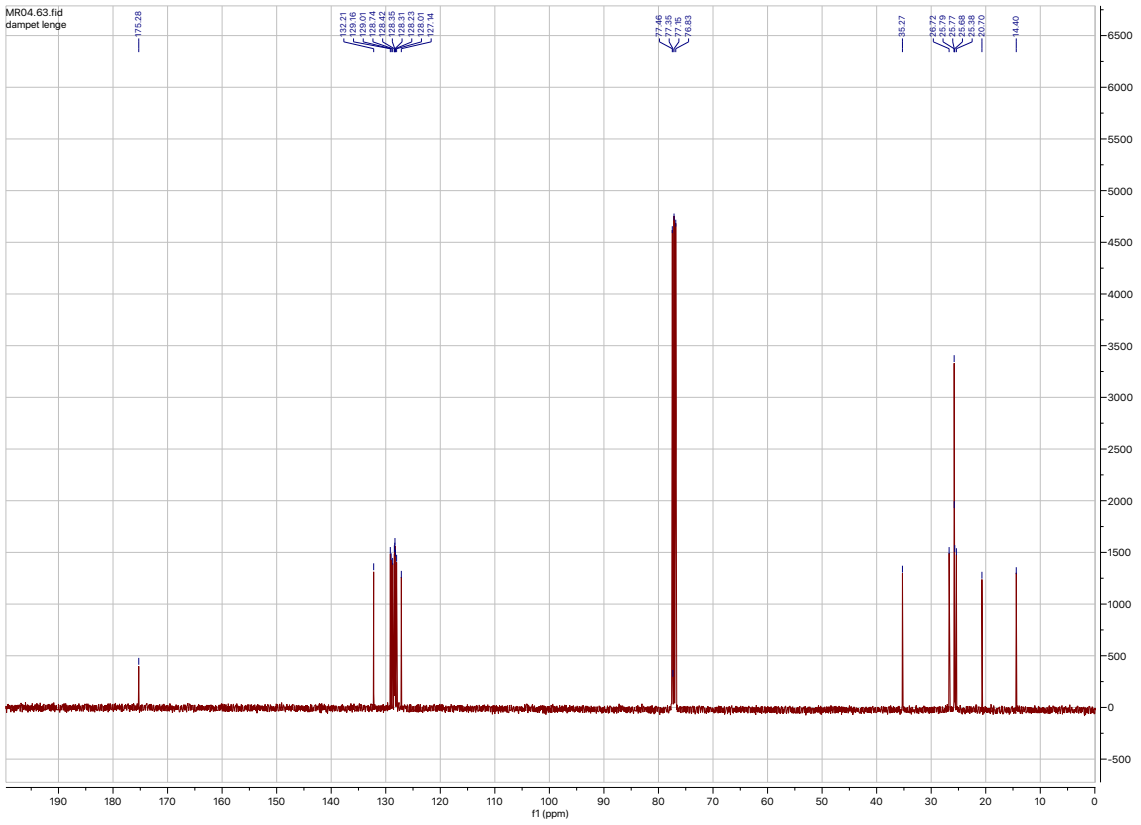


Figure 6-96 ^{13}C NMR spectrum of **43**.

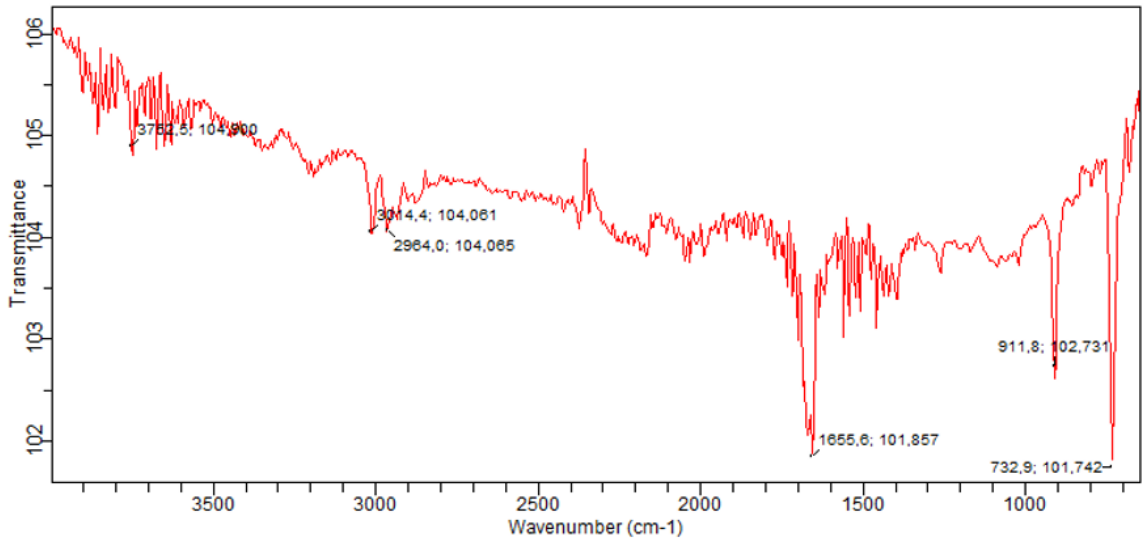
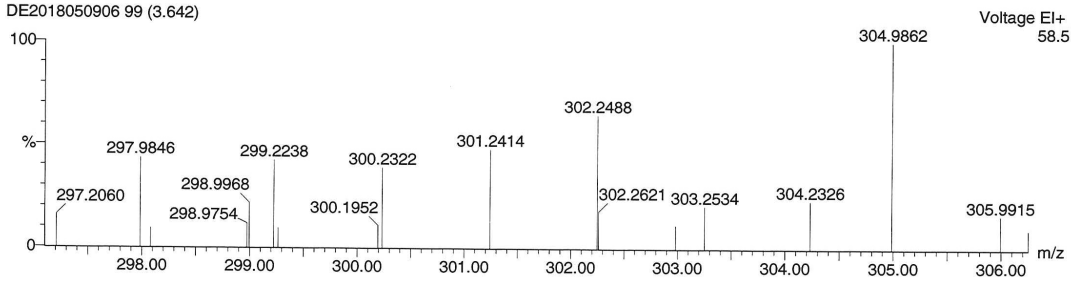


Figure 6-97 IR-spectrum of **43**.

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

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

EPA amid
DE2018050906 99 (3.642)



Minimum:				-1.5		
Maximum:		200.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
301.2414	301.2406	0.8	2.8	6.0	1	C20 H31 N O
	301.2392	2.2	7.2	6.5	2	C18 H29 N4

Figure 6-98 HR-MS spectrum of **43**.

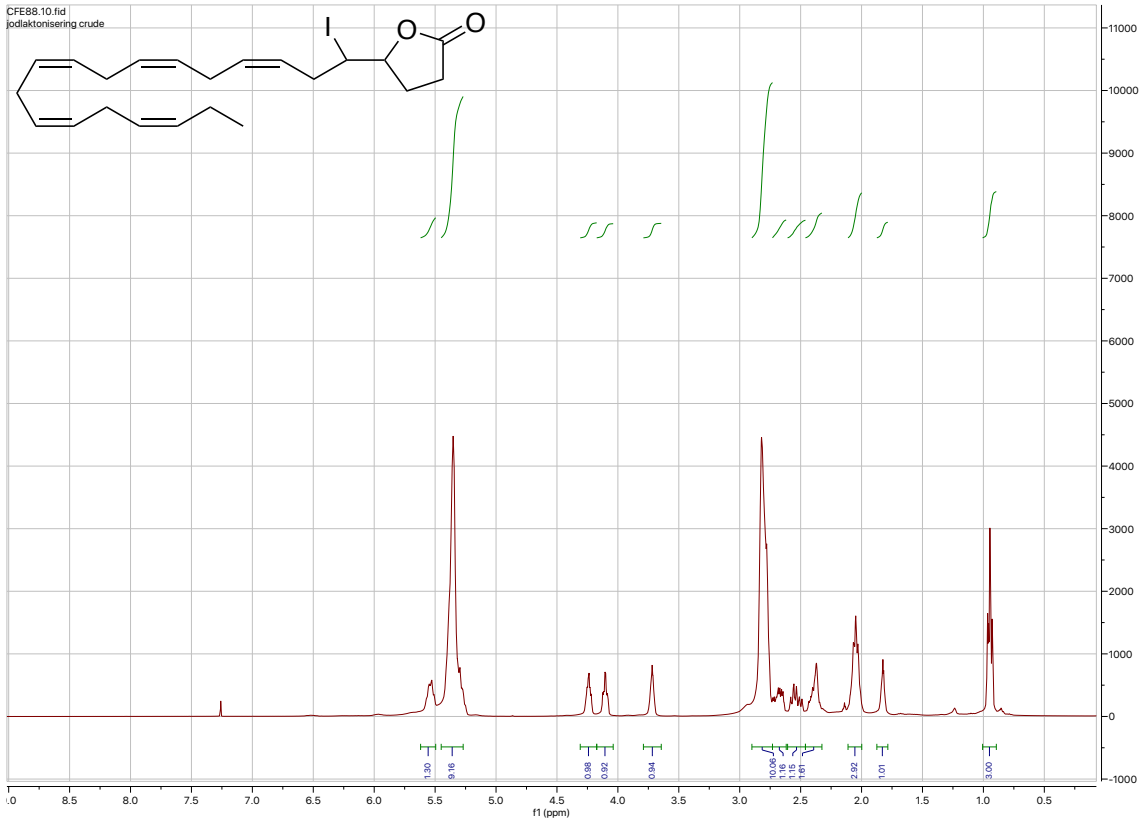


Figure 6-99 ^{1}H NMR spectrum of **27**.

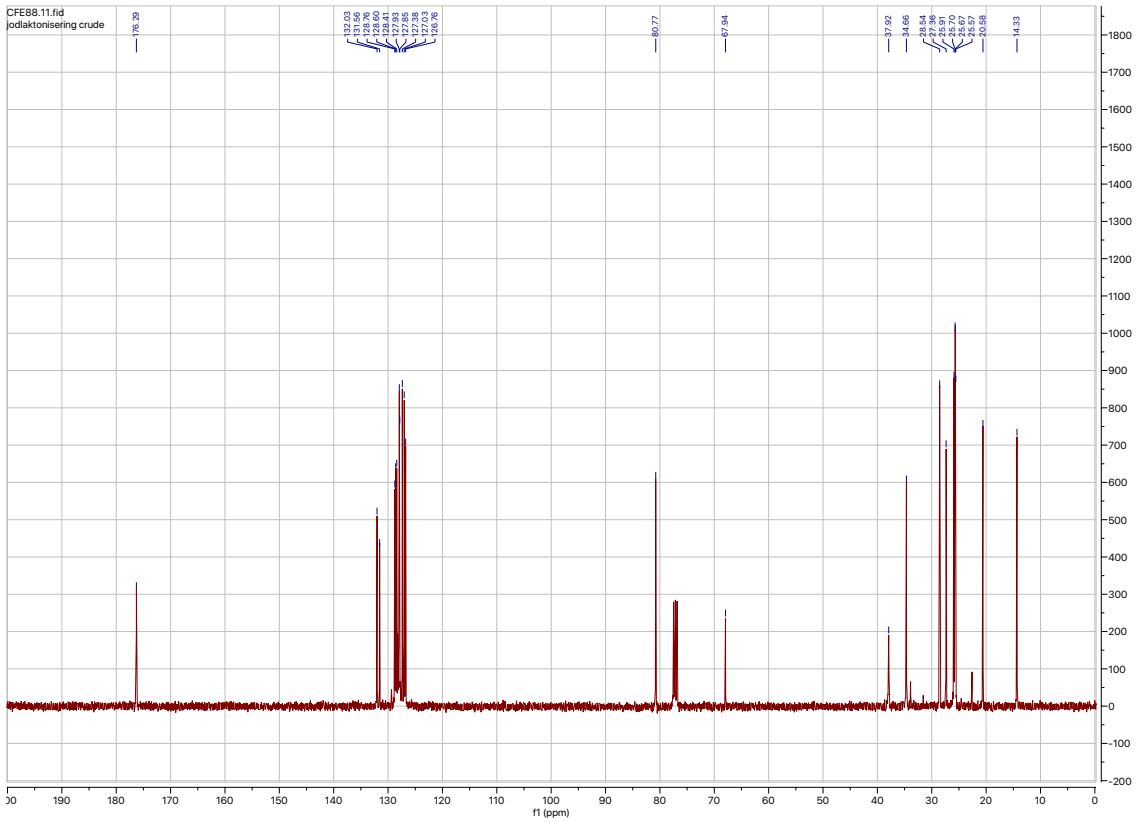


Figure 6-100 ^{13}C NMR spectrum of **27**.

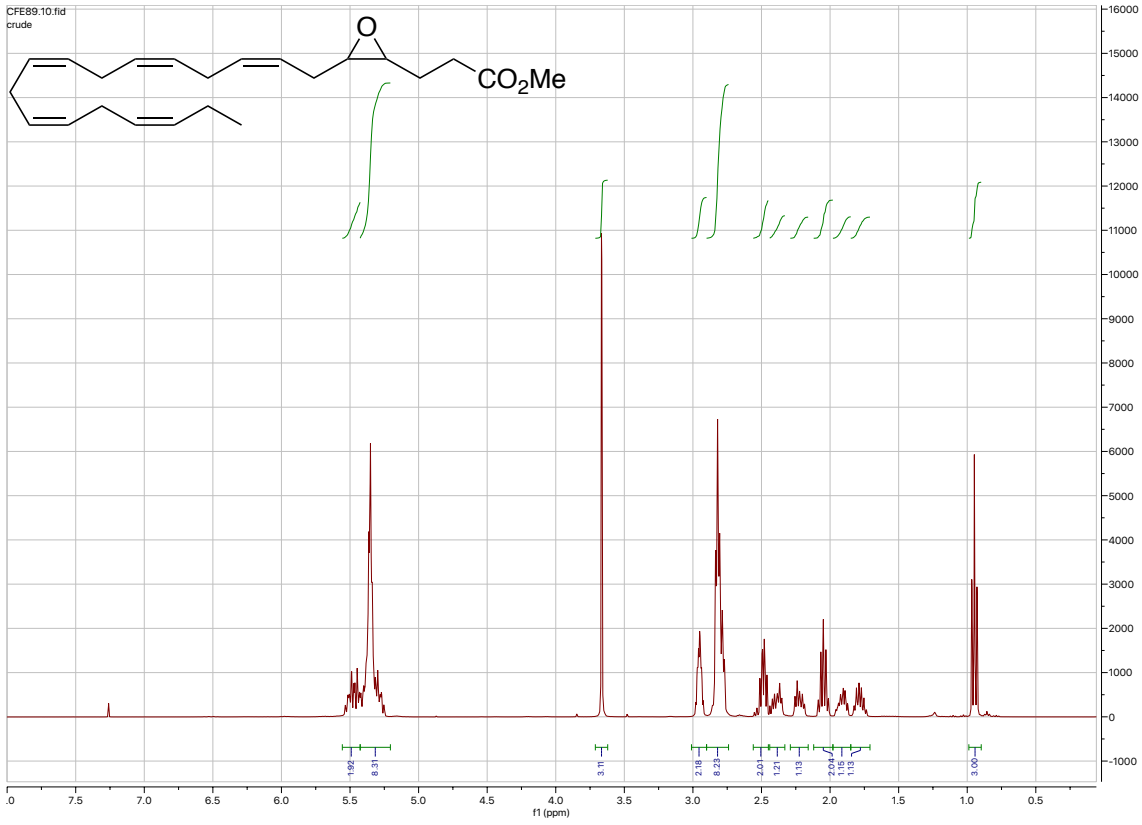


Figure 6-101 ^{1}H NMR spectrum of **28**.

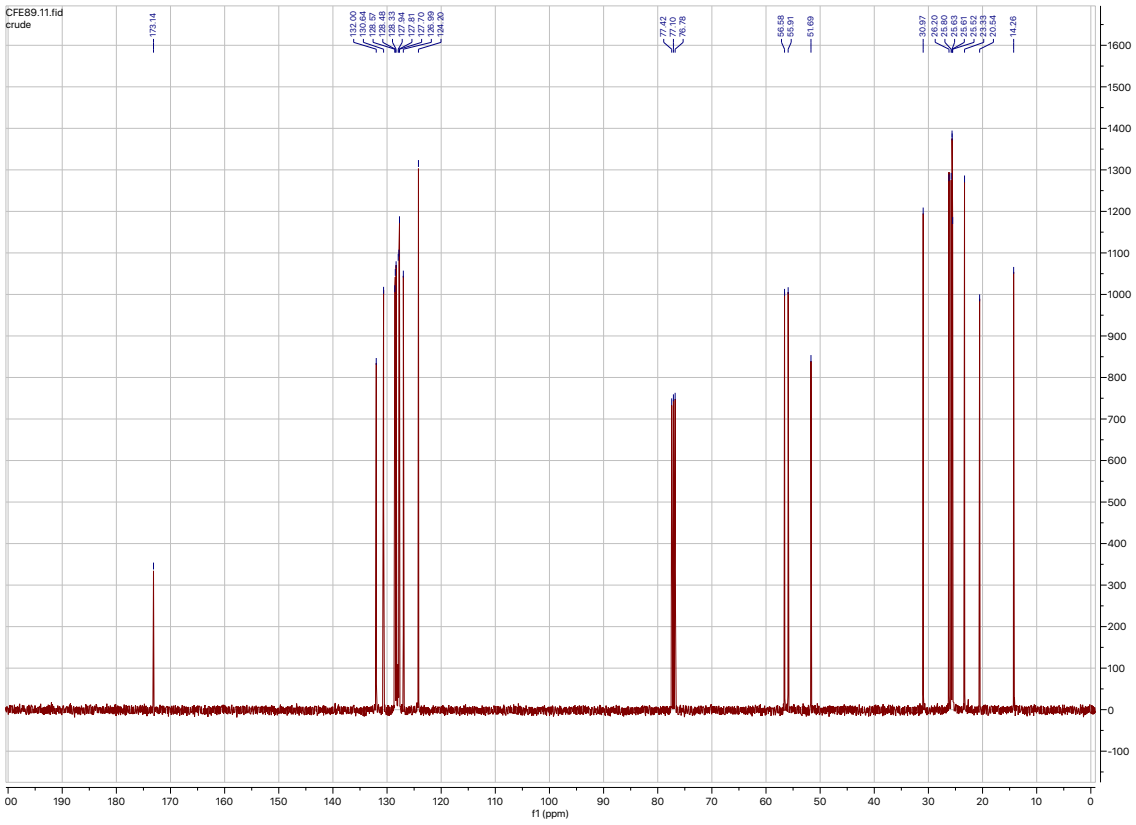


Figure 6-102 ^{13}C NMR spectrum of **28**.

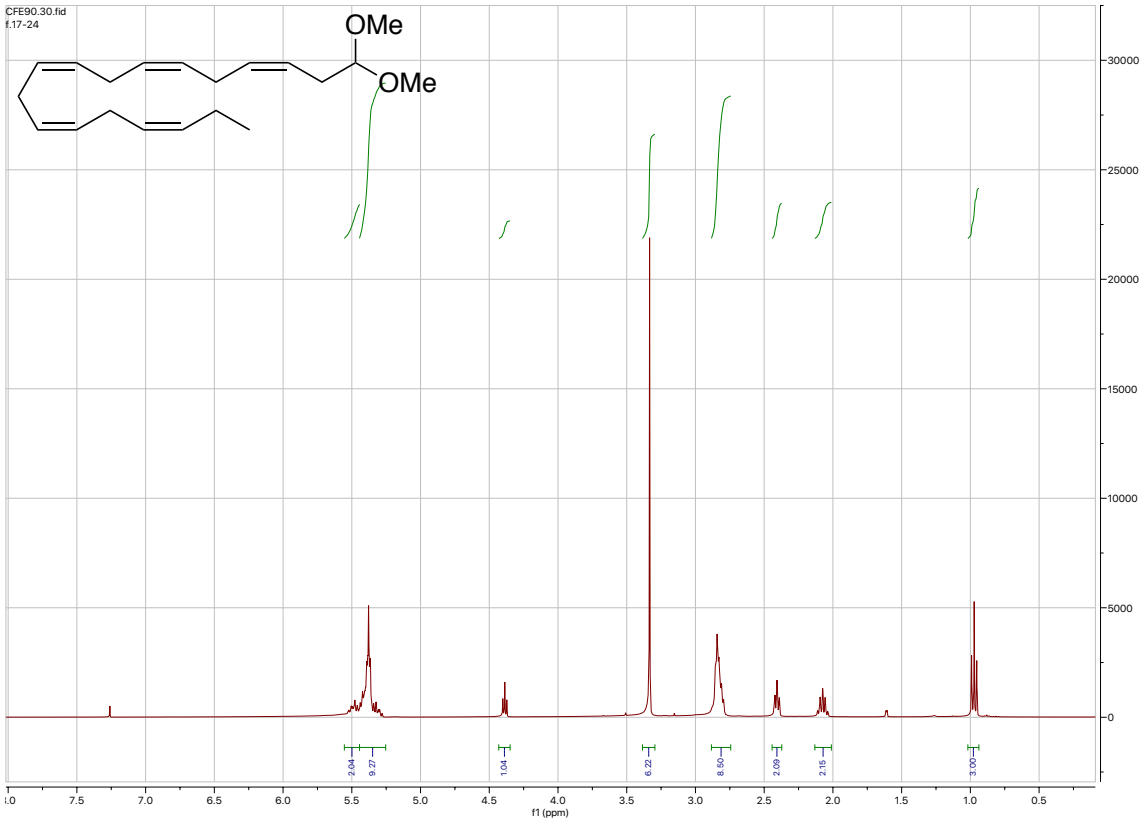


Figure 6-103 ^{1}H NMR spectrum of **29a**.

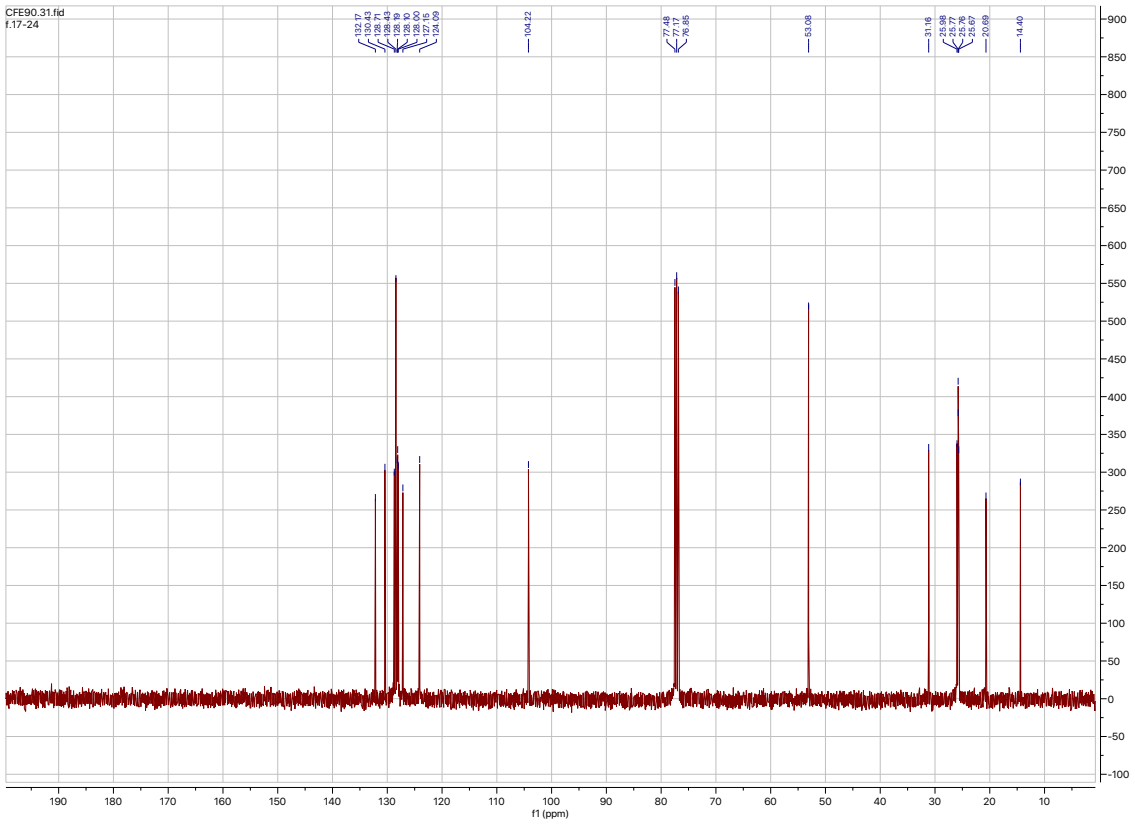


Figure 6-104 ^{13}C NMR spectrum of **29a**.

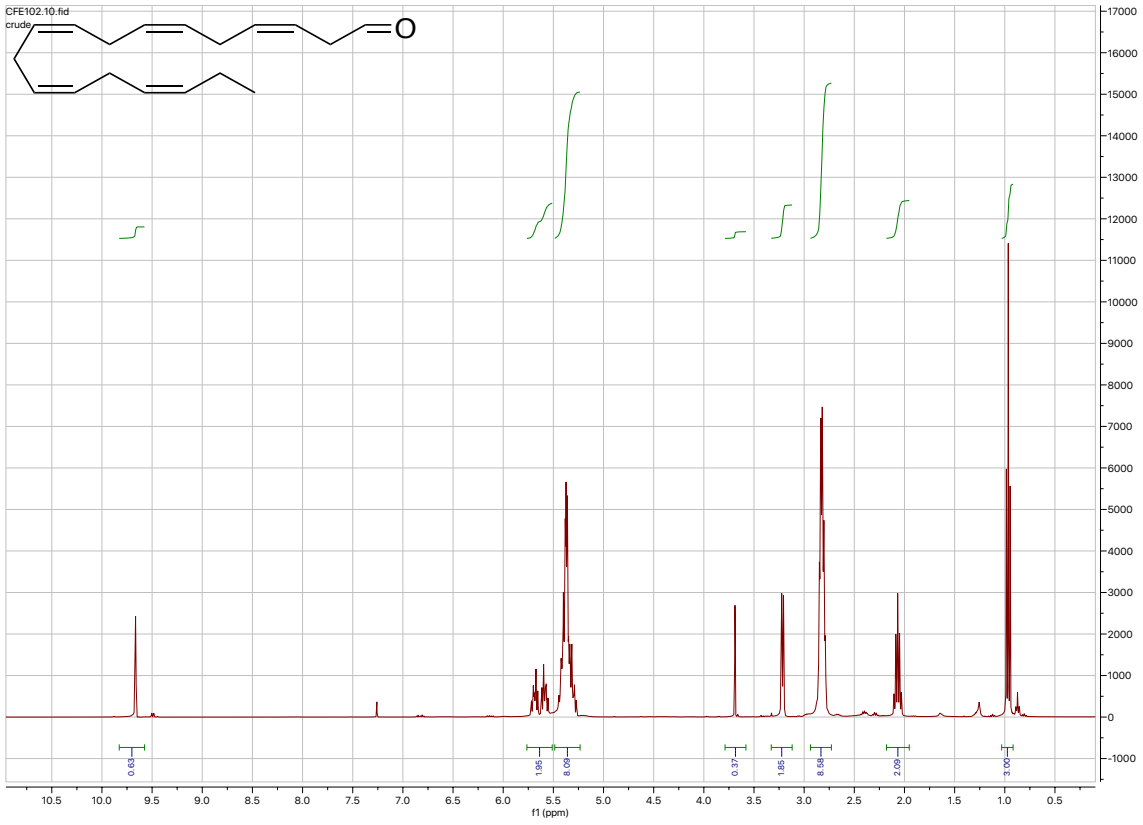


Figure 6-105 ^{1}H NMR spectrum of **29**.

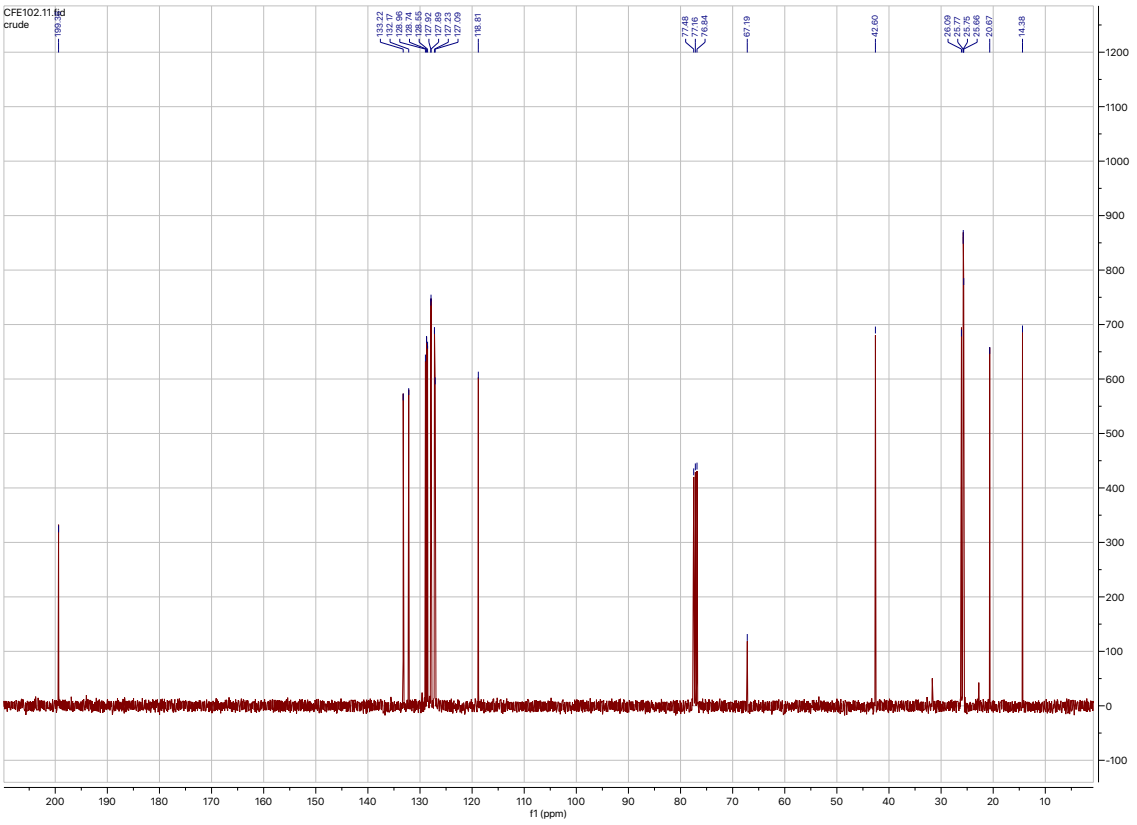


Figure 6-106 ^{13}C NMR spectrum of **29**.

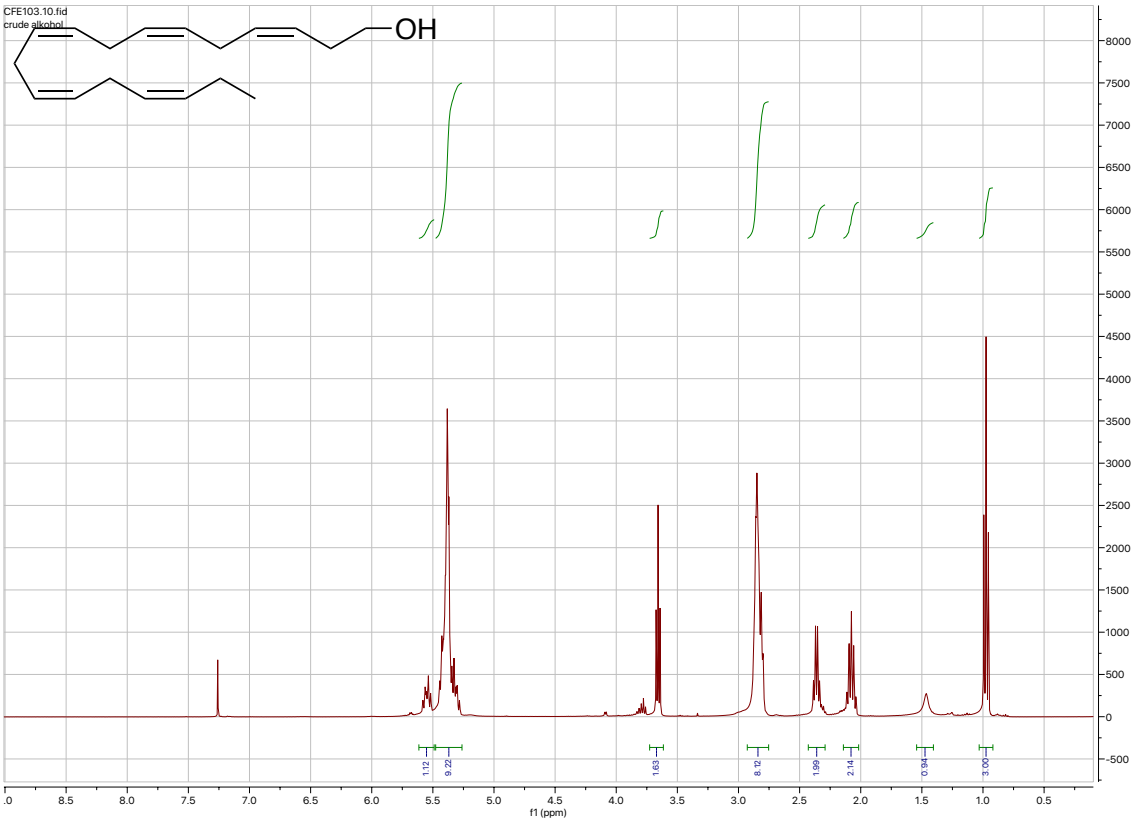


Figure 6-107 1H NMR spectrum of **44**.

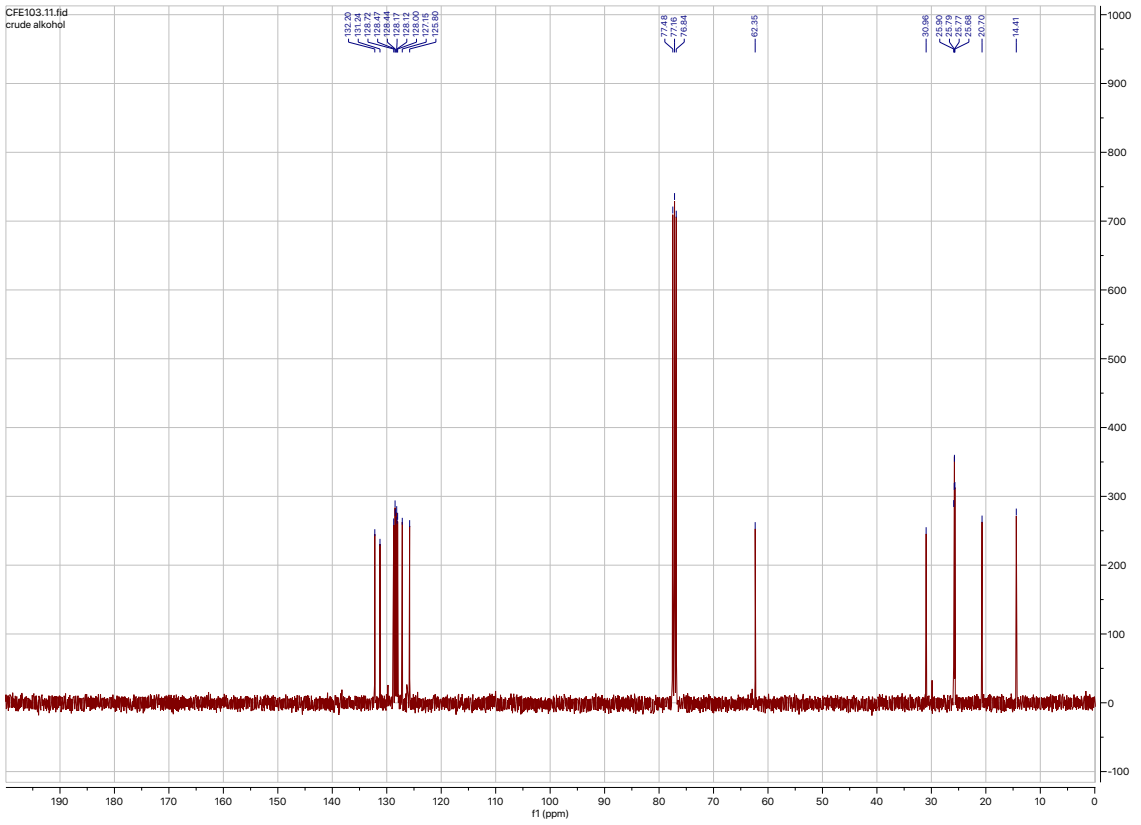


Figure 6-108 13C NMR spectrum of **44**.

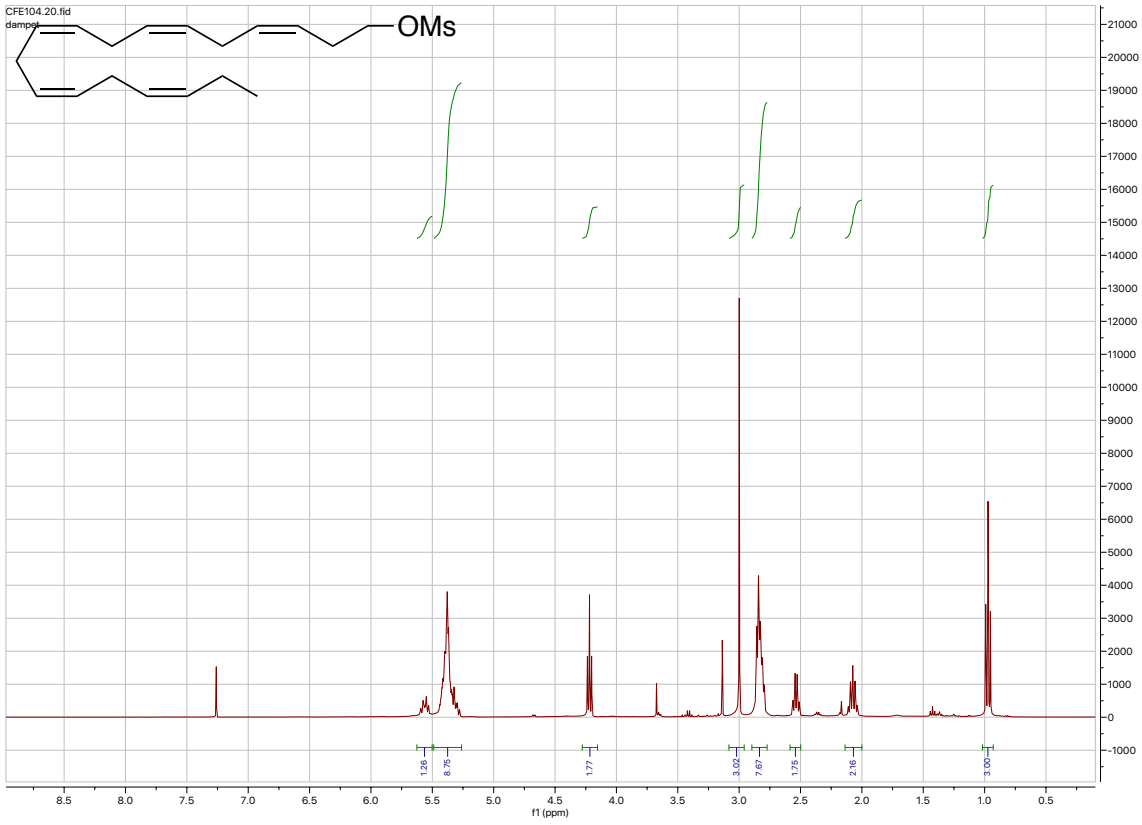


Figure 6-109 ^{1}H NMR spectrum of **45**.

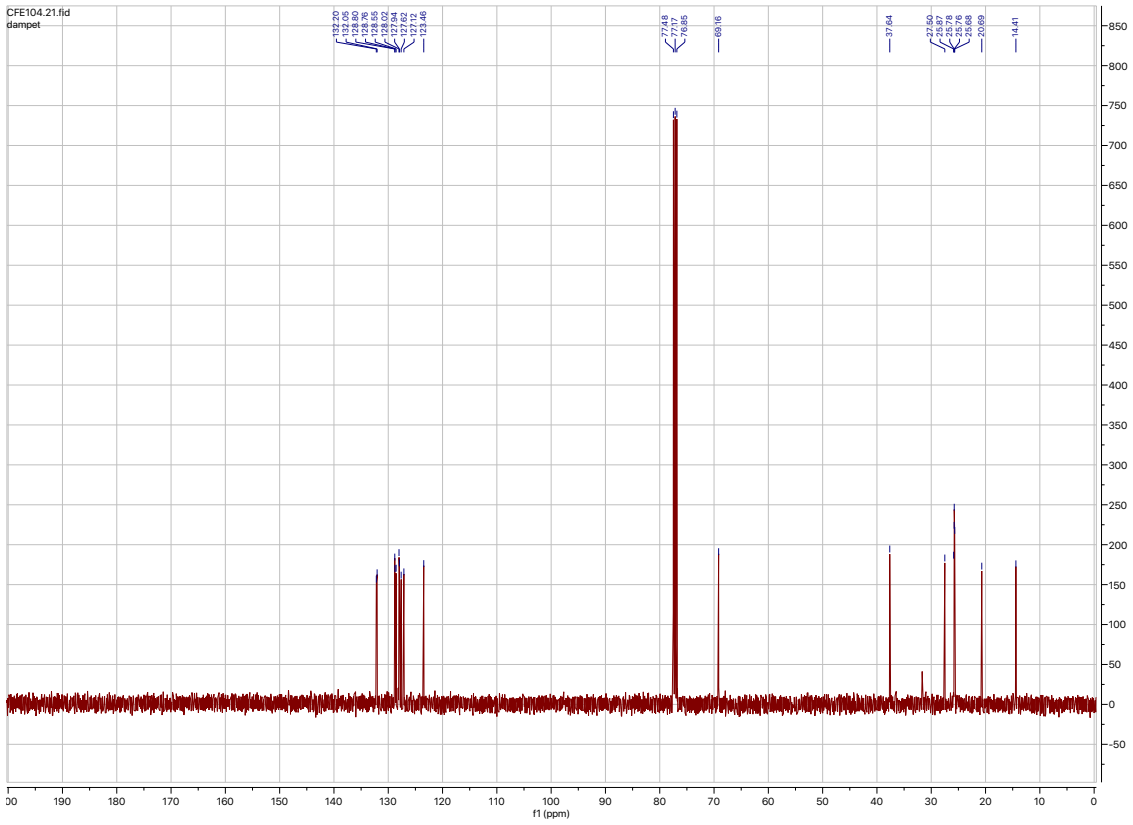


Figure 6-110 13C NMR spectrum of **45**.

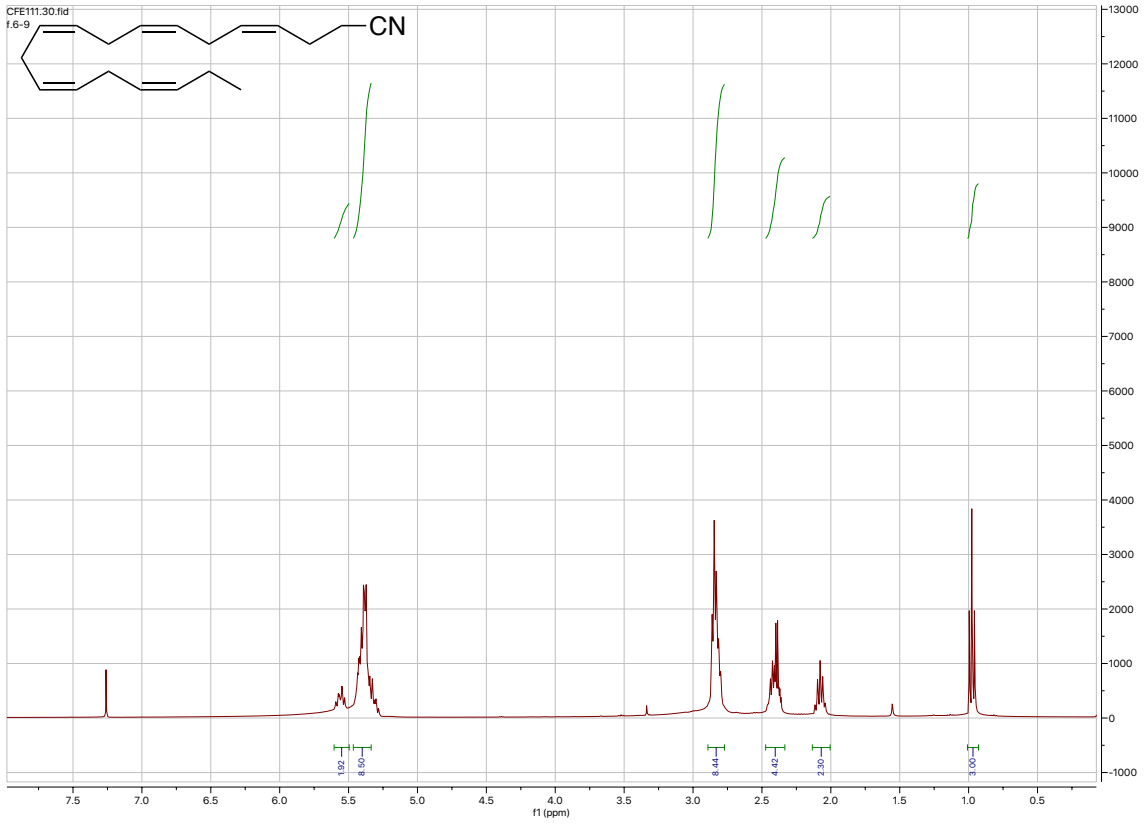


Figure 6-111 ^{1}H NMR spectrum of **46**.

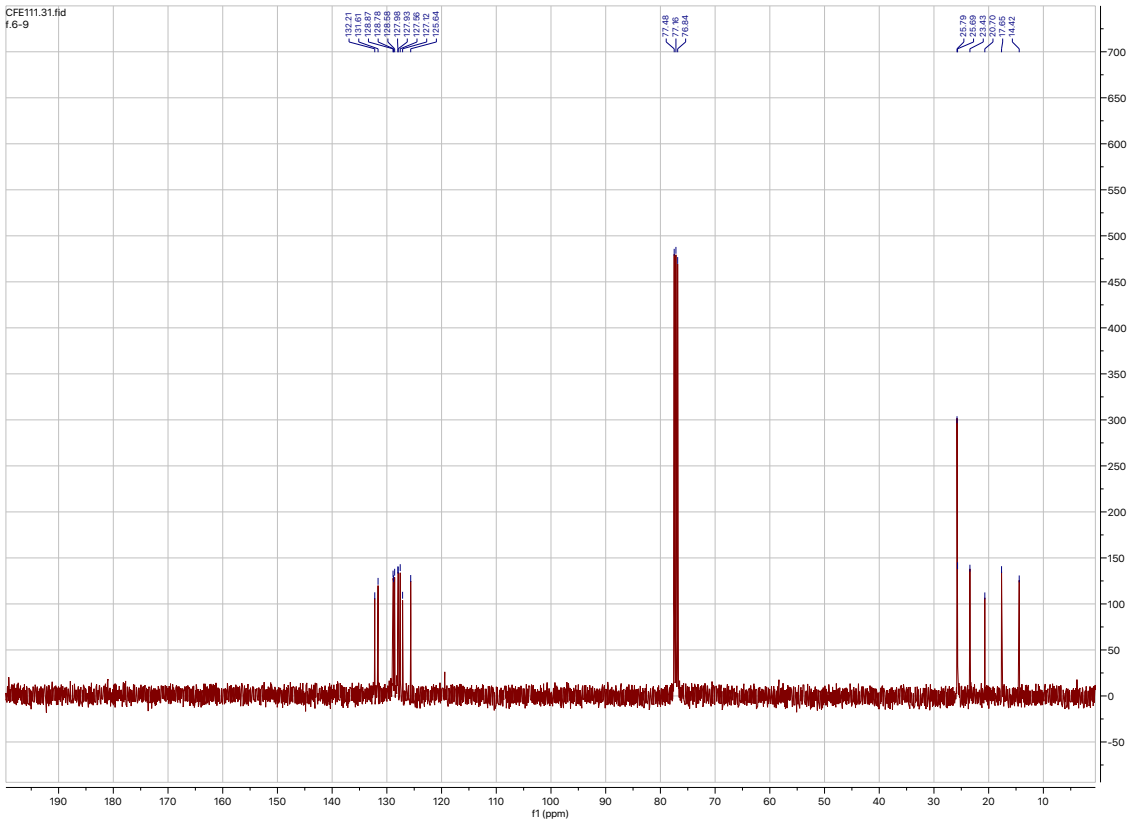


Figure 6-112 ^{13}C NMR spectrum of **46**.

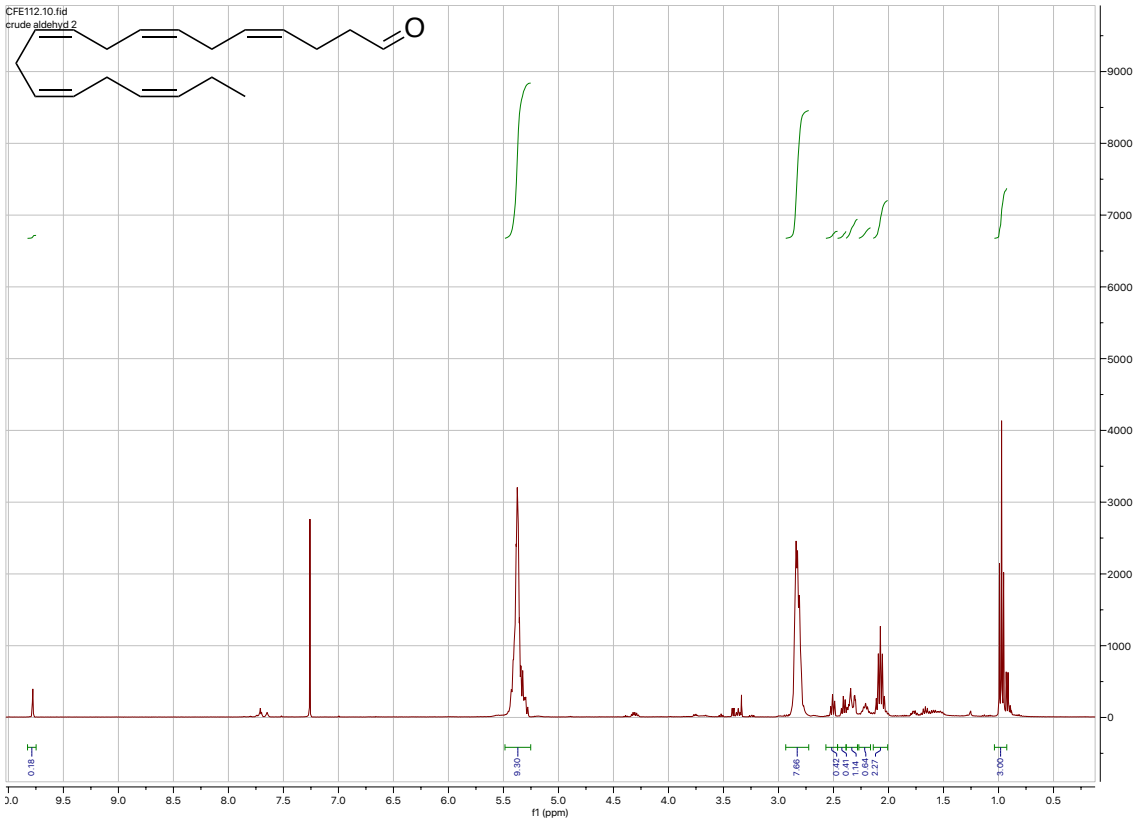


Figure 6-113 ^{1}H NMR spectrum of **47**.

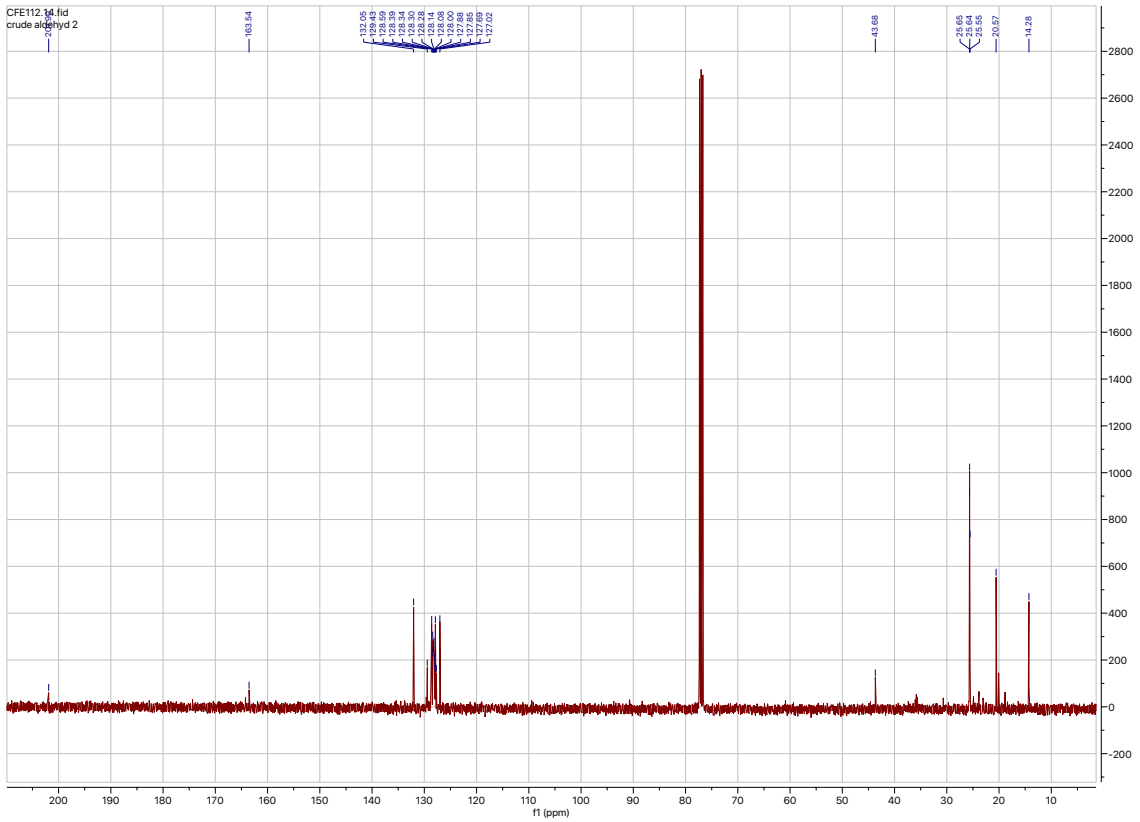


Figure 6-114 ^{13}C NMR spectrum of **47**.

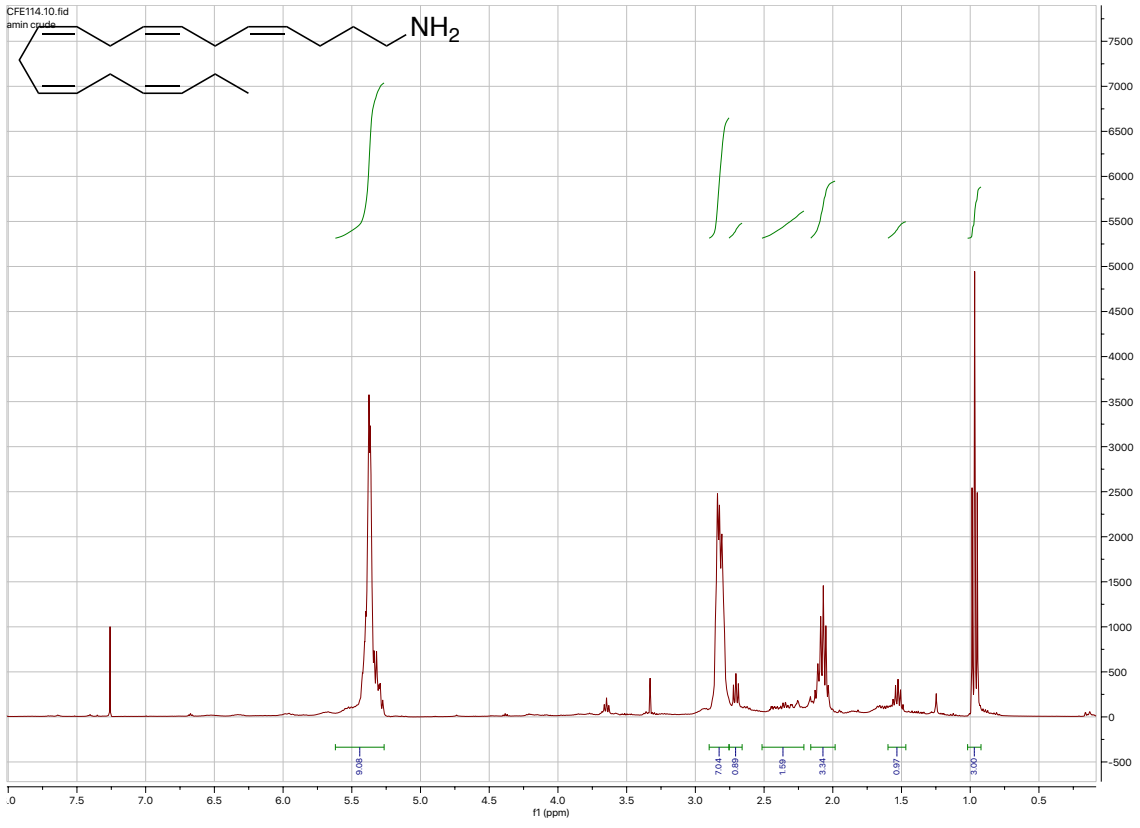


Figure 6-115 ^{1}H NMR spectrum of **48**.

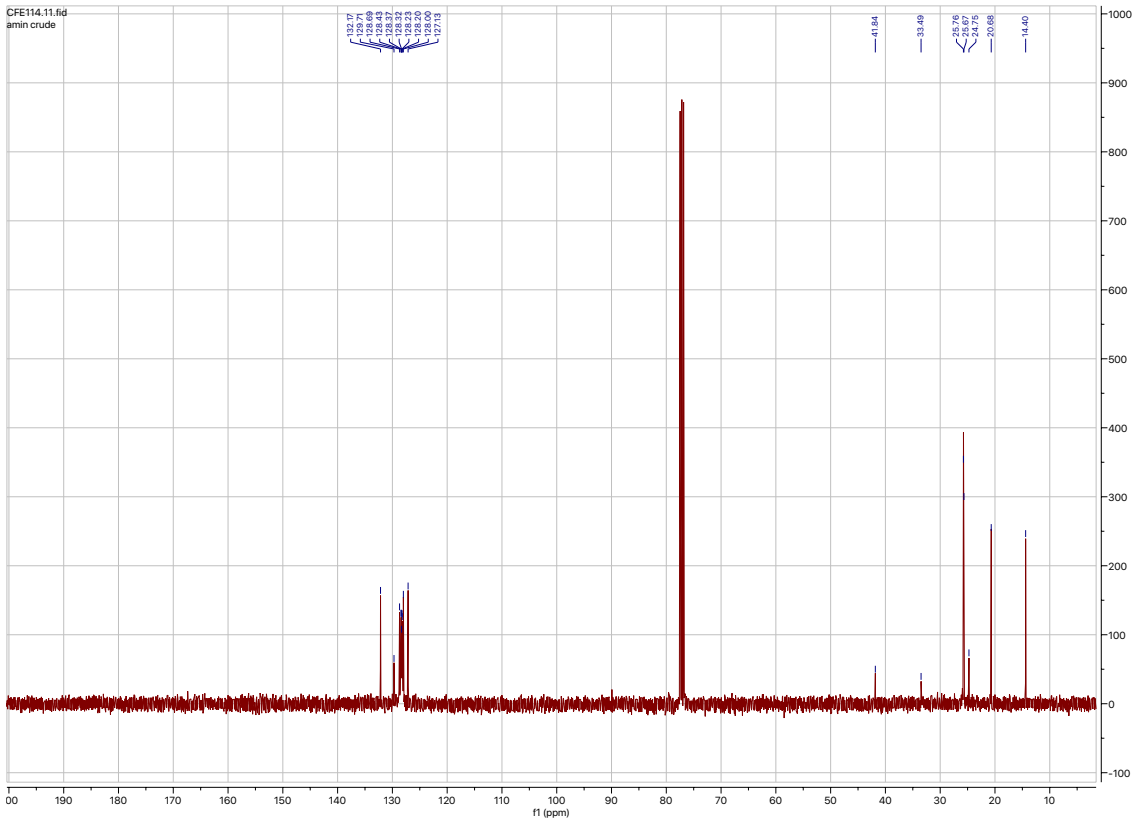


Figure 6-116 ^{13}C NMR spectrum **48**.

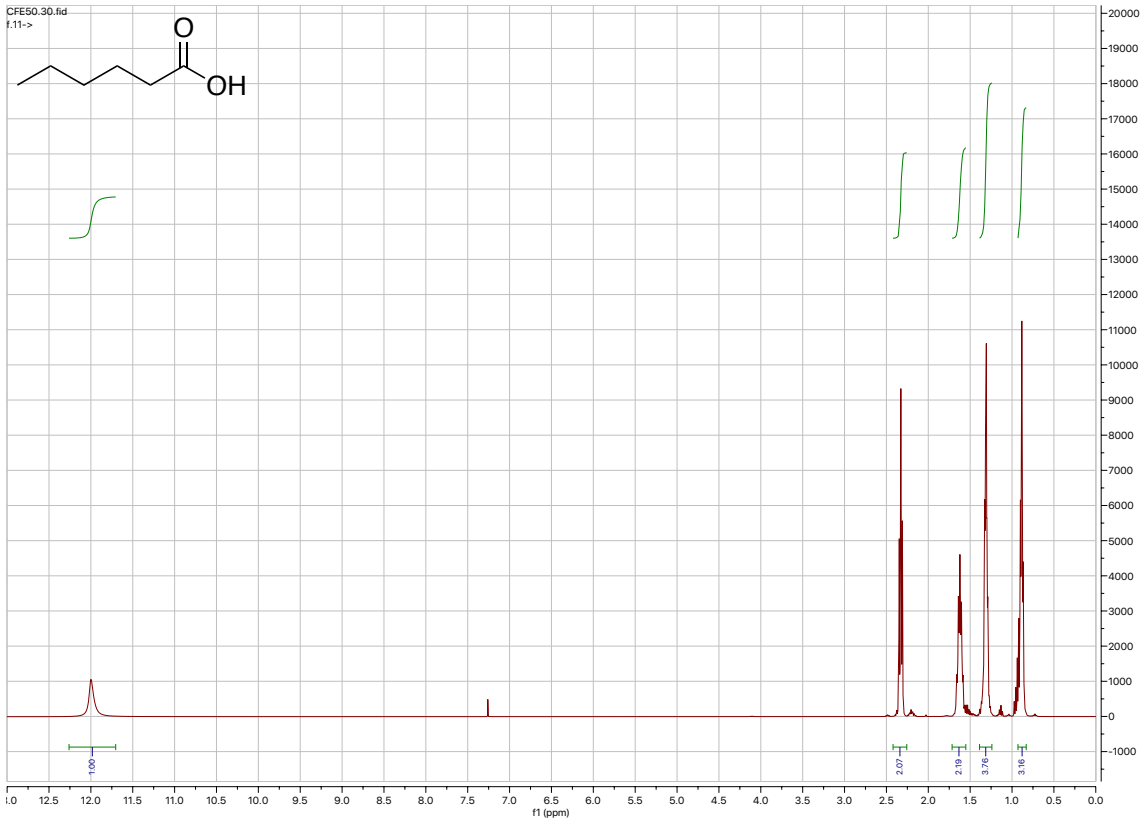


Figure 6-117 ^{1}H NMR spectrum of **54**.

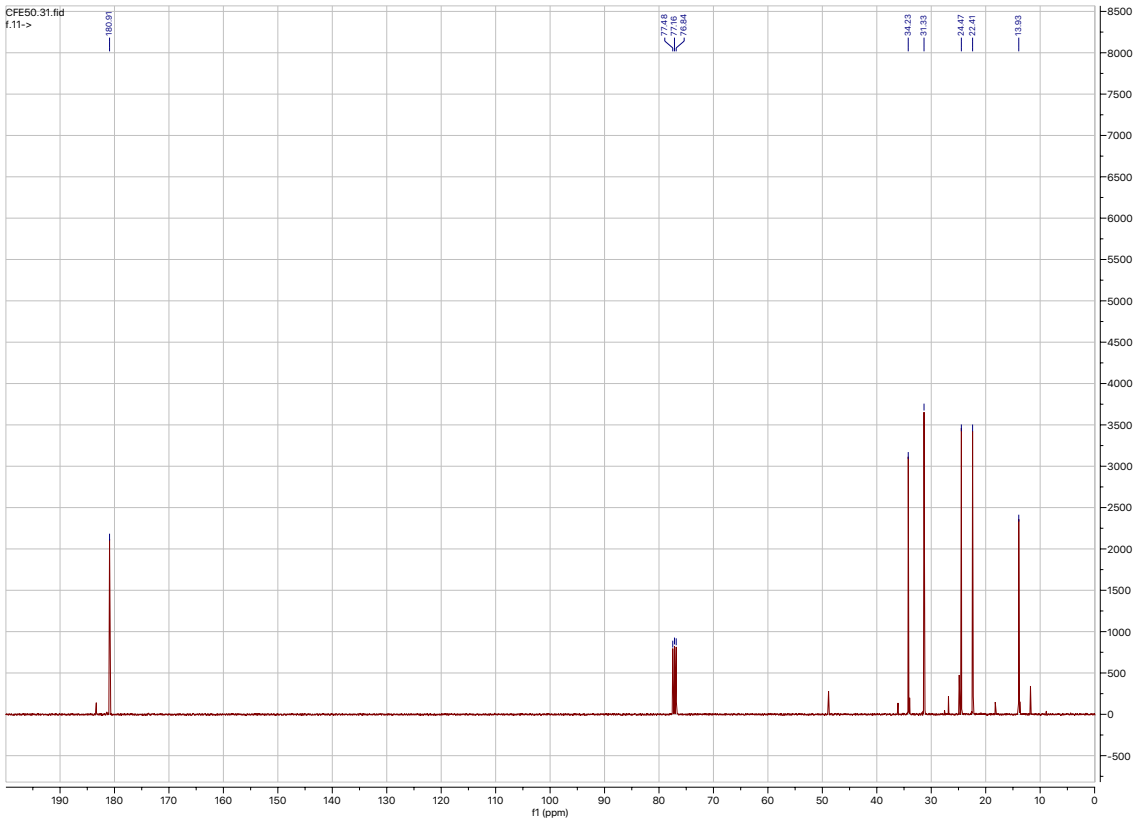


Figure 6-118 ^{13}C NMR spectrum of **54**.

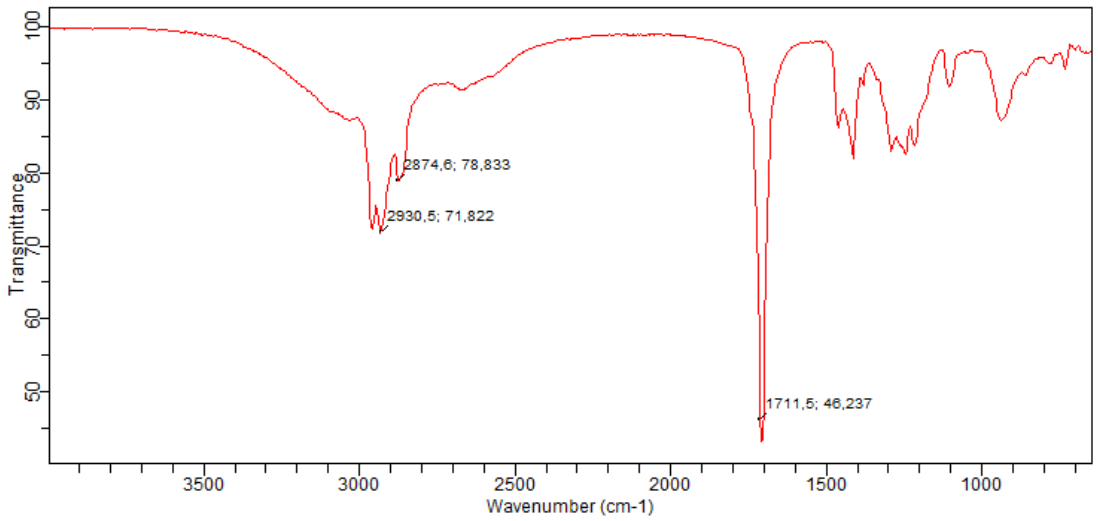


Figure 6-119 IR-spectrum of **54**.

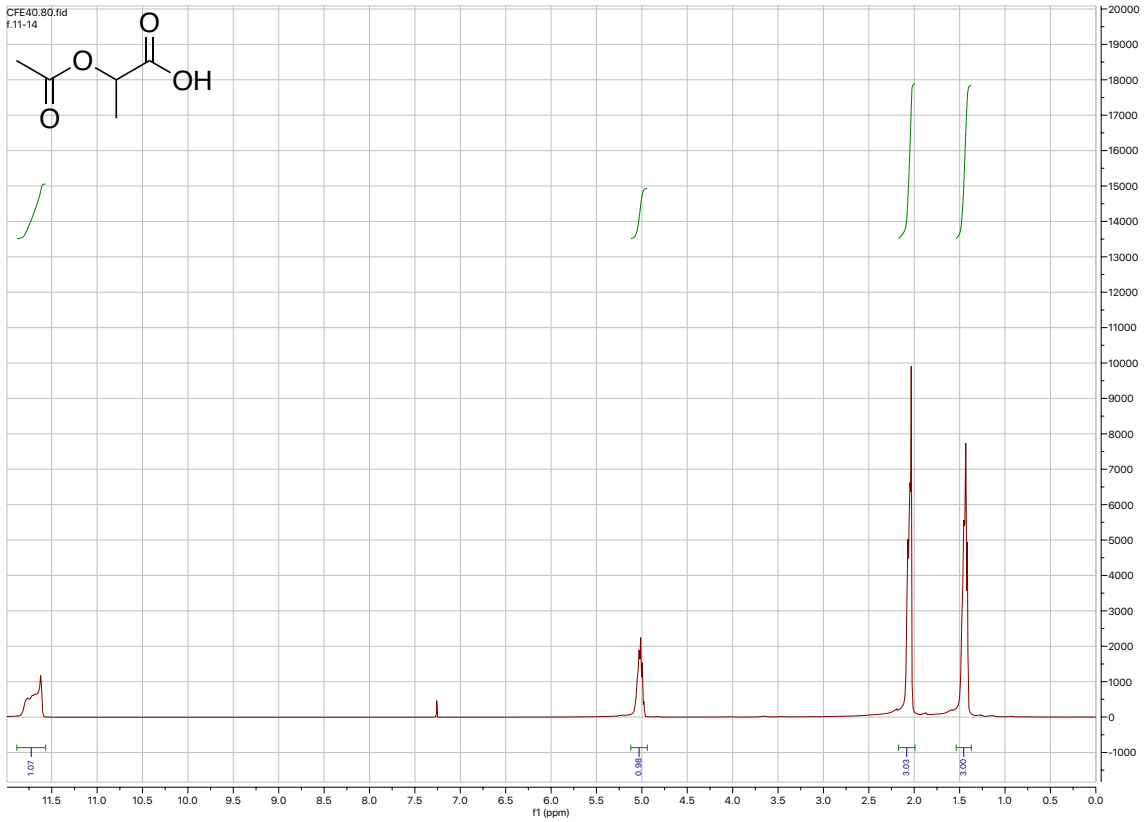


Figure 6-120 ^{1}H NMR spectrum of **55**.

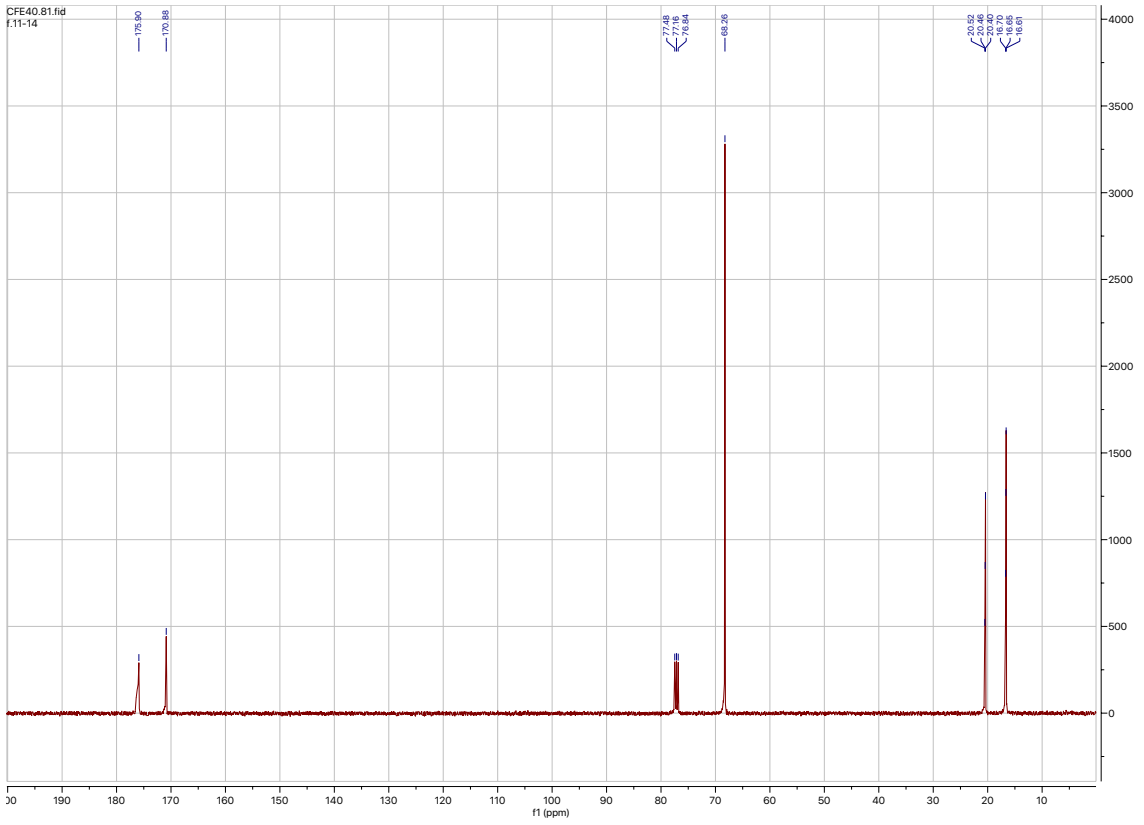


Figure 6-121 ^{13}C NMR spectrum of **55**.

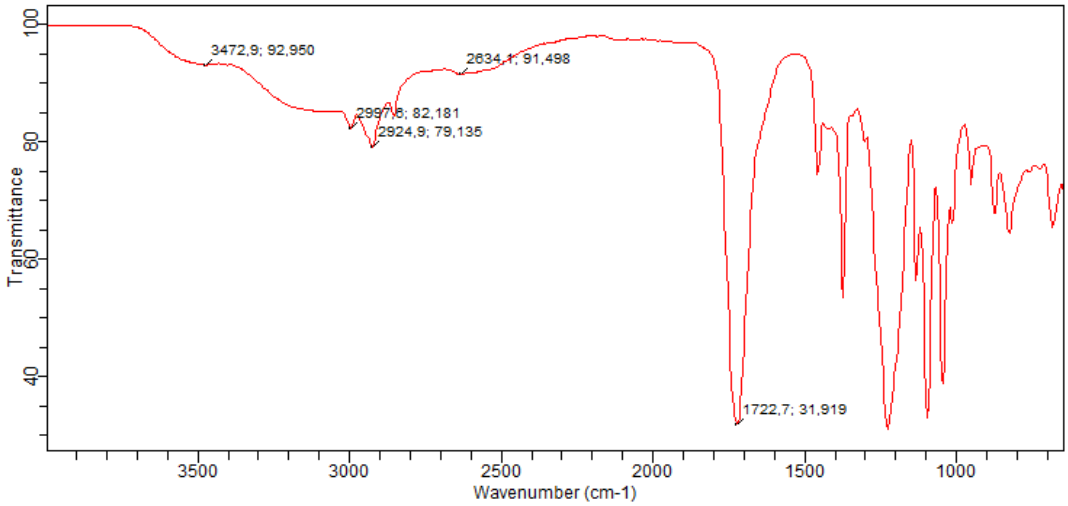


Figure 6-122 IR-spectrum of **55**.

Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway