Synthesis and applications of homoallyl hydroxy fatty acid derivatives. Novel LCNVAs as TRPV1 and FAAH modulators

Silvia López Chinarro

Universitat de Lleida

TESI DOCTORAL

Synthesis and applications of homoallyl hydroxy fatty acid derivatives. Novel LCNVAs as TRPV1 and FAAH modulators

Silvia López Chinarro

Memòria presentada per optar al grau de Doctor per la Universitat de Lleida
Programa de Doctorat en Ciència i Tecnologia Agrària i Alimentària

Director:
Dr. Ramon Canela

2019

"Defiende tu derecho a pensar, porque incluso

pensar de manera errónea es mejor que no pensar"

Hipatia de Alejandría

AGRADECIMIENTOS

En primer lugar quisiera agradecer de todo corazón a mi director, Dr. Ramon Canela, la oportunidad que me dio para formar parte de su equipo y realizar esta tesis bajo su supervisión. Gracias por el apoyo mostrado en todo momento, por las ideas compartidas y los consejos en momentos de duda. También quiero agradecer a la Dra. Mercè Balcells, al Dr. Jordi Eras y a la Dra. Gemma Villorbina por toda la ayuda recibida.

Quiero dar las gracias, desde lo más profundo de mi corazón, a mis padres, Lorenzo y Aurora. Gracias por confiar y creer en mis expectativas, por los consejos, valores y principios que me habéis inculcado. Vuestro amor infinito, apoyo incondicional y ayuda constante a lo largo de estos años han hecho posible esta tesis y todo lo que soy. Jamás podré agradeceros lo suficiente todo lo que hacéis por mí. Sois unas personas maravillosas y enormemente trabajadoras a las que admiro profundamente. Es un gran orgullo ser vuestra hija. Os quiero de todo corazón.

A mis hermanos, Alberto y Ana, por acompañarme y apoyarme durante todos estos años. Y a mis sobrinos, Alejandro y Álvaro que también han estado ahí y a los que quiero mucho.

A todos mis compañeros de laboratorio Edinson, Carmen, Albert, Toni, Pau y Remei, gracias por vuestra ayuda. De manera más personal me gustaría agradecerles a Olalla, a Alexis y a Anna toda su ayuda, apoyo, las risas compartidas de todos estos años y lo más importante de todo, vuestra amistad.

A Mireia Oromí, Montse Llovera y Paquita Vilaró, personal técnico de los Serveis Cientificotècnics de la UdL por su colaboración y disponibilidad siempre que ha sido necesario.

To Dr. Vincenzo Di Marzo and Dr. Luciano De Petrocellis of the Endocannabinoid Research Group at Istituto per la Chimica di Molecole di Interesse Biologico (Pozzuoli, Italy), who gave me the opportunity to join his group, and the staff working there, who received me as one more in the group.

A ti amore, Carme Guerris, cómo puedo agradecerte todo lo que me has ayudado y hemos vivido en estos años. Cómo darte gracias por las risas, las lágrimas, las cervezas, los vermuts… Soy realmente afortunada de tenerte como mi mejor amiga y deseo que esto sea sólo el principio. Además quiero agradecer a tu familia todo su apoyo y cariño recibido. Especialmente a Carmelo Guerris y Antonia Castillo que desde el primer momento me acogieron y me hicieron sentir una más de la familia. No tengo palabras para expresar lo mucho que os agradezco todo. Daría lo que fuera

por poder celebrarlo todos juntos. A Javi Galindo, gracias por tu amistad. Y a Iker Jiménez, por ser tan maravilloso. ¡Os quiero muchísimo!

A Janire Lamariano, gracias por tu amistad, tus consejos y por nuestras, tan necesarias, escapadas.

Me gustaría dar las gracias también a la Dra. Mariluz Martín de los Laboratorios Janssen en Toledo. Gracias por todas tus enseñanzas, pasión, rigor y exigencia por el trabajo bien hecho. Gracias por creer en mí.

Toni, gracias por estar ahí día tras día. Por apoyarme y acompañarme en estos duros meses. Tú me has dado la fuerza que necesitaba para terminar este trabajo. Gracias por hacerme tan feliz. Te quiero.

Finalmente y no por ello menos importante, quiero agradecer a todos mis amigos por su preocupación e interés por esta tesis. A Marival, a Montse Guiu, a Enric Franch, a Jordi Arroyos, a Eduard Palacios, a Belén López, a Rocío Garro y a Mireia Terés.

¡Muchas gracias a todos!

TABLE OF CONTENTS

SUMMARY

Vegetable oils are an important renewable raw material for the chemical industry. Homoallyl hydroxy fatty acids such as ricinoleic or lesquerolic acid are highlighted within this oils. They lead to a large number of derivatives with multiple applications, both industrial and pharmaceutical. This work was focused on obtaining and using α,β-unsaturated carbonyl fatty acids through selective processes. First at all, one-step oxidations of the hydroxy group of these acids were studied. For this, both transition-metal and metal-free oxidants were evaluated. Finally, a methodology based on the use of the Shvo's catalyst, a ruthenium(II) complex, and acrolein, which was employed as hydrogen acceptor, was developed. The optimization of the reaction conditions allowed the synthesis and isolation with good yields of the corresponding α,β-unsaturated carbonyl fatty acids from ricinoleic and lesquerolic acid and also their derivatives. Subsequently, sebacic acid was obtained from the α,β-unsaturated carbonyl fatty acids previously synthesized. This dicarboxylic acid has a great industrial interest. Usually, sebacic acid is obtained by the alkaline fission of castor oil. This process requires of high concentrations of sodium hydroxide and high temperatures making it inefficient from an energy point of view. In the present thesis, various synthesis routes were explored to carry out the oxidative cleavage of α,β-unsaturated carbonyl fatty acids to the corresponding dicarboxylic acid. Hence, environmentally friendly catalysts and oxidants, as well as more efficient heating systems such as microwave ovens or ultrasound reactors were studied. Among them, the Oxone™/$NaIO_4$ system was the one that allowed the synthesis of sebacic acid in good yield. This procedure shows to be a more efficient way than the one currently used in the industry.

Finally, new long-chain *N*-vanillyl acylamides (LCNVAs) were designed and synthesized from ricinoleic and lesquerolic acid with the aim of developing a new family of molecules exhibiting dual biological activity against pain receptors such as the TRPV1 receptor and the FAAH enzyme. For this purpose, chemical modifications were introduced in the homoallylic alcohol, leading to 22 new *N*-vanillyl acylamides. These new molecules were biologically tested against the TRPV1 receptor and the FAAH enzyme. In addition, their selectivity was evaluated by testing them in other receptors also expressed in nociceptive neurons.

RESUMEN

Las grasas vegetales son una importante materia prima renovable para la industria química. Dentro de este grupo destacan los ácidos grasos homoalílicos hidroxilados como el ácido ricinoléico o el ácido lesquerólico, los cuales dan lugar a una gran cantidad de derivados con múltiples aplicaciones tanto industriales como farmacéuticas. Esta tesis se centró, por un lado, en la obtención de ácidos grasos carbonilo α,β-insaturados a través de oxidaciones más selectivas y en un solo paso del grupo hidroxilo de dichos ácidos. Para ello, se evaluaron oxidantes tanto basados en metales de transición como libres de estos metales. Finalmente, se desarrolló una metodología basada en el uso del catalizador de Shvo, un complejo de rutenio(II), y acroleína, que se empleó como aceptor de hidrógeno. La optimización de las condiciones de reacción permitió la síntesis y aislamiento con buenos rendimientos de los correspondientes ácidos grasos carbonilo α,β-insaturados procedentes de los ácidos ricinoléico y lesquerólico y también de sus derivados. Por otro lado, se procedió a la obtención del ácido sebácico, un ácido dicarboxílico con gran interés industrial, a partir de los ácidos grasos carbonilo α,β-insaturados anteriormente sintetizados. Tradicionalmente, el ácido sebácico se obtiene mediante la fusión alcalina del aceite de ricino. Este proceso utiliza altas concentraciones de hidróxido de sodio y elevadas temperaturas haciendo que sea poco eficiente desde un punto de vista energético. En la presente tesis se exploraron diferentes rutas de síntesis empleando catalizadores y oxidantes ambientalmente respetuosos, además de sistemas de calentamiento más eficientes como hornos microondas o reactores de ultrasonidos, para llevar a cabo la ruptura oxidativa de los ácidos grasos carbonilo α,β-insaturados. Entre todos ellos, el sistema Oxono™/$NaIO_4$ fue el que permitió obtener el ácido sebácico con buen rendimiento de una forma más eficiente que la usada actualmente en la industria.

Por último, se diseñaron y sintetizaron nuevas *N*-vanillil acilamidas de cadena larga (LCNVAs) a partir de los ácidos ricinoléico y lesquerólico con el objetivo de desarrollar una nueva familia de moléculas que presentaran actividad biológica dual frente a receptores del dolor como son el receptor TRPV1 y la enzima FAAH. Para ello, se introdujeron modificaciones químicas en el alcohol homoalílico dando lugar a 22 nuevas *N*-vanillil acilamidas. Estas nuevas moléculas fueron testadas biológicamente frente al receptor TRPV1 y la enzima FAAH. Además se evaluó su selectividad testándolas en otros receptores también expresados en las neuronas nociceptivas.

RESUM

Els greixos vegetals són una important matèria primera renovable per a la indústria química. Dins d'aquest grup destaquen els àcids grassos homoalílics hidroxilats com l'àcid ricinoleic o l'àcid lesqueròlic, els quals donen lloc a una gran quantitat de derivats amb múltiples aplicacions tant industrials com farmacèutiques. Aquesta tesi es va centrar, d'una banda, en l'obtenció d'àcids grassos carbonil α,β-insaturats mitjançant oxidacions més selectives i en un sol pas del grup hidroxil d'aquests àcids. Per a això, es van avaluar oxidants tant basats en metalls de transició com lliures d'aquests metalls. Finalment, es va desenvolupar una metodologia basada en l'ús del catalitzador de Shvo, un complex de ruteni (II), i acroleïna, que es va fer servir com a acceptor d'hidrogen. L'optimització de les condicions de reacció va permetre la síntesi i aïllament amb bons rendiments dels corresponents àcids grassos carbonil α,β-insaturats procedents dels àcids ricinoleic i lesqueròlic, i també dels seus derivats. D'altra banda, es va procedir a l'obtenció de l'àcid sebàcic, un àcid dicarboxílic amb gran interès industrial, a partir dels àcids grassos carbonil α,β-insaturats anteriorment sintetitzats. Tradicionalment, l'àcid sebàcic s'obté mitjançant una fusió alcalina de l'oli de ricí. Aquest procés utilitza grans concentracions d'hidròxid de sodi i elevades temperatures fent que sigui poc eficient des d'un punt de vista energètic. En la present tesi es van explorar diferents rutes de síntesi fent servir catalitzadors i oxidants ambientalment respectuosos, a més de sistemes d'escalfament més eficients com forns microones o reactors d'ultrasons, per dur a terme la ruptura oxidativa dels àcids grassos carbonil α,β-insaturats. Entre tots ells, el sistema Oxono™/$NaIO_4$ va ser el que va permetre obtenir l'àcid sebàcic amb més bon rendiment i d'una forma més eficient que la usada actualment a la indústria.

Finalment, es van dissenyar i sintetitzar noves *N*-vanillil acilamides de cadena llarga (LCVNAs) a partir dels àcids ricinoleic i lesqueròlic amb l'objectiu de desenvolupar una nova família de molècules que presentessin activitat biològica dual davant de receptors del dolor com són el receptor TRPV1 i l'enzim FAAH. Per a això, es van introduir modificacions químiques en l'alcohol homoalílic donant lloc a 22 noves *N*-vanillil acilamides. Aquestes noves molècules van ser testades biològicament davant del receptor TRPV1 i l'enzim FAAH. A més es va avaluar la seva selectivitat testant-les a altres receptors també expressats en les neurones nociceptives.

OVERVIEW INFORMATION

The present doctoral thesis was developed within the UdL-Impuls program (13100) of the University of Lleida and Banco Santander. The global title of this project was "Obtenció de derivats d'àcids grassos hidroxilats d'interés industrial mitjançant mètodes quimioenzimàtics i química verda". The work was carried out in the Organic Chemistry Laboratory located at Escola Superior d'Enginyeria Agrària i Alimentària (ETSEA) of University of Lleida.

The biological activity of the new synthetized LCNVAs was evaluated during a stay in the Endocannabinoid Research Group (Istituto per la Chimica di Molecole di Interesse Biologico, CNR, Pozzuoli, Italy) under the supervision of the Professors Vincenzo di Marzo and Luciano De Petrocellis. This stay was partially granted by the University of Lleida

This thesis work was the subject of a patent and a publication to date:

- Procedimiento para la obtención de ácidos dicarboxílicos saturados. Ruben Torregrosa, Javier Hijós Bitrián, **Silvia López Chinarro**, Mercé Balcells, Gemma Villorbina, Jordi Eras and Ramon Canela. WO2014080059A1, November 20, 2013.
- Elongation of the hydrophobic chain as a molecular switch: discovery of capsaicin derivatives and endogenous lipids as potent Transient Receptor Potential Vanilloid Channel 2 antagonists. Aniello Schiano Moriello, **Silvia López Chinarro**, Olalla Novo Fernández, Jordi Eras, Pietro Amodeo, Ramon Canela-Garayoa, Rosa Maria Vitale, Vincenzo Di Marzo, and Luciano De Petrocellis. *J. Med. Chem.*, **2018**, *61* (18), 8255-8281.

ABBREVIATIONS AND ACRONYMS

AA-5-HT	*N*-Arachidonoylserotonin
AcOH	Acetic acid
AEA	*N*-Arachidonylethanolamine or Anandamide
AKAP150	A-Kinase anchoring protein 150
Anh.	Anhydrous
Ar	Aryl
AS	Amidase signature
ATP	Adenosin triphosphate
ATR	Attenuated Total Reflectance
Boc	*tert*-Butyloxycarbonyl
CaM	Calmodulin
CAMKII	Ca^{2+}/calmodulin-dependent protein kinase
CAN	Cerium Ammonium Nitrate
CB	Cannabinoid receptor
CMAI	Chemical Market Associates, Inc.
CNS	Central Nervous System
cryo-EM	Cryo-electron microscopy
CTH	Catalytic Transfer Hydrogenation
CuAAC	Copper-catalyzed azide–alkyne cycloaddition
DBS	Dibutyl Sebacate
DCA	Dioctanol adipate
DCC	*N*,*N*'-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DCO	Dehydrated Castor Oil
DCP	Dioctanol phthalate
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DFT	Density Functional Theory
DIPE	Diisopropyl ether
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine

DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DOS	Dioctyl sebacate
EC_{50}	Half-maximal response
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
2-EH	2-Ethylhexanoic acid
EKODE	Epoxyketooctadecenoic acid
equiv.	Equivalent
ESI	Electrospray ionization
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
EtOH	Ethanol
FAAH	Fatty Acid Amide Hydrolase
FAPAs	Fatty Acid Primary Amides
FT-IR	Fourier Transform Infrared Spectroscopy
GC	Gas Chromatography
GC-MS	Gas Chromatography Mass Spectrometry
Gly	Glycerol
HATU	1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HCO	Hydrogenated Castor Oil
HEK293	Human Embryonic Kidney 293 cell
HFA	Hydroxy Fatty Acid
HPETE	Hydroperoxyeicosatetranoic acid
HPLC	High Performance Liquid Chromatography
HR-MS	High Resolution Mass Spectrometry
HTS	High-Throughput Screening
IBA	2-Iodobenzoic acid
IBX	2-Iodoxybenzoic acid
IC_{50}	Half maximal inhibitory concentration

IPA	Isopropyl alcohol
IPN	Interpenetrating Polymer Networks
IR	Infrared spectroscopy
LCNVAs	Long Chain *N*-Vanillyl Acylamides
MAPF	Methoxyarachidonylfluorophosphonate
m-CPBA	*meta*-Chloroperoxybenzoic acid
MeCN	Acetonitrile
MeOH	Methanol
mp	Melting point
MW	Molecular Weight
NaAc	Sodium acetate
NADA	*N*-Arachidonoyl-dopamine
NAEs	*N*-Acyl ethanolamines
NMDA	*N*-Methyl-*D*-aspartate
NMR	Nuclear Magnetic Resonance
OAt	*O*-Acyl(tetramethyl)isouronium
OLEA	*N*-Oleylethanolamine
Ph	Phenyl
PIP_2	Phosphatidylinositol 4,5-bisphosphate
PKA	Protein Kinase A
PKC	Protein Kinase C
ppm	Parts per million
PTFE	Polytetrafluoroethylene
p-TsOH	*para*-Toluenesulfonic acid
PVC	Polyvinyl chloride
quant.	Quantitative
rt	Room temperature
RTX	Resiniferatoxin

SAR	Structure-Activity Relationship
SPB	Sodium perborate
SPC	Sodium percarbonate
TBAF	Tetra-*n*-butylammonium fluoride
TBDMS	*tert*-Butyldimethylsilyl
TBHP	*tert*-butyl hydroperoxide
tert-BuO	*tert*-Butoxide
TEA	Triethylamine
tert-BuOH	*tert*-Butanol
THF	Tetrahydrofuran
TLC	Thin Liquid Chromatography
TM	Trademark
TMS	Trimethylsilane
TOF	Time of Flight
Tol	Tolyl
TRP	Transient Receptor Potential
TRPA	Transient Receptor Potential Ankyrin
TRPV	Transient Receptor Potential Vanilloid
UV	Ultraviolet spectroscopy
v/v	Volume/volume
w/v	Weight/volume
w/w	Weight/weight

General Introduction

The use of vegetable oils for edible and industrial purposes has a long history as mankind itself.[1] Vegetable oils have low price, renewable and naturally raw materials with usually low toxicity. Vegetable oils are extracted primarily from the seed of oilseed plants. Their competitive cost, worldwide availability, and built-in functionality make them attractive. In recent years, there has been a growing trend in using vegetable oils as renewable resources, especially in oleochemical products. **Figure 1** shows the distribution of nine major vegetable oils (coconut, cottonseed, olive, palm, palm kernel, peanut, rapeseed, soybean, and sunflower) for non-food and food purposes over the recent years.[2] Going from 1999 to 2012, the non-food portion has increased from 8.7 to 35.8 million of tons. This increase is particularly remarkable from 2003/04 onwards. The data confirms the increasing rate of non-food use of vegetable oils in the world, particularly during the last decade.

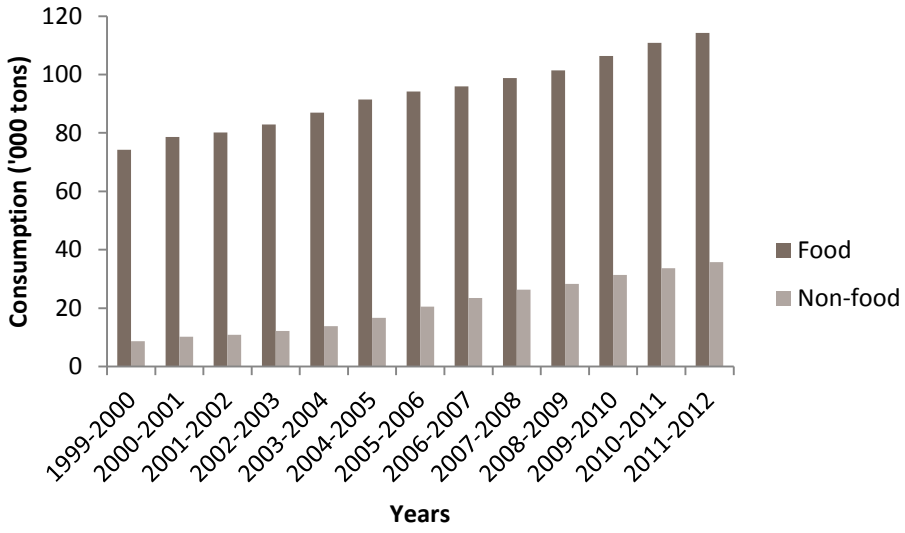


Figure 1. Food and non-food consumption (million tons) of nine major vegetable oils between 1999- and 2012.[2]

Vegetable oils consist of mainly triglycerides, esters of glycerol with various fatty acids, which have a three-armed star structure (**Figure 2**). The chemical structures of triglycerides are very complex owing to the combination and permutations of fatty acids that can be esterified at the three (enzymatically non-equivalent) hydroxy groups of glycerol.

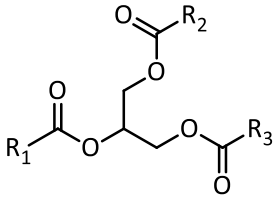


Figure 2. The triglyceride molecular structure of vegetable oils.

When $R_1=R_2=R_3$, the trivial name of the triglyceride is derived from the parent acid by means of a termination -in (e.g., for stearic acid where $R_1=R_2=R_3= C_{17}H_{35}$, the triglyceride is called tristearin). If R_1 and R_3 are different, the glyceride molecule can exist in two enantiomeric forms.[3] Most of the common oils contain fatty acids that vary from 14 to 22 carbons in length, with 0 to 3 double bonds per fatty acids. **Table 1** summarizes the most common fatty acids present in vegetable oil with their principal natural vegetable source.[4,5]

Table 1. Representative fatty acids.

Fatty acid	Common name (designation)[a]	Source
Octanoic acid	Caprylic acid (C8:0)	Coconut
Decanoic acid	Capric acid (C10:0)	Coconut
Dodecanoic acid	Lauric acid (C12:0)	Coconut, palm kernel
Tetradecanoic acid	Myristic acid (14:0)	Coconut, palm kernel
Hexadecanoic acid	Palmitic acid (C16:0)	Palm, cotton,
cis-9-Octadecenoic	Oleic acid (C18 :1 9c)	Olive, tall, peanut
cis,*cis*-9,12-Octadecadienoic acid	Linoleic (C18 :2 9c,12c) acid	Safflower, sunflower, corn, soy, cotton
cis,*cis*,*cis*-9,12,15-Octadecatrienoic acid	Linolenic acid (C18 :3 9c,12c,15c)	Linseed
12-Hydroxy-*cis*-9-octadecenoic acid	Ricinoleic acid (C18:1 9c, 12-OH)	Castor
cis-12-Epoxyoctadeca-*cis*-9-enoic acid	Vernolic acid (C18:1 9c, 12-O)	Vernonia

[a]Number of carbon atoms:number/location of unsaturation

1. HOMOALLYL HYDROXY FATTY ACIDS

The naturally occurring oxygenated fatty acids include the hydroxy, keto, and epoxy groups, of which the hydroxyl-substituted are the most common. The hydroxy fatty acids (HFA) are composed of a series of straight-chain carboxylic acids that contain one or more hydroxyl groups substituted on the acyl portion of the molecule. Since the hydroxy fatty acids possess at least one asymmetrical carbon atom, they are capable of being resolved into their optical isomers. Most of the naturally occurring hydroxy acids are optically active. The carbon-carbon bonds in the hydroxy fatty acids may be all saturated, or the acyl may contain one or more carbon-carbon unsaturated bonds. Those hydroxy acids that contain an ethylenic bond exhibit geometrical isomerism and can be obtained in either *cis* or *trans* form. These types of hydroxy fatty acids are important industrial materials. Castor and lesquerella oils are the two major sources.

1.1. Castor oil

The castor-oil plant, which is apparently indigenous in Africa, is now a native of India[6] and is extensively cultivated in the warmer regions throughout the world. The oil is obtained by extracting or expressing the seed of *Ricinus communis,* a plant of the family Eurphorbiacae (**Figure 3**).[7]

Figure 3. Castor plant (left) and castor seeds (right).[8,9]

Castor oil is a viscous, pale yellow non-volatile and non-drying oil with a bland taste and is sometimes used as a purgative. It has a slight characteristic odour while the crude oil tastes slightly acrid with a nauseating after-taste. Relative to other vegetable oils, it has a good shelf life and it does not turn rancid unless subjected to excessive heat. Asia can be considered as the main player for oils and fats used for the oleochemical industry. India is the world's largest exporter of castor oil, with a share of 70% of the total exports, followed at considerable distance by China, Brazil and Thailand, as shown in **Figure 4**. The major importing countries are USA, Russia and Japan.[10]

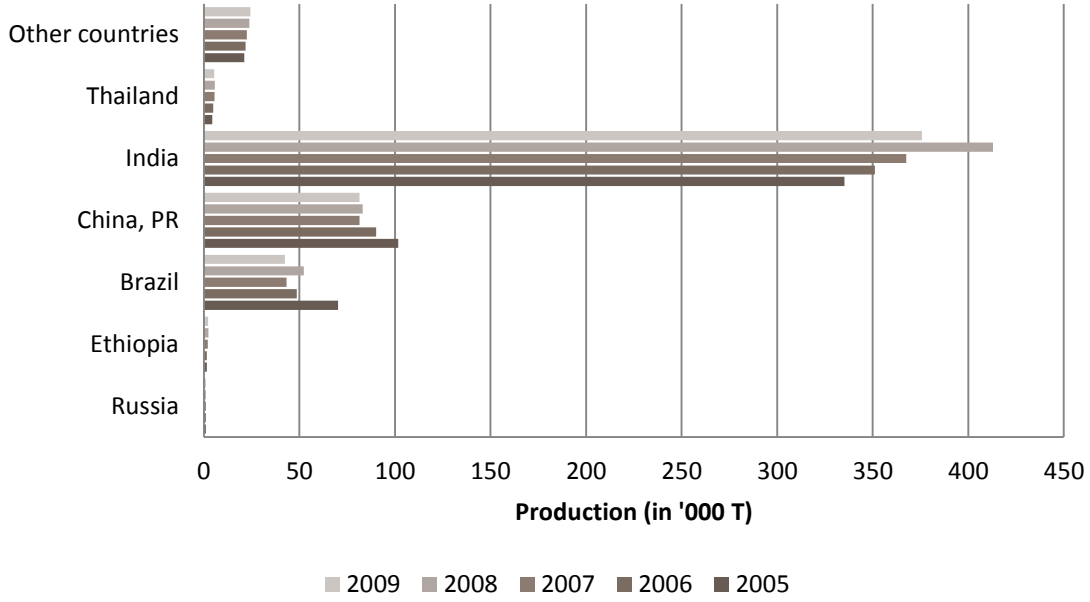


Figure 4. Annual production of castor oil by origin.[11]

1.1.1. Properties

Castor oil has different physical and chemical properties that vary with the method of extraction. Cold-pressed castor oil has low acid value, similar iodine value and a slightly higher saponification value than solvent-extracted oil, and it is lighter in colour (**Table 2**). It was already discovered in 1845 that heating castor oil to high temperature gives interesting results and broadens the application possibilities of the oil, and the value of castor has ever since increased greatly.[12] Moreover, certain characteristics of castor oil, like high lubricity, high viscosity over a wide range of temperatures, and insolubility in aliphatic petrochemical fuels and solvents, make it directly applicable as lubricant for equipment operating under extreme conditions.

Table 2. Properties of castor oil grades.

Properties	Cold-pressed oil	Solvent-extracted oil
Specific gravity	0.961 – 0.963	0.957 – 0.937
Acid value	3	10
Iodine value (W_{ij})	82 – 88	80 – 88
Saponification value	179 – 185	177 – 182

Castor seeds are poisonous to humans and animals because they contain ricin, ricinine and certain allergens that are toxic. However, processed or refined castor oil is free from any of these substances and can be safely used in pharmaceutical and industrial applications.

1.1.2. Composition and chemistry

Like other vegetable oils, castor oil is a triacylglycerol composed of various fatty acids and glycerol. Castor oil is unique among all fats and oils in that:

I. It is the only commercial source of ricinoleic acid, an 18-carbon hydroxylated fatty acid with one carbon-carbon double bond.

II. Ricinoleic acid comprises approximately 87% of the fatty acid composition.

III. Product uniformity and consistency are relatively high for a naturally occurring material.

A part of ricinoleic acid, castor oil contains varying small amounts of various saturated and unsaturated fatty acids.[13] **Table 3** shows the chemical composition of castor oil.

Table 3. Castor oil composition.

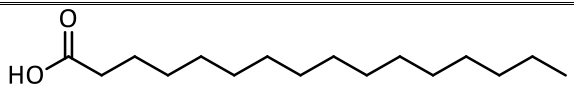
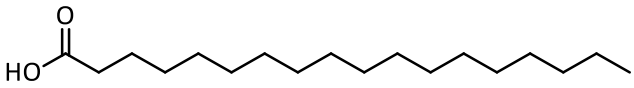
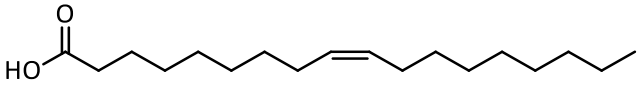
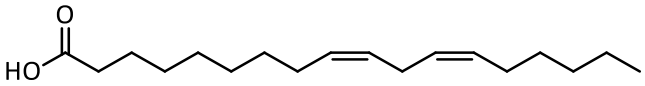
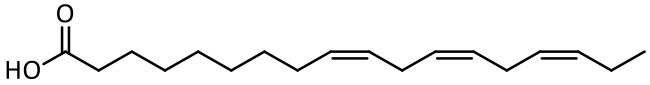
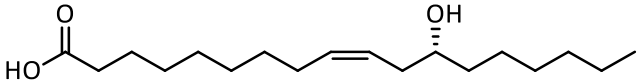
Fatty acid	Structure	Percentage (%)
Palmitic		0.8 – 1.1
Stearic		0.7 – 1.0
Oleic		2.2 – 3.3
Linoleic		4.1 – 4.7
Linolenic		0.5 – 0.7
Ricinoleic		87.7 – 90.4

Figure 5 shows the high number of chemical processes applied on castor oil to prepare various commercial products.

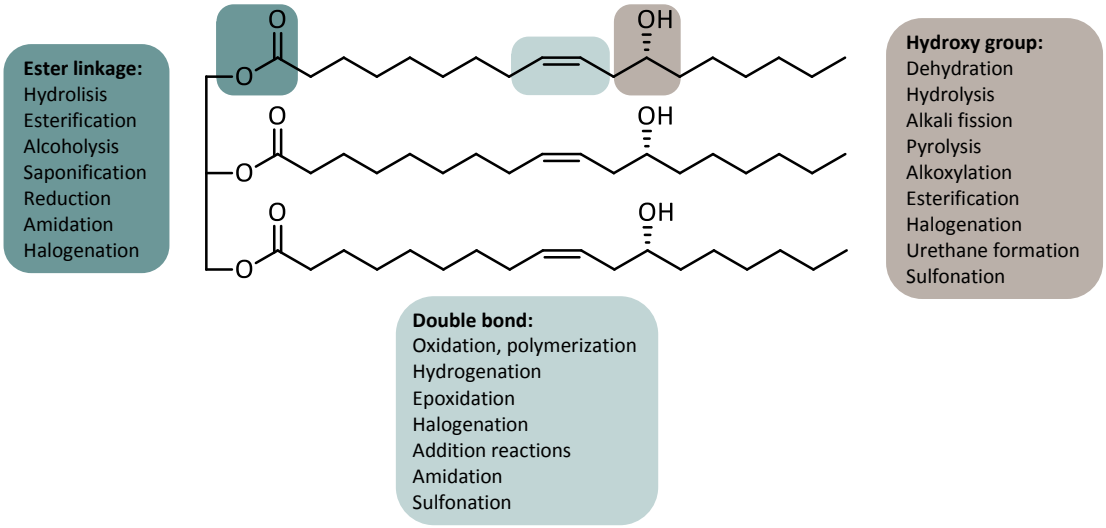


Figure 5. Chemical structure of ricinoleic acid triglyceride, the primary component of castor oil. Chemical reactions for the preparation of a variety of derivatives of castor oil are indicated.

Generally, the three functional groups in ricinoleic acid provide multitude of possibilities of converting or modifying castor oil into many other useful products depending on the intended specific uses. For example, the presence of a carboxylic group allows the transformation of castor oil via several reactions such as esterification, amidation[14-16] whereas the presence of a carbon-carbon double bond, affords the transformation of the oil through reactions such as hydrogenation,[15,17] carbonylation[18] and epoxidation.[19] Furthermore, the hydroxyl functional group can be acetylated[19,20] alkoxylated[21,22] or removed by dehydration[23,24] to increase the unsaturation number of the oil.

1.1.3. Applications of castor oil and its derivatives

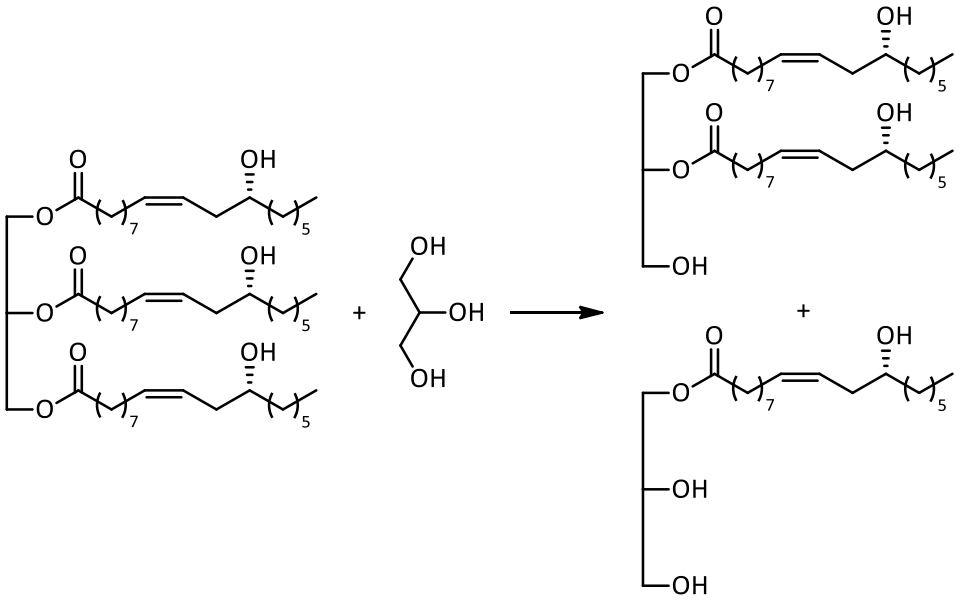
- *Fuel and biodiesel*

Few studies have been done regarding castor fuel-related properties in pure form or a blend with diesel fuel, primarily due to the extremely high content of ricinoleic acid. Berman *et al.*[25] described that methyl esters of castor oil can be used as a biodiesel alternative feedstock when blended with diesel fuel. However, the maximum blending level is limited to 10% due to the high levels of ricinoleic acid present in the oil, which directly affects to the biodiesel kinematic viscosity and distillation temperature. Shojaeefard *et al.*[26] examined the effects of castor oil biodiesel blends on diesel engine performance and emissions. They found that a 15% blend of castor oil–biodiesel was an optimized blend of biodiesel–diesel proportions. The results indicated that lower blends of

biodiesel provide acceptable engine performance and even improve it. Similar to this study, Panwar *et al.*[27] prepared the castor methyl ester by transesterification using potassium hydroxide (KOH) as catalyst. This methyl ester was tested in a four-stroke, single cylinder variable compression ratio type diesel engine. It was concluded that the lower blends of biodiesel increased the break thermal efficiency and reduced the fuel consumption. Further, the exhaust gas temperature increased with increasing biodiesel concentration. Results of their study proved that the use of biodiesel from castor seed oil in a compression ignition engine is a viable alternative to diesel. As a result, biodiesel can be obtained by transesterification of castor oil using either ethanol or methanol as the transesterification agents.[28]

- *Polymer materials*

The depletion of fossil fuels and environmental issues has pushed researchers to focus their attention and efforts on the utilization of renewable resources as raw materials for the synthesis of polymeric materials. Biobased polymers offer a number of advantages over polymers prepared from petroleum-based monomers as they are cheaper, readily available from renewable natural resources and they possess comparable or better properties. Some bio-based polymers are biodegradable, nontoxic and have low carbon footprints.[29] Castor oil and its derivatives can be used in the synthesis of renewable monomers and polymers.[30] Castor oil allows the synthesis of a variety of polyurethane products, ranging from coatings, cast elastomers, thermoplastic elastomers, rigid foams, semi-rigid foams, sealants and adhesives to flexible foams. Concerning the direct utilization in rigid polyurethanes, castor oil has advantages (water resistant and flexible) as well as some disadvantages, such as low functionality, low reactivity due to the secondary hydroxy groups, thus leading to semi-flexible and semi-rigid materials.[31] By reacting castor oil with polyols, such as glycerol, a higher hydroxyl number is obtained, which leads to rigid polyurethanes foams with good physico-mechanical properties (**Scheme 1**). Glycerol-modified polyurethanes products clearly exhibited higher tensile strength, lower elongation at break and chemical resistance as compared to polyurethanes prepared from pure castor oil. Interestingly, the thermal degradation of polyurethanes was more or less similar, indicating that the polyol modification of castor oil has not influenced the thermal stability. Moreover, castor oil was polymerized and cross-linked with sulfur or diisocyanates to form the vulcanized and urethane derivatives, respectively.[32]



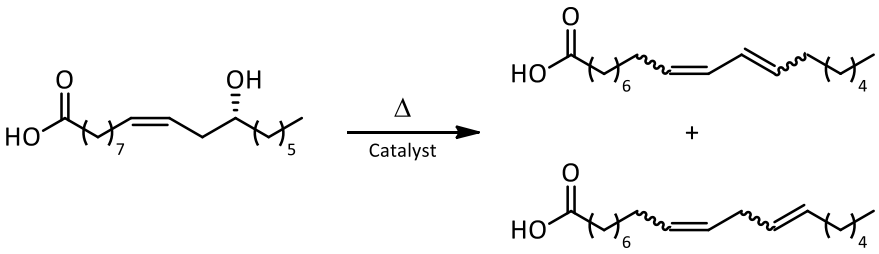
Scheme 1. Schematic representation of the formation of glycerol-modified, castor oil-based polyols for polyurethane synthesis.

Biodegradable polyesters are one of the most common applications using castor oil.[33] The presence of this hydroxyl group provides additional functionality for the preparation of polyesters or polyester-anhydrides. The dangling chains of the ricinoleic acid impart hydrophobicity to the resulting polyesters, thereby influencing the mechanical and physical property of the polymers. These chains act as plasticizers by reducing the glass transition temperatures of the polyesters.[33,34] Castor oil can be combined with other monomers to produce an array of copolymers. Fine-tuning these copolymers can provide materials with different properties useful in products ranging from solid implants to in situ injectable hydrophobic gel.[33]

Recently the use of castor oil to synthetize Interpenetrating polymer networks (IPN) is receiving great interest. These materials present numerous technological applications in diverse fields, such as ion-exchange resins, reinforced elastomers, thermoplastic IPN, piezodialysis membranes, high-temperature alloys, coatings and adhesives, noise- and vibration-damping materials, and sheet-moulding compounds.[35-37] Castor oil as such, after hydrogenation, or transesterification with glycerol or modification with linseed oil, or in combination with polyethylenglycol, was extensively used as precursor in the preparation of IPN.[29,32,38,39]

- *Coatings*

Coatings and paints are also another application of castor oil. Castor oil is classified as a non-drying oil due to its low iodine value. However, it can be dehydrated to give semi-drying or drying oils. The dehydration process is usually carried out at about 250 °C in the presence of catalysts under an inert atmosphere or vacuum.[40-43] The most widely catalysts used are sulfuric acid, sodium bisulfate, phosphoric acid, phthalic anhydride and acid-activated clays.[23,44,45] During the dehydration process of ricinoleic acid, the hydroxy group and one of its α-hydrogen atoms are removed, yielding a regioisomeric mixture of two acids (**Scheme 2**). The ratio of the two regioisomers and also the cis/trans ratio of the formed double bonds depend on the dehydration conditions.[44] The conjugated acid is likely to undergo polymerization. To prevent such a polymerization, an antipolymerization agent (sodium bisulfite, zinc chloride or aluminum chloride) can be used together with the catalyst.[46]



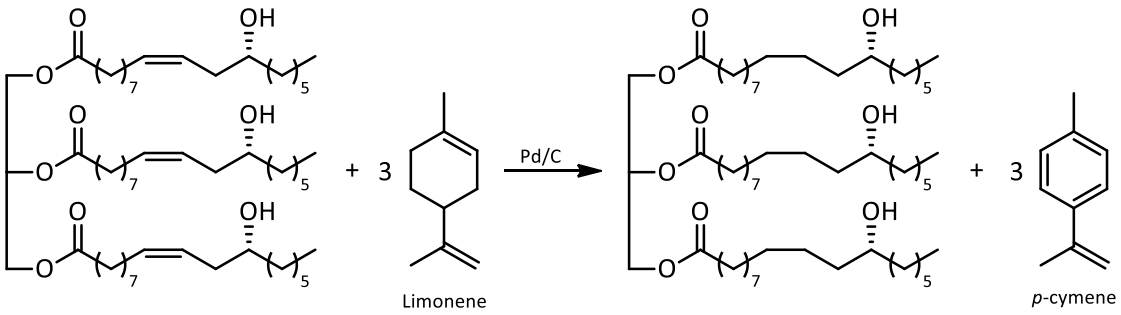
Scheme 2. Isomeric mixture of unsaturated fatty acids from the dehydration of castor oil.

Dehydrated Castor Oil (DCO) is well known for its non-yellowing and outstanding color retention characteristics; it has better drying properties than linseed oil and its water and alkali resistance can be almost as good as for tung oil.[47] Varnishes, alkyds and resin systems based on dehydrated castor oil offer properties like fast drying, flexibility, excellent chemical resistance, adhesion to metals, high gloss and water resistance.[48-52]

- *Waxes*

Hydrogenated Castor Oil (HCO) is a wax like compound obtained by the controlled hydrogenation of pure castor oil. HCO is a hard, brittle, high melting point product (86 °C) that is practically odourless and tasteless. Moreover, the hydrogenated oil has an improved oxidative and thermal stability. Commercial preparation of this product must be accomplished by careful introduction of hydrogen at lower temperatures (150 °C) in the presence of nickel catalysts (0.2–0.6%) or an Adkins copper chromite catalyst (1%), thus avoiding the decomposition of the hydroxy group.[53] It is reported that the catalyst can be repeatedly reused if 10% of fresh catalyst is added before each run. A good quality hydrogenated castor with high hydroxyl value and low iodine value is

obtained at 423 K; $1.034 \cdot 10^6$ Pa; in 5 h with 2 % (weight of oil) Raney nickel catalyst.[53] Hydrogenation of castor oil at low pressure ($1.96 - 2.45 \cdot 10^5$ Pa) and low temperature (398–408 K) requires high catalyst concentration.[54,55] Castor oil hydrogenation can also be done by catalytic transfer hydrogenation (CTH) (**Scheme 3**).[15,56]



Scheme 3. Catalytic transfer hydrogenation of castor oil.

Hydrogenated Castor Oil (HCO) is insoluble in water and in most organic solvents but it is soluble in hot organic solvents like ether and chloroform.[54] This property makes HCO valuable for lubricant industries because of water resistance and retention of its lubricity. Moreover, the polarity and surface wetting properties of HCO are useful in cosmetics, hair dressing, solid lubricant, paint additives, manufacture of waxes, polishes, carbon paper, candles and crayons.[53]

- *Sulfonation of castor oil*

Another product formed from the modification of castor oil is sulfonated castor oil, which is also known as ‘‘Turkey red oil’’. Sulfonation of castor oil produces sulphuric acid esters in which the hydroxy group of ricinoleic acid has been esterified (**Figure 6**).[57] The reaction is performed by treating raw castor oil at room temperature or at temperature less than 308 K with concentrated sulphuric acid for several hours, followed by washing and neutralizing with sodium hydroxide solution.

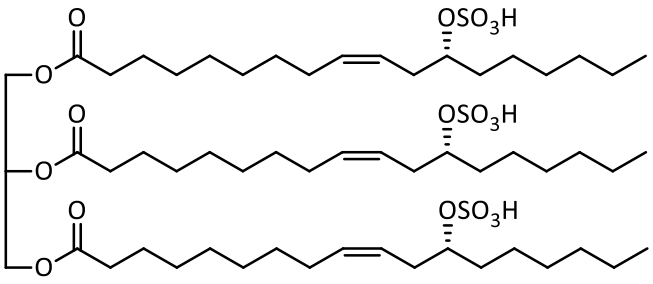


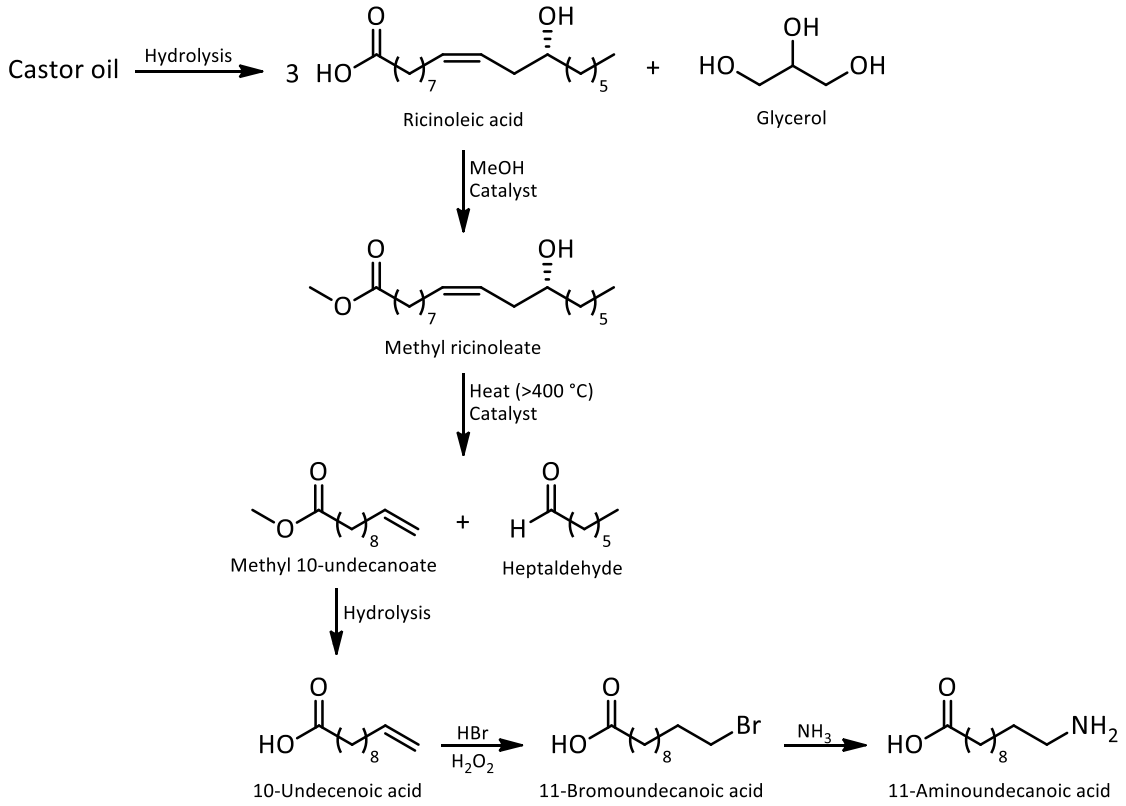
Figure 6. Chemical structure of Turkey red oil.

Turkey red oil is widely used in textile and cosmetics industries by producing synthetic detergents in the recipes/formulations of lubricants, softeners, and dyes. In addition, Turkey red oil is

an active wetting agent in dyeing and in finishing of cotton and linen. It is also used in bath oil recipes along with natural or synthetic fragrance or essential oils or in shampoos.[58] Finally, the action of sulphuric acid on castor oil produces a useful emulsifier for certain insecticidal oils.

- *Pyrolisis*

The pyrolysis of castor oil at a high temperature (>400 °C) splits the ricinoleate molecule at the hydroxy group to form heptaldehyde and 10-undecenoic acid. The two main products, heptaldehyde and 10-undecenoic acid are important raw materials for cosmetics (C11 and C7 aldehydes are used in soaps, shampoos, talcum powders and perfume formulations), pharmaceuticals, and polymeric compounds).[53,59-61] Furthermore, heptaldehyde is used as a solvent for rubber, resins, and plastics.[53] For the 10-undecenoic acid, it serves as a source of bactericides and fungicides[53] but also further reactions on the acid can produce monomers for the formation of nylon 11.[62] Castor oil is hydrolyzed to give ricinoleic acid and glycerol, which are separated. High temperature treatment of the methyl ester of ricinoleic acid produces methyl 10-undecenoate and heptaldehyde. The methyl 10-undecenoate is hydrolysed to give 10-undecenoic acid, which is treated with hydrogen bromide in a non-polar solvent in the presence of peroxide. Under these conditions, reverse Markownikoff addition occurs and the main product is 11-bromoundecanoic acid. The product is then treated with ammonia to give 11-aminoundecanoic acid, which is a crystalline solid. 11-Aminoundecanoic acid is the starting material for nylon-11.[63] It is claimed that Arkema,[64] a French company, produces nylon-11, commercially known as Rilsan® 11, by this route. The overall process is shown in **Scheme 4**.

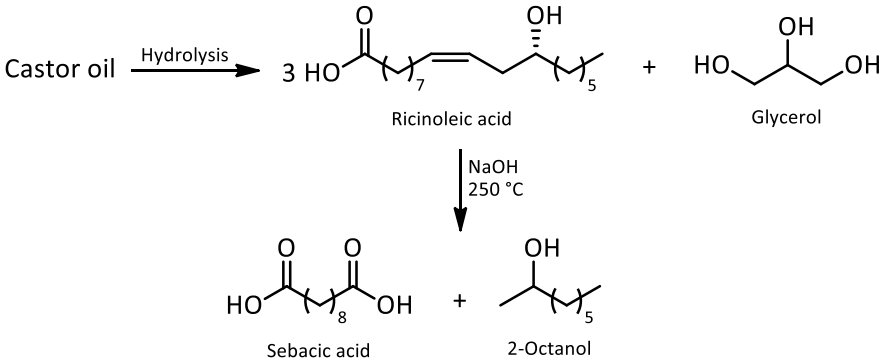


Scheme 4. Conversion of ricinoleic acid into monomers for nylon 11.

- *Hydrolysis*

Hydrolysis of castor oil by slow addition of castor oil to 80 % caustic solution (sodium hydroxide) produces ricinoleic acid and glycerol. Upon heating at 523 K in the presence of NaOH, sebacic acid (a 10 carbon dicarboxylic acid) and 2-octanol are produced (**Scheme 5**). Both sebacic acid and 2-octanol have many uses. One of the largest uses of sebacic acid is in the manufacture of Nylon 6,10. Sebacic acid and its derivatives have a variety of industrial uses in plasticizers, lubricants, hydraulic fluids, cosmetics, candles, etc. They are used in the synthesis of polyamide and alkyd resins. An isomer, isosebacic acid, has other applications in the manufacture of extrusion plastics, adhesives, polyesters, polyurethane resins and synthetic rubber. Sebacic acid is also used as an intermediate for aromatics, antiseptics and painting materials. A large number of esters can be obtained from thousands of potential starting materials. It is used as a corrosion inhibitor in metalworking fluids and as a complexing agent in greases. When mixed with amines, sebacic acid can give a very effective water soluble corrosion inhibitor for metal working fluids. Lithium hydroxystearate complex greases often utilize dibasic acids such as sebacic acid for the more unusual performance parameters. These greases require the esters of sebacic acid, which were developed for specific performance criteria under varying conditions. The esters of sebacic acid also are used as plasticizers for vinyl resins and in

the manufacture of dioctyl sebacate, a jet lubricant and lubricant in air-cooled combustion motors. On the other hand, 2-octanol is used as a solvent dehydrater and anti-bubbling agent. In coal industry, it is used as floatation agent; finds uses as a frother in mineral flotation. The refined derivative can be used to produce plasticizers such as dioctanol phthalate (DCP), dioctanol adipate (DCA). It can be used as a possible alternate for 2-ethylhexanol or isooctyl alcohol in the preparation of diesters, monomeric and polymeric plasticizers. 2-Octanol also finds applications in personal care and cosmetic products such as personal creams and ointments.



Scheme 5. Conversion of ricinoleic acid into sebacic acid and 2-octanol.

1.2. Lesquerella oil

The genus *Lesquerella* is a New World genus native to the U.S., Mexico, and some species are found throughout the Americas and belongs to the mustard family.[65] The genus consists of about 70 species which are widely genetically variable, about 30% of species are annuals.[66] Producing abundant nonshattering seed, the species, *Lesquerella fendleri*, has been found to have superior agronomic potential (**Figure 7**).

Figure 7. *Lesquerella fendleri* plant.[67]

The species is native to the states of Arizona, New Mexico, Oklahoma, and Texas and found in regions of poor soil and low rainfall (25 cm/yr). Its low water demand may make it an attractive potential substitute for certain heavily irrigated crops in these regions. Limited commercial data is available on current production and yields. However, trials are currently being carried out in a number of countries across Europe, particular scientific interest and development is currently taking place in Belgium, Italy, Netherlands and UK. From these trials it has been established that *Lesquerella fendleri* is not well adapted to temperate western European climate, the crop showed very poor establishment and germination. In USA where the crop is better suited the seed yields are currently 950-1120 kg/ha at 21% oil content, this figure needs to be improved through breeding to allow commercialisation to occur. In small scale trial plots yields of up to 1.600 kg/ha have been achieved whereas on large scale field trials this has been reduced to only 800-900 kg/ha. Lesquerella has not reached commercial production however, 16.2 hectare field plots have been grown in 2003 and 2004 for market development. The key aspects of lesquerella have centered on improving the agronomics of the crop through breeding[68] and best management practices. Improved lesquerella breeding lines with seed oil contents of 35% compared with 30% are currently under development and seed yield of 2040 kg/ha versus current releases of 1360 kg/ha will help lesquerella to become competitive in the hydroxy oil market.[69]

1.2.1 Properties

The *L. fendleri* seed contains approximately 21% triglyceride oil. Crude lesquerella oil is the colour of molasses and has a distinct odour. Oil recovery from lesquerella is difficult due to the small size of the seed. Pre-treatment of small seeds may result in large losses and percolation of solvents may block the process. This problem can be overcome by performing oil extraction; however this method is not commercially viable when large quantities are to be extracted. The physical properties of the crude oil are shown in **Table 4**.

Table 4. Physical properties of crude lesquerella oil.

Properties	Crude oil
Hydroxyl value	102
Acid value	1.5
Iodine value (W_{ij})	107
Saponification value	168

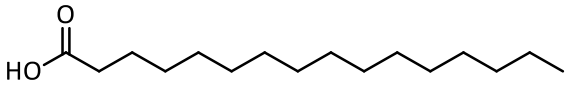
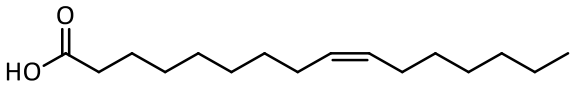
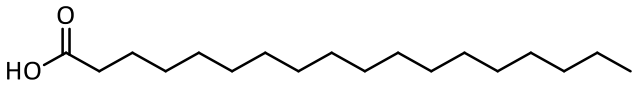
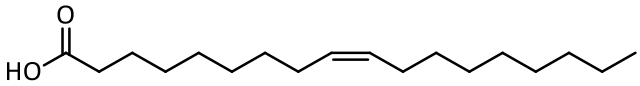
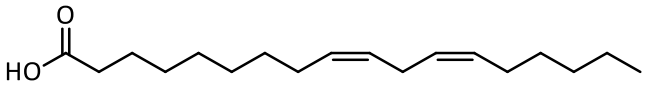
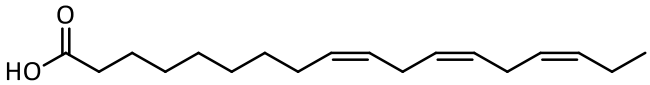
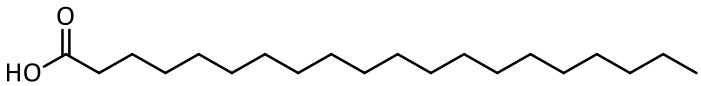
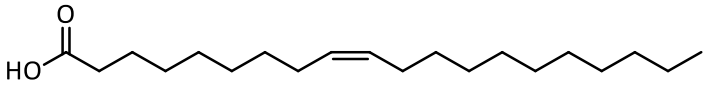
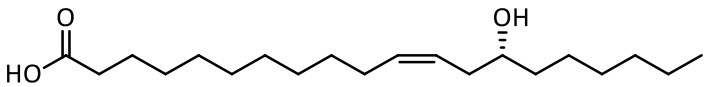
The meal resulting from oil extraction has an excellent distribution of amino acids being particularly high in lysine.[70] Preconditioning the seed is necessary for deactivation of the thioglucosidase enzyme system so that the meal can be used for animal feed. Meal feeding studies are in progress for a variety of livestock. Unlike castor, lesquerella does not contain toxic moieties like the very lethal protein, ricin; the poisonous alkaloid, ricinine; or the very potent allergen, CB-1A. There is significant interest in the lesquerella meal as a source of natural antioxidants, pigments, gums, and protein extracts as well as for animal feed.

The seed coat of several Lesquerella species, including *L. fendleri*, contains a natural unique gum that can be separated either before or after the oil is extracted. The gum could be as valuable as the oil. Possible uses include cosmetics, plasticizers, lubricants, coatings, and thickening agents for foods and for crude oil recovery.

1.2.2. Composition and chemistry

Lesquerella fendleri was discovered for its unique industrial seed oil in 1960 (Jones and Wolf, 1960; Smith et al., 1961).[71,72] *Lesquerella fendleri* contains a seed oil which is approximately 55% lesquerolic acid (14-hydroxy-*cis*-11-eicosenoic acid), a 20-carbon long fatty acid with a single hydroxy group and a double bond, and has a similar hydroxy fatty acid (HFA) profile as castor oil. **Table 5** shows the main chemical composition of lesquerella oil. Aurolic acid (14-hydroxy-11,17-eicosadienoic), epoxy compounds have also been discovered in *L. fendleri* and very long chain fatty acids to C-30 albeit at low levels.[73] Oil from *L. fendleri* is compositionally similar to and in some ways structurally superior to castor oil. Lesquerella has several novel properties absent in other oilseeds. The oil contains natural, unique molecules (estolides), which are rare in other seed oils. These molecules promote natural ease of flow of the oil under many different conditions. Naturally occurring estolides allow lesquerella oil to flow more easily than petroleum at cold temperatures.

Table 5. Lesquerella oil composition.

Fatty acid	Structure	Percentage (%)
Palmitic		0.4
Palmitoleic		0.3
Stearic		1.1
Oleic		18.1
Linoleic		9.3
Linolenic		14.0
Arachidic		0.2
Gadoleic		1.2
Lesquerolic		55.4

Because the structure of lesquerolic acid is homologous to that of ricinoleic acid, the chemical modifications of lesquerolic acid, thus far have closely duplicated derivatizations of ricinoleic acid (**Figure 8**).

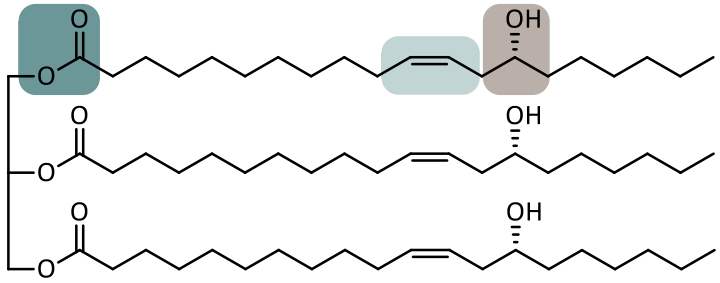


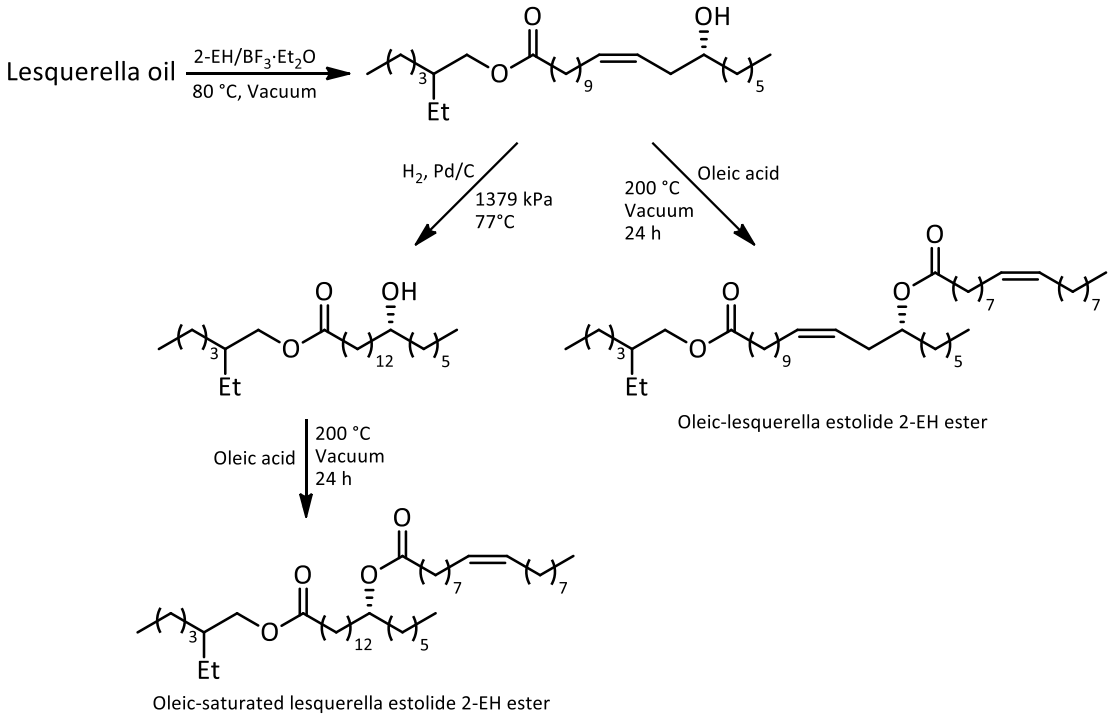
Figure 8. Chemical structure of lesquerolic acid tryglyceride. The functionality is marked with colours: ester linkage (green), double bond (blue) and hydroxy group (grey).

1.2.3. Applications of lesquerella oil and its derivatives

- *Lubricants*

A number of lesquerella derivatives and products have been synthesized utilizing the hydroxy moiety of the oil with a particular focus on the development of estolides. Hydroxy fatty acids such as lesquerella can be readily converted to estolides[74] either as triglycerides in the presence of free fatty acid or from homopolymerization of the split fatty acids. Lawate[75] synthesized triglyceride estolides from lesquerella oil by capping the hydroxy moiety with heptanoic, isostearic, adipic and fumaric acids. The fumaric acid capped estolides had the largest impact on viscosity where a 5% mixture of estolides in the high oleic vegetable oil increased the solution viscosity by 23%. Mono and fully capped triglyceride estolides were synthesized from lesquerella oil by condensation with a range of (C2–C18) fatty acid capping groups and their physical properties.[76,77] Lesquerella oil has a pour point of −21 °C with a 40 °C viscosity of 127.7 cST. The high viscosity of the oil precludes the pure oil from a number of lubricant applications. The pour points of the estolides were significantly reduced in comparison to the parent oil where a C6:0 fully capped estolide had a pour point of −36 °C and a viscosity of 87.9 cSt. These materials may find suitable applications as gear lubricants or as thickening agents in other base oils.

Further improvements in the physical properties of the oil can be made by synthesizing estolides directly from lesquerella fatty acids.[78] Oleic and 2-ethylhexanoic acid (2-EH) capping groups gave excellent pour points of −48 °C and −54 °C, respectively as their 2-ethylhexyl esters (**Scheme 6**). The viscosities of these estolides are sufficiently low enough to meet many lubricating applications that the triglyceride estolides and the parent oil could not meet. Lesquerella estolides have been synthesized using clays and enzymes as catalysts. [79,80]

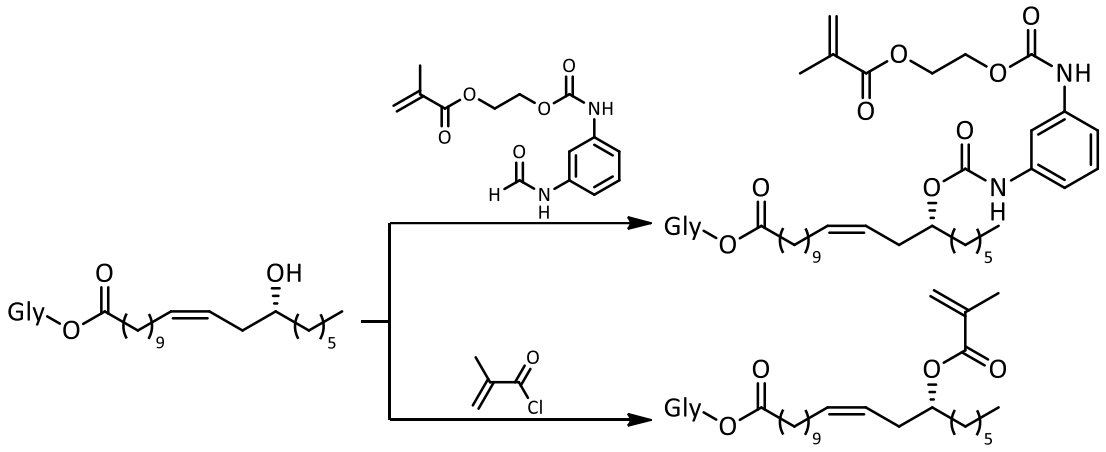


Scheme 6. Reaction scheme for the formation of lesquerella estolides.

- *Coatings*

Thames *et al.*[81-83] have reported the application of lesquerella oil for industrial coating purposes. Novel polyesters containing lesquerella oil have been synthesized and characterized. Moreover, the functional lesquerella oil polyesters have been used in the preparation of polyester-polyurethanes that have subsequently been evaluated for their performance as coating compositions of high quality. Thames and Yu[82] reported the synthesis, characterization, and application of lesquerella oil and its derivatives in water-reducible coatings. Polyesters of acid values approaching 50 were synthesized from lesquerella oil. Aqueous solutions of the polyesters were prepared and used as ingredients in the formulation of industrial melamine-polyester baked coatings and air-dried polyester coatings.

Polyesters obtained from lesquerella oil have variations in colors and have low solution viscosity as compared with castor oil polyesters. The viscosity variations are usually associated with the structural differences of the oil. Solution viscosity of castor oil-based polyesters is usually high because ricinoleic acid in castor oil allows extensive hydroxyl hydrogen bonding. Furthermore, lesquerella oil-based polyesters are effectively plasticized because of longer C20 fatty acid. The hydroxy group of lesquerella oil can be exploited to make acrylates (**Scheme 7**).[84]



Scheme 7. Synthesis of lesquerella oil acrylates.

Lesquerella oil acrylates impart excellent gloss to wood, aluminum, and steel and have good adhesive properties. The hydroxy groups of lesquerella and castor oil also react with cycloethers such as propylene oxide, epichlorohydrin, and ethylene oxide. As a result of these reactions, novel polyhydroxy compounds of much improved reactivity can be obtained. Epichlorohydrin-modified lesquerella oil has increased reactivity characteristics. The coatings developed from epichlorohydrin and lesquerella oil form harder films in shorter dry time (**Figure 9**).[84]

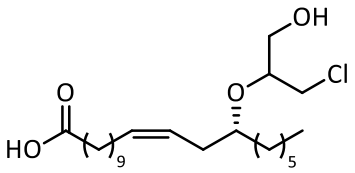


Figure 9. Epichlorohydrin modification of lesquerella oil.

- *Hydrolysis*

As in the case of ricinoleic acid, hydrolysis of lesquerolic acid by addition of a sodium hydroxide solution leads to the formation of 1,12-dodecanedioic acid. This compound is the major ingredient in the synthesis of nylon 12,12 and nylon 6,12 and other molded plastics.[85]

2. REFERENCES

(1) Hoffmann, G. In *The Chemistry and Technology of Edible Oils and Fats and Their High Fat Products*; Taylor, S.L., Ed.; Academic Press Limited: London, 1989, p 1-28.

(2) Gunstone, F. D., The AOCS Lipid Library. http://lipidlibrary.aocs.org/ (Accessed 17 May 2018).

(3) Gunstone, F. D. *Topics in lipid chemistry*; Gunstone, F.D., Ed.; Logos: London, 1970; Vol. 1.

(4) Xia, Y.; Larock, R. C. *Green Chem.* **2010**, *12*, 1893-1909.

(5) Stevens, C.; Verhé, R. In *Renewable Bioresources: Scope and Modification for Non-Food Applications*; Stevens, C., Verhé, R., Eds.; Wiley: West Sussex, 2004, p 208-250.

(6) Andes, L. E. *Vegetable Fats and Oils: Their Practical Preparation, Purification, Properties, Adulteration and Examination.*; 4 ed.; Stocks, H. B., Ed.; Scott, Greenwood & son: London, 1925.

(7) Kirk-Othmer *Kirk-Othmer Encyclopedia of Chemical Technology*; Kirk-Othmer, Ed.; John Wiley & Sons: New York, 1979; Vol. 5.

(8) Alvesgaspar, Leaves and flowers of Ricinus communis (Castor oil plant). https://commons.wikimedia.org/wiki/File:Ricinus_March_2010-1.jpg (Accessed 27 May 2018).

(9) Indiamart, Castor oil seed. https://www.indiamart.com/proddetail/castor-oil-seed-15713926955.html (Accessed 27 May 2018).

(10) FAO, http://faostat.fao.org (Accessed 18 May 2018).

(11) Castoroil Inc., Comprehensive Castor Oil Report. www.castoroil.in/reference/report/report.html (Accessed 22 January 2015).

(12) Hargreaves, G. H.; Owen, L. N. *J. Chem. Soc,* **1947**, 753-756.

(13) Binder, R. G.; Applewhite, T. H.; Kohler, G. O.; Goldblatt, L. A. *J. Am. Oil Chem. Soc.* **1962**, *39*, 513-517.

(14) G. R. O'Shea Company. www.groshea.com (Accessed 20 May 2018).

(15) Alwaseem, H.; Donahue, C. J.; Marincean, S. *J. Chem. Educ.* **2014**, *91*, 575-578.

(16) Deshpande, D. P.; Haral, S. S.; Gandhi, S. S.; Ganvir, V. N. *Res. J. Engineering Sci.* **2012**, *1*, 2-7.

(17) Sreenivasan, B.; Kamath, N. R.; Kane, J. G. *J. Am. Oil Chem. Soc.* **1957**, *34*, 302-307.

(18) Furst, M. R. L.; Goff, R. L.; Quinzler, D.; Mecking, S.; Botting, C. H.; Cole-Hamilton, D. J. *Green Chem.* **2012**, *14*, 472-477.

(19) Sinadinović-Fišer, S.; Janković, M.; Borota, O. *Chem. Eng. Process. Process Intensif.* **2012**, *62*, 106-113.

(20) Grummitt, O.; Fleming, H. *Ind. Eng. Chem.* **1945**, *37*, 485-491.

(21) Mukherjea, R. N.; Saha, K. K.; Sanyal, S. K. *J. Am. Oil Chem. Soc.* **1978**, *55*, 653-656.

(22) Zhang, Q.; Sun, Y.; Zhi, L.; Zhang, Y.; Di Serio, M. *J Surfactants Deterg.* **2015**, *18*, 365-370.

(23) Bhowmick, D. N.; Sarma, S. A. N. *Ind. Eng. Chem. Prod. Res. Dev.* **1977**, *16*, 107-111.

(24) Nezihe, A.; Elif, D.; Özlem, Y.; Tunçer, E. A. *Ind. Eng. Chem. Res.* **2011**, *50*, 398-403.

(25) Berman, P.; Nizri, S.; Wiesman, Z. *Biomass Bioenergy* **2011**, *35*, 2861-2866.

(26) Shojaeefard, M. H.; Etgahni, M. M.; Meisami, F.; Barari, A. *Environ. Technol.* **2013**, *34*, 2019-2026.

(27) Panwar, N. L.; Shrirame, H. Y.; Rathore, N. S.; Jindal, S.; Kurchania, A. K. *Appl. Therm. Eng.* **2010**, *30*, 245-249.

(28) Meneghetti, S. M. P.; Meneghetti, M. R.; Wolf, C. R.; Silva, E. C.; Lima, G. E. S.; de Lira Silva, L.; Serra, T. M.; Cauduro, F.; de Oliveira, L. G. *Energy Fuels* **2006**, *20*, 2262-2265.

(29) Nayak, P. L. *J. Macromol. Sci. Polymer Rev.* **2000**, *40*, 1-21.

(30) Hatice, M.; R., M. M. A. *Eur. J. Lipid Sci. Tech.* **2010**, *112*, 10-30.

(31) Petrović, Z. S. *Polym. Rev.* **2008**, *48*, 109-155.

(32) Yenwo, G. M.; Manson, J. A.; Pulido, J.; Sperling, L. H.; Conde, A.; Devia, N. *J. Appl. Polym. Sci.* **1977**, *21*, 1531-1541.

(33) Kunduru, K. R.; Basu, A.; Haim Zada, M.; Domb, A. J. *Biomacromolecules* **2015**, *16*, 2572-2587.

(34) Petrović, Z. S.; Cvetković, I.; Hong, D.; Wan, X.; Zhang, W.; Abraham, T.; Malsam, J. *J. Appl. Polym. Sci.* **2008**, *108*, 1184-1190.

(35) Sperling, L. H.; Mishra, V. *Polym. Adv. Technol.* **1996**, *7*, 197-208.

(36) Nidhi, G.; K., S. A. *Polym. Int.* **1994**, *35*, 109-118.

(37) Rogovina, L. Z.; Dembo, A. T.; Skitanth Sharma, P. R.; Frisch, H. L.; Schulz, M. *Polymer* **2000**, *41*, 2893-2898.

(38) Yeping, Y.; Shuren, Y.; Xueyong, Z. *J. Appl. Polym. Sci.* **2003**, *88*, 1840-1842.

(39) Seniha Güner, F.; Yağcı, Y.; Tuncer Erciyes, A. *Prog. Polym. Sci.* **2006**, *31*, 633-670.

(40) Priest, G. W.; Mikusch, J. D. V. *Ind. Eng. Chem.* **1940**, *32*, 1314-1319.

(41) Terrill, R. L. *J. Am. Oil Chem. Soc.* **1950**, *27*, 477-481.

(42) Grummitt, O.; Marsh, D. *J. Am. Oil Chem. Soc.* **1953**, *30*, 21-25.

(43) Böeseken, J.; Hoevers, R. *Recl. Trav. Chim. Pays-Bas* **1930**, *49*, 1165-1168.

(44) Forbes, W. C.; Neville, H. A. *Ind. Eng. Chem.* **1940**, *32*, 555-558.

(45) Don, B. S. *J. Am. Oil Chem. Soc.* **1959**, *36*, 518-523.

(46) Achaya, K. T. *J. Am. Oil Chem. Soc.* **1971**, *48*, 758-763.

(47) Weiss, E. A. *Castor, Sesame and Safflower*; Barnes&Noble, Ed.; Leonard-Hill: London, 1971.

(48) Bishai, A. M.; Hakim, I. K. *Polym. Int.* **1995**, *36*, 315-324.

(49) Majumdar, S.; Kumar, D.; Nirvan, Y. P. S. *J. Coatings Technol.* **1998**, *70*, 27-33.

(50) Ogunniyi, D.; Njikang, G. *Pak. J. Sci. Ind. Res.* **2000**, *43*, 378-380.

(51) Shende, P. G.; Jadhav, A. B.; Dabhade, S. B. *Pigm. Resin. Technol.* **2002**, *31*, 310-314.

(52) Onukwli, O. D.; Igbokwe, P. K. *J. Eng. Appl. Sci.* **2008**, *3*, 161-165.

(53) Naughton, F. C. *J. Am. Oil Chem. Soc.* **1974**, *51*, 65-71.

(54) Rao, S. H.; Chari, K. S.; Aggarwal, J. S. *J. Sci. Ind. Res.* **1962**, 21D:89-92.

(55) Trivedi, R. K.; Vasishtha, A. K. *J. Am. Oil Chem. Soc.* **1988**, *65*, 1467-1469.

(56) Kulkarni, M. G.; Sawant, S. B. *Eur. J. Lipid Sci. Tech.* **2003**, *105*, 214-218.

(57) Rangarajan, S.; Palaniappan, N. P. *Ind. Eng. Chem.* **1958**, *50*, 1787-1788.

(58) Santhanam, N.; Balu, M.; Sreevatsan, S., Production and uses of key castor oil oleochemicals. Comprehensive castor oil report. www.castoroil.com (Accessed 17 March 2015).

(59) Anderson, D. In *Bailey's Industrial Oil and Fat Products*; Shahidi, F., Ed.; John Wiley& Sons: New Jersey, 2005, p 1-56.

(60) Domsch, A. *SÖFW J.* **1994**, *120*, 322-329.

(61) Hunting, A. L. L. *Cosmet. Toiletrics* **1981**, *96*, 29-34.

(62) Lligadas, G.; Ronda, J. C.; Galià, M.; Cádiz, V. *Polymers* **2010**, *2*, 440-453.

(63) Herzog, B.; Kohan, M. I.; Mestemacher, S. A.; Pagilagan, R. U.; Redmond, K. In *Ullmann's Encyclopedia of Industrial Chemistry*; Wiley&VCH Verlag GmbH & Co: Weinheim, 2013, p 25-50.

(64) Arkema, Rilsan-11. https://www.arkema.com/en/products/product-finder/product-viewer/Rilsan-Polyamide-11-Resin/ (Accessed 26 May 2018).

(65) Rollins, R. C.; Shaw, E. A. *The Genus Lesquerella (Cruciferae) in North America*; Rollins, R.C., Shaw, E.A., Eds.; Harvard University Press: Massachusetts, 1973.

(66) Arthur, S. B.; Gentry, H. S.; Quentin, J. *Econ. Bot.* **1962**, *16*, 95-100.

(67) Oil Seed Crops, Lesquerella Fendleri http://www.oilseedcrops.org/lesquerella/ (Accessed 27 May 2018).

(68) Dierig, D. A.; Thompson, A. E.; Nakayama, F. S. *Ind. Crops Prod.* **1992**, *1*, 289-293.

(69) Brahim, K.; Stumpf, D. K.; Ray, D. T.; Dierig, D. A. *Ind. Crops Prod.* **1996**, *5*, 245-252.

(70) Carlson, K. D.; Chaudhry, A.; Bagby, M. O. *J. Am. Oil Chem. Soc.* **1990**, *67*, 438-442.

(71) Jones, Q.; Wolff, I. A. *Econ. Bot.* **1960**, *14*, 56-68.

(72) Smith, C. R.; Wilson, T. L.; Miwa, T. K.; Zobel, H.; Lohmar, R. L.; Wolff, I. A. *J. Org. Chem.* **1961**, *26*, 2903-2905.

(73) Hayes, D. G.; Kleiman, R.; Phillips, B. S. *J. Am. Oil Chem. Soc.* **1995**, *72*, 559-569.

(74) Penoyer, C. E.; Fischer, W.; Bobalek, E. G. *J. Am. Oil Chem. Soc.* **1954**, *31*, 366-370.

(75) Lawate, S. S. Triglyceride oils thickened with estolides of hydroxy-containing triglycerides. US5427704A. January 28, 1994.

(76) Isbell, T. A.; Cermak, S. C. *J. Am. Oil Chem. Soc.* **2002**, *79*, 1227-1233.

(77) Isbell, T. A.; Lowery, B. A.; DeKeyser, S. S.; Winchell, M. L.; Cermak, S. C. *Ind. Crops Prod.* **2006**, *23*, 256-263.

(78) Cermak, S. C.; Brandon, K. B.; Isbell, T. A. *Ind. Crops Prod.* **2006**, *23*, 54-64.

(79) Burg, D. A.; Kleiman, R.; Erhan, S. M. Production of hydroxy fatty acids and estolide intermediates. US5380894A. March 1, 1991.

(80) Hayes, D. G.; Kleiman, R. *J. Am. Oil Chem. Soc.* **1995**, *72*, 1309-1316.

(81) Thames, S. F.; Bautista, M. O.; Watson, M. D.; Wang, M. D. In *Polymers from Agricultural Coproducts*; Fishman, Marshall L., Friedman, Robert B., Eds.; ACS Symposium Series 575; American Chemical Society: Connecticut, 1994, p 212-222.

(82) Thames, S. F.; Yu, H. B. *J. Coatings Technol.* **1996**, *8*, 63-67.

(83) Thames, S. F.; Yu, H.; Schuman, T. P.; Wang, M. D. *Prog. Organic Coat.* **1996**, *28*, 299-305.

(84) Narine, S. S.; Kong, X. In *Bailey's Industrial Oil and Fat Products*; Shahidi, F., Ed.; John Wiley & Sons, Inc.: 2005, p 279-306.

(85) Babb, D. A. *Adv. Polym. Sci.* **2012**, *245*, 315-360.

General Objectives

AIM AND SCOPE OF THIS THESIS

The aims of this thesis were to i) develop chemical modifications of homoallyl hydroxy fatty acids to obtain derivatives with industrial interest, ii) apply first selective oxidations using non-transition metal and transition metal oxidants to synthetize α,β-unsaturated carbonyl fatty acids; iii) investigate alternative methods energetically favorable for the oxidative cleavage of homoallyl hydroxy fatty acids into dicarboxylic acids and iv) design and synthetize new long chain *N*-vanillyl acylamides with biological response in pain receptors starting from homoallyl hydroxy fatty acids.

In the introduction of this thesis a general overview on the homoallyl hydroxy fatty acids, castor and lesquerella oil, is presented. Origin, abundance, production and market, chemical nature and physical-chemical characteristics are explained in detail. Then, the main chemical reactions that the homoallyl hydroxy fatty acids undergo are disclosed, emphasizing the large number of derivatives that can be obtained through them and their wide applications in several industries.

Chapter 1 contains the results, discussion and conclusions for the experiments on selective oxidation of homoallyl hydroxy fatty acids to α,β-unsaturated carbonyl fatty acids. Already available transition metal-free oxidants and oxidants based on transition metals are compared. Special emphasis is placed on the Oppenauer-type oxidation mediated by the Shvo's catalyst, a ruthenium(II) complex, in presence of easily reducible hydrogen acceptors. The combination of the Shvo's catalyst and acrolein is shown to be capable of oxidize homoallyl hydroxy fatty acids to α,β-unsaturated carbonyl fatty acids in a one-step and highly selective manner. The method involves a straightforward experimental procedure and allows for the facile isolation of the product, making it a convenient and readily applicable synthetic protocol for the lab-scale synthesis of α,β-unsaturated carbonyl fatty acids from homoallyl hydroxy fatty acids.

Chapter 2 presents the research done on the oxidative cleavage of α,β-unsaturated carbonyl fatty acids derived from ricinoleic acid to obtain sebacic acid, a dicarboxylic acid widely used in industry. A system based on Oxone™ and sodium periodate is capable to the oxidative cleavage of the α,β-unsaturated carbonyl compounds to sebacic acid without the use of transition metal reagents. This system allows to operate at milder reaction conditions and lower temperatures than the classical procedures used in industry. The applicability of ultrasound technology to this system is also included at the end of this chapter.

In **Chapter 3** the synthesis of new long chain *N*-vanillyl acylamides (LCNVAs) starting from homoallyl hydroxy fatty acids is presented. These new compounds are envisaged to evaluate their

"dual" biological activity in pain receptors (TRPV1 receptor and FAAH enzyme) and therefore, act as analgesics drugs. Based on structure-activity relationship studies of the natural agonists of these pain receptors, structural transformations over the homoallyl hydroxy moiety of the homoallyl hydroxy fatty acids are carried out. As a result, 22 new capsaicin-like compounds derived from homoallyl hydroxy fatty acids are synthetized.

Chapter 4 contains the biological evaluation of the new long chain *N*-vanillyl acylamides (LCNVAs) described in Chapter 3. These compounds are tested to determine the agonist behaviour for TRPV1 and the inhibitory potential in the FAAH enzyme with the target to discover new acyl templates with potential "dual" mechanism of action against pain. In addition, the activity results in other related TRP receptor such as TRPV2 and TRPA1 are included to assess their selectivity. The introduction of this chapter includes an explanation about the biological assays carried out to determine the activity in this type of receptors.

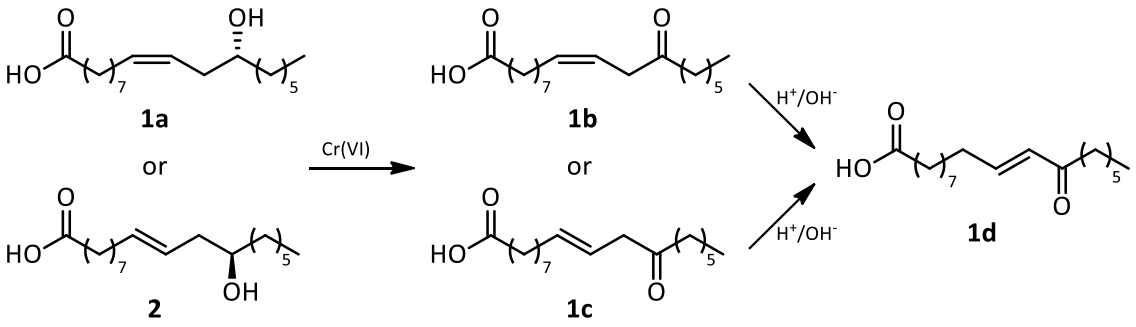
Chapter 1

Selective Oxidation of Homoallyl Hydroxy Fatty Acids: Synthesis of α,β-Unsaturated Carbonyl Fatty Acids

1.1. INTRODUCTION

α,β-Unsaturated carbonyl fatty acids are important building blocks in organic synthesis.[1,2] They are often reported as metabolites of oxidation of fatty acids produced by microorganisms or as by-products from the autooxidation of monounsaturated fatty acids but relatively rarely as natural fatty acids in their own right.[3-5]

The α,β-unsaturated carbonyl fatty acid from castor oil was firstly synthetized in 1950 by Ellis.[6] Based on the study of Kon *et al*.[7] Ellis described the oxidation of ricinoleic acid **1a** into (*E*)-12-oxo-10-octadecenoic acid **1d**. The general synthetic route used to synthesize the α,β-enone **1d** consists of two steps. First, the β,ϒ-unsaturated enones **1b** and **1c** are synthesized through chromium(VI)-mediated oxidation of ricinoleic **1a** or ricinelaidic acid **2**, respectively. The α,β-enone **1d** is in equilibrium with the β,ϒ-enones **1b** and **1c** due to the small energy differences among them.[8] A semiempirical quantum chemical calculation demonstrated that the heat formation of **1d** is only about 1 kcal/mol lower than the one of **1b**.[9] The catalytic treatment of unconjugated unsaturated ketones **1b** and **1c** with acids or bases causes a tautomeric change which results in the conjugation of the double bond with the carbonyl group.[10] $BF_3{\cdot}Et_2O$, H_2SO_4 or NaOH are the most common reagents employed to carry out the tautomeric change to the α,β-enone **1d** (**Scheme 1.1**).[9,11-15]



Scheme 1.1. General procedure to prepare the unsaturated ketones **1b**, **1c**, **1d** from ricinoleic acid **1a** and ricinelaidic acid **2**.

This synthetic route requires an excess of reagents such as CrO_3 or PCC, which generates large amounts of hazardous waste. Furthermore, the fact that it is a two-step synthesis and the poor selectivity due to the formation of by-products prompts to an unattractive process. All of these drawbacks lead to search for a synthesis more selective and efficient, which would allow the preparation of α,β-unsaturated carbonyl compounds in one-step from homoallyl hydroxy fatty acids. In the recent years, a variety of synthetically important oxidation methodologies based on greener reagents have been extensively developed.[16-19] Secondary alcohol oxidations can be classified

according to the nature of the oxidant as: (i) transition metal-free oxidations or (ii) transition metal-mediated oxidations.

This chapter was focused on the development of an alternative one-step synthetic route to synthetize α,β-unsaturated carbonyl fatty acids. For this, selective oxidations based on non-transition metal and transition metal oxidants were assessed in the oxidation of homoallyl hydroxy fatty acids.

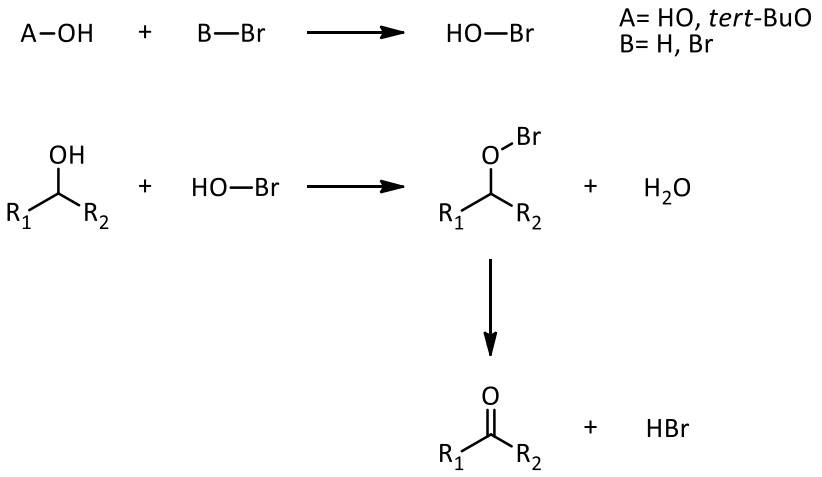
1.2. TRANSITION METAL-FREE ALCOHOL OXIDATIONS

The development of catalytic synthetic methodologies using clean oxidants is an area of current interest with a view to replace stoichiometric oxidants, which are unreasonable from both economic and environmental point of views. Increasing emphasis is being placed toward the development of transition metal free synthetic methodologies to avoid the use of toxic and expensive metals and their complexes. Oxidation of secondary alcohols to ketones have been performed using dimethyl sulfoxide,[20] dicyclohexylcarbodiimide and various acids,[21] dimethyl sulfoxide activated with oxalyl chloride,[20] sodium hypochlorite in acetic acid,[22] $NaBrO_3$-NH_4Cl,[23] *N*-chlorosuccinimide with catalytic amounts of *N*-*tert*-butylbenzecensulfenamide[24]. Among the clean oxidants, the hydroperoxides such as aqueous hydrogen peroxide and *tert*-butyl hydroperoxide (TBHP) have a prominent position.[25,26] Both of them are relatively stable, easy to store, commercially available, relatively cheap reagents of low molecular weight that can be used for both laboratory and industrial purposes. They contain relatively high amounts of active oxygen (H_2O_2 – 47% and TBHP – 17.8% weight), and are environmentally friendly since the products of their reduction are water and *tert*-butanol, respectively.

The use of hydrogen peroxide and TBHP without an activator or catalyst is very limited.[27,28] In alkaline medium, hydroperoxides act as nucleophiles, because formed peroxide anion ROO^- can react with electron-deficient substrates.[29] On the other hand, relatively strong carboxylic acids such as formic, succinic and trifluoroacetic react with hydrogen peroxide producing peroxycarboxylic acids. Peroxycarboxylic acids oxidize simple alkenes, alkenes carrying a variety of functional groups (such as ethers, alcohols, esters, ketones and amides), some aromatic compounds, furans and *N*-azaheterocycles, sulfides and amines.

In 2003, Neumann *et al*.[30] used a combination of hydrogen peroxide and hydrohalic acid as a green halogenating agent. This inspired Sain *et al*.[31] to explore the potential of this transition metal-free system for oxidation of secondary alcohols. Hydroperoxides coordinates the proton of hydrohalic acids and HO^+ cations are produced. The plausible mechanistic pathway for these

reactions may involve the formation of hypobromous acid by the reaction of peroxide oxidant with hydrobromic acid,[31] and its reaction with secondary alcohol to afford hypobromite species that promotes on abstraction of hydrogen yielding the corresponding ketone as shown in **Scheme 1.2**.



Scheme 1.2. Mechanistic pathway of secondary alcohol oxidation by hydroperoxides-HBr system proposed by Sain.[31]

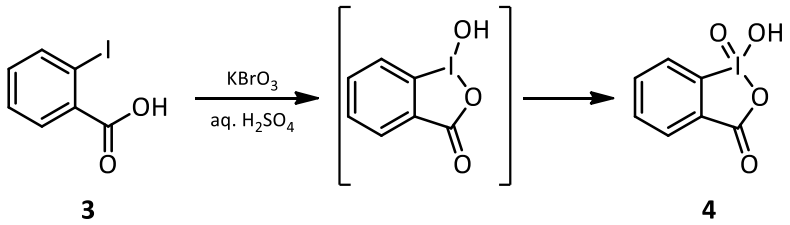
The oxidation of a range of activated and non-activated secondary alcohols to ketones in absence of organic solvent was achieved with this method. Moreover, the oxidation of secondary alcohols was selective in presence of primary alcohols and other functional groups.

The main drawback of the use of hydroperoxides is the instability of the peroxide bond. The decomposition of hydroperoxides liberates oxygen and heat; this can be dangerous, as spilling high-concentration hydrogen peroxide on a flammable substance can cause an immediate fire. For this reason, in recent years the use of solid peroxy compounds, such as sodium perborate (SPB) and sodium percarbonate (SPC) has gained considerable attention in various chemical transformations[32-35] due to their storage stability, crystalline nature, ease of handling, and higher hydrogen peroxide contents. Solid peroxy compounds can be used in organic synthesis as a convenient source of anhydrous H_2O_2, in particular in solvents that cannot dissolve the carbonate but can leach the H_2O_2 out of it. In presence of acids, SPB and SPC are able to successfully oxidize secondary alcohols to ketones under mild conditions.[36]

In the past two decades, bismuth(III) derivatives such as $BiCl_3$, $BiBr_3$ or Bi_2O_3 have attracted growing interests as versatile catalysts in diverse organic synthesis owing to their remarkable chemical and physical properties such as relevant stability, air- and moisture-tolerance and low toxicity. Chakraborty and Malik have deeply investigated about the oxidizing character of Bi_2O_3 in

presence of *tert*-butyl hydroperoxide.[37,38] Primary alcohols and aldehydes are oxidized to carboxylic acids using this mild catalytic system. In addition, secondary alcohols are easily converted to the corresponding ketones. This system shows sufficient selectivity to enable the expected oxidation to take place without affecting other functionalities like phenol and amine groups.

Hypervalent iodine compounds have also been focus of great attention due to their mild and chemoselective oxidizing properties and their environmentally benign character in contrast to toxic metal reagents.[39-41] One of the most representative hypervalent iodine compounds is 2-iodoxybenzoic acid (IBX, **4**). Santagostino was the first to report the use of **4** in DMSO for alcohol oxidation in 1994.[42] IBX oxidations are easily conducted in DMSO solution at room temperature, with yields ranging from good to quantitative. IBX **4** was first synthesized in 1893 by the treatment of 2-iodobenzoic acid (IBA, **3**) with potassium bromate and sulfuric acid (**Scheme 1.3**).[43]



3 **4**

Scheme 1.3. Typical synthesis of IBX **4**.

Santagostino also developed an alternative one-step synthesis of IBX **4** using Oxone™.[44] This allowed the elimination of bromate or other impurity present in the reagent, which might cause some explosions when using IBX **4**.[45] Oxone™ is a triple salt with the formula $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$. It is an incredibly inexpensive reagent, which compares favourably with hydrogen peroxide.[46] The reaction of 2-iodobenzoic acid **3** with Oxone™ in hot water gives **4**. This finding inspired Vinod and co-workers who demonstrated the first catalytic use of **4** in the presence of Oxone™ as a co-oxidant for the oxidation of primary and secondary alcohols in aqueous acetonitrile.[47] Secondary alcohols were oxidized to the corresponding ketones. Indeed, the presence of Oxone™ avoided the formation of Bayer–Villiger oxidation products.

1.3. TRANSITION METAL-MEDIATED ALCOHOL OXIDATIONS

1.3.1. Heterogeneous oxidations

1.3.1.1. $KMnO_4/CuSO_4·5H_2O$ system

The $KMnO_4$-promoted oxidation process is recognized as friendly to the environment because manganese dioxide, MnO_2, the coproduct formed by the reduction of $KMnO_4$, can be recycled.[48] In 1977, Regen and Koteel communicated their discovery that molecular sieves impregnated with $KMnO_4$ oxidize alcohols efficiently in benzene at 70 °C.[49] Two years later, Menger and Lee reported that secondary alcohols in particular can be transformed, even more efficiently, into the corresponding ketones upon treatment with $KMnO_4$ absorbed on solid copper(II) sulfate pentahydrate ($CuSO_4·5H_2O$) in refluxing benzene.[50] Since then, several papers have appeared presenting $KMnO_4$ dispersed copper(II) sulfate pentahydrate as a green oxidant of alcohols.[51,52] These reactions are mostly assisted by microwave radiation. Based on this, Luu and co-workers demonstrated that the $KMnO_4/CuSO_4·5H_2O$ system (PP/4CSP) was able to oxidize several secondary alcohols, which under solvent-free reaction conditions and using microwave irradiation are smoothly converted to the corresponding ketones in high yields.[53]

1.3.1.2. Metal oxides

Metal oxides are able to catalyse alcohol oxidation in order to produce highly selective products using mild oxidants like H_2O_2 and TBHP.[54] Nickel oxide hydroxide has been known to oxidize a host of organic substrates such as alcohols, aldehydes, phenols, amines, and oximes. However, an extensive review covering the major part of these reactions demonstrated the requirement of stoichiometric amounts of nickel oxide hydroxide (1-1.5 equiv. of nickel).[55] Recent findings have led to a new oxidation method utilizing catalytic amounts of nickel(II) salts and excess of bleach (5% aqueous sodium hypochlorite) under ambient conditions.[56] This system is compatible with allylic double bounds.

Manganese dioxide very soon became a widely used standard oxidant for the transformation of allylic alcohols into ketones. It offers very mild conditions and is extremely selective for allylic alcohols when it is not employed at a high temperature. During the oxidation of alcohols with active MnO_2, water is produced that can partially inactivate the MnO_2 or generate a brown mud. In 2007, Zárraga and Sánchez described a green method to oxidize alcohols in mild conditions using water as

solvent.[57] The treatment of $MnSO_4$ with Oxone™ generates active MnO_2 in the reaction media, which leads the oxidation of amines, alcohols and benzylic methyl groups.

Magtrieve™ is a trademark of DuPont for the oxidant based on tetravalent chromium dioxide (CrO_2).[58] Magtrieve™ is a cheap ($1/g), nontoxic, easily disposed chromium(IV) oxide that can be readily removed from the reaction due to its heterogeneous nature and ferromagnetic properties. Reactivity is comparable to activated manganese dioxide,[59] but in many cases the yields are higher, the reactions are cleaner and the work-up dramatically simpler with Magtrieve™. The reduced trivalent chromium oxyhydroxide surface can be reconverted to CrO_2 by heating in air at 300-350 °C for 1-2 h, thus providing a chance to avenue for recyclability and cost-effectiveness. Since CrO_2 is a magnetically retrievable material, it makes it a very well suited reagent for microwave synthesis. Bogdal and coworkers demonstrated that Magtrieve™ carries a benefit of efficient conversion of electromagnetic energy in heat according to the dielectric heating mechanism.[60] In an experiment, the irradiation of Magtrieve™ (2.5 g) with a continuous power of microwave reactor (30%) in an open vessel (5 cm diameter) led to quick heating of the material up to 360 °C within 2 min. They recorded the temperature by means of a thermovision camera and the center of the reaction vessel showed the highest value as it was expected (a, **Figure 1.3**). This drawback was solved using toluene as solvent. In the presence of toluene (15 mL), which is a weak microwave absorber, the temperature of Magtrieve™ reached ca. 140 °C within 2 min and was more uniformly distributed (b, **Figure 1.3**).

a) b)

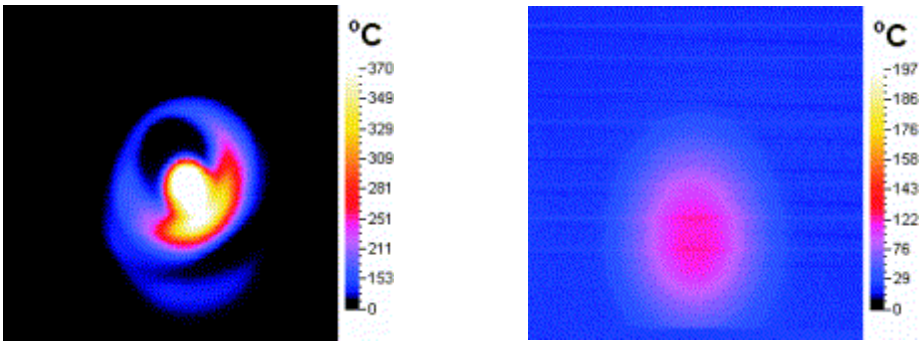


Figure 1.3. The temperature profile for Magtrieve™ irradiated by microwaves *vs* the temperature profile for Magtrieve™ in a toluene solution irradiated by microwaves (both 2 min of irradiation).[60]

Magtrieve™ allows the oxidation of alcohols to the carbonyl compounds with satisfactory yield and in a short time. The primary alcohols were converted to the desired aldehydes without over oxidizing them to carboxylic acids. The reactivity of the secondary alcohols seemed to be slightly lower and the addition of higher amount of the oxidant did not increase the yield of desired ketones.

The advantages of this procedure include a simple reaction work-up because of the magnetically retrievable oxidant.

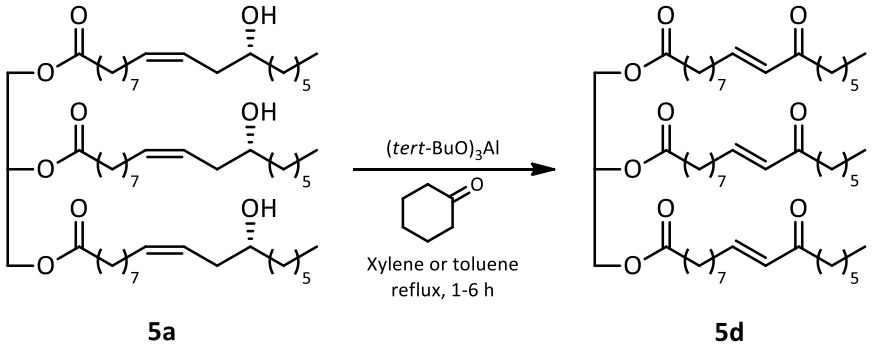
1.3.1.3. Metallic nitrates

Metallic nitrates can be used as mild oxidizing agent, but little attention has been paid to this potential in organic synthesis. Laszlo and Cornelis, have reported on appreciably unstable Fe(III) nitrate impregnated on K10 bentnite clay, as a supported metal nitrato complex for the oxidation of alcohol to carbonyl compound.[61,62] They have reported that, satisfactory results have been obtained with a variety of secondary aliphatic, alicyclic, and benzylic alcohols, and with benzyl alcohols. With primary aliphatic alcohol, complex mixtures of products were obtained. In 1997, a report by Varma and Dahiya outlined that clayfen can oxidize alcohols upon exposure to microwaves under solvent-free conditions.[63] Ceric ammonium nitrate (CAN) is one of the widely used oxidizing agents. Oxidation of alcohols with CAN was first mentioned in the 1960s. It was found that benzylic alcohols can be oxidized to aldehydes,[64] pentan-1-ol to 2-methyltetrahydrofuran,[65] and cyclopropylmethanol, to cyclopropanecarbaldehyde.[66] Depending on the structure, secondary alcohols can either be oxidized to ketones or gave aldehydes due to the C—C(OH) bond cleavage.[67] All the reactions with participation of CAN and other Ce(IV) salts were carried out in solutions of acetonitrile, water, methanol, or acetic acid. In 2007, Kapustina *et al*.[68] easily oxidized 2-octanol to 2-octanone upon the solvent-free contact with CAN.

Nishiguchi and Asano supported some metal nitrates on silica gel and used those as oxidizing agents for the oxidations of benzylic alcohols under nitrogen atmosphere in 1988.[69] The activity of several supported metallic nitrates such as $Cu(NO_3)_2$, $Zn(NO_3)_2$, $Fe(NO_3)_3$ or $Co(NO_3)_3$ was evaluated in the oxidation of cyclododecanol using CCl_4 or *n*-hexane as solvent. The oxidation with metallic nitrates supported in silica can be accelerated by microwave irradiation and used under solvent conditions. Rafati and co-workers selectively oxidized alcohols to carbonyl compounds using supported cobalt nitrate hexahydrate in silica gel.[70] This oxidizing agent was stable and could be stored in air at room temperature without losing activity. It is noteworthy that cleavage of double bonds of α,β-unsaturated alcohols were not observed. The absence of silica was detrimental to yield. As previous reports,[62] it is probable that cobalt nitrate hexahydrate supported on silica gel is a source of nitrosonium ions and the oxidation reaction presumably proceeds via the formation of nitrous esters.

1.3.2. Homogeneous oxidation: Oppenauer-Type Oxidation mediated by Shvo's catalyst

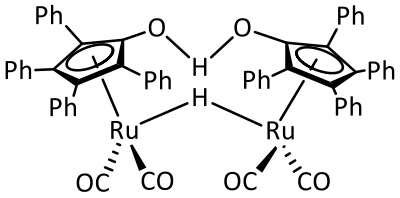
Homogeneous catalyst has been extensively used in the oxidative process for the manufacturing of bulk as well as fine chemicals. This is because of its efficiency in bringing huge influences in chemical conversion via the same phase catalysis reaction.[71] The development of efficient and selective homogeneous catalysts contributes to decreasing the environmental impact of oxidation by making possible the use of more environmentally acceptable oxidizing agents such as H_2O_2 or O_2 instead of hazardous and toxic stoichiometric reagents.[72,73] In this regard, the hydrogen transfer type oxidation of alcohols[74,75] is a very appealing oxidant free process that is attracting interest for the synthesis of carbonyl compounds. In 1958, Nichols described the Oppenauer oxidation of castor oil **5a** using aluminium *tert*-butoxide as oxidant and cyclohexanone to produce α,β-unsaturated ketones (**Scheme1.4**).[76] This process is based on a hydrogen transfer reaction.



Scheme 1.4. Oppenauer oxidation of castor oil **5a** carried out by Nichols.[76]

Oppenauer oxidations with non-transition metals need stoichiometric quantities of the alkoxide and it is not a catalytic cycle thus, they produce substantial amounts of by-products and generate environmentally undesired salts during work up.[77] The use of transition metals (palladium, iridium and rhodium) to catalyse transfer hydrogenation reactions is a familiar concept, with examples from as early as the 1950s.[78] Nevertheless, ruthenium catalysts have contributed significantly to the recent development of such oxidation reactions.[79-82] One of the most important ruthenium-based catalysts is the Shvo's catalyst **6**.[83-86]

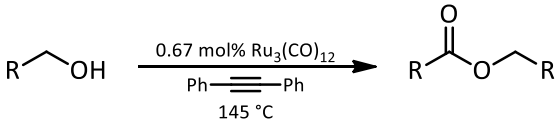
Shvo's catalyst **6**, $\{[(\eta^5\text{-}Ph_4C_4COH)(CO)]H_2\}Ru_2(CO)_4(\mu\text{-}H)$, is a hydroxycyclopentadienyl ruthenium dimer bearing a bridging hydride ligand that which was first synthesized in 1984 by Shvo *et al*. (**Figure 1.4**).[85,87]



6

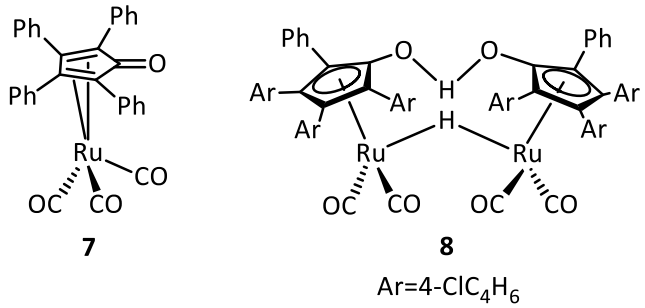
Figure 1.4. The Shvo's catalyst **6**.

In preliminary studies Blum and Shvo showed that solutions of $Ru_3(CO)_{12}$ exhibited catalytic activity for the disproportionation of primary alcohols to esters and that the catalytic activity was highest rather than ketones or aldehydes when diphenylacetylene was added as the hydrogen acceptor (**Scheme 1.5**).[87]



Scheme 1.5. The ruthenium-catalysed formation of esters from alcohols.

Eventually the Shvo's catalyst **6**, which was originally incorrectly assigned as $[(\eta^4\text{-}Ph_4C_4CO)(CO)_2Ru]_2$, and a related complex, **7**, were isolated from the reaction mixture and found to be catalysts for the formation of esters from alcohols.[87,88] Re-examination of the ^{1}H NMR spectrum of the isolated ruthenium dimer revealed a resonance at -17.75 ppm indicative of a hydride ligand.[85] This prompted a more detailed examination of the catalyst so the X-ray crystal structure of analogous complex **8** was determined and revealed the bridging hydride and hydroxyl proton (**Figure 1.5**).



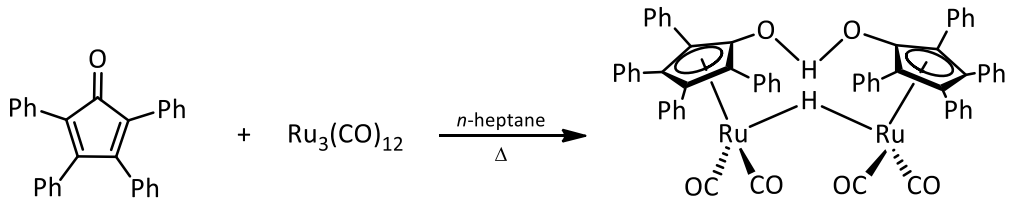
7 **8**

Ar=4-ClC_4H_6

Figure 1.5. Complexes synthesized by Shvo.

Complex **7** had been synthesised previously[89] and the cyclopentadienone ligand is formed by a [2+2+1] cycloaddition reaction between 2 molecules of diphenylacetylene and 1 molecule of CO mediated by the ruthenium cluster.[87,89,90] Complex **8** was found to serve as a precursor to the Shvo's catalyst **6**, the latter complex being formed upon reaction with aqueous sodium carbonate.[91] The

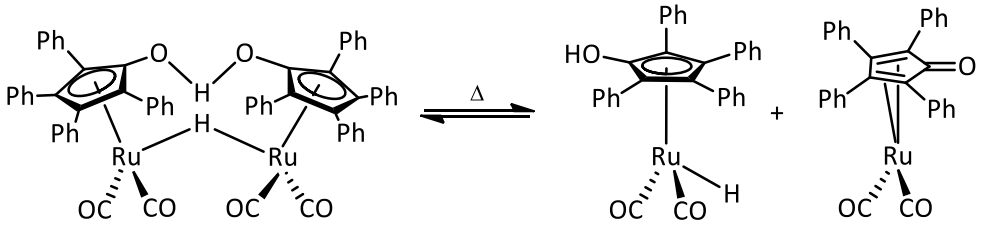
dimeric ruthenium complex {[(η^5-Ph_4C_4COH)(CO)]H_2}$Ru_2(CO)_4$(μ-H) **6** can also be synthesized directly by reacting $Ru_3(CO)_{12}$ with tetracyclone **9** as shown in **Scheme 1.6**.[92,93]



9 **6**

Scheme 1.6. Synthesis of {[(η^5-Ph_4C_4COH)(CO)]H_2}$Ru_2(CO)_4$(μ-H) **6** from $Ru_3(CO)_{12}$ and tetralone **9**.

The Shvo's catalyst **6** is known to catalyse a number of processes including the reduction of ketones, imines, alkenes and alkynes, the Oppenauer-type oxidation of alcohols and the dynamic kinetic resolution of alcohols and amines among others.[84,86] The dimer **6** is activated by heat and dissociates into the two catalytically active monomers, one isolable Ru(II) 18 electron complex **6a** and one proposed Ru(0) 16 electron complex **6b** (**Scheme 1.7**). The 18 electron species **6a** acts as a hydrogenation catalyst, whereas the 16 electron species **6b** acts as a dehydrogenation catalyst.[80,83]



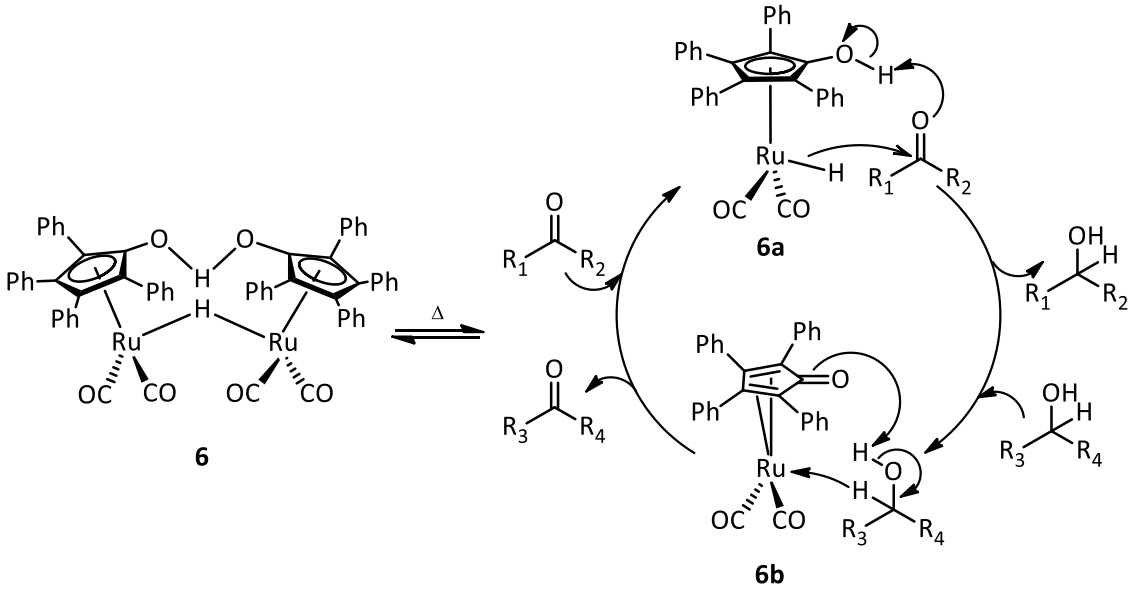
6 **6a** **6b**

Scheme 1.7. Thermal activation of Shvo's catalyst **6** in monomers **6a** and **6b**.

1.3.2.1. Proposed mechanisms

- *The outer-sphere mechanism*

The hydrogenation of ketones and the dehydrogenation of alcohols mediated by Shvo's catalyst **6** have been proposed to operate through a concerted *outer-sphere* mechanism, i.e. the proton and the hydride are transferred to the substrate simultaneously.[83,94] The pendant ketone functionality of **6b** attacks the hydroxy proton of the substrate and the metal centre abstracts the α-proton in a concerted manner to give the oxidised product and another molecule of **6a**. Complex **6a** donates a proton and hydride to the hydrogen acceptor to reform **6b** and complete the catalytic cycle. Although **6b** is too reactive to be isolated, its existence has been inferred from trapping experiments with triphenylphosphine (**Scheme 1.8**).[87,95]

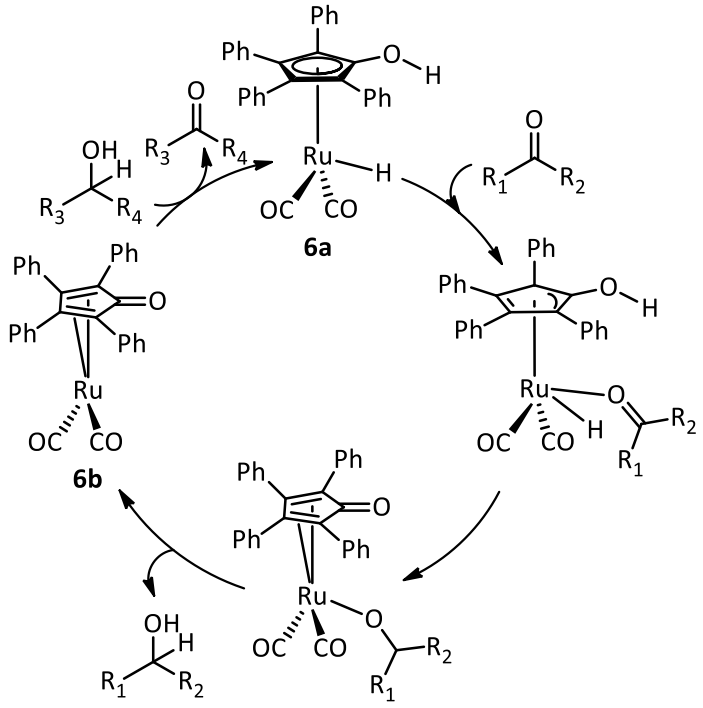


Scheme 1.8. The *outer-sphere* mechanism for the oxidation of alcohols by the Shvo's catalyst **6**.

The reaction mechanism for the hydrogen-transfer process, however, is a matter of controversy. Studies by Casey by means of primary deuterium isotope effects on the hydrogenation of PhCHO *via* the active reducing form **6a** of the Shvo tolyl analogue catalyst, [2,5-Ph_2-3,4-Tol_2(η^5-C_4COH)]Ru$(CO)_2$H, concluded that carbonyl hydrogenation is concerted without substrate coordination (*outer-sphere* mechanism, **Scheme 1.8**),[83] this conclusion was also supported by DFT calculations performed by the same authors.[96]

- *The inner-sphere mechanism*

Bäckvall, using a similar methodology, also reported a concerted mechanism for alcohol dehydrogenation using species **6b**.[94] Nevertheless, although there is agreement in that catalytic reaction using the Shvo's catalyst **6** is concerted, Bäckvall suggested that the substrate coordinates the metal *via* a $\eta^5 \rightarrow \eta^3$ ring slippage of the aromatic ligand,[80,94] followed by a simultaneous β-hydride addition and a proton transfer to the unsaturated organic substrate (**Scheme 1.9**).



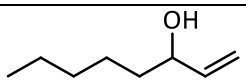
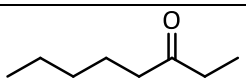
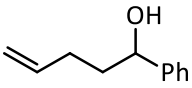
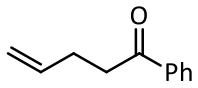
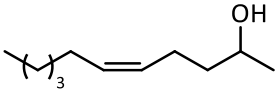
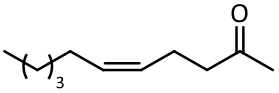
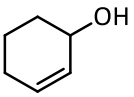
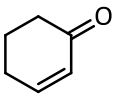
Scheme 1.9. The *inner-sphere* mechanism for ketone reductions by the Shvo's catalyst **6**. One of several possible ring-slips is shown.

This mechanism involves the coordination of the ketone prior to reduction therefore a ring-slip of the hydroxycyclopentadienyl ligand **6a** is necessary to provide a vacant coordination site. Alternatively the temporary loss of a CO ligand could also take place to provide a vacant coordination site, however this is unlikely since Casey demonstrated that CO exchange with ^{13}CO is a slow process.[83]

1.3.2.2. Influence of the hydrogen acceptor in the oxidation of secondary alcohols

Bäckvall *et al*.[97] developed highly efficient ruthenium-catalyzed Oppenauer-type oxidations of secondary alcohols to ketones using the Shvo's catalyst **6**. With catalyst loadings from 0.1-0.5% in refluxing acetone, a range of secondary alcohols including aliphatic, allylic and benzylic alcohols were oxidised (**Table 1.1**).

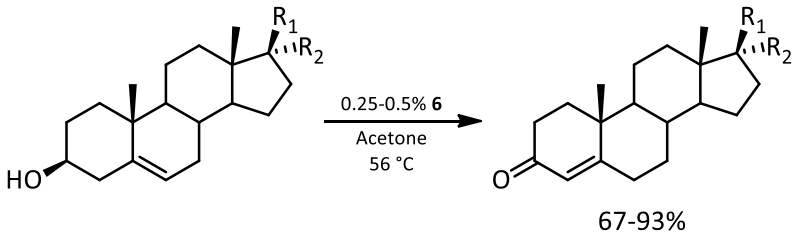
Table 1.1. Oxidation of unsaturated secondary alcohols using Shvo's catalyst **6** carried out by Bäckvall.[a]

Entry	Substrate	Time (h)	Product	Yield (%)[b]
1		24		96[c]
2		24		73
3		24		74
4		4		76

[a]The oxidations were carried out in refluxing acetone.
[b]Isolated yield.
[c]Determined by GC.

The results obtained with the Shvo's catalyst **6** were worthy for the oxidation of aliphatic alcohols. Despite the cyclic allyl alcohol gave the α,β-unsaturated ketone in a good yield (entry 4), the allyl alcohol was quantitatively converted to the saturated ketone (entry 1).

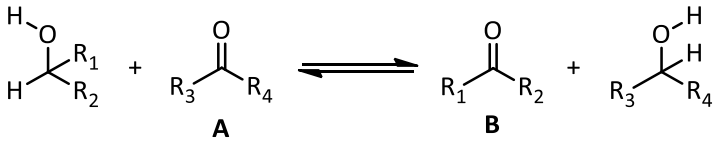
This method was also applied to the oxidation of 5-en-3β-hydroxysteroids.[98] Substrates with various different pendant functionalities were tested and the expected 4-en-3-ones were obtained in yields of 67-93% (**Scheme 1.10**). This method significantly improves upon a previously developed catalytic system for the oxidation of homoallyl alcohols using the Shvo's catalyst **6** instead of the use of 2,6-di-*tert*-butylbenzoquinone and MnO_2 as the terminal oxidant in which only poor conversions were obtained for steroidal substrates.[99]



Scheme 1.10. Ruthenium-catalyzed oxidation of steroidal 5-en-3β-ols to 4-en-3-ones.

As it can be observed, acetone is an excellent hydrogen acceptor in the oxidization of allyl and homoallylic cyclic secondary alcohols to ketones. However, when linear allyl alcohols are oxidized, acetone cannot avoid the reduction of the double bond and thus, the saturated ketones are obtained.

Insight into the thermodynamics of the hydrogen transition-metal transfer equilibria may be helpful in selecting either the reductant or the oxidant properly and in determining the quantity of oxidant. The equilibrium of the Oppenauer-type oxidation can be estimated from the redox potentials of the two carbonyls **A** and **B** (**Scheme 1.11**).[100,101]



Scheme 1.11. The equilibrium can be calculated from the redox potentials of **A** and **B**.

Adkins and Cox have determined redox potentials for a wide range of carbonyl compounds.[102] Carbonyl compounds with high redox potentials like aromatic and aliphatic aldehydes are particularly suitable as oxidants in Oppenauer oxidations. An increase in conversion can be realized by applying: (i) a hydride acceptor with a higher redox potential than the product; (ii) a larger amount of oxidant, which is often achieved by using the oxidant as solvent; (iii) the selective removal of the resulting alcohol or product by evaporation.[103]

Acetone in combination with benzene as solvent was used exclusively by Oppenauer in his original studies and this ketone has remained one of the most widely used hydrogen acceptors.[104] In spite of its undesirable low oxidation potential (0.129 V) acetone is often selected since it is cheap and can be used in large excess. Even its condensation product (mesityl oxide) can be removed fairly readily. Cyclohexanone not only has a higher oxidation potential (0.162 V) than acetone but a higher boiling point, which allows a shorter reaction time. Cyclohexanone is also readily available and is particularly useful in steroid oxidation since it can be removed from the reaction products by steam distillation. Aldehydes have only rarely been used as hydrogen acceptors. The Tishchenko condensation of them and the tendency for condensation of the resulting products hamper the processes.[105]

1.4. OBJECTIVE

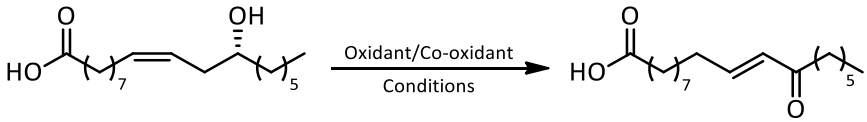
The main objective of this chapter was to carried out selective oxidations of homoallyl hydroxy fatty acids to synthetize α,β-unsaturated carbonyl fatty acids. For this, two types of oxidants were employed. Firstly, transition metal-free oxidations were studied to evaluate their ability to transform ricinoleic acid **1a** into its corresponding α,β-unsaturated keto isomer **1d**. Secondly, the oxidation of ricinoleic acid **1a** mediated by transition metals was attempted. On the one hand, several heterogeneous catalysts were assessed. On the other hand, Oppenauer-type oxidation in presence of several easily reducible hydrogen acceptors has been investigated. Finally, acids, esters and even the triglycerides of the castor and lesquerella oil were submitted to oxidation under the optimal conditions in order to isolate the corresponding α,β-unsaturated carbonyl fatty acids.

1.5. RESULTS AND DISCUSSION

1.5.1. Transition metal-free homoallyl hydroxy fatty acid oxidations

We chose ricinoleic acid **1a** as starting material for optimising the reaction conditions (**Table 1.2**). In carrying out our optimisation studies, we focused our attention on the choice of the transition metal-free oxidation system. The reagents and conditions used and the conversions are shown in the following table.

Table 1.2. Model reaction used for procedure optimization.



1a **1d**

Entry	Oxidant	Co-oxidant	Conditions			Conversion (%)[c]	Yield (%)[c]
			Solvent	Temperature (°C)	Time		
1[36]	30% H_2O_2 2 equiv.	48% HBr 0.2 equiv.	-	120[a]	20 min	100	0
2[36]	70% *tert*-BuOOH 2.5 equiv.	48% HBr 0.2 equiv.	-	80[b]	16 h	100	0
3[36]	$NaBO_3 \cdot 4H_2O$ 2.5 equiv.	48% HBr 0.2 equiv.	AcOH	50[b]	16 h	100	0
4[36]	$Na_2CO_3 \cdot 1.5H_2O_2$ 2.5 equiv.	48% HBr 0.2 equiv.	AcOH	50[b]	16 h	100	0
5[38]	Bi_2O_3 0.1 equiv.	70% *tert*-BuOOH 5 equiv.	EtOAc	90[a]	40 min	100	0
6[47]	*o*-Iodobenzoic acid (IBA) 0.2 equiv.	Oxone™ 1.0 equiv.	MeCN/H_2O (2:1)	70[b]	16 h	100	0

[a]Microwave-assisted heating.
[b]Classical heating.
[c]Determined by ^{1}H NMR.

Work started by studying various hydroperoxides in presence of hydrobromic acid (entries 1 and 2), which is a green method for the oxidation a range of secondary alcohols to ketones in absence of solvent. Our preliminary results showed that hydrogen and *tert*-butyl peroxides were not active in this reaction. We observed negligible conversion of ricinoleic acid **1a** after heating reagents both at 120 °C 20 min and 80 °C overnight in the presence of hydrobromic acid as co-oxidant. Using the same co-oxidant, we also tested solid peroxy compounds. SPC and SPB did not show activity after heating the starting material **1a** at 50 °C in presence of 48% HBr and using acetic acid as solvent (entries 3 and 4).

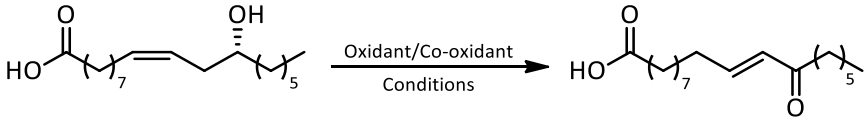
Therefore, we focused our attention on other oxidant systems based on bismuth(III) and hypervalent iodine compounds (entries 5 and 6). Unfortunately, both Bi_2O_3/*tert*-BuOOH and IBA/Oxone™ systems did not lead to the formation of the desired α,β-unsaturated carbonyl fatty acid **1d**.

1.5.2. Transition metal-mediated homoallyl hydroxy fatty acid oxidations

1.5.2.1. Heterogeneous oxidations

We started studying the oxidation reaction of ricinoleic acid **1a** in presence of different heterogeneous catalysts (**Table 1.3**).

Table 1.3. Model reaction used for procedure optimisation.



1a **1d**

Entry	Oxidant	Co-oxidant	Conditions			Conversion (%)[b]	Yield (%)[b]
			Solvent	Temperature (°C)	Time		
1[53]	$KMnO_4/CuSO_4·5H_2O$ (1:4) 1 equiv.	-	-	100	20 min	100	0
2[56]	$NiCl_2·6H_2O$ 0.025 equiv.	5% NaClO 4 equiv.	-	100	24 h	100	0
3[57]	$MnSO_4·5H_2O$ 1.5 equiv.	Oxone™ 2.5 equiv.	H_2O	100	30 min	100	0
4[60]	Magtrieve™ 7.7 equiv.	-	Toluene	100	30 min	100	0
5[68]	$Ce(NH_4)NO_3$ 3 equiv.	-	-	100	20 min	100	0
6[70]	$Co(NO_3)_2/SiO_2$ 2 equiv.	-	-	100	20 min	100	0

[a]Microwave-assisted heating.
[b]Determined by ^1H NMR.

First, we carried out the oxidation using the $KMnO_4/CuSO_4·5H_2O$ system (entry 1), performing the reaction under microwave heating in absence of solvent. We did not observe reaction after 20 min of heating at 120 °C. The same result was achieved when metallic oxides were used (entries 2-4). The system $NiCl_2·6H_2O$/NaClO used to produce *in situ* nickel oxide hydroxide able of oxidizing secondary alcohols in a mild way could not oxidize the ricinoleic acid **1a**. The system $MnSO_4·5H_2O$/Oxone™ which generates MnO_2 in the reaction media did not work either. Magtrieve™, the ionic and magnetic chromium(IV) oxide, did not lead to the formation of the α,β-unsaturated

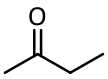
carbonyl fatty acid **1d** despite the high temperature achieved in the microwave oven. Finally, metallic nitrates such CAN or $Co(NO_3)_2$ supported in silica (entries 5 and 6) did not show any positive result in the oxidation of ricinoleic acid **1a**.

1.5.2.2. Homogeneous oxidation: Oppenauer-type homoallyl fatty acid oxidation mediated by Shvo's catalyst.

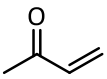
In view of the high activity of the Shvo's catalyst **6** in combination with acetone,[97,98] several easily reducible hydrogen acceptors were tested with methyl ricinoleate **10a** as substrate and $\{[(\eta^5\text{-}Ph_4C_4COH)(CO)]H_2\}Ru_2(CO)_4(\mu\text{-}H)$ **6** as catalyst. The hydrogen acceptors were chosen on the basis of their relative reduction potentials, their facility of being removed from the reaction medium or by their natural origin. **Figure 1.6** shows the hydrogen acceptors studied. They are divided in three main groups: ketones, aldehydes, and esters showing an allyl or/and acryl moiety. As it was indicated above, ketones and aldehydes are regularly used as hydrogen acceptors in Oppenauer reactions, in addition; terminal alkenes have been described as a good hydrogen acceptors.[106,107]

1. Ketones:

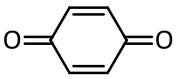
Acetone



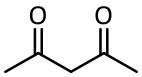
2-Butanone



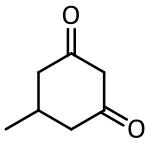
Methyl vinyl ketone



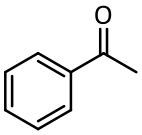
p-Benzoquinone



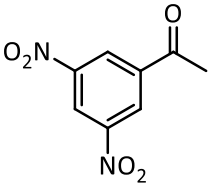
2,4-Pentanedione



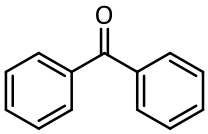
5-Methyl-1,3-cyclohexanedione



Acetophenone

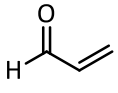


3,5-Dinitro acetophenone

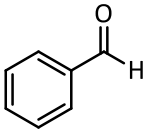


Benzophenone

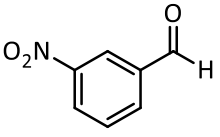
2. Aldehydes:



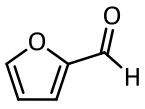
Acrolein



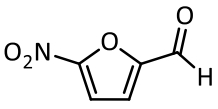
Benzaldehyde



3-Nitrobenzaldehyde

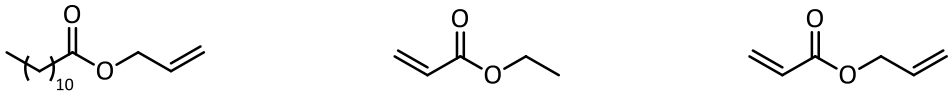


Furfural



5-Nitrofurfural

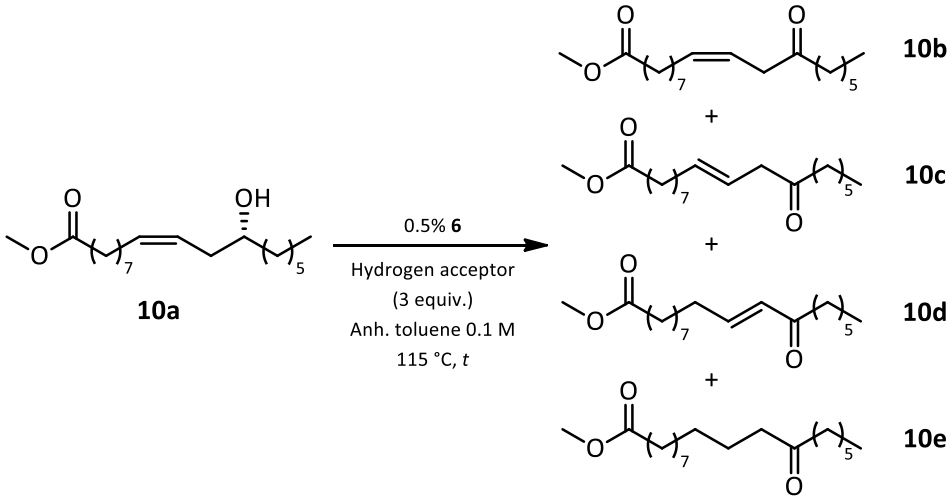
3. Terminal alkenes and unsaturated esters:



Allyl dodecanoate | Ethyl acrylate | Allyl acrylate

Figure 1.6. List of investigated hydrogen acceptor molecules.

The relative percentages of oxidation of methyl ricinoleate **10a** to its corresponding carbonyl fatty esters such as methyl (*Z*)-12-oxo-9-octadecenoate **10b**, methyl (*E*)-12-oxo-9-octadecenoate **10c**, methyl (*Z*)-12-oxo-10-octadecenoate **10d** and methyl 12-oxooctadecanoate **10e** (**Scheme 1.12**) were determined from the corresponding gas chromatography peak area.



Scheme 1.12. Carbonyl fatty acids determined in the oxidation methyl ricinoleate **10a** by the Shvo's catalyst **6**.

Based on the original works of Nichols and Bäckvall,[76,97,98] all experiments were conducted in anhydrous toluene at reflux using 3 equiv. of the hydrogen acceptor and 0.5% of Shvo's catalyst **6**. All reactions were performed for 24 h, aliquots collected at 1, 4, 8 and 24 h. **Table 1.4** summarise the relative percentages of each ester resulting from the oxidation of methyl ricinoleate **10a**.

Table 1.4 Effect of selected carbonyl compounds as hydrogen acceptors on the time-course oxidation of methyl ricinoleate **10a** using Shvo's catalyst **6**.[a]

Entry	Hydrogen acceptor	Time (h)	Conversion (%)[c]	Relative percentage (%)[c]				Yield (%)[d]			
				10b	**10c**	**10d**	**10e**	**10b**	**10c**	**10d**	**10e**
1	No acceptor	1	80	12	12	17	39	-	-	-	-
		4	100	9	11	3	77	-	-	-	-
		8	100	6	9	1	84	-	-	-	-
		24	100	6	9	1	84	-	-	-	-
2	Acetone	1	96	9	21	50	16	-	-	-	-
		4	100	7	10	33	50	-	-	-	-
		8	100	7	8	23	62	-	-	-	-
		24	100	6	7	20	67	-	-	-	-
3	2-Butanone	1	97	7	18	48	24	-	-	-	-
		4	100	5	10	30	55	-	-	-	-
		8	100	4	10	27	59	-	-	-	-
		24	100	4	9	26	61	-	-	-	-
4[b]	Methyl vinyl ketone	1	92	8	26	56	2	7	19	56	2
		4	100	7	21	70	2	7	19	56	2
		8	100	7	21	70	2	6	18	58	2
		24	100	7	21	70	2	6	18	58	2
5	*p*-Benzoquinone	1	100	5	19	74	2	3	11	61	1
		4	100	4	17	76	2	2	8	42	1
		8	100	5	11	82	2	2	5	30	2
		24	100	5	7	86	2	1	1	24	2
6	2,4-Pentanedione	1	26	10	10	6	0	-	-	-	-
		4	27	11	11	5	0	-	-	-	-
		8	27	10	11	6	0	-	-	-	-
		24	27	8	11	8	0	-	-	-	-
7	5-Methyl-1,3-cyclohexanedione	1	41	10	18	10	3	-	-	-	-
		4	47	10	20	14	3	-	-	-	-
		8	47	9	20	15	3	-	-	-	-
		24	50	8	19	20	3	-	-	-	-
8	Acetophenone	1	100	6	10	25	59	-	-	-	-
		4	100	5	4	13	78	-	-	-	-
		8	100	5	4	10	81	-	-	-	-
		24	100	5	3	9	83	-	-	-	-
9	3,5-Dinitro acetophenone	1	100	6	18	64	12	-	-	-	-
		4	100	7	17	57	19	-	-	-	-
		8	100	7	16	56	21	-	-	-	-
		24	100	7	16	56	21	-	-	-	-
10	Benzophenone	1	69	12	22	24	11	-	-	-	-
		4	98	12	15	33	38	-	-	-	-
		8	100	12	13	31	44	-	-	-	-
		24	100	11	12	30	47	-	-	-	-
11[b]	Acrolein	1	100	7	22	69	2	6	16	60	1
		4	100	7	21	70	2	5	17	60	2
		8	100	7	21	70	2	5	17	60	2
		24	100	6	21	71	2	5	17	59	2

Table 1.4. Effect of selected carbonyl compounds as hydrogen acceptors on the time-course oxidation of methyl ricinoleate **10a** using Shvo's catalyst **6** (*continued*).[a]

Entry	Hydrogen acceptor	Time (h)	Conversion (%)[c]	Relative percentage (%)[c]				Yield (%)[d]			
				10b	10c	10d	10e	10b	10c	10d	10e
12	Benzaldehyde	1	100	6	20	64	10	-	-	-	-
		4	100	6	19	58	17	-	-	-	-
		8	100	6	19	56	19	-	-	-	-
		24	100	6	19	54	21	-	-	-	-
13	3-Nitro benzaldehyde	1	100	5	14	74	7	-	-	-	-
		4	100	5	15	68	12	-	-	-	-
		8	100	5	17	63	15	-	-	-	-
		24	100	6	17	59	18	-	-	-	-
14	Furfural	1	100	6	20	66	8	4	12	56	10
		4	100	6	20	60	15	5	15	56	15
		8	100	5	19	60	16	4	14	50	17
		24	100	6	19	53	22	4	14	48	23
15	5-Nitrofurfural	1	100	6	20	72	2	5	16	61	2
		4	100	6	20	72	2	5	16	59	2
		8	100	6	20	72	2	5	16	56	2
		24	100	6	20	72	2	4	13	51	2
16	Allyl dodecanoate	1	33	11	12	7	3	-	-	-	-
		4	53	11	18	16	8	-	-	-	-
		8	56	10	17	20	9	-	-	-	-
		24	61	8	15	28	10	-	-	-	-
17[b]	Ethyl acrylate	1	11	6	5	0	0	-	-	-	-
		4	19	7	4	8	0	-	-	-	-
		8	31	8	9	14	0	-	-	-	-
		24	40	8	8	24	0	-	-	-	-
18[b]	Allyl acrylate	1	14	8	4	2	0	-	-	-	-
		4	18	9	6	3	0	-	-	-	-
		8	20	8	7	5	0	-	-	-	-
		24	33	9	10	14	0	-	-	-	-

[a]Methyl ricinoleate **10a** (0.32 mmol), Shvo's catalyst **6** (0.5%), hydrogen acceptor (3 mmol) in refluxing anhydrous toluene (3.2 mL).
[b]Distilled.
[c]The conversion and relative percentages were estimated from the corresponding GC peaks areas.
[d]Yields were determined by gas chromatography using ethyl dodecanoate as the internal standard.

Once the data were analyzed, the best results in terms of relative product formation were selected to determine the yield of the corresponding carbonyl fatty acid methyl esters (**10b-d** and **10e**). Initially, the reaction was carried out in absence of hydrogen acceptor to assess the evolution of the system (entry 1). Percentages were 17% for compound **10d** and 22% of methyl 12-oxostearate **10e** for 1 h reaction (80% of conversion). The percentage for both non-conjugated isomers **10b** and **10c** was 12% yield. α,β-Enone **10d** percentage was 3%, and percentages for **10b** and **10c** decreased slightly (9% and 11%, respectively) and the percentage for the oxo saturated ester **10e** was 77% for 4 h reaction. No differences were observed for 8 and 24 h reaction, with 6%, 9% and 84% percentages

for **10b**, **10c** and **10e**, respectively. These data indicated the absence of a hydrogen acceptor led to the fast formation of the oxo saturated ester **10e**.

Hence, a set of ketones were screened. Acetone (entry 2) yielded 50% of **10d** and 16% of **10e** for 1 h reaction, although the conversion was not complete. The relative percentage of the α,β-enone **10d** decreased as the reaction progressed, while the relative percentage of **10e** increased, reaching 33 and 67% for **10d** and **10e**, respectively for 24 h reaction. Similar percentages were determined for 2-butanone (entry 3). The high yields of the saturated fatty ester **10e** obtained in both cases indicated the redox potentials of acetone and 2-butanone,[108] were not enough to prevent the over-reduction of α,β-enone **10d**.

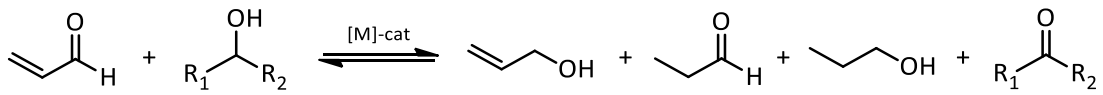
House and coworkers determined the reduction potential of several α,β-unsaturated ketones in aprotic media.[109] They concluded that their reduction potentials in aprotic media were lower negative than in the protic media. These more accurate values make the unsaturated ketones good reagents in reactions that involve organometallic catalyst. Methyl vinyl ketone and *p*-benzoquinone (entries 4 and 5) were selected to evaluate their ability to accept hydrogen. The use of quinones has been widely studied in the oxidation of Δ^5-3-hydroxysteroids *via* Oppenauer.[110] Methyl vinyl ketone is commercially stabilized with hydroquinone, which produced the inhibition of the catalyst. Thus, methyl vinyl ketone was distilled at atmospheric pressure before carrying out the time-depending study. Methyl vinyl ketone (entry 4) allowed the whole conversion of the starting material **10a**, yielding 70% α,β-enone **10d** and 2% of the saturated ketone **10e** after 4 h. These relative percentages remained unchanged until 24 h. The quantification by gas chromatography using ethyl dodecanoate as the internal standard confirmed the low amount of **10e** formed and a 58% of the α,β-enone **10d**. Although *p*-benzoquinone (entry 5) showed satisfactory relative percentages for the α,β-enone **10d** (74%) and compound **10e** (2%), the quantification showed a progressive decrease in yield of the α,β-enone **10d** and its non-conjugated isomers **10b** and **10c**, reaching up to a 49% reduction of the total carbonyl unsaturated fatty acids (**10b-d**) for 24 h reaction. However, the amount of **10e** remained unchanged. This decrease could be explained by the presence of a black solid in the reaction medium. Moreover, NMR studies indicated the formation of addition compounds between α,β-enone **10d** and *p*-hydroquinone, which resulted from the reduction of *p*-benzoquinone.[111]

The behaviour of 2,4-pentadione (entry 6) and 5-methyl-1,3-cyclohexadione (entry 7) were investigated based on the original works of Adkins and Cox,[112] which measured the redox potential of several 1,3-diketones,. While the 1,3-diketones appeared to prevent the formation of the saturated compound **10e**, both ketones exhibited a nearly constant behaviour along the reaction (entries 6 and

7). This prevention was more severe for 2,4-pentadione (entry 6) than for the cyclic ketone (entry 7), which showed a 50% conversion for 24 h reaction.

Beaupère *et al*.[113-115] used benzalacetone as hydrogen acceptor to transfer hydrogenation reaction between alcohols and α,β-unsaturated ketones. Accordingly, a series of acetophenone and derivatives was tested. After 1 h, the conversion of the starting material **10a** was completed in the case of acetophenone (entry 8) but the main product obtained was **10e** in 59%. The relative percentage was increasing over time at the same time that the percentage of the α,β-enone **10d** decreased. The presence of two nitro groups in 3,5-dinitroacetophenone (entry 9) yielded a higher relative percentage of the desired product **10d** (64%) and reduced the relative percentage of **10e** (12%) for 1 h reaction. Benzophenone (entry 10) led to a lowest conversion (69%) for 1 h reaction. The reaction was nearly complete at 4 h, yielding 33% of **10d** and 38% of **10e**. The reduction of the α,β-enone **10d** was observed during the end of the reaction. The redox potential of benzophenone seems does not prevent the reduction of the double bond.[116]

Schinz and coworkers found that aldehydes are effective hydrogen acceptors when used for the oxidation of primary alcohols and aldehydes.[117-119] In spite of the side aldol condensation reactions that can occur between the hydrogen acceptor and the final product, recently aromatic aldehydes have been used for the oxidation of secondary alcohols *via* Oppenauer.[120] This aromatic aldehydes allowed the selective oxidation of the alcohol moiety in α,β-unsaturated alcohols. Acrolein, the simplest α,β-unsaturated aldehyde has been widely studied studied within catalytic hydrogen transfer reactions due to the interest of obtaining its corresponding α,β-unsaturated alcohol. Several studies based on thermodynamic calculations describe of the reactivity of various aliphatic alcohols as hydrogen donors to acrolein in its vapour-phase transfer hydrogenation.[121-123] **Scheme 1.13** shows the resulting products of this last reaction. Hence, a series of aldehydes were screened as hydrogen acceptors in the *O*-oxidation of methyl ricinoleate **10a**.



Scheme 1.13. Reduction products obtained in the catalytic hydrogen transfer reaction of acrolein.

Acrolein was distilled before use due to the presence of hydroquinone. Acrolein (entry 11) allowed the complete conversion of the starting material **10a**, yielding 69% α,β-enone **10d**, 7% *Z*-isomer **10b**, 22% *E*-isomer **10c** and 2% **10e** for 1 h reaction, which remained unchanged to the end of the reaction. Acrolein (entry 11) presented better results than methyl vinyl ketone (entry 4), which

had a similar behavior for 4 h reaction. Additionally, acrolein has a low boiling point and can be easily removed from the reaction medium by vacuum distillation. The quantification by gas chromatography of the crude of reaction confirmed these worthy results.

The aromatic aldehydes behavior is described in entries 12-15. *meta*-Nitro electron attracting substituent improved the relative percentage of α,β-enone **10d** for 1 h reaction. As reaction time progressed, benzaldehyde and 3-nitrobenzaldehyde (entries 12 and 13) showed similar relative percentages for the all unsaturated carbonyl fatty acids **10b**, **10c** and **10d** and for the oxo saturated ester **10e**. In both cases, the α,β-enone **10d** was reduced to reach 20% of compound **10e**. 5-Nitrofurfural (entry 15) yielded a lower relative percentage of **10e** (2%) than furfural (entry 14), whose value of **10e** increased as α,β-enone **10d** decreased. Nevertheless, 5-nitrofurfural had the same problem as *p*-benzoquinone, a solid black precipitate was produced as the reaction proceeded. Quantification by gas chromatography confirmed the drop in α,β-enone **10d** yield as the reaction progressed, though the yield of **10e** remained constant.

The last group investigated compiled the hydrogen acceptors that had a terminal double bond or were α,β-unsaturated esters. Terminal alkenes acts as good hydrogen acceptors in the dehydration reactions of alcohols mediated by transition metals.[106,124] Moreover, based on the work of House,[109] which empirically measured the reduction potential of α,β-unsaturated esters, allyl dodecanoate, ethyl acrylate and allyl acrylate (entries 16-18) were evaluated. Ethyl acrylate and allyl acrylate (entries 17 and 18) exhibited similar behaviors, though conversions were lower than 50% for 24 h reaction. It is important to remark that the keto saturated fatty ester **10e** was not detected. This indicated conjugated esters prevented the formation of **10e** from the α,β-enone **10d**, though conversions were low and needed longer reaction times. Allyl dodecanoate (entry 16) yielded an improvement in relative percentages of the all unsaturated carbonyl compounds (61% conversion, 28% **10d**, 8% **10b** and 17% **10c** for 24 h reaction) compared to allyl acrylate (entry 18). However, the terminal double bond did not prevent the formation of **10e** (10% at 24 h reaction).

Summary

I. Classical and widely-used hydrogen acceptors such as acetone and butanone were not suitable for the synthesis of methyl (*E*)-12-oxo-10-octadecenoate **10d** as they could not prevent its over-reduction and transformation to the corresponding oxo saturated ester **10e**.

II. α,β-Unsaturated carbonyl compounds (ketones, aldehydes and esters) were able to avoid the reduction of methyl (*E*)-12-oxo-10-octadecenoate **10d** due to their lower reduction potentials. The best results of both conversion of methyl ricinolate **10a**, time and yield of the α,β-enone **10d** were achieved using acrolein, which can also be easily removed from the reaction medium by distillation due to its low boiling point

III. 1,3-Diketones behaved as good hydrogen acceptors avoiding the reduction of **10d** although long reaction times were required.

IV. In spite of the good relative percentages obtained in the case of *p*-benzoquinone and 5-nitrofurfural, the quantification revealed the dramatic drop in the final yield of **10d**.

V. The rest of aromatic carbonyls compounds (except acetophenone and benzophenone) gave good conversions and relative percentages of **10d** but they were not able to avoid the over-reduction.

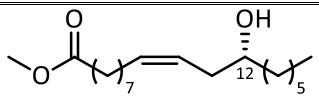
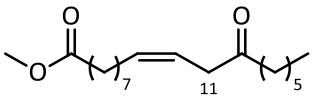
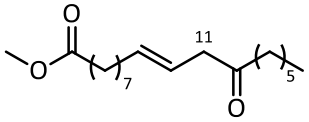
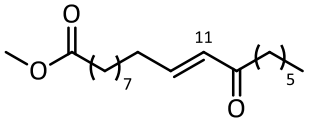
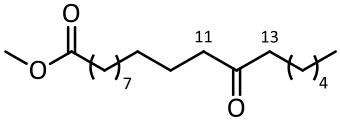
VI. Allyl dodecanoate presented low conversions and did not prevent the reduction of the α,β-enone **10d** despite the presence of the terminal double bond.

Considering the highest conversion and the yields obtained at 1 h listed in **Table 1.4**, acrolein was adopted as the most suitable hydrogen acceptor for the synthesis of methyl (*E*)-12-oxo-10-octadecenoate **10d** in the ruthenium-catalyzed Oppenauer-type oxidation of methyl ricinoleate **10a**.

1.5.3. Reaction Condition Requirements

Once the hydrogen acceptor was selected, several reaction conditions were studied taking as starting point the conditions used in the previous works. ^{1}H NMR was used to determine the relative percentages of each oxidized compound. **Table 1.5** shows the protons of each compound used to perform this analysis, the expected integration values, its chemical displacement and the signal multiplicity between.

Table 1.5. Selected proton signals used to quantify the carbonyl derivatives from methyl ricinoleate **10a**.

Entry	Compound	Structure	H	Integration	δ (ppm)
1	**10a**		H-12	1	3.53-3.61 (m)
2	**10b**		H-11	2	3.13 (d)
3	**10c**		H-11	2	3.07 (d)
4	**10d**		H-11	1	6.07 (d)
5	**10e**		H-11&H-13	4	2.38-2.44 (m)

1.5.3.1. Influence of the temperature

The reaction temperature is a critical variable in hydrogen transfer reactions. Early studies have employed hydrogen atom transfer at higher temperatures (usually >150 °C), particularly, in the classical Oppenauer oxidations. Toluene or xylene are the typical high-boiling solvents. Nevertheless, with the advances in the design and synthesis of efficient homogeneous transition metal catalysts coupled with effective hydrogen atom donors, much lower temperatures have been used for effective hydrogen transfer reactions of many organic compounds. The work of Bäckvall in the 1990s, particularly allowed the hydrogen transfer reactions over a mild range of temperatures; including ambient to refluxing temperatures with noted increased rates of reactions.[97,98,125] For studying this parameter, the reaction conditions above used were maintained and only the temperature was modified (**Table 1.6**).

Table 1.6. Effect of the temperature on the oxidation of methyl ricinoleate **10a**.[a]

Entry	Temperature (°C)	Conversion (%)[b]	10b (%)[b]	10c (%)[b]	10d (%)[b]	10e (%)[b]
1	115	100	12	11	72	5
2	90	45	8	13	11	13
3	80	43	9	11	8	15
4	53[c]	0	0	0	0	0

[a]Methyl ricinoleate **10a** (0.32 mmol), Shvo's catalyst **6** (0.5%), distilled acrolein (3 mmol) in refluxing anhydrous toluene (3.2 mL) for 1 h.
[b]Relative percentage determined by ^{1}H NMR.
[c]Acrolein boiling point. Reaction carried out without solvent.

First, the oxidation of methyl ricinoleate **10a** was carried out under the general conditions and the relative percentages of reaction products (**10b-d** and **10e**) were quantified by ^{1}H NMR. The conversion was complete after 1 h and the results were in accordance with the chromatographic results obtained above (entry 11, **Table 1.4**). Then, experiments at 53, 80 and 90 °C were performed. The lowest temperature corresponds to the boiling point of acrolein and therefore, this experiment was carried out without solvent but maintaining the concentration of reagents and catalyst. Temperature had a positive effect on the conversion. In refluxing acrolein (entry 4), only starting material **10e** was observed. There was any transformation in the α,β-enone **10d**. At temperatures higher than 53 °C (entries 2 and 3), carbonyl fatty acids were synthesized. The higher the temperature was, higher conversion of α,β-enone **10d** obtained. Refluxing toluene (115° C, entry 1), temperature seemed to be the optimal reaction temperature with regards to acrolein selectivity. These results were in accordance with the effect of the temperature in the equilibria between conjugated and unconjugated keto esters described by Fuller.[8] These thermal studies demonstrated that only a small energy difference existed between conjugated and unconjugated keto compounds and also, high temperatures 180-200 °C are necessary to shift the equilibrium towards the α,β-enone **10d** formation.

1.5.3.2. Influence of the volume of solvent

Oppenauer oxidations usually are carried out at high solvent dilution, which generally acts also as hydrogen acceptor.[104] However, in chemical industry the reduction of solvent used is worth to develop commercial synthesis. Hence, in this section several experiments were attempted decreasing the amount of toluene used. **Table 1.7** summarizes the results of the reactions performed without change the hydrogen acceptor (acrolein), the percentage of the Shvo's catalyst **6**, the temperature (refluxing toluene) and the reaction time (1 h).

Table 1.7. Effect of the concentration on the oxidation of methyl ricinoleate **10a**.[a]

Entry	Solvent volume (mL)	Concentration (M)	Conversion (%)[b]	10b (%)[b]	10c (%)[b]	10d (%)[b]	10e (%)[b]
1	3.20	0.10	100	12	11	72	5
2	1.60	0.20	100	5	12	72	11
3	1.00	0.34	100	5	12	69	14
4	0.50	0.65	100	5	15	66	14
5	0.30	1.12	100	6	16	62	16
6	0.10	3.32	100	8	16	58	18

[a]Methyl ricinoleate **10a** (0.32 mmol), Shvo's catalyst **6** (0.5%), distilled acrolein (3 mmol) in refluxing anhydrous toluene for1 h.
[b]Relative percentage determined by ^{1}H NMR.

The reduction by half of the amount of volume used (0.2 M, entry 2) was possible, obtaining similar percentages as carrying out the reaction with the initial conditions (entry 1). Although the percentage of **10e** increased (11%), the amount of α,β-enone **10d** was equivalent to the 0.1 M concentration (entry 1). Afterwards (entries 3-6), the increase in the reagent concentration resulted in higher relative percentages of oxo saturated **10e** and carbonyl unconjugated fatty esters (**10b** and **10c**). Hence, a clear increase in the amount of oxo saturated compound **10e** was observed as the volume of solvent decreased. In all cases, conversion of the starting product **10a** was complete after 1 h refluxing. Due to the lower relative percentage of methyl 12-oxooctadecanoate **10e**, the selected concentration for the oxidation of methyl ricinoleate **10a** through ruthenium-catalyzed Oppenauer-type reaction was 0.1 M.

1.5.3.3. Influence of the amount of hydrogen acceptor

The reduction of the mass of all the materials involved in a chemical process improves the sustainability of the reaction. With this in mind, several experiments were performed to reduce the amount of hydrogen acceptor. In the previous section, it was concluded that the reduction of the amount of solvent was possible to carry out the oxidation of methyl ricinoleate **10a**. Excepting the amount of acrolein employed, the rest of reaction conditions were the same as those used in the hydrogen acceptor screening (**Table 1.4**). The results are shown in **Table 1.8**.

Table 1.8. Effect of the hydrogen acceptor amount on the oxidation of methyl ricinoleate **10a**.[a]

Entry	Equiv.[b]	Conversion (%)[c]	10b (%)[c]	10c (%)[c]	10d (%)[c]	10e (%)[c]
1	3.00	100	5	12	72	11
2	2.00	100	7	14	64	15
3	1.50	100	8	17	61	14
4	1.20	100	7	16	58	19

[a]Methyl ricinoleate **10a** (0.32 mmol), Shvo's catalyst **6** (0.5%), distilled acrolein in refluxing anhydrous toluene (3.2 mL) for 1 h.
[b]Molar equivalents of acrolein with regard to methyl ricinoleate **10a**.
[c]Relative percentage determined by ^{1}H NMR.

A decrease in the amount of hydrogen acceptor dramatically reduced the relative percentage of α,β-enone **10d** at the same time as the oxo saturated fatty ester **10e** increased. Moreover, an increase of the amount of the unconjugated carbonyl fatty esters (**10b** and **10c**) was observed as equivalents dropped. The reduction in the amount of hydrogen acceptor was detrimental to the formation of the desired product **10d** and therefore, the use of 3 equivalents of acrolein (entry 1) was the most suitable for carrying out this reaction.

1.5.3.4. Influence of the amount of catalyst

The main drawback in homogeneous catalysis is that in many cases is not possible to recover the catalyst. The amount of catalyst required to achieve the largest amount of α,β-enone **10d** in the oxidation of methyl ricinoleate **10a** was evaluated. First, half of the amount of catalyst employed up to now was checked. Thereafter, a value in between them was chosen. Finally, the progress of the reaction doubling the amount of catalyst was assessed (**Table 1.9**).

Table 1.9. Effect of the Shvo's catalyst **6** amount on the oxidation of methyl ricinoleate **10a**.[a]

Entry	6 (%)[b]	Conversion (%)[c]	10b (%)[c]	10c (%)[c]	10d (%)[c]	10e (%)[c]
1	1.00	100	7	14	67	12
2	0.50	100	5	12	72	11
3	0.34	100	4	10	65	21
4	0.25	90	7	16	49	18

[a]Methyl ricinoleate **10a** (0.32 mmol), Shvo's catalyst **6**, distilled acrolein (3 mmol) in refluxing anhydrous toluene (3.2 mL) for 1 h.
[b]Molar percentage of Shvo's catalyst **6** with regard to methyl ricinoleate **10a**.
[c]Relative percentage determined by ^{1}H NMR.

The use of 1% of the Shvo's catalyst **6** (entry 1) did not lead to an increase in the relative percentage of α,β-enone **10d** mantaining the general conditions (entry 2). Quantities less than 0.5% resulted in a larger quantity of oxo saturated compound **10e** (entries 3 and 4). The conversion of the starting material **10a** was incomplete (entry 4) when the amount of catalyst was halved.

1.5.3.5. Influence of the presence of a base

There are several ruthenium-based catalytic systems that showed excellent activities but needed a promoter or a basic condition. $[Ru(OCOCF_3)(CO)(PPh_3)_2]$ with CF_3COOH as a promoter,[126] base-promoted ruthenium hydride-phosphine systems,[74,127,128] $RuCl_3$ hydrate/phosphine and $[RuCl_2(p\text{-cymene})]_2$/nitrogen-containing ligands in a basic medium,[129,130] Grubbs catalyst and $[RuCl_2(p\text{-cymene})]_2/PPh_3$ system with LiOH,[131] and pincer diamine and diphosphane Ru complexes with a catalytic amount of *tert*-BuOK[132] are some examples. The influence of the promoters in the catalytic efficiencies of homogeneous transition metal catalysts is exerted through their role as catalyst preactivation agents. Their presence in the reaction mixture affords a highly active and reproducible catalyst system for effective transfer of hydrogen atoms. Although it is known that the Shvo's catalyst **6** transforms allyl alcohols into ketones without any base,[133] in order to investigate the

influence of a base on the catalytic oxidation of methyl ricinoleate **10a** several experiments were performed. Cesium carbonate was chosen as promoter because of its application in the oxidation of allyl alcohols mediated by ruthenium.[134] All experiments were performed in anhydrous toluene at 115 °C (0.1 M) in the presence of 3.0 molar equivalents of acrolein as hydrogen acceptor and 0.5% of the Shvo's catalyst **6**. Three different amounts of Cs_2CO_3 were screened and the results are summarized in **Table 1.10**.

Table 1.10. Effect of the presence of Cs_2CO_3 on the oxidation of methyl ricinoleate **10a**.[a]

Entry	Cs_2CO_3 (%)[b]	Conversion (%)[c]	10b (%)[c]	10c (%)[c]	10d (%)[c]	10e (%)[c]
1	15.0	68	7	19	24	18
2	10.0	100	6	18	56	20
3	5.0	100	6	20	58	16

[a]Methyl ricinoleate **10a** (0.32 mmol), Shvo's catalyst **6** (0.5%), Cs_2CO_3 (%), distilled acrolein (3 mmol) in refluxing anhydrous toluene (3.2 mL) for1 h.
[b]Molar percentage of Cs_2CO_3 with regard to methyl ricinoleate **10a**.
[c]Relative percentage determined by ^{1}H NMR.

The presence of Cs_2CO_3 decreased the relative percentages of α,β-enone **10d** drastically. Adding 15% of Cs_2CO_3 (entry 1) led to an incomplete conversion of the starting material **10a**. The decrease in the amount of base (entries 2 and 3) resulted in complete conversions but the selectivity was affected. Higher amounts of the oxo saturated compound **10e** were formed in comparison with the total absence of base. This upward trend was also observed in the case of the *E*-isomer **10c**.

1.5.3.6. Optimization of the reaction time

Considering all the results attained above and trying to increase the efficiency of the reaction, reaction times shorter than 1 h were screened. All experiments were performed in anhydrous toluene at 115 °C (0.1 M) in the presence of acrolein (3 equiv.) as hydrogen acceptor and 0.5% of the Shvo's catalyst **6** (**Table 1.11**).

Table 1.11. Effect of the reaction time on the oxidation of methyl ricinoleate **10a**.[a]

Entry	Time (min)	Conversion (%)[b]	10b (%)[b]	10c (%)[b]	10d (%)[b]	10e (%)[b]
1	60	100	5	12	72.0	11
2	30	100	3	11	78	8
3	15	84	5	13	56	10

[a]Methyl ricinoleate **10a** (0.32 mmol), Shvo's catalyst **6** (0.5%), distilled acrolein (3 mmol) in refluxing anhydrous toluene (3.2 mL).
[b]Relative percentage determined by ^{1}H NMR.

Table 1.11 shows that (entry 2), complete conversion was achieved for 30 min reaction. Surprisingly, the relative percentage of the α,β-enone **10d** was the highest obtained of the whole series of tests carried out. The decrease in reaction time in half allowed very few α,β-enone **10d** to be reduced to the oxo saturated compound **10e** in the reaction medium. In addition, the relative percentages of the non-conjugated compounds (**10c** and **10d**) were slightly lower. However, 15 min of reaction (entry 3) were not enough to oxidize the methyl ricinoleate **10a** completely.

1.5.3.7. Efficient continuous flow oxidation

Continuous flow chemistry is the process of performing chemical reactions in a tube or pipe. Reactive components are pumped together at a mixing junction and flowed down a temperature controlled pipe or tube.[135-137] The main advantages associated with the flow processes performed in microreactors can be comprised in two broad classes. The first one is associated with the small dimensions of the channels and includes the precise control of the reaction conditions, the efficient mass and heat transfer, the possibility of working under superheating conditions. The second aspect is related to the continuous nature of the process and includes the simplicity in reaction scale-up, the possibility of performing sequential synthetic steps with independent control of reaction conditions, the possibility of introducing in-line purification by means of supported scavengers or sorbents and the possibility of interfacing the reactor with in-line analysis devices for real time monitoring. In view of the advantages shown by the continuous flow chemistry and more specifically that referred to scaling, the ruthenium-catalyzed Oppenauer-type oxidation of methyl ricinoleate **10a** was carried out using this technique. The continuous flow system consisted in a combination of FRX Pump Module and an etched glass chip microreactor of 250 µL. A mixture of methyl ricinoleate **10a**, distilled acrolein (3 equiv.) and the Shvo's catalyst **6** (0.5%) dissolved in anhydrous toluene (0.1 M) was pumped with a flow rate of 10 µL/min into etched glass chip microreactor heated at 120 °C. The residence time was 25 min. The relative percentages were determined by ^{1}H NMR (**Table 1.12**).

Table 1.12. Oxidation of methyl ricinoleate **10a** by continuous flow.[a]

Entry	Residence time (min)	Conversion (%)[b]	10b (%)[b]	10c (%)[b]	10d (%)[b]	10e (%)[b]
1	25	85	5	19	47	14

[a]Methyl ricinoleate **10a** (0.64 mmol), Shvo's catalyst **6** (0.5%), distilled acrolein (3 mmol) in refluxing anhydrous toluene (6.4 mL).
[b]Relative percentage determined by ^{1}H NMR.

After completion of the reaction, the conversion of the starting product **10a** was 85%. The relative percentages of all the oxidized compounds were not as good as in comparison with the results obtained in batch but were in accordance with the conversion.

1.5.3.8. Laboratory scaling up

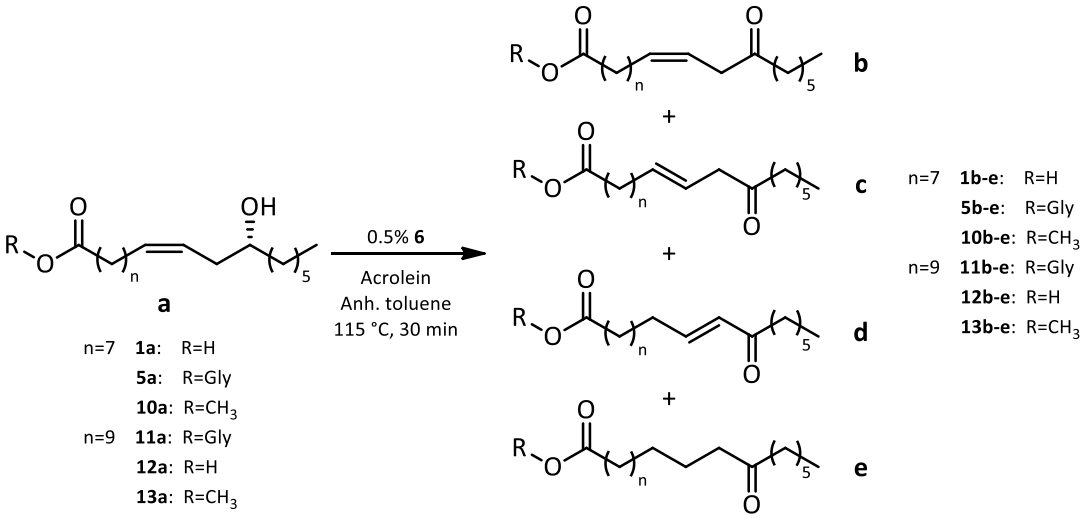
Once the synthetic route and the optimum conditions for carrying out oxidation of methyl ricinoleate **10a** to methyl (*E*)-12-oxo-10-octadenoate **10d** were defined, the next step was to verify that the process was scalable and to determine the recovery of the isolated α,β-enone **10d**. The ruthenium-catalyzed Oppenauer oxidation was performed in 1000 mL three-necked flask. Methyl ricinoleate **10a** (10 g) were dissolved in 320 mL of anhydrous toluene and 6.4 mL of freshly distilled acrolein were added. The flask was fitted with a condenser and was heated under vigorously stirring in a preheated and stabled-temperature oil bath (150 °C) to compensate the dissipation of heat to the reaction flask and the environment. The necessary reaction time for the complete conversion of the starting material **10a** was 40 min. The hydrogen acceptor and the solvent were distilled under reduced pressure. The ^{1}H NMR analysis revealed the following relative percentages: 71% **10d** and 10% **10e** (also 6% **10b** and 13% of **10c**). After the purification by liquid column chromatography using SiO_2 and petroleum ether/Et_2O (9:1), the α,β-enone **10d** was isolated in 68% yield.

1.5.4. Scope of the reaction

With the optimized reaction conditions in hand, the substrate scope was extended to other derivatives of methyl ricinoleate **10a** as ricinoleic acid **1a** and its corresponding triglyceride, castor oil **5a,** and also was applied to lesquerella oil **11a** and its derivatives lesquerolic acid **12a** and methyl lesquerolate **13a**. The homoallyl hydroxy fatty compounds were dissolved in anhydrous toluene and the Shvo's catalyst **6** and acrolein were added. The reaction mixture was heated under reflux for 30 min and the crude reaction mixtures were analyzed by ^{1}H NMR. Finally, both methyl esters and acid

α,β-enones were isolated by liquid column chromatography or recrystallization. The results are shown below (**Table 1.13**).

Table 1.13. Synthesis of α,β-unsaturated carbonyl fatty acids.[a]



Entry	Substrate	Conversion (%)[b]	b (%)[b]	c (%)[b]	d (%)[b]	e (%)[b]	d (%)[c]
1	**1a**	100	1	18	71	10	68
2	**5a**	100	5	14	69	12	n.i.[d]
3	**10a**	100	3	18	73	6	70
4	**11a**	100	4	11	60	24	n.i.
5	**12a**	100	1	21	66	12	50
6	**13a**	100	4	18	65	13	53

[a]Homoallyl hydroxy fatty acid (0.32 mmol), Shvo's catalyst **6** (0.5%), distilled acrolein (3 mmol) in refluxing anhydrous toluene (3.2 mL) for 30 min.
[b]Relative percentage determined by ^{1}H NMR.
[c]Isolated yield of the pure product.
[d]Not isolated.

The conversion was 100% in all cases. Castor oil **5a** (entry 1) and its corresponding derivatives ricinoleic acid **1a** (entry 2) and methyl ricinoleate **10a** (entry 3) gave better yields than lesquerella oil **11a**, lesquerolic acid **12a**, and methyl lesquerolate **13a** (entries 4-6). Except for triglycerides, the α,β-enone compounds **1d**, **10d**, **12d** and **13d** were isolated and characterized. The α,β-enone fatty acids **1d** and **12d** were isolated by crystallization in EtOH (68 and 50% yield respectively) whereas the corresponding α,β-enone methyl esters **10d** and **13d** were isolated by silica gel liquid column chromatography (70 and 53% yield respectively). The castor and lesquerella α,β-enones **5d** and **11d** were not isolated due to the crude mixture complexity.

1.5. CONCLUSIONS

The assayed transition metal-free catalysis and heterogeneous transition metal catalysis failed to transform the ricinoleic acid **1a**, a homoallyl hydroxy fatty acid, to its α,β-unsaturated carbonyl derivative. However, the ruthenium-catalyzed Oppenauer-type oxidation proved to be an excellent reaction to achieve this sort of compounds. For this, it was necessary to ascertain what hydrogen acceptor was the most suitable to avoid the over-reduction drawback. Several hydrogen acceptors were screened with the Shvo's catalyst **6** as oxidant. α,β-Unsaturated carbonyl compounds were found to avoid the over-reduction of the α,β-enone in the reaction media. Indeed, homoallyl hydroxy fatty acids could be oxidized to their corresponding α,β-unsaturated carbonyl fatty acids with acrolein, which presented a higher tendency to be reduced than the α,β-enone. Moreover, acrolein could be easily removed because of its low boiling point. Once selected acrolein as the most suitable hydrogen acceptor, the reaction condition requirements were studied in order to make the process more efficient. Finally, the free acids, methyl esters and triglycerides of these homoallyl hydroxy fatty acids were subjected to oxidation under the optimal conditions. Except for the triglycerides, the rest of α,β-enones were successfully isolated at the end of this process.

1.6. REFERENCES

(1) Mustafa, J.; Ahmad, M.; Rauf, A.; M. Osman, S. *J. Am. Oil Chem. Soc.* **1984**, *61*, 555-558.

(2) Mustafa, J.; Ahmad, M. S.; Rauf, A.; Osman, S. M. *J. Am. Oil Chem. Soc.* **1984**, *61*, 941-944.

(3) Mungur, R.; Glass, A. D. M.; Goodenow, D. B.; Lightfoot, D. A. *J. Biomed. Biotechnol.* **2005**, *2005*, 198-214.

(4) Kim, M.; Furuzono, T.; Yamakuni, K.; Li, Y.; Kim, Y.-I.; Takahashi, H.; Ohue-Kitano, R.; Jheng, H.-F.; Takahashi, N.; Kano, Y.; Yu, R.; Kishino, S.; Ogawa, J.; Uchida, K.; Yamazaki, J.; Tominaga, M.; Kawada, T.; Goto, T. *FASEB J.* **2017**, *31*, 5036-5048.

(5) Ellis, G. W. *Biochem. J.* **1936**, *30*, 753-761.

(6) Ellis, G. W. *J. Chem. Soc.* **1950**, 9-12.

(7) Birch, S. F.; Kon, G. A. R.; Norris, W. S. G. P.; Thorpe, J. F. *J. Chem. Soc., Trans.* **1923**, *123*, 1361-1374.

(8) Taylor, P. M.; Fuller, G. *J. Org. Chem.* **1969**, *34*, 3627-3630.

(9) Maletz, R.; Schäfer, H. J.; Quermann, R. *Fett/Lipid* **1996**, *98*, 370-379.

(10) Achaya, K. T. *J. Am. Oil Chem. Soc.* **1971**, *48*, 758-763.

(11) Nichols, J.; Schipper, E. *J. Am. Chem. Soc.* **1958**, *80*, 5705-5710.

(12) Davletbakova, A. M.; Baibulatova, N. Z.; Dokichev, V. A.; Yunusova, S. G.; Yunusov, M. S. *Russ. J. Org. Chem.* **2001**, *37*, 781-783.

(13) Lie Ken Jie, M. S. F.; Lam, C. N. W. *Lipids* **2004**, *39*, 583-587.

(14) aus dem Kahmen, M.; Schäfer, H. J. *Fett/Lipid* **1998**, *100*, 227-235.

(15) Conacher, H. B. S.; Gunstone, F. D. *Chem. Phys. Lipids* **1969**, *3*, 203-220.

(16) Mallat, T.; Baiker, A. *Chem. Rev.* **2004**, *104*, 3037-3058.

(17) Schultz, M. J.; Sigman, M. S. *Tetrahedron* **2006**, *62*, 8227-8241.

(18) Matsumoto, T.; Ueno, M.; Wang, N.; Kobayashi, S. *Chem. Asian J.* **2008**, *3*, 196-214.

(19) Vinod, C. P.; Wilson, K.; Lee, A. F. *J. Chem. Technol. Biotechnol.* **2011**, *86*, 161-171.

(20) Omura, K.; Swern, D. *Tetrahedron* **1978**, *34*, 1651-1660.

(21) Pfitzner, K. E.; Moffatt, J. G. *J. Am. Chem. Soc.* **1963**, *85*, 3027-3028.

(22) Stevens, R. V.; Chapman, K. T.; Weller, H. N. *J. Org. Chem.* **1980**, *45*, 2030-2032.

(23) Shaabani, A.; Ameri, M. *J. Chem. Res., Synop.* **1998**, 100-101.

(24) Mukaiyama, T.; Matsuo, J.-I.; Lida, D.; Kitagawa, H. *Chem. Lett.* **2001**, *30*, 846-847.

(25) Sheldon, R. A. In *Organic Peroxygen Chemistry*; Herrmann, Wolfgang A., Ed.; Topics in Current Chemistry 162; Springer-Verlag: Heidelberg, 1993, p 21-43.

(26) Sanderson, R. W. *Pure Appl. Chem.* **2000**, *72*, 1289-1304.

(27) Jones, A. K.; Wilson, T. E. In *Encyclopedia of Reagents for Organic Synthesis*; Paquette, L.A., Ed.; v. 2; John Wiley & Sons: Chichester, 1995, p 880-888.

(28) Berkessel, A.; Voglt, N. In *The Chemistry of Peroxides*; Rappoport, Z., Ed.; v 2, Part 1; John Wiley & Sons: Chichester, 1996, p 307-595.

(29) Zwanenburg, B.; ter Wiel, J. *Tetrahedron Lett.* **1970**, *11*, 935-936.

(30) Ben-Daniel, R.; de Visser, S. P.; Shaik, S.; Neumann, R. *J. Am. Chem. Soc.* **2003**, *125*, 12116-12117.

(31) Sharma, V. B.; Jain, S. L.; Sain, B. *Synlett* **2005**, *2005*, 173-175.

(32) McKillop, A.; Sanderson, W. R. *J. Chem. Soc. Perkin Trans. I* **2000**, 471-476.

(33) Muzart, J. *Synthesis* **1995**, *1995*, 1325-1347.

(34) McKillop, A.; Sanderson, W. R. *Tetrahedron* **1995**, *51*, 6145-6166.

(35) Gomez, M. V.; Caballero, R.; Vazquez, E.; Moreno, A.; de la Hoz, A.; Diaz-Ortiz, A. *Green Chem.* **2007**, *9*, 331-336.

(36) Jain, S. L.; Sharma, V. B.; Sain, B. *Tetrahedron* **2006**, *62*, 6841-6847.

(37) Malik, P.; Chakraborty, D. *Tetrahedron Lett.* **2010**, *51*, 3521-3523.

(38) Malik, P.; Chakraborty, D. *Synthesis* **2010**, *2010*, 3736-3740.

(39) Wirth, T. *Hypervalent Iodine Chemistry*; Wirth, T., Ed.; Springer-Verlag Berlin Heidelberg: Berlin, Heidelberg, 2003; Vol. 224.

(40) Wirth, T. *Angew. Chem., Int. Ed.* **2001**, *40*, 2812-2814.

(41) Zhdankin, V. V.; Stang, P. J. *Chem. Rev.* **2002**, *102*, 2523-2584.

(42) Frigerio, M.; Santagostino, M. *Tetrahedron Lett.* **1994**, *35*, 8019-8022.

(43) Hartmann, C.; Meyer, V. *Ber. Dtsch. Chem. Ges.* **1893**, *26*, 1727-1732.

(44) Frigerio, M.; Santagostino, M.; Sputore, S. *J. Org. Chem.* **1999**, *64*, 4537-4538.

(45) Plumb, J. B.; Harper, D. J. *Chem. Eng. News,* **1990**, *68*, 2-3,45.

(46) Sigma-Aldrich, C. *Aldrich Chemistry 2009-2010: Handbook of Fine Chemicals*; Sigma-Aldrich: Sant Louise, 2008.

(47) Thottumkara, A. P.; Bowsher, M. S.; Vinod, T. K. *Org. Lett.* **2005**, *7*, 2933-2936.

(48) Singh, N.; Lee, D. G. *Org. Process Res. Dev.* **2001**, *5*, 599-603.

(49) Regen, S. L.; Koteel, C. *J. Am. Chem. Soc.* **1977**, *99*, 3837-3838.

(50) Menger, F. M.; Lee, C. *J. Org. Chem.* **1979**, *44*, 3446-3448.

(51) Noureldin, N. A.; Bellegarde, J. W. *Synthesis* **1999**, *1999*, 939-942.

(52) Luu, T. X. T.; Lam, T. T.; Le, T. N.; Duus, F. *Molecules* **2009**, *14*, 3411-3424.

(53) Luu, T. X. T.; Christensen, P.; Duus, F.; Le, T. N. *Synth. Commun.* **2008**, *38*, 2011-2024.

(54) Mandi, U.; Pramanik, M.; Roy, A. S.; Salam, N.; Bhaumik, A.; Islam, S. k. *RSC Adv.* **2014**, *4*, 15431-15440.

(55) George, M. V.; Balachandran, K. S. *Chem. Rev.* **1975**, *75*, 491-519.

(56) Grill, J. M.; Ogle, J. W.; Miller, S. A. *J. Org. Chem.* **2006**, *71*, 9291-9296.

(57) Vázquez Sánchez, A.; Ávila Zárraga, J. G. *J. Mex. Chem. Soc.* **2007**, *51*, 213-216.

(58) Lee, R. A.; Donald, D. S. *Tetrahedron Lett.* **1997**, *38*, 3857-3860.

(59) Bhosale, S.; Kurhade, S.; Vyas, S.; Palle, V. P.; Bhuniya, D. *Tetrahedron* **2010**, *66*, 9582-9588.

(60) Bogdal, D.; Lukasiewicz, M.; Pielichowski, J.; Miciak, A.; Bednarz, S. *Tetrahedron* **2003**, *59*, 649-653.

(61) Cornelis, A.; Laszlo, P. *Synthesis* **1980**, 849-850.

(62) Cornelis, A.; Laszlo, P. *Synthesis* **1985**, 909-918.

(63) Varma, R. S.; Dahiya, R. *Tetrahedron Lett.* **1997**, *38*, 2043-2044.

(64) Trahanovsky, W. S.; Young, L. B. *J. Chem. Soc.* **1965**, 5777-5778.

(65) Trahanovsky, W. S.; Young, M. G.; Nave, P. M. *Tetrahedron Lett.* **1969**, *10*, 2501-2504.

(66) Young, L. B.; Trahanovsky, W. S. *J. Org. Chem.* **1967**, *32*, 2349-2350.

(67) Trahanovsky, W. S. In *Methods in Free Radical Chemistry*; Huyser, E. S., Ed.; Marcel Dekker: New York, 1973, p 133-169.

(68) Kapustina, N. I.; Sokova, L. L.; Nikishin, G. I. *Russ. Chem. Bull.* **2010**, *59*, 1284-1288.

(69) Nishiguchi, T.; Asano, F. *J. Org. Chem.* **1989**, *54*, 1531-1535.

(70) Kiasat, A. R.; Kazemi, F.; Rafati, M. *Synth. Commun.* **2003**, *33*, 601-605.

(71) Cole-Hamilton, D. J.; Tooze, R. P. In *Catalyst Separation, Recovery and Recycling: Chemistry and Process Design*; Cole-Hamilton, D.J., Tooze, R.P., Eds.; Springer Science & Business Media: Netherlands, 2006, p 1-8.

(72) Cardona, F.; Parmeggiani, C. *Transition Metal Catalysis in Aerobic Alcohol Oxidation*; Cardona, F., Parmeggiani, C., Eds.; Royal Society of Chemistry: Cambridge, 2014; Vol. 28.

(73) Strukul, G. *Catalytic Oxidations with Hydrogen Peroxide as Oxidant*; Strukul, G., Ed.; Kluwer Academic Publisher: Dordrecht, 2013.

(74) Johnson, T. C.; Morris, D. J.; Wills, M. *Chem. Soc. Rev.* **2010**, *39*, 81-88.

(75) Dobereiner, G. E.; Crabtree, R. H. *Chem. Rev.* **2010**, *110*, 681-703.

(76) Nichols, J. Oxidation of castor oil. US2851471 A. September 9, 1958.

(77) Strauss, C. R. *Aust. J. Chem.* **1999**, *52*, 83-96.

(78) Djerassi, C.; Manson, A. J.; Gorman, M. *J. Am. Chem. Soc.* **1955**, *77*, 4925-4926.

(79) Naota, T.; Takaya, H.; Murahashi, S.-I. *Chem. Rev.* **1998**, *98*, 2599-2660.

(80) Csjernyik, G.; Éll, A. H.; Fadini, L.; Pugin, B.; Bäckvall, J.-E. *J. Org. Chem.* **2002**, *67*, 1657-1662.

(81) Noyori, R.; Yamakawa, M.; Hashiguchi, S. *J. Org. Chem.* **2001**, *66*, 7931-7944.

(82) Seddon, E. A.; Seddon, K. R. *The Chemistry of Ruthenium*; Clark, R. J. H., Ed.; Elsevier: Amsterdam, 1984; Vol. 19.

(83) Casey, C. P.; Singer, S. W.; Powell, D. R.; Hayashi, R. K.; Kavana, M. *J. Am. Chem. Soc.* **2001**, *123*, 1090-1100.

(84) Conley, B. L.; Pennington-Boggio, M. K.; Boz, E.; Williams, T. J. *Chem. Rev.* **2010**, *110*, 2294-2312.

(85) Shvo, Y.; Czarkie, D.; Rahamim, Y.; Chodosh, D. F. *J. Am. Chem. Soc.* **1986**, *108*, 7400-7402.

(86) Karvembu, R.; Prabhakaran, R.; Natarajan, K. *Coord. Chem. Rev.* **2005**, *249*, 911-918.

(87) Blum, Y.; Shvo, Y. *Isr. J. Chem.* **1984**, *24*, 144-148.

(88) Blum, Y.; Shvo, Y. *J. Organomet. Chem.* **1985**, *282*, C7-C10.

(89) Bruce, M. I.; Knight, J. R. *J. Organomet. Chem.* **1968**, *12*, 411-413.

(90) Washington, J.; McDonald, R.; Takats, J.; Menashe, N.; Reshef, D.; Shvo, Y. *Organometallics* **1995**, *14*, 3996-4003.

(91) Shvo, Y.; Czarkie, D. *J. Organomet. Chem.* **1986**, *315*, C25-C28.

(92) Menashe, N.; Shvo, Y. *Organometallics* **1991**, *10*, 3885-3891.

(93) Mays, M. J.; Morris, M. J.; Raithby, P. R.; Shvo, Y.; Czarkie, D. *Organometallics* **1989**, *8*, 1162-1167.

(94) Johnson, J. B.; Bäckvall, J.-E. *J. Org. Chem.* **2003**, *68*, 7681-7684.

(95) Casey, C. P.; Johnson, J. B.; Singer, S. W.; Cui, Q. *J. Am. Chem. Soc.* **2005**, *127*, 3100-3109.

(96) Casey, C. P.; Bikzhanova, G. A.; Cui, Q.; Guzei, I. A. *J. Am. Chem. Soc.* **2005**, *127*, 14062-14071.

(97) Almeida, M. L. S.; Beller, M.; Wang, G.-Z.; Bäckvall, J.-E. *Chem. Eur. J.* **1996**, *2*, 1533-1536.

(98) Almeida, M. L. S.; Kočovský, P.; Bäckvall, J.-E. *J. Org. Chem.* **1996**, *61*, 6587-6590.

(99) Karlsson, U.; Wang, G. Z.; Bäckvall, J.-E. *J. Org. Chem.* **1994**, *59*, 1196-1198.

(100) Denmark, S. E.; Stavenger, R. A.; Wong, K.-T.; Su, X. *J. Am. Chem. Soc.* **1999**, *121*, 4982-4991.

(101) Krohn, K.; Knauer, B.; Küpke, J.; Seebach, D.; Beck, A. K.; Hayakawa, M. *Synthesis* **1996**, *1996*, 1341-1344.

(102) Ahmad, M. U.; Sinha, S.; Husain, S. K.; Osman, S. M. *J. Sci. Food Agric.* **1984**, *35*, 408-414.

(103) de Graauw, C. F.; Peters, J. A.; van Bekkum, H.; Huskens, J. *Synthesis* **1994**, *1994*, 1007-1017.

(104) Oppenauer, R. V. *Recl. Trav. Chim. Pays-Bas* **1937**, *56*, 137-144.

(105) Mahrwald, R. *Curr. Org. Chem.* **2003**, *7*, 1713-1723.

(106) Cho, C. S.; Kim, D. Y.; Shim, S. C. *Bull. Korean Chem. Soc.* **2005**, *26*, 802-804.

(107) Takahashi, M.; Oshima, K.; Matsubara, S. *Tetrahedron Lett.* **2003**, *44*, 9201-9203.

(108) Adkins, H.; Elofson, R. M.; Rossow, A. G.; Robinson, C. C. *J. Am. Chem. Soc.* **1949**, *71*, 3622-3629.

(109) House, H. O.; Huber, L. E.; Umen, M. J. *J. Am. Chem. Soc.* **1972**, *94*, 8471-8475.

(110) Mandell, L. *J. Am. Chem. Soc.* **1956**, *78*, 3199-3201.

(111) Desiraju, G. R.; Curtin, D. Y.; Paul, I. C. *Mol. Cryst. Liq. Cryst.* **1979**, *52*, 259-266.

(112) Adkins, H.; Cox, F. W. *J. Am. Chem. Soc.* **1938**, *60*, 1151-1159.

(113) Beaupere, D.; Bauer, P.; Nadjo, L.; Uzan, R. *J. Organomet. Chem.* **1982**, *238*, C12-C14.

(114) Isaac, I.; Aizel, G.; Stasik, I.; Wadouachi, A.; Beaupère, D. *Synlett* **1998**, *1998*, 475-476.

(115) Isaac, I.; Staslk, I.; Beaupère, D.; Uzan, R. *Tetrahedron Lett.* **1995**, *36*, 383-386.

(116) Michielli, R. F.; Elving, P. J. *J. Am. Chem. Soc.* **1968**, *90*, 1989-1995.

(117) Schinz, H.; Rossi, A. *Helv. Chim. Acta* **1948**, *31*, 1953-1962.

(118) Rüegg, R.; Jeger, O. *Helv. Chim. Acta* **1948**, *31*, 1753-1759.

(119) Zobrist, F.; Schinz, H. *Helv. Chim. Acta* **1949**, *32*, 1192-1197.

(120) Graves, C. R.; Zeng, B.-S.; Nguyen, S. T. *J. Am. Chem. Soc.* **2006**, *128*, 12596-12597.

(121) Gliński, M.; Ulkowska, U. *Catal. Letters* **2011**, *141*, 293-299.

(122) Ballard, S. A.; Finch, H. D.; Winkler, D. E. In *Advances in Catalysis*; Adalbert, Farkas, Ed.; Academic Press: New York, 1957, p 754-763.

(123) Tuokko, S.; Pihko, P. M.; Honkala, K. *Angew. Chem., Int. Ed.* **2016**, *55*, 1670-1674.

(124) Muzart, J. *European J. Org. Chem.* **2015**, *2015*, 5693-5707.

(125) Chowdhury, R. L.; Backvall, J.-E. *J. Chem. Soc. Chem. Commun.* **1991**, 1063-1064.

(126) Dobson, A.; Robinson, S. D. *Inorg. Chem.* **1977**, *16*, 137-142.

(127) Morton, D.; Cole-Hamilton, D. J. *J. Chem. Soc. Chem. Commun.* **1988**, 1154-1156.

(128) Morton, D.; Cole-Hamilton, D. J.; Utuk, I. D.; Paneque-Sosa, M.; Lopez-Poveda, M. *J. Chem. Soc., Dalton Trans.* **1989**, 489-495.

(129) Junge, H.; Beller, M. *Tetrahedron Lett.* **2005**, *46*, 1031-1034.

(130) Junge, H.; Loges, B.; Beller, M. *Chem. Comm.* **2007**, 522-524.

(131) Adair, G. R. A.; Williams, J. M. J. *Tetrahedron Lett.* **2005**, *46*, 8233-8235.

(132) Baratta, W.; Bossi, G.; Putignano, E.; Rigo, P. *Chem. Eur. J.* **2011**, *17*, 3474-3481.

(133) Bäckvall, J.-E.; Andreasson, U. *Tetrahedron Lett.* **1993**, *34*, 5459-5462.

(134) Ren, K.; Hu, B.; Zhao, M.; Tu, Y.; Xie, X.; Zhang, Z. *J. Org. Chem.* **2014**, *79*, 2170-2177.

(135) Fanelli, F.; Parisi, G.; Degennaro, L.; Luisi, R. *Beilstein J. Org. Chem.* **2017**, *13*, 520-542.

(136) Jas, G.; Kirschning, A. *Chem. Eur. J.* **2003**, *9*, 5708-5723.

(137) Wiles, C.; Watts, P. *Green Chem.* **2012**, *14*, 38-54.

Chapter 2

Synthesis of Sebacic Acid by Oxidative Cleavage of α,β-Unsaturated Carbonyl Fatty Acids

2.1. INTRODUCTION

α,ω-Dicarboxylic acids derived from basic oleochemicals are of great interest. The general formula for this class of compound is $HOOC(CH_2)_nCOOH$ where n represents the number of methylene groups. Typically, n is between 0 and 22. In general, α,ω-dicarboxylic acids are industrially important chemicals due to their potential in the production of various intermediates.[1] α,ω-Dicarboxylic acids are classified in three groups according to the size of the carbon chain: a) short-chain α,ω-dicarboxylic acids where n=0-6; b) medium-chain α,ω-dicarboxylic acids (n=7-9) and; c) long-chain α,ω-dicarboxylic acids (n≥10). Long-chain diacids provide greater flexibility and strength than short-chain diacids due to their ability to bend, which minimizes breakage and reduces the number of links in a polymer.

Sebacic acid **14**, a medium-chain α,ω-dicarboxylic acid, is a very unique chemical with a wide diversity of applications and performance benefits (**Figure 2.1**).

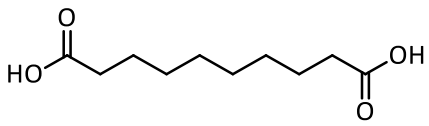
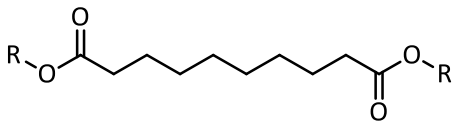


Figure 2.1. Chemical structure of sebacic acid **14**.

Sebacic acid **14** was named from the Latin sebaceus (tallow candle) or sebum (tallow) in reference to its use in the manufacture of candles. One of the largest uses of sebacic acid **14** is the manufacture of Nylon 6,10. Sebacic acid **14** and hexamethylene diisocyanate polymerize through condensation to produce Nylon 6,10. More than 70% of global sebacic acid **14** demand is for nylons 10,10 and 6,10 according to CMAI (2010). In the industrial setting, sebacic acid **14** can be used as such or as an intermediate in lubricants, hydraulic fluids, cosmetics, candles, aromatics, antiseptics and painting materials. Two derivatives of sebacic acid **14** are used in a range of applications as well (**Figure 2.2**):

- Dibutyl sebacate (DBS, **15**) is a transparent oil liquid, dissolves ethanol and ether. This product is widely used as rocket propellant. Being non-toxic, used in food and pharma industry as packing material. It is also used as cold resistant plasticizer for synthetic resin and synthetic rubber.
- Dioctyl sebacate (DOS, **16**) is a transparent light yellow oil liquid with a distinct smell. This product with low volatility and high-resistant, lightproof, and electrical insulation dissolve in hydrocarbons, alcohol, ether, benzene and other organic solvents. It is mainly used by PVC,

chloroethylene copolymer, nitrocellulose, ethyl cellulose and synthetic rubber industries as plasticizer and suitable for cold resistant cables, leatherette, thin film, sheet material, etc.



15: R=C_4H_9
16: R=C_4H_{17}

Figure 2.2. Chemical structure of DBS **15** and DOS **16**.

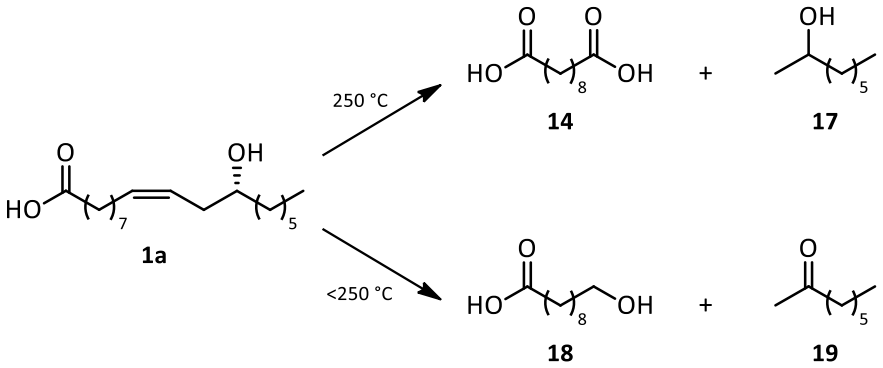
In 2010, the global demand for high performance sebacic acid **14** was about 58,700 metric tons with more than 90% produced in China, where the installed capacity reaches 125,000 metric tons/year. Sebacic acid **14** can be commercially produced by electro oxidation of adipic acid, by oxycarbonylation of butadiene or by oxidation of stearic acid with N_2O_4. However, alkaline oxidative cleavage is the most applied industrially process to produce sebacic acid **14**. This process requires the use of a high amount of oxidant and energy besides the use of heavy metals to split the ricinoleic acid **1a** in either a batch or continuous process.[2-6]

α,β-Unsaturated carbonyl fatty acids are very suitable intermediates to synthesize dicarboxylic acids due to the high reactivity of the conjugated double bond. This chapter was mainly focused on the search of a more selective and energetically favorable synthetic route to afford sebacic acid **14** by oxidative cleavage of the α,β-unsaturated carbonyl fatty acids derived from ricinoleic acid **1a**.

2.2. OXIDATIVE CLEAVAGE OF UNSATURATED FATTY ACIDS

2.2.1. Alkaline oxidative cleavage

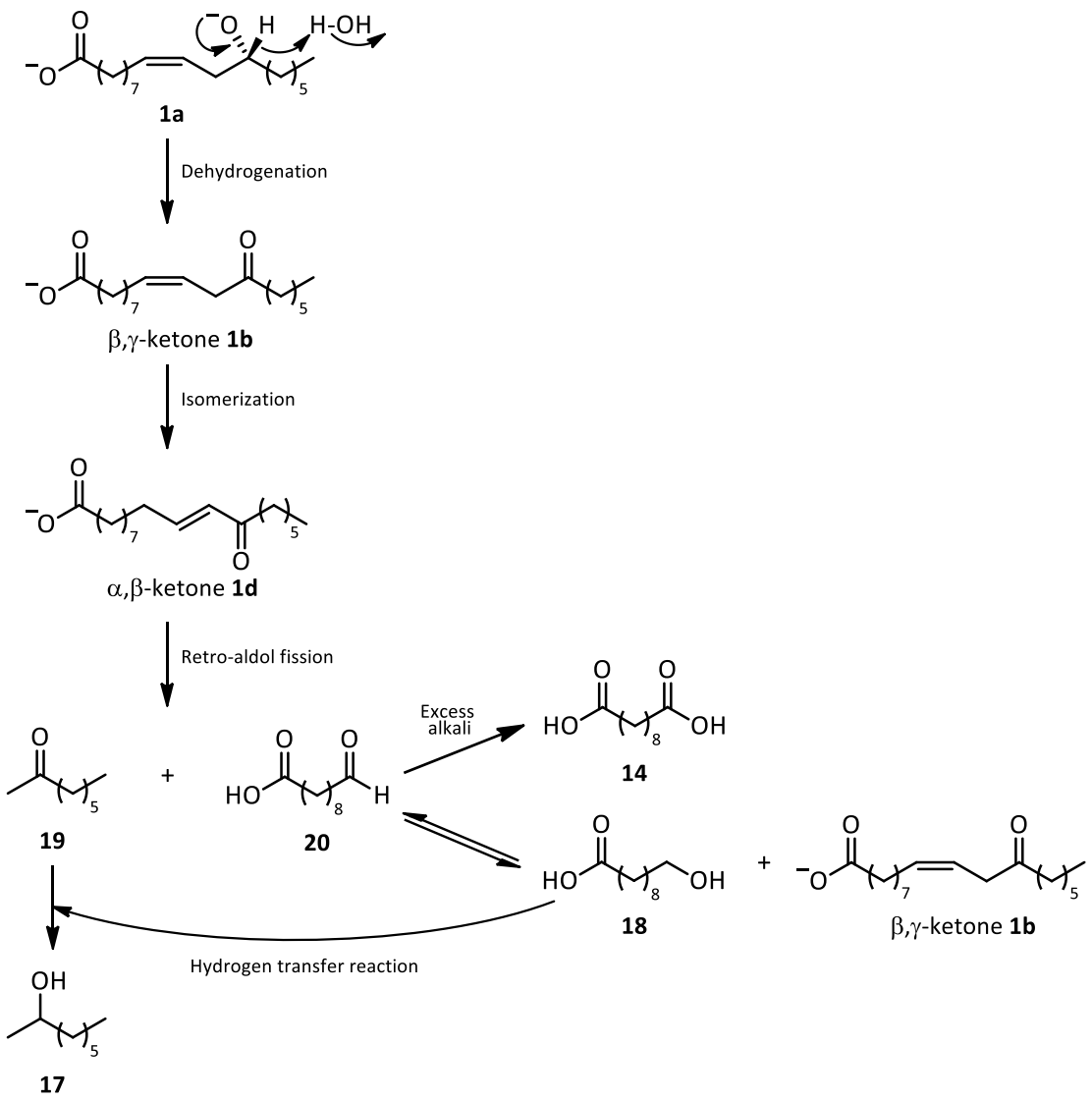
As it was indicated above, alkaline oxidative cleavage is one of the industrially processes to convert ricinoleic acid **1a** to chemical intermediates with 8 and 10 atoms. It is accomplished with sodium hydroxide or potassium hydroxide at elevated temperatures and in the presence of catalysts to split the ricinoleate molecule. Dependent upon reaction conditions, different products can be obtained (**Scheme 2.1**).



Scheme 2.1. Alkaline oxidative cleavage of ricinoleic acid **1a**.

At reaction temperatures of 180-200 °C, 10-hydroxydecanoic acid **18** and 2-octanone **19** are achieved using a molar ratio of alkali and long reaction times (~13 h).[4-6] The 10-hydroxydecanoic acid **18** is formed in good yield if the reaction is performed in the presence of a high-boiling unhindered primary or secondary alcohol.[7,8]

The increase of two the mol ratio of alkali/mol ricinoleate with an increased temperature range of 250-275 °C and a shorter reaction cycle produces sebacic acid **14** and 2-octanol **17**. A detailed reaction mechanism has been proposed by Dyntham *et al*.[6] in 1960 to explain the alkaline oxidative cleavage. During the alkaline oxidative cleavage, ricinoleic acid **1a** is converted into the β,ϒ-unsaturated carbonyl acid **1b** which isomerizes in alkaline media to the α,β-unsaturated carbonyl acid **1d**. The α,β-unsaturated ketone undergoes a retro-aldol reaction to yield the 2-octanone **19** and the C10 formil acid **20**. These products become starting points for a complex series of reactions (**Scheme 2.2**). The formil acid **20** can react in one of two ways: it can go irreversibly to the dicarboxylic sebacic acid **14** or the C10 formil acid **20** can accept hydrogen from ricinoleic acid **1a** and convert it to β,ϒ-unsaturated carbonyl acid **1b**, which recycles into the system, and gives rise to the 10-hydroxydecanoic acid **18**.



Scheme 2.2. Mechanism of the alkaline oxidative cleavage of ricinoleic acid **1a**.

In the actual reaction, the castor oil **5a** and sodium hydroxide are fed to a reactor at a temperature of 180 to 270 °C where the ricinoleic acid **1a** undergoes a series of reactions with evolution of hydrogen to give disodium sebacate and 2-octanol **17**. The disodium sebacate is then partially neutralized to the half acid salt which is water soluble. The oil and aqueous layers are separated. The aqueous layer containing the half salt is acidulated to a pH of about 2, causing the resulting sebacic acid **14** to precipitate from the solution. It is then filtered, water washed, and finally dried.

The literature reveals that the yield of sebacic acid **14** in earlier attempts have been low and far from satisfactory. Adams *et al*.[9] synthetized 2-octanol **17** by alkaline oxidative cleavage in 1921.

As a result, 2-octanol **17** was obtained in 23-42% conversion based on castor oil **5a** whereas sebacic acid **14** was obtained as by-product in a low yield. In subsequent years, several US patents described changes in the reaction conditions to improve the yield of sebacic acid **14**. Higher amounts of NaOH and the use of lubricants or mineral oils to control the formation of foam are some of the changes introduced.[10-12] Verma *et al*.[13] introduced the lead tetroxide (Pb_3O_4) as catalyst, which increased the yield of sebacic acid **14** to 70%. In view of the performance improvement, other metal oxides such as cadmium oxide, barium oxide or nickel oxides were used as contemplated in several patents.[14-17] Vasishtha *et al*.[18] investigated the alkali pyrolysis of castor oil **5a** in the presence of white mineral oil (mixture of alkanes from 15–40 carbons and cyclic paraffins) and 1% lead tetroxide catalyst, which yielded 70.1% 2-octanol **17** and 72.5% sebacic acid **14**. The use of sodium nitrate as a catalyst is present in a US patent.[19] This patent describes the use of heat transfer fluids such as an aromatic oil, glycol oil or petroleum oil as a diluent to allow the mechanical agitation of the reaction mixture. Three years after, a process for the synthesis of sebacic acid **14** and 2-octanol **17** at high temperature accomplished by using a chemically inert thinning agent was disclosed. The use of this agent changed the fluidity of the solution, thus allowing for improved yield and efficiency of the reaction.[20]

In 2008, Azcan *et al*.[21] introduced the use of the microwave technology on the alkaline oxidative cleavage of castor oil **5a**. In this study, different parameters, such as NaOH/oil ratio, reaction temperature, and reaction time were evaluated. The results of the study suggested that the alkali fission of methylated, presaponifed castor oil containing 1 wt-% Pb_3O_4 in a 250-mL quartz reactor at 240 °C for 20 min reaction time can yield 76.2% sebacic acid **14** and 62.6% 2-octanol **17**. According to the results, it could be concluded that microwave heating reduced the reaction time from hours (5 h) to minutes (20 min). Moreover, the reaction temperature was reduced (20 K decrease) compared to literature data carried out with conventional heating techniques.[18]

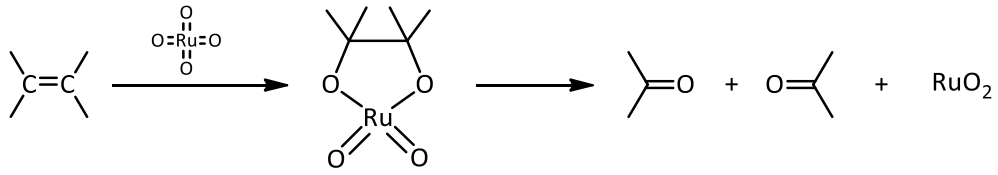
2.2.2. Alkaline sodium hypochlorite cleavage

Zaidman *et al*.[22] presented different oxidizing agents, including potassium permanganate, dichromate, chromic acid, hydrogen peroxide, ozone, and sodium hypochlorite, in the oxidative cleavage of unsaturated fatty acid double bonds, analyzed their economic performance, and developed a manufacturing computer-simulation based on sodium hypochlorite-$RuCl_3$ oxidation technology. The economic parameters obtained showed that sodium hypochlorite is the most commercially efficient oxidant. Although the ruthenium-hypochlorite system is widely known to break down unsaturated fatty acids, Tao *et al*.[23] achieved dicarboxylic acids by the treatment of soy fatty acid with sodium hypochlorite in absence of ruthenium catalyst. The data showed that the

ruthenium catalyst accelerated the initial double-bond oxidation rate but consumed more active chlorine and retarded the completion of oxidation. At 25°C without catalyst, only 24 h were required to transform all double bonds, consuming only 51.4% of the active chlorine (close to the stoichiometric amount required). Thus, the absence of catalyst decreased the consumption of active chlorine by 31.4% and completed the oxidation faster. During the reaction, C_8 diacid oxidation products were detected by GC-MS analysis.

2.2.3. Ruthenium-catalyzed oxidative cleavage

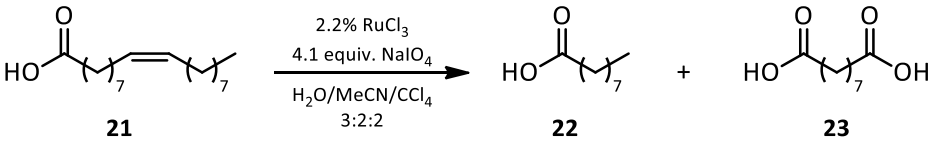
Transition metal-based catalytic systems are considered to be most suitable for the oxidative cleavage of olefins. Their high catalytic activities make it possible to use more gentle oxidants. Ruthenium is one of the oldest transition metals employed in the oxidative cleavage of C–C double bonds.[24,25] Ruthenium tetroxide is a powerful oxidant in organic synthesis for the oxidative cleavage of olefins. The stoichiometric oxidation of double bonds by RuO_4 is fast and very selective.[26-29] This resulting from a reaction mechanism that does not involve epoxides or hydroxylated intermediates. This mechanism involves the formation of a cyclic perruthenate ester.[30] RuO_4 can also be used as a catalyst when $RuCl_3$ is employed with a secondary oxidant like $NaIO_4$, NaClO, *tert*-BuOOH or RCOOOH. These oxidants can perform the re-oxidization of RuO_2 to RuO_4 (**Scheme 2.3**).



Scheme 2.3. Oxidative cleavage of C–C double bonds by RuO_4.

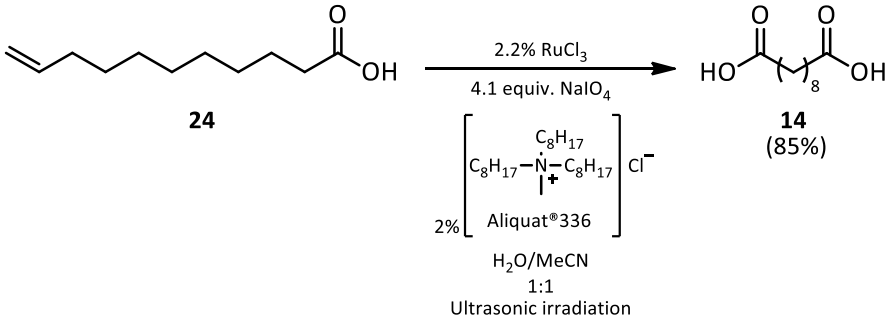
Sharpless and co-workers have reported on the use of $RuCl_3$ in combination with $NaIO_4$ for oxidative cleavage of alkenes. RuO_4 is generated in situ by the reaction between $RuCl_3$ (or RuO_2) and $NaIO_4$.[31] Usually, a catalytic amount of ruthenium is used in a biphasic media water/organic solvent(s). RuO_4 is generated in this biphasic mixture, and the resulting RuO_2 remains dissolved in the solvent mixture after the oxidation of the substrate. Otherwise, RuO_2 will precipitate in the organic solvents[32] inhibiting the catalytic cycle. Sharpless underline the important role of (i) carbon tetrachloride for RuO_4 solubilization and (ii) acetonitrile to avoid the catalytic cycle inactivation due to low-valent ruthenium carboxylate complexes.[33] For these considerations and because RuO_4 is a vigorous oxidant and the number of co-solvents is limited, the substitution of CCl_4 or MeCN by another co-solvent is not easy.

The Sharpless system has been widely explored and further developed by other groups.[34] Catalytic amounts of $RuCl_3$ with stoichiometric amounts of $NaIO_4$ in CCl_4/MeCN/H_2O,[35,36] can also be used to oxidatively cleave oleic acid **21** into nonanoic acid **22** and azelaic acid **23**, as reported by Nakano and co-workers (**Scheme 2.4**).[37]



Scheme 2.4. Oxidative cleavage of oleic acid **21** carried out by Nakano and co-workers.[37]

Optimization of the system involved the replacement of CCl_4 by ethyl acetate to give the system EtOAc/MeCN/H_2O 2:2:3 (v/v) in order to nearly quantitatively cleave a series of unsaturated fatty acids within 2-4 h at room temperature, using 2.2% catalyst loading and 4.1 equiv. $NaIO_4$.[38] Sebacic acid **14** was isolated in 78% yield from 10-undecylenic acid **24**. Later, the same group carried out a study to assess the influence of the mixture of solvents.[39] The substitution of CCl_4 by the emulsifier Aliquat® 336 and the use of ultrasound technology increased the reaction rate of oleic acid **21** cleavage to yield 96% nonanoic acid **22** and 81% azelaic acid **23** in MeCN/H_2O 1:1 (v/v) in only 45 min at room temperature. Under these conditions, *trans*-unsaturated fatty acids of different chain lengths can also be readily cleaved with this system (**Scheme 2.5**). Alternatively, the use of organic solvents with this system can even be omitted. 10-Undecylenic acid **24** under 20 kHz ultrasonic irradiation and with Aliquat® 336 in aqueous solution for 6 h gives 85% of sebacic acid **14** after 45 min.[40]

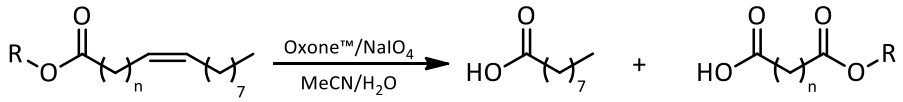


Scheme 2.5. General oxidative cleavage of 10-undecylenic acid **24** into sebacic acid **14** carried out by Zimmermann *et al*.[39]

2.2.4. Oxone™/periodate oxidative cleavage

Adaptation of systems known to catalyze epoxidation or dihydroxylation of internal olefins can therefore be of interest for the development of new catalytic systems for selective oxidative C=C bond scission. Oxone™ is an attractive oxidant for alkene oxidation, as it is cheap and readily accessible. Epoxidations with Oxone™ have been reported in water at neutral pH and without organic solvent, but the activity for electron-rich aliphatic olefins proved negligible under these conditions.[41] Other common solvents for epoxidations with Oxone™ are aqueous MeCN or hexafluoroisopropanol. Ketones are commonly added in such olefin oxidations with Oxone™,[42,43] to yield dioxirane intermediates, which are the active species in the epoxidation.[44,45] Recently, Gebbink *et al.*[46] observed significant amounts of cleavage products in reactions with a combination of sacrificial oxidants only, i.e. without any metal complex. They demonstrated that the combination of Oxone™ and periodate in a MeCN/H_2O reaction mixture shows a preference for the cleavage of electron-rich internal olefins. Moreover, industrially interesting unsaturated fatty acids such oleic acid **21** and derivatives can be oxidatively cleaved into mono- and diacids and a series of terpenes can be transformed into diacids (**Table 2.1**).

Table 2.1. Oxidative cleavage of unsaturated fatty acids.[a]



22

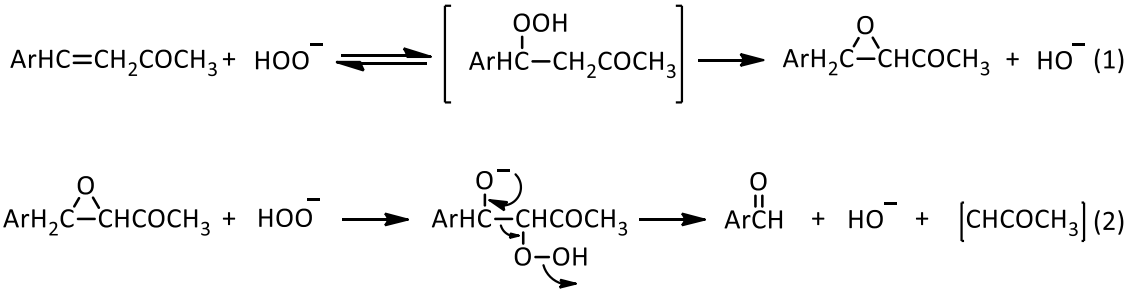
Entry	n	R	Time (h)	Conversion (%)[b]	Yield (%)[b]
1	8	Me	6	83	70
2	8	Me	18	96	95
3	8	H	6	100	99
4	11	Me	18	65	52
5	8	H	18	100	99

[a]Oxone™ (2 equiv.), $NaIO_4$ (1.5 equiv.). MeCN/H_2O (3:1 v/v), 0.18 M, 2-16 h reflux, then. MeCN/H_2O (3:9 v/v), 0.07 M, 2 h reflux.
[b]Yield of nonanoic acid **22** determined by GC.

2.2.5. Alkaline hydrogen peroxide cleavage

Oxidation with alkaline hydrogen peroxide, as described by Weitz and Scheffer in 1921,[47] has been used successfully for preparation of the epoxides of many α,β-unsaturated ketones and aldehydes. The knowledge of the mechanism of this reaction is largely due to the work of Bunton and Minkoff.[48] Based on this study, the first work referred to the cleavage of α,β-unsaturated ketones in presence of alkaline hydrogen peroxide was described by Sapper and coworkers in 1955.[49] They reported that alkaline hydrogen peroxide oxidation can follow quite a different course in the case of α,β-unsaturated ketones which present functional groups next the ketone. Their experiments led to the hypothesis that α,β-unsaturation and a rather readily enolizable hydrogen on the α'-carbon were necessary and sufficient to render a ketone susceptible to the cleavage. They proposed that the attack of the peroxide must be preceded by the attack that occurs on the α,β-unsaturation. Such a requirement would seem to be explained best by the assumption that the oxidation at the α'-position is accomplished by interaction with a hydroperoxide group initially introduced at the β-position. The procedure used with this reaction mixture was designed primarily for the separation of the aldehyde fraction; the quantity of acid obtained was too small.

Later, Temple carried out the cleavage of a variety of α,β-unsaturated ketones, which were converted into their corresponding acids in good yields.[50] The oxidative cleavage reaction is composed by a two-step mechanism. The first step is the epoxidation of the double bond, after that the opening of the epoxide ring in basic media followed by the elimination of hydroxide from β-hydroxy ketone leads to the retro aldol cleavage (**Scheme 2.6**).



Scheme 2.6. Epoxidation and cleavage of α,β-unsaturated ketones mechanism.

2.3. OBJECTIVE

The objective of this chapter was to synthetize sebacic acid **14** through the oxidative cleavage of the α,β-unsaturated carbonyl fatty acids derived from ricinoleic acid **1a** and obtained in **Chapter 1**. This method was designed to be more selective, benign and energetically favourable than the current ones.

2.4. RESULTS AND DISCUSSION

2.4.1. Alkaline oxidative cleavage

Initially, (*E*)-12-oxo-10-octadecenoic acid **1d** was selected as substrate to study the oxidative cleavage of the conjugated double bond. Microwave technology was chosen as heating system due to the advantages of speed, selectivity, precise control and improved economics owing to consumption of less energy.[51]

Alkaline oxidative cleavage of (*E*)-12-oxo-10-octadecenoic acid **1d** was carried out according to a known procedure,[10] in which sodium hydroxide is used in excess (50% w/v). A series of reaction in which the temperature was changing was carried out to find the minimum required temperature. Four temperatures were analyzed: 100, 120, 140 and 150 °C. The results are shown in **Figure 2.3**. The yields and conversion were calculated by GC-MS. For this, the reaction mixture was derivatized using methanol and catalytic H_2SO_4. The conversion of the starting material was complete in all the cases. With a temperature of 100 °C, sebacic acid **14** was no observed although the (*E*)-12-oxo-10-octadecenoic acid **1d** was totally consumed. Since the temperature was increased, a higher percentage of sebacic acid **14** was obtained. At 120 °C only 19% yield of sebacic acid **14** was detected but when the temperature was increased 20 °C, the percentage of sebacic acid **14** was almost doubled. Finally, with a temperature of 150 °C, 90% of sebacic acid **14** was achieved.

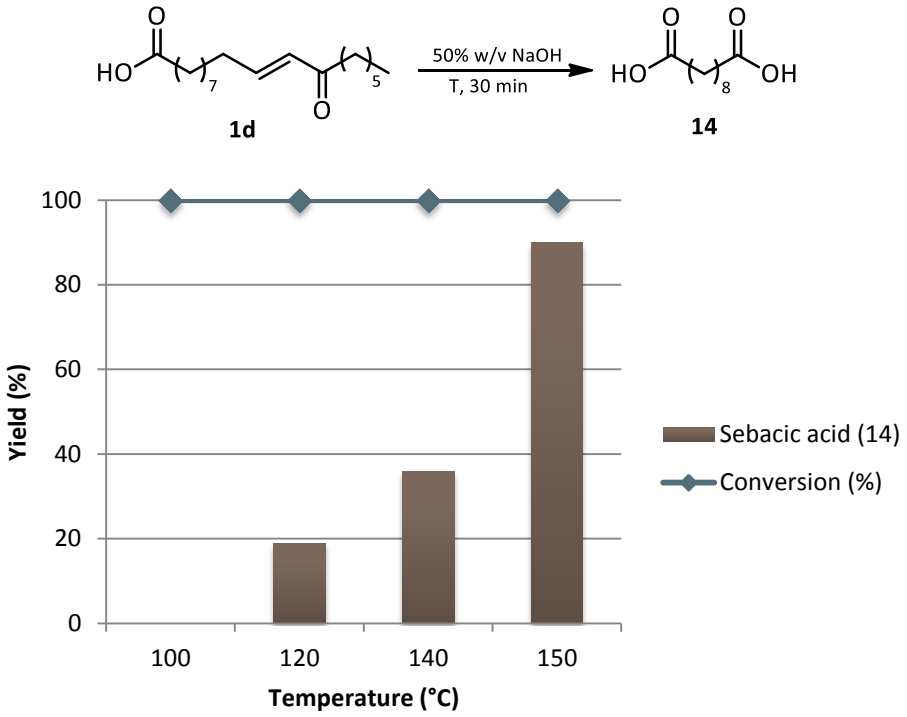


Figure 2.3. Effect of temperature in the alkaline oxidative cleavage of (*E*)-12-oxo-10-octadecenoic acid **1d**. Reaction conditions: (*E*)-12-oxo-10-octadecenoic acid **1d** (0.16 mmol) and 50% w/v NaOH (6.25 mmol) under microwave irradiation for 30 min.

From these results it is clear that the alkali splitting of the α,β-unsaturated carbonyl fatty acid **1d** could be performed at a lower temperature than the necessary to cleavage ricinoleic acid **1a**, but the excessive amount of alkali was still the major drawback. Different concentrations of alkali were studied with the aim of determining if lower amounts of alkali allowed the alkali splitting of α,β-unsaturated carbonyl fatty acids. **Figure 2.4** shows the results after evaluating five different concentrations. Interestingly, as the alkali concentration was decreased, an increase in the yield of the 12-oxooctadecanoic acid **1e** was detected. As seen in **Chapter 1**, the α,β-unsaturated carbonyl fatty acid **1d** tended to be reduced quickly and easily due to its large hydrogen acceptor capacity. These results were in accordance with the mechanism of alkali fission described by Dytham.[6] However, sebacic acid **14** was obtained in 80% yield when 6% w/v of sodium hydroxide was used. The oxidation of the C10 formil acid **20** was faster than the reduction of the (*E*)-12-oxo-10-octadecenoic acid **1d**. This implied that the alkaline oxidative cleavage of α,β-unsaturated carbonyl fatty acids can be carried out with lower amounts of sodium hydroxide.

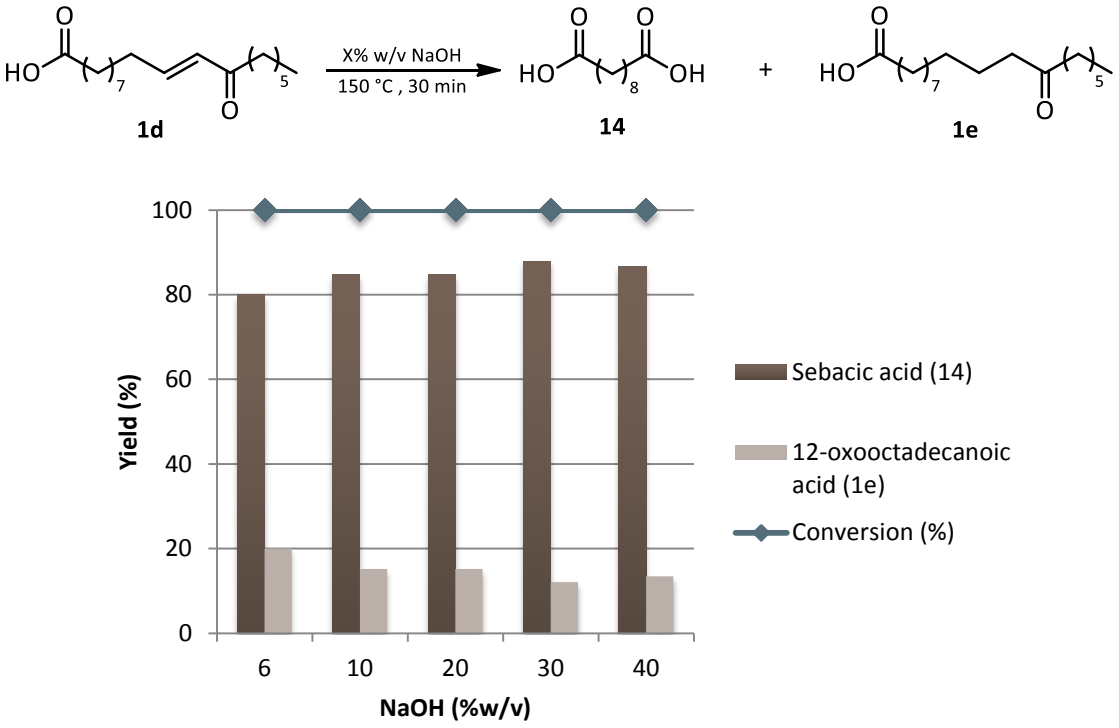


Figure 2.4. Effect of concentration of sodium hydroxide in the alkaline oxidative cleavage of (*E*)-12-oxo-10-octadecenoic acid **1d**. Reaction conditions: (*E*)-12-oxo-10-octadecenoic acid **1d** (0.16 mmol) and NaOH under microwave irradiation for 30 min at 150 °C.

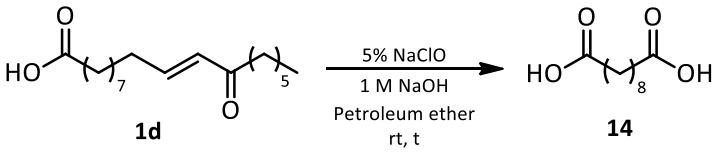
Although the yield of sebacic acid **14** was slightly lower using 6% sodium than the other tests, the results were quite satisfactory. Therefore, the reaction was scaled up using this concentration of sodium hydroxide with the objective of isolating the dicarboxylic acid **14**. The microwave used was a Milestone Ethos-Touch Control equipped with a temperature sensor. Two grams of (*E*)-12-oxo-10-octadecenoic acid **1d** and 68 mL of 6% w/v NaOH were placed in a 100 mL-PTFE tube. After 30 min heating at 150 °C, the reaction mixture was allowed to room temperature. The work up of the reaction was carried out based on the procedure described by Emslie in a patent slightly modified.[52] 75% H_2SO_4 was added until pH 6.5 was reached. At this point, an oily layer was formed at the top of the aqueous solution. This top layer was withdrawn (16% of total weight), derivatized and analyzed by GC-MS. This first isolated layer contained a 62% of sebacic acid **14**. Next, the remaining aqueous system was adjusted to pH 5.6 by addition of 75% H_2SO_4. A second oily layer appeared and was also withdrawn. This portion constituted the 45% of the total weight. Despite being the most abundant part, only 36% of sebacic acid **14** was present in this layer. Finally, the pH was reduced to 2.5 appearing a yellow solid which was filtered (39% of total weight). The GC-MS analysis revealed that it was formed by a mixture of dicarboxylic acid in which azelaic acid **23** was the main product.

As it was indicated above, the alkaline oxidative cleavage of castor oil **5a** at a temperatures of 185-195 °C is favored by the presence of primary or secondary alcohols like 2-octanol **17**. In addition, at temperatures of the order of 240 °C, the presence of ketones or formil acids improves the yield of the alkali fission.[53] In view of the achieved results, we performed the microwave-assisted scaling up in presence of 2-octanone **19**, a ketone with a high boiling point. The reaction conditions were the same that the used before: 2 g of the α,β-unsaturated ketone **1d**, 68 mL of 6% NaOH and 4 equiv. of 2-octanone **19**. The analysis of the oily layers obtained at pH 6.5 and 5.6 revealed that the main product was the starting material **1d**. On the other hand, the yellow solid from pH 2.5 resulted a mixture of monocarboxylic acids.

2.4.2. Alkaline sodium hypochlorite oxidative cleavage

Initially, the cleavage with alkaline sodium hypochlorite of the compound **1d** was accomplished at room temperature. To favor the solubility of the starting material **1d**, petroleum ether was added in a small volume (2% v/v). The amount of active chlorine used in the reaction was a ratio 1:9 α,β-unsaturated ketone/5% NaClO. It was added in twice in order to minimize the loss of active chlorine. The pH of the reaction was taken to 8 by the addition of 1 M NaOH. **Table 2.2** shows the results achieved performing the reaction for 24, 48 and 72 h. At 24 h (entry 1), the conversion of the starting material **1d** was 70% however, only 7% of sebacic acid **14** was afforded. Conversion was complete after 48 h of reaction (entry 2) and the yield increased to 11%. Finally, despite the 72 h of reaction (entry 3) and the 100% of conversion, the yield of sebacic acid **14** was 13%.

Table 2.2. Time evolution of cleavage of (*E*)-12-oxo-10-octadecenoic acid **1d** by alkaline sodium hypochlorite.[a]



Entry	Time(h)	Conversion (%)[b]	Yield (%)[b]
1	24	70	7
2	48	100	11
3	72	100	13

[a](*E*)-12-oxo-10-octadecenoic acid **1d** (1.68 mmol), 5% NaClO (15.1 mmol), 1 M NaOH/petroleum ether (3:7, 1125 µL).
[b]Conversion and yield determined by HPLC-UV using standard calibration curves of (*E*)-12-oxo-10-octadecenoic acid **1d** and sebacic acid **14**.

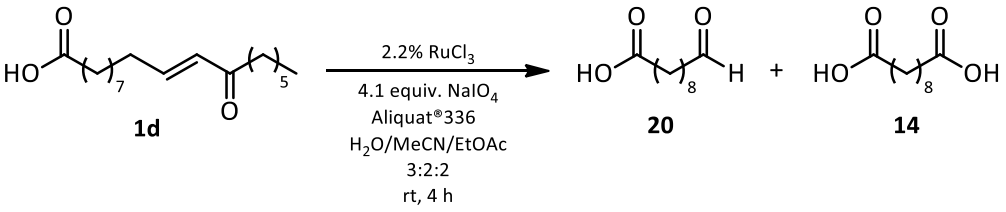
In view of these results, we performed the reaction adding slowly the bleach through an automatic pump. The amounts and conditions were the same as the in the previous experiments. The bleach was added with a flux of 20 µL/min. After 24 h of reaction, a white solid was formed on top of the reaction. This solid was filtered (42% recovery) and analyzed by ^{1}H NMR. Despite the presence of small signals of starting material, the remaining signals indicated the presence of monocarboxylic acids. The rest of reaction was extracted first with petroleum ether and later with dichloromethane. Both fractions were analyzed by ^{1}H NMR. The apolar fraction was totally the starting material **1d** and corresponded to half of the recovered material (~50% conversion). The dichloromethane fraction (7% recovery) was a mixture of the α-chloroketone **25** and the diol **26** (**Figure 2.5**).

25 **26**

Figure 2.5. Chemical structures of compounds **25** and **26**.

2.4.3. Ruthenium-catalyzed oxidative cleavage

With the mind set on the work of Zimmermann *et al.*[38] who optimized the Sharpless system replacing toxic solvents by greener solvents, we decided to apply these conditions to the oxidative cleavage of α,β-unsaturated fatty acids. (*E*)-12-Oxo-10-octadecenoic acid **1d** was treated with the catalyst $RuCl_3 \cdot H_2O$ (2.2%) and $NaIO_4$ (4.1 equiv.) (**Scheme 2.7**). The mixture of solvents used was H_2O/MeCN/EtOAc in a ratio 3:2:2 and 2% w/w of the emulsifier Aliquat® 336 was added. The reaction mixture was stirred at room temperature and after 4 h the starting material **1d** was consumed. The ^{1}H NMR analysis showed a mixture of the formil acid **20** (33%) and the sebacic acid **14** (57%).



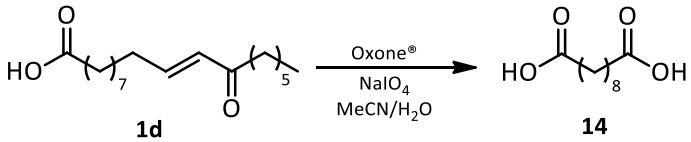
1d **20** **14**

Scheme 2.7. Ruthenium-catalyzed oxidative cleavage of (*E*)-12-oxo-10-octadecenoic acid **1d**.

2.4.4. Oxone™/periodate oxidative cleavage

Based on the studies of Gebbink *et al*.[46] we carried out the reaction between (*E*)-12-oxo-10-octadecenoic acid **1d** and a combination of Oxone™ and sodium periodate (**Table 2.3**). Initially, the proportion of MeCN/H_2O used was 1:3 and the mixture was heated at reflux for 24 h (entry 1). After that time, an abundant black solid in suspension appeared in the reaction media. The GC-MS analysis of a derivatized fraction of the reaction mixture revealed, apart the sebacic acid **14** as the main compound (76%), the presence of heptanoic acid and azelaic acid **23**. In addition, the conversion of the starting material **1d** was complete. After several recrystallizations in hot water, pure sebacic acid **14** was isolated in 41% yield. To improve the solubility of the starting material **1d**, the reaction was tested with a lower amount of H_2O. The proportion of solvents was MeCN/H_2O 1:0.17. A catalytic amount of the emulsifier Aliquat® 336 was also added (entry 2). These changes produced a decrease in the amount of heptanoic acid formed, which resulted into an increase in the yield of the desired compound **14** (89% yield). However, the recovery of sebacic acid **14** (40%) after the recrystallizations in hot water was practically identical to the previous result. On the other hand, the submission of the reaction mixture to continuous ultrasounds for 30 min (entry 3) gave worse results than the classical heating. The conversion was 90% and the crude reaction was more complex. Even though sebacic acid **14** was the main compound, the yield only was 43%.

Table 2.3. Oxidative cleavage of (*E*)-12-oxo-10-octadecenoic acid **1d** by the Oxone™/$NaIO_4$ system.[a]



1d **14**

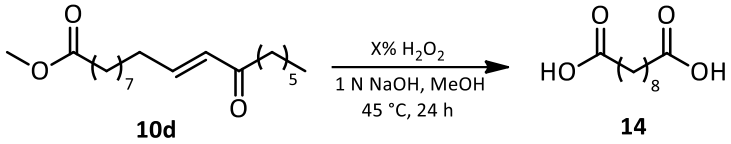
Entry	MeCN/H_2O (v/v)	T (°C)	Aliquat® 336 (µL)	Time (h)	Conversion (%)[b]	Yield (%)[b]	Isolated yield (%)
1[c]	1:3	reflux	-	24	100	76	41
2[c]	1:0.17	reflux	10	24	100	89	40
3[d]	1:3	-	-	0.5	90	43	-

[a](*E*)-12-Oxo-10-octadecenoic acid **1d** (0.67 mmol), Oxone™ (1.35 mmol), $NaIO_4$ (1.0 mmol), MeCN/H_2O.
[b]Conversion and yield determined by GC-MS.
[c]Reflux.
[d]Ultrasound reactor (output level 40%).

2.4.5. Alkaline hydrogen peroxide oxidative cleavage

The oxidative cleavage of methyl (*E*)-12-oxo-10-octadecenoate **10d** mediated by alkaline hydrogen peroxide was investigated using 30, 50 and 60% concentrations of hydrogen peroxide (**Table 2.4**).

Table 2.4. Time evolution alkaline hydrogen peroxide of methyl (*E*)-12-oxo-10-octadecenoate **10d**.[a]

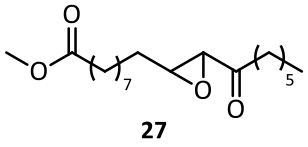


10d **14**

Entry	H_2O_2 (%)	Conversion (%)[b]	Yield (%)[b]
1	30	100	0
2	50	100	0
3	60	100	0

[a]Methyl (*E*)-12-oxo-10-octadecenoate **10d** (0.32 mmol), X% H_2O_2 (386 µL), 1 N NaOH/methanol (2:3, 2.57 mL).
[b]Conversion and yield determined ^{1}H NMR.

In all the cases, the starting material **10d** was completely consumed and the ^{1}H NMR revealed its transformation into the epoxyketone **27** (**Figure 2.6**). Any experiment led to the desired compound, the sebacic acid **14**.



27

Figure 2.6. Chemical structure of the epoxyketone **27**.

2.5. CONCLUSIONS

First, the microwave technology was applied to the alkali fission of the compound (*E*)-12-oxo-10-octadecenoic acid **1d** to obtain sebacic acid **14**. The optimization of these reaction conditions allowed to achieve sebacic acid **14** with a decrease of the temperature and the amount of oxidant compared to the industrial processes. However, the scaling up of the reaction with the new conditions did not lead to the dicarboxylic acid **14**. After the evaluation of all the tests performed, we could ensure that the best results were obtained with the Oxone™/periodate system despite the price of sodium periodate. This system allowed the one-pot oxidative cleavage of α,β-unsaturated fatty acids and did not require expensive or toxic transition metal reagents or catalysts. The dicarboxylic acid **14** could be isolated by recrystallizations in non-toxic solvent (H_2O). In addition, it was proved that oxidative cleavage can be carried out by using ultrasound reactors decreasing the reaction time and avoiding conventional heating.

2.6. REFERENCES

(1) Kadesch, R. G. *J. Am. Oil Chem. Soc.* **1954**, *31*, 568-573.

(2) Anderson, D. In *Bailey's Industrial Oil and Fat Products. Edible Oil and Fat Products: Processing Technologies.*; Shahidi, F., Ed.; John Wiley & Sons: New Jersey, 2005, p 1–56.

(3) Hargreaves, G. H.; Owen, L. N. *J. Chem. Soc.* **1947**, 753-756.

(4) Naughton, F. C. *J. Am. Oil Chem. Soc.* **1974**, *51*, 65-71.

(5) McKetta, J. J.; Dekker, M. In *Encyclopedia of Chemical Processing and Design*; McKetta, J.J., Dekker, M., Eds.; Taylor & Francis: New York, 1978, p 401–420.

(6) Dytham, R. A.; Weedon, B. C. L. *Tetrahedron* **1960**, *8*, 246-260.

(7) Diamond, M. J.; Binder, R. G.; Applewhite, T. H. *J. Am. Oil Chem. Soc.* **1965**, *42*, 882-884.

(8) Diamond, M. J.; Applewhite, T. H. *J. Am. Oil Chem. Soc.* **1967**, *44*, 656-658.

(9) Adams, R.; Marvel, C. S. In *Organic Syntheses*; Adams, Roger, Marvel, C. S., Eds.; John Wiley & Sons: Chichester, 1921, p 61-66.

(10) Bruson, H. A.; Covert, L. W. Process for manufacturing sebacic acid. US2182056 A. December 5, 1939.

(11) Bernard, B.; Holden, C. J. Process for the production of organic compounds. US 2212175 A. August. 20, 1940.

(12) Davis, G. D.; Dombrow, B. A. Process for treating ricinoleic acid compounds. US2318762. May 11, 1943.

(13) Verma, J.; Aggarwal, J. *J. Sci. Ind. Res.* **1949**, *8B*, 183-184.

(14) Werner, S. Production of sebacic acid. US 2696500 A. December 7, 1954.

(15) Naughton, F. C. Sebacic acid production in the presence of barium compounds. US2734916 A. February 14, 1956.

(16) Daidone, P. C.; Naughton, F. C. Lead-catalyzed sebacic acid production in the presence of barium compounds. US2851492 A. September 9, 1958.

(17) Naughton, F. C. Nickel-catalyzed sebacic acid production in the presence of barium compounds. US2851493 A. September 9, 1958.

(18) Vasishtha, A. K.; Trivedi, R. K.; Das, G. *J. Am. Oil Chem. Soc.* **1990**, *67*, 333-337.

(19) Ries, C. D.; Totah, T. S. Method for preparing cleaved products from castor oil or derivatives thereof. WO1998035925 A1. August 20, 1998.

(20) Logan, R. L.; Udeshi, S. V. Method for preparing sebacic acid and octanol-2. US 6392074 B1. May 21, 2002.

(21) Azcan, N.; Demirel, E. *Ind. Eng. Chem. Res.* **2008**, *47*, 1774-1778.

(22) Zaldman, B.; Kisilev, A.; Sasson, Y.; Garti, N. *J. Am. Oil Chem. Soc.* **1988**, *65*, 611-615.

(23) Wang, P.; Tao, B. Y. *J. Am. Oil Chem. Soc.* **1998**, *75*, 9-14.

(24) Dean, F. M.; Knight, J. C. *J. Chem. Soc.* **1962**, 4745-4752.

(25) Djerassi, C.; Engle, R. R. *J. Am. Chem. Soc.* **1953**, *75*, 3838-3840.

(26) Naota, T.; Takaya, H.; Murahashi, S.-I. *Chem. Rev.* **1998**, *98*, 2599-2660.

(27) Freitag, J.; Nuchter, M.; Ondruschka, B. *Green Chem.* **2003**, *5*, 291-295.

(28) Bataille, C. J. R.; Donohoe, T. J. *Chem. Soc. Rev.* **2011**, *40*, 114-128.

(29) Yu, W.; Mei, Y.; Kang, Y.; Hua, Z.; Jin, Z. *Org. Lett.* **2004**, *6*, 3217-3219.

(30) Whitehead, D. C.; Travis, B. R.; Borhan, B. *Tetrahedron Lett.* **2006**, *47*, 3797-3800.

(31) Lee, D. G.; van den Engh, M. In *Organic Chemistry*; Trahanovsky, Walter S., Ed.; Academic Press: New York, 1973, p 177-227.

(32) Berkowitz, L. M.; Rylander, P. N. *J. Am. Chem. Soc.* **1958**, *80*, 6682-6684.

(33) Murahashi, S.-I. In *Modern Oxidation Methods*; Bäckvall, J.-E., Ed.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, 2006, p 165-190.

(34) Rossiter, B. E.; Katsuki, T.; Sharpless, K. B. *J. Am. Chem. Soc.* **1981**, *103*, 464-465.

(35) Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936-3938.

(36) Rajagopalan, A.; Lara, M.; Kroutil, W. *Adv. Synth. Catal.* **2013**, *355*, 3321-3335.

(37) Nakano, Y.; Foglia, T. A. *J. Am. Oil Chem. Soc.* **1982**, *59*, 163-166.

(38) Zimmermann, F.; Meux, E.; Mieloszynski, J.-L.; Lecuire, J.-M.; Oget, N. *Tetrahedron Lett.* **2005**, *46*, 3201-3203.

(39) Rup, S.; Zimmermann, F.; Meux, E.; Schneider, M.; Sindt, M.; Oget, N. *Ultrason. Sonochem.* **2009**, *16*, 266-272.

(40) Rup, S.; Sindt, M.; Oget, N. *Tetrahedron Lett.* **2010**, *51*, 3123-3126.

(41) Zhu, W.; Ford, W. T. *J. Org. Chem.* **1991**, *56*, 7022-7026.

(42) Legros, J.; Crousse, B. t.; Bourdon, J.; Bonnet-Delpon, D.; Bégué, J.-P. *Tetrahedron Lett.* **2001**, *42*, 4463-4466.

(43) Legros, J.; Crousse, B.; Bonnet-Delpon, D.; Bégué, J.-P. *Tetrahedron* **2002**, *58*, 3993-3998.

(44) Yang, D.; Wong, M.-K.; Yip, Y.-C. *J. Org. Chem.* **1995**, *60*, 3887-3889.

(45) Yang, D.; Yip, Y.-C.; Tang, M.-W.; Wong, M.-K.; Cheung, K.-K. *J. Org. Chem.* **1998**, *63*, 9888-9894.

(46) Spannring, P.; Bruijnincx, P. C. A.; Weckhuysen, B. M.; Gebbink, R. J. M. K. *RSC Adv.* **2013**, *3*, 6606-6613.

(47) Weitz, E.; Scheffer, A. *Chem. Ber.* **1921**, *54*, 2327-2344.

(48) Bunton, C. A.; Minkoff, G. J. *J. Chem. Soc.* **1949**, 665-670.

(49) Southwick, P. L.; Dimond, H. L.; Moores, M. S.; Sapper, D. I. *J. Am. Chem. Soc.* **1956**, *78*, 101-104.

(50) Temple, R. D. *J. Org. Chem.* **1970**, *35*, 1275-1280.

(51) Tierney, J.; Lidström, P. *Microwave Assisted Organic Synthesis*; Tierney, J., Lidström, P., Eds.; Blackwell Publishing: Oxford, 2009.

(52) Emslie, R. S. Production of Sebacic Acid. US2731495A. Jan. 17.

(53) Achaya, K. T. *J. Am. Oil Chem. Soc.* **1971**, *48*, 758-763.

Chapter 3

Synthesis of New Long Chain N-Vanillyl Acylamides (LCNVAs) with biological response in pain receptors

3.1. INTRODUCTION

Approximately 20% of the adult European population have chronic pain[1] and, in addition to the physical and emotional burden it brings, the financial cost to society is huge, currently estimated at more than €200 billion per annum in Europe. The same chronic pain leads to $150 billion per annum in the USA.[2] Fewer than 2% of sufferers ever attend a pain clinic[1] with the remainder managed mainly in primary care, if anywhere.[3] Management of pain is essential for patients and the discovery of new analgesics constitutes a prime economical interest for pharmaceutical companies. Pain is integrated as an electric signal on sensory neurons, known as nociceptors, sensitive to a wide range of stimuli. Mechanical changes (pressure, cuts, and injuries), variations in temperature (cold/hot) and chemical stimuli[4] are enabled due to specialized sensory neurons. Activation of these receptors induces a gradual depolarisation of the membrane that can initiate action potentials when the membrane potential (at -70 mV during resting) reaches a threshold of intensity (-55 mV). Action potentials are conducted by the axon to the central nervous system (CNS) for further processing.

From a neurobiological perspective, three types of pain are distinguished:

- **Nociceptive pain** is essential to detect and minimize contact with damaging or noxious stimuli.
- **Inflammatory pain** that accompany inflammation has also a protective role and usually resolves spontaneously due to healing. However, in cases of ongoing inflammation or severe injure it needs to be reduced.
- **Neuropathic pain** occurs after damage of nervous system.

Over the years, the study of transient receptors potential (TRP) channels has provided insight into the mechanisms of nociception and has helped to understand the detection and processing of painful stimuli. TRP channels from various species participate in all five of the Aristotelian senses (sight, hearing, touch, smell and taste). They are tetrameric integral membrane proteins grouped in 8 subfamilies, namely TRPC (Canonical), TRPP (Polycystin), TRPV (Vanilloid), TRPM (Mucolipin), TRPN (or NOMPC= NO Mechanoreceptor Potential C), TRPA (Ankyrin), TRPML (Melastatin), and recently identified TRPY in yeast.[5] (**Figure 3.1**)

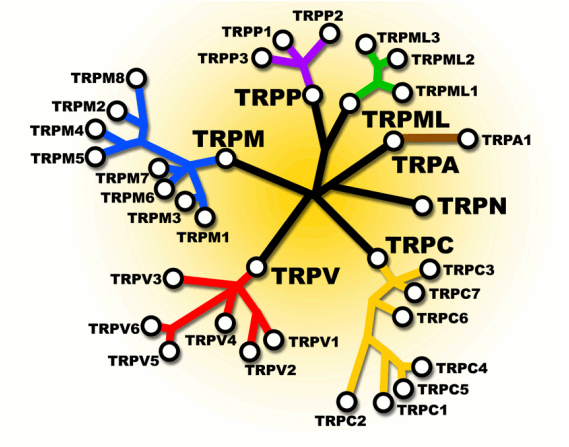


Figure 3.1. TRP subfamilies tree.[6]

They have been recognized as key transducers of sensory signals and as particularly important noxious stimuli detectors. Various TRP family members participate in thermosensation, are gated by temperature changes and are therefore known as thermoTRPs: TRPV1,[7] TRPV2,[8] TRPV3,[9,10] TRPV4,[11] TRPM8,[12,13] and TRPA1[14] (**Figure 3.2**).

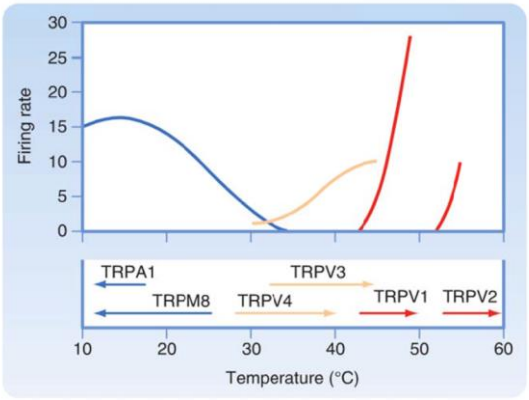


Figure 3.2. The temperature dependency of the firing rate of different thermoTRP channels.[15]

These channels may respond to chemical compounds, voltage and some of them are activated by mechanical stimuli. ThermoTRPs are activated by naturally occurring compounds. TRPV1 is stimulated by capsaicin, the burning molecule in red hot chilli peppers and camphor. TRPV3 is activated by camphor and TRPV4 by bisandrographolide (*Andrographis paniculata*).[16] TRPM8 is a channel responding to menthol (mint), the active ingredient in green mint and TRPA1 responds to pungent ingredients such as isothiocyanates (mustard oil, horseradish), cinnamaldehyde (cinnamon), allicin (garlic) and eugenol (cloves) (**Figure 3.3**).

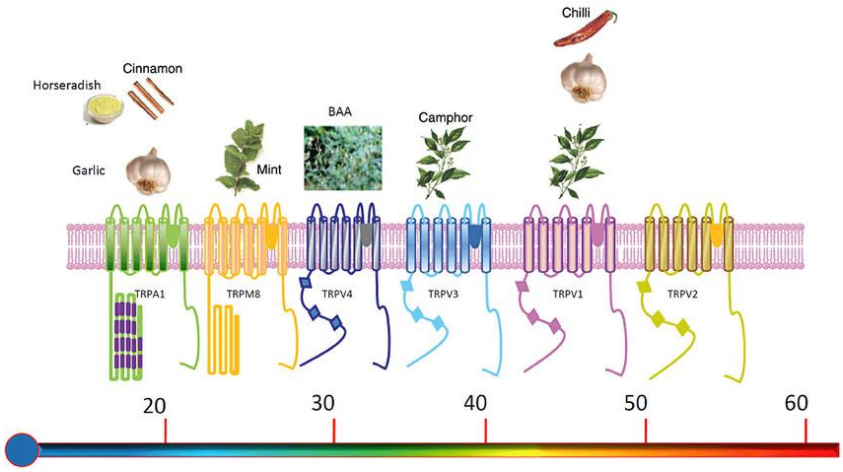
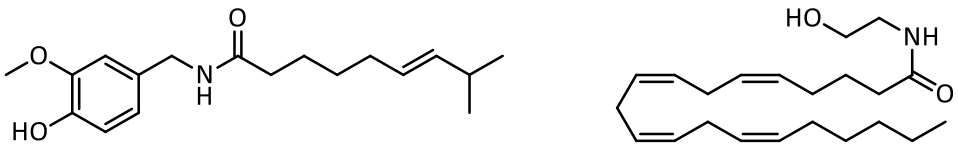


Figure 3.3. Schematic depiction of the predicted membrane topology of the thermoTRPs and their activation by natural ligands. BAA, bisandrographolide A.[17]

TRPV1 was the first receptor linked with a pain and thermosensation in 1997, it was cloned and described as both capsaicin **28** (hot chili pepper substance) receptor and noxious heat detector (temperatures above 43 °C). Over the last decade, TRPV1 has become the model receptor for pain signal integration. This receptor constitutes an alternative for pain treatment to the commonly targeted COX pathway. The endocannabinoid *N*-arachidonoylethanolamine **29** (anandamide, AEA) was the first endogenous modulator of TRPV1 identified (**Figure 3.4**). There is a large body of evidence suggesting that the endocannabinoid anandamide **29** acts as a sensitizer, partial agonist, or full agonist of TRPV1 depending on various metabolism- or disease-related factors (e.g. receptor reserve, phosphorylation level and the presence of other modulators).[18,19]



28 **29**

Figure 3.4. Structures of capsaicin **28** and anandamide **29**.

Fatty acid amide hydrolase (FAAH) is the major catabolic enzyme of anandamide **29**, which also hydrolyses other endogenous fatty acid ethanolamides with different degrees of efficiency. FAAH is increasingly being considered a relevant therapeutic target, especially in models of inflammatory pain.[20] When FAAH enzyme is inhibited, anandamide **29** is not hydrolysed and it is bounded to TRPV1 receptor activating the corresponding processes and finally, the analgesia effect is produced. Therefore, FAAH inhibitors have in fact definitely demonstrated therapeutic benefit in a variety of pain models.[21]

The present study was aimed at testing both the TRPV1 receptor and FAAH enzyme response through various capsaicin-like compounds derived from homoallyl hydroxy fatty acids (ricinoleic **1a** and lesquerolic acid **12a**). The synthesis of several compounds was envisaged to evaluate their activity and discover new acyl templates with potential "dual" mechanism of action against pain.

3.2. TRANSIENT RECEPTOR POTENTIAL VANILLOID I (TRPV1)

3.2.1. Structure and activity of TRPV1

The vanilloid receptor I is a non-selective Ca^{2+} channel. It is a homotetrameric membrane protein whose structure features 839 amino acids, six predicted α-transmembrane domains and six ankyrin repeat domains.[22] It presents the *N*- and *C*- termini in the cytosol, and has a relatively conserved hydrophobic pore domain between the fifth and sixth transmembrane domains. This topology is characteristic of ion channels and has already been reported in voltage-gated K^+ channels. The *C*- terminus contains the characteristic TRP domain, a 25 amino acid segment highly conserved in the TRP superfamily. Temperature sensing domains are located at this terminus. At the other side, the *N*-terminus contains the three ankyrin domains, which are composed of 33 residues forming two helices separated by a loop region. This motif accommodates protein-protein interactions. In the case of TRPV1 has been found to bind to calmodulin (CaM), the calcium-binding protein (**Figure 3.5**).[23]

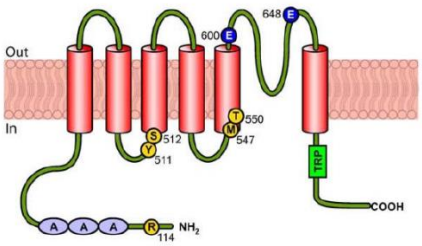


Figure 3.5. Schematic representation of the topology of TRPV1 protein subunit (modified).[24]

The high resolution three-dimensional structure of full TRPV1 is not available, only the structure of ankyrin domain has been solved.[25] In 2008, a low resolution structure of rat TRPV1 was obtained by electron cryomicroscopy.[26] Gao *et al.*[27] reported a much improved resolution structures of TRPV1, alone and in a complex with different modulators combining electron cryomicroscopy with the nanodisc reconstitution of the TRPV1 protein, in 2016 (**Figure 3.6**).

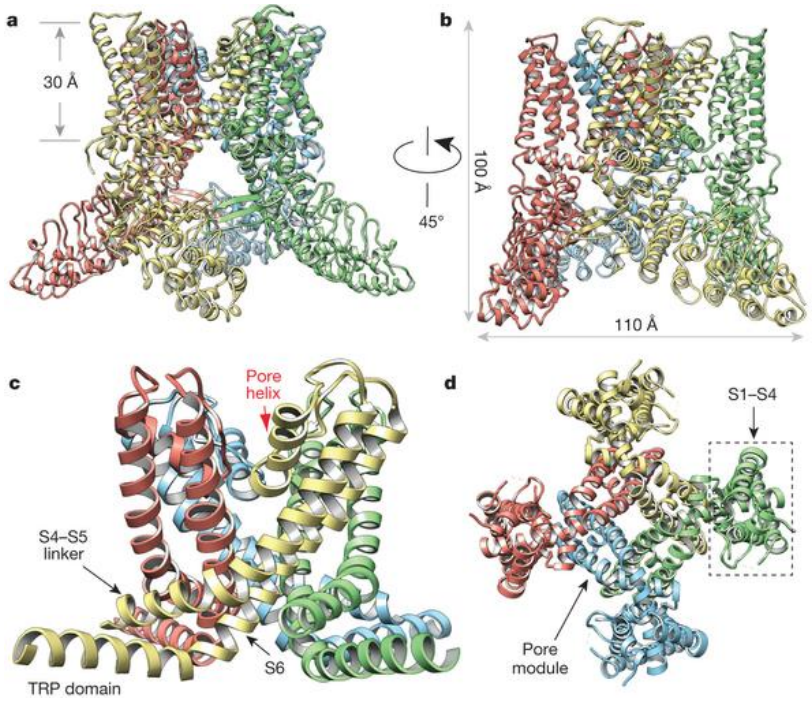


Figure 3.6. Ribbon diagram of TRPV1 atomic model with each of the four identical units color-coded.[28]

The main mechanism for the activation of TRPV1 is based on phosphorylation, which acts to potentiate capsaicin- or proton-evoked responses and reduces the temperature threshold for TRPV1 activation.[29-31] Several second messenger pathways are associated with this mechanism. Residues S502 and S800 are phosphorylated by protein kinase C (PKC). Calmodulin binds to the first ankyrin domain and to the *C*- terminus, on the 767-801 segment. The system Ca^{2+}/calmodulin-dependent protein kinase (CAMKII) also phosphorylates the residues S502, T370 and T704. The phosphorylation of S502, S116, T144 and T370 leads to the activation of protein kinase A (PKA).[29,32] A-Kinase anchoring protein 150 (AKAP150) is required for the phosphorylation of TRPV1 by PKA or PKC in sensory neurons and, hence, affects TRPV1-dependent hyperalgesia under pathological conditions. The activation of *N*-methyl-*D*-aspartate (NMDA) receptors sensitizes TRPV1 by enhancing serine phosphorylation through PKC in trigeminal nociceptors (**Figure 3.7**).[33]

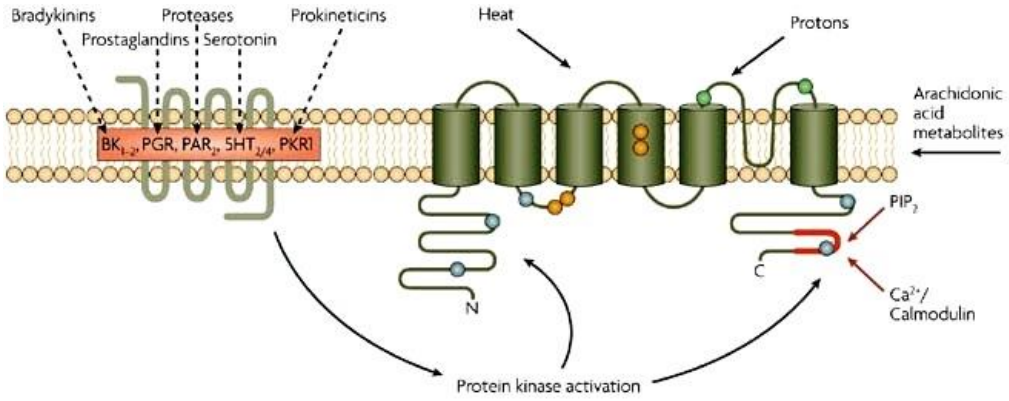


Figure 3.7. TRPV1 is a polymodal integrator in the peripheral nervous system. Blue arrows represent TRPV1-sensitizing stimuli via protein-kinase activation and red arrows indicate negative regulation by PIP_2, Ca^{2+} and calmodulin. Coloured circles represent amino-acid residue for vanilloid binding (orange, Y511, S512, L547, T550), phosphorylation sites (blue, S116, T370, S502, T704, S800) and proton activation sites (green, E600, E648). The *C*- terminal part of TRPV1 (red line) interacts with PIP_2 and calmodulin.[34]

To sum up, when damage is produced inflammation occurs. Proinflammatory compounds and the activated kinases lower the threshold of activation of TRPV1. It produces an excitability of the peripheral terminal membrane of the neuron increasing its sensitivity.

3.2.2. Regulation of TRPV1 activity

According to their mode of action there are three major types of compounds that can modulate the activity of TRPV1: agonists, competitive antagonists, and non-competitive antagonists. Agonists can interact at the binding site producing a desensitization of the receptor after a fast onset. A competitive antagonist binds to a receptor but does not activate the receptor. These antagonists will compete with available agonists for receptor binding sites on the same receptor. Sufficient antagonist will displace the agonist from the binding sites, resulting in a lower frequency of receptor activation. Non-competitive antagonists remove the receptor or its response potential from the system; this may be by preventing the agonist from producing its effect at a receptor site by irreversible change to the receptor or its capacity to respond. This antagonism is not reversible by increasing the concentration of the agonist. Only agonists and their role in nociception are described below in detail because they are of particular relevance to this dissertation. Depending on their origin, agonists can be divided in two groups: endogenous and exogenous agonists. An endogenous agonist is a compound naturally produced by the body which binds to and activates that receptor. An exogenous agonist is not produced by the body and they can be obtained from natural sources, semisynthetically or synthetically.

3.2.2.1. Endogenous agonists

A wide range of stimuli are responsible for TRPV1 activation including noxious heat, low extracellular pH and a variety of chemical mediators. TRPV1 is thought to mediate the phenomenon of peripheral sensitization that involves a reduction in the threshold of activation and an increase in the responsiveness of the peripheral termini of nociceptors. Several studies have been presented on the existence of endogenous vanilloid agonists, a class of compounds referred to endovanilloids.[18] Various lipids from fatty acid derivatives have also been identified as TRPV1 activators. These compounds can be divided in two groups:

- **Biogenic amides:** anandamide **29**, *N*-oleylethanolamine[35,36] (**30**, OLEA), *N*-arachidonoyldopamine[37] (**31**, NADA), *N*-arachidonolylserine **32**, *N*-arachidonoylserotonin (**33**, AA-5-HT)[38] and various *N*-acyltaurines and *N*-acylsalsolinols (**Figure 3.8**).[39]

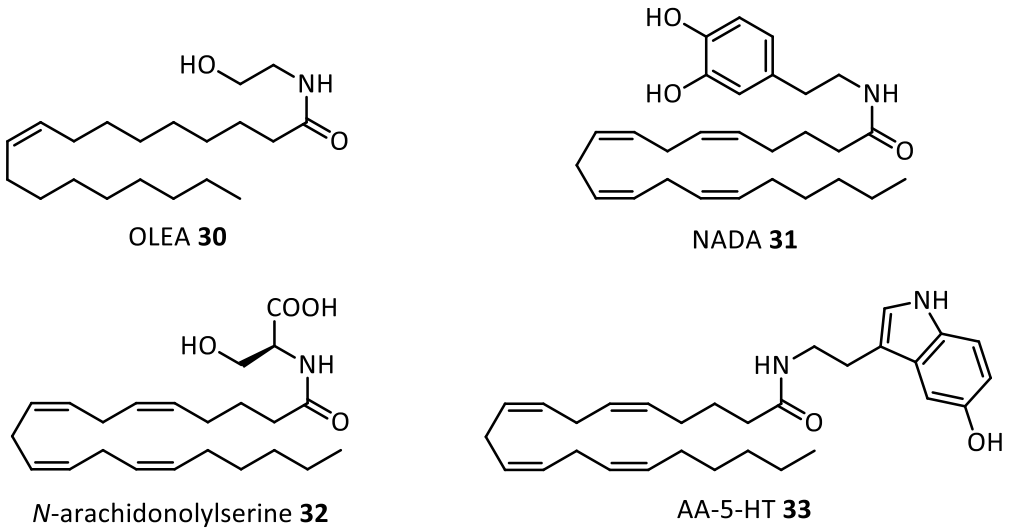


Figure 3.8. Structures of compounds **30**-**33**.

- **Oxygenated eicosatetraenoic acids:** 5-, 12-, and 15-hydroperoxyeicosatetranoic acids (5S-, 12S-, 15S-HPETE, **34**-**36**), their reduced hydroxylic analogues (**37**-**39**), prostaglandins, leukotriene B4,41 adenosine, ATP, and polyamines (such as spermine, spermidine and putrescine) (**Figure 3.9**).[42]

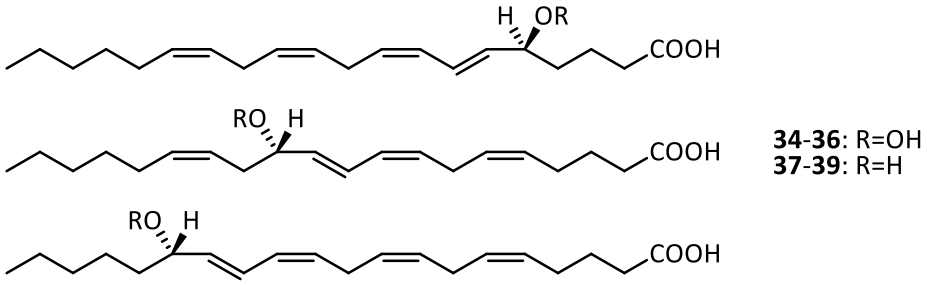


Figure 3.9. Structures of compounds **34**-**39**.

3.2.2.2. Exogenous agonists

TRPV1 can be activated by a heterogeneous array of natural products that includes dietary compounds (capsaicin **28**, capsaicinoids and capsinoids, piperine, eugenol, and gingerol), plant toxins (resiniferatoxin **40**) and animal toxins.[43] TRPV1 activation by capsaicin **28** is followed by nociceptor desensitization, a state characterized by the inability of the receptor to respond to the capsaicin **28** or other noxious stimuli. TRPV1 desensitization is a process markedly depending on Ca^{2+} and involves various intracellular signalling pathways.[25,44-47] Thus, dephosphorylation by the phosphatase calcineurin of TRPV1 previously phosphorylated by cAMP-dependent protein kinase A (PKA) or Ca^{2+}-calmodulin-dependent kinase II (CaMKII) leads to TRPV1 desensitization.[29,30,48,49] Conversely, phosphorylations at several consensus sites for protein kinase C (PKC), cAMP-dependent protein kinase A (PKA) and Ca^{2+}/calmodulin dependent kinase II (CaMKII) have been shown to reduce the Ca^{2+}-mediated desensitization of TRPV1. There are a number of putative phosphorylation sites at which the TRPV1 channel can be regulated: S502 and S800 have been implicated as targets of PKC-dependent phosphorylation and S116, T144, T370, and S502 were identified as the key sites at which PKA phosphorylation increases the open probability and reverses desensitization of the channel. Later, evidence was presented that the TRPV1 desensitization might involve a much more complex Ca^{2+}-dependent pathways. Acute Ca^{2+}-dependent TRPV1 desensitization has been shown to be accompanied by a profound change in voltage dependence,[50,51] loss of capsaicin binding,[30] and depletion of membrane phosphatidylinositol 4,5-bisphosphate (PIP_2), indicative of the involvement of Ca^{2+}-dependent PLC activity in this process. This desensitisation explains the paradoxical analgesic effect of capsaicin **28**.[34]

After the discovery that a specific and selective receptor for capsaicin **28** was present in nociceptive neurons[52] and the subsequent characterization of such receptor as the TRPV1 ion channel,[7] efforts have been made to understand the molecular basis of the interaction between

capsaicin **28** and TRPV1. For SAR studies, capsaicin **28** has been traditionally divided in three parts (**Figure 3.10**):[53-55]

- **Aromatic region.** It is the 4-hydroxy-3-methoxybenzyl moiety characteristic of homovanillyn derivatives. This polar head is essential for exciting sensory neurons.
- **Benzylamidic region.** The amide bond linker.
- **Lipophilic acyl region.** This hydrophobic part interacts with a hydrophobic region in the receptor.

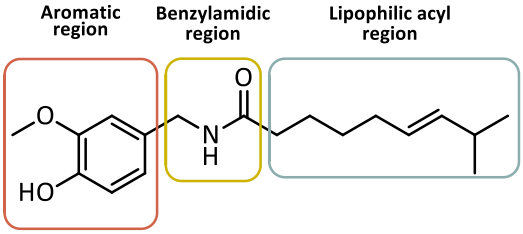


Figure 3.10. Chemical structure of capsaicin **28** showing relevant regions: polar (red), linker (green) and hydrophobic (blue) moieties.

Liao *et al.*[28] reported the structure of TRPV1 partially solved by cryoelectron microscopy, which describes the density assigned to capsaicin **28** located in a pocket involving residues from helices S3 (Y511) and S4 (M547 and T550).[56-58] Further contacts were identified including E570 from the S4-S5 linker, and L669 from S6 of an adjacent unit. The vanillyl ring would point upward and would interact with T550 and W549, and the aliphatic tail would point toward Y511 (**Figure 3.11**).

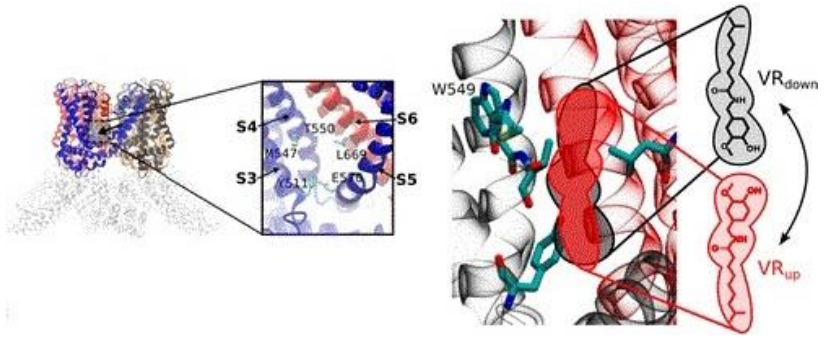


Figure 3.11. Structural representation of the TRPV1 channel resolved by cryo-EM corresponding highlighting the transmembrane domain colored by chain: A (blue), B (red), C (gray), D (orange). The black arrow indicates the binding site of capsaicin **28** at the interface between two monomers (A and B), which is magnified on the right. Four identical capsaicin **28** binding sites are present in TRPV1. Two orientations of capsaicin **28** denoted VR_{down} and VR_{up} are depicted at the binding site observed in the cryo-EM structure (modified).[59]

The extremely irritant diterpene present in the dried latex of the plant *Euphorbia resinifera*, resiniferatoxin (RTX, **40**) is a very specific agonist for the TRPV1 channel. *E. resinifera* is a cactus-like plant native to the Anti-Atlas Mountains of Morocco.[60] Early reports of the medical use of dried latex of *E. resinifera* describe its direct application to dental cavities to mitigate toothache or to suppress chronic pain.[61] Importantly, resiniferatoxin **40** is about 3 to 4 magnitude more potent than capsaicin **28** as well in dose-response curve as for the effect on thermoregulation and neurogenic inflammation.[62] Resiniferatoxin **40** has been useful for the treatment of urinary urge incontinence and pain associated with diabetic neuropathy, two human pathologies mediated by TRPV1 dysfunction (**Figure 3.12**).[63]

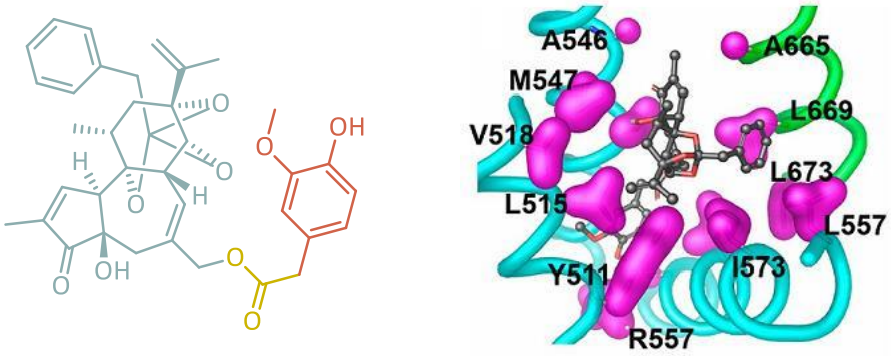
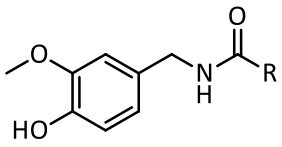


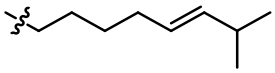
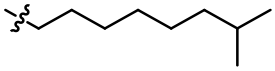
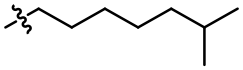
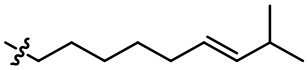
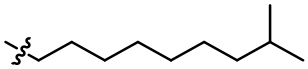
Figure 3.12. Right: Chemical structure of resiniferatoxin **40** showing relevant regions: polar (red), linker (green) and hydrophobic (blue) moieties. Left: RTX **40** binding mode with TRPV1 receptor as balls and sticks, and the surrounding amino acids as surface (modified).[64]

- *Capsaicinoids: N-Vanillyl-Acylamides (NVAs) and Long Chain N-Vanillyl Acylamides (LCNVAs)*

Capsaicinoids are the name given to a large family of natural compounds found in members of the *Capsicum* family (also known as peppers), discovered in 1961 by Japanese chemists.[65] They were the first exogenous agonists identified. The most common capsaicinoid is 8-methyl-*N*-vanillyl-6-nonenamide (capsaicin, **28**). Capsaicin **28** was first purified in 1846[66] but its structure started to be described only in 1919.[67] Nelson showed the pungency of chillies resulted from vanillyl amides[68] and contributed to solving the structures of capsaicin **28** in 1923.[69] All capsaicinoids share structural and activity similarities with capsaicin **28**,[70,71] but they are not as abundant as capsaicin **28** that can account for up to 80% of capsaicinoid content of chili peppers. The pungency of all these molecules emphasizes the fact that this activity is defined mainly by the benzene ring region; however, the length of acyl chain can modify it.[72] Besides the five natural capsaicinoids (**Table 3.1**), one synthetic member of the capsaicinoid family exists: vanillylamide of *n*-nonanoic acid (**45**, VNA, also PAVA) used as a reference substance for determining the relative pungency of capsaicinoids.

Table 3.1. Structure and pungency of some capsaicinoids.



Entry	Capsaicinoid	R	Pungency (x10^6)[a]	Typical relative amount (%)
1	Capsaicin **28**		16	69
2	Dihydrocapsaicin **41**		15	22
3	Nordihydrocapsaicin **42**		9	7
4	Homocapsaicin **43**		8.6	1
5	Homodihydrocapsaicin **44**		8.6	1
6	Pseudocapsaicin **45**		9.2	-

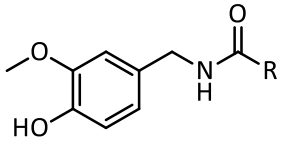
[a]Measured by Scoville scale.

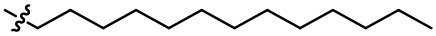
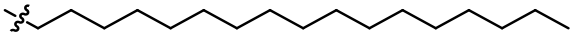
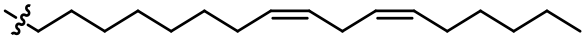
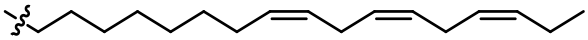
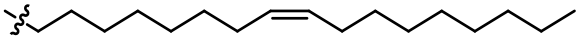
N-vanillyl-acylamides (NVAs) are a group of compounds that are encompassed within the capsaicinoids. NVAs have been at the centre of intense research activity aimed at elucidating the basis of their antinociceptive properties and exploiting their therapeutic potential. These studies provided evidence of definite structure-activity relationships; however, the potency of the parent compound could not be increased significantly, and no better clinical lead emerged from these investigations.[73] The discovery of alternative templates with improved potency and/or pharmaceutical profiles (resiniferonoids,[74] *N*-vanillyl-*N*'-(3-acyloxy-2-benzylpropyl)thioureas,[75] *N*-alkylhomovanillamides,[76] and *N*-vanillyl-*N*'-benzylureas[77]), shifted the focus of vanilloid research from NVAs.

Certain observations suggest that lipophilic domain of capsaicin receptor has elements capable of binding polar groups or, alternatively, that certain structural elements can preorganize, in conformational terms, the lipophilic acyl moiety of NVAs for binding. Studies on the relationship between the acyl chain length and the pungency of NVAs revealed that a chain length of around nine carbons, as capsaicin **28** and dihydrocapsaicin **41**, causes the strongest sensation of pungency in humans.[78,79] However, those with longer or shorter acyl chain than that of capsaicin's are less pungent.[78] Although NVAs with a chain length of more than 18 carbons do not generate any stimulus, some capsaicin-like useful activities remain in these long chain *N*-vanillyl acyl amides (LCNVAs). Long

chain *N*-vanillyl acylamides (LCNVAs) have been developed as synthetic capsaicin **28** analogues with capsaicin-like physiological activities and with no, or less, harmful stimuli.[80] LCNVAs with ubiquitously occurring natural fatty acid moieties, such as myristic (C14:0), palmitic (C16:0), stearic (C18:0), linoleic (C18:2), and linolenic (C18:3) acids, have been developed as myrvanil, palvanil, stevanil, livanil, and linvanil, respectively (**46-50**).[81-83] Since the late 1980s, olvanil, *N*-vanillyl-9-(*E*)-octadecenamide (also known NE-19550) **51**, has mostly been studied as an attractive LCNVA because of its high capsaicin-like activities: it is anti-inflammatory[84] and antinociceptive,[85] and it enhances adrenaline secretion,[86] despite its lack of irritancy or pungency. Furthermore, several studies have shown that the potency of olvanil **51** (EC_{50}=3.7 nM) to activate TRPV1 is comparable to that of capsaicin **28** (EC_{50}=9.1 nM).[7,87,88] The paradoxical relationship between the high potency of olvanil **51** to activate TRPV1 and its lack of pungency might be due to its lower accessibility to TRPV1 in the tongue because of its higher lipophilicity than capsaicin **28**.[88] LCNVAs with arachidonic (C20:4) (arvanil, **52**) and docosahexaenoic (C22:6) **53** acids have also been investigated (**Table 3.2**).[89,90]

Table 3.2. Chemical structure of some LCNVAs.



Entry	R	LCNVA
1		Myrvanil **46**
2		Palvanil **47**
3		Stevanil **48**
4		Livanil **49**
5		Linvanil **50**
6		Olvanil **51**
7		Arvanil **52**
8		Docosahexaenoic **53**

Palvanil **47** exhibited kinetics of activation of human TRPV1 slower than that of capsaicin **28**. Slow kinetics of TRPV1 agonists were previously found to be associated with stronger potencies as TRPV1 desensitizing agents, lower pungency and stronger anti-hyperalgesic activity.[91] Long lipophilic sequences resembling the acyl moiety of capsaicin **28** but constrained in a covalent or conformational way and occasionally interrupted by polar groups (hydroxy, ketone carbonyl, double

bond(s)) are present in a host of compounds from the natural products pool, mostly fatty and terpenoid acids. Many of them are easily available and could provide interesting probes for characterizing the lipophilic domain of the capsaicin binding site. An analogue of olvanil **51** is the rinvanil **54** (**Figure 3.13**).[92] This compound is easily available from commercial and cheap ricinoleic acid **1a** and it presents a hydroxy group on the homoallylic carbon. Its potency (EC_{50}=6.0 nM) is comparable to capsaicin **28** and olvanil **51**. This finding suggested the presence of polar elements within the apolar pocket accommodating the acyl residue of capsaicin **28**.

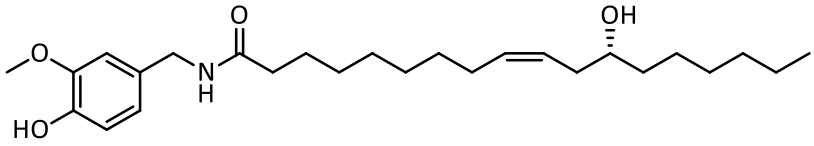
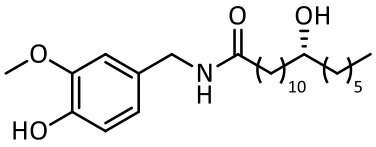
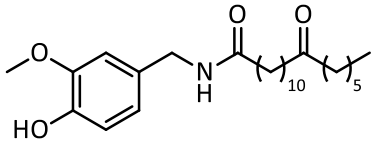
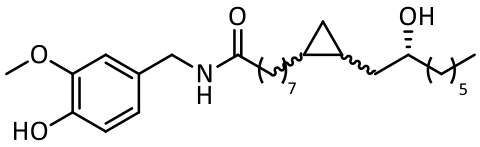
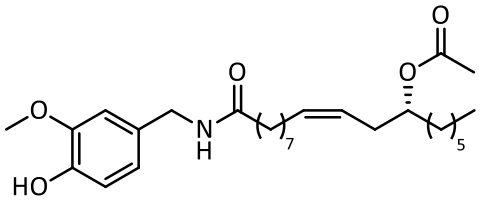


Figure 3.13. Chemical structure of rinvanil **54**.

The fact that the vanilloid activity was tolerant to the presence of oxygen on the distal side of the double bond led to the synthesis of new ricinoleic *N*-acylamides derivatives. Appendino and Di Marzo were the first to prepare derivatives from ricinoleic acid **1a**.[92] The first approach was to confirm the need of the presence of the double bond. The resulting product from hydrogenation of rinvanil **55** and its corresponding saturated ketone **56** showed a reduction of efficacy but not potency. The efficacy was especially increased when a cyclopropanation of de double bound **57** was performed. Another approach was the study of the existence of groups that can establish hydrogen bounds starting from observation that resiniferatoxin **40** shows an aromatic moiety on its lipophilic diterpenoid core. The acetylation of hydroxy group **58** led to a significantly increment of activity which suggest that polar element(s) as hydroxy group does not establish a hydrogen bond within the apolar pocket. Hence, there should be a second pocket, capable of interacting with ester groups, which might also be present in the apolar cleft accommodating the acyl residue of LCNVAs (**Table 3.3**).

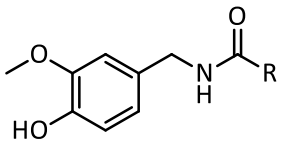
Table 3.3. TRPV1 biological evaluation of the vanillamides **55**-**58**.

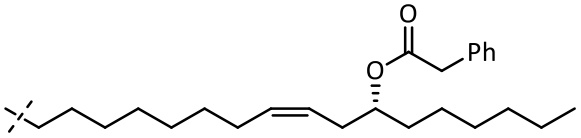
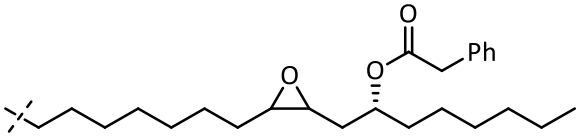
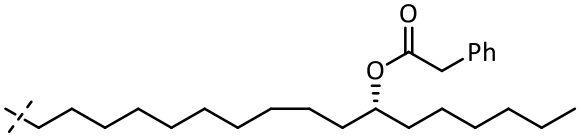
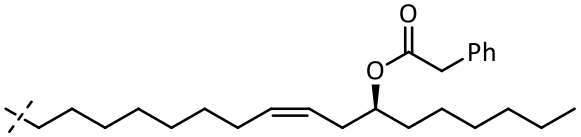
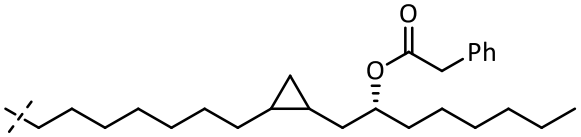
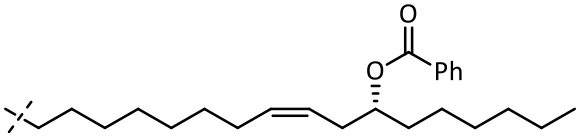
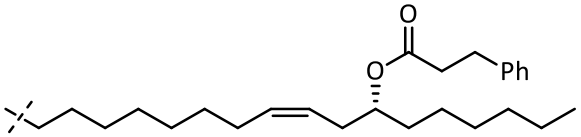
Entry	Compound	Structure	Efficacy (%)	pEC_{50}
1	**55**		69.7±0.9	8.3±0.2
2	**56**		67.0±1.2	8.2±0.1
3	**57**		98.0±7.1	8.3±0.2
4	**58**		93.5±5.9	9.4±0.5

Data from Appendino and Di Marzo.[92]

The introduction of an hydroxy group in the acyl chain of olvanil **51** yielded rinvanil **54**, which was subsequently esterified with several acids containing different aryl moieties.[93] The synthesis of phenylacetylrinvanil **59** (IDN5890) yielded what is considered the most potent capsaicinoid reported to date because it is approximately 500-fold more active than capsaicin **29** and less pungent. Phenylacetylrinvanil **59** was the first compound structurally unrelated to resiniferatoxin **41** that shows efficacy comparable with the natural product in assays of vanilloid activity. Although its potency was 8-fold lower, the activity of phenylacetylrinvanil **59** is in the same two-digit picomolar EC_{50} range. The ultra-potency of **59** was decreased by further chemical modifications (epoxidation **60** or hydrogenation **61**) of its fatty acid chain. Chemical modifications as the configurational inversion of the chiral center **62** and cyclopropanation **63** of the double bond were tolerated (**Table 3.4**). Benzoyl- **64** and phenylpropionylrinvanil **65** were as potent and less potent than **59**, respectively.

Table 3.4. Structures and biological results in TRPV1 receptor of the vanillamides **59**-**65**.

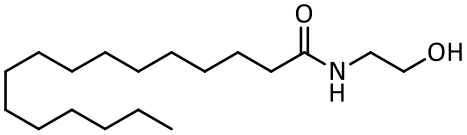


Entry	Compound	R	Efficacy (%)	EC_{50} (nM)
1	**59**		98.2±2.1	0.09±0.02
2	**60**		71.3±3.2	5.0±0.2
3	**61**		72.2±3.5	0.59±0.03
4	**62**		95.2±2.3	0.10±0.02
5	**63**		80.1±2.3	0.13±0.02
6	**64**		86.1±2.2	0.13±0.02
7	**65**		98.4±2.7	0.20±0.02

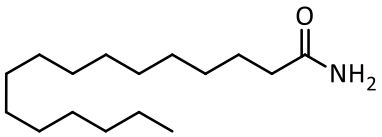
Data from Appendino and Di Marzo.[93]

3.3. FATTY ACID AMINO HYDROLASE (FAAH)

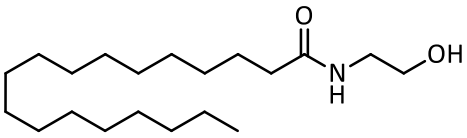
In addition to serve as the core structural components of cell membranes, lipids also function as signalling molecules that regulate a number of physiological processes by acting on specific receptor in the nervous system and periphery. One of the most important groups of lipid transmitters is the fatty acid amides (FAAs). The fatty acid amide bond has long been recognized in nature, being important in the structure of ceramides[94] and sphingolipids.[95] The first non-sphingosine based fatty acid amide isolated from a natural source was *N*-palmitoylethanolamine **66** from egg yolk in 1957.[96]Two general classes of endogenous FAAs have been identified: the *N*-acyl ethanolamines (NAEs) and fatty acid primary amides (FAPAs) (**Figure 3.14**).



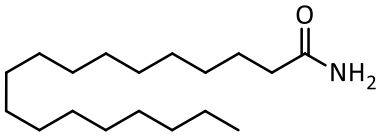
N-palmitoylethanolamide **66**



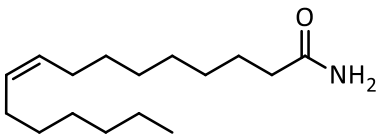
Palmitamide **68**



N-stearoylethanolamide **67**



Stearamide **69**



Oleamide **70**

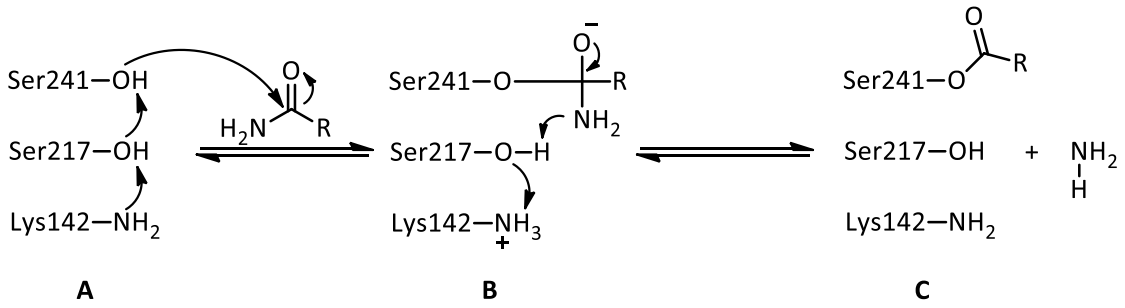
Figure 3.14. Structures of some endogenous NAEs and FAPAs.

Anandamide **29** is one of the best-known and best understood members of NAEs. Anandamide **29** is an endogenous ligand for the cannabinoid receptors in the mammalian brain[97] and has been shown to regulate numerous neurobehavioral processes, including pain, motility, cognition, and feeding. The production of anandamide **29** takes place in postsynaptic neurons in response to a stimulus, e.g. elevation of intracellular Ca^{2+}-levels. Thus, anandamide **29** is not stored but synthesized "on demand".[98] Anandamide **29** is simultaneously released to extracellular space where its binding sites (CB-receptors) are located. Reuptake is thought to be carried out by either passive diffusion,[99] facilitated diffusion,[100] endocytosis,[101] or by transport protein.[102,103] The existence of these different

theories[104] inherits from the fact that as a very lipophilic molecule, anandamide **29** is most likely able to diffuse through membranes readily without aiding mechanisms.

3.3.1. Structure and activity of FAAH

In 1996, an "oleamide hydrolase" activity was affinity purified to near homogeneity from rat liver membrane.[105] After the cloning of its cDNA and transfection into COS-7 cells, a high anandamide hydrolase activity was observed, confirming that a single enzyme was indeed capable of degrading both NAEs and FAPAs. Hence, this enzyme was named Fatty Acid Amide Hydrolase (FAAH), in recognition of the large number of endogenous FAAs that it accepted as substrates. FAAH is comprised of 579 amino acids and it was characterized by sequence analysis as the first mammalian member of amidase signature (AS) enzyme family, containing over 100 mainly fungal and bacterial enzymes. AS enzymes share a characteristic feature of having a highly conserved region that is rich in serine, glycine, and alanine residues.[106] Unlike most AS enzymes, FAAH is an integral membrane protein, having a transmembrane domain of first 29 amino acids. However, deletion of these amino acids (ΔTM-FAAH) resulted in an enzyme that had the same catalytic activity and binding property to membranes as wild type FAAH. These results indicate that FAAH possesses multiple domains for membrane association and is capable of being active in other than its natural configuration. In 2002 the X-ray crystalline structure of ΔTM-FAAH was determined in 2.8 Å resolution opening a new era in FAAH study.[107] The results revealed FAAH to be a dimeric enzyme. The crystallization of the enzyme by binding with methoxyarachidonylfluorophosphonate (MAPF) revealed that the residue Serine 241 (Ser241) plays a very important role in the FAAH active site. Ser241 forms an unusual Ser-Ser-Lys catalytic triad with Ser217 and Lys142 which is thought to carry out the hydrolysis of the amide bond (**Scheme 3.1**).



Scheme 3.1. Proposed mechanism for the FAAH active site acylation step in the amide hydrolysis.[108]

The FAAH structure contains channels that allow the enzyme to access both the membrane and cytosolic compartments of the cell. One of these channels is called the acyl chain-binding (AB)

channel leading from the membrane-binding surface of the protein to the enzyme active site. AB channel is comprised almost entirely of hydrophobic residues and is thought to participate in substrate recognition.[109] Another channel emerges from the active site at an angle of approximately 80° from the substrate-binding cavity to create a solvent-exposed "cytoplasmic port" (CP). FAAH structure suggests a model where fatty acid amide substrates gain access to the active site by first entering into the membrane. After hydrolysis, the fatty acid would exit the enzyme via AB channel to membrane. Hydrophilic amine products would be excreted directly to cytosolic space via the cytoplasmic port (also called membrane access (MA) channel).[107] The cytoplasmic port may also play a role by providing an entry for the water required in the hydrolysis (**Figure 3.15**).[106]

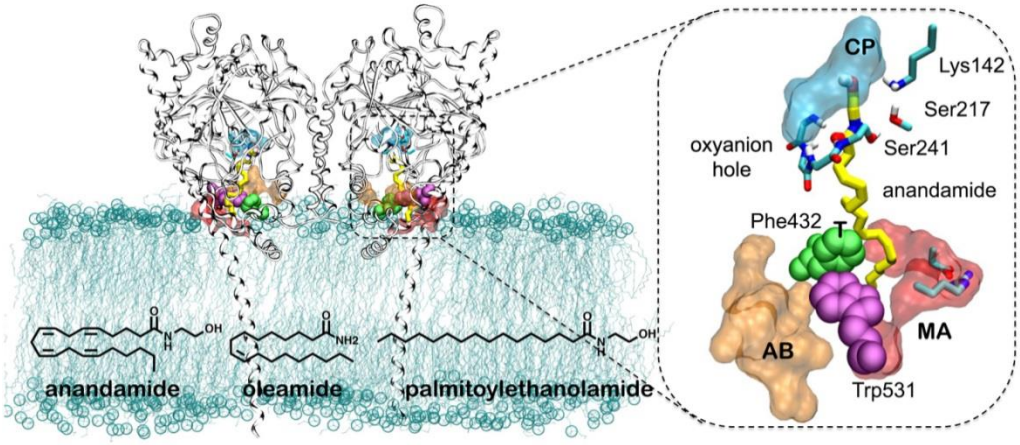
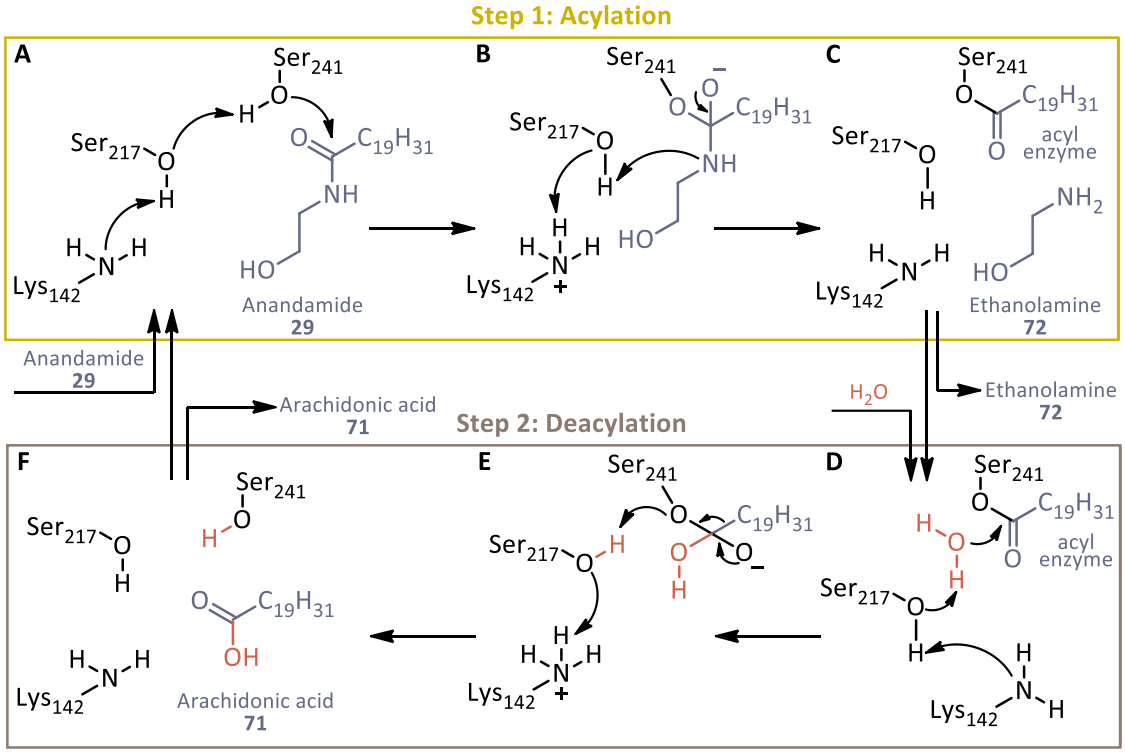


Figure 3.15. Overview of the FAAH protein in complex anandamide **29**, embedded in a 1-palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE) lipid bilayer. The interface region between the MA and AB channels is indicated as transition region (T). The chemical structures of anandamide **29**, *N*-palmitoylethanolamide **66** and, oleamide **70** is also shown.[110]

Regarding anandamide **29**, it is hydrolysed by FAAH in neurons and astrocytes into breakdown products arachidonic acid **71** and ethanolamine **72**. The catalytic mechanism for anandamide **29** hydrolysis comprises two main chemical steps: enzyme acylation (Step 1; A→C in **Scheme 3.2**) and enzyme deacylation (Step 2; D→F in **Scheme 3.2**). During the acylation reaction, the neutral form of Lys142 activates the Ser241 nucleophile via proton transfers that involve Ser217 (A). The activated Ser241 attacks the anandamide carbonyl group, leading to the formation of a tetrahedral intermediate (B). At this point, a reversed proton transfer, from Lys142 through Ser217, leads to the protonation of the leaving group (i.e., ethanolamine **72**), thus obtaining the acyl−enzyme adduct (C). The deacylation reaction takes place upon exit of the ethanolamine **72** leaving group from the catalytic site. During the deacylation reaction, in fact, a water molecule acts as a nucleophilic agent, attacking the carbonyl group of the acyl−enzyme adduct. The restoration of the initial

protonation state and ordered H-bond network of the catalytic triad terminates the catalytic loop, with the catalyst FAAH ready for a new cycle.[111,112]



Scheme 3.2. The catalytic reaction has two main steps: formation of the acyl-enzyme complex (Acylation or Step 1-top), and subsequent deacylation through a H_2O molecule that enters into the catalytic cycle (Deacylation or Step 2-bottom).

3.3.2. Regulation of FAAH activity

By inhibiting the main endocannabinoid hydrolysing enzyme FAAH the effect of endocannabinoids could be enhanced and more selective therapeutic effects achieved.[113-115] The increase of concentration of endocannabinoids in the extracellular space (**29**, **66**) can lead to several beneficial therapeutic effects[116] such as alleviation of pain[117,118] and anxiety,[119] reduction of intraocular pressure,[120] as well as increase of appetite.[121] Thus the inhibition of this enzyme is of great interest to medicinal chemists nowadays. The development of FAAH inhibitors that raise their endogenous levels and sustain their duration of action by blocking their hydrolysis, has emerged as an approach that may avoid the undesired side effects of a conventional cell surface receptor agonist. Since FAAH inhibition only potentiates an activated signalling pathway, increasing the endogenous levels of the released lipid signalling molecules at their sites of action, it provides a temporal and spatial pharmacological control not available to a classical blunt force receptor agonist.

3.3.2.1. Anandamide as TRPV1 ligand

It was a breakthrough for vanilloid field when anandamide **29** itself was shown to be an activator of TRPV1 in vitro. Anandamide **29** metabolism plays an important role in the regulation of in vivo anandamide **29** concentrations. The inhibition of anandamide **29** breakdown may result in high-enough anandamide **29** concentrations to activate TRPV1 receptor. The efficacy of anandamide **29** as a TRPV1 agonist is influenced by a succession of factors including receptor reserve, phosphorylation, metabolism and uptake, CB1 receptor activation, voltage, temperature, pH and bovine serum albumin. Anandamide **29** may be synthetized and released in significant quantities, potentially sufficient to activate TRPV1 (0.5-10 μM). The affinity of anandamide **29** for TRPV1 binding seems to be similar or about fivefold weaker than capsaicin **28**. An important consequence of these data is that, to activate TRPV1, anandamide **29** must be present in relatively high concentrations. Accordingly, anandamide **29** is a partial activator of TRPV1 when the receptor expression is low, while it is a full agonist when receptor expression is high. Inhibition of FAAH enzyme leads to an increment of the anandamide **29** concentration inside of the neuron, which can interact with the cannabinoid receptors CB1 and CB2 and TRPV1. Low concentrations of anandamide **29** usually evoke analgesia by activating CB1 and by decreasing TRPV1 responsiveness (**Figure 3.16**).

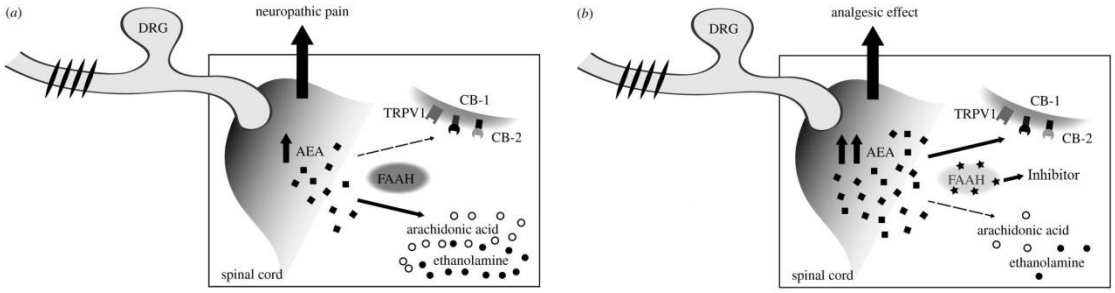
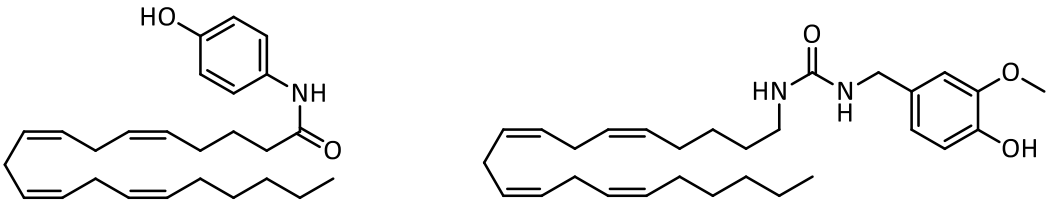


Figure 3.16. Schematic of the manipulation of spinal anandamide **29** levels. (a) The effects of anandamide **29** are mediated through the activation of cannabinoid CB1, CB2 receptors. (b) The presence of a FAAH inhibitor increases **29** levels, as a result TRPV1 receptor is activated (modified).[122]

3.3.2.2. FAAH inhibitors

A considerable body of research has demonstrated that pharmacological blockade of FAAH reduces nociceptive and hyperalgesic behaviour in a wide range of acute, inflammatory, and neuropathic pain model.[123] A popular and logical approach to FAAH inhibition is the modification of substrates to create inhibitors (**Figure 3.17**).



AM404 **73** O-1987 **74**

Figure 3.17. Structures of AM404 **73** and O-1987 **74** FAAH inhibitors.

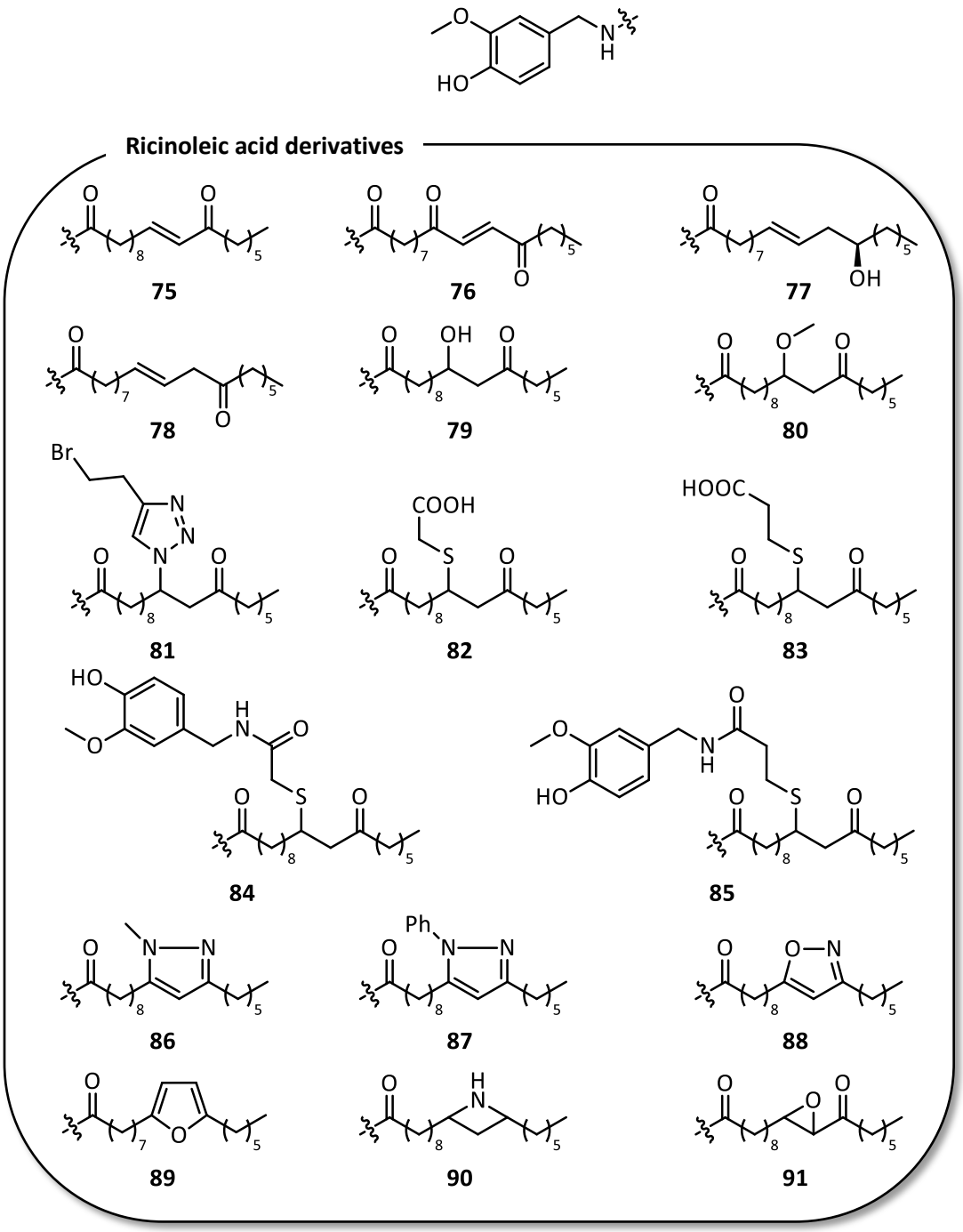
Early work in this area identified a number of FAAH inhibitors including AA-5-HT **33**,[124] *N*-arachidonoylaminophenol (AM404, **73**),[125,126] and arvanil **52**.[127] In 2001 Di Marzo reported a SAR study on arvanil **52** (IC_{50}=32.0 μM) looking at a combination of TRPV1 and CB1 agonism, anandamide **29** transport, and FAAH inhibition.[128] The urea analogue **74** (O-1987) was the most potent FAAH inhibitor of these analogues (IC_{50}=2.0±0.4 μM).[128] Intravenous administration of **74** to mice produced effects in all of the typical cannabinoid tetrad tests: decreased spontaneous activity, decreased rectal temperature, and antinociception in the tail flick test. However, the lack of selectivity in these molecules makes it difficult to verify that the observed pharmacology is a result of FAAH inhibition.

3.4. OBJECTIVE

To take advantage of the experience gained in the synthetic modification of ricinoleic **1a** and lesquerolic **12a** acids, a new objective was envisage. As discussed previously, there is a critical need for effective new pharmacotherapies for pain. The challenge of designing receptor-selective compounds that preserve the physiological activity of TRPV1 as well as the design of new inhibitors of FAAH is still open. The preparation of new compounds that allows the development of SAR databases to gain insight on their mechanism of action and the determinants of activity are essential for the development of new TRPV1-based and FAAH-based analgesic leads. Consequently, the main objective of this chapter was the design and synthesis of a set of new long chain vanillyl acylamides (LCNVAs) introducing modifications at the lipophilic region of the capsaicin. These modifications were carried out through of chemical transformations over the homoallyl hydroxy moiety of the ricinoleic **1a** and lesquerolic **12a** acids.

3.5. RESULTS AND DISCUSSION

This section describes the different procedures employed to synthesise new long chain *N*-vanillyl acylamides derived from ricinoleic **1a** and lesquerolic acid **12a**, which were designed on the basis of bibliographical SAR studies (**Figure 3.18**).



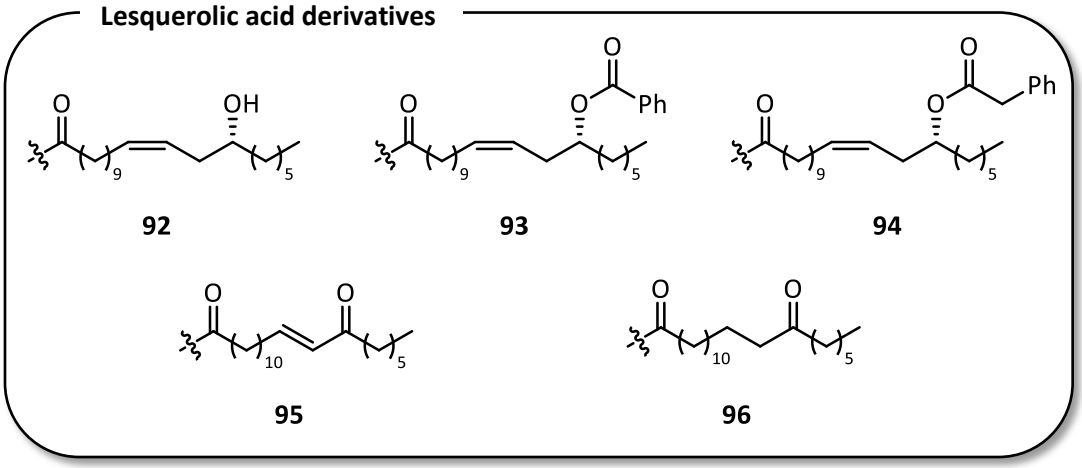
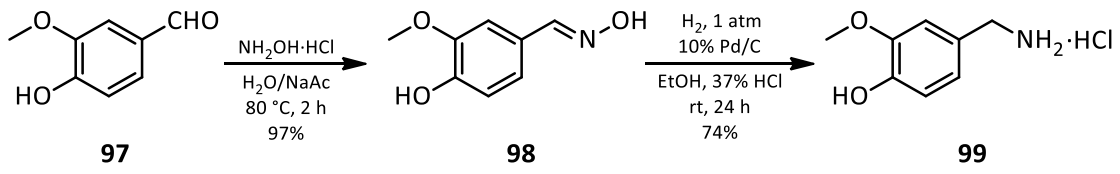


Figure 3.18. Target molecules derived from ricinoleic **1a** and lesquerolic **12a** acid planned to synthetize.

3.5.1. Synthesis of vanillylamine

Since the objective of this work is the preparation of new capsaicin-like fatty acid derivatives, it is obvious that the first step was to carry out the synthesis of the aromatic part for later, to perform the amidation with the different carboxylic acids to yield the derivatives with the vanillylamine moiety.

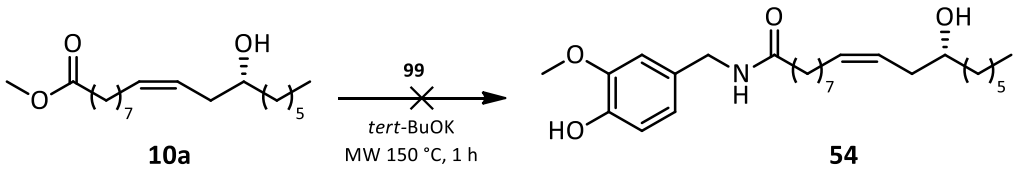
Nelson prepared vanillyl oxime **98** by heating vanillin **97** with hydroxylamine. The resultant oxime was reduced with sodium amalgam to generate the amine, which was isolated as the hydrochloride salt **99**.[67] Ganett assayed palladium and platinum to catalyse the reduction reaction.[129] PtO_2 led to partial over-reduction whereas palladium over carbon provided the vanillylamine hydrochloride **99**.[130] This last method was carried out in this thesis. The synthesis of oxime **49** was performed by adding hydroxylamine hydrochloride (1.03 equiv.) to an aqueous solution of vanillin **97** at 80 °C, using NaAc (1 equiv.) as a base. The corresponding oxime **98** was achieved (97%). Vanillylamine **99** was prepared by direct hydrogenation of the oxime **98** using 10% loading Pd/C. The reaction was carried out in EtOH under acidic conditions. H_2 was used at atmospheric pressure to avoid over-reduction of the aromatics and finally, vanillylamine hydrochloride **99** was obtained (74% yield) (**Scheme 3.3**).



Scheme 3.3. Synthesis of vanillylamine hydrochloride **99**.

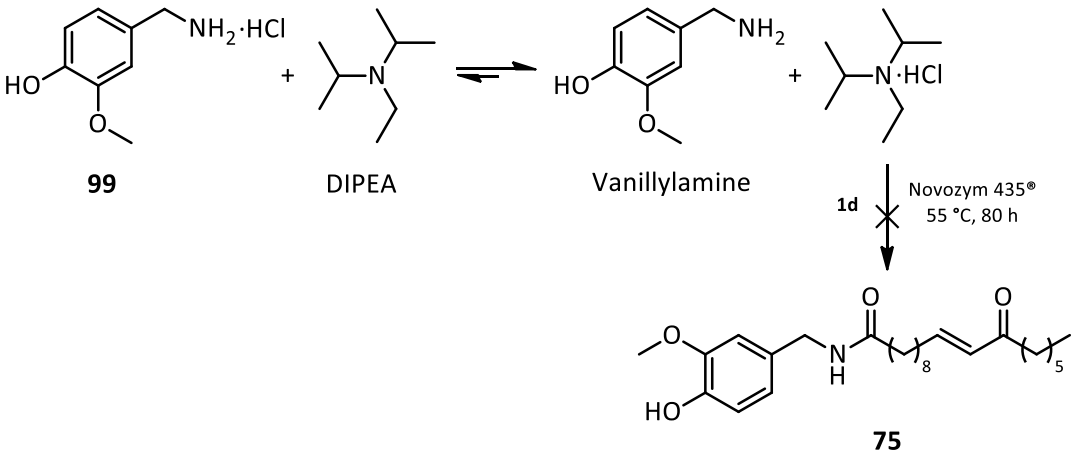
3.5.2. Preliminary attempts to amide bond formation

Amide bonds are found ubiquitously in natural or synthetic molecules of biologic interest. Since the early days of synthetic organic chemistry, methods for the formation of amides have been described. Methods range from the rather straightforward use of acyl halides, anhydrides, and carbodiimides, to the more elaborate as enzyme-catalysed methods. As stated in the Introduction, the benzylamidic region of capsaicinoids is very important in terms of TRPV1 potency. Despite the pharmacological relevance of vanillamides, there is still a need for a simple and mild method to prepare these compounds, especially in a single step and with an easy isolation procedure. The most suitable method for preparation of capsaicinoids is by selective acylation of vanillylamine **99** with acyl chloride. The major problem is the chemoselectivity of this acylation. Moreover, vanillylamine **99** is poorly soluble in anhydrous systems, and acyl chloride is easily hydrolysed when water is present, so it is very difficult to achieve a satisfactory yield. The first analogues of capsaicin, vanillylamides of saturated C2–C12 fatty acids and 10-undecylenic acid, were synthesised by acylation of vanillylamine **99** with acyl chlorides or acid anhydrides in dry ether.[68] Although 2 equiv. of vanillylamine **99** were used, the yields ranged 44 to 83%. Acylation under Schotten-Baumann conditions showed excellent selectivity for *N*- versus *O*-acylation. However, the activation of the acid as a chloride is required, and formation of emulsions can take place with long-chain acids. Janusz *et al*.[131] proposed using an aqueous soda solution vanillylamine HCl **99** to avoid the presence of an excess of vanillylamine, although the yields ranged 27 to 69% for DMF, THF or ether as co-solvents. Furthermore, while formation of acyl chlorides is a routine operation for simple unfunctionalized acids, carefully controlled conditions are required with polyunsaturated acids, and protection on the hydroxy group is necessary in hydroxy fatty acids. Perreux *et al*.[132] studied the effect of the microwave activation in the ester aminolysis in a free-solvent media. They concluded that different esters and amines in presence of potassium *tert*-butoxide led to the corresponding amides under microwave heating. This approach was attempted using methyl ricinoleate **10a** and vanillylamine **99** to evaluate the effect of the microwave heating in the synthesis of new LCNVAs. After 1 h in a microwave oven at 150 °C and with *tert*-BuOK as a base, only starting material was obtained. This approach was discarded (**Scheme 3.4**).



Scheme 3.4. Microwave-mediated synthesis of **54**.

In reference to the enzyme-catalysed synthesis, Kobata *et al*.[133] reported the enzymatic synthesis of capsaicin analogues by reaction of vanillylamine HCl **99** with fatty acid methyl esters, but only 8–28% yield was achieved. Later, some studies reported the acylase- or lipase-catalysed synthesis of capsaicins with higher yields.[134,135] These last conditions were tested in order to synthetize the corresponding first compound planned **75**. **75** was envisaged based on two reasons: first, its structure has an α,β-unsaturated ketone which is also presented in shogaols (compounds presents in ginger and active in TRPV1, **Figure 3.19**) and second, α,β-unsaturated carbonyl compounds can be transformed in several compounds because of their reactivity. Vanillylamine hydrochloride **99** (1 equiv.) was treated with DIPEA (18 equiv.) and Novozym 435® (10 mg/ml) was added. Finally, a solution of **1d** (1 equiv.) in 2-methyl-2-butanol was added and the reaction was carried out under stirring at 55 °C for 80 h. The reaction did not result in the desired product (**Scheme 3.5**).



Scheme 3.5. Enzymatic synthesis of compound **75**.

In view of the direct amidation did not give us any result, we decided to use coupling agents in order to activate the carboxylic acid. This reaction is described in the following section since it was the chosen approach to synthesize the proposed molecules.

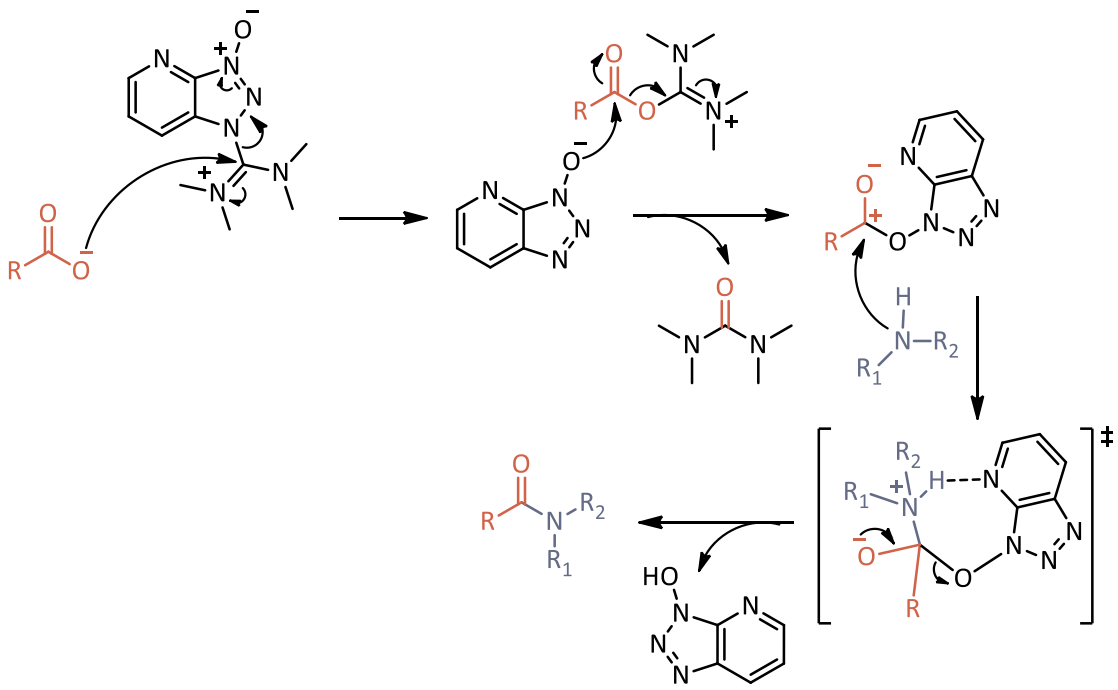
3.5.3. Synthesis of *N*-vanillyl acylamides of ricinoleic acid-related compounds

3.5.3.1. Oxidized and isomerized amides

- *(E)-N-(4-Hydroxy-3-methoxybenzyl)-12-oxooctadec-10-enamide*

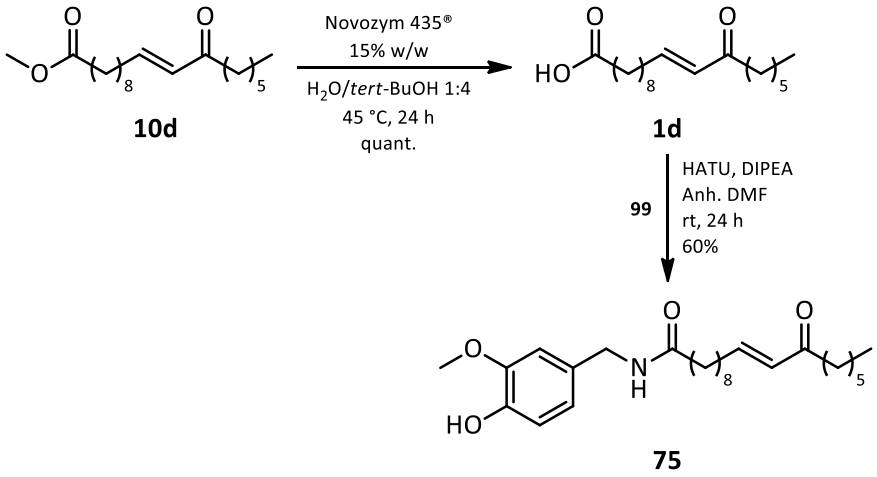
Recently, numerous coupling reagents have been described including those based on phosphonium salts, uronium (iminium) salts, preformed anhydrides, active esters and acid chlorides and fluorides. In situ activation with carbodiimide condensing agents (DCC, EDCI) requires protection of the phenolic hydroxyl of vanillamine[55] because the discrimination between the amino group and the phenolic hydroxyl is poor. *N*,*O*-Diacyl derivatives formed in the reaction can be selectively *O*-deacylated with pyrrolidine,[54] but this strategy is untenable with expensive acids, with one full equivalent being wasted, and problems can arise with base-labile functionalities. Protection of vanillamine is also necessary when the acid is activated ex situ as a hydroxysuccinimide derivative or as a mixed anhydride.

Couplings with aminium/uronium salts in the presence of base have proved to be more effective than those carried out with phosphonium reagents or carbodiimides in the presence of hydroxylamine derivatives. HATU (1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate) is a reagent used in peptide coupling chemistry to generate an active ester from a carboxylic acid. HATU in presence of Hünig's base (*N*,*N*-diisopropylethylamine, DIPEA) allows the formation of amide bonds. HATU has been proven to be very efficient in difficult sterically hindered condensations and usually gives a low level of racemisation.[136,137] It involves the formation of 7-azabenzotriazol-1-yl esters, very highly reactive species towards amines, probably because of intramolecular general base catalysis. In the first step, the carboxylate anion (formed by DIPEA deprotonation of the carboxylic acid) reacts with HATU to form the unstable *O*-acyl(tetramethyl)isouronium salt (OAt salt). The OAt anion rapidly reacts with the isouronium salt, affording the OAt-active ester and liberating a stoichiometric quantity of tetramethylurea. The addition of a nucleophile, as an amine, to the OAt-active ester yields the corresponding amide. The high coupling efficiencies and fast reaction rates associated with HATU coupling are thought to arise from a neighbouring group effect brought about by the pyridine nitrogen atom, which stabilises the incoming amine through a hydrogen-bonded 7-membered cyclic transition state (**Scheme 3.6**).



Scheme 3.6. Mechanism of amide formation mediated by HATU.

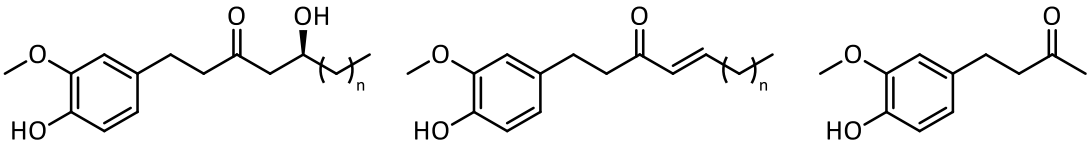
The synthesis of the desired amide **75** started with the enzymatic hydrolysis of methyl (*E*)-12-oxo-10-octadeneoate **10d** using Novozym 435® as catalyst to yield the carboxylic acid **1d** quantitatively, which was used without any further purification. The corresponding acid **1d** was mixed with the vanillylamine **99** (1.1 equiv.), HATU (1.5 equiv.) and DIPEA (3 equiv.) in anhydrous DMF. Finally, the reaction mixture was washed with sat. NaCl, 1 M HCl and sat. $NaHCO_3$. The crude reaction mixture was purified by column chromatography yielding the (*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-12-oxooctadec-10-enamide **75** (60% yield) (**Scheme 3.7**).



Scheme 3.7. Synthesis of compound **75**.

- *(E)-N-(4-Hydroxy-3-methoxybenzyl)-9,12-dioxooctadec-10-enamide*

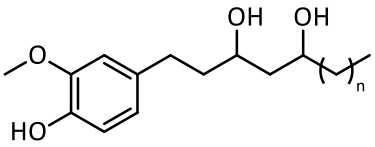
Ginger (*Zingiber officinale Roscoe*) has a characteristic flavor and pungency and is used worldwide. It has a variety of biological effects such as analgesia, antiinflammatory effects, antiplatelet effects, antipyretic effects, and antioxidation.[138-141] The components of Ginger mainly comprise [6]-, [8]- and [10]-gingerols, [6]-, [8]- and [10]-shogaols and zingerone (**100**-**106**).[142,143] Other compounds include [6]-, [8]- and [10]-gingerdiols, [6]- and [10]-gingerdiones, etc. in less quantity (**107**-**111**) (**Figure 3.19**).



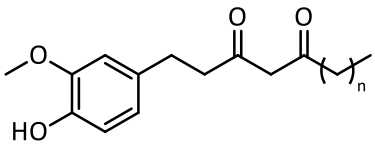
100: n=4 [6]-gingerol
101: n=6 [8]-gingerol
102: n=8 [10]-gingerol

103: n=4 [6]-shogaol
104: n=6 [8]-shogaol
105: n=8 [10]-shogaol

106: zingerone



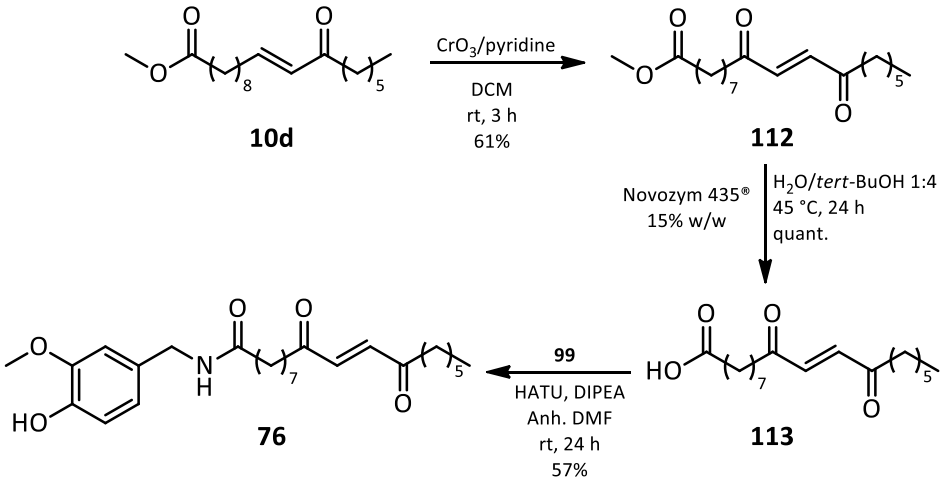
107: n=4 [6]-gingerdiol
108: n=6 [8]-gingerdiol
109: n=8 [10]-gingerdiol



110: n=4 [6]-gingerdione
111:n=8 [10]-gingerdione

Figure 3.19. Ginger pungent constituents.

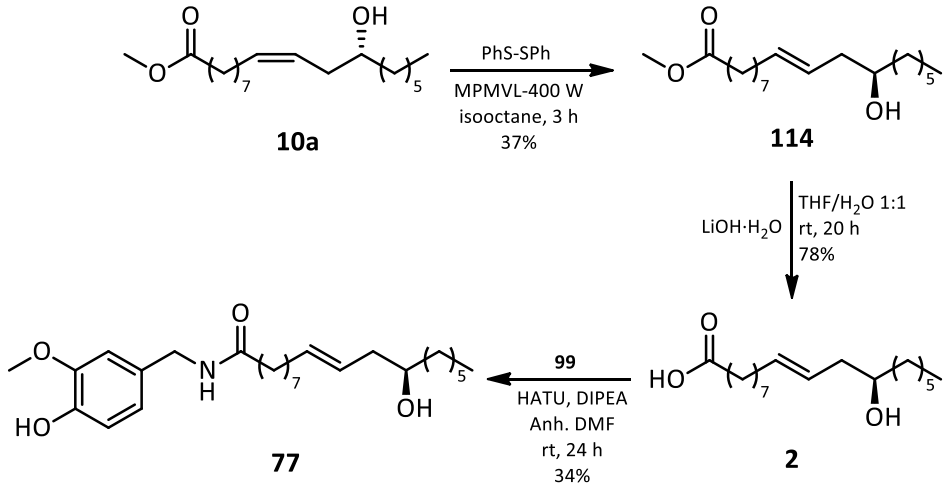
[6,8,10]-Shogaols **103**-**105** can increase the intracellular calcium concentration in TRPV1-expresing HEK293 cells through TRPV1.[144] Shogaols appear to be more potent than gingerols, and most of the compounds cause aversive or nociceptive responses mediated by TRPV1.[144] In view of this background, we decided to synthetize an α,β-unsaturated diketone **76** in order to evaluate if the increment of conjugation affects to activity. The treatment of methyl (*E*)-12-oxooctadec-10-enoate **10d** with CrO_3 in excess (19 equiv.) led to the methyl (*E*)-9,12-dioxooctadec-10-enoate **112** in a good yield after purification by column chromatography (61%). The enzymatic hydrolysis of this compound with Novozym 435® and posterior HATU-mediated coupling with the vanillyl amine **99** gave the corresponding (*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-9,12-dioxooctadec-10-enamide **76** (57% yield) (**Scheme 3.8**).



Scheme 3.8. Synthetic route to compound **76**.

- *(R,E)-N-(4-Hydroxy-3-methoxybenzyl)-12-hydroxyoctadec-9-enamide*

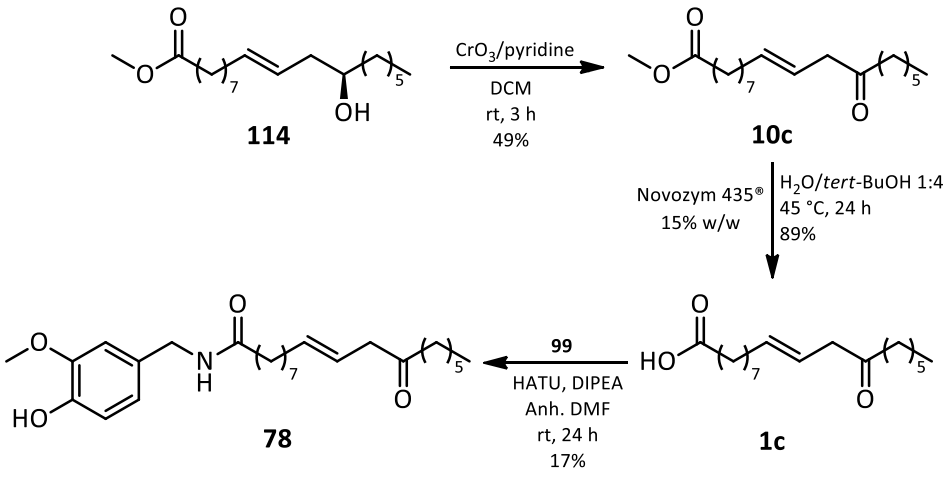
As stated at the beginning of this chapter, rinvanil **54** was the first compound comparable in efficacy with olvanil **51** (EC_{50}=8.5 nM). It was synthesized by Appendino *et al*.[93] by amidation of ricinoleic acid **1a** with vanillyl amine **99**. Despite configuration of double bond of capsaicin **28** does not seem relevant for receptor interaction;[145] we wanted to inquire if, in the case of ricinoleic acid **1a**, the configuration of double bond is crucial for the efficacy. Hence, to synthetize the *E*-analogue of the ricinoleic acid or ricinelaidic acid **2**, we had to carry out an isomerization of the double bond under irradiation. The isomerization protocol was based on the work published by Thalmann *et al.*[146] that used the diphenyl disulphide (2%) and Philips HP(L) 400-W medium-pressure mercury lamp. After 3 h under irradiation, methyl ricinelaidate **114** was obtained pure after several recrystallizations a low temperature (37% yield). The methyl ester was hydrolysed under basic conditions and the corresponding ricinelaidic acid **2** was treated with vanillyl amine **99** and HATU to give the (*R*,*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-12-hydroxyoctadec-9-enamide **77** (34%) (**Scheme 3.9**).



Scheme 3.9. Synthesis of compound **77**.

- *(E)-N-(4-hydroxy-3-methoxybenzyl)-12-oxooctadec-9-enamide*

Additionally, the compound **114** was oxidized by treatment with CrO_3 (6 equiv.) to obtain the *trans* ketone **10c** which was enzymatically hydrolysed and subsequently coupled with the vanillyl amine **99** to achieve the (*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-12-oxooctadec-9-enamide **78** after purification by liquid column chromatography (17% yield) (**Scheme 3.10**).

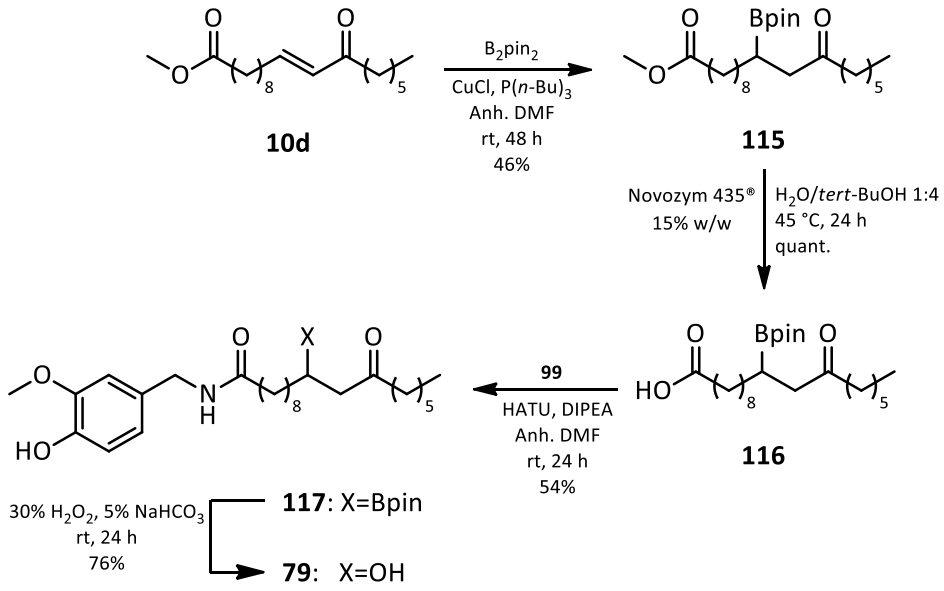


Scheme 3.10. Synthesis of compound **78**.

3.5.3.2. 1,4-Ketosubstituted amides

- *N-(4-Hydroxy-3-methoxybenzyl)-10-hydroxy-12-oxooctadecanamide*

In 2002, some studies reported that some gingerols (**100** and **101**) activated TRPV1 receptor with a lower potency than capsaicin **28**.[147,148] With this background we decided to synthetized the *N*-vanillyl acyl β-hydroxyketone **79**. Methyl (*E*)-12-oxo-10-octadeneoate **10d** was treated with bis(pinacolato)diboron (1.2 equiv.), tri-*n*-butylphosphine (0.11 equiv.) and copper chloride (0.11 equiv.) to generate the boron intermediate **115** after purification by liquid column chromatography.[149] This product was immediately hydrolysed enzymatically using Novozym 435® to obtain the corresponding carboxylic acid **116** (quant.). The HATU-mediated coupling with the vanillyl amine **99** gave the amide **117** (54% yield), which was finally treated with 30% hydrogen peroxide and 5% $NaHCO_3$ to lead to the racemic mixture of the β-hydroxyketone **79** (76% yield) (**Scheme 3.11**).

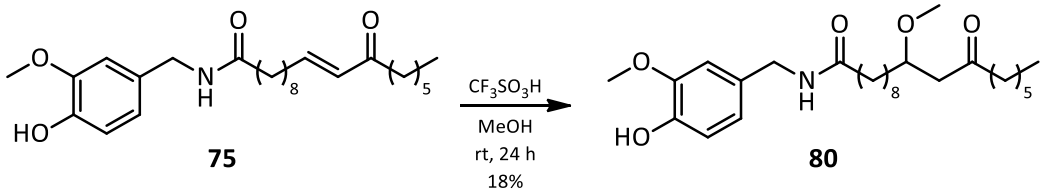


Scheme 3.11. Synthesis of compound **79**.

- *N-(4-Hydroxy-3-methoxybenzyl)-10-methoxy-12-oxooctadecanamide*

As it was explained before, some evidences suggest the existence of a second apolar pocket in the TRPV1 receptor. This cleft accommodates the acyl residue of LCNVAs and does not establish hydrogen bonds with polar groups.[92] In order to corroborate the existence of this second pocket, the synthesis of a β-alkoxyketone analogue to **79** was planned. Oxa-Michael reactions (alkoxylation) normally only require catalytic amounts of a strong base or a Lewis acid to activate either the nucleophile or the acceptor center. With most weak nucleophiles as alcohols, oxa-Michael additions are far difficult. In 2003, Spencer *et al*.[150] developed an universal method for acid-catalyzed oxa-

Michael additions. They hypothesized that activation of Michael acceptors by protonation of the carbonyl group (pK_a ca. -5 for α,β-unsaturated ketones) should be feasible in the presence of weakly basic nucleophiles, with levelling effects limiting efficiency for more basic Michael donors. Brønsted acid catalysts such as triflic acid or trifluoromethanesulphonic acid gave rapid conversions. Accordingly, (*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-12-oxooctadec-10-enamide **75** was treated with trifluoromethanesulphonic acid (0.1 equiv.) at room temperature for 24 h obtaining the β-methoxyketone **80** (18% yield) (**Scheme 3.12**).

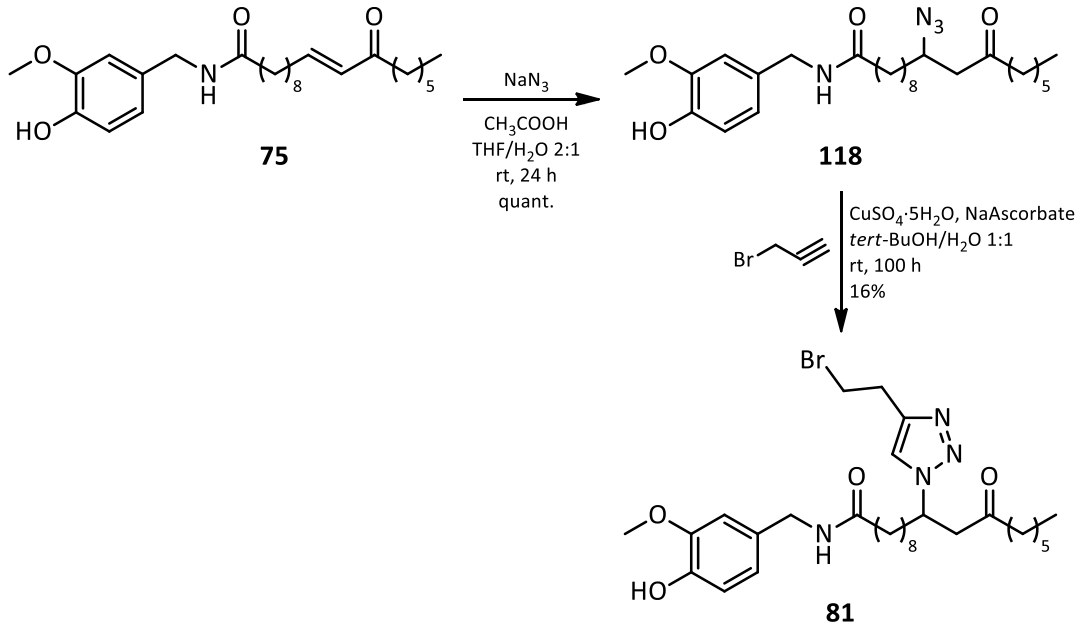


Scheme 3.12. Synthesis of compound **80**.

- *10-[4-(2-Bromoethyl)-1H-1,2,3-triazol-1-yl]-N-(4-hydroxy-3-methoxybenzyl)-12-oxooctadecanamide*

The 1,2,3-triazole ring features a combination of H-bond donor and acceptor sites capable of mimicking the hydrogen bonding acidity and basicity of a peptide bond.[151] There are studies that demonstrate that 1,2,3-triazole ring are potentially bioequivalent to an amide bond.[152] Appendino *et al*.[153] showed that the presence of a triazole ring on the alkyl chain resulted a agonist behaviour at TRPV1 receptor. Thus, we planned the synthesis of a β-triazoleketone derivative. The general method for the preparation of triazoles is based on Copper-catalyzed azide–alkyne cycloaddition (CuAAC). This process emerged as the first example of click chemistry.[154] It transforms organic azides and terminal alkynes exclusively into the corresponding 1,4-disubstituted 1,2,3-triazoles, in contrast to the uncatalyzed reaction, which requires much higher temperatures and provides mixtures of 1,4- and 1,5-triazole regioisomers. In regard to our case, the first step is the introduction of the azide group in the β-position from the ketone. Sodium azide (8 equiv.) and glacial acetic acid (54 equiv.) were added to a solution of **75** in THF/H_2O 2:1. After 24 h at room temperature and without any purification the β-azidoketone **118** was obtained as a racemic mixture (quant.). Then, we proceeded to carry out the copper-catalyzed azide–alkyne cycloaddition. We observed that when the solvent used was methanol the reaction did not work. However, when *tert*-butanol was used instead of methanol, the solubility of the starting material **118** improved and the triazole was synthetized. Compound **118** was treated with 1-bromo-3-butin (1 equiv.) in presence of $CuSO_4 \cdot 5H_2O$ (0.001

equiv.) and sodium ascorbate (reducing agent, 0.01 equiv.). After 100 h at room temperature the compound **81** was obtained as a racemic mixture (16% yield) (**Scheme 3.13**).



Scheme 3.13. Synthesis of the β-triazolketone **81**.

- *β-Thioketones derivatives*

In 2012, Sang *et al*.[155] demonstrated that [6]-shogaol **103** is extensively metabolized in mice and in cancer cells in thirteen metabolites. Among these metabolites, derivatives with a sulphur atom directly attached to the aliphatic chain were found (**Figure 3.20**). It was proved that α,β-unsaturated keto group of [6]-shogaol **103** was a good substrate for thiol conjugation through the mercapturic acid pathway.

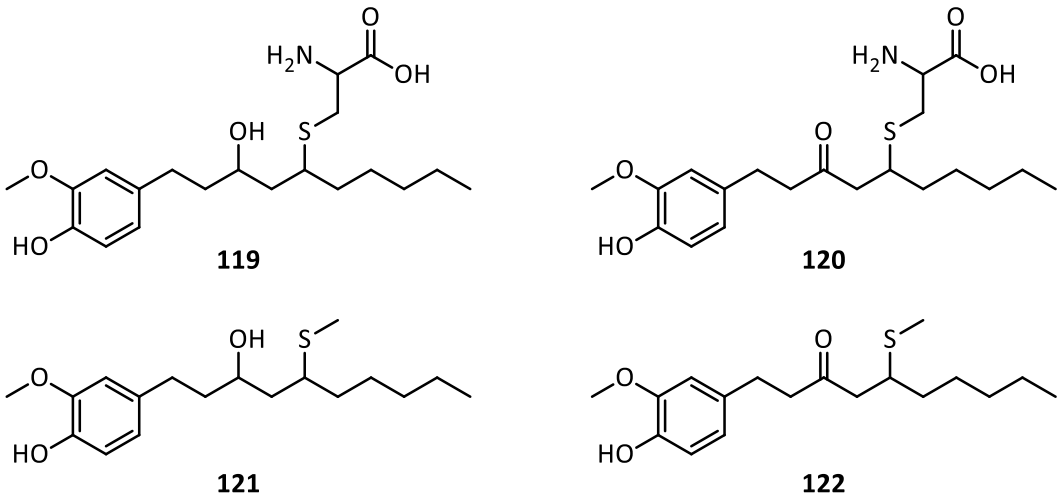
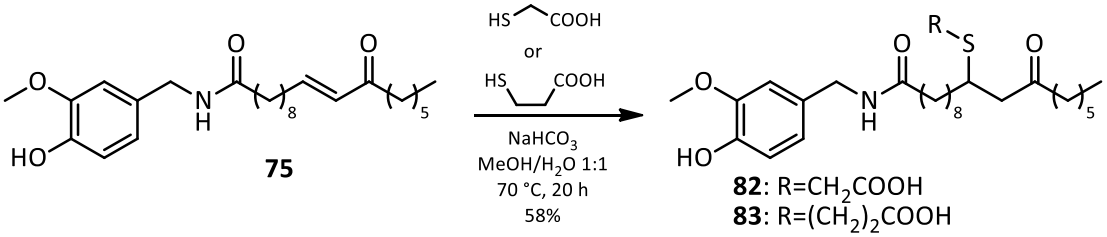


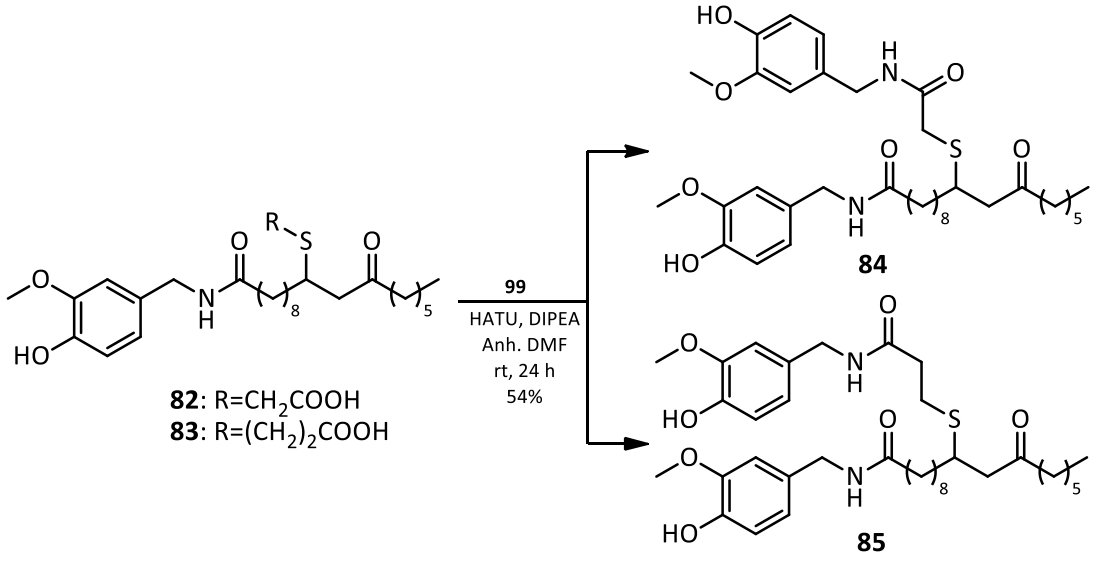
Figure 3.20. Structures of some thiol-conjugated metabolites from [6]-shogaol **103**.

Based on the aforementioned structures, we designed a small library of compounds that include structural similarity in the side-chain of the *N*-vanillyl amide, in order to obtain SAR information. We chose thioglycolic and 3-mercaptopropionic acid to evaluate the presence of a sulphur atom in the β-position respecting the ketone group and the effect of the length of the substituent in the activity. Michael reaction of (*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-12-oxooctadec-10-enamide **75** was carried out using thioglycolic or 3-mercaptopropionic acid (3 equiv.) in presence of solid $NaHCO_3$ (0.04 equiv.). After 20 h at 70 °C the corresponding β-thioketones **82** and **83** were obtained (58% yield) (**Scheme 3.14**).



Scheme 3.14. Synthesis of β-thioketones **82** and **83**.

Because the TRPV1 is a multimeric receptor,[63] we decided to introduce another vanillylamine **99** to test if double vanillamides could improve the activity in the TRPV1 receptor. Hence, the synthesis of the new amides **82** and **83** was attempted using the general procedure of HATU-mediated coupling above described. Each of the compounds **82** and **83** were separately treated with vanillylamine **99** (1.1 equiv.), HATU (1.5 equiv.) and DIPEA (3 equiv.). After 24 h at room temperature the diamides **84** and **85** were isolated (both 54% yield) (**Scheme 3.15**).



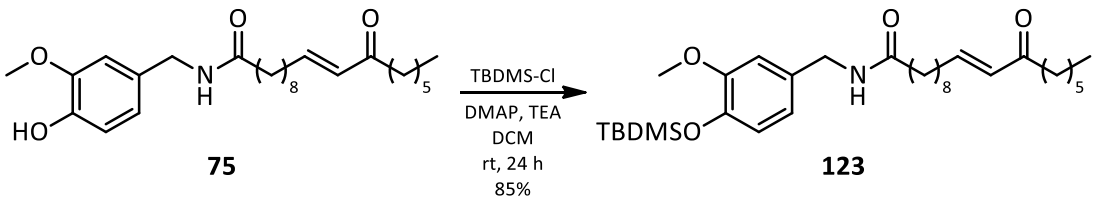
Scheme 3.15. Synthesis of compounds **84** and **85**.

3.5.3.3. Side-chain conformational constrained amides

In 2002, Appendino *et al*.[92] designed a serie of *N*-vanillyl acylamides cyclopropanated with the aim of dissecting the conformational and polar contributions of unsaturations to the activity. Cyclopropanation of olvanil **51** and other monounsaturated vanillamides led to increase efficacy, suggesting that the double bond essentially has a conformational effect, preorganizing the long chain for bonding. Later, Appendino and Di Marzo, demonstrated that a triazole ring (flat bivalent element) could also mimic the conformational constraint imposed on alkyl chains by a double bound.[153] In the attempt to investigate the influence of conformational effect of the alkyl chain, a family of new olefin-bioisosteres derivatives was designed.

- *Pyrazole derivatives*

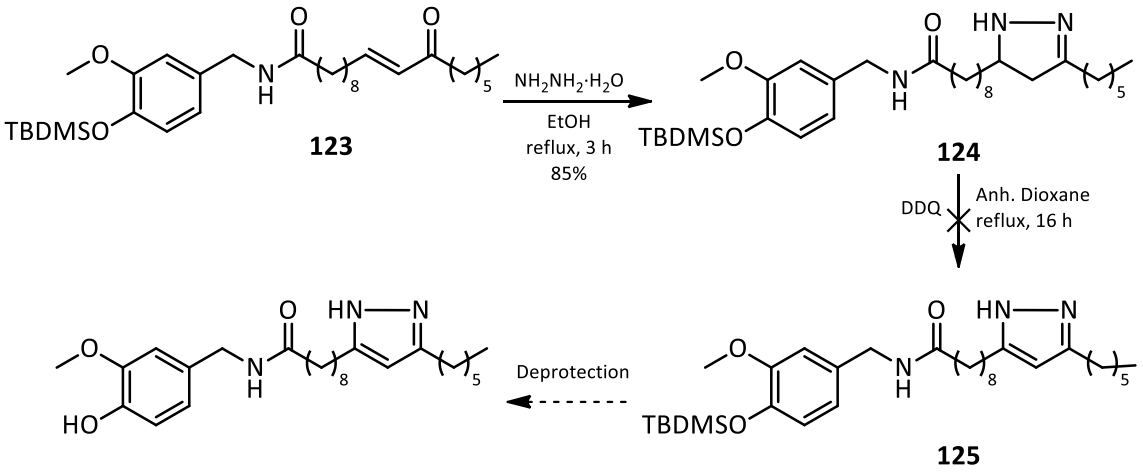
The pyrazole ring is an important central template for a wide variety of biologically active compounds including anti-inflamatory.[156] α,β-Unsaturated ketones have been widely used in organic synthesis for the production of heterocyclic compounds such as pyrazole derivatives. The reaction of α,β-unsaturated aldehydes and ketones with different types of hydrazines followed by oxidation is one of the most used and easy methods. Thus, we decided to synthetized a small library of LCNVAs derivate from ricinoleic acid **1a** with a pyrazole moiety in the alkyl chain to evaluate the effect of the substituent and the effect of the conformational constraint of the ring. First, we attempted the synthesis of the simplest pyrazole (no substituents). The protection of the phenol group was necessary because it could be also oxidized during the ring aromatization. (*E*)-*N*-(4-Hydroxy-3-methoxybenzyl)-12-oxooctadec-10-enamide **75** (1 equiv.) was treated with *tert*-butyldimethylsilane chloride (2 equiv.) in presence of DMAP (1.7 equiv.) and TEA (6 equiv.). After 24 h at room temperature and isolation by liquid column chromatography the compound **123** was obtained (85% yield) (**Scheme 3.16**).



Scheme 3.16. Synthesis of compound **123**.

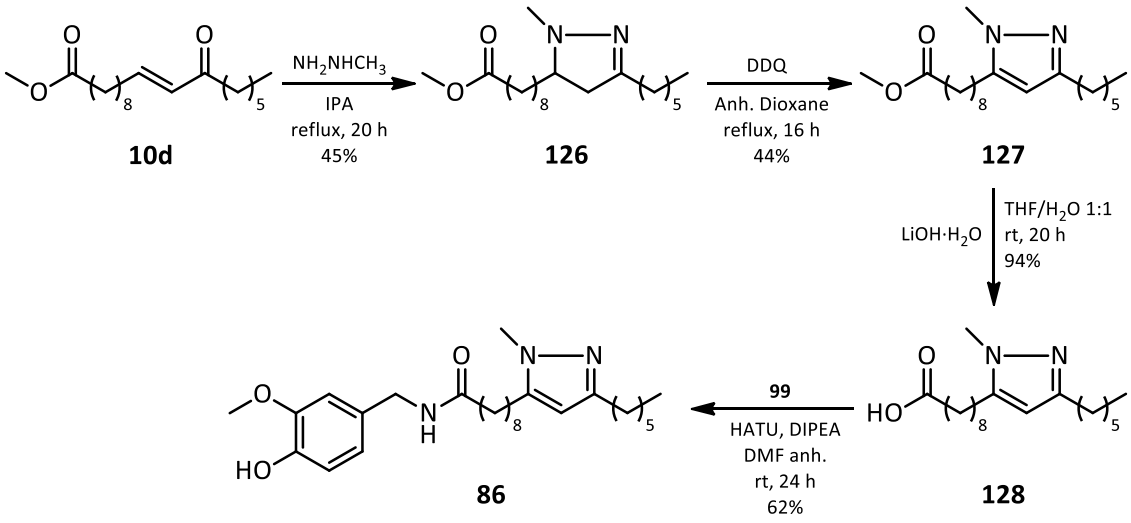
The next step was the reaction of compound **123** with hydrazine monohydrate (6 equiv.) under nitrogen atmosphere at reflux for 3 h in an alcoholic media. After removing the solvent, the corresponding dihydropyrazole **124** was immediately oxidized in presence of DDQ (1 equiv.) under

reflux conditions. Unfortunately, after several attempts, the pyrazole **125** was not achieved with enough purity and this synthetic route was discarded (**Scheme 3.17**).



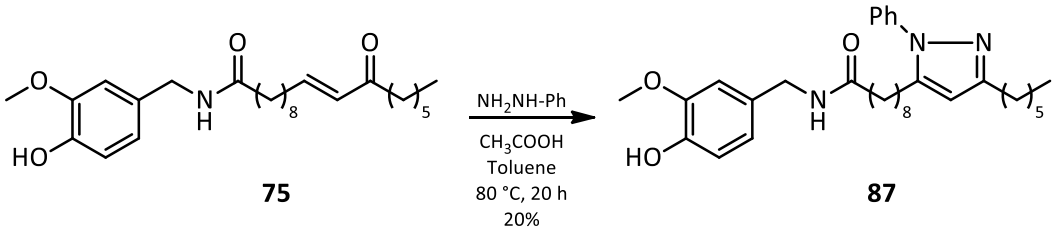
Scheme 3.17. Synthetic route attempted.

Considering the purification problems described above, we decided to start with the synthesis of the next pyrazole derivative, the 9-(3-hexyl-1-methyl-1*H*-pyrazol-5-yl)-*N*-(4-hydroxy-3-methoxybenzyl)nonanamide **86**. The synthesis started from the methyl ester enone **10d**. This approach could not be used for the previous derivative because methyl esters react with hydrazine monohydrate yielding hydrazides.[157] Methyl-(*E*)-12-oxo10-octadecenoate **10d** was treated with methyl hydrazine (8 equiv.) under nitrogen atmosphere at reflux for 20 h The methyldihydropyrazole **126** was purified by liquid column chromatography just after the reaction ended and used in the next step (45% yield). The oxidation of compound **126** with DDQ using the above conditions gave pure methyl 9-(3-hexyl-1-methyl-1*H*-pyrazol-5-yl)nonanoate **127,** after purification by column chromatography (44% yield). The alkaline hydrolysis of the methyl ester **127** led to the carboxylic acid **128** in an excellent yield (94%), which was amidated with the vanillylamine **99** by HATU-mediated coupling conditions yielding the amide of the methylpyrazole **86** (62%) (**Scheme 3.18**).



Scheme 3.18. Synthetic route to compound **86**.

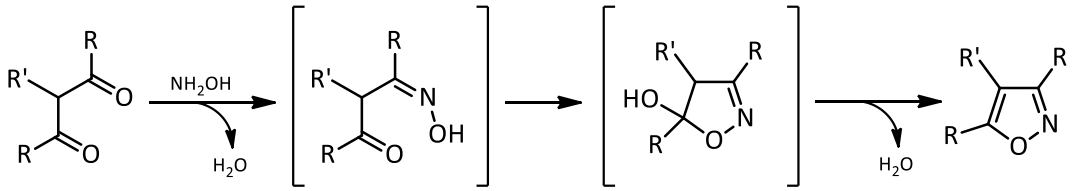
The pyrazole ring can be obtained by condensation of an α,β-unsaturated carbonyl compound with hydrazines under air atmosphere.[158] It is important to remark that as the reaction occurs at 80 °C in an open vessel, the boiling point of the hydrazine used must be higher than the boiling point of solvent. Otherwise, the hydrazine would be lost. Phenylhydrazine has a higher boiling point than methylhydrazine (243 *vs* 87 °C), which is very close to the reaction temperature. Hence, the synthesis of the phenylpyrazole **87** was easier because we were able to perform an one pot reaction whereas this synthetic route was not able to be applied for compound **86**. (*E*)-*N*-(4-Hydroxy-3-methoxybenzyl)-12-oxooctadec-10-enamide **75** was treated with phenylhydrazine (4 equiv.) in presence of glacial acetic acid (35 equiv.) heating at 80 °C in an open vessel to lead the phenylpyrazole compound **87** after purification by liquid column chromatography (21%) (**Scheme 3.19**).



Scheme 3.19. Synthesis of compound **87**.

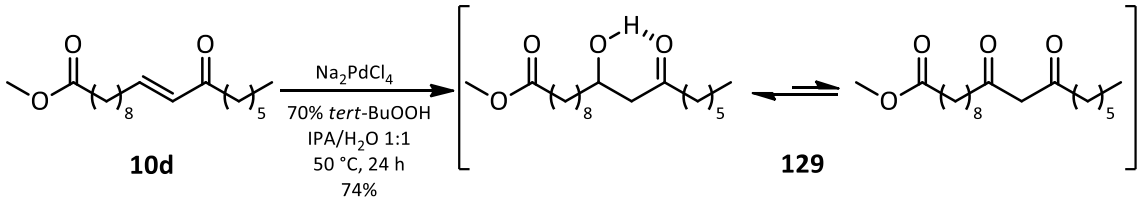
- *Isoxazole derivative*

Isoxazoles are a large group of heterocyclic compounds that display interesting medicinal, agricultural and some other industrial utilities. Isoxazoles possess important biological activities, such as anti-inflammatory, antimicrobial, anticancer, and antinociceptive activities. In 2011 and after an HTS campaign, Palin *et al*.[159] identified a series of isoxazole-3-carboxamide derivatives with moderate potency at TRPV1. The introduction of an isoxazole ring led to an improving of oral bioavailability and *in vivo* efficacy of the TRPV1 antagonists. In view of these results, we decided to synthetize a LCNVA based on the isoxazole ring and evaluate its effect at TRPV1 receptor and FAAH enzyme. In 1888, Claisen described the first general synthesis of isoxazoles.[160] The process involved the reaction of β-diketones with hydroxylamine followed by the cyclization-dehydration of the intermediate oxime (**Scheme 3.20**).



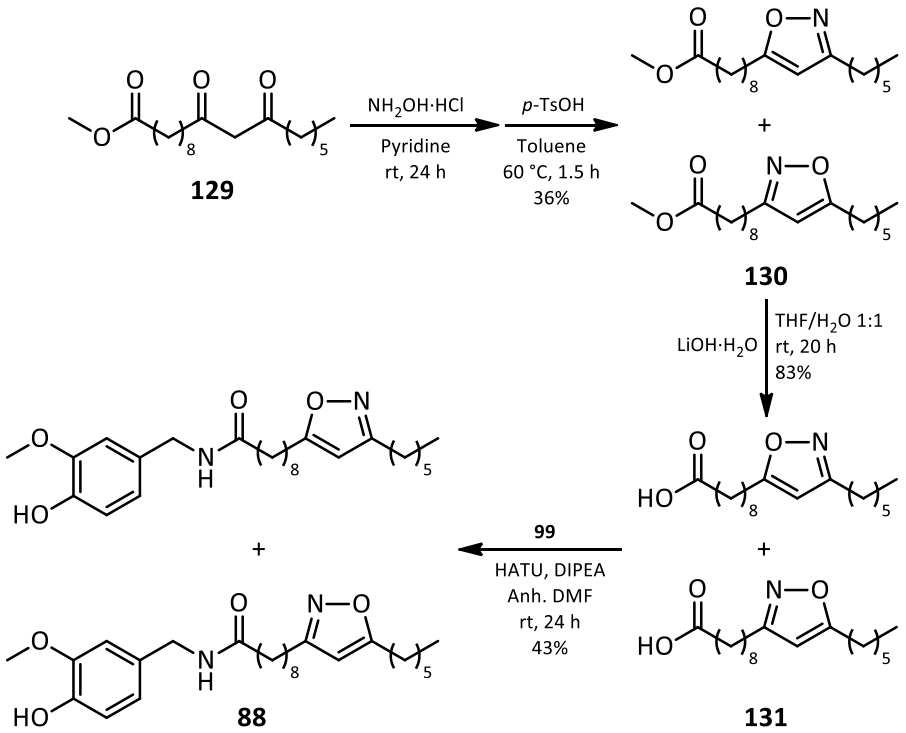
Scheme 3.20. Claisen's mechanism for the formation of isoxazole rings.

Thus, the first step of the synthesis was the preparation of the starting material, the β-diketone. The preparation has been effected usually by condensation reactions of carbonyl compounds under such strongly basic or acidic conditions that cause serious side reactions. However, in recent years new catalytic methods have been developed. One of the most used is the oxidation of α,β-unsaturated carbonyl catalysed by palladium using a peroxide as oxidizing agent.[161] Accordingly, methyl 12-oxo-10-octadecenoate **10d** was treated with Na_2PdCl_4 (0.1 equiv.) and 70% *tert*-butylhydroperoxide (2 equiv.). After 24 h at 50 °C and subsequent purification by liquid column chromatography, the β-diketone **129** was obtained as a mixture of tautomers (74% yield) (**Scheme 3.21**).



Scheme 3.21. Synthesis of the β-diketone **129**.

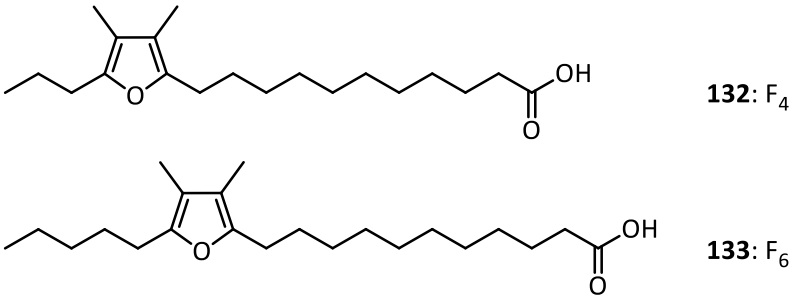
The β-diketones are principally in the enol form and the "inert" solvents favour this form, since the internally hydrogen-bonded enol molecule is less polar than the keto molecule. In polar solvents the fraction of keto molecules increases.[162] The electrostatic repulsion between carbonyl groups of the keto form is reduced in a polar solvent. Also, the solvation energy of keto molecules is presumably greater than for enol molecules in polar solvents and the solubility of the keto tautomer is greater in these solvents; hence, one might expect the equilibrium to shift toward the keto tautomer on dilution in polar solvents. The reaction of β-diketone **129** in pyridine (polar solvent) with hydroxylamine hydrochloride (1.9 equiv.) followed by a dehydration mediated by *p*-toluenesulphonic acid (1 equiv.) gave the isoxazole **130** as a mixture of isomers (36%) which could be differentiated by ^{13}C NMR. The alkaline hydrolysis of the methyl esters **130** and the posterior amide coupling with the vanillylamine **99** mediated by HATU led to the compound **88** as a mixture of isomers (43% yield) (**Scheme 3.22**).



Scheme 3.22. Synthesis of isoxazole **88**.

- *8-(5-Hexylfuran-2-yl)-N-(4-hydroxy-3-methoxybenzyl)octanamide*

Furans are 5-membered planar ring that have important feature of a variety of medicinal agents. During the last few decades a considerable attention has been put on the synthesis of furan derivatives and their pharmacological activities. The furan ring system is the basic skeleton of numerous compounds possessing biological activities such as cardiovascular, antibacterial, antiviral, analgesic, antifungal, etc.[163] In 2011, Abe *et al*.[164] isolated a lipid extract from *Perna canaliculus* (New Zealand green-lipped mussel) displaying anti-inflammatory effects in animal models and in human controlled studies. This extract is constituted of furan fatty acids (F-acids), which exhibited potent anti-inflammatory activity (**Figure 3.21**).

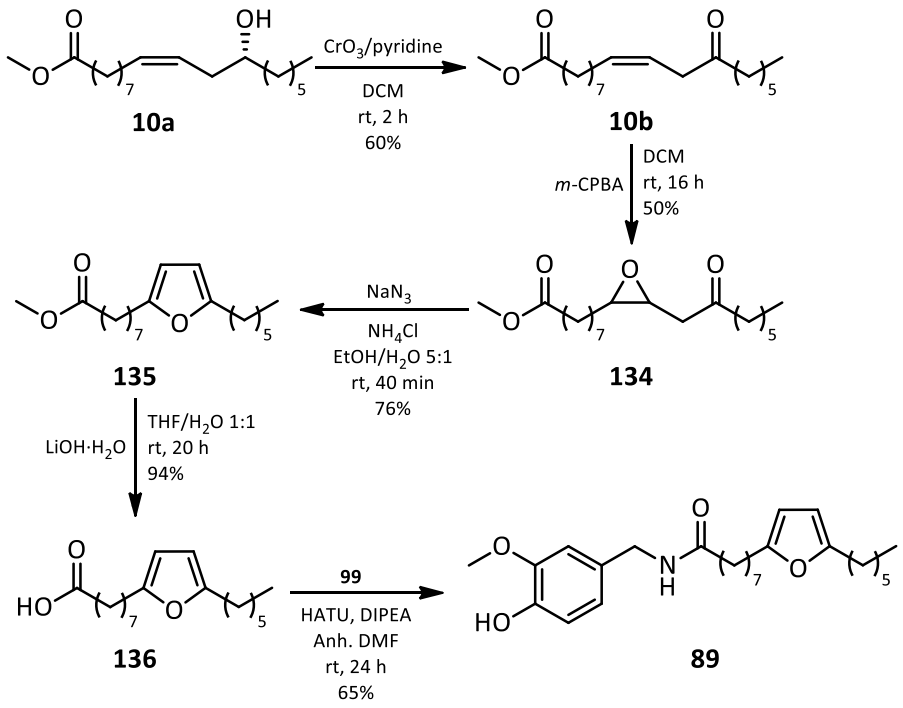


132: F_4

133: F_6

Figure 3.21. Structures of the representative furan fatty acids detected in the green-lipped mussel.

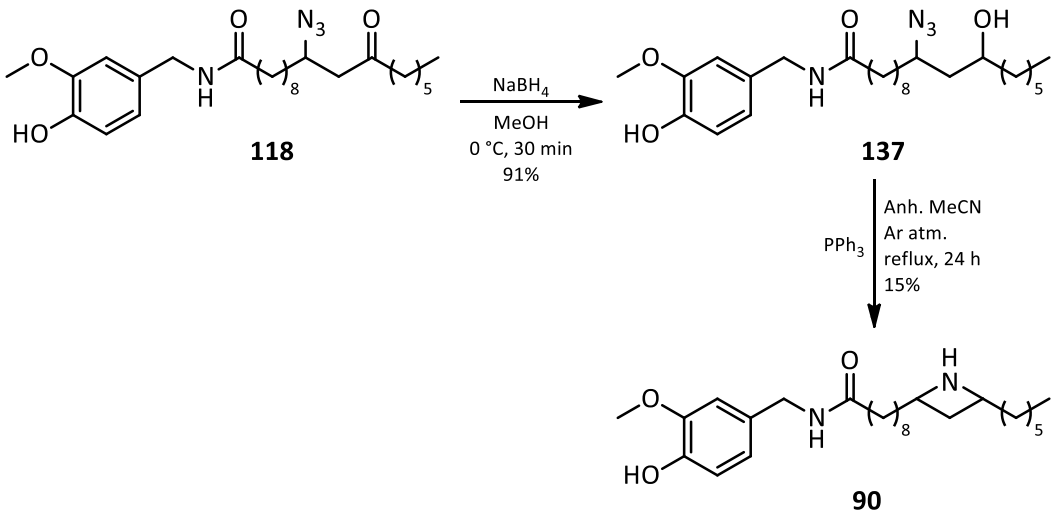
Furan amide **89** was synthetized using the methodology previously described by Lie Ken Jie e*t al.*[165] The general synthetic method involved the oxidation of the methyl ricinoleate **10a** to the β,ϒ-unsaturated ketone **10b** using CrO_3 (6 equiv.) and pyridine (12 equiv.). After the purification of this compound, the double bond was epoxidated with *m*-CPBA (1.8 equiv.) obtaining the β-epoxyketone **134** (50% yield). The treatment of compound **134** with NaN_3 (3.1 equiv.) and NH_4Cl (3.1 equiv.) gave the methyl ester **135** (76% yield). Posterior alkaline hydrolysis followed by amidation with vanillyl amine **99** led to the 8-(5-hexylfuran-2-yl)-*N*-(4-hydroxy-3-methoxybenzyl)octanamide **89** (65%) (**Scheme 3.23**).



Scheme 3.23. Synthetic route to compound **89**.

- *9-(4-Hexylazetidin-2-yl)-N-(4-hydroxy-3-methoxybenzyl)nonanamide*

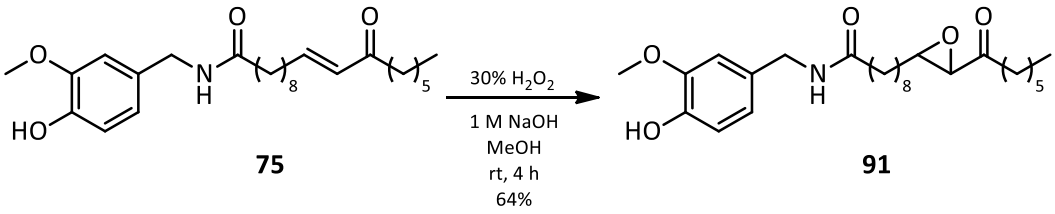
Azetidine-based ring systems have had enormous application in medicinal chemistry in the form of β-lactams.[166,167] The use of the fully reduced form of this four-membered heterocycle in the context of drug discovery has been less common. This can be attributed in part to the apparent difficulty in accessing azetidines in their enantioenriched form. Nevertheless, the synthesis of spirocyclic azetidines for their potential use in drug discovery has recently been described.[168-170] To introduce the azetidine ring in the alkyl chain of the *N*-vanillyl amide, we started using the reaction conditions described by Lie Ken Jie e*t al*.[171] 10-Azido-*N*-(4-hydroxy-3-methoxybenzyl)-12-oxooctadecanamide **118** was reduced with $NaBH_4$ (5 equiv.) to the corresponding alcohol **137**, which led to the 9-(4-hexylazetidin-2-yl)-*N*-(4-hydroxy-3-methoxybenzyl)nonanamide **90** (15% yield) by treatment with PPh_3 (1 equiv.) (**Scheme 3.24**).



Scheme 3.24. Synthesis of compound **90**.

- *9-(3-Heptanoyloxiran-2-yl)-N-(4-hydroxy-3-methoxybenzyl)nonanamide*

Epoxyketooctadecenoic acids (EKODEs) are a class of linoleic acid oxidation products that are formed *in vivo* and *in vitro* by a free radical mechanism initiated by either enzymatic or nonenzymatic pathways. There is major interest in these compounds owing to their highly potent biological activities and their ability to covalently modify proteins. Various isomers of α,β-unsaturated epoxyketooctadecenoic acid (EKODE)[172-174] have pronounced biological activity in stimulating the production and secretion of steroid hormone [175] and in increasing cellular calcium.[176] In view of these facts, we decided to introduce an epoxide ring and synthesize an *N*-vanillyl amide based on an EKODE to improve the structure-activity knowledge on these highly potent bioactive molecules. The epoxidation of (*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-12-oxooctadec-10-enamide **75** was performed using 30% H_2O_2 (5.6 equiv.) and 1 M NaOH (1 equiv.). After the purification by liquid column chromatography the α,β-epoxyketone **91** was obtained (65% yield) (**Scheme 3.25**).



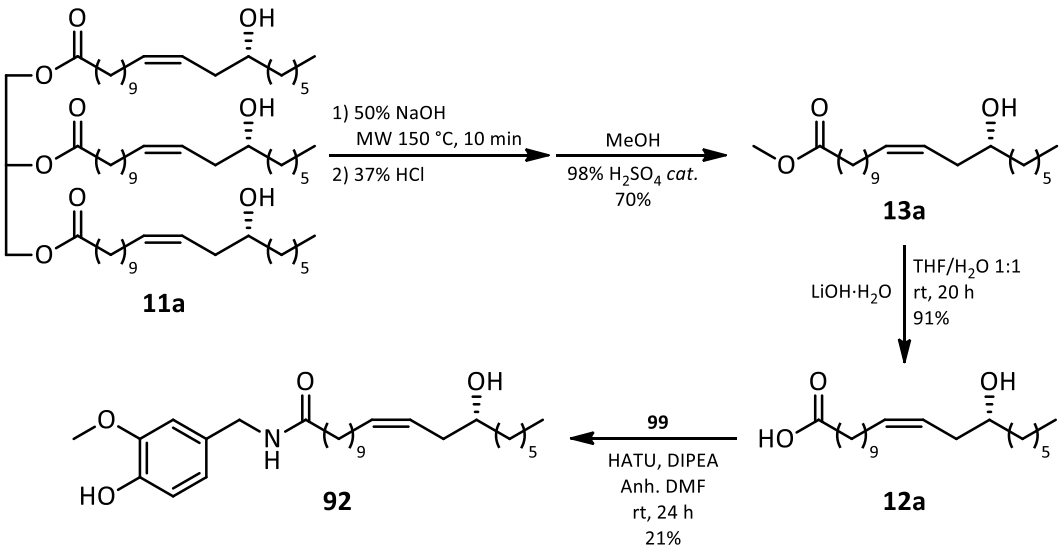
Scheme 3.25. Synthesis of compound **91**.

3.5.4. Synthesis of *N*-vanillyl acylamides of lesquerolic acid-related compounds

3.5.4.1. Modifications at the homoallyl hydroxy group

- *(R,Z)-14-Hydroxy-N-(4-hydroxy-3-methoxybenzyl)eicos-11-enamide*

The first compound synthetized was the *N*-vanillyl amide of lesquerolic acid. Thus, firstly we had to purify the lesquerolic acid **12a** in order to using it for the further syntheses. Lesquerella oil **11a** was hydrolysed in basic media under microwave irradiation followed by the esterification with methanol and 98% H_2SO_4 to achieve methyl lesquerolate **13a** after purification by liquid column chromatography (70%). The compound **13a** was hydrolysed using lithium hydroxide and the pure lesquerolic acid **12a** was achieved. This acid was treated with HATU and vanillyl amine **99** to yield the (*R,Z*)-14-hydroxy-*N*-(4-hydroxy-3-methoxybenzyl)eicos-11-enamide **92** (21% yield) (**Scheme 3.26**).

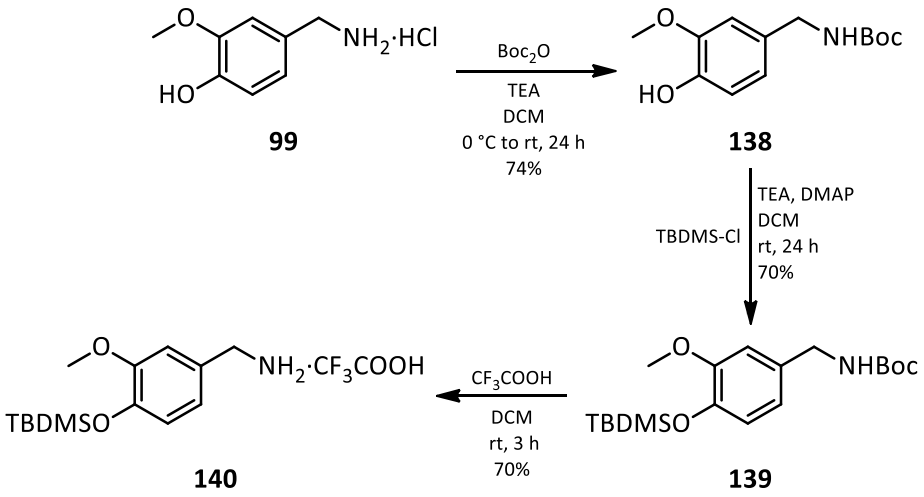


Scheme 3.26. Synthesis of compound **92**.

- *Acyllesquevanil derivatives*

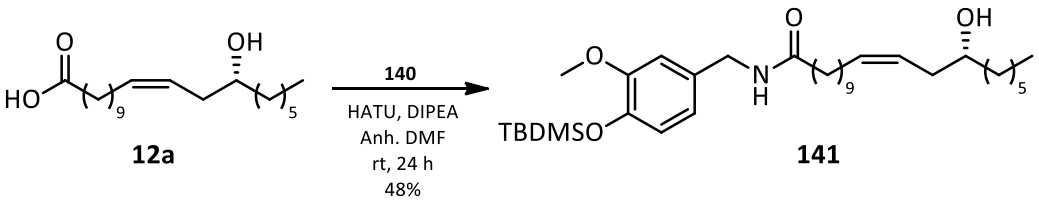
As already mentioned above, Appendino *et al*.[93] discovered that rinvanil **54** exhibited less potency than olvanil **51** but its activity could be modulated by acetylation of the hydroxy group on the fatty acid moiety. Phenylacetylrinvanil **59** and benzoylrinvanil **64** exhibited significantly higher potency and efficacy than olvanil **51**. Additionally, benzoylrinvanil **64** was active as antagonist on FAAH enzyme. To investigate the possibility to identify new potent and selective modulators of TRPV1 channel and FAAH enzyme, we synthesized the benzoyl- and phenylacetyl- lesquerolic analogues (**93** and **94**). The synthesis of these analogues was performed using the HATU-mediated coupling conditions above described. In the case of the vanillyl amine **99**, two orthogonal protecting

groups were used. First, the amine group of the vanillyl amine hydrochloride **99** was protected as a carbamate **138** using di-*tert*-butyl dicarbonate (1 equiv.). Then, *tert*-butyldimethylsilyl chloride (1.5 equiv.) was used to protect the phenolic group to give the silyl ether **139**. The carbamate group was removed using trifluoroactic acid yielding the vanillyl amino trifluoroacetate salt **140** (**Scheme 3.27**).



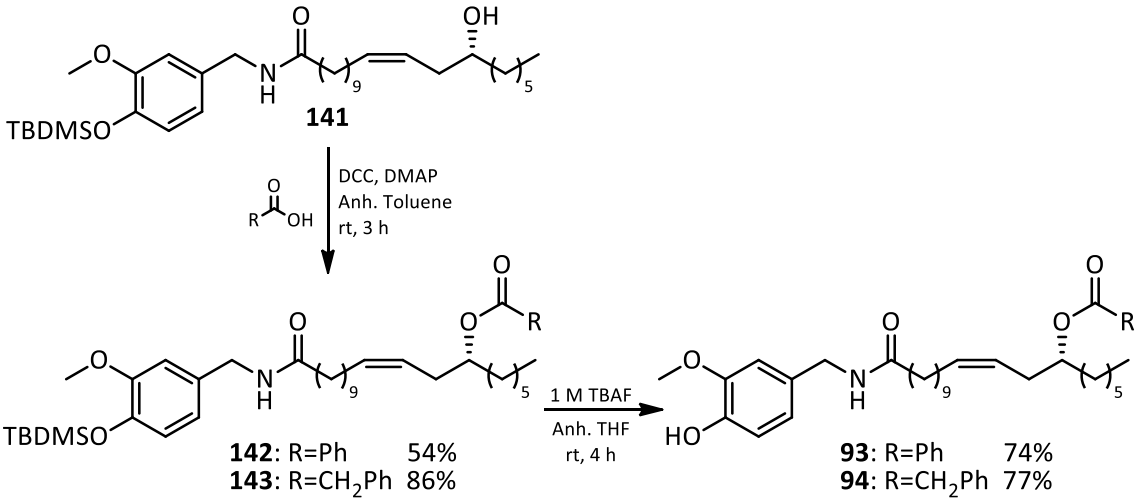
Scheme 3.27. Synthetic route to protect the phenolic group of **99**.

Coupling of the lesquerolic acid **12a** with protected vanillyl amine **140**, again with HATU, allowed the recovery of amide **141** after purification by liquid column chromatography (48% yield) (**Scheme 3.28**).



Scheme 3.28. Synthesis of the protected amide **141**.

The hydroxy group of the side chain of compound **141** was esterificated with benzoic acid (1.5 equiv.) **142** or phenylacetic acid (1.5 equiv.) **143** under Steglich conditions using DCC as coupling agent (2.4 equiv.) and DMAP as acyl-transfer agent (1.5 equiv.).[177] The corresponding esters **142** and **143** were isolated (54 and 86%, respectively) after liquid column chromatography. Finally, both compounds **142** and **143** were deprotected by treatment with TBAF (2 equiv.) at room temperature yielding the *N*-vanillyl acylamides **93** and **94** (74 and 77% yield, respectively) (**Scheme 3.29**).

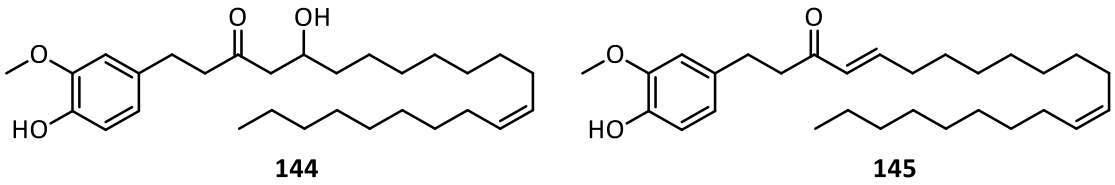


Scheme 3.29. Synthesis of acyllesquevanils **93** and **94**.

3.5.4.2. Oxidized derivatives

- *(E)-N-(4-Hydroxy-3-methoxybenzyl)-14-oxoeicos-12-enamide*

In 2007, Morita *et al*.[178] synthetized a two new shogaol derivatives containing an oleyl moiety. These compounds were the oleylgingerol **144** and oleylshogaol **145** (**Figure 3.22**). They concluded that the EC_{50} values of gingerol-related compounds decreased with increases in the carbon chain length. However, in the case of shogaols was determined the opposite. To explain these results, they established the position of the hydroxy group may have an important role in activating the ion channel.

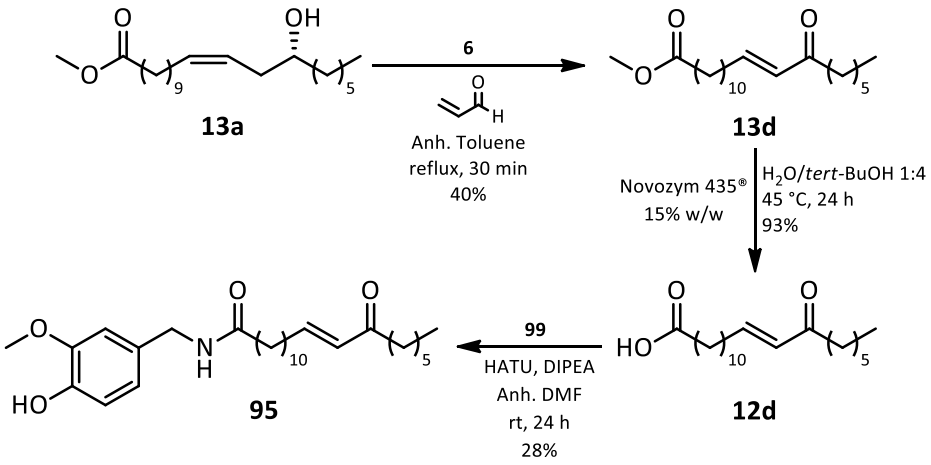


144 **145**

Figure 3.22. Structures of oleylgingerol **144** and oleylshogaol **145**.

Thus, we proposed that the position of the α,β-unsaturated carbonyl moiety may have influence in the agonist behaviour. For that reason, we decided to synthetize the *N*-vanillyl acylamide of the enone of lesquerolic acid **95** to compare it with the enone of ricinoleic acid **1d**. As it was described in **Chapter 1**, the synthesis of the α,β-unsaturated carbonyl compound from methyl lesquerolate **13a** was carried out through an hydrogen transfer reaction catalyzed by ruthenium. Methyl lesquerolate **13a** was treated with Shvo's catalyst **6** (0.5%) and acrolein (3 equiv.) as hydrogen acceptor, after 45 min under reflux of toluene and followed by purification by liquid

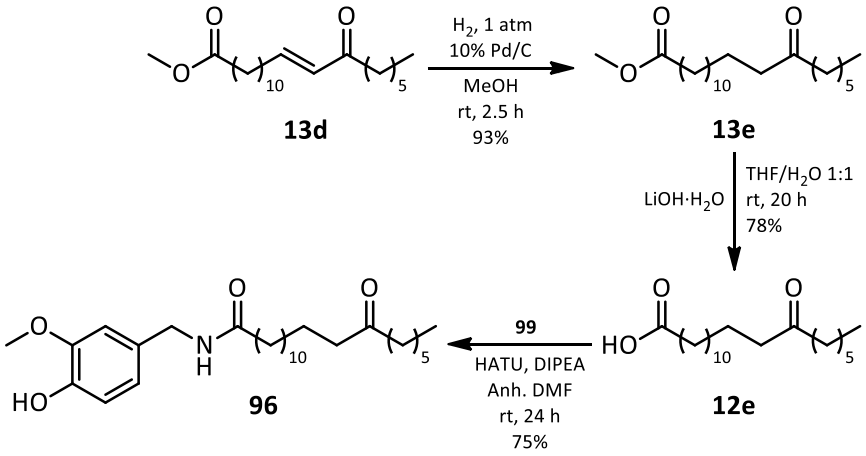
column chromatography, methyl (*E*)-14-oxoeicos-12-enoate **13d** was obtained (40% yield). The enzymatically hydrolysis of compound **13d** gave the corresponding carboxylic acid **12d**, which was directly amidated, under HATU conditions, with vanillyl amine **99** to obtain the amide **95** (28% yield) (**Scheme 3.30**).



Scheme 3.30. Synthesis of compound **95**.

- *N-(4-Hydroxy-3-methoxybenzyl)-14-oxoeicosanamide*

Vanilloid activity of linear fatty acids peaks at C-9 and then fades with a further increase of the chain length.[131] However, the introduction of polar groups (hydroxy, ketones, etc.) in the saturated side-chain contributes to fully restore of activity.[92] With the aim of dissecting the polar contributions to the activity saturated fatty acid amides, we synthetized the saturated ketone *N*-vanillylacyl amide **96**. It was synthetized by the hydrogenation of the compound **13d** using 10% loading Pd/C under atmospheric pressure. Methyl 14-oxoeicosanoate **13e** was obtained (93% yield) and was used in the next step without any purification. The alkaline hydrolysis of the methyl ester **13e** gave the 14-oxoeicosanoic acid **12e** (78% yield), which was treated with vanillyl amide **99** and HATU to obtain the *N*-(4-hydroxy-3-methoxybenzyl)-14-oxoicosanamide **96** (75% yield) (**Scheme 3.31**).



Scheme 3.31. Synthesis of the saturated *N*-vanillyl acylamide **96**.

3.6. CONCLUSIONS

In this chapter we focused our attention on the synthesis of a series of new long chain *N*-vanillyl acylamides (LCNVAs) starting from homoallyl hydroxy fatty acids as ricinoleic and lesquerolic acid. We performed structural transformations over the homoallyl hydroxy moiety. As a result, structural variants of the hydrophobic side chain of the capsaicin molecule were incorporated into a series of vanillylamides. A total of 22 new molecules were synthetized, which biological response in pain receptors was evaluated. The corresponding biological results are explained in detail in **Chapter 4**.

3.7. REFERENCES

(1) Breivik, H.; Collett, B.; Ventafridda, V.; Cohen, R.; Gallacher, D. *Eur. J. Pain* **2006**, *10*, 287-287.

(2) Tracey, I.; Bushnell, M. C. *J. Pain* **2009**, *10*, 1113-1120.

(3) Smith, B. H.; Torrance, N. *Curr. Opin. Support Palliat. Care* **2011**, *5*, 137-142.

(4) Belmonte, C.; Cervero, F. *Neurobiology of Nociceptors*; Belmonte, C., Cervero, F., Eds.; Oxford University Press: Oxford, 1996.

(5) Venkatachalam, K.; Montell, C. *Annu. Rev. Biochem.* **2007**, *76*, 387-417.

(6) Latorre, R.; Zaelzer, C.; Brauchi, S. *Q. Rev. Biophys.* **2009**, *42*, 201-246.

(7) Caterina, M. J.; Schumacher, M. A.; Tominaga, M.; Rosen, T. A.; Levine, J. D.; Julius, D. *Nature* **1997**, *389*, 816-824.

(8) Caterina, M. J.; Rosen, T. A.; Tominaga, M.; Brake, A. J.; Julius, D. *Nature* **1999**, *398*, 436-441.

(9) Smith, G. D.; Gunthorpe, M. J.; Kelsell, R. E.; Hayes, P. D.; Reilly, P.; Facer, P.; Wright, J. E.; Jerman, J. C.; Walhin, J. P.; Ooi, L.; Egerton, J.; Charles, K. J.; Smart, D.; Randall, A. D.; Anand, P.; Davis, J. B. *Nature* **2002**, *418*, 186-190.

(10) Xu, H.; Ramsey, I. S.; Kotecha, S. A.; Moran, M. M.; Chong, J. A.; Lawson, D.; Ge, P.; Lilly, J.; Silos-Santiago, I.; Xie, Y.; DiStefano, P. S.; Curtis, R.; Clapham, D. E. *Nature* **2002**, *418*, 181-186.

(11) Guler, A. D.; Lee, H.; Iida, T.; Shimizu, I.; Tominaga, M.; Caterina, M. *J. Neurosci.* **2002**, *22*, 6408-6414.

(12) McKemy, D. D.; Neuhausser, W. M.; Julius, D. *Nature* **2002**, *416*, 52-58.

(13) Peier, A. M.; Moqrich, A.; Hergarden, A. C.; Reeve, A. J.; Andersson, D. A.; Story, G. M.; Earley, T. J.; Dragoni, I.; McIntyre, P.; Bevan, S.; Patapoutian, A. *Cell* **2002**, *108*, 705-715.

(14) Story, G. M.; Peier, A. M.; Reeve, A. J.; Eid, S. R.; Mosbacher, J.; Hricik, T. R.; Earley, T. J.; Hergarden, A. C.; Andersson, D. A.; Hwang, S. W.; McIntyre, P.; Jegla, T.; Bevan, S.; Patapoutian, A. *Cell* **2003**, *112*, 819-829.

(15) Koeppen, B. M.; Stanton, B. A. *Berne & Levy Physiology, Updated Edition*; Koeppen, B.M., Stanton, B.A., Eds.; Mosby Elsevier: Philadelphia, 2009.

(16) Smith, P. L.; Maloney, K. N.; Pothen, R. G.; Clardy, J.; Clapham, D. E. *J. Biol. Chem.* **2006**, *281*, 29897-29904.

(17) Vay, L.; Gu, C.; McNaughton, P. A. *Br. J. Pharmacol.* **2012**, *165*, 787-801.

(18) Van der Stelt, M.; Di Marzo, V. *Eur. J. Biochem.* **2004**, *271*, 1827-1834.

(19) Szolcsányi, J. *Neuropeptides* **2004**, *38*, 377-384.

(20) Otrubova, K.; Ezzili, C.; Boger, D. L. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4674-4685.

(21) Petrosino, S.; Di Marzo, V. *Curr. Opin. Investig. Drugs* **2010**, *11*, 51-62.

(22) Nilius, B.; Vennekens, R.; Owsianik, G. *Curr. Pharm. Des.* **2008**, *14*, 18-31.

(23) Rosenbaum, T.; Gordon-Shaag, A.; Munari, M.; Gordon, S. E. *J. Gen. Physiol.* **2004**, *123*, 53-62.

(24) Belmonte, C.; Viana, F. *Mol. Pain* **2008**, *4*, 14-14.

(25) Lishko, P. V.; Procko, E.; Jin, X.; Phelps, C. B.; Gaudet, R. *Neuron* **2007**, *54*, 905-918.

(26) Moiseenkova-Bell, V. Y.; Stanciu, L. A.; Serysheva, I. I.; Tobe, B. J.; Wensel, T. G. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 7451-7455.

(27) Gao, Y.; Cao, E.; Julius, D.; Cheng, Y. *Nature* **2016**, *534*, 347-351.

(28) Liao, M.; Cao, E.; Julius, D.; Cheng, Y. *Nature* **2013**, *504*, 107-112.

(29) Bhave, G.; Zhu, W.; Wang, H.; Brasier, D. J.; Oxford, G. S.; Gereau, R. W. t. *Neuron* **2002**, *35*, 721-731.

(30) Jung, J.; Shin, J. S.; Lee, S.-Y.; Hwang, S. W.; Koo, J.; Cho, H.; Oh, U. *J. Biol. Chem.* **2004**, *279*, 7048-7054.

(31) Woo, D. H.; Jung, S. J.; Zhu, M. H.; Park, C. K.; Kim, Y. H.; Oh, S. B.; Lee, C. J. *Mol. Pain* **2008**, *4*, 1744-8069.

(32) Rathee, P. K.; Distler, C.; Obreja, O.; Neuhuber, W.; Wang, G. K.; Wang, S. Y.; Nau, C.; Kress, M. *J. Neurosci.* **2002**, *22*, 4740-4745.

(33) Lee, J.; Chung, M. K.; Ro, J. Y. *Biochem. Biophys. Res. Commun.* **2012**, *424*, 358-363.

(34) Szallasi, A.; Cortright, D. N.; Blum, C. A.; Eid, S. R. *Nat. Rev. Drug Discov.* **2007**, *6*, 357-372.

(35) Ahern, G. P. *J. Biol. Chem.* **2003**, *278*, 30429-30434.

(36) Wang, X.; Miyares, R. L.; Ahern, G. P. *J. Physiol.* **2005**, *564*, 541-547.

(37) Price, T. J.; Patwardhan, A.; Akopian, A. N.; Hargreaves, K. M.; Flores, C. M. *Br. J. Pharmacol.* **2004**, *141*, 1118-1130.

(38) Maione, S.; De Petrocellis, L.; de Novellis, V.; Moriello, A. S.; Petrosino, S.; Palazzo, E.; Rossi, F. S.; Woodward, D. F.; Di Marzo, V. *Br. J. Pharmacol.* **2007**, *150*, 766-781.

(39) Appendino, G.; Minassi, A.; Pagani, A.; Ech-Chahad, A. *Curr. Pharm. Des.* **2008**, *14*, 2-17.

(40) Hwang, S. W.; Cho, H.; Kwak, J.; Lee, S.-Y.; Kang, C.-J.; Jung, J.; Cho, S.; Min, K. H.; Suh, Y.-G.; Kim, D.; Oh, U. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6155-6160.

(41) 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. U.S.A.* **2002**, *99*, 8400-8405.

(42) Ahern, G. P.; Wang, X.; Miyares, R. L. *J. Biol. Chem.* **2006**, *281*, 8991-8995.

(43) Vriens, J.; Nilius, B.; Vennekens, R. *Curr. Neuropharmacol.* **2008**, *6*, 79-96.

(44) Vyklický, L.; Nováková-Tousová, K.; Benedikt, J.; Samad, A.; Touska, F.; Vlachová, V. *Physiol. Res.* **2008**, *57*, S59.

(45) Rohacs, T. *Pflugers Arch.* **2015**, *467*, 1851-1869.

(46) Sanz-Salvador, L.; Andres-Borderia, A.; Ferrer-Montiel, A.; Planells-Cases, R. *J. Biol. Chem.* **2012**, *287*, 19462-19471.

(47) Cao, E.; Cordero-Morales, J. F.; Liu, B.; Qin, F.; Julius, D. *Neuron* **2013**, *77*, 667-679.

(48) Mohapatra, D. P.; Nau, C. *J. Biol. Chem.* **2005**, *280*, 13424-13432.

(49) Mohapatra, D. P.; Nau, C. *J. Biol. Chem.* **2003**, *278*, 50080-50090.

(50) Piper, A. S.; Yeats, J. C.; Bevan, S.; Docherty, R. J. *J. Physiol.* **1999**, *518*, 721-733.

(51) Gunthorpe, M. J.; Harries, M. H.; Prinjha, R. K.; Davis, J. B.; Randall, A. *J. Physiol.* **2000**, *525*, 747-759.

(52) Szallasi, A.; Blumberg, P. M. *Brain Res.* **1990**, *524*, 106-111.

(53) Walpole, C. S. J.; Wrigglesworth, R.; Bevan, S.; Campbell, E. A.; Dray, A.; James, I. F.; Perkins, M. N.; Reid, D. J.; Winter, J. *J. Med. Chem.* **1993**, *36*, 2362-2372.

(54) Walpole, C. S. J.; Wrigglesworth, R.; Bevan, S.; Campbell, E. A.; Dray, A.; James, I. F.; Masdin, K. J.; Perkins, M. N.; Winter, J. *J. Med. Chem.* **1993**, *36*, 2373-2380.

(55) Walpole, C. S. J.; Wrigglesworth, R.; Bevan, S.; Campbell, E. A.; Dray, A.; James, I. F.; Masdin, K. J.; Perkins, M. N.; Winter, J. *J. Med. Chem.* **1993**, *36*, 2381-2389.

(56) Gavva, N. R.; Klionsky, L.; Qu, Y.; Shi, L.; Tamir, R.; Edenson, S.; Zhang, T. J.; Viswanadhan, V. N.; Toth, A.; Pearce, L. V.; Vanderah, T. W.; Porreca, F.; Blumberg, P. M.; Lile, J.; Sun, Y.; Wild, K.; Louis, J. C.; Treanor, J. J. *J. Biol. Chem.* **2004**, *279*, 20283-20295.

(57) Yang, F.; Xiao, X.; Cheng, W.; Yang, W.; Yu, P.; Song, Z.; Yarov-Yarovoy, V.; Zheng, J. *Nat. Chem. Biol.* **2015**, *11*, 518-524.

(58) Jordt, S. E.; Julius, D. *Cell* **2002**, *108*, 421-430.

(59) Darré, L.; Domene, C. *Mol. Pharm.* **2015**, *12*, 4454-4465.

(60) Hergenhahn, M.; Kusumoto, S.; Hecker, E. *Experientia* **1974**, *30*, 1438-1440.

(61) Appendino, G.; Szallasi, A. *Life Sci.* **1997**, *60*, 681-696.

(62) Szallasi, A.; Blumberg, P. M. *Neuroscience* **1989**, *30*, 515-520.

(63) Szallasi, A. *Drugs Aging* **2001**, *18*, 561-573.

(64) Elokely, K.; Velisetty, P.; Delemotte, L.; Palovcak, E.; Klein, M. L.; Rohacs, T.; Carnevale, V. *Proc. Natl. Acad. Sci. U.S.A.* **2016**, *113*, E137-E145.

(65) Kosuge, S.; Inagaki, Y.; Okumura, H. *Nippon Nogei Kagaku Kaishi* **1961**, *35*, 923-927.

(66) Thresh, L. *Pharm. J.* **1846**, *6*, 941.

(67) Nelson, E. K. *J. Am. Chem. Soc.* **1919**, *41*, 1115-1121.

(68) Nelson, E. K. *J. Am. Chem. Soc.* **1919**, *41*, 2121-2130.

(69) Nelson, E. K.; Dawson, L. E. *J. Am. Chem. Soc.* **1923**, *45*, 2179-2181.

(70) Blum, E.; Mazourek, M.; O'connell, M.; Curry, J.; Thorup, T.; Liu, K.; Jahn, M.; Paran, I. *Theor. Appl. Genet.* **2003**, *108*, 79-86.

(71) Contreras-Padilla, M.; Yahia, E. M. *J. Agric. Food Chem.* **1998**, *46*, 2075-2079.

(72) Reyes-Escogido, M. d. L.; Gonzalez-Mondragon, E. G.; Vazquez-Tzompantzi, E. *Molecules* **2011**, *16*, 1253-1270.

(73) Wrigglesworth, R.; Walpole, C. S. *Drugs Future* **1998**, *23*, 531–538.

(74) Walpole, C. S. J.; Bevan, S.; Bloomfield, G.; Breckenridge, R.; James, I. F.; Ritchie, T.; Szallasi, A.; Winter, J.; Wrigglesworth, R. *J. Med. Chem.* **1996**, *39*, 2939-2952.

(75) Lee, J.; Lee, J.; Kim, J.; Kim, S. Y.; Chun, M. W.; Cho, H.; Hwang, S. W.; Oh, U.; Park, Y. H.; Marquez, V. E.; Beheshti, M.; Szabo, T.; Blumberg, P. M. *Bioorg. Med. Chem.* **2001**, *9*, 19-32.

(76) Park, N. S.; Seong, C. M.; Jung, Y. S.; Kim, W. B.; Kim, S. H. *Drugs Future* **2000**, *25*, 1131-1137.

(77) Urban, L.; Campbell, E. A.; Panesar, M.; Patel, S.; Chaudhry, N.; Kane, S.; Buchheit, K.-H.; Sandells, B.; James, I. F. *PAIN* **2000**, *89*, 65-74.

(78) Watanabe, T.; Kawada, T.; Kato, T.; Harada, T.; Iwai, K. *Life Sci.* **1994**, *54*, 369-374.

(79) Todd, P. H.; Bensinger, M. G.; Biftu, T. *J. Food Sci.* **1977**, *42*, 660-665.

(80) Szallasi, A.; Di Marzo, V. *Trends Neurosci.* **2000**, *23*, 491-497.

(81) Kim, K. M.; Kawada, T.; Ishihara, K.; Inoue, K.; Fushiki, T. *J. Nutr.* **1998**, *128*, 1978-1983.

(82) Melck, D.; Bisogno, T.; De Petrocellis, L.; Chuang, H.-H.; Julius, D.; Bifulco, M.; Di Marzo, V. *Biochem. Biophys. Res. Commun.* **1999**, *262*, 275-284.

(83) Di Marzo, V.; Lastres-Becker, I.; Bisogno, T.; De Petrocellis, L.; Milone, A.; Davis, J. B.; Fernandez-Ruiz, J. J. *Eur. J. Pharmacol.* **2001**, *420*, 123-131.

(84) Brand, L.; Berman, E.; Schwen, R.; Loomans, M.; Janusz, J.; Bohne, R.; Maddin, C.; Gardner, J.; Lahann, T.; Farmer, R.; et al. *Drugs Exp. Clin. Res.* **1987**, *13*, 259-265.

(85) Sietsema, W. K.; Berman, E. F.; Farmer, R. W.; Maddin, C. S. *Life Sci.* **1988**, *43*, 1385-1391.

(86) Watanabe, T.; Sakurada, N.; Kobata, K. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 2443-2447.

(87) Ralevic, V.; Jerman, J. C.; Brough, S. J.; Davis, J. B.; Egerton, J.; Smart, D. *Biochem. Pharmacol.* **2003**, *65*, 143-151.

(88) Iida, T.; Moriyama, T.; Kobata, K.; Morita, A.; Murayama, N.; Hashizume, S.; Fushiki, T.; Yazawa, S.; Watanabe, T.; Tominaga, M. *Neuropharmacology* **2003**, *44*, 958-967.

(89) Sharkey, K. A.; Cristino, L.; Oland, L. D.; Van Sickle, M. D.; Starowicz, K.; Pittman, Q. J.; Guglielmotti, V.; Davison, J. S.; Di Marzo, V. *Eur. J. Neurosci.* **2007**, *25*, 2773-2782.

(90) Tuoya; Baba, N.; Shimoishi, Y.; Murata, Y.; Tada, M.; Koseki, M.; Takahata, K. *Life Sci.* **2006**, *78*, 1515-1519.

(91) De Petrocellis, L.; Guida, F.; Moriello, A. S.; De Chiaro, M.; Piscitelli, F.; de Novellis, V.; Maione, S.; Di Marzo, V. *Pharmacol. Res.* **2011**, *63*, 294-299.

(92) Appendino, G.; Minassi, A.; Morello, A. S.; De Petrocellis, L.; Di Marzo, V. *J. Med. Chem.* **2002**, *45*, 3739-3745.

(93) Appendino, G.; De Petrocellis, L.; Trevisani, M.; Minassi, A.; Daddario, N.; Moriello, A. S.; Gazzieri, D.; Ligresti, A.; Campi, B.; Fontana, G.; Pinna, C.; Geppetti, P.; Di Marzo, V. *J. Pharmacol. Exp. Ther.* **2005**, *312*, 561-570.

(94) Thudichum, J. L. W. *A Treatise on the Chemical Constitution of the Brain*; Thudichum, J.L.W., Ed.; Archon Books: Connecticut, 1962.

(95) Levene, P.; West, C. *J. Biol. Chem.* **1916**, *24*, 111-116.

(96) Kuehl, F. A.; Jacob, T. A.; Ganley, O. H.; Ormond, R. E.; Meisinger, M. A. P. *J. Am. Chem. Soc.* **1957**, *79*, 5577-5578.

(97) Devane, W.; Hanus, L.; Breuer, A.; Pertwee, R.; Stevenson, L.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. *Science* **1992**, *258*, 1946-1949.

(98) Mechoulam, R.; Fride, E.; Di Marzo, V. *Eur. J. Pharmacol.* **1998**, *359*, 1-18.

(99) Kaczocha, M.; Hermann, A.; Glaser, S. T.; Bojesen, I. N.; Deutsch, D. G. *J. Biol. Chem.* **2006**, *281*, 9066-9075.

(100) Hillard, C. J.; Edgemond, W. S.; Jarrahian, A.; Campbell, W. B. *J. Neurochem.* **1997**, *69*, 631-638.

(101) McFarland, M. J.; Porter, A. C.; Rakhshan, F. R.; Rawat, D. S.; Gibbs, R. A.; Barker, E. L. *J. Biol. Chem.* **2004**, *279*, 41991-41997.

(102) Beltramo, M.; Stella, N.; Calignano, A.; Lin, S. Y.; Makriyannis, A.; Piomelli, D. *Science* **1997**, *277*, 1094-1097.

(103) Ligresti, A.; Morera, E.; Van Der Stelt, M.; Monory, K.; Lutz, B.; Ortar, G.; Di Marzo, V. *Biochem. J.* **2004**, *380*, 265-272.

(104) Hillard, C. J.; Jarrahian, A. *Br. J. Pharmacol.* **2003**, *140*, 802-808.

(105) Cravatt, B. F.; Giang, D. K.; Mayfield, S. P.; Boger, D. L.; Lerner, R. A.; Gilula, N. B. *Nature* **1996**, *384*, 83-87.

(106) Ahn, K.; McKinney, M. K.; Cravatt, B. F. *Chem. Rev.* **2008**, *108*, 1687-1707.

(107) Bracey, M. H.; Hanson, M. A.; Masuda, K. R.; Stevens, R. C.; Cravatt, B. F. *Science* **2002**, *298*, 1793-1796.

(108) Patricelli, M. P.; Lovato, M. A.; Cravatt, B. F. *Biochemistry* **1999**, *38*, 9804-9812.

(109) Patricelli, M. P.; Cravatt, B. F. *Biochemistry* **2001**, *40*, 6107-6115.

(110) Palermo, G.; Bauer, I.; Campomanes, P.; Cavalli, A.; Armirotti, A.; Girotto, S.; Rothlisberger, U.; De Vivo, M. *PLoS Comput. Biol.* **2015**, *11*, e1004231.

(111) McKinney, M. K.; Cravatt, B. F. *Annu. Rev. Biochem.* **2005**, *74*, 411-432.

(112) Mileni, M.; Kamtekar, S.; Wood, D. C.; Benson, T. E.; Cravatt, B. F.; Stevens, R. C. *J. Mol. Biol.* **2010**, *400*, 743-754.

(113) Lambert, D. M.; Fowler, C. J. *J. Med. Chem.* **2005**, *48*, 5059-5087.

(114) Seierstad, M.; Breitenbucher, J. G. *J. Med. Chem.* **2008**, *51*, 7327-7343.

(115) Cravatt, B. F.; Lichtman, A. H. *Curr. Opin. Chem. Biol.* **2003**, *7*, 469-475.

(116) Pertwee, R. G.; Ross, R. A. *Prostaglandins Leukot. Essent. Fatty Acids* **2002**, *66*, 101-121.

(117) Kinsey, S. G.; Long, J. Z.; O'Neal, S. T.; Abdullah, R. A.; Poklis, J. L.; Boger, D. L.; Cravatt, B. F.; Lichtman, A. H. *J. Pharmacol. Exp. Ther.* **2009**, *330*, 902-910.

(118) Walker, J. M.; Huang, S. M.; Strangman, N. M.; Tsou, K.; Sañudo-Peña, M. C. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 12198-12203.

(119) Kathuria, S.; Gaetani, S.; Fegley, D.; Valino, F.; Duranti, A.; Tontini, A.; Mor, M.; Tarzia, G.; Rana, G. L.; Calignano, A.; Giustino, A.; Tattoli, M.; Palmery, M.; Cuomo, V.; Piomelli, D. *Nat. Med.* **2003**, *9*, 76-81.

(120) Järvinen, T.; Pate, D. W.; Laine, K. *Pharmacol. Ther.* **2002**, *95*, 203-220.

(121) Di Marzo, V.; Goparaju, S. K.; Wang, L.; Liu, J.; Batkai, S.; Jarai, Z.; Fezza, F.; Miura, G. I.; Palmiter, R. D.; Sugiura, T.; Kunos, G. *Nature* **2001**, *410*, 822-825.

(122) Starowicz, K.; Przewlocka, B. *Phil. Trans. R. Soc. B* **2012**, *367*, 3286-3299.

(123) Schlosburg, J. E.; Carlson, B. L. A.; Ramesh, D.; Long, J. Z.; Cravatt, B. F.; Lichtman, A. H.; Abdullah, R. A. *AAPS J.* **2009**, *11*, 342-352.

(124) Bisogno, T.; Melck, D.; De Petrocellis, L.; Bobrov, M. Y.; Gretskaya, N. M.; Bezuglov, V. V.; Sitachitta, N.; Gerwick, W. H.; Marzo, V. D. *Biochem. Biophys. Res. Commun.* **1998**, *248*, 515-522.

(125) Di Marzo, V.; Bisogno, T.; De Petrocellis, L.; Melck, D.; Orlando, P.; Wagner, J. A.; Kunos, G. *Eur. J. Biochem.* **1999**, *264*, 258-267.

(126) Hillard, C. J.; Shi, L.; Tuniki, V. R.; Falck, J. R.; Campbell, W. B. *J. Mol. Neurosci.* **2007**, *33*, 18-24.

(127) Jarrahian, A.; Manna, S.; Edgemond, W. S.; Campbell, W. B.; Hillard, C. J. *J. Neurochem.* **2000**, *74*, 2597-2606.

(128) Di Marzo, V.; Griffin, G.; De Petrocellis, L.; Brandi, I.; Bisogno, T.; Williams, W.; Grier, M. C.; Kulasegram, S.; Mahadevan, A.; Razdan, R. K.; Martin, B. R. *J. Pharmacol. Exp. Ther.* **2002**, *300*, 984-991.

(129) Gannett, P. M.; Nagel, D. L.; Reilly, P. J.; Lawson, T.; Sharpe, J.; Toth, B. *J. Org. Chem.* **1988**, *53*, 1064-1071.

(130) Meyer, O.; Heddesheimer, I. Process for preparing vanillylamine hydrochloride. US2003065223 (A1). April 3, 2003.

(131) Janusz, J. M.; Buckwalter, B. L.; Young, P. A.; LaHann, T. R.; Farmer, R. W.; Kasting, G. B.; Loomans, M. E.; Kerckaert, G. A.; Maddin, C. S.; Berman, E. F.; et al. *J. Med. Chem.* **1993**, *36*, 2595-2604.

(132) Perreux, L.; Loupy, A.; Delmotte, M. *Tetrahedron* **2003**, *59*, 2185-2189.

(133) Kobata, K.; Yoshikawa, K.; Kohashi, M.; Watanabe, T. *Tetrahedron Lett.* **1996**, *37*, 2789-2790.

(134) Koreishi, M.; Zhang, D.; Imanaka, H.; Imamura, K.; Adachi, S.; Matsuno, R.; Nakanishi, K. *J. Agric. Food Chem.* **2006**, *54*, 72-78.

(135) Castillo, E.; Torres-Gavilán, A.; Severiano, P.; Arturo, N.; López-Munguía, A. *Food Chem.* **2007**, *100*, 1202-1208.

(136) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397-4398.

(137) Carpino, L. A.; El-Faham, A.; Albericio, F. *Tetrahedron Lett.* **1994**, *35*, 2279-2282.

(138) Suekawa, M.; Ishige, A.; Yuasa, K.; Sudo, K.; Aburada, M.; Hosoya, E. *J. Pharmacobiodyn.* **1984**, *7*, 836-848.

(139) Park, K.-K.; Chun, K.-S.; Lee, J.-M.; Lee, S. S.; Surh, Y.-J. *Cancer Lett.* **1998**, *129*, 139-144.

(140) Guh, J.-H.; Ko, F.-N.; Jong, T.-T.; Teng, C.-M. *J. Pharm. Pharmacol.* **1995**, *47*, 329-332.

(141) Aeschbach, R.; Löliger, J.; Scott, B. C.; Murcia, A.; Butler, J.; Halliwell, B.; Aruoma, O. I. *Food Chem. Toxicol.* **1994**, *32*, 31-36.

(142) Govindarajan, V. S. *CRC Crit. Rev. Food Sci. Nutr.* **1982**, *17*, 189-258.

(143) Govindarajan, V. S.; Connell, D. W. *CRC Crit. Rev. Food Sci. Nutr.* **1983**, *17*, 1-96.

(144) Iwasaki, Y.; Morita, A.; Iwasawa, T.; Kobata, K.; Sekiwa, Y.; Morimitsu, Y.; Kubota, K.; Watanabe, T. *Nutr. Neurosci.* **2006**, *9*, 169-178.

(145) Wong, G. Y.; Gavva, N. R. *Brain Res. Rev.* **2009**, *60*, 267-277.

(146) Thalmann, A.; Oertle, K.; Gerlach, H. *Org. Synth.* **2003**, *63*, 192-195.

(147) Witte, D. G.; Cassar, S. C.; Masters, J. N.; Esbenshade, T.; Hancock, A. A. *J. Biomol. Screen.* **2002**, *7*, 466-475.

(148) Dedov, V. N.; Tran, V. H.; Duke, C. C.; Connor, M.; Christie, M. J.; Mandadi, S.; Roufogalis, B. D. *Br. J. Pharmacol.* **2002**, *137*, 793-798.

(149) Ito, H.; Yamanaka, H.; Tateiwa, J.-i.; Hosomi, A. *Tetrahedron Lett.* **2000**, *41*, 6821-6825.

(150) Wabnitz, T. C.; Spencer, J. B. *Org. Lett.* **2003**, *5*, 2141-2144.

(151) Kolb, H. C.; Sharpless, K. B. *Drug Discov. Today* **2003**, *8*, 1128-1137.

(152) Brik, A.; Alexandratos, J.; Lin, Y.-C.; Elder, J. H.; Olson, A. J.; Wlodawer, A.; Goodsell, D. S.; Wong, C.-H. *Chembiochem* **2005**, *6*, 1167-1169.

(153) Appendino, G.; Bacchiega, S.; Minassi, A.; Cascio, M. G.; De Petrocellis, L.; Di Marzo, V. *Angew. Chem. Int. Ed.* **2007**, *46*, 9312-9315.

(154) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2001**, *40*, 2004-2021.

(155) Chen, H.; Lv, L.; Soroka, D.; Warin, R. F.; Parks, T. A.; Hu, Y.; Zhu, Y.; Chen, X.; Sang, S. *Drug Metab. Dispos.* **2012**, *40*, 742-753.

(156) Badawey, E. A. M.; El-Ashmawey, I. M. *Eur. J. Med. Chem.* **1998**, *33*, 349-361.

(157) Yale, H. L.; Losee, K.; Martins, J.; Holsing, M.; Perry, F. M.; Bernstein, J. *J. Am. Chem. Soc.* **1953**, *75*, 1933-1942.

(158) Yu, X.; Zhang, J. *Chem. Eur. J.* **2012**, *18*, 12945-12949.

(159) Palin, R.; Abernethy, L.; Ansari, N.; Cameron, K.; Clarkson, T.; Dempster, M.; Dunn, D.; Easson, A.-M.; Edwards, D.; Maclean, J.; Everett, K.; Feilden, H.; Ho, K.-K.; Kultgen, S.; Littlewood, P.; McArthur, D.; McGregor, D.; McLuskey, H.; Neagu, I.; Neale, S.; Nisbet, L.-A.; Ohlmeyer, M.; Pham, Q.; Ratcliffe, P.; Rong, Y.; Roughton, A.; Sammons, M.; Swanson, R.; Tracey, H.; Walker, G. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 892-898.

(160) Li, J. J. In *Name Reactions: A Collection of Detailed Reaction Mechanisms*; Springer, Ed.; Springer: Heidelberg, 2006, p 138-139.

(161) Tsuji, J.; Nagashima, H.; Hori, K. *Chem. Lett.* **1980**, *9*, 257-260.

(162) Allen, G.; Dwek, R. A. *J. Chem. Soc. B* **1966**, 161-163.

(163) Lukevits, É.; Demicheva, L. *Chem. Heterocycl. Compd.* **1993**, *29*, 243-267.

(164) Wakimoto, T.; Kondo, H.; Nii, H.; Kimura, K.; Egami, Y.; Oka, Y.; Yoshida, M.; Kida, E.; Ye, Y.; Akahoshi, S.; Asakawa, T.; Matsumura, K.; Ishida, H.; Nukaya, H.; Tsuji, K.; Kan, T.; Abe, I. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 17533-17537.

(165) Jie, M. S. F. L. K.; Syed-rahmatullah, M. S. K.; Wong, K. P. *Nat. Prod. Lett.* **1992**, *1*, 93-98.

(166) von Nussbaum, F.; Brands, M.; Hinzen, B.; Weigand, S.; Häbich, D. *Angew. Chem. Int. Ed.* **2006**, *45*, 5072-5129.

(167) Singh, G. S. *Mini Rev. Med. Chem.* **2004**, *4*, 69-92.

(168) Wuitschik, G.; Rogers-Evans, M.; Buckl, A.; Bernasconi, M.; Märki, M.; Godel, T.; Fischer, H.; Wagner, B.; Parrilla, I.; Schuler, F.; Schneider, J.; Alker, A.; Schweizer, W. B.; Müller, K.; Carreira, E. M. *Angew. Chem. Int. Ed.* **2008**, *47*, 4512-4515.

(169) Burkhard, J. A.; Wagner, B.; Fischer, H.; Schuler, F.; Müller, K.; Carreira, E. M. *Angew. Chem. Int. Ed.* **2010**, *49*, 3524-3527.

(170) Burkhard, J. A.; Guérot, C.; Knust, H.; Carreira, E. M. *Org. Lett.* **2012**, *14*, 66-69.

(171) Lie Ken Jie, M. S. F.; Syed-Rahmatullah, M. S. K. *J. Am. Oil Chem. Soc.* **1992**, *69*, 359-362.

(172) Gardner, H. W.; Kleiman, R.; Weisleder, D. *Lipids* **1974**, *9*, 696-706.

(173) Gardner, H. W.; Kleiman, R. *Biochim. Biophys. Acta, Lipids Lipid Metab.* **1981**, *665*, 113-125.

(174) Gardner, H. W.; Crawford, C. G. *Biochim. Biophys. Acta, Lipids Lipid Metab.* **1981**, *665*, 126-133.

(175) Goodfriend, T. L.; Ball, D. L.; Raff, H.; Bruder, E. D.; Gardner, H. W.; Spiteller, G. *Endocr. Res.* **2002**, *28*, 325-330.

(176) Payet, M. D.; Goodfriend, T. L.; Bilodeau, L.; Mackendale, C.; Chouinard, L.; Gallo-Payet, N. *Am. J. Physiol. Endocrinol. Metab.* **2006**, *291*, E1160-E1167.

(177) Neises, B.; Steglich, W. *Angew. Chem. Int. Ed.* **1978**, *17*, 522-524.

(178) Morita, A.; Iwasaki, Y.; Kobata, K.; Yokogoshi, H.; Watanabe, T. *Biosci. Biotechnol. Biochem.* **2007**, *71*, 2304-2307.

Chapter 4

Biological Evaluation of the LCNVAs

4.1. INTRODUCTION

As explained in **Chapter 3**, TRPV1 is an ion channel which, when is activated, leads to the gating of cations, including Ca^{2+}, thus generating changes in intracellular Ca^{2+} concentration. The use of Ca^{2+} fluorescent indicators is a tool for monitoring intracellular Ca^{2+} concentration when TRPV1 receptor is activated. Because of its advantages of information, throughput, sensitivity and temporal response described it was the assay chosen to evaluate the agonist behaviour of the new molecules described in **Chapter 3**.

4.2. INTRACELLULAR Ca^{2+} MEASUREMENT USING FLUORESCENT DYES

A multitude of Ca^{2+} signals, varying from nanoscopic, subcellular domains to whole-cell Ca^{2+} waves and lasting for microseconds to hours, are used by different types of cells.[1,2] The characteristics of these cellular Ca^{2+} signals depend on the expression of tissue specific Ca^{2+} transport systems.[3] The diversity of cellular Ca^{2+} signalling means that there is no single technique that can be used to monitor Ca^{2+} changes in all situations. However, fluorescent Ca^{2+} indicators and imaging provide the most versatile and widely used method for analyzing cellular Ca^{2+} responses. Since their introduction by Tsien and colleagues,[4] fluorescent Ca^{2+} indicators have underpinned the investigation of Ca^{2+} signalling in a host of different experimental settings. Using appropriate technology and suitable indicators, it is possible to monitor Ca^{2+} signals spanning from subcellular to multicellular, at high speed or time lapse, within living cells. Ca^{2+}-sensitive fluorescent indicators fall into one of two groups: dual-wavelength ratiometric indicators and single-wavelength non-ratiometric indicators. Single wavelength indicators exhibit significant Ca^{2+} dependent changes in fluorescence intensity without shifting their excitation or emission wavelengths.

Ca^{2+} indicator dyes are commercially available in three chemical forms: salts, dextran conjugates or acetoxymethyl (AM) esters.[5] Salts are the simplest form of Ca^{2+} indicators, but because of their hydrophilic nature, they are membrane impermeable and require invasive loading procedures. Dextran conjugates have high water solubility, low toxicity, and exhibit essentially no compartmentalization over very long recording periods up to days in length. Dextran conjugates are available for all of the most common and popular Ca^{2+} indicators including, Fluo-3, Fluo-4, Rhod-2, Fura-2, Calcium-Green 1, Indo-1 and Oregon Green 488 BAPTA-1. All incorporate a Ca^{2+}-chelating moiety, that usually presents 1,2-bis(2-amino-phenoxy)-ethane-*N*,*N*,*N*',*N*'-tetraacetic acid (BAPTA).[6] The affinity of the BAPTA moiety towards Ca^{2+} can be tuned by introduction of some groups (increased by electron substituents and decreased by electron withdrawing ones). The main

inconvenient of these dyes is that they are hydrophilic compounds, and therefore, they present difficulties to be loaded into cells. Because of this fact, Ca^{2+} indicator dyes were engineered with acetoxymethyl esters (AM) to offer a more convenient method for loading hydrophilic dyes into cells. AM dyes are sufficiently hydrophobic that they are membrane permeable and can be passively loaded into cells simply by adding them to the extracellular medium. Intracellular esterases then cleave the AM group and trap the dye inside cells. This method of dye loading also effectively concentrates Ca^{2+} indicators inside cells such that a bath concentration of 1–5 μM results in a cytosolic concentration greater than 100 μM. Within the latter group it should be highlighted Fluo-4-AM, which is essentially a brighter, more photostable derivative than its precursor Fluo-3-AM. Its Ca^{2+} affinity is a little lower (K_d~345 nM) making it more suitable for 488 nm excitation using an argon laser.[7-9] This makes Fluo-4-AM brighter at a lower dye concentration and consequently, less phototoxic. Lower concentrations of dye can yield almost double the amount of fluorescence, which is advantageous in cell lines plated at lower densities. As importantly, Fluo-4-AM has very low background absorbance and lower dye concentrations require shorter incubation times (**Figure 4.1**).

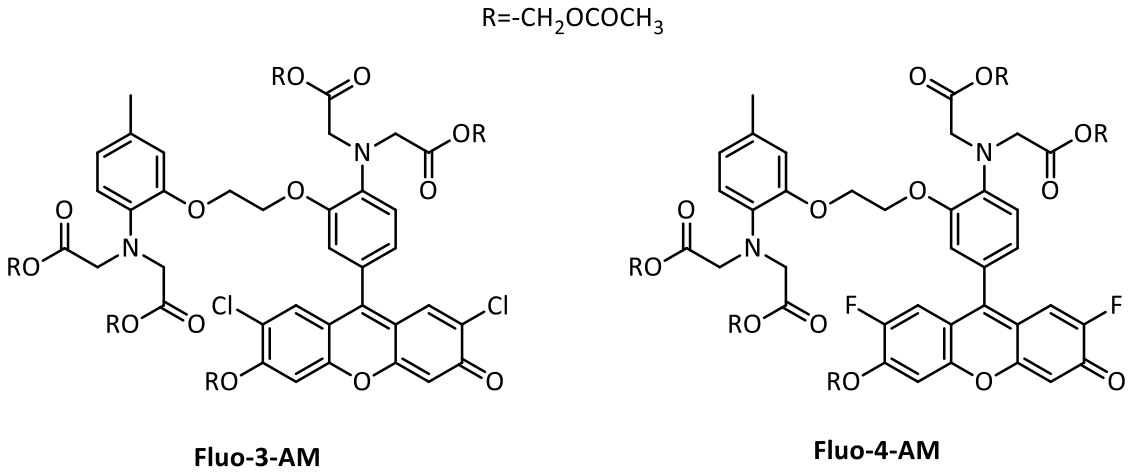


Figure 4.1. Structure of the membrane-permeable precursor fluorimetric dyes Fluo-3-AM and Fluo-4-AM.

Generally, each dye incorporates a fluorophore, and selectively chelate calcium ions. The action mechanism is as follows: each dye was loaded into the cells by incubation with the membrane-soluble acetoxymethyl ester form; once incorporated into the cell; cytosolic esterases hydrolyse the ester groups, to leave the membrane-impermeable form of the dye "trapped" within the cell. The free form chelates the intracellular calcium. The chelation of a calcium ion causes a shift in the wavelength and an increase in the intensity of the fluorescence. The change in fluorescence is proportional to $[Ca^{2+}]_i$, thereby the measurement of changes in fluorescence allows the measurement of changes in $[Ca^{2+}]_i$ (**Figure 4.2**).

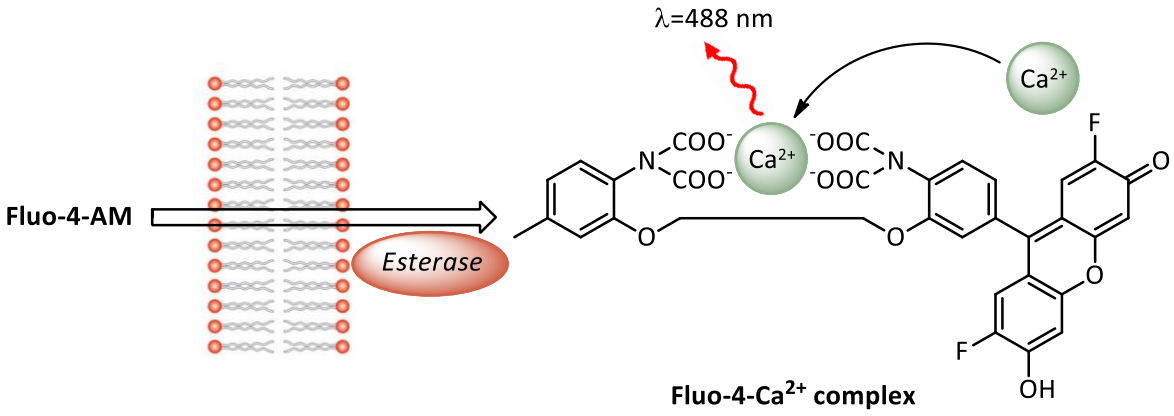


Figure 4.2. Hydrolysis of Fluo-4-AM and complexation of intracellular Ca^{2+}.

4.3. FAAH ACTIVITY RADIOMETRIC ASSAY

FAAH is a membrane-bound protein found predominantly in microsomal and mitochondrial fractions.[10-12] The enzymatic activity was highest in the liver, followed by the brain. The enzyme can be solubilized from the membrane with the aid of detergents such as sodium taurodeoxycholate[10] and Triton X-100.[12,13] As mentioned in the **Chapter 3**, anandamide **29** is the main substrate of the FAAH enzyme. Anandamide **29** was shown to be hydrolyzed by a single enzyme in mouse neuroblastoma cells.[14] This finding was confirmed with recombinant rat FAAH.[13] The rate of anandamide **29** hydrolysis by the recombinant enzyme was shown to be the highest against the hydrolysis rate of oleamide **70** and other primary amides from different fatty acids.[15,16] The catalytic action of FAAH can be determined by Scintillation Proximity Assay (SPA). The technique requires a labelled molecule with a weakly emitting radioisotope and a ligand. The molecule becomes adsorbed to minute beads that contain a scintillant that is excited by the energy of radioactive decay and a recognition site for the ligand. Secondary emission of light from the scintillant is quantified in a scintillation counter. Because a weakly emitting radioisotope is used, only when the radioactive decay occurs in close proximity to the scintillant (i.e. when the molecule is bound to the bead) is a visible emission produced.[17-19]

Omeir *et al.*[20] developed an assay based on the distinct physicochemical and binding properties of the FAAH substrate anandamide **29** and its two products, arachidonic acid **71** and ethanolamine **72**. In this study, arachidonoyl ethanolamide labelled in the ethanolamine portion of anandamide **29** ([1,2-^{14}C]arachidonoyl ethanolamide). After incubation of the FAAH enzyme with this substrate, the reaction mixture is stopped by the addition of organic solvent. The radiolabelled anandamide substrate partitions into the organic phase while the product ([1,2-^{14}C]ethanolamine) conveniently partitions into the aqueous phase which is measured by liquid scintillation counting.

The use of [1,2-^{14}C]arachidonoyl ethanolamide provides a simple method to monitor the breakdown of anandamide that does not require a thin layer chromatography separation step.

To evaluate the FAAH inhibition of a new molecule, the rat brain membrane suspension is incubated with the new compound and [1,2-^{14}C]-arachidonoyl ethanolamide. At the time indicated, the tissue suspension is extracted with a mixture of MeOH/CH_3Cl 1:1 and to this upper-phase is added the scintillation liquid. The no β direct emission radioactivity indicates that the new molecule avoids the hydrolysis of [1,2-^{14}C]-arachidonoyl ethanolamide into arachidonic acid and [1,2-^{14}C]-ethanolamine and therefore, it presents an excellent inhibitory behaviour.

4.4. OBJECTIVES

The aim of this chapter was to evaluate the library of compounds synthesized in **Chapter 3** to determine the agonist behaviour for TRPV1 and the inhibitory potential in the FAAH enzyme. Furthermore, these compounds were tested in other TRP receptors (TRPV2 and TRPA1) to assess their selectivity for TRPV1 receptor. These results allowed the elaboration of a library of new compounds with biological activity in pain receptors in function of the structure–activity relationships.

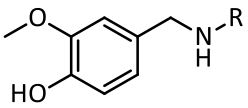
4.5. RESULTS AND DISCUSSION

The assays for determining the biological activities in TRPV1 receptor and FAAH enzyme of the new LCNVAs described in **Chapter 3** were performed in collaboration with the Endocannabinoid Research Group of the Institute of Biomolecular Chemistry (ICB-CNR) in Pozzuoli (Italy) under the supervision of Dr. Vincenzo Di Marzo and Dr. Luciano de Petrocellis. Cell-based assay was chosen to evaluate the agonist behaviour in TRPV1 (ion channel) of the new compounds whereas that the ability to inhibit the FAAH enzyme activity by these same molecules was determined by a biochemical assay.

4.5.1. TRP activity evaluation

The activity at human TRPV1 of the 22 new compounds synthetized in **Chapter 3** was measured by the fluorescence-based intracellular Ca^{2+} assays. In addition to evaluating the biological activity of these new synthesized compounds, another objective of this chapter was to evaluate their selectivity evaluated towards other TRP channels as TRPV2 and TRPA1 that are also abundant in sensory neurons. In **Table 4.1** are shown the chemical structures of the molecules to be tested.

Table 4.1. The set of molecules submitted to biological evaluation.



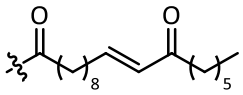
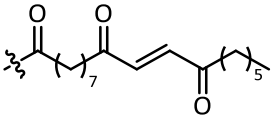
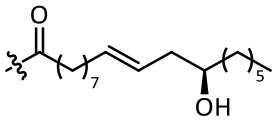
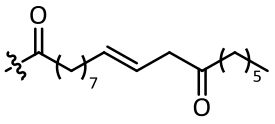
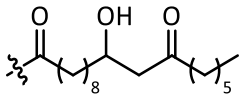
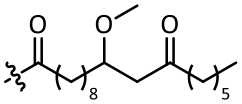
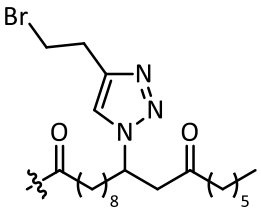
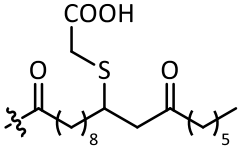
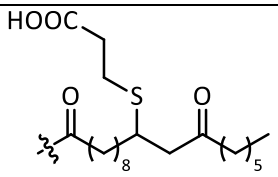
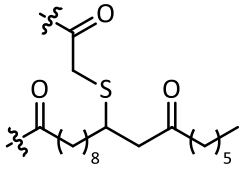
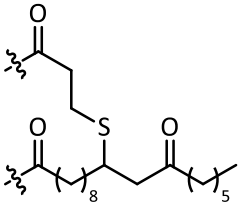
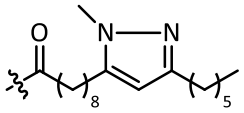
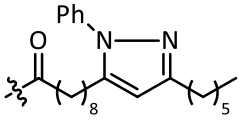
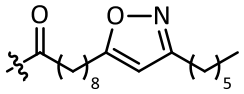
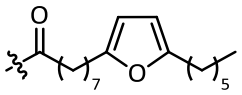
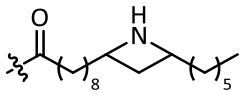
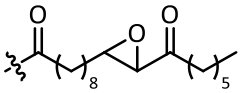
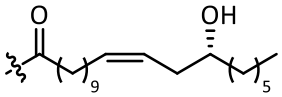
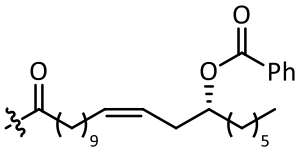
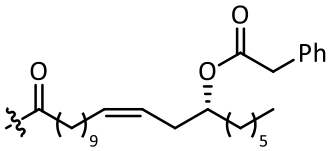
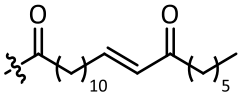
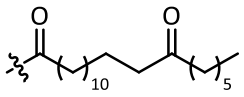
Compound	R	Compound	R
75		**76**	
77		**78**	
79		**80**	
81		**82**	

Table 4.1. The set of molecules submitted to biological evaluation (*continued*).

Compound	R	Compound	R
83		**84**	
85		**86**	
87		**88**	
89		**90**	
91		**92**	
93		**94**	
95		**96**	

4.5.1.1. Measure of the agonist behaviour at TRPV1

The agonist behaviour of each new long chain *N*-vanillyl acylamide was evaluated by recording and monitoring Ca^{2+} signals using the single wavelength fluorescence indicator Fluo-4 acetoxymethyl (AM) in HEK-293 cells transfected with human TRPV1. Once the cells were loaded with the Ca^{2+} indicator Fluo-4-AM were transferred to the quartz cuvette of the espectrofluorimetre (λ_{EX}=488 nm; λ_{EM}=516 nm) under continuous stirring. Experiments were performed by measuring cell fluorescence before and after addition of various concentrations of the compounds to evaluate. The potency of the test compounds (EC_{50} values) was determined as the concentration of the test substances required to produce half-maximal increases in $[Ca^{2+}]_{intracellular}$. The efficacy of the agonists was determined by comparing their effect with the analogous effect observed with 4 µM of ionomycin, which is an ionophore produced by the bacterium *Streptomyces conglobatus* that increases the $[Ca^{2+}]_i$. Finally, the desensitizing behaviour (antagonism) was evaluated against

capsaicin **28** (0.1 μM) by adding the capsaicin **28** in the quartz cuvette 5 min before stimulation of cells with the test compounds. Data are expressed as the concentration exerting a half-maximal inhibition of the agonist effect (IC_{50}). **Table 4.2** collects the EC_{50} values determined for each compound, as well as their efficacy and desensitizing behaviour against capsaicin **28**.

Table 4.2. Summary of the TRPV1 biological data of new LCNVAs synthetized determined by measure of $[Ca^{2+}]_i$ fluorescence methodology.[a]

Entry	Compound	EC_{50} (nM)	Efficacy[b]	IC_{50} (nM)
1	**75**	4.1±0.3	71.1±1.2	2.7±0.1
2	**76**	5.8±0.3	77.2±0.9	8.7±0.4
3	**77**	3.87±0.2	77.7±0.9	3.93±0.03
4	**78**	1.7±0.1	73.7±0.6	1.84±0.08
5	**79**	78.3±3.2	63.5±0.7	57.7±2.7
6	**80**	14.3±1.1	65.4±1.0	6.2±0.3
7	**81**	9.04±0.29	73.6±0.6	9.11±0.33
8	**82**	37.9±2.4	77.4±0.9	23.7±1.5
9	**83**	49.3±1.9	75.2±0.7	54.2±1.7
10	**84**	24.4±0.5	70.5±0.4	29.9±1.2
11	**85**	29.5±0.6	69.5±0.4	38.9±2.7
12	**86**	5.05±0.25	75.3±0.9	3.37±0.15
13	**87**	1.7±0.04	76.8±0.3	1.8±0.1
14	**88**	2.15±0.10	76.2±0.8	2.29±0.08
15	**89**	0.24±0.02	73.8±0.2	0.26±0.07
16	**90**	61.6±1.3	60.6±0.3	81.9±5.0
17	**91**	31.6±0.9	59.7±0.4	21.5±0.9
18	**92**	1.63±0.09	76.4±0.7	1.00±0.05
19	**93**	0.26±0.02	78.9 ±1.0	0.24±0.02
20	**94**	0.31±0.04	69.3±2.0	0.26±0.02
21	**95**	0.88±0.07	74.0±0.9	0.73±0.06
22	**96**	0.6±0.1	64.4±0.4	0.92±0.05

[a]Data are means ±SEM of *n*=3 determinations.
[b]% at maximal tested concentration.

Table 4.2 shows the agonist TRPV1 potency of the 22 compounds synthesized containing a vanillyl amide bond. These data allowed the extraction of qualitative SAR information. Firstly, all the new compounds showed agonist character in concentrations of nanomolar scale, which is in

agreement with the presence of the pharmacophore (vanillyl ring). Therefore, these results reaffirmed the requirement of the vanillyl ring and the amide bond (in comparison with the capsiates which are at least 1 order of magnitude less potent TRPV1 activators than capsaicin **28**) to achieve high TRPV1 potencies as agonists.

The structure-activity study of the α,β-unsaturated ketones revealed, in general terms, that both the enone of ricinoleic acid **75** and enone of lesquerolic acid **95** were strongly more active than any of the natural shogaols[21] ([6]-shogaol **103** EC_{50}=0.611 μM, [8]-shogaol **104** EC_{50}=0.711 μM and [10]-shogaol **105** EC_{50}=0.874 μM). **95** was more active (>4-fold) and better desensitizer than **75**. This behaviour could be explained by the influence of the molecule lipophilicity on the TRPV1 activity. **95**, with 20 carbon atoms in the acyl chain, presents higher lipophilicity than **75**. An increment of the lipophilicity of a molecule facilities the transport through the cell membrane, which improves the activity.[22]

The effect of the conjugation was studied considering the compound **76**. The EC_{50} of the α,β-unsaturated diketone **76** remained within the order of magnitude of the enone **75**. The increment of the conjugation in the side chain through the presence of an α,β-unsaturated diketone moiety did not result in an improvement in activity.

Capsaicin **28** is a moderately flexible molecule characterized by a double bond, whose stereochemistry does not affect for receptor interaction.[23] Rinvanil **54**, the *N*-vanillyl acylamide of ricinoleic acid **1a**, is a TRPV1 agonist (EC_{50}=6.0 nM) that has a *Z*-double bond. **77** was synthesized with the purpose of checking if the stereochemistry of the double bond of rinvanil **54** is crucial for the potency of the molecule. It was apparent that the replacement of the stereochemistry of the double bond from the *Z*-configuration to the *E*-configuration resulted in a decrease of potency, and thus an improvement of TRPV1 activity. The synthesis of the β,ϒ-unsaturated ketone **78** resulted in a better compound than its precursor **77** in terms of potency and inhibition. **78** had also a higher activity than **75**, which is its conjugated analogue. The agonist behaviour of **78** was also better than olvanil **51** (EC_{50}=3.7 nM),[24] the commercial analogue without the keto group. It seems that the presence in the alkyl side chain of an *E*-double bond and a carbonyl group in the δ-position relative of the double bond led to the improvement of the agonist behaviour.

The compound **79** was synthetized as *N*-vanillyl acylamide analogue of [6,8]-gingerols, which activate both native and recombinant TRPV1.[25,26]. Although the activity increased over 8-fold than natural gingerols, it was the worst result of the whole series. As it was described in **Chapter 3**, there

are some evidences of the existence of a second pocket capable to interact with apolar groups. This pocket cannot form hydrogen bonds with hydroxy groups.[27] **80** was synthezised to corroborate the existence of this apolar cleft. The substitution of the hydroxy group of the β-hydroxyketone **79** by a methoxy group **80** in the β-position resulted in a substantial increase of activity. Although the inhibition was also improved, the efficacy was maintained at the same values. As a result, we could assure that the replacement of the hydrogen-donor polar group(s) in the side-chain by non-hydrogen-donor groups generated higher potencies.

A good mimic of an amide bond is the 1,4-substituted 1,2,3-triazole ring.[28,29] Appendino *et al*.[30] synthetized several 1,2,3-triazolcannabinoids that showed TRPV1 activity. They conclude 1,2,3-triazole ring can act as a peptidomimetic element capable of both sustaining and modulating amide-related bioactivity. This effect was reflected in the biological activity of **81**. The introduction of an 1,2,3-triazole ring in the β-position led to a better potency respecting other β-substituted ketones such as **82** and **83**. On the other hand, this highly polar group could have a detrimental effect on bioactivity when implanted in a lipophilic moiety as an isosteric surrogate for a double bond. Compound **81** showed worse potency compared to derivatives with double bond in the side chain.

[6]-Shogaol **103** is metabolized by mice cells. L-Cysteine conjugates with [6]-shogaol **103** in form of β-thioketones through the mercapturic acid pathway in order to be excreted of the organism.[31] Based on these metabolites, we synthetized four β-thioketones to evaluate their activity and design of a small library about this moiety. Compound **82** presented slightly better potency and inhibition than **83** against capsaicin **28**. With respect to the double *N*-vanillyl acylamides **84** and **85**, these compounds showed higher activity than their carboxylic acid precursors **82** and **83** but worse efficacy values. The slight increase of potency evidenced the importance of the substitution of hydroxy groups present in the carboxylic acids by other acid derivatives as esters or amides due to the presence of the second pocket. Although TRPV1 is a multimeric receptor,[32] the presence of the phenol group in the second vanillin ring leads to the formation of hydrogen bonds. This fact hinders the accommodation of the side chain in the apolar pocket and therefore, which is detrimental to the activity.

Respecting to compounds with conformational constrained side-chain, we were able to draw several conclusions. First, the increment of the conformational constraint on the side-chain either as pyrazoles **86** and **87**, isoxazoles **88** and furans **89** resulted in better activities and efficacies than the derivatives with more of freedom of movement as **90** and **91**. Within the *N*-pyrazole derivatives, the presence of an *N*-phenyl (**87**) instead of an *N*-methyl group (**86**) increased the values of potency and

inhibition, although the efficacy was similar. This behaviour of compound **87** could be explained by establishing a hydrophobic π-π stacking[33] with the phenyl group of the aminoacid Y511 of the S3 helix, which is known to interact with the aliphatic tail of **28**.[34] This resulted in a better accommodation of **87** in the apolar cleft of TRPV1. π-π Interactions are usually energetically important when the ligand has either positive charge or an aromatic ring, and are involved in control of ion channels, G-protein-coupled receptors, transporters, and enzymatic catalysis.[35] However, the methyl substituent of **86** would not stablish this interaction and it would result in a decrease of potency. Secondly, the replacement of the substituted nitrogen atom by an oxygen atom (**88**) led to slightly improvement of potency and inhibition compared to the *N*-methylpyrazole **86**. Nevertheless, this change did not allow **86** to achieve the activity of **87**. This fact reinforced the hypothesis that the presence of the phenyl substituent generates π-π interactions with the aminoacids of the apolar pocket positively influencing the activity of the molecule in TRPV1.

Furan derivative **89** showed the best results in term of potency of the whole series. Based on our activity data, we could deduce that the presence of a furan ring in the side chain of the vanillyl amide improve the activity in TRPV1 receptor. It seems that the conformational constraining of the furan favors the accommodating of the aliphatic chain in the apolar pocket. Having in mind the design a library of LCNVAs based on SAR studies, the synthesis of new furan derivatives in the side-chain could be a good starting point to explore the activity around this moiety. The introduction of an azetidine ring **90** into the chain, gave the worst results of the whole series of conformational limited compounds. The presence of the NH-group did not favor the accommodation of the side-chain in the apolar cleft. In addition, the azetidine derivative **90** gave the worst inhibitory result of the whole series. This result implies that capsaicin **28** has greater affinity for the receptor than **90** and so, this compound would be rapidly replaced by the natural agonist **28**. However, the change of the azetidine ring **90** by a β-epoxyketone **91** led approximately to an activity twice higher as well as over 4-fold in inhibition.

Previous studies had established that the introduction of an allylic hydroxy group on the distal homoallylic position increases the efficacy without greatly altering potency.[27] This is particularly the case of rinvanil **54** (EC_{50}=6.0 nM), that suggests the presence of polar elements within the apolar pocket accommodating the acyl residue of capsaicin **28**. **92**, the *N*-vanillyl acylamide of lesquerolic acid **12a**, showed better potency than rinvanil **54**. As it was indicated above, the increment of the number of carbon atoms in the side-chain results in an increase of the lipophilicity of the molecule improving the activity. The derivatization of the hydroxy group of **54** into the

homoallyl esters (**59** and **64**) led to a spectacular increment of potency respecting rinvanil **54**. This same effect was observed in the case of the acyllesquevanils **93** and **94**. The replacement of the hydroxy group of the lesquerolic amide **92** by an ester group (benzoyl- **93** and phenylacetyllesquevanil **94**) produced a higher potency (≈15-fold) and inhibition. Therefore, polar elements as hydroxy group do not establish a hydrogen bond within the apolar pocket. The existence of a second pocket capable of interacting with ester groups might also be present in the apolar cleft accommodating the acyl residue of LCNVAs.[27] This new acyllesquevanils **93** and **94** showed slightly worse activity and efficacy than the ricinoleic ester analogues, benzoyl- **64** and phenylacetylrinvanil **59**.[36] **93** showed the best value of efficacy of the whole series and it was similar to capsaicin **28**. Finally, these acyllesquevanils **93** and **94** presented good values of inhibition against capsaicin **28**.

In 2002, Appendino *et al*.[27] synthetized the saturated alcohol **55** (EC_{50}=5.0 nM) and ketone **56** (EC_{50}=6.3 nM) from ricinoleic acid **1a**, which reduced efficacy but not potency regarding rinvanil **54**. Our compound **96**, the fully saturated ketone from **95**, followed the same tendency. **96** showed lower efficacy but higher potency (EC_{50}=0.6 nM) than **92** and **95** (EC_{50}=1.63 and 0.88 nM respectively). It is interesting to highlight that **96** showed over 10-fold activity than **56** and similar efficacy. As is known, polar elements such as hydroxy or ketone groups present in the saturated side-chain contribute positively to the activity by better accommodation of the acyl residue of LCNVAs.

4.5.1.2. Measure of the agonist behaviour in TRPV2

Transient receptor potential vanilloid type 2 (TRPV2) is a calcium-permeable cation channel belonging to the TRPV family and it was independently identified by two groups in 1999 using different approaches.[37,38] It shares ~50% sequence identity with TRPV1.[39] Nevertheless, it differs from TRPV1 in its overall cellular function.[39] Heterologously expressed TRPV2 predominantly localizes to intracellular membranes.[40] In addition, TRPV2 does not respond to heat or vanilloids in vivo.[41,42] By Northern blotting, the expressing of TRPV2 is high in brain, lung and spleen.[43] TRPV2 consists of a large *N*-terminal cytoplasmic domain (~390 residues), followed by six transmembrane segments (~250 residues) containing a pore-forming loop, and a *C*-terminal cytoplasmic domain (~100 residues). The *N*-terminal cytoplasmic region of TRPV2 can be further subdivided into a distal *N*-terminal region, an ankyrin repeat domain, and a membrane-proximal linker region. Recently, structure of the full-length TRPV2 channel by cryo-EM was resolved.[44]

In heterologous expression systems, TRPV2 activity was shown to be increased by endogenous ligands as lysophosphatidylcholine **146** and lysophosphatidylinositol **147** and by

exogenous small molecules such as 2-aminoethoxydiphenyl borate (**148**, 2-APB) and probenecid **149** (**Figure 4.3**), and blocked by ruthenium red and Gd.[39,45,46] However, physiological regulators of TRPV2 and other TRPV channels remain unknown.

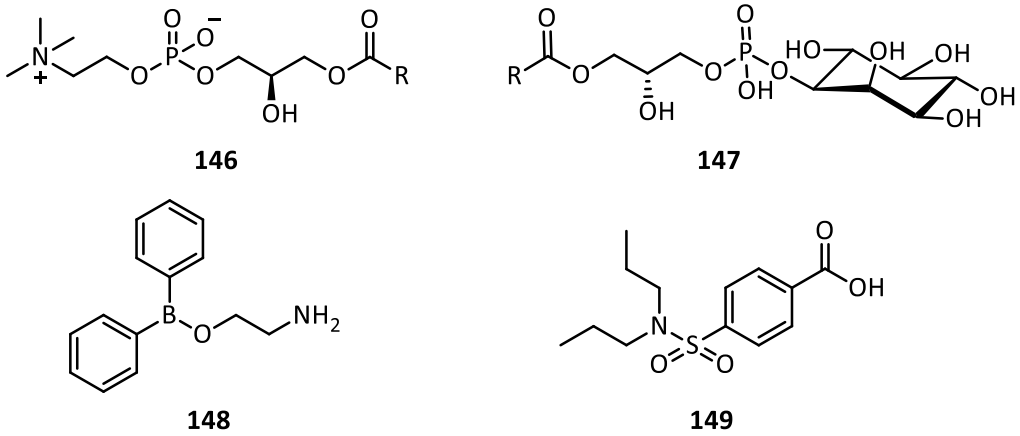


Figure 4.3. Chemical structures endogenous and exogenous TRPV2 agonists.

TRPV2 has been proposed as a potential pain target, but very little is known about its activation mechanism or possible candidates for specific or endogenous TRPV2 activators. The method employed to measure the effect on TRPV2 of the compounds was the same as the case of TRPV1. The increase of intracellular calcium was measured by fluorescence in HEK-293 cells stably over-expressing the rat recombinant TRPV2 channel. Data are expressed as EC_{50}. The efficacy was calculated as a percentage of the effect achieved with ionomycin (4 μM). In the antagonism-desentization experiments, the compounds were given to cells 5 min prior to lysophosphatidylcholine **146** (LPC) (3 μM) before stimulation of cells with agonists. Data are expressed as the concentration exerting a half-maximal inhibition of the agonist effect (IC_{50}). These results are given in **Table 4.3**.

Table 4.3. Summary of the TRPV2 biological data of new LCNVAs synthetized determined by measure of $[Ca^{2+}]_i$ fluorescence methodology.[a]

Entry	Compound	EC_{50} (nM)	Efficacy[b]	IC_{50} (nM)
1	**75**	n.a.[c]	<10	>50
2	**76**	n.a.	<10	47.3±0.01
3	**77**	n.a.	<10	>10
4	**78**	n.a.	<10	>10
5	**79**	n.a.	<10	49.1±4.7
6	**80**	n.a.	<10	>50
7	**81**	n.a.	<10	>10
8	**82**	n.a.	<10	>50
9	**83**	n.a.	<10	>50
10	**84**	n.a.	<10	46.3±0.01
11	**85**	n.a.	<10	44.3±0.01
12	**86**	n.a.	<10	>10
13	**87**	n.a.	<10	>50
14	**88**	n.a.	<10	>50
15	**89**	n.a.	<10	2.32±0.25
16	**90**	n.a.	<10	>10
17	**91**	n.a.	<10	>50
18	**92**	n.a.	<10	>50
19	**93**	n.a.	<10	12.0±1.7
20	**94**	n.a.	<10	>50
21	**95**	n.a.	<10	>10
22	**96**	n.a.	<10	>10

[a]Data are means ±SEM of *n*=3 determinations.
[b]% at maximal tested concentration.
[c]Not active

Table 4.3 shows that any compound of all synthesized presented activity against the TRPV2 receptor. In addition, the efficacy of all of them was less than 10%. With regard to compound **89**, the most potent of the series against the TRPV1 receptor, despite not showing TRPV2 activity and efficacy, a certain antagonism was observed in presence of its endogenous agonist lisophosphatidylcholine **146**. These data confirmed that compound **89** exhibits selectivity for TRPV1 *vs* TRPV2.

4.5.1.3. Measure of the agonist behaviour in TRPA1

TRPA1 is the only TRPA protein present in humans and other mammals[47] and was previously called ANKTM1 because the protein consists of many *N*-terminal ankyrin repeats. This channel is found expressed highly expressed in dorsal root and trigeminal ganglion neurons, where it is co-expressed with TRPV1.[48] There, it has a variety of sensory roles, especially sensation of painful or irritating stimuli.[49-51] TRPA1 has six transmembrane domains with a reentrant loop between the fifth and sixth that creates the pathway for ion permeation,[52] and intracellular *N*- and *C*-termini.[53] Recently, the atomic structure of the human TRPA1 (hTRPA1) channel was solved by cryo-EM, revealing channel organization, pore architecture and key regulatory interactions.[54] The best-known activators of TRPA1 are a set of exogenous electrophilic agonists that form covalent adducts with cysteine and lysine residues contained within the *N*-terminal domain. TRPA1 is chemically activated by the psychoactive component in marijuana, [55] environmental irritants, and pungent compounds. Examples of electrophilic agonists are allyl isothiocyanate **150** (AITC) from wasabi,[56,57] cinnamaldehyde **151** from cinnamon extracts,[56,57] allicin **152** from garlic extract,[58,59] acrolein **153** in diesel exhaust,[50] and other noxious substances such as tear gases.[60] TRPA1 is also activated by menthol **154** (a cooling agent),[61] 4-hydroxynonenal **155** (a product of oxidative stress),[62] *N*-methylmaleimide **156** (a cysteine-modifying reagent), Ca^{2+} ions,[63] and alkalization (**Figure 4.5**).

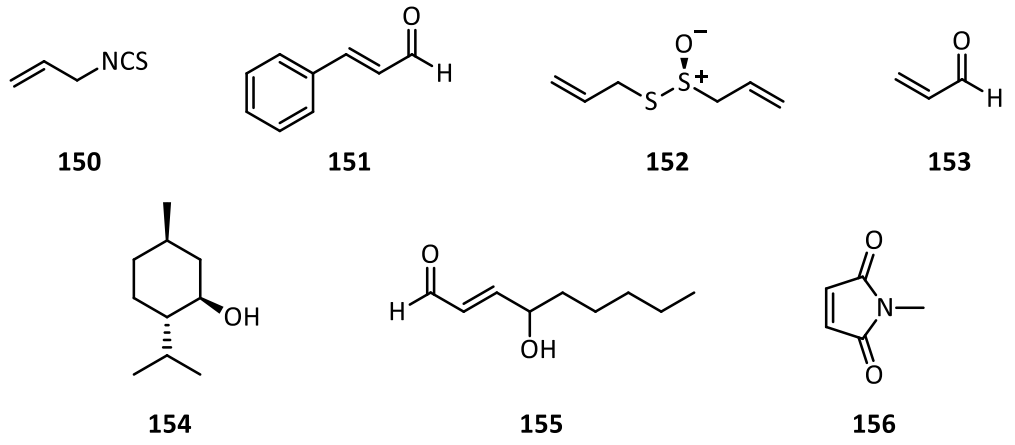


Figure 4.5. Chemical structures of some TRPA1 exogenous agonists.

Two studies[64,65] have proposed that allyl isothiocyanate **150** and cinnamaldehyde **151**, both electrophilic compounds, activate TRPA1 through covalent binding on specific cysteine residues present in the ankyrin repeats of the proteins. These findings could be extended to alkyl chains inferior to 8 carbons possessing α,β-unsaturated double bond,[65] showing that the reactive cysteine residues could attack these compounds through a Michael addition. Finally, it is downstream of receptors that activate the phospholipase C (PLC) pathway, such as the bradykinin receptor.

The method used to measure the effect on TRPA1 of the synthesized compounds was the same as the case of TRPV1. The increase of intracellular calcium was measured by fluorescence in HEK-293 cells stably over-expressing the rat recombinant TRPA1 channel. Data are expressed as EC_{50}. The efficacy was calculated as a percentage of the effect achieved with allylisothiocianate **150** (100 μM). In the antagonism-desentization experiments, the compounds were given to cells 5 min prior to allyl isothiocianate **150** (100 μM) before stimulation of cells with agonists. **Table 4.4** shows the concentration exerting a half-maximal inhibition of the agonist effect (IC_{50}).

Table 4.4. Summary of the TRPA1 biological data of new LCNVAs synthetized determined by measure of $[Ca^{2+}]_i$ fluorescence methodology.[a]

Entry	Compound	EC_{50} (nM)	Efficacy[b]	IC_{50} (nM)
1	**75**	1.5±0.1	71.0±0.3	3.7±0.3
2	**76**	0.13±0.01	88.7±1.9	0.22±0.02
3	**77**	>10	119.8±7.8	37.0±3.3
4	**78**	>10	93.1±10.8	48.3±15.6
5	**79**	10.0±0.1	15.2±0.1	>50
6	**80**	4.5±0.4	55.1±0.4	30.2±1.0
7	**81**	>10	92.9±14.1	>50
8	**82**	11.1±0.1	17.7±0.1	>50
9	**83**	10.0±0.1	18.8±0.1	>50
10	**84**	n.a.[c]	<10	30.6±0.3
11	**85**	n.a.	<10	27.5±0.3
12	**86**	n.a.	<10	30.3±6.2
13	**87**	8.6±0.1	50.1±0.1	16.8±0.2
14	**88**	5.10±0.01	34.6±0.1	>50
15	**89**	10.5±0.01	84.1±0.3	13.1±0.1
16	**90**	n.a.	<10	>50
17	**91**	1.1±0.1	20.9±0.3	>50
18	**92**	n.a.	<10	28.4±2.7
19	**93**	9.8±0.1	21.9±0.1	35.9±0.8
20	**94**	n.a.	0	>50
21	**95**	>10	98.1±14.1	6.9±0.8
22	**96**	n.a.	<10	>50

[a] Data are means ±SEM of *n*=3 determinations.
[b] % at maximal tested concentration.
[c] Not active

Screening of the components of the library of new LCNVAs to evaluate their selectivity at TRPV1 against TRPA1 showed that the found activity of these compounds was of the micromolar order or practically null. Whereby, the synthetized agonist were selective for the TRPV1 instead TRPA1. The double α,β-unsaturated ketone **76** exhibited the highest activity among the most active compounds. This behaviour was expected because it is the compound with the highest electrophilic character of the whole series. This molecule could be attacked by the cysteine residues through a Michael addition. Moreover **76** behaved as rather selective TRPA1 antagonist in as much as it inhibited the response to allyl isothiocyanate **150**. The loss of a ketone group led to a decrease of the electrophilic behaviour and so, the potency was affected. **75** showed 10-fold lower activity than **76**. Efficacy and inhibition were also worsened. On the other hand, **75** presented better activity than its ginger analogue [6]-shogaol **103** (EC_{50}=16.0 μM).

The *Z*-isomer of rinvanil **77** showed the 100% of efficacy at TRPA1 receptor, although the potency was higher than 10 μM. The absence of electrophilicity in this molecule was detrimental of activity and inhibition but no of efficacy. Oxidation of compound **77** to give the compound **78** with higher electrophilicity did not increase the potency but maintained an elevated efficacy. It seems that the presence of conjugation in the side-chain is crucial for the TRPA1 activity.

Compound **80**, one of the 1,4-ketosubstituted amides studied, showed an unexpected value of potency considering that it does not present a very electrophilic character as **75** or **76**. However, the values of efficacy and inhibition were not good. On the other hand, the β-hydroxyketone **79** was less active and showed lower efficacy than **80**. The replacement of a hydrogen donor group by the methoxy group in the side-chain highlighted the marginal importance of the β-hydroxy group for the activity. **79** gave very similar values than the ginger derivative [6]-gingerol **100**,[66] whereas the efficacy was the highest among the whole series of 1,4-ketosubstituted amides The presence of an 1,2,3-triazole ring gave acceptable values of TRPA1 activity and allowed the inhibition against allyl isothiocyanate **150**.

Within the series of conformational constrained side-chain compounds, we could observe, in the case of pyrazoles, the same phenomenon that occurred in TRPV1. While *N*-phenylpyrazole **87** showed activity for TRPA1 and some efficacy, *N*-methylpyrazole **86** was not active for the same receptor. This suggested that phenyl ring could establish π-π interactions with certain residues of the activation site.[54] The isoxazole mixture **88** showed the best results between all the conformational constrained compounds in terms of potency. However, the efficacy was low and did not inhibit the response of allyl isothiocyanate **150**. The furan compound **89**, the most active molecule in TRPV1, did

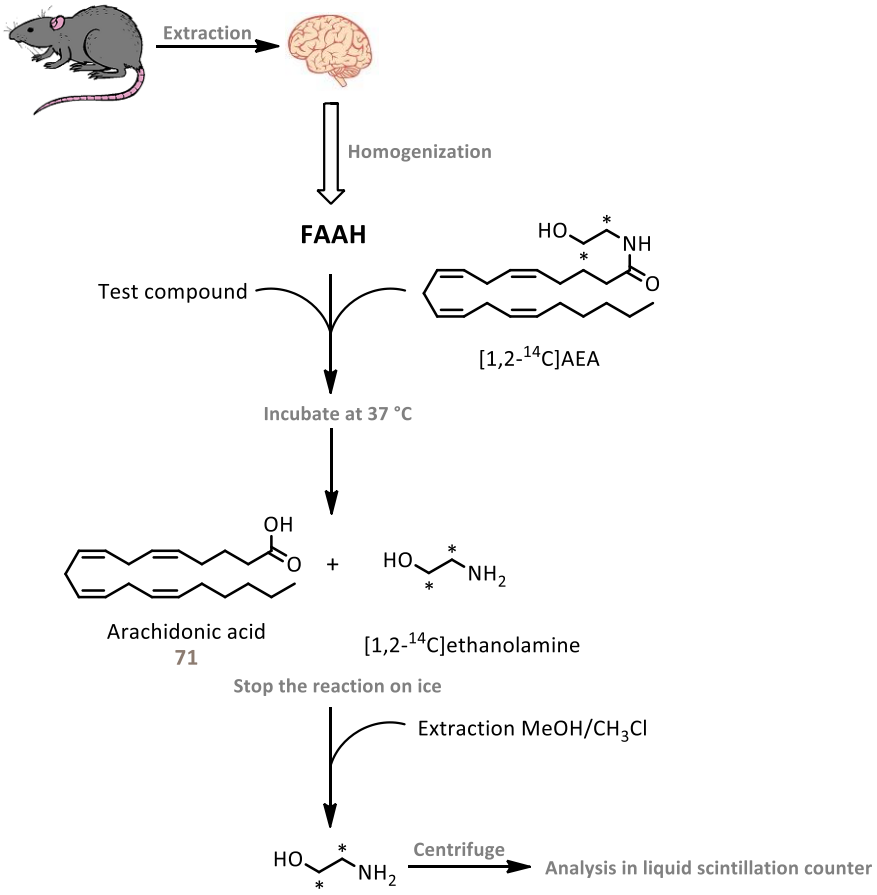
not show good values of potency. In contrast, the efficacy was similar to **76**. So; these results allowed us to assure that **89** is selective for TRPV1 against TRPA1. The azetidine ring **90** did not prove to be a good moiety to incorporate to the side-chain due to the complete absence of activity. Unlike **90**, the β-epoxyketone **91** presented the second best activity of the tested compounds. However, its efficacy and desensitizer behaviour were not suitable.

Almost all lesquerolic acid derivatives were practically inactive. The lesquerolic *N*-vanillyl acylamide **92** did not show potency and its efficacy was lower than 10%. The benzyllesquevanil **93** resulted more active than the corresponding phenyllesquevanil **94**, which was completely inactive. In the case of **95**, the efficacy was almost 100%. However, an activity >10 μM was achieved. In comparison with **75**, it seems to indicate that the elongation of the chain containing the α,β-unsaturated ketone did not favour TRPA1 activity but improved the efficacy. Finally, the total absence of double bonds and the increase in the number of carbons **96** produced a deep drop in the values.

To sum up, we could conclude that the furan *N*-vanillyl acylamide **89** was selective for TRPV1 and did not show activity towards other TRP channels as TRPV2 and TRPA1.

4.5.2. FAAH inhibition evaluation

The inhibitory behaviour in the FAAH enzyme of each new long chain *N*-vanillyl acylamide previously synthetized in **chapter 3** was determined using a radiometric assay. This assay was carried out by measuring the effect of increasing concentrations of these compounds on the enzymatic hydrolysis of [^{14}C]anandamide using membranes prepared from frozen brains of CD rats.[14] The test compounds were incubated with the membranes (70-100 µg) and [^{14}C]anandamide (10000 cpm, 1.8 µM) in 50 mM Tris-HCl, pH 9, for 30 min at 37 °C. [^{14}C]Ethanolamine produced was used to calculate FAAH activity and was measured by scintillation counting of the aqueous phase after extraction of the incubation mixture with 2 volumes of $CHCl_3$/CH_3OH (1:1 by volume) (**Scheme 4.1**).



Scheme 4.1. The assay for FAAH activity carried out in this work. The asterisk indicates the position of the ^{14}C.

Data are expressed as the concentration exerting a half-maximal inhibition (IC_{50}). The efficacy was calculated as a percentage of the effect obtained with URB597, which is an inhibitor of FAAH (0.1 µM). **Table 4.5** shows the IC_{50} values determined for each compound, as well as their efficacy.

Table 4.5. Summary of the FAAH biological data of new LCNVAs synthetized determined by scintillation.[a]

Entry	Compound	IC_{50} (μM)	Efficacy[b]
1	**75**	19.34±7.24	84.96
2	**76**	>10	1.17±1.17
3	**77**	>10	33.44±12.31
4	**78**	~10	51.62±3.62
5	**79**	27.78±11.00	74.00
6	**80**	16.22±6.15	85.00
7	**81**	6.00±1.17	63.21±4.96
8	**82**	>10	5.20±0.93
9	**83**	>10	9.28±1.20
10	**84**	>10	25.27±9.90
11	**85**	>10	4.77±4.16
12	**86**	3.08±0.92	84.22±0.33
13	**87**	4.19±0.09	69.02±1.45
14	**88**	4.06±0.98	73.88±0.43
15	**89**	4.49±1.17	65.68±3.02
16	**90**	>10	1.66±0.16
17	**91**	20.06±6.02	82.81
18	**92**	4.17±0.16	68.96±9.65
19	**93**	>10	0.00
20	**94**	>10	1.13±1.12
21	**95**	>10	33.20±1.84
22	**96**	>10	6.62±4.57

[a]Data are means ±SEM of *n*=2 determinations.
[b]% at maximal tested concentration.

As can be seen from **Table 4.5**, only half of the new synthesized compounds presented FAAH inhibitory activity. Most FAAH inhibitors utilize electrophilic carbonyls to achieve potency.[67] Because electrophilic ketones/carbamates typically inhibit serine hydrolases by formation of either a reversible or irreversible covalent adduct with the conserved serine nucleophile, these agents may interact with multiple members of this large enzyme class. The relative potency of the inhibitors depends of the electrophilic behaviour of the ketone. The more electrophilic the ketone is, the more inhibition is achieved. This hypothesis could explain the activity values showed by our ketones. The α,β-unsaturated ketone **75** showed more activity than other ketones such as **76** and **95**, which

presented >10 μM potency. The non-conjugation of the ketone **78** improved the activity respecting **75** but was in detrimental of the efficacy. The presence of an unsaturated diketone **76** resulted in an improvement of the inhibition of [^{14}C]anandamide hydrolysis either.

The rinvanil *Z*-isomer **77** was not active, which was in accordance with the literature. The incorporation of the *Z* double bond increases the potency of the inhibitors, and its replacement with a *E*-double bond or its removal results in incremental reductions in potency.[68]

The ketones with *O*-substituents in the β-position **79** and **80** showed activity and good efficacies in FAAH enzyme, while the presence of *S*-substituents **82**-**85** is detrimental to the inhibitory activity. The higher electronegativity of the oxygen atom than the sulphur atom provided higher electrophilicity to the ketone and thus, improved the inhibition. However, the presence of a 1,2,3-triazole ring **81** gave better potency values. This behaviour could be explained by the electronic structure of the 1,2,3-triazole ring. The three nitrogen atoms of the 1*H*-1,2,3-triazole cause a strong polarization of the aromatic π and it produces that the nitrogen atom in the 1-position is positively charged.[69-71]

It seems that the presence of a heterocycle ring in the side-chain brought activity to the molecules as is observed in the cases of **86**-**89**. **86** was the compound with the highest activity and inhibition efficacy among all compounds with constrained conformation in the side-chain. The *N*-phenylpyrazole amide **87** showed lower activity and efficacy than the *N*-methylpyrazole **86**. The isoxazole mixture **88** and the furan **89** derivatives exhibited equipotent antagonistic activities compared to **87**. These data revealed the sort of heterocyclic ring in the side-chain did not influence critically the activity of the *N*-vanillyl acylamides on FAAH unlike the α-ketoheterocycles inhibitors.[72-74] Special attention should be paid to compound **89**, which behaves as FAAH inhibitor (IC_{50}=4.49±1.17 μM) and TRPV1 agonist (EC_{50}=0.24±0.02 nM). In the case where the aromatic ring is a substituent of the side chain and not forming part of it **81**, the values of potency and efficacy decreased slightly but were maintained in the order of the above values. The β-epoxyketone **91** gave lower potency than the rest of heterocycles amides; however the presence of the azetidine ring **90** in the lipophilic part resulted in a decrease of potency and efficacy.

With respect the lesquerolic acid, amide **92** and all their derivatives (esters **93** and **94**, unsaturated **95** and saturated ketone **96**) did not present FAAH inhibitory activity except **92**, which was active but its efficacy was practically null. None of the acyllesquevanils **93** and **94**, which had shown good activities as TRPV1 agonists, behaved as FAAH inhibitors. Homoallylic alcohol and ester

functionalities did not show FAAH activity although they are good motifs for the agonism at TRPV1. Finally, the activity was lost when α,β-unsaturated ketone **95** was combined with an increase in the side chain.

In summary, the presence of a heterocycle ring in the side-chain might be suitable for the interaction with FAAH enzyme. Compound **86** showed the best results of the whole series. The furan amide **89** seems to serve as a good template for the design of new compounds with "dual" action as TRPV1 agonist and FAAH inhibitor.

4.6. CONCLUSIONS

In the present chapter, we have described the pharmacological evaluation of the 22 new long chain *N*-vanillyl acylamides synthesized. Compound **89** exhibited the highest capability of activing TRPV1 and inhibiting FAAH. Moreover, compound **89** showed poor activity for the TRPV2 and TRPA1 receptor, so it can be said to be selective for TRPV1 and FAAH.

4.7. REFERENCES

(1) Berridge, M. J. *Cell Calcium* **2006**, *40*, 405-412.

(2) Bootman, M. D.; Lipp, P.; Berridge, M. J. *J. Cell. Sci.* **2001**, *114*, 2213-2222.

(3) Berridge, M. J.; Lipp, P.; Bootman, M. D. *Nat. Rev. Mol. Cell. Biol.* **2000**, *1*, 11-21.

(4) Tsien, R. Y.; Pozzan, T.; Rink, T. J. *Nature* **1982**, *295*, 68-71.

(5) Paredes, R. M.; Etzler, J. C.; Watts, L. T.; Zheng, W.; Lechleiter, J. D. *Methods* **2008**, *46*, 143-151.

(6) Tsien, R. Y. *Biochemistry* **1980**, *19*, 2396-2404.

(7) Gee, K. R.; Brown, K. A.; Chen, W. N. U.; Bishop-Stewart, J.; Gray, D.; Johnson, I. *Cell Calcium* **2000**, *27*, 97-106.

(8) Miriel, V. A.; Mauban, J. R. H.; Blaustein, M. P.; Gil Wier, W. *J. Physiol.* **1999**, *518*, 815-824.

(9) Chambers, J.; Ames, R. S.; Bergsma, D.; Muir, A.; Fitzgerald, L. R.; Hervieu, G.; Dytko, G. M.; Foley, J. J.; Martin, J.; Liu, W.-S.; Park, J.; Ellis, C.; Ganguly, S.; Konchar, S.; Cluderay, J.; Leslie, R.; Wilson, S.; Sarau, H. M. *Nature* **1999**, *400*, 261-265.

(10) Schmid, P. C.; Zuzarte-Augustin, M. L.; Schmid, H. H. *J. Biol. Chem.* **1985**, *260*, 14145-14149.

(11) Desarnaud, F.; Cadas, H.; Piomelli, D. *J. Biol. Chem.* **1995**, *270*, 6030-6035.

(12) Ueda, N.; Kurahashi, Y.; Yamamoto, S.; Tokunaga, T. *J. Biol. Chem.* **1995**, *270*, 23823-23827.

(13) Cravatt, B. F.; Giang, D. K.; Mayfield, S. P.; Boger, D. L.; Lerner, R. A.; Gilula, N. B. *Nature* **1996**, *384*, 83-87.

(14) Maurelli, S.; Bisogno, T.; De Petrocellis, L.; Di Luccia, A.; Marino, G.; Di Marzo, V. *FEBS Lett.* **1995**, *377*, 82-86.

(15) Kurahashi, Y.; Ueda, N.; Suzuki, H.; Suzuki, M.; Yamamoto, S. *Biochem. Biophys. Res. Commun.* **1997**, *237*, 512-515.

(16) Lang, W.; Qin, C.; Lin, S.; Khanolkar, A. D.; Goutopoulos, A.; Fan, P.; Abouzid, K.; Meng, Z.; Biegel, D.; Makriyannis, A. *J. Med. Chem.* **1999**, *42*, 896-902.

(17) Cook, N. D. *Drug Discov. Today* **1996**, *1*, 287-294.

(18) Park, Y.-W.; Cummings, R. T.; Wu, L.; Zheng, S.; Cameron, P. M.; Woods, A.; Zaller, D. M.; Marcy, A. I.; Hermes, J. D. *Anal. Biochem.* **1999**, *269*, 94-104.

(19) Picardo, M.; Hughes, K. T. In *High Throughput Screening: The Discovery of Bioactive Substances*; Devlin, J. P., Ed.; Marcel Dekker: New York, 1997, p 307-316.

(20) Omeir, R. L.; Chin, S.; Hong, Y.; Ahern, D. G.; Deutsch, D. G. *Life Sci. J.* **1995**, *56*, 1999-2005.

(21) Morita, A.; Iwasaki, Y.; Kobata, K.; Yokogoshi, H.; Watanabe, T. *Biosci. Biotechnol. Biochem.* **2007**, *71*, 2304-2307.

(22) Lazar, J.; Braun, D. C.; Tóth, A.; Wang, Y.; Pearce, L. V.; Pavlyukovets, V. A.; Blumberg, P. M.; Garfield, S. H.; Wincovitch, S.; Choi, H.-K.; Lee, J. *Mol. Pharmacol.* **2006**, *69*, 1166-1173.

(23) Wong, G. Y.; Gavva, N. R. *Brain Res. Rev.* **2009**, *60*, 267-277.

(24) Brand, L.; Berman, E.; Schwen, R.; Loomans, M.; Janusz, J.; Bohne, R.; Maddin, C.; Gardner, J.; Lahann, T.; Farmer, R.; et al. *Drugs Exp. Clin. Res.* **1987**, *13*, 259-265.

(25) Dedov, V. N.; Tran, V. H.; Duke, C. C.; Connor, M.; Christie, M. J.; Mandadi, S.; Roufogalis, B. D. *Br. J. Pharmacol.* **2002**, *137*, 793-798.

(26) Witte, D. G.; Cassar, S. C.; Masters, J. N.; Esbenshade, T.; Hancock, A. A. *J. Biomol. Screen.* **2002**, *7*, 466-475.

(27) Appendino, G.; Minassi, A.; Morello, A. S.; De Petrocellis, L.; Di Marzo, V. *J. Med. Chem.* **2002**, *45*, 3739-3745.

(28) Valverde, I. E.; Bauman, A.; Kluba, C. A.; Vomstein, S.; Walter, M. A.; Mindt, T. L. *Angew. Chem. Int. Ed.* **2013**, *52*, 8957-8960.

(29) Pedersen, D. S.; Abell, A. *European J. Org. Chem.* **2011**, *2011*, 2399-2411.

(30) Appendino, G.; Bacchiega, S.; Minassi, A.; Cascio, M. G.; De Petrocellis, L.; Di Marzo, V. *Angew. Chem. Int. Ed.* **2007**, *46*, 9312-9315.

(31) Zhu, Y.; Warin, R. F.; Soroka, D. N.; Chen, H.; Sang, S. *PLoS ONE* **2013**, *8*, e54677.

(32) Szallasi, A. *Drugs Aging* **2001**, *18*, 561-573.

(33) Bissantz, C.; Kuhn, B.; Stahl, M. *J. Med. Chem.* **2010**, *53*, 5061-5084.

(34) Darré, L.; Domene, C. *Mol. Pharm.* **2015**, *12*, 4454-4465.

(35) Zacharias, N.; Dougherty, D. A. *Trends Pharmacol. Sci.* **2002**, *23*, 281-287.

(36) Appendino, G.; De Petrocellis, L.; Trevisani, M.; Minassi, A.; Daddario, N.; Moriello, A. S.; Gazzieri, D.; Ligresti, A.; Campi, B.; Fontana, G.; Pinna, C.; Geppetti, P.; Di Marzo, V. *J. Pharmacol. Exp. Ther.* **2005**, *312*, 561-570.

(37) Caterina, M. J.; Rosen, T. A.; Tominaga, M.; Brake, A. J.; Julius, D. *Nature* **1999**, *398*, 436-441.

(38) Kanzaki, M.; Zhang, Y.-Q.; Mashima, H.; Li, L.; Shibata, H.; Kojima, I. *Nat. Cell Biol.* **1999**, *1*, 165-170.

(39) Perálvarez-Marín, A.; Doñate-Macian, P.; Gaudet, R. *FEBS J.* **2013**, *280*, 5471-5487.

(40) Cohen, M. R.; Huynh, K. W.; Cawley, D.; Moiseenkova-Bell, V. Y. *PLoS ONE* **2013**, *8*, e85392.

(41) Park, U.; Vastani, N.; Guan, Y.; Raja, S. N.; Koltzenburg, M.; Caterina, M. J. *J. Neurosci.* **2011**, *31*, 11425-11436.

(42) Cohen, M. R.; Johnson, W. M.; Pilat, J. M.; Kiselar, J.; DeFrancesco-Lisowitz, A.; Zigmond, R. E.; Moiseenkova-Bell, V. Y. *Mol. Cell Biol.* **2015**, *35*, 4238-4252.

(43) Kanzaki, M.; Nagasawa, M.; Kojima, I.; Sato, C.; Naruse, K.; Sokabe, M.; Iida, H. *Science* **1999**, *285*, 882-886.

(44) Huynh, K. W.; Cohen, M. R.; Jiang, J.; Samanta, A.; Lodowski, D. T.; Zhou, Z. H.; Moiseenkova-Bell, V. Y. *Nat. Commun.* **2016**, *7:11130*, 1-8.

(45) Caterina, M. J.; Schumacher, M. A.; Tominaga, M.; Rosen, T. A.; Levine, J. D.; Julius, D. *Nature* **1997**, *389*, 816-824.

(46) Bang, S.; Kim, K. Y.; Yoo, S.; Lee, S.-H.; Hwang, S. W. *Neurosci. Lett.* **2007**, *425*, 120-125.

(47) Montell, C. *Sci. STKE* **2005**, *2005*, re3.

(48) Story, G. M.; Peier, A. M.; Reeve, A. J.; Eid, S. R.; Mosbacher, J.; Hricik, T. R.; Earley, T. J.; Hergarden, A. C.; Andersson, D. A.; Hwang, S. W.; McIntyre, P.; Jegla, T.; Bevan, S.; Patapoutian, A. *Cell* **2003**, *112*, 819-829.

(49) Nagata, K.; Duggan, A.; Kumar, G.; García-Añoveros, J. *J. Neurosci.* **2005**, *25*, 4052-4061.

(50) Bautista, D. M.; Jordt, S.-E.; Nikai, T.; Tsuruda, P. R.; Read, A. J.; Poblete, J.; Yamoah, E. N.; Basbaum, A. I.; Julius, D. *Cell* **2006**, *124*, 1269-1282.

(51) Kwan, K. Y.; Glazer, J. M.; Corey, D. P.; Rice, F. L.; Stucky, C. L. *J. Neurosci.* **2009**, *29*, 4808-4819.

(52) Owsianik, G.; Talavera, K.; Voets, T.; Nilius, B. *Annu. Rev. Physiol.* **2006**, *68*, 685-717.

(53) Samad, A.; Sura, L.; Benedikt, J.; Ettrich, R.; Minofar, B.; Teisinger, J.; Vlachova, V. *Biochem. J.* **2011**, *433*, 197-204.

(54) Paulsen, C. E.; Armache, J.-P.; Gao, Y.; Cheng, Y.; Julius, D. *Nature* **2015**, *520*, 511-517.

(55) Cavanaugh, E. J.; Simkin, D.; Kim, D. *Neuroscience* **2008**, *154*, 1467-1476.

(56) Bandell, M.; Story, G. M.; Hwang, S. W.; Viswanath, V.; Eid, S. R.; Petrus, M. J.; Earley, T. J.; Patapoutian, A. *Neuron* **2004**, *41*, 849-857.

(57) Jordt, S.-E.; Bautista, D. M.; Chuang, H.-h.; McKemy, D. D.; Zygmunt, P. M.; Hogestatt, E. D.; Meng, I. D.; Julius, D. *Nature* **2004**, *427*, 260-265.

(58) Bautista, D. M.; Movahed, P.; Hinman, A.; Axelsson, H. E.; Sterner, O.; Högestätt, E. D.; Julius, D.; Jordt, S.-E.; Zygmunt, P. M. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 12248-12252.

(59) Macpherson, L. J.; Geierstanger, B. H.; Viswanath, V.; Bandell, M.; Eid, S. R.; Hwang, S.; Patapoutian, A. *Curr. Biol.* **2005**, *15*, 929-934.

(60) Bessac, B. F.; Sivula, M.; von Hehn, C. A.; Escalera, J.; Cohn, L.; Jordt, S.-E. *J. Clin. Invest.* **2008**, *118*, 1899-1910.

(61) Karashima, Y.; Damann, N.; Prenen, J.; Talavera, K.; Segal, A.; Voets, T.; Nilius, B. *J. Neurosci.* **2007**, *27*, 9874-9884.

(62) Trevisani, M.; Siemens, J.; Materazzi, S.; Bautista, D. M.; Nassini, R.; Campi, B.; Imamachi, N.; Andrè, E.; Patacchini, R.; Cottrell, G. S.; Gatti, R.; Basbaum, A. I.; Bunnett, N. W.; Julius, D.; Geppetti, P. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 13519-13524.

(63) Zurborg, S.; Yurgionas, B.; Jira, J. A.; Caspani, O.; Heppenstall, P. A. *Nat. Neurosci.* **2007**, *10*, 277-279.

(64) Hinman, A.; Chuang, H.-h.; Bautista, D. M.; Julius, D. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 19564-19568.

(65) Macpherson, L. J.; Xiao, B.; Kwan, K. Y.; Petrus, M. J.; Dubin, A. E.; Hwang, S.; Cravatt, B.; Corey, D. P.; Patapoutian, A. *J. Neurosci.* **2007**, *27*, 11412-11415.

(66) Morera, E.; De Petrocellis, L.; Morera, L.; Schiano Moriello, A.; Nalli, M.; Di Marzo, V.; Ortar, G. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1674-1677.

(67) Cravatt, B. F.; Lichtman, A. H. *Curr. Opin. Chem. Biol.* **2003**, *7*, 469-475.

(68) Boger, D. L.; Sato, H.; Lerner, A. E.; Hedrick, M. P.; Fecik, R. A.; Miyauchi, H.; Wilkie, G. D.; Austin, B. J.; Patricelli, M. P.; Cravatt, B. F. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5044-5049.

(69) Adam, W.; Grimison, A. *Theor. Chim. Acta* **1967**, *7*, 342-351.

(70) Palmer, M. H.; Parsons, S. *Acta Crystallogr. C Struct. Chem.* **1996**, *52*, 2818-2822.

(71) Schulze, B.; Schubert, U. S. *Chem. Soc. Rev.* **2014**, *43*, 2522-2571.

(72) Ezzili, C.; Mileni, M.; McGlinchey, N.; Long, J. Z.; Kinsey, S. G.; Hochstatter, D. G.; Stevens, R. C.; Lichtman, A. H.; Cravatt, B. F.; Bilsky, E. J.; Boger, D. L. *J. Med. Chem.* **2011**, *54*, 2805-2822.

(73) Duncan, K. K.; Otrubova, K.; Boger, D. L. *Bioorg. Med. Chem.* **2014**, *22*, 2763-2770.

(74) Otrubova, K.; Cravatt, B. F.; Boger, D. L. *J. Med. Chem.* **2014**, *57*, 1079-1089.

Chapter 5

Experimental part

5.1. GENERAL METHODS

Reactions requiring anhydrous conditions were performed in blazed or oven-dried glassware using anhydrous solvents and under inert atmosphere (argon). The solvents and reagents were purchase from Acros Organics, Sigma Aldrich, Fluka, Merk, Panreac, Strem Chemicals or TCI Chemicals. Petroleum ether, EtOAc and MeOH were used without further purification. In case of anhydrous reactions, solvent and reagents were properly dried: MeCN was dried over activated molecular sieves 3 Å,[1] acrolein, 2-methyl vinyl ketone, ethyl acrylate and allyl acrylate were distilled at atmospheric pressure and used immediately.

Saponification and transesterification reactions were carried out using a Discover LabMate microwave reactor (CEM, Matthews, USA). Scaling up of oxidative cleavage was carried out using Milestone Pyro 320 Touch Control.

The continuous flow system consisted in a combination of FRX Pump Module and an etched glass chip microreactor of 250 µL of 3 inputs (Syrris Ltd, Royston, UK). The glass chip was placed on a digitally controlled RCT basic hotplate (IKA-Werke GmbH&Co., KG, Staufen, Germany) with an external Pt 100 sensor for optimum control of temperature.

The reactions were monitored by TLC on Silica Gel 60F-254 precoated plates (Merck). Visualization of the compounds was made by UV light (254 nm) and stained was performed either by immersion in a 5% solution of concentrated H_2SO_4 in methanol or 5% w/v phosphomolibdic acid in ethanol followed by heating.

Flash column chromatography was performed over silica gel (technical grade, 60 Å, 40-63 µm) (Sigma Aldrich) under air pressure.

NMR spectra were recorded on a MERCURYplus AS400 MHz Varian spectrometer using TMS as the internal standard when needed. Chemical shifts are reported in parts per million (ppm, δ units). Coupling constants (*J*) are reported and expressed in hertz (Hz), splitting patterns are designated as: br (broad), s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), dt (double triplet), td (triple doublet), ddd (double double doublet) and m (multiplet). All ^{13}C NMR spectra were proton decoupled.

GC analyses were performed on an Agilent HP6890 series gas chromatograph coupled with a flame ionization detector.

High resolution mass spectra (HR-MS) were recorded on at the Serveis Cientificotècnics of Universitat de Lleida (SCT-UdL) and Servei de Recursos Científics i Tècnics of Universitat Rovira I Virgili (URV) with an Agilent G6510AA Q-TOF MS spectrometer in positive electrospray ionisation (ESI^+) and Agilent LC1200 Series coupled to MS6210 TOF spectrometer in electrospray ionisation (ESI^+) respectively. Mobile phase was composed of MeCN/MeOH 50:50. Flow rate: 0.6 mL/min.

Infrared spectra were recorded on Jasco FT-IR 6300 using a diamond ATR crystal cell or NaCl cell using CS_2 as solvent.

Melting points were measured using Gallenkamp capillary apparatus and are uncorrected.

Optical rotations were measured at 20 °C with a Perkin Elmer 241 nc polarimeter (λ=589 Na, path length 1 dm). Some recorded values were within the error limit of the polarimeter and therefore were not possible to determine them. They are indicated as $[\alpha]_D^{20}<1°$.

5.2. GENERAL PROCEDURES

5.2.1. General procedure for saponification

Vegetable oil and 50% w/v aqueous solution of NaOH (1 equiv.) were placed in a pressure vial. The vial was microwave irradiated (100W) at 150 °C for 10 min. The mixture was acidified until pH 1 with 6 M HCl, and extracted with EtOAc. The organic layer was recovered, washed with H_2O, dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated under reduced pressure.

5.2.2. General procedure for esterification

To a 1 M solution of the corresponding fatty acid in MeOH was added 98% H_2SO_4 (0.1% w/v). The reaction mixture was stirred at reflux for 24 h. The mixture was taken up in H_2O and extracted into Et_2O. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure.

5.2.3. General procedure for ruthenium (II)-catalyzed alcohol oxidation

To a 0.1 M solution of the corresponding homoallylic fatty acid in anhydrous toluene was added acrolein freshly distilled (3 equiv.) and the Shvo's catalyst **6** (0.5% mol). The reaction mixture was purgued with N_2 and stirred under reflux for 30 min. The solvent was evaporated under reduced pressure. The crude reaction mixture was purified by silica gel column chromatography or by recrystallization.

5.2.4. General procedure for enzymatic ester hydrolysis

To a 0.35 M solution of the corresponding methyl ester in H_2O/*tert*-BuOH 1:4 was added Novozym 435® (50% w/w). The reaction mixture was stirred at 45 °C for 24 h. The mixture was filtered and the solvent was evaporated under reduced pressure.

5.2.5. General procedure for amide coupling

To a 0.07 M solution of starting material in anhydrous DMF was added HATU (1.5 equiv.). The reaction mixture was stirred at room temperature for 30 min. Then the corresponding amine salt (1.1 equiv.) and DIPEA (3 equiv.) were added successively. The reaction mixture was stirred at room temperature for 24 h. The mixture was taken up in brine and extracted into EtOAc. The organic extract was washed sequentially with 1 M HCl, saturated $NaHCO_3$ and brine. The organic phase was

dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography.

5.2.6. General procedure for chemical ester hydrolysis

To a 0.2 M solution of the starting material in THF/H_2O 1:1 was added $LiOH \cdot H_2O$ (3 equiv.). The reaction mixture was stirred at room temperature for 20 h. The mixture was acidified until pH 1 with 1 M HCl, and extracted with DCM. The organic layer was recovered, washed with H_2O, dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated under reduced pressure.

5.2.7. General procedure for alcohol oxidation

To a 0.3 M solution of the corresponding homoallylic fatty acid in DCM was added pyridine (12 equiv.) and CrO_3 (6 equiv.). The mixture was vigorously stirred at room temperature for 2 h. The reaction mixture was filtered over Celite® and washed with 1 M HCl. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude reaction mixture residue was purified by silica gel column chromatography.

5.2.8. General procedure for Michael addition

To a 0.07 M solution of (*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-12-oxooctadec-10-enamide **75** in MeOH/H_2O 1:1 was added the corresponding thioacid (3 equiv.) and $NaHCO_3$ (0.04 equiv.). The reaction mixture was stirred at 70 °C for 20 h. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography.

5.2.9. General procedure for DCC-mediated esterification

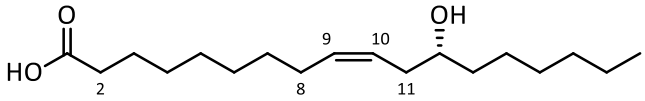
To a 0.05 M solution of (*R,Z*)-*N*-(3-{[*tert*-butyl(dimethyl)silyl]oxy}-4-methoxybenzyl)-14-hydroxyeicos-11-enamide **141** in anhydrous toluene was added DCC (2.6 equiv.), DMAP (1.6 equiv.) and the corresponding acid (1.2 equiv.). The reaction mixture was stirred at room temperature for 3 h. The reaction was filtered and the solvent was evaporated under reduced pressure. The crude was taken up in 1 M HCl and extracted into EtOAc. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography.

5.2.10. General procedure for TBDMS deprotection

To a 0.03 M solution of the starting material in anhydrous THF was added TBAF (1.5 equiv. of a commercial 1 M solution in THF). The reaction mixture was stirred at room temperature for 4 h. EtOAc was added and the organic extract was washed sequentially with 1 M HCl and brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography.

5.3. COMPOUND DESCRIPTION

Ricinoleic acid (**1a**)



Chemical Formula: $C_{18}H_{34}O_3$
MW=298.46 g·mol^{-1}
[141-22-0]

Hydrolysis of castor oil **5a** (100 mg, 0.11 mmol) was performed according to the general procedure **5.2.1.** for saponification using 50% w/v NaOH (0.5 mL, 6.25 mmol) to yield the fatty acid **1a** as a yellowish oil (98 mg, quantitative). The percentage of ricinoleic acid **1a** in the oil was assessed by ^{1}H NMR using dimethylsulphone as internal standard (82%). The spectral data were in accordance with the literature:[2]

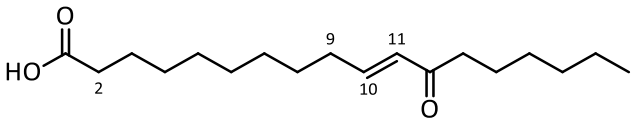
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.25 – 1.37 (m, 16H, **CH_2**), 1.43 – 1.50 (m, 2H, **CH_2**), 1.58 – 1.68 (m, 2H, **CH_2**), 2.04 (q, 2H, *J* = 6.4 Hz, **H-8**), 2.21 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.34 (t, 2H, *J* = 6.9 Hz, **H-11**), 3.57 – 3.67 (m, 1H, **C*H*OH**), 5.33 – 5.44 (m, 1H, **H-10**), 5.50 – 5.61 (m, 1H, **H-9**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.19 (**CH_3**), 22.72 (**CH_2**), 24.84 (**CH_2**), 25.76 (**CH_2**), 27.42 (**CH_2**), 29.07 (**CH_2**), 29.11 (**CH_2**), 29.15 (**CH_2**), 29.44 (**CH_2**), 29.59 (**CH_2**), 31.93 (**CH_2**), 34.34 (**C-2**), 35.29 (**CH_2**), 36.78 (**CH_2**), 71.77 (**CHOH**), 125.27 (**C-10**), 133.28 (**C-9**), 179.52 (**COOH**).

IR (ATR) ν =2927, 2855, 1725, 1454, 1382, 1192, 1143, 841, 708 cm^{-1}.

$[\alpha]_D^{20}$=+6.5° (c 2, acetone)

(*E*)-12-Oxooctadec-10-enoic acid (**1d**)



Chemical Formula: $C_{18}H_{32}O_3$
MW=296.44 g·mol^{-1}
[69727-30-6][3]

Oxidation of ricinoleic acid **1a** (500 mg, 1.68 mmol) was performed according to the general procedure **5.2.3.** for ruthenium (II)-catalyzed alcohol oxidation using Shvo's catalyst **6** (9 mg, 8 µmol) and acrolein freshly distilled (420 µL, 5.04 mmol) in anhydrous toluene (15 mL) to obtain a mixture of oxidized compounds. The residue was dissolved in hot petroleum ether (20 mL), allowed to stand at

room temperature for 1 h and the insoluble material removed by filtration. The filtrate was cooled at -30 °C, the precipitate was collected by vacuum filtration and recrystallized from 70% aqueous solution of EtOH, yielding the enone **1d** (300 mg, 60%) as a white solid. mp=50-51 °C.

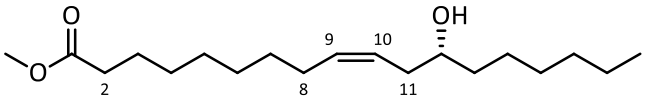
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.25 – 1.34 (m, 14H, **CH_2**), 1.41 – 1.51 (m, 2H, **CH_2**), 1.55 – 1.68 (m, 4H, **CH_2**), 2.20 (q, 2H, *J* = 6.4 Hz, **H-9**), 2.35 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.52 (t, 2H, *J* = 6.9 Hz, **$COCH_2$**), 6.09 (dt, 1H, *J* = 15.9, 1.5 Hz, **H-11**), 6.82 (dt, 1H, *J* = 15.9, 6.9 Hz, **H-10**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.20 (**CH_3**), 22.67 (**CH_2**), 24.47 (**CH_2**), 24.77 (**CH_2**), 28.19 (**CH_2**), 29.09 (**CH_2**), 29.15 (**CH_2**), 29.20 (**CH_2**), 29.21 (**CH_2**), 29.27 (**CH_2**), 31.78 (**CH_2**), 32.56 (**CH_2**), 34.02 (**C-2**), 40.27 (**$CO\underline{C}H_2$**), 130.47 (**C-11**), 147.48 (**C-10**), 179.22 (**COOH**), 201.34 (**$\underline{C}OCH_2$**)**.**

IR (ATR) ν=3029, 2925, 2917, 2850, 2645, 1689, 1628, 1285, 974, 723, 681 cm^{-1}.

HR-MS (ESI^+): *m/z*: $[M+H]^+$ Calcd. for $C_{18}H_{33}O_3$ 297.2424; Found 297.2435.

Methyl ricinoleate (**10a**)



Chemical Formula: $C_{19}H_{36}O_3$
MW=312.49 g·mol^{-1}
[141-24-2]

Esterification of ricinoleic acid **1a** (500 mg, 1.67 mmol) was performed according to the general procedure **5.2.2.** for esterification using 98% H_2SO_4 (100 µL) in MeOH (5 mL) to yield the methyl ester **10a** (365 mg, 70%) as a yellow oil after purification by silica gel column chromatography (petroleum ether/Et_2O 9:1). R_f=0.54 (petroleum ether/Et_2O 8:2). The spectral data were in accordance with the literature:[4]

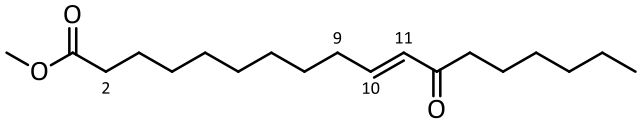
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.25 – 1.36 (m, 16H, **CH_2**), 1.43 – 1.48 (m, 2H, **CH_2**), 1.59 – 1.66 (m, 2H, **CH_2**), 2.05 (q, 2H, *J* = 6.4 Hz, **H-8**), 2.21 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.30 (t, 2H, *J* = 6.9 Hz, **H-11**), 3.57 – 3.65 (m, 1H, **C*H*OH**), 3.66 (s, 3H, **CH_3O**), 5.36 – 5.45 (m, 1H, **H-10**), 5.52 – 5.60 (m, 1H, **H-9**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.18 (**CH_3**), 22.72 (**CH_2**), 25.01 (**CH_2**), 25.81 (**CH_2**), 27.46 (**CH_2**), 29.17 (2x**CH_2**), 29.21 (**CH_2**), 29.45 (**CH_2**), 29.66 (**CH_2**), 31.94 (**CH_2**), 34.17 (**C-2**), 35.42 (**CH_2**), 36.91 (**CH_2**), 51.56 (**CH_3O**), 71.61 (**CHOH**), 125.33 (**C-10**), 133.36 (**C-9**), 174.45 (**$\underline{C}OOCH_3$**).

IR (ATR) ν=3439, 2925, 2854, 1740, 1457, 1436, 1171, 1017, 857, 724 cm^{-1}.

$[\alpha]_D^{20}$=+7.1° (c 1.7, MeOH)

Methyl (*E*)-12-oxooctadec-10-enoate (**10d**)



Chemical Formula: $C_{19}H_{34}O_3$
MW=310.47 g·mol^{-1}
[21308-79-2]

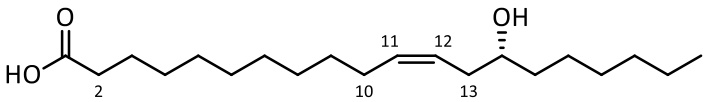
Oxidation of methyl ricinoleate **10a** (500 mg, 1.60 mmol) was performed according to the general procedure **5.2.3.** for ruthenium (II)-catalyzed alcohol oxidation using Shvo's catalyst **6** (9 mg, 8 µmol) and acrolein freshly distilled (390 µL, 4.80 mmol) in anhydrous toluene (15 mL) to yield the enone **10d** (348 mg, 70%) as a yellowish oil after purification by silica gel column chromatography (petroleum ether/Et_2O 95:5). R_f=0.50 (petroleum ether/Et_2O 9:1). The spectral data were in accordance with the literature:[5]

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.25 – 1.34 (m, 14H, **CH_2**), 1.41 – 1.49 (m, 2H, **CH_2**), 1.57 – 1.66 (m, 4H, **CH_2**), 2.20 (q, 2H, *J* = 6.4 Hz, **H-9**), 2.30 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.52 (t, 2H, *J* = 6.9 Hz, **$COCH_2$**), 3.67 (s, 3H, **CH_3O**), 6.08 (dt, 1H, *J* = 15.9, 1.5 Hz, **H-11**), 6.80 (dt, 1H, *J* = 15.9, 6.9 Hz, **H-10**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.17 (**CH_3**), 22.64 (**CH_2**), 25.02 (**CH_2**), 28.21 (**CH_2**), 29.23 (**CH_2**), 29.23 (5x**CH_2**), 31.76 (**CH_2**), 32.54 (**CH_2**), 34.19 (**C-2**), 40.24 (**$CO\underline{C}H_2$**), 51.57 (**CH_3O**), 130.45 (**C-11**), 147.37 (**C-10**), 174.40 (**$\underline{C}OOCH_3$**), 201.15 (**$\underline{C}OCH_2$**).

IR (ATR) ν=2927, 2855, 1736, 1709, 1436, 1195, 1169, 1104, 979, 880, 752 cm^{-1}.

Lesquerolic acid (**12a**)



Chemical Formula: $C_{20}H_{38}O_3$
MW=326.51 g·mol^{-1}
[4103-20-2][3]

Hydrolysis of lesquerella oil **11a** (200 mg, 0.2 mmol) was performed according to the general procedure **5.2.1.** for saponification using an aqueous solution of NaOH (50% w/v, 0.9 mL, 11.4 mmol) to yield the fatty acid **12a** as an orange oil (190 mg, quantitative). The percentage of lesquerolic acid

12a in the oil was assessed by ^{1}H NMR using dimethylsulphone as internal standard (72%). The spectral data were in accordance with the literature:[6]

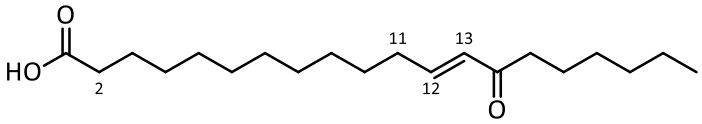
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.26 – 1.34 (m, 20H, **CH_2**), 1.44 – 1.49 (m, 2H, **CH_2**), 1.59 – 1.66 (m, 2H, **CH_2**), 2.05 (q, 2H, *J* = 6.4 Hz, **H-10**), 2.22 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.34 (t, 2H, *J* = 6.9 Hz, **H-13**), 3.59 – 3.67 (m, 1H, **C*H*OH**), 5.34 – 5.45 (m, 1H, **H-12**), 5.52 – 5.61 (dd, 1H, *J* = 18.1, 7.2 Hz, **H-11**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.23 (**CH_3**), 22.75 (**CH_2**), 24.96 (**CH_2**), 25.83 (**CH_2**), 27.55 (**CH_2**), 29.22-29.83 (8x**CH_2**), 31.96 (**CH_2**), 34.16 (**C-2**), 36.72 (**CH_2**), 71.76 (**CHOH**), 125.18 (**C-12**), 147.01 (**C-11**), 173.48 (**COOH**).

IR (ATR) ν=2923, 2854, 1709, 1458, 1408, 1282, 1190, 1084, 935, 724 cm^{-1}.

$[\alpha]_D^{20}$=+6° (c 1.6, $CHCl_3$)

(*E*)-14-Oxoeicos-12-enoic acid (**12d**)



Chemical Formula: $C_{20}H_{36}O_3$
MW=324.50 g·mol^{-1}

Oxidation of lesquerolic acid **12a** (100 mg, 0.31 mmol) was performed according to the general procedure **5.2.3.** for ruthenium (II)-catalyzed alcohol oxidation using Shvo's catalyst **6** (1.6 mg, 0.5% mol) and acrolein freshly distilled (75 µL, 0.93 mmol) in anhydrous toluene (3.2 mL) to obtain a mixture of oxidized compounds. The residue was dissolved in hot petroleum ether (10 mL), allowed to stand at room temperature for 1 h and the insoluble material removed by filtration. The filtrate was cooled at -30 °C, the precipitate was collected by vacuum filtration and recrystallized from petroleum ether, yielding the enone **12d** (50 mg, 50%) as a white solid. mp=58-59 °C.

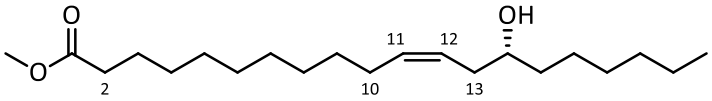
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.25 – 1.34 (m, 18H, **CH_2**), 1.41 – 1.50 (m, 2H, **CH_2**), 1.55 – 1.67 (m, 4H, **CH_2**), 2.20 (q, 2H, *J* = 6.4 Hz, **H-2**), 2.35 (t, 2H, *J* = 6.9 Hz, **H-10**), 2.52 (t, 2H, *J* = 6.9 Hz, **$COCH_2$**), 6.09 (dt, 1H, *J* = 15.9, 1.5 Hz, **H-13**), 6.82 (dt, 1H *J* = 15.9, 6.9 Hz, **H-12**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.18 (**CH_3**), 22.65 (**CH_2**), 24.47 (**CH_2**), 24.80 (**CH_2**), 28.23 (**CH_2**), 29.13 (**CH_2**), 29.15 (**CH_2**), 29.29 (**CH_2**), 29.32 (**CH_2**), 29.46 (**CH_2**), 29.49 (**CH_2**), 29.54 (**CH_2**), 31.76 (**CH_2**), 32.58 (**CH_2**), 34.16 (**C-2**), 40.22 (**CO*C*H_2**), 130.43 (**C-13**), 147.60 (**C-12**), 179.97 (**COOH**), 201.37 (***C*OCH$_2$**).

IR (ATR) ν=3108, 3047, 2953, 2912, 2848, 1690, 1633, 1470, 1298, 975, 899, 716 cm^{-1}.

HR-MS (ESI$^+$): *m/z*: $[M+K]^+$ Calcd. for $C_{20}H_{36}O_3K$ 363.2269; Found 363.2301.

Methyl lesquerolate (**13a**)



Chemical Formula: $C_{21}H_{40}O_3$
MW=340.54 g·mol^{-1}
[4102-96-9]

Esterification of lesquerolic acid **12a** (100 mg, 0.31 mmol) was performed according to the general procedure **5.2.2.** for esterification using 98% H_2SO_4 (10 µL) in MeOH (310 µL) to yield the methyl ester **13a** (73 mg, 70%) as an orange oil after purification by silica gel column chromatography (petroleum ether/Et_2O 9:1). R_f=0.56 (petroleum ether/Et_2O 8:2). The spectral data were in accordance with the literature:[7]

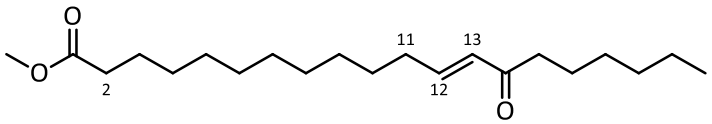
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.25 – 1.32 (m, 20H, **CH_2**), 1.44 – 1.48 (m, 2H, **CH_2**), 1.59 – 1.64 (m, 2H, **CH_2**), 2.05 (q, 2H, *J* = 6.4 Hz, **H-10**), 2.21 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.30 (t, 2H, *J* = 6.9 Hz, **H-13**), 3.58 – 3.63 (m, 1H, **C*H*OH**), 3.66 (s, 3H, **CH_3O**), 5.36 – 5.44 (m, 1H, **H-12**), 5.52 – 5.61 (m, 1H, **H-11**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.20 (**CH_3**), 22.73 (**CH_2**), 25.05 (**CH_2**), 25.82 (**CH_2**), 27.52 (**CH_2**), 29.24 (**CH_2**), 29.34 (**CH_2**), 29.38 (**CH_2**), 29.46 (**CH_2**), 29.49 (**CH_2**), 29.55 (**CH_2**), 29.76 (**CH_2**), 31.95 (**CH_2**), 34.22 (**C-2**), 35.41 (**CH_2**), 36.90 (**CH_2**), 51.59 (**CH_3O**), 71.68 (**CHOH**), 125.21 (**C-12**), 133.56 (**C-11**), 174.54 (***C*OOCH$_3$**).

IR (ATR) ν=2922, 2853, 1741, 1458, 1435, 1362, 1170, 1079, 1029, 855, 723 cm^{-1}.

$[\alpha]_D^{20}$=+10.1° (c 1.8, MeOH)

Methyl (*E*)-14-oxoeicos-12-enoate (**13d**)



Chemical Formula: $C_{21}H_{38}O_3$
MW=338.52 g·mol^{-1}

Oxidation of methyl lesquerolate **13a** (384 mg, 1.13 mmol) was performed according to the general procedure **5.2.3.** for ruthenium (II)-catalyzed alcohol oxidation using Shvo's catalyst **6** (6 mg, 5.6 µmol) and acrolein freshly distilled (275 µL, 3.4 mmol) in anhydrous toluene (11 mL) to yield the

enone **13d** (203 mg, 53%) as a colorless oil after purification by silica gel column chromatography (petroleum ether/Et_2O 94:6). R_f=0.49 (petroleum ether/Et_2O 9:1).

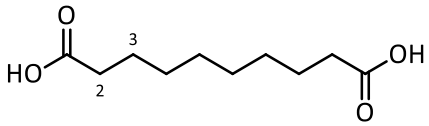
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.24 – 1.31 (m, 18H, **CH_2**), 1.40 – 1.49 (m, 2H, **CH_2**), 1.55 – 1.65 (m, 4H, **CH_2**), 2.19 (q, 2H, *J* = 6.4 Hz, **H-2**), 2.29 (t, 2H, *J* = 6.9 Hz, **H-11**), 2.51 (t, 2H, *J* = 6.9 Hz, **$COCH_2$**), 3.65 (s, 3H, **CH_3O**), 6.07 (dt, 1H, *J* = 15.9, 1.5 Hz, **H-13**), 6.81 (dt, 1H, *J* = 15.9, 6.9 Hz, **H-12**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.18 (**CH_3**), 22.65 (**CH_2**), 24.45 (**CH_2**), 25.07 (**CH_2**), 28.24 (**CH_2**), 29.13 (**CH_2**), 29.25 (**CH_2**), 29.30 (**CH_2**), 29.35 (**CH_2**), 29.48 (**CH_2**), 29.51 (**CH_2**), 29.56 (**CH_2**), 31.76 (**CH_2**), 32.58 (**CH_2**), 34.22 (**C-2**), 40.24 (**CO*C*H_2**), 51.58 (**CH_3O**), 130.44 (**C-13**), 147.47 (**C-12**), 174.46 (***C*$OOCH_3$**), 201.20 (***C*OCH_2**).

IR (ATR) ν=2925, 2855, 1735, 1707, 1436, 1170, 1104, 935, 724 cm^{-1}.

HR-MS (ESI^+): *m/z*: $[M+H]^+$ Calcd. for $C_{21}H_{39}O_3$ 339.2867; Found 339.2896.

Sebacic acid (**14**)



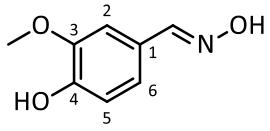
Chemical Formula: $C_{10}H_{18}O_4$
MW=202.25 g·mol^{-1}
[111-20-6]

To a 0.18 M solution of (*E*)-12-oxo-10-octadecenoic acid **12a** (1g, 3.37 mmol) in MeCN/H_2O (3:1) was added Oxone™ (4.15 g, 6.75 mmol) and $NaIO_4$ (1.08 g, 5.06 mmol). The mixture was stirred at reflux for 24 h. Then, a volume of 24 mL of H_2O was added and the mixture was stirred at reflux for 2h. The reaction mixture was filtered and was extracted with EtOAc (4x20 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude reaction mixture residue was recrystallized in hot H_2O to obtain the sebacic acid **14** (228.1 mg, 41%) as a white solid. mp=133-137 °C. The spectral data were in accordance with the literature:[8]

^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ = 1.29 – 1.37 (m, 8H, **CH_2**), 1.53 – 1.63 (m, 4H, **H-3**), 2.27 (t, *J* = 6.9 Hz, 4H, **H-2**), 10.38 (br s, 2H, **COOH**).

IR (ATR) ν=3024, 1932, 2917, 2869, 2850, 2662, 1685, 1407, 1297, 1187, 923, 676 cm^{-1}.

(*E*)-4-Hydroxy-3-methoxybenzaldehyde oxime (**98**)



Chemical Formula: $C_8H_9NO_3$
MW=167.16 g·mol^{-1}
[2874-33-1]

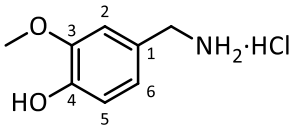
Hydroxylamine hydrochloride (2.37 g, 34.0 mmol) in H_2O (10 mL) and sodium acetate trihydrate (4.48 g, 32.9 mmol) in H_2O (10 mL) were successively added to a solution of vanillin **97** (5.00 g, 32.9 mmol) in H_2O (30 mL). The reaction mixture was stirred at 80 °C for 2 h. The crude reaction was extracted with EtOAc, the organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated under reduced pressure to yield the oxime **98** (5.26 g, 97%) as a white-off solid. mp=118-119 °C. The spectral data were in accordance with the literature:[9]

^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 3.77 (s, 3H, **CH_3O**), 6.78 (d, 1H, *J* = 8.1 Hz, **H-5**), 6.97 (dd, 1H, *J* = 8.1, 1.9 Hz, **H-6**), 7.16 (d, 1H, *J* = 1.9 Hz, **H-2**), 7.99 (s, 1H, **CH=N**), 9.33 (s, 1H, **OH**), 10.84 (s, 1H, **N-OH**).

^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ = 55.49 (**CH_3O**), 109.20 (**C-2**), 115.48 (**C-5**), 120.51 (**C-6**), 124.46 (**C-1**), 147.84 (**C-4**), 148.00 (**C-3**), 148.09 (**CH=N**).

IR (ATR) v=3444, 3213, 3008, 2941, 1596, 1513, 1428, 1027, 969 cm^{-1}.

4-Hydroxy-3-methoxybenzylamine hydrochloride (**99**)



Chemical Formula: $C_8H_{12}ClNO_2$
MW=189.64 g·mol^{-1}
[7149-10-2]

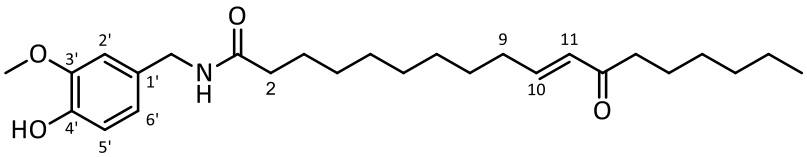
A volume of 37% HCl (20 mL, 0.26 mol) and Pd/C (10 wt. % loading) (20% w/w, 1.05 g) were added to a solution of (*E*)-4-hydroxy-3-methoxybenzaldehyde oxime **98** (5.2 g, 0.03 mol) in EtOH (150 mL). The reaction mixture was hydrogenated at 1 atm at room temperature for 24 h. The crude reaction was filtered over Celite® and the solvent volume was reduced under pressure. The residue was crystallised from EtOAc and filtered to yield the amine hydrochloride salt **99** (4.2 g, 74%) as a white solid. mp=219-222 °C. The spectral data were in accordance with the literature:[10]

^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 3.77 (s, 3H, **CH_3O**), 3.83 – 3.90 (m, 2H, **CH_2NH_2**), 6.79 (d, 1H, *J* = 8.1 Hz, **H-5**), 6.85 (dd, 1H, *J* = 8.1, 1.9 Hz, **H-6**), 7.18 (d, 1H, *J* = 1.9 Hz, **H-2**), 8.40 (br, s, 3H, **NH_2**, **HCl**), 9.19 (s, 1H, **OH**).

^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ = 42.18 (**CH_2**), 55.69 (**CH_3O**), 113.44 (**C-2**), 115.26 (**C-5**), 121.73 (**C-6**), 124.64 (**C-1**), 146.80 (**C-4**), 147.51 (**C-3**).

IR (ATR) ν=3112, 3024, 2805, 1763, 1377, 1033, 828, 670 cm^{-1}.

(*E*)-*N*-(4-Hydroxy-3-methoxybenzyl)-12-oxooctadec-10-enamide (**75**)



Chemical Formula: $C_{26}H_{41}NO_4$
MW=431.61 g·mol^{-1}

Amidation of (*E*)-12-oxo-10-octadecenoic acid **1d** (100 mg, 0.34 mmol) was performed according to the general procedure **5.2.5.** for amide coupling using HATU (193 mg, 0.51 mmol), 4-hydroxy-3-methoxybenzylamine hydrochloride **99** (57 mg, 0.37 mmol) and DIPEA (177 µL, 1.0 mmol) in anhydrous DMF (5 mL) to yield the amide **75** (85 mg, 60%) as a white solid after purification by silica gel flash column chromatography (petroleum ether/EtOAc 7:3). R_f=0.48 (petroleum ether/EtOAc 4:6). mp=64-66 °C.

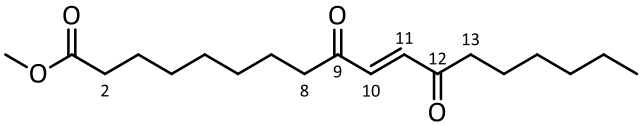
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.24 – 1.31 (m, 14H, **CH_2**), 1.39 – 1.48 (m, 2H, **CH_2**), 1.54 – 1.69 (m, 4H, **CH_2**), 2.14 – 2.23 (m, 4H, **H-2**, **H-9**), 2.51 (t, 2H, *J* = 6.9 Hz, **$COCH_2$**), 3.86 (s, 3H, **CH_3O**), 4.34 (d, 2H, *J* = 5.7 Hz, **CH_2NH**), 5.75 (s, 1H, **OH**, **CH_2NH**), 6.08 (d, 1H, *J* = 15.9 Hz, **H-11**), 6.72 – 6.89 (m, 4H, **H_{Ar}**, **H-10**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.18 (**CH_3**), 22.65 (**CH_2**), 24.43 (**CH_2**), 25.85 (**CH_2**), 28.17 (**CH_2**), 29.12 (**CH_2**), 29.19 (**CH_2**), 29.31 (**CH_2**), 29.33 (**CH_2**), 29.36 (**CH_2**), 31.76 (**CH_2**), 32.52 (**CH_2**), 36.93 (**C-2**), 40.25 (**$COCH_2$**), 43.65 (**CH_2NH**), 56.05 (**CH_3O**), 110.82 (**C-2'**), 114.49 (**C-5'**), 120.90 (**C-6'**), 130.45 (**C-1'**), 130.48 (**C-11**), 145.23 (**C-4'**), 146.81 (**C-3'**), 147.44 (**C-10**), 173.01 (**NHCO**), 201.28 (**$COCH_2$**).

IR (ATR) ν=3301, 2913, 2849, 1644, 1554, 1271, 1154, 1038, 714 cm^{-1}.

HR-MS (ESI^+): *m/z*: $[M+Na]^+$ Calcd. for $C_{26}H_{41}NO_4Na$ 454.2928; Found 454.2929.

Methyl (*E*)-9,12-dioxooctadec-10-enoate (**112**)



Chemical Formula: $C_{19}H_{32}O_4$
MW=324.45 g·mol^{-1}
[91482-34-7]

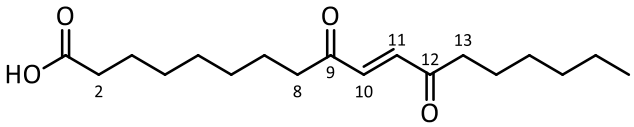
A solution of methyl (*E*)-12-oxooctadec-10-enoate **1d** (1g, 3.22 mmol) in DCM (6 mL) was slowly added to a solution of CrO_3 (1.93 g, 19 mmol) and pyridine (3.1 mL, 39 mmol) in DCM (6 mL). The reaction mixture was vigorously stirred at room temperature for 3 h. The crude reaction was filtered over Celite® and washed with 1 M HCl. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to yield the 9,12-diketone **112** (650 mg, 61%) as a white solid after purification by silica gel column chromatography (petroleum ether/Et_2O 95:5). R_f=0.44 (petroleum ether/Et_2O 9:1). mp=56-59 °C. The spectral data were in accordance with the literature:[11]

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.26 – 1.34 (m, 12H, **CH_2**), 1.58 – 1.66 (m, 6H, **CH_2**), 2.29 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.63 (t, 4H, *J* = 6.9, **H-8**, **H-13**), 3.66 (s, 3H, **CH_3O**), 6.86 (s, 2H, **H-10**, **H-11**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.14 (**CH_3**), 22.60 (**CH_2**), 23.76 (**CH_2**), 23.85 (**CH_2**), 24.97 (**CH_2**), 28.94 (**CH_2**), 29.04 (2x**CH_2**), 29.13 (**CH_2**), 31.68 (**CH_2**), 34.16 (**C-2**), 41.70 (**C-8**), 41.80 (**C-13**), 51.60 (**CH_3O**), 136.34 (**C-11**), 136.42 (**C-10**), 174.35 (**$\underline{C}OOCH_3$**), 200.83 (**C-9**), 200.95 (**C-12**).

IR (ATR) v=2954, 2926, 2851, 1728, 1675, 1379, 1170, 975, 882, 722 cm^{-1}.

(*E*)-9,12-dioxooctadec-10-enoic acid (**113**)



Chemical Formula: $C_{18}H_{30}O_4$
MW=310.43 g·mol^{-1}
[28833-56-9][12]

The hydrolysis of methyl (*E*)-9,12-dioxooctadec-10-enoate **112** (40 mg, 0.12 mmol) was performed according to the general procedure **5.2.4.** for enzymatic ester hydrolysis using Novozym 435® (30 mg) in H_2O (86 µL) and *tert*-BuOH (256 µL) to yield the acid **113** (37 mg, quantitative) as a white sticky solid. mp=99-101 °C.

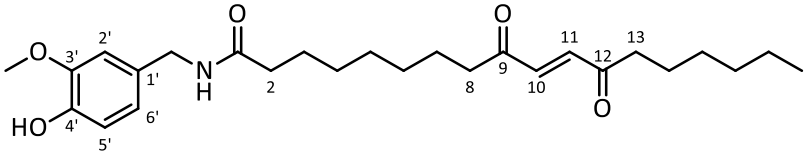
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.23 – 1.34 (m, 12H, **CH_2**), 1.58 – 1.65 (m, 6H, **CH_2**), 2.33 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.62 (t, 4H, *J* = 6.9 Hz, **H-8**, **H-13**), 6.85 (s, 2H, **H-10**, **H-11**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.12 (**CH_3**), 22.57 (**CH_2**), 23.72 (**CH_2**), 23.83 (**CH_2**), 24.68 (**CH_2**), 28.90 (**CH_2**), 28.92 (**CH_2**), 28.98 (**CH_2**), 29.07 (**CH_2**), 31.65 (**CH_2**), 34.06 (**C-2**), 41.65 (**C-8**), 41.76 (**C-13**), 136.35 (**C-11**), 136.43 (**C-10**), 179.72 (**COOH**), 200.91 (**C-9**), 201.06 (**C-12**).

IR (ATR) ν=3373, 2914, 1697, 1678, 1379, 1263, 1079, 995, 931, 724 cm^{-1}.

HR-MS (ESI^+): *m/z*: $[M+Na]^+$ Calcd. for $C_{18}H_{30}O_4Na$ 333.2036; Found 333.2064.

(*E*)-*N*-(4-Hydroxy-3-methoxybenzyl)-9,12-dioxooctadec-10-enamide (**76**)



Chemical Formula: $C_{26}H_{39}NO_5$
MW=445.59 g·mol^{-1}

Amidation of (*E*)-9,12-dioxooctadec-10-enoic acid **113** (40 mg, 0.18 mmol) was performed according to the general procedure **5.2.5.** for amide coupling using HATU (73 mg, 0.19 mmol), 4-hydroxy-3-methoxybenzylamine hydrochloride **99** (27 mg, 0.14 mmol) and DIPEA (67 µL, 0.31 mmol) in anhydrous DMF (2.6 mL) to yield the amide **76** (36 mg, 57%) as a yellow oil after purification by silica gel flash column chromatography (petroleum ether/EtOAc 1:1). R_f=0.4 (petroleum ether/EtOAc 1:1).

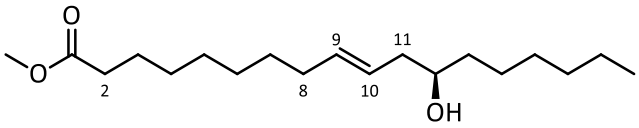
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.24 – 1.34 (m, 12H, **CH_2**), 1.56 – 1.68 (m, 6H, **CH_2**), 2.19 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.63 (t, 4H, *J* = 6.9 Hz, **H-8**, **H-13**), 3.87 (s, 3H, **CH_3O**), 4.35 (d, 2H, *J* = 5.7 Hz, **C$\underline{H_2}$NH**), 5.74 (br s, 1H, **OH**), 6.72 – 6.88 (m, 5H, **H_{Ar}**, **H-10**, **H-11**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.15 (**CH_3**), 22.61 (**CH_2**), 23.72 (**CH_2**), 23.84 (**CH_2**), 25.75 (**CH_2**), 28.93 (**CH_2**), 29.01 (**CH_2**), 29.14 (**CH_2**), 29.16 (**CH_2**), 31.68 (**CH_2**), 36.83 (**C-2**), 41.66 (**C-8**), 41.81 (**C-13**), 43.72 (**CH_2NH**), 56.09 (**CH_3O**), 110.86 (**C-2'**), 114.51 (**C-5'**), 120.96 (**C-6'**), 130.45 (**C-1'**), 136.32 (**C-11**), 136.44 (**C-10**), 145.27 (**C-4'**), 146.82 (**C-3'**), 172.99 (**NHCO**), 200.87 (**C-9**), 200.99 (**C-12**).

IR (ATR) ν=3503, 3310, 2953, 2927, 2851, 1677, 1640, 1518, 1257, 1024, 856, 722 cm^{-1}.

HR-MS (ESI^+): *m/z*: $[M+H]^+$ Calcd. for $C_{26}H_{40}NO_5$ 446.2901; Found 446.2936.

Methyl (*R,E*)-12-hydroxyoctadec-9-enoate (**114**)



Chemical Formula: $C_{19}H_{36}O_3$
MW=312.49 g·mol^{-1}
[7706-01-6]

Diphenyl disulfide (56 mg, 0.26 mmol) was added to a solution of methyl ricinoleate **10a** (4 g, 12.8 mmol) in isooctane (120 mL). The reaction mixture was placed in a photochemical reactor and irradiated for 3 h with a Philips HP(L) 400-W medium-pressure mercury lamp. After irradiation the solvent was removed under reduced pressure and the crude reaction mixture was dissolved in hot petroleum ether (185 mL). The filtrate was cooled at -30 °C and after 48 h a white solid appeared. This solid was quickly filtered and recovered at -30 °C to yield the isomer **114** (1.49 g, 37%) as a yellowish oil at room temperature. The spectral data were in accordance with the literature:[13]

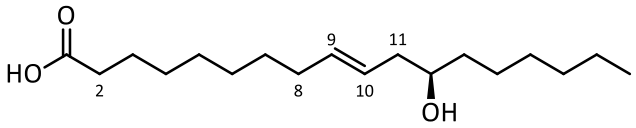
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.23 – 1.39 (m, 16H, **CH_2**), 1.39 – 1.48 (m, 3H, **CH_2**), 1.55 – 1.71 (m, 2H, **CH_2**), 1.97 – 2.09 (m, 3H, **H-8**, **H-11a**), 2.18 – 2.26 (m, 1H, **H-11b**), 2.29 (t, 2H, *J* = 6.9 Hz, **H-2**), 3.53 – 3.61 (m, 1H, **C*H*OH**), 3.65 (s, 3H, **CH_3O**), 5.35 – 5.44 (m, 1H, **H-10**), 5.47 – 5.56 (m, 1H, **H-9**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.22 (**CH_3**), 22.75 (**CH_2**), 25.05 (**CH_2**), 25.79 (**CH_2**), 29.06 (**CH_2**), 29.20 (**CH_2**), 29.22 (**CH_2**), 29.49 (2x**CH_2**), 31.97 (**CH_2**), 32.75 (**CH_2**), 34.22 (**C-2**), 36.88 (**CH_2**), 40.85 (**C-11**), 51.57 (**CH_3O**), 71.06 (**CHOH**), 126.07 (**C-10**), 134.69 (**C-9**), 174.44 (**C**$COOCH_3$).

IR (ATR) ν=3431, 2924, 2854, 1740, 1435, 1197, 1171, 969, 860, 724 cm^{-1}.

$[\alpha]_D^{20}$=-0.2° (c 2.44, $CHCl_3$)

(*R,E*)-12-Hydroxyoctadec-9-enoic acid (**2**)



Chemical Formula: $C_{18}H_{34}O_3$
MW=298.46 g·mol^{-1}
[540-12-5][14]

The hydrolysis of methyl (*R,E*)-12-hydroxyoctadec-9-enoate **114** (200 mg, 0.64 mmol) was performed according to the general procedure **5.2.6.** for chemical ester hydrolysis using LiOH·H_2O (46 mg, 1.92 mmol) in THF/H_2O 1:1 (3 mL) to yield the fatty acid **2** (150 mg, 78%) as a yellowish solid after recrystallization in hot petroleum ether. mp=49-51 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.22 – 1.40 (m, 16H, **CH_2**), 1.40 – 1.50 (m, 4H, **CH_2**), 1.58 – 1.68 (m, 2H, **CH_2**), 1.97 – 2.11 (m, 3H, **H-8**, **H-11a**), 2.19 – 2.28 (m, 1H, **H-11b**), 2.33 (t, 2H, *J* = 6.9 Hz, **H-2**), 3.54 – 3.63 (m, 1H, **C*H*OH**), 5.35 – 5.46 (m, 1H, **H-10**), 5.47 – 5.58 (m, 1H, **H-9**).

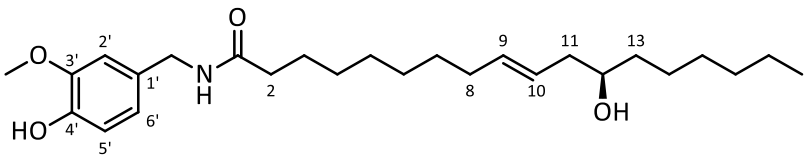
^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.24 (**CH_3**), 22.77 (**CH_2**), 24.79 (**CH_2**), 25.79 (**CH_2**), 29.02 (**CH_2**), 29.11 (**CH_2**), 29.15 (**CH_2**), 29.47 (**CH_2**), 29.50 (**CH_2**), 31.98 (**CH_2**), 32.73 (**CH_2**), 34.06 (**C-2**), 36.86 (**CH_2**), 40.81 (**C-11**), 71.17 (**CHOH**), 126.05 (**C-10**), 134.74 (**C-9**), 179.27 (**COOH**).

IR (ATR) ν=3321, 3221, 3040, 2955, 2916, 2848, 1690, 1466, 1072, 959, 720, 682 cm^{-1}.

HR-MS (ESI$^+$): *m/z*: $[M+Na]^+$ Calcd. for $C_{18}H_{34}O_3Na$ 321.240; Found 321.2411.

$[\alpha]_D^{20}$=+6.6° (c 1, EtOH)

(*R,E*)-*N*-(4-Hydroxy-3-methoxybenzyl)-12-hydroxyoctadec-9-enamide (**77**)



Chemical Formula: $C_{26}H_{43}NO_4$
MW=433.62 g·mol^{-1}

Amidation of (*E*)-12-hydroxy-9-octadecenoic acid **2** (70 mg, 0.23 mmol) was performed according to the general procedure **5.2.5.** for amide coupling using HATU (133 mg, 0.35 mmol), 4-hydroxy-3-methoxybenzylamine hydrochloride **99** (53 mg, 0.28 mmol) and DIPEA (122 µL, 0.70 mmol) in anhydrous DMF (3.3 mL) to yield the amide **76** (35 mg, 34%) as a white-off solid after purification by

silica gel flash column chromatography (petroleum ether/EtOAc 6:4). R_f=0.37 (petroleum ether/EtOAc 6:4). mp=73-75 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.24 – 1.35 (m, 15H, **CH_2**, **H-13a**), 1.37 – 1.46 (m, 3H, **CH_2**, **H-13b**), 1.59 – 1.71 (m, 2H, **CH_2**), 1.96 – 2.09 (m, 3H, **H-8**, **H-11a**), 2.14 – 2.27 (m, 3H, **H-2**, **H-11b**), 3.53 – 3.61 (m, 1H, **C*H*OH**), 3.87 (s, 3H, **CH_3O**), 4.34 (d, *J* = 5.7 Hz, 2H, **C*H*$_2$NH**), 5.35 – 5.44 (m, 1H, **H-10**), 5.47 – 5.56 (m, 1H, **H-9**), 5.73 (br s, 2H, **CH_2NH**, **OH**), 6.79 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, **H_{Ar}**).

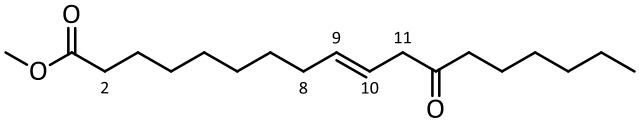
^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.23 (**CH_3**), 22.75 (**CH_2**), 25.79 (**CH_2**), 25.86 (**CH_2**), 29.06 (**CH_2**), 29.26 (**CH_2**), 29.35 (**CH_2**), 29.46 (**CH_2**), 29.49 (**CH_2**), 31.97 (**C-8**), 32.73 (**CH_2**), 36.91 (**C-13**), 36.96 (**C-2**), 40.82 (**C-11**), 43.65 (**CH_2NH**), 56.07 (**CH_3O**), 71.07 (**CHOH**), 110.86 (**C-2'**), 114.53 (**C-5'**), 120.91 (**C-6'**), 126.12 (**C-10**), 130.54 (**C-1'**), 134.68 (**C-9**), 145.26 (**C-4'**), 146.84 (**C-3'**), 173.01 (**NHCO**).

IR (ATR) ν=3295, 2920, 2849, 1631, 1515, 1463, 1270, 1030, 959 cm^{-1}.

HR-MS (ESI^+): *m/z*: $[M+H]^+$ Calcd. for $C_{26}H_{44}NO_4$ 434.3265; Found 434.3293.

$[\alpha]_D^{20}$<+1° (c 0.5, DCM)

Methyl (*E*)-12-oxooctadec-9-enoate (**10c**)



Chemical Formula: $C_{19}H_{34}O_3$
MW=310.47 g·mol^{-1}
[21994-17-2]

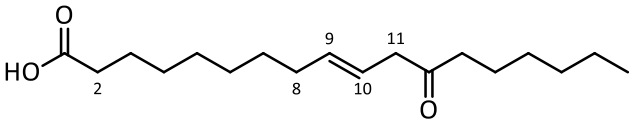
Oxidation of methyl (*R,E*)-12-hydroxyoctadec-9-enoate **114** (500 mg, 1.6 mmol) was performed according to the general procedure **5.2.7.** for alcohol oxidation using CrO_3 (960 mg, 9.6 mmol) and pyridine (1.5 mL, 19.2 mmol) in DCM (6 mL) to yield the ketone **10c** (246 g, 49%) as a yellowish oil after purification by silica gel column chromatography (petroleum ether/Et_2O 98:2). R_f=0.48 (petroleum ether/Et_2O 9:1). The spectral data were in accordance with the literature:[15]

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.21 – 1.39 (m, 14H, **CH_2**), 1.51 – 1.69 (m, 4H, **CH_2**), 1.98 – 2.05 (m, 2H, **H-8**), 2.29 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.41 (t, 2H, *J* = 6.9 Hz, **$COCH_2$**), 3.07 (d, 2H, *J* = 5.7 Hz, **H-11**), 3.66 (s, 3H, **CH_3O**), 5.48 – 5.52 (m, 2H, **H-9**, **H-10**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.16 (**CH_3**), 22.63 (**CH_2**), 23.84 (**CH_2**), 25.06 (**CH_2**), 29.03 (**CH_2**), 29.06 (**CH_2**), 29.21 (2x**CH_2**), 29.27 (**CH_2**)**,** 31.73 (**CH_2**), 32.67 (**CH_2**), 34.22(**C-2**), 42.31 (**CO$\underline{C}H_2$**), 46.95 (**C-11**), 51.57 (**CH_3O**), 122.13 (**C-10**), 135.16 (**C-9**), 174.42 (**$\underline{C}OOCH_3$**), 209.95 (**$\underline{C}OCH_2$**).

IR (ATR) ν=2925, 2854, 1738, 1715, 1435, 1362, 1195, 1170, 968, 725 cm^{-1}.

(*E*)-12-Oxooctadec-9-enoic acid (**1c**)



Chemical Formula: $C_{18}H_{32}O_3$
MW=296.44 g·mol^{-1}
[6629-55-6][12]

The hydrolysis of methyl (*E*)-12-oxooctadec-9-enoate **10c** (20 mg, 0.06 mmol) was performed according to the general procedure **5.2.4.** for enzymatic ester hydrolysis using Novozym 435® (20 mg) in H_2O (31 µL) and *tert*-BuOH (138 µL) to yield the ketone **1c** (17 mg, 89%) as a white solid. mp=71-73 °C.

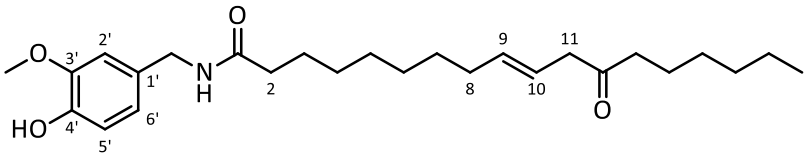
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.24 – 1.36 (m, 14H, **CH_2**), 1.50 – 1.58 (m, 2H, **CH_2**), 1.58 – 1.66 (m, 2H, **CH_2**), 1.98 – 2.05 (m, 2H, **H-8**), 2.34 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.41 (t, 2H, *J* = 6.9 Hz, **$COCH_2$**), 3.08 (d, 2H, *J* = 5.7 Hz, **H-11**), 5.44 – 5.57 (m, 2H, **H-9**, **H-10**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.17 (**CH_3**), 22.63 (**CH_2**), 23.85 (**CH_2**), 24.79 (**CH_2**), 29.03 (2x**CH_2**), 29.12 (**CH_2**), 29.18 (**CH_2**), 29.26 (**CH_2**), 31.73 (**CH_2**), 32.66 (**CH_2**), 34.09 (**C-2**), 42.32 (**CO$\underline{C}H_2$**), 46.95 (**C-11**), 122.13 (**C-10**), 135.17 (**C-9**), 179.59 (**COOH**), 210.13 (**$\underline{C}OCH_2$**).

IR (ATR) ν=3121, 2954, 2918, 2848, 1701, 1263, 1082, 962, 720, 689 cm^{-1}.

HR-MS (ESI^+): *m/z*: $[M+Na]^+$ Calcd. for $C_{18}H_{32}O_3Na$ 319.2244; Found 319.2267.

(*E*)-*N*-(4-Hydroxy-3-methoxybenzyl)-12-oxooctadec-9-enamide (**78**)



Chemical Formula: $C_{26}H_{41}NO_4$
MW=431.61 g·mol^{-1}

Amidation of (*E*)-12-oxo-9-octadecenoic acid **1c** (210 mg, 0.71 mmol) was performed according to the general procedure **5.2.5.** for amide coupling using HATU (404 mg, 1.06 mmol), 4-hydroxy-3-methoxybenzylamine hydrochloride **99** (148 mg, 0.78 mmol) and DIPEA (400 µL, 2.1 mmol) in anhydrous DMF (10 mL) to yield the amide **78** (52 mg, 17%) as a white-off solid after purification by silica gel flash column chromatography (petroleum ether/EtOAc 7:3). R_f=0.36 (petroleum ether/EtOAc 7:3). mp=71-73 °C.

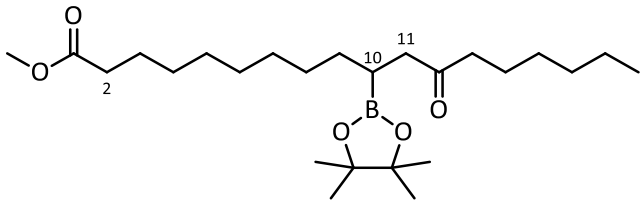
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.22 – 1.38 (m, 14H, **CH_2**), 1.50 – 1.58 (m, 2H, **CH_2**), 1.59 – 1.69 (m, 2H, **CH_2**), 1.97 – 2.04 (m, 2H, **H-8**), 2.19 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.40 (t, 2H, *J* = 6.9 Hz, **$COCH_2$**), 3.08 (d, 2H, *J* = 5.7 Hz, **H-11**), 3.87 (s, 3H, **CH_3O**), 4.35 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 5.47 – 5.52 (m, 2H, **H-9**, **H-10**), 5.67 (s, 1H, **CH_2NH**), 5.73 (br s, 1H, **OH**), 6.79 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, **H_{Ar}**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.17 (**CH_3**), 22.63 (**CH_2**), 23.86 (**CH_2**), 25.86 (**CH_2**), 29.03 (**CH_2**), 29.05 (**CH_2**), 29.23 (**CH_2**), 29.26 (**CH_2**), 29.36 (**CH_2**), 31.73 (**CH_2**), 32.64 (**CH_2**), 36.96 (**C-2**), 42.37 (**CO*C*H$_2$**), 43.66 (**CH_2NH**), 46.89 (**C-11**), 56.07 (**CH_3O**), 110.84 (**C-2'**), 114.50 (**C-5'**), 120.92 (**C-6'**), 122.12 (**C-10**), 130.56 (**C-1'**), 135.11 (**C-9**), 145.25 (**C-4'**), 146.83 (**C-3'**), 172.99 (**NHCO**), 210.08 (***C*OCH$_2$**).

IR (ATR) ν=3393, 3312, 2917, 2850, 1703, 1636, 1554, 1509, 1242, 1125, 967, 705 cm^{-1}.

HR-MS (ESI$^+$): *m/z*: $[M+H]^+$ Calcd. for $C_{26}H_{42}NO_4$ 432.3108; Found 432.3137.

Methyl 12-oxo-10-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)octadecanoate (**115**)

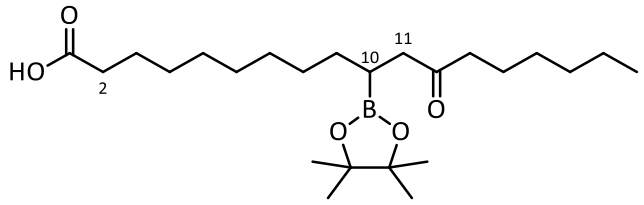


Chemical Formula: $C_{25}H_{47}BO_5$
MW=438.45 g·mol^{-1}

Tri-*n*-butylphosphine (26 µL, 0.10 mmol) was added to a solution of anhydrous CuCl (10 mg, 0.10 mmol) in anhydrous DMF (4.5 mL) was added, under argon atmosphere. In another reaction vessel, bis(pinacolato)diboron (283 mg, 1.12 mmol) was added, under argon atmosphere, to a solution of methyl (*E*)-12-oxooctadec-10-enoate **10d** (290 mg, 0.93 mmol) in anhydrous DMF (4.5 mL). This solution was transferred to the catalyst solution. The reaction mixture was stirred at room temperature for 48 h. The crude was taken up in H_2O and extracted into petroleum ether. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to yield the β-boronketone **115** (190 mg, 46%) as a yellow oil after purification by silica gel column chromatography (petroleum ether/EtOAc 95:5). R_f=0.49 (petroleum ether/Et_2O 9:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.84 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.18 – 1.28 (m, 30H, **$(CH_3)_4$**, **CH_2**), 1.34 – 1.39 (m, 1H, **H-10**), 1.49 – 1.60 (m, 4H, **CH_2**), 2.27 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.33 (td, 2H, *J* = 7.4, 3.7 Hz, **H-11**), 2.50 (d, 2H, *J* = 6.8 Hz, **$COCH_2$**), 3.64 (s, 3H, **CH_3O**).

12-Oxo-10-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)octadecanoic acid (**116**)

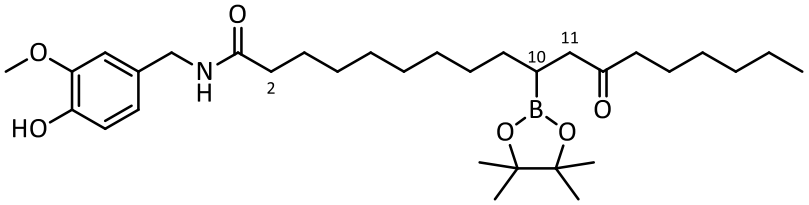


Chemical Formula: $C_{24}H_{45}BO_5$
MW=424.42 g·mol^{-1}

The hydrolysis of methyl 12-oxo-10-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)octadecanoate **115** (190 mg, 0.43 mmol) was performed according to the general procedure **5.2.4.** for enzymatic ester hydrolysis using Novozym 435® (83 mg) in H_2O (308 µL) and *tert*-BuOH (922 µL) to yield the acid **116** (180 mg, quantitative) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.20 – 1.33 (m, 30H, **$(CH_3)_4$**, **CH_2**), 1.38 – 1.44 (m, 1H, **H-10**), 1.51 – 1.58 (m, 2H, **CH_2**), 1.59 – 1.66 (m, 2H, **CH_2**), 2.29 – 2.41 (m, 2H, **H-2**), 2.53 (d, 2H, *J* = 6.8 Hz, **H-11**).

N-(4-Hydroxy-3-methoxybenzyl)-12-oxo-10-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)octadecanamide (**117**)

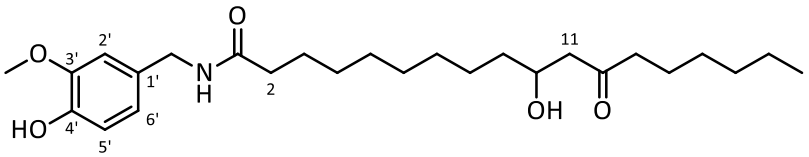


Chemical Formula: $C_{32}H_{54}BNO_6$
MW=559.59 g·mol^{-1}

Amidation of 12-oxo-10-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)octadecanoic acid **116** (175 mg, 0.41 mmol) was performed according to the general procedure **5.2.5.** for amide coupling using HATU (235 mg, 0.62 mmol), 4-hydroxy-3-methoxybenzylamine hydrochloride **99** (69 mg, 0.45 mmol) and DIPEA (200 µL, 1.24 mmol) in anhydrous DMF (6 mL) to yield the amide **117** (125 mg, 54%) as a brown oil after purification by silica gel flash column chromatography (petroleum ether/EtOAc 6:4). R_f=0.55 (petroleum ether/EtOAc 3:7).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.21 – 1.31 (m, 30H, **$(CH_3)_4$**, **CH_2**), 1.35 – 1.41 (m, 1H, **H-10**), 1.61 – 1.67 (m, 2H, **CH_2**), 2.18 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.32 – 2.39 (m, 2H, **$COCH_2$**), 2.52 (d, 2H, *J* = 6.8 Hz, **H-11**), 3.88 (s, 3H, **CH_3O**), 4.35 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 5.64 – 5.71 (m, 1H, **CH_2N*H***), 6.82 (ddd, 3H, J = 12.5, 9.9, 5.5 Hz, **H_{Ar}**)

N-(4-Hydroxy-3-methoxybenzyl)-10-hydroxy-12-oxooctadecanamide (**79**)



Chemical Formula: $C_{26}H_{43}NO_5$
MW=449.62 g·mol^{-1}

A volume of 5% w/v $NaHCO_3$ (2.5 mL, 1.49 mmol) was added to a solution of *N*-(4-hydroxy-3-methoxybenzyl)-12-oxo-10-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)octadecanamide **117** (125 mg, 0.22 mmol) and 2.5 mL of 30% H_2O_2 (0.02 mmol). The reaction mixture was stirred at room temperature for 24 h. Saturated aqueous $Na_2S_2O_4$ (0.25 mL) was added to decompose any remaining

peroxide keeping the temperature below 40 °C. The reaction mixture was diluted with H_2O and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated under reduced pressure to yield the β-hydroxyketone **79** (75 mg, 76%) as a rosaceous solid after recrystallization from Et_2O. mp=73-75 °C.

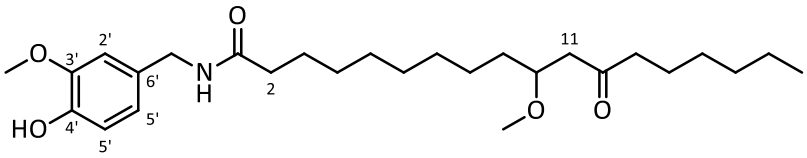
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9, Hz, **CH_3**), 1.20 – 1.41 (m, 18H, **CH_2**), 1.40 – 1.50 (m, 2H, **CH_2**), 1.52 – 1.60 (m, 2H, **CH_2**), 1.60 – 1.68 (m, 2H, **CH_2**), 2.18 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.41 (t, 2H, *J* = 6.9 Hz, **$COCH_2$**), 2.46 – 2.52 (m, 1H, **H-11a**), 2.59 (dd, 1H, *J* = 17.3, 1.8 Hz, **H-11b**), 3.08 (br s, 1H, **CHO*H***), 3.87 (s, 3H, **CH_3O**), 3.94 – 4.05 (m, 1H, **C*H*OH**), 4.35 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 5.69 (br s, 2H, O*H*, **CH_2N*H***), 6.67 – 6.88 (m, 3H, **H_{Ar}**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.16 (**CH_3**), 22.61 (**CH_2**), 23.73 (**CH_2**), 25.53 (**CH_2**), 25.87 (**CH_2**), 28.97 (**CH_2**), 29.34 (**CH_2**), 29.35 (**CH_2**), 29.48 (**CH_2**), 29.55 (**CH_2**), 31.70 (**CH_2**), 36.52 (**CH_2**), 36.96 (**CH_2**), 43.66 (**CH_2NH**), 43.84 (**CO*C*H_2**), 49.06 (**C-11**), 56.08 (**CH_3O**), 67.77 (***C*HOH**), 110.85 (**C-2'**), 114.53 (**C-5'**), 120.93 (**C-6'**), 130.56 (**C-1'**), 145.25 (**C-4'**), 146.84 (**C-3'**), 172.99 (**NHCO**), 212.84 (**$COCH_2$**).

IR (ATR) ν=3318, 2912, 2849, 1705, 1638, 1513, 1267, 1240, 1122, 718 cm^{-1}.

HR-MS (ESI^+): *m/z*: $[M+Na]^+$ Calcd. for $C_{26}H_{43}NO_5Na$ 472.3033; Found 472.3042.

N-(4-Hydroxy-3-methoxybenzyl)-10-methoxy-12-oxooctadecanamide (**80**)



Chemical Formula: $C_{27}H_{45}NO_5$
MW=463.65 g·mol^{-1}

A volume of trifluoromethanesulphonic acid (6 µL, 0.05 mmol) was added to a solution of (*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-12-oxooctadec-10-enamide **75** (200 mg, 0.46 mmol) in MeOH (1 mL, 85 mmol). The reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated under reduced pressure to yield the β-methoxyketone **80** (20 mg, 18%) as a yellowish oil after purification by silica gel flash column chromatography ($CHCl_3$/EtOAc 9:1). R_f=0.4 ($CHCl_3$/EtOAc 8:2).

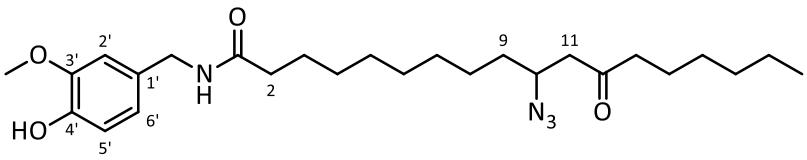
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.25 – 1.32 (m, 16H, **CH_2**), 1.38 – 1.52 (m, 2H, **CH_2**), 1.53 – 1.56 (m, 2H, **CH_2**), 1.64 (dt, 2H, *J* = 14.9, 7.5 Hz, **CH_2**), 2.19 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.37 – 2.45 (m, 3H, **H-11a**, **$COCH_2$**), 2.61 – 2.68 (m, 1H, **H-11b**), 3.30 (s, 3H, **CHOC*H*$_3$**), 3.61 – 3.69 (m, 1H, **C*H*OCH_3**), 3.88 (s, 3H, **CH_3O**), 4.35 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 5.61 (s, 1H, **CH_2N*H***), 5.65 (br s, 1H, **OH**), 6.80 (ddd, 3H, *J* = 21.0, 9.7, 4.8 Hz, **H_{Ar}**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.17 (**CH_3**), 22.64 (**CH_2**), 23.68 (**CH_2**), 25.20 (**CH_2**), 25.88 (**CH_2**), 29.00 (**CH_2**), 29.37 (**CH_2**), 29.39 (**CH_2**), 29.51 (**CH_2**), 29.85 (**CH_2**), 31.75 (**CH_2**), 34.11 (**CH_2**), 36.99 (**C-2**), 43.69 (**CH_2NH**), 44.21 (**CO*C*H_2**), 47.46 **(C-11**), 56.10 (**CH_3O**), 57.14 (**CHO*C*H_3**), 77.47 (***C*HOCH$_3$**), 110.86 (**C-2'**), 114.52 (**C-5'**), 120.97 (**C-6'**), 130.59 (**C-1'**), 145.28 (**C-4'**), 146.85 (**C-3'**), 172.99 (**NHCO**), 210.34 (***C*OCH$_2$**).

IR (ATR) ν=3316, 2924, 2854, 1709, 1645, 1514, 1272, 1034, 723 cm^{-1}.

HR-MS (ESI^+): *m/z*: $[M+Na]^+$ Calcd. for $C_{27}H_{45}NO_5Na$ 486.319; Found 486.3192.

10-Azido-*N*-(4-hydroxy-3-methoxybenzyl)-12-oxooctadecanamide (**118**)



Chemical Formula: $C_{26}H_{42}N_4O_4$
MW=474.64 g·mol^{-1}

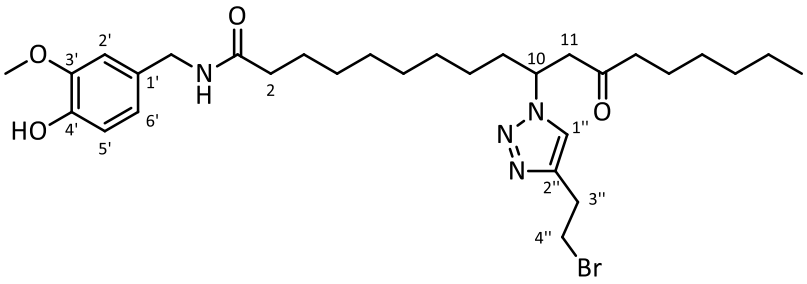
NaN_3 (205 mg, 3.15 mmol) and acetic acid (1.2 mL, 21 mmol) were successively added to a solution of (*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-12-oxooctadec-10-enamide **75** (170 mg, 0.39 mmol) in THF/H_2O 2:1 (1.8 mL). The reaction mixture was stirred at room temperature for 24 h. The mixture was poured to a saturated solution of $NaHCO_3$ and extracted into EtOAc. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to yield the β-azidoketone **118** (180 mg, quantitative) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.23 – 1.35 (m, 16H, **CH_2**), 1.45 – 1.53 (m, 2H, **CH_2**), 1.53 – 1.61 (m, 2H, **CH_2**), 1.60 – 1.70 (m, 2H, **CH_2**), 2.20 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.42 (t, 2H, *J* = 6.9 Hz, **COCH$_2$**), 2.49 (dd, 1H, *J* = 17.1, 4.6 Hz, **H-11a**), 2.64 (dd, 1H, *J* = 17.1, 8.2 Hz, **H-11b**), 3.80 – 3.87 (m, 1H, **CHN_3**), 3.88 (s, 3H, **CH_3O**), 4.36 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 5.64 (br s, 1H, **CH_2N*H***), 5.69 (br s, 1H, **OH**), 6.80 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, **H_{Ar}**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.16 (**CH_3**), 22.62 (**CH_2**), 23.71 (**CH_2**), 25.85 (**CH_2**), 26.06 (**CH_2**), 28.95 (**CH_2**), 29.28 (**CH_2**), 29.29 (**CH_2**), 29.33 (**CH_2**), 29.38 (**CH_2**), 31.70 (**CH_2**), 34.62 (**CH_2**), 36.94 (**C-2**), 43.73 (**CH_2NH**), 43.76 (**CO*C*H_2**), 47.25 (**C-11**), 56.10 (**CH_3O**), 58.38 (**CHN**), 110.87 (**C-2'**), 114.51 (**C-5'**), 120.98 (**C-6'**), 130.47 (**C-1'**), 145.28 (**C-4'**), 146.83 (**C-3'**), 173.14 (**NHCO**), 208.60 (***C*OCH$_2$**).

IR (ATR) ν=3290, 3082, 2926, 2855, 2101, 1712, 1644, 1514, 1272, 844, 724 cm^{-1}.

10-[4-(2-Bromoethyl)-1*H*-1,2,3-triazol-1-yl]-*N*-(4-hydroxy-3-methoxybenzyl)-12-oxooctadecanamide (**81**)



Chemical Formula: $C_{30}H_{47}BrN_4O_4$
MW=607.62 g·mol^{-1}

1-Bromo-3-butin (30 µL, 0.32 mmol) was added to a solution of 10-azido-*N*-(4-hydroxy-3-methoxybenzyl)-12-oxooctadecanamide **118** (150 mg, 0.32 mmol) in *tert*-BuOH (2.1 mL) and H_2O (1.7 mL). Then, a solution of $CuSO_4 \cdot 5H_2O$ (6 mg, 3.16 µmol) in H_2O (200 µL) and a solution of sodium ascorbate (6 mg, 31.6 µmol) in H_2O (200 µL) were successively added to the above solution. The reaction mixture was stirred at room temperature for 100 h. The solvent was evaporated under reduced pressure to yield the triazol **81** (30 mg, 16%) as a yellow oil after purification by silica gel flash column chromatography (petroleum ether/EtOAc 7:3). R_f=0.35 (petroleum ether/EtOAc 6:4).

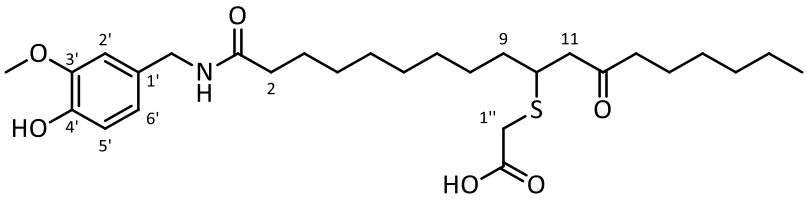
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.85 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.19 – 1.27 (m, 16H, **CH_2**), 1.42 – 1.51 (m, 2H, **CH_2**), 1.57 – 1.65 (m, 2H, **CH_2**), 1.73 – 1.83 (m, 2H, **CH_2**), 2.17 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.25 – 2.42 (m, 2H, **$COCH_2$**), 2.87 (dd, 1H, *J* = 17.1, 4.6 Hz, **H-11a**), 3.17 – 3.35 (m, 3H, **H-3''**, **H-11b**), 3.61 (t, 2H, *J* = 6.9 Hz, **H-4''**), 3.86 (s, 3H, **CH_3O**), 4.35 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 4.86 (ddd, 1H, *J* = 12.5, 9.9, 5.0 Hz, **H-10**), 5.78 – 5.88 (m, 2H, **CH_2N*H*, OH**), 6.79 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, **H_{Ar}**), 7.45 (s, 1H, **C-1''**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.13 (**CH_3**), 22.56 (**CH_2**), 23.57 (**CH_2**), 25.75 (**CH_2**), 25.80 (**CH_2**), 28.86 (**C-3''**), 29.04 (**CH_2**), 29.09 (**CH_2**), 29.18 (**CH_2**), 29.46 (**CH_2**), 29.83 (**CH_2**), 31.62 (**C-4''**), 31.74 (**CH_2**), 35.19 (**CH_2**), 36.83 (**C-2**), 43.48 (**CO*C*H$_2$**), 43.62 (**CH_2NH**), 47.62 (**C-11**), 56.08 (**CH_3O**), 57.30 (**C-10**), 110.89 (**C-2'**), 114.54 (**C-5'**), 120.88 (**C-6'**), 122.84 (**C-1''**), 130.57 (**C-1'**), 144.04 (**C-2''**), 145.26 (**C-4'**), 146.86 (**C-3'**), 173.00 (**NHCO**), 207.99 (***C*OCH$_2$**).

IR (ATR) ν=3275, 2917, 2850, 1645, 1514, 1273, 1034, 720 cm^{-1}.

HR-MS (ESI$^+$): *m/z*: $[M+H]^+$ Calcd. for $C_{30}H_{48}BrN_4O_4$ 607.2853; Found 607.2888.

{[10-[(4-Hydroxy-3-methoxybenzyl)amino]-10-oxo-1-(2-oxooctyl)decyl]thio} acetic acid (**82**)



Chemical Formula: $C_{28}H_{45}NO_6S$
MW=523.73 g·mol^{-1}

Thioalkilation of (*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-12-oxooctadec-10-enamide **75** (100 mg, 0.23 mmol) was performed according to the general procedure **5.2.8.** for Michael addition using thioglycolic acid (50 µL, 0.69 mmol) and $NaHCO_3$ (1 mg, 0.01 mmol) in MeOH/H_2O 1:1 (3.2 mL) to yield the compound **82** (70 mg, 58%) as a yellowish oil after purification by silica gel column chromatography (Et_2O). R_f=0.4 (Et_2O).

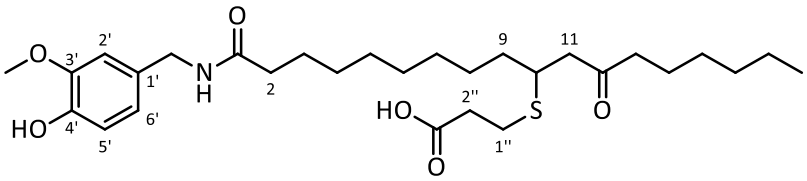
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.24 – 1.34 (m, 14H, **CH_2**), 1.37 – 1.47 (m, 2H, **CH_2**), 1.51 – 1.60 (m, 4H, **CH_2**), 1.60 – 1.69 (m, 2H, **CH_2**), 2.24 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.41 (t, 2H, *J* = 6.9 Hz, **$COCH_2$**), 2.64 (dd, 1H, *J* = 17.1, 4.6 Hz, **H-11a**), 2.75 (dd, 1H, *J* = 17.1, 8.2 Hz, **H-11b**), 3.21 – 3.32 (m, 3H, **CHS**, **H-1''**), 3.88 (s, 3H, **CH_3O**), 4.36 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 5.76 (br s, 1H, **CH_2N*H***), 5.90 (br s, 1H, **OH**), 6.79 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, **H_{Ar}**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.18 (**CH_3**), 22.63 (**CH_2**), 23.77 (**CH_2**), 25.47 (**CH_2**), 26.21 (**CH_2**), 28.28 (**CH_2**), 28.37 (**CH_2**), 28.39 (**CH_2**), 28.52 (**CH_2**), 28.99 (**CH_2**), 31.73 (**CH_2**), 33.28 (**C-9**), 34.71 (**C-1''**), 36.72 (**C-2**), 41.63 (**CO*C*H_2**), 43.70 (**CH_2NH**), 43.91 (**CHS**), 48.64 (**C-11**), 56.13 (**CH_3O**), 110.95 (**C-2'**), 114.53 (**C-5'**), 121.03 (**C-6'**), 130.06 (**C-1'**), 145.33 (**C-4'**), 146.87 (**C-3'**), 173.11 (**NHCO**), 174.18 (**COOH**), 209.45 (***C*OCH_2**).

IR (ATR) ν=3356, 2925, 2854, 1708, 1612, 1514, 1272, 1033, 723 cm^{-1}.

HR-MS (ESI$^+$): *m/z*: $[M+H]^+$ Calcd. for $C_{28}H_{46}NO_6S$ 524.304; Found 524.3078.

{[10-[(4-Hydroxy-3-methoxybenzyl)amino]-10-oxo-1-(2-oxooctyl)decyl]thio} propanoic acid (**83**)



Chemical Formula: $C_{29}H_{47}NO_6S$
MW=537.75 g·mol^{-1}

Thioalkilation of (*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-12-oxooctadec-10-enamide **75** (100 mg, 0.23 mmol) was performed according to the general procedure **5.2.8.** for Michael addition using 3-mercaptopropionic acid (60 µL, 0.69 mmol) and $NaHCO_3$ (1 mg, 0.01 mmol) in MeOH/H_2O 1:1 (3.2 mL) to yield the compound **83** (73 mg, 58%) as a yellowish oil after purification by silica gel column chromatography (Et_2O). R_f=0.48 (petroleum ether/Et_2O 1:9).

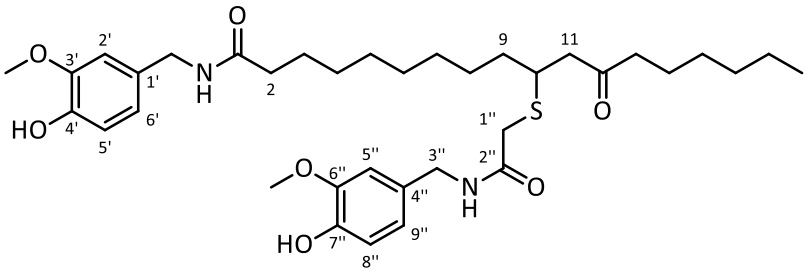
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.23 – 1.33 (m, 14H, **CH_2**), 1.34 – 1.43 (m, 2H, **CH_2**), 1.43 – 1.59 (m, 4H, **CH_2**), 1.59 – 1.68 (m, 2H, **CH_2**), 2.22 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.40 (t, 2H, *J* = 6.9 Hz, **$COCH_2$**), 2.55 – 2.68 (m, 3H, **H-11a**, **H-1''**), 2.68 – 2.79 (m, 3H, **H-11b**, **H-2''**), 3.07 – 3.15 (m, 1H, **CHS**), 3.86 (s, 3H, **CH_3O**), 4.34 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 5.96 (br s, 1H, **OH**), 6.78 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, **H_{Ar}**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.16 (**CH_3**), 22.61 (**CH_2**), 23.73 (**CH_2**), 25.82 (**CH_2**), 25.94 (**CH_2**), 26.59 (**C-1''**), 28.94 (**CH_2**), 28.97 (2x**CH_2**), 29.06 (**CH_2**), 29.07 (**CH_2**), 31.71 (**CH_2**), 34.75, 35.24 (**C-2''**), 36.77 (**C-2**), 40.91 (**CO*C*H$_2$**), 43.79 (**CH_2NH**), 43.92 (**C-11**), 49.02 (**CHS**), 56.09 (**CH_3O**), 110.92 (**C-2'**), 114.54 (**C-5'**), 120.95 (**C-6'**), 130.20 (**C-1'**), 145.28 (**C-4'**), 146.87 (**C-3'**), 173.86 (**NHCO**), 175.73 (**COOH**), 209.65 (***C*OCH$_2$**).

IR (ATR) ν=3350, 2926, 2854, 1708, 1614, 1514, 1272, 1236, 1033, 796, 723 cm^{-1}.

HR-MS (ESI$^+$): *m/z*: $[M+H]^+$ Calcd. for $C_{29}H_{48}NO_6S$ 538.3197; Found 538.3244.

N-(4-Hydroxy-3-methoxybenzyl)-10-({2-[(4-hydroxy-3-methoxybenzyl)amino]-2-oxoethyl}thio)-12-oxooctadecanamide (**84**)



Chemical Formula: $C_{36}H_{54}N_2O_7S$
MW=658.89 g·mol^{-1}

Amidation of {[10-[(4-hydroxy-3-methoxybenzyl)amino]-10-oxo-1-(2-oxooctyl)decyl]thio}acetic acid **82** (35 mg, 0.07 mmol) was performed according to the general procedure **5.2.5.** for amide coupling using HATU (62 mg, 0.16 mmol), 4-hydroxy-3-methoxybenzylamine hydrochloride **99** (14 mg, 0.07 mmol) and DIPEA (35 µL, 0.2 mmol) in anhydrous DMF (1 mL) to yield the amide **84** (24 mg, 54%) as a yellow oil after purification by silica gel flash column chromatography (petroleum ether/EtOAc 2:8). R_f=0.4 (petroleum ether/EtOAc 1:9).

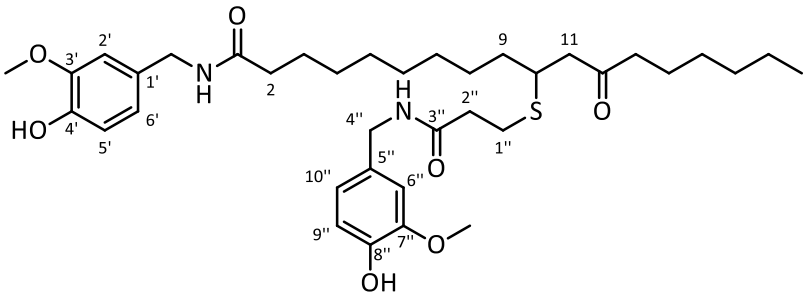
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.18 – 1.29 (m, 16H, **CH_2**), 1.45 – 1.53 (m, 4H, **CH_2**), 1.60 – 1.67 (m, 2H, **CH_2**), 2.20 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.36 (t, 2H, *J* = 6.9 Hz, **$COCH_2$**), 2.61 (d, 2H, *J* = 5.7 Hz, **H-11**), 3.00 – 3.09 (m, 1H, **CHS**), 3.25 (q, 2H, *J* = 17.0 Hz, **H-1''**), 3.85 – 3.88 (m, 6H, **CH_3O**), 4.34 (dd, 3H, *J* = 14.4, 5.4 Hz, **C*H*$_2$NH**, **H-3''a**), 4.47 (dd, 1H, *J* = 14.4, 5.4 Hz, **H-3''b**), 5.91 (s, 1H, **OH**), 6.88 – 6.74 (m, 6H, **H_{Ar}**), 7.52 (t, 1H, *J* = 6.9 Hz, **NH**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.17 (**CH_3**), 22.61 (**CH_2**), 23.78 (**CH_2**), 25.75 (**CH_2**), 26.83 (**CH_2**), 28.95 (**CH_2**), 29.18 (**CH_2**), 29.21 (**CH_2**), 29.28 (**CH_2**), 29.29 (**CH_2**), 29.87 (**CH_2**), 31.70 (**CH_2**), 35.01 (**CH_2**), 36.84 (**C-2**), 41.17 (**CO*C*H$_2$**), 43.70 (**CH_2NH**, **C-3''**), 43.98 (**CHS**), 47.65 (**C-11**), 56.07 (**CH_3O**), 56.09 (**CH_3O**), 110.93 (**C-2'**), 111.15 (**C-5''**), 114.54 (**C-5'**), 114.57 (**C-8''**), 120.94 (**C-9''**), 121.11 (**C-6'**), 130.11 (**C-1'**), 130.48 (**C-4''**), 145.28 (**C-7''**), 145.32 (**C-4'**), 146.86 (**C-6''**), 146.87 (**C-3'**), 168.63 (**C-2''**), 173.22 (**NHCO**), 209.33 (***C*OCH$_2$**).

IR (ATR) ν=3289, 2926, 2854, 1707, 1641, 1514, 1273, 1124, 1033, 729 cm^{-1}.

HR-MS (ESI$^+$): *m/z*: $[M+H]^+$ Calcd. for $C_{36}H_{55}N_2O_7S$ 659.3724; Found 659.3778.

N-(4-Hydroxy-3-methoxybenzyl)-10-({3-[(4-hydroxy-3-methoxybenzyl)amino]-3-oxopropyl}thio)-12-oxooctadecanamide (**85**)



Chemical Formula: $C_{37}H_{56}N_2O_7S$
MW=672.91 g·mol^{-1}

Amidation of 3-{[10-[(4-hydroxy-3-methoxybenzyl)amino]-10-oxo-1-(2-oxooctyl)decyl]thio} propanoic acid **83** (58 mg, 0.11 mmol) was performed according to the general procedure **5.2.5.** for amide coupling using HATU (62 mg, 0.16 mmol), 4-hydroxy-3-methoxybenzylamine hydrochloride **99** (22 mg, 0.12 mmol) and DIPEA (56 µL, 0.32 mmol) in anhydrous DMF (1.6 mL) to yield the amide **85** (24 mg, 54%) as a yellow oil after purification by silica gel flash column chromatography (petroleum ether/EtOAc 2:8). R_f=0.4 (petroleum ether/EtOAc 1:9).

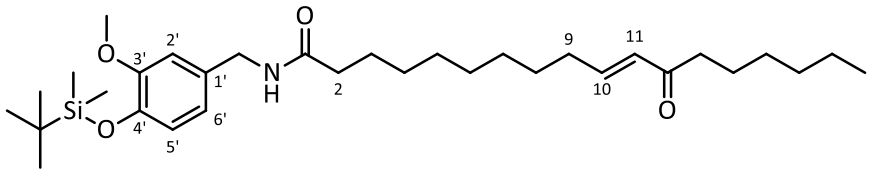
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.22 – 1.30 (m, 16H, **CH_2**), 1.43 – 1.54 (m, 4H, **CH_2**), 1.58 – 1.68 (m, 2H, **CH_2**), 2.19 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.24 – 2.42 (m, 2H, **H-2''**), 2.44 – 2.59 (m, 3H, **H-11a**, **$COCH_2$**), 2.66 (dd, 1H, *J* = 17.1, 8.2 Hz, **H-11b**), 2.77 – 2.85 (t, 2H, *J* = 6.9 Hz, **H-1''**), 3.02 – 3.14 (m, 1H, **CHS**), 3.86 – 3.89 (m, 6H, **CH_3O**), 4.29 – 4.42 (m, 4H, **C*H*$_2$NH**, **H-4''**), 5.63 – 5.71 (m, 1H, **CH_2N*H***), 5.77 (s, 1H, **OH**), 6.21 (s, 1H, **OH**), 6.74 – 6.87 (m, 6H, **H_{Ar}**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.18 (**CH_3**), 22.64 (**CH_2**), 23.70 (**CH_2**), 25.81 (**CH_2**), 26.92 (**CH_2**), 27.19 (**C-1''**), 28.97 (**CH_2**), 29.24 (**CH_2**), 29.25 (**CH_2**), 29.33 (**CH_2**), 29.36 (**CH_2**), 31.73 (**CH_2**), 35.74 (**CH_2**), 36.77 (**C-2''**), 36.91 (**C-2**), 40.29 (**CO*C*H$_2$**), 43.68 (**C-4''**), 43.72 (**CH_2NH**), 43.98 (**CHS**), 48.51 (**C-11**), 56.10 (**CH_3O**), 56.14 (**CH_3O**), 110.84 (**C-6''**), 110.87 (**C-2'**), 114.44 (**C-5'**), 114.52 (**C-9''**), 120.89 (**C-10''**), 120.93 (**C-6'**), 130.31 (**C-1'**), 130.54 (**C-5''**), 145.20 (**C-8''**), 145.26 (**C-4'**), 146.81 (**C-7''**), 146.84 (**C-3'**), 171.33 (**C-3''**), 173.08 (**NHCO**), 210.00 (***C*OCH$_2$**).

IR (ATR) ν=3288, 2925, 2853, 1707, 1640, 1514, 1273, 1033, 724 cm^{-1}.

HR-MS (ESI$^+$): *m/z*: $[M+H]^+$ Calcd. for $C_{37}H_{57}N_3O_4S_2$ 673.3942; Found 673.3936.

(*E*)-*N*-{4-[(*tert*-butyldimethylsilyl)oxy]-3-methoxybenzyl}-12-oxooctadec-10-enamide (**123**)



Chemical Formula: $C_{32}H_{55}NO_4Si$
MW=545.87 g·mol^{-1}

tert-Butyldimethylsilyl chloride (509 g, 3.38 mmol) was slowly added to a solution of (*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-12-oxooctadec-10-enamide **75** (500 mg, 1.69 mmol), DMAP (8 mg, 0.07 mmol) and TEA (590 µL, 4.22 mmol) in DCM (15 mL). The reaction was stirred at room temperature for 24 h. The mixture was acidified until pH 1 with 1 M aqueous solution of HCl and organic layer was recovered, washed again with 1 M HCl and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to yield the protected phenol **123** (784 mg, 85%) as a yellow oil after purification by silica gel flash column chromatography (petroleum ether/EtOAc 9:1). R_f=0.58 (petroleum ether/EtOAc 7:3).

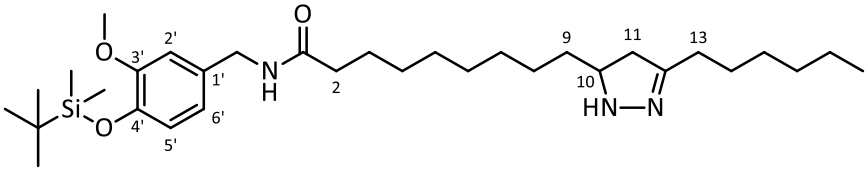
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.14 (s, 6H, **Si(CH$_3$)$_2$**), 0.88 (t, 3H, *J* = 6.9 Hz, **CH$_3$**), 0.98 (s, 9H, **SiC(CH$_3$)$_3$**), 1.26 – 1.31 (m, 14H, **CH$_2$**), 1.39 – 1.48 (m, 2H, **CH$_2$**), 1.55 – 1.67 (m, 4H, **CH$_2$**), 2.15 – 2.22 (m, 4H, **H-2**, **H-9**), 2.51 (t, 3H, *J* = 6.9 Hz, **COCH$_2$**), 3.78 (s, 3H, **CH$_3$O**), 4.35 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 5.68 (br s, 1H, **CH$_2$N*H***), 6.07 (dt, 1H, *J* = 15.9, 1.5 Hz, **H-11**), 6.67 – 6.87 (m, 4H, **H$_{Ar}$**, **H-10**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = -4.50 (**Si(CH$_3$)$_2$**), 14.18 (**CH$_3$**), 18.58 (**Si*C*(CH$_3$)$_3$**), 22.65 (**CH$_2$**), 24.45 (**CH$_2$**), 25.85 (**CH$_2$**, **SiC(*C*H$_3$)$_3$**), 25.88 (**CH$_2$**), 28.21 (**CH$_2$**), 29.14 (**CH$_2$**), 29.23 (**CH$_2$**), 29.33 (**CH$_2$**), 29.35 (**CH$_2$**), 29.39 (**CH$_2$**), 31.77 (**CH$_2$**), 32.54 (**CH$_2$**), 36.97 (**C-2**), 40.27 (**CO*C*H$_2$**), 43.65 (**CH$_2$NH**), 55.63 (**CH$_3$O**), 112.11 (**C-2'**), 120.32 (**C-6'**), 121.00 (**C-5'**), 130.48 (**C-11**), 131.93 (**C-1'**), 144.65 (**C-4'**), 147.37 (**C-10**), 151.22 (**C-3'**), 172.97 (**NHCO**), 201.19 (***C*OCH$_2$**).

IR (ATR) ν=3328, 2955, 2928, 2856, 1712, 1654, 1511, 1463, 1418, 1361, 1281, 907, 728 cm^{-1}.

HR-MS (ESI$^+$): *m/z*: [M+Na]$^+$ Calcd. for $C_{32}H_{55}NO_4SiNa$ 568.3793; Found 568.3835.

N-{4-[(*tert*-butyldimethylsilyl)oxy]-3-methoxybenzyl}-9-(3-hexyl-4,5-dihydro-1*H*-pyrazol-5-yl)nonanamide (**124**)

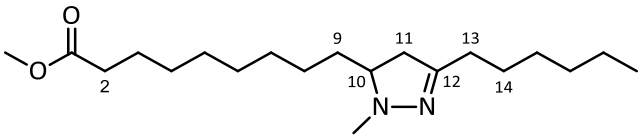


Chemical Formula: $C_{32}H_{57}N_3O_3Si$
MW= 559,42 g·mol^{-1}

64% Hydrazine (240 µL, 3.07 mmol) was added to a solution of (*E*)-*N*-{4-[(*tert*-butyldimethylsilyl)oxy]-3-methoxybenzyl}-12-oxooctadec-10-enamide **123** (284 mg, 0.52 mmol) in EtOH (3 mL) under nitrogen atmosphere. The reaction mixture was stirred at reflux for 20 h. The solvent was evaporated under reduced pressure to yield the compound **124** (247 mg, 83%) as a yellow oil. The ^{1}H NMR spectra revealed the partial deprotection of the phenol group (50%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.14 (s, 6H, **Si(CH$_3$)$_2$**), 0.87 (t, 3H, *J* = 6.9 Hz, **CH$_3$**), 0.98 (s, 9H, **SiC(CH$_3$)$_3$**), 0.98 (s, 9H, **SiC(CH$_3$)$_3$**), 1.24 – 1.33 (m, 16H, **CH$_2$**), 1.43 – 1.56 (m, 4H, **CH$_2$**), 1.59 – 1.69 (m, 2H, **H-9**), 2.11 – 2.22 (m, 3H, **H-11a**, **H-13**), 2.26 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.65 (dd, 1H, *J* = 16.8, 9.4 Hz, **H-11b**), 2.55 – 2.66 (m, 1H, **H-10**), 3.78 (s, 3H, **CH$_3$O**), 4.35 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 5.68 (br s, 1H, **CH$_2$N*H***), 6.67 – 6.88 (m, 3H, **H$_{Ar}$**).

Methyl 9-(3-hexyl-4,5-dihydro-1-methyl-1*H*-pyrazol-5-yl)nonanoate (**126**)



Chemical Formula: $C_{20}H_{38}N_2O_2$
MW=338.53 g·mol^{-1}

Methylhydrazine (407 µL, 7.73 mmol) was added to a solution of methyl (*E*)-12-oxo-10-octadecenoate **10d** (300 mg, 0.97 mmol) in IPA (4 mL) under nitrogen atmosphere. The reaction mixture was stirred at reflux for 20 h. The solvent was evaporated under reduced pressure to yield the compound **126** (148 mg, 45%) as a yellow oil after purification by silica gel flash chromatography (petroleum ether/EtOAc 9:1). R_f=0.5 (petroleum ether/EtOAc 9:1).

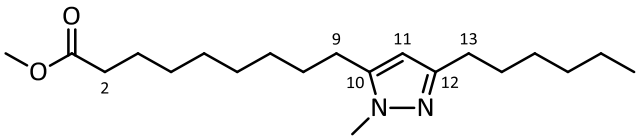
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.8 Hz, **CH$_3$**), 1.24 – 1.33 (m, 16H, **CH$_2$**), 1.41 – 1.54 (m, 3H, **H-9a**, **H-14**), 1.55 – 1.67 (m, 2H, **CH$_2$**), 1.68 – 1.80 (m, 1H, **H-9b**), 2.20 – 2.34 (m, 5H, **H-2**, **H-11a**, **H-13**), 2.60 – 2.78 (m, 5H, **H-10**, **H-11b**, **NCH$_3$**), 3.66 (s, 3H, **CH$_3$CO**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.20 (**CH_3**), 22.67 (**CH_2**), 25.06 (**CH_2**), 26.90 (**CH_2**), 27.29 (**CH_2**), 29.17 (**CH_2**), 29.25 (**CH_2**), 29.31 (**CH_2**), 29.51 (**CH_2**), 29.85 (**CH_2**), 30.95 (**CH_2**), 31.72 (**CH_2**), 33.05 (**CH_2**), 34.23 (**C-2**), 41.65 (**NCH_3**), 42.45 (**C-11**), 51.61 (**CH_3O**), 69.88 (**C-12**), 156.35 (**C-10**), 174.44 (**$\underline{C}OOCH_3$**).

IR (ATR) ν=2925, 2855, 2780, 1735, 1674, 1434, 1169, 1008, 725 cm^{-1}.

HR-MS (ESI^+): *m/z*: $[M+H]^+$ Calcd. for $C_{20}H_{39}N_2O_2$ 339.3006; Found 339.3013

Methyl 9-(3-hexyl-1-methyl-1*H*-pyrazol-5-yl)nonanoate (**127**)



Chemical Formula: $C_{20}H_{36}N_2O_2$
MW=336.51 g·mol^{-1}
[250643-19-7]

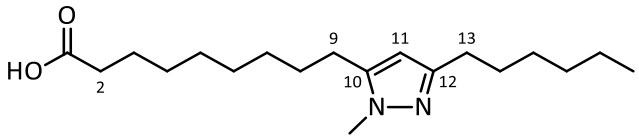
DDQ (184 mg, 0.81 mmol) was added to a solution of methyl 9-(3-hexyl-4,5-dihydro-1-methyl-1*H*-pyrazol-5-yl)nonanoate **126** (250 mg, 0.74 mmol) in anhydrous dioxane (23 mL). The reaction mixture was stirred at reflux for 16 h and then, filtered over Celite® to yield the compound **127** (110 mg, 44%) as a yellow oil after purification by silica gel column chromatography (petroleum ether/EtOAc 8:2). R_f=0.5 (petroleum ether/EtOAc 7:3). The spectral data were in accordance with the literature:[16]

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H *J* = 6.9 Hz, **CH_3**), 1.28 – 1.34 (m, 14H, **CH_2**), 1.55 – 1.67 (m, 6H, **CH_2**), 2.29 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.48 – 2.55 (m, 4H, **H-9**, **H-13**), 3.65 (s, 3H, **CH_3O**), 3.69 (s, 3H, **CH_3N**), 5.78 (s, 1H, **H-11**).

^{13}C NMR {^{1}H} (101 MHz, $CDCl_3$) δ = 14.21 (**CH_3**), 22.72 (**CH_2**), 25.03 (**CH_2**), 25.76 (**CH_2**), 28.44 (**CH_2**), 28.55 (**CH_2**), 29.21 (**CH_2**), 29.26 (**CH_2**), 29.27 (**CH_2**), 29.34 (2x**CH_2**), 30.02 (**CH_2**), 31.82 (**CH_2**), 34.19 (**C-2**), 35.78 (**CH_3N**), 51.57 (**CH_3O**), 102.67 (**C-11**), 143.75 (**C-10**), 152.17 (**C-12**), 174.38 (**$\underline{C}OOCH_3$**).

IR (ATR) ν=2926, 2854, 1737, 1435, 1195, 1006, 776, 725 cm^{-1}.

9-(3-Hexyl-1-methyl-1*H*-pyrazol-5-yl)nonanoic acid (**128**)



Chemical Formula: $C_{19}H_{34}N_2O_2$
MW=322.49 g·mol^{-1}

The hydrolysis of methyl 9-(3-hexyl-1-methyl-1*H*-pyrazol-5-yl)nonanoate **127** (50 mg, 0.15 mmol) was performed according to the general procedure **5.2.6.** for chemical ester hydrolysis using LiOH·H_2O (19 mg, 0.45 mmol) in THF/H_2O 1:1 (700 µL) to yield the acid **128** (45 mg, 94%) as a yellow oil.

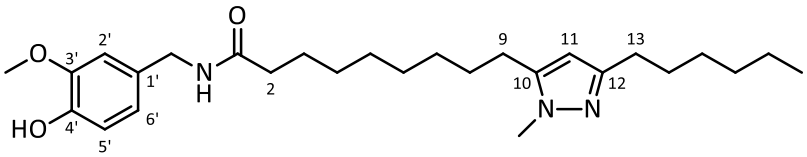
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.28 – 1.34 (m, 14H, **CH_2**), 1.55 – 1.66 (m, 6H, **CH_2**), 2.33 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.48 – 2.56 (m, 4H, **H-9**, **H-13**), 3.72 (s, 3H, **CH_3N**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.21 (**CH_3**), 22.70 (**CH_2**), 24.89 (**CH_2**), 25.66 (**CH_2**), 28.04 (**CH_2**), 28.46 (**CH_2**), 29.16 (**CH_2**), 29.23 (**CH_2**), 29.24 (2x**CH_2**), 29.30 (**CH_2**), 29.87 (**CH_2**), 31.78 (**CH_2**), 34.31 (**C-2**), 35.52 (**CH_3N**), 102.80 (**C-11**), 144.04 (**C-10**), 152.09 (**C-12**), 178.65 (**COOH**).

IR (ATR) ν=2925, 2854, 2537, 1711, 1458, 1240, 1194, 780, 725 cm^{-1}.

HR-MS (ESI$^+$): *m/z*: $[M+H]^+$ Calcd. for $C_{19}H_{35}N_2O_2$ 323.2693; Found 323.2695.

9-(3-Hexyl-1-methyl-1*H*-pyrazol-5-yl)-*N*-(4-hydroxy-3-methoxybenzyl) nonanamide (**86**)



Chemical Formula: $C_{27}H_{43}N_3O_3$
MW=457.65 g·mol^{-1}

Amidation of 9-(3-hexyl-1-methyl-1*H*-pyrazol-5-yl)nonanoic acid **128** (45 mg, 0.14 mmol) was performed according to the general procedure **5.2.5.** for amide coupling using HATU (80 mg, 0.21 mmol), 4-hydroxy-3-methoxybenzylamine hydrochloride **99** (30 mg, 0.15 mmol) and DIPEA (73 µL, 0.42 mmol) in anhydrous DMF (2 mL) to yield the compound **86** (40 mg, 62%) as a yellow oil after purification by silica gel flash column chromatography (petroleum ether/EtOAc 6:4). R_f=0.36 (petroleum ether/EtOAc 6:4).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.26 – 1.36 (m, 14H, **CH_2**), 1.55 – 1.68 (m, 6H, **CH_2**), 2.19 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.47 – 2.57 (m, 4H, **H-9**, **H-13**), 3.70 (s, 3H, **N-CH_3**), 3.87 (s, 3H,

CH_3O), 4.35 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 5.70 (br s, 1H, **OH**), 5.79 (s, 1H, **H-11**), 6.78 (ddd, 3H, *J* = 12.5, 9.90, 5.0 Hz, **H_{Ar}**).

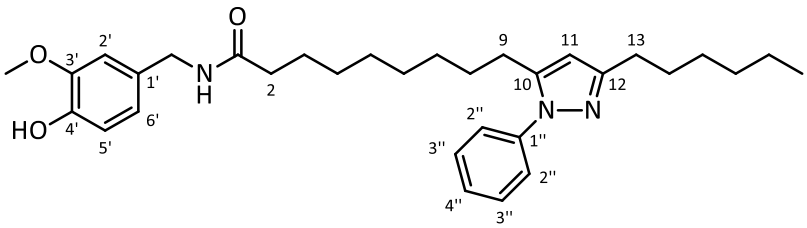
^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.23 (**CH_3**), 22.73 (**CH_2**), 25.74 (**CH_2**), 25.85 (**CH_2**), 28.40 (**CH_2**), 28.55 (**CH_2**), 29.34 (3x**CH_2**), 29.37 (**CH_2**), 29.38 (**CH_2**), 30.00 (**CH_2**), 31.82 (**CH_2**), 35.77 (**N-CH_3**), 36.94 (**C-2**), 43.66 (**CH_2NH**), 56.07 (**CH_3O**), 102.75 (**C-11**), 110.88 (**C-2'**), 114.53 (**C-5'**), 120.89 (**C-6'**), 130.48 (**C-1'**), 143.87 (**C-10**), 145.31 (**C-4'**), 146.88 (**C-3'**), 152.17 (**C-12**), 172.94 (**NHCO**).

IR (ATR) ν=3257, 2925, 2854, 1643, 1514, 1461, 1429, 1274, 1035, 794, 724 cm^{-1}.

R_f=0.36 (petroleum ether/EtOAc 6:4)

HR-MS (ESI^+): *m/z*: $[M+H]^+$ Calcd. for $C_{27}H_{44}N_3O_3$ 458.3377; Found 458.3376.

9-(3-Hexyl-1-phenyl-1*H*-pyrazol-5-yl)-*N*-(4-hydroxy-3-methoxybenzyl) nonanamide (**87**)



Chemical Formula: $C_{32}H_{45}N_3O_3$
MW=519.72 g·mol^{-1}

Phenylhydrazine (34 µL, 0.35 mmol) was added to a solution of (*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-12-oxooctadec-10-enamide **75** (75 mg, 0.17 mmol) and glacial acetic acid (341 µL) in anhydrous toluene (850 µL). The reaction mixture was stirred at 80 °C for 20 h in an open vessel. The solvent was evaporated under reduced pressure to yield the compound **87** (23 mg, 20%) as a yellowish oil after purification by silica gel flash column chromatography (petroleum ether/Et_2O 1:9). R_f=0.4 (petroleum ether/Et_2O 2:8).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.89 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.24 – 1.33 (m, 12H, **CH_2**), 1.52 – 1.72 (m, 8H, **CH_2**), 2.17 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.59 (t, 2H, *J* = 6.9 Hz, **H-13**), 2.66 (t, 2H, *J* = 6.9 Hz, **H-9**), 3.87 (s, 3H, **CH_3O**), 4.35 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 5.60 (br s, 2H, **CH_2N*H*, OH**), 6.03 (s, 1H, **H-11**), 6.79 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, **H_{Ar}**), 7.32 – 7.48 (m, 5H, **H_{Phenyl}**).

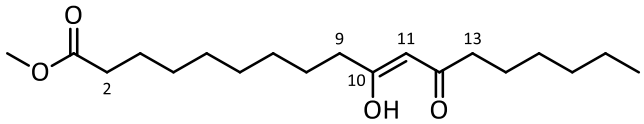
^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.25 (**CH_3**), 22.76 (**CH_2**), 25.85 (**CH_2**), 26.42 (**CH_2**), 28.90 (**CH_2**), 29.25 (**CH_2**), 29.31 (**CH_2**), 29.32 (**CH_2**), 29.36 (2x**CH_2**), 29.81 (**CH_2**), 29.85 (**CH_2**), 31.83 (**CH_2**), 36.96 (**C-2**), 43.69 (**CH_2NH**), 56.09 (**CH_3O**), 104.38 (**C-11**), 110.84 (**C-2'**), 114.50 (**C-5'**), 120.96 (**C-6'**), 125.57 (2x**C-2''**),

127.81 (**C-4''**), 129.19 (2x**C-3''**), 130.54 (**C-1'**), 144.77 (**C-12**), 145.27 (**C-4'**), 146.81 (**C-3'**), 153.81 (**C-10**, **C-1''**), 172.88 (**NHCO**).

IR (ATR) ν=3274, 3066, 2925, 2854, 1720, 1644, 1504, 1274, 1035, 795, 695 cm^{-1}.

HR-MS (ESI^+): *m/z*: $[M+H]^+$ Calcd. for $C_{32}H_{46}N_3O_3$ 520.3534; Found 520.357.

Methyl (*Z*)-10-hydroxy-12-oxooctadec-10-enoate (**129**)



Chemical Formula: $C_{19}H_{34}O_4$
MW=326.47 g·mol^{-1}
[17414-55-0][17]

Na_2PdCl_4 (95 mg, 0.32 mmol) and 70% *tert*-butylhydroperoxide (1.34 mL, 0.97 mmol) were added to a solution of the methyl (*E*)-12-oxooctadec-10-enoate **10d** (1g, 3.22 mmol) in IPA/H_2O 1:1 (3.3 mL). The reaction mixture was stirred at 50 °C for 24 h. The mixture was taken up in H_2O and extracted into EtOAc. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to yield the β-hydroxyketone **129** (780 mg, 74%) as a yellow oil after purification by silica gel column chromatography (petroleum ether/EtOAc 98:2). R_f=0.4 (petroleum ether/EtOAc 95:5). β-Diketone tautomer was observed in NMR (ca. 12%), only the most representative signals are described.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.86 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.24 – 1.30 (m, 14H, **CH_2**), 1.53 – 1.61 (m, 6H, **CH_2**), 2.22 – 2.30 (m, 6H, **H-2**, **H-9**, **H-13**), 3.64 (s, 3H, **CH_3O**), 5.45 (s, 1H, **H-11**). **β-diketone:** 3.52 (s, 2H, **H-11**), 2.39 – 2.50 (m, 4H, **H-9**, **H-13**).

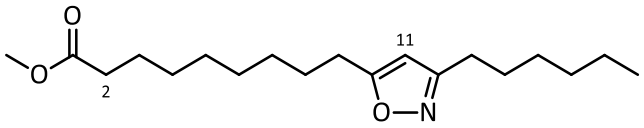
^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.13 (**CH_3**), 22.59 (**CH_2**), 25.00 (**CH_2**), 25.77 (**CH_2**), 25.80 (**CH_2**), 29.01 (**CH_2**), 29.15 (**CH_2**), 29.16 (**CH_2**), 29.24 (**CH_2**), 29.24 (**CH_2**), 31.64 (**CH_2**), 34.16 (**C-2**), 38.48 (**C-13**), 38.49 (**C-9**), 51.52 (**CH_3O**), 99.15 (**C-11**), 174.35 (***C*OOCH$_3$**), 194.59 (**C-10**), 194.60 (***C*OCH$_2$**). **β-diketone:** 43.85 (**C-9**), 43.92 (**C-13**), 57.28 (**C-11**), 204.48 (***C*OCH$_2$**), 204.54 (***C*OCH$_2$**).

IR (ATR) ν=2927, 2856, 1737, 1603, 1435, 1170, 776, 725 cm^{-1}.

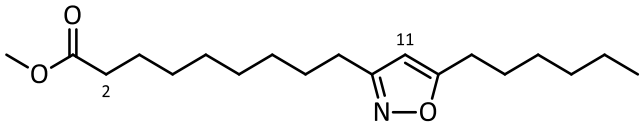
HR-MS (ESI^+): *m/z*: $[M+H]^+$ Calcd. for $C_{19}H_{35}O_4$ 327.2535; Found 327.2532.

Methyl 9-(3 or 5-hexylisoxazol-5 or 3-yl)nonanoate (**130**)

130a



130b



Chemical Formula: $C_{19}H_{33}NO_3$
MW=323.47 g·mol^{-1}

Hydroxylamine chloride (132 mg, 1.30 mmol) was added to a solution of the methyl (*Z*)-10-hydroxy-12-oxooctadec-10-enoate **129** (222 mg, 0.68 mmol) in pyridine (6.5 mL). The reaction mixture was stirred at room temperature for 24 h. The mixture was taken up in H_2O and extracted into EtOAc. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The resulting material was dissolved in toluene and *p*-toluenesulphonic acid (127 mg, 0.67 mmol) was added. This solution was stirred at 60 °C for 1.5 h. H_2O and EtOAc were added and the resulting organic layer was separated. The aqueous layer was extracted with EtOAc and the combined organic layers were dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to yield a mixture of isomers **130** (80 mg, 36%) as a yellow oil after purification by silica gel flash column chromatography (DCM/MeOH 98:2). R_f=0.45 (DCM). The ^{1}H NMR signals for both isomers were indistinguishable and they were elucidated by ^{13}C NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.26 – 1.37 (m, 14H, **CH_2**), 1.58 – 1.71 (m, 6H, **CH_2**), 2.30 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.60 (td, 2H, *J* = 7.9, 2.0 Hz, **CHC=NC*H*$_2$**), 2.69 (td, 2H, *J* = 7.9, 20 Hz, , **C*H*$_2$COC=H**), 3.66 (s, 3H, **CH_3O**), 5.79 (s, 1H, **H-11**).

130a:

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.17 (**CH_3**), 22.63 (**CH_2**), 25.04 (**CH_2**), 26.20 (**CH_2**), 26.85 (**CH_2**), 27.62 (**CH_2**), 28.43 (**CH_2**), 28.91 (**CH_2**), 29.04 (**CH_2**), 29.15 (**CH_2**), 29.21 (**CH_2**), 29.27 (**CH_2**), 31.56 (**CH_2**), 34.21 (**C-2**), 51.60 (**CH_3O**), 100.35 (**C-11**), 164.14 (**CH*C*=NCH$_2$**), 173.37 (**CH$_2$*C*OC=H**), 174.41 (***C*OOCH$_3$**).

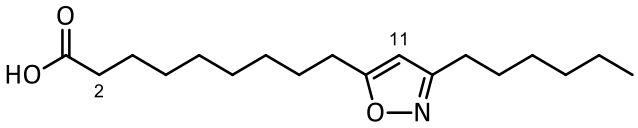
130b:

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.18 (**CH_3**), 22.66 (**CH_2**), 25.06 (**CH_2**), 26.22 (**CH_2**), 26.87 (**CH_2**), 27.62 (**CH_2**), 28.45 (**CH_2**), 28.91 (**CH_2**), 29.04 (**CH_2**), 29.16 (**CH_2**), 29.23 (**CH_2**), 29.27 (**CH_2**), 31.64 (**CH_2**), 34.22 (**C-2**), 51.60 (**CH_3O**), 100.38 (**C-11**), 164.23 (**CH*C*=NCH$_2$**), 173.49 (**CH$_2$*C*OC=H**), 174.43 (***C*OOCH$_3$**).

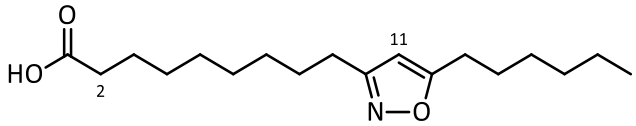
IR (ATR) ν=2926, 2855, 1735, 1601, 1435, 1170, 790, 725 cm^{-1}.

9-(3 or 5-Hexylisoxazol-5 or 3-yl)nonanoic acid (**131**)

131a



131b



Chemical Formula: $C_{18}H_{31}NO_3$
MW=309.44 g·mol^{-1}

The hydrolysis of the mixture of isomers **130** (80 mg, 0.24 mmol) was performed according to the general procedure **5.2.6.** for chemical ester hydrolysis using LiOH·H_2O (31 mg, 0.74 mmol) in THF/H_2O 1:1 (1 mL) to yield the corresponding mixture of isomers **131** (64 mg, 83%) as a yellowish solid. mp=55-57 °C. The ^{1}H NMR signals for both isomers were indistinguishable and they were elucidated by ^{13}C NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.29 – 1.36 (m, 14H, **CH_2**), 1.60 – 1.70 (m, 6H, **CH_2**), 2.34 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.61 (td, 2H, *J* = 7.9, 2.0 Hz, **CHC=NC*H*$_2$**), 2.69 (td, 2H, *J* = 7.9, 20 Hz, , **C*H*$_2$COC=H**), 5.80 (s, 1H, **H-11**).

131a:

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.17 (**CH_3**), 22.64 (**CH_2**), 24.78 (**CH_2**), 26.18 (**CH_2**), 26.84 (**CH_2**), 27.62 (**CH_2**), 28.41 (**CH_2**), 28.92 (**CH_2**), 29.05 (**CH_2**), 29.14 (**CH_2**), 29.25 (**CH_2**), 29.85 (**CH_2**), 31.57 (**CH_2**), 33.97 (**C-2**), 100.37 (**C-11**), 164.15 (**CH*C*=NCH$_2$**), 173.37 (**CH$_2$*C*OC=H**), 178.88 (**COOH**).

131b:

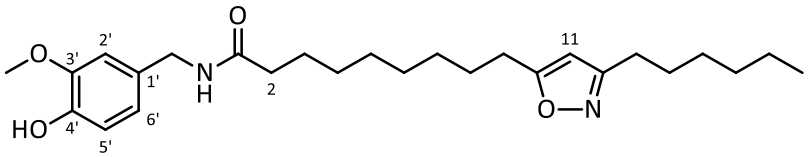
^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.19 (**CH_3**), 22.67 (**CH_2**), 24.78 (**CH_2**), 26.21 (**CH_2**), 26.88 (**CH_2**), 27.62 (**CH_2**), 28.45 (**CH_2**), 28.92 (**CH_2**), 29.10 (**CH_2**), 29.19 (**CH_2**), 29.25 (**CH_2**), 29.85 (**CH_2**), 31.65 (**CH_2**), 33.97 (**C-2**), 100.42 (**C-11**), 164.24 (**CH*C*=NCH$_2$**), 173.52 (**CH$_2$*C*OC=H**), 178.91 (**COOH**).

IR (ATR) ν=3111, 2919, 2851, 1693, 1601, 1411, 937, 720 cm^{-1}.

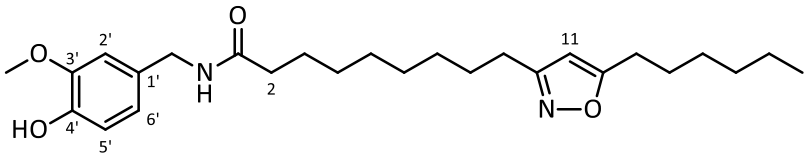
HR-MS (ESI$^+$): *m/z*: $[M+H]^+$ Calcd. for $C_{18}H_{32}NO_3$ 310.2377; Found 310.2402.

N-(4-Hydroxy-3-methoxybenzyl)-9-(3 or 5-hexylisoxazol-5 or 3-yl)nonanamide (**88**)

88a



88b



Chemical Formula: $C_{26}H_{40}N_2O_4$
MW=444.61 g·mol^{-1}

Amidation of the mixture of isomers **131** (65 mg, 0.21 mmol) was performed according to the general procedure **5.2.5.** for amide coupling using HATU (119 mg, 0.31 mmol), 4-hydroxy-3-methoxybenzylamine hydrochloride **99** (43 mg, 0.23 mmol) and DIPEA (110 µL, 0.63 mmol) in anhydrous DMF (3 mL) to yield a mixture of the two isomers **88** (40 mg, 43%) as a white solid after purification by silica gel flash column chromatography (petroleum ether/EtOAc 7:3). R_f=0.37 (petroleum ether/EtOAc 7:3). mp=56-59 °C. The ^{1}H NMR signals for both isomers were indistinguishable and they were elucidated by ^{13}C NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.26 – 1.38 (m, 14H, **CH_2**), 1.57 – 1.71 (m, 6H,**CH_2**), 2.20 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.59 (td, 2H, *J* = 7.9, 2.0 Hz, **CHC=NC*H*$_2$**), 2.68 (td, 2H, *J* = 7.9, 2.0 Hz, **C*H*$_2$COCH**), 3.87 (s, 3H, **CH_3O**), 4.35 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 5.80 (s, 1H, **H-11**), 5.89 (br s, 1H, **OH**), 6.79 (ddd, 3H, *J* = 12.50, 9.9, 5.0 Hz, **H$_{Ar}$**).

88a:

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.16 (**CH_3**), 22.62 (**CH_2**), 25.88 (**CH_2**), 26.10 (**CH_2**), 26.78 (**CH_2**), 27.60 (**CH_2**), 28.33 (**CH_2**), 28.90 (**CH_2**), 29.03 (**CH_2**), 29.12 (**CH_2**), 29.20 (**CH_2**), 31.55 (**CH_2**), 31.62 (**CH_2**), 36.63 (**C-2**), 43.84 (**CH_2NH**), 56.08 (**CH_3O**), 100.41 (**C-11**), 110.89 (**C-2'**), 114.53 (**C-5'**), 120.96 (**C-6'**), 130.16 (**C-1'**), 145.33 (**C-4'**), 146.86 (**C-3'**), 164.13 (**CH*C*=NCH$_2$**), 173.38 (**NHCO**), 173.57 (**CH$_2$*C*OC=H**).

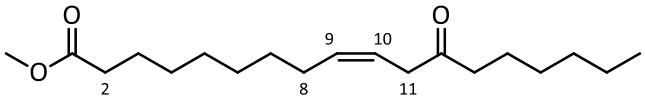
88a:

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.17 (**CH_3**), 22.65 (**CH_2**), 25.88 (**CH_2**), 26.19 (**CH_2**), 26.86 (**CH_2**), 27.60 (**CH_2**), 28.42 (**CH_2**), 28.90 (**CH_2**), 29.03 (**CH_2**), 29.15 (**CH_2**), 29.26 (**CH_2**), 31.55 (**CH_2**), 31.62 (**CH_2**), 36.63 (**C-2**), 43.84 (**CH_2NH**), 56.08 (**CH_3O**), 100.47 (**C-11**), 110.89 (**C-2'**), 114.53 (**C-5'**), 120.96 (**C-6'**), 130.16 (**C-1'**), 145.33 (**C-4'**), 146.86 (**C-3'**), 164.26 (**CH*C*=NCH$_2$**), 173.38 (**NHCO**), 173.57 (**CH$_2$*C*OC=H**).

IR (ATR) ν=3510, 3325, 2916, 2850, 1703, 1642, 1519, 1292, 1031, 846, 721 cm^{-1}.

HR-MS (ESI$^+$): *m/z*: $[M+H]^+$ Calcd. for $C_{26}H_{41}N_2O_4$ 445.3061; Found 445.3088.

Methyl (*Z*)-12-oxooctadec-9-enoate (**10b**)



Chemical Formula: $C_{19}H_{34}O_3$
MW=310.47 g·mol^{-1}
[3047-65-2]

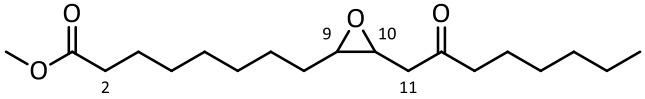
Oxidation of methyl ricinoleate **10a** (10 g, 32 mmol) was performed according to the general procedure **5.2.7.** for alcohol oxidation using CrO_3 (19.2 g, 192 mmol) and pyridine (31 mL, 384 mmol) in DCM (120 mL) to yield the ketone **10b** (5.96 g, 60%) as a yellowish oil after purification by silica gel column chromatography (petroleum ether/Et_2O 98:2). R_f=0.48 (petroleum ether/Et_2O 9:1). The spectral data were in accordance with the literature:[18]

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.86 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.24 – 1.33 (m, 14H, **CH_2**), 1.51 – 1.63 (m, 4H, **CH_2**), 2.01 (q, 2H, *J* = 6.4 Hz, **H-8**), 2.29 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.41 (t, 2H, *J* = 6.9 Hz, **$COCH_2$**), 3.13 (d, 2H, *J* = 5.7 Hz, **H-11**), 3.65 (s, 3H, **CH_3O**), 5.48 – 5.61 (m, 2H, **H-9**, **H-10**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.14 (**CH_3**), 22.61 (**CH_2**), 23.90 (**CH_2**), 25.03 (**CH_2**), 27.59 (**CH_2**), 29.01 (**CH_2**), 29.18 (**CH_2**), 29.19 (**CH_2**), 29.23 (**CH_2**), 29.38 (**CH_2**), 31.72 (**CH_2**), 34.19 (**C-2**), 41.77 (**C-11**), 42.49 (**$CO\underline{C}H_2$**), 51.55 (**CH_3O**), 121.14 (**C-10**), 133.63 (**C-9**), 174.37 (**$\underline{C}OOCH_3$**), 209.35 (**$\underline{C}OCH_2$**).

IR (ATR) ν=2926, 2855, 1738, 1716, 1435, 1195, 1170, 1072, 842, 725 cm^{-1}.

Methyl 8-[3-(2-oxooctyl)oxiran-2-yl]octanoate (**134**)



Chemical Formula: $C_{19}H_{34}O_4$
MW=326.47 g·mol^{-1}
[34724-40-8]

m-Chloroperbenzoic acid (214 mg, 1.24 mmol) was added to a solution of methyl (*Z*)-12-oxooctadec-9-enoate **10b** (210 mg, 0.68 mmol) in DCM (75 mL). The reaction mixture was stirred at room temperature for 16 h. An aqueous solution of 10% Na_2SO_3 was added and the organic layer was recovered, washed sequentially with saturated solution of $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated under reduced pressure to yield the epoxide **134** (110 mg, 50%) as a yellow oil after purification by silica gel column chromatography (petroleum

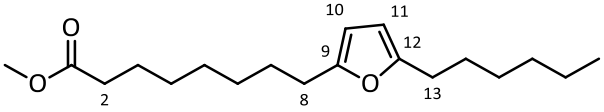
ether/Et_2O 8:2). R_f=0.8 (petroleum ether/Et_2O 7:3). The spectral data were in accordance with the literature:[19]

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.24 – 1.38 (m, 14H, **CH_2**), 1.43 – 1.49 (m, 2H, **CH_2**), 1.58 – 1.66 (m, 4H, **CH_2**), 2.30 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.47 (t, 2H, *J* = 6.9 Hz, **$COCH_2$**), 2.57 (dd, 1H, *J* = 17.2, 6.0 Hz, **H-11a**), 2.68 (dd, 1H, *J* = 17.2, 6.0 Hz, **H-11b**), 2.95 – 3.01 (m, 1H, **H-10**),3.26 – 3.32 (m, 1H, **H-9**), 3.67 (s, 3H, **CH_3O**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.17 (**CH_3**), 22.63 (**CH_2**), 23.71 (**CH_2**), 25.03 (**CH_2**), 26.55 (**CH_2**), 28.12 (**CH_2**), 28.98 (**CH_2**), 29.16 (**CH_2**), 29.29 (**CH_2**), 29.41 (**CH_2**), 31.72 (**CH_2**), 34.20 (**C-2**), 41.77 (**C-10**), 43.51 (**$CO\underline{C}H_2$**), 51.62 (**CH_3O**), 52.50 (**C-11**), 56.67 (**C-9**), 174.40 (**$\underline{C}OOCH_3$**), 208.63 (**$\underline{C}OCH_2$**).

IR (ATR) v=2951, 2917, 2850, 1736, 1702, 1174, 847, 717 cm^{-1}.

Methyl 8-(5-hexylfuran-2-yl)octanoate (**135**)



Chemical Formula: $C_{19}H_{32}O_3$
MW=308.46 g·mol^{-1}
[10038-16-1]

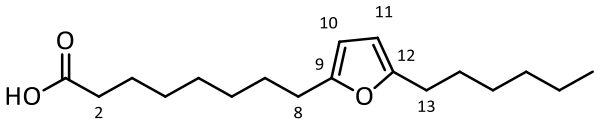
NaN_3 (36.5 mg, 0.56 mmol) and NH_4Cl (30 mg, 0.56 mmol) were added to a solution of methyl 8-[3-(2-oxooctyl)oxiran-2-yl]octanoate **134** (60 mg, 0.18 mmol) in EtOH/H_2O 5:1 (7.8 mL). The reaction mixture was stirred under reflux for 40 min. The mixture was taken up in H_2O and extracted into Et_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to yield the furan **135** (43 mg, 76%) as a yellowish oil after purification by silica gel column chromatography (petroleum ether/Et_2O 98:2). R_f=0.7 (petroleum ether/Et_2O 95:5). The spectral data were in accordance with the literature:[19]

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.29 – 1.34 (m, 12H, **CH_2**), 1.56 – 1.65 (m, 6H, **CH_2**), 2.30 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.55 (t, 2H, *J* = 6.9 Hz, **H-8**, **H-13**), 3.66 (s, 3H, **CH_3O**), 5.83 (s, 2H, **H-10**, **H-11**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.20 (**CH_3**), 22.71 (**CH_2**), 25.05 (**CH_2**), 28.15 (**CH_2**), 28.20 (**C-8**, **C-13**), 28.24 (**CH_2**), 29.02 (**CH_2**), 29.12 (2x**CH_2**), 29.18 (**CH_2**), 31.73 (**CH_2**), 34.21 (**C-2**), 51.55 (**CH_3O**), 104.91 (**C-11**), 104.98 (**C-10**), 154.57 (**C-12**), 154.79 (**C-9**), 174.39 (**$\underline{C}OOCH_3$**).

IR (ATR) v=2927, 2856, 1740, 1566, 1434, 1170, 1012, 778, 726 cm^{-1}.

8-(5-Hexylfuran-2-yl)octanoic acid (**136**)



Chemical Formula: $C_{18}H_{30}O_3$
MW=294.43 g·mol^{-1}
[4179-44-6][20]

The hydrolysis of methyl 8-(5-hexylfuran-2-yl)octanoate **135** (40 mg, 0.13 mmol) was performed according to the general procedure **5.2.6.** for chemical ester hydrolysis using LiOH·H_2O (17 mg, 0.39 mmol) in THF/H_2O 1:1 (700 µL) to yield the acid **136** (36 mg, 94%) as a yellow oil.

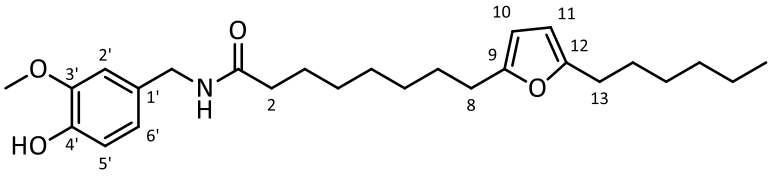
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.89 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.28 – 1.39 (m, 12H, **CH_2**), 1.56 – 1.68 (m, 6H, **CH_2**), 2.35 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.56 (t, 2H, *J* = 6.9 Hz, **H-8**, **H-13**), 5.84 (s, 2H, **H-10**, **H-11**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.21 (**CH_3**), 22.73 (**CH_2**), 24.78 (**CH_2**), 28.16 (**CH_2**), 28.21 (**C-8**), 28.22 (**C-13**), 28.25 (**CH_2**), 29.03 (**CH_2**), 29.11 (**CH_2**), 29.12 (**CH_2**), 31.75 (**CH_2**), 34.18 (**C-2**), 104.93 (**C-11**), 105.00 (**C-10**), 154.57 (**C-12**), 154.82 (**C-9**), 180.25 (**COOH**).

IR (ATR) v=2926, 2856, 1707, 1566, 1219, 1012, 953, 777, 725 cm^{-1}.

HR-MS (ESI$^+$): *m/z*: $[M+H]^+$ Calcd. for $C_{18}H_{31}O_3$ 295.2268; Found 295.2256.

8-(5-Hexylfuran-2-yl)-*N*-(4-hydroxy-3-methoxybenzyl)octanamide (**89**)



Chemical Formula: $C_{26}H_{39}NO_4$
MW=429.59 g·mol^{-1}

Amidation of 8-(5-hexylfuran-2-yl)octanoic acid **136** (40 mg, 0.14 mmol) was performed according to the general procedure **5.2.5.** for amide coupling using HATU (78 mg, 0.20 mmol), 4-hydroxy-3-methoxybenzylamine hydrochloride **99** (29 mg, 0.15 mmol) and DIPEA (71 µL, 0.41 mmol) in anhydrous DMF (2 mL) to yield the amide **89** (38 mg, 65%) as a yellow oil after purification by silica gel flash column chromatography (petroleum ether/EtOAc 7:3). R_f=0.41 (petroleum ether/EtOAc 7:3).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.26 – 1.36 (m, 12H, **CH_2**), 1.54 – 1.70 (m, 6H, **CH_2**), 2.19 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.55 (t, 2H, *J* = 6.9 Hz, **H-8**, **H-13**), 3.87 (s, 3H, **CH_3O**), 4.35 (d, 2H,

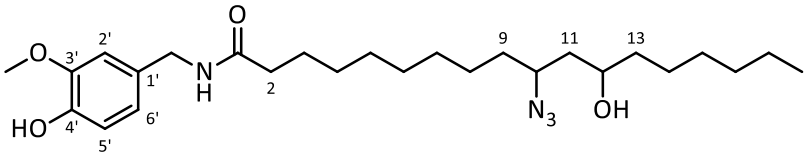
J = 5.7 Hz, **C*H*$_2$NH**), 5.65 (s, 1H, **CH$_2$N*H***), 5.66 (br s, 1H, **OH**), 5.83 (s, 2H, **H-10**, **H-11**), 6.79 (ddd, 3H, *J* = 12.5, 9.9, 5.0 H, **H$_{Ar}$**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.22 (**CH$_3$**), 22.72 (**CH$_2$**), 25.88 (**CH$_2$**), 28.16 (**CH$_2$**), 28.20 (**CH$_2$**), 28.21 (**CH$_2$**), 28.24 (**CH$_2$**), 29.03 (**CH$_2$**), 29.17 (**CH$_2$**), 29.22 (**CH$_2$**), 29.35 (**CH$_2$**), 31.74 (**CH$_2$**), 36.97 (**C-2**), 43.68 (**CH$_2$NH**), 56.07 (**CH$_3$O**), 104.92 (**C-11**), 104.99 (**C-10**), 110.83 (**C-2'**), 114.50 (**C-5'**), 120.95 (**C-6'**), 130.52 (**C-1'**), 145.26 (**C-4'**), 146.83 (**C-3'**), 154.56 (**C-9**), 154.83 (**C-12**), 172.97 (**NHCO**).

IR (ATR) ν=3312, 2918, 2851, 1640, 1550, 1381, 1256, 1153, 1124, 796, 722 cm^{-1}.

R$_f$=0.41 (petroleum ether/EtOAc 7:3)

10-Azido-12-hydroxy-*N*-(4-hydroxy-3-methoxybenzyl)octadecanamide (**137**)



Chemical Formula: $C_{26}H_{44}N_4O_4$
MW=476.65 g·mol^{-1}

$NaBH_4$ (68 mg, 1.79 mmol) was added in portions to a solution of the 10-azido-*N*-(4-hydroxy-3-methoxybenzyl)-12-oxooctadecanamide **118** (170 mg, 0.36 mmol) in MeOH (4 mL) previously cooled at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. The solvent was evaporated under reduced pressure. The mixture was taken up in H_2O and extracted into EtOAc. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to yield the β-hydroxyazide **137** (156 mg, 91%, yellow oil) as the mixture of stereoisomers. Diastereoisomers could be elucidated by NMR.

First diastereoisomer:

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH$_3$**), 1.25 – 1.34 (m, 17H, **CH$_2$**, **H-11a**), 1.43 – 1.47 (m, 3H, **CH$_2$**, **H-11b**), 1.51 – 1.66 (m, 6H, **CH$_2$**), 2.19 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.81 (s, 1H, **CHO*H***), 3.42 – 3.51 (m, 1H, **CHN$_3$**), 3.68 – 3.76 (m, 1H, **C*H*OH**), 3.88 (s, 3H, **CH$_3$O**), 4.35 (d, 2H, *J* = 5.7 Hz, **C*H$_2$*NH**), 5.65 (br s, 1H, **OH**), 6.80 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, **H$_{Ar}$**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.22 (**CH$_3$**), 22.75 (**CH$_2$**), 25.55 (**CH$_2$**), 25.83 (**CH$_2$**), 25.93 (**CH$_2$**), 29.29 (**CH$_2$**), 29.32 (**CH$_2$**), 29.35 (**CH$_2$**), 29.40 (**CH$_2$**), 29.42 (**CH$_2$**), 31.95 (**CH$_2$**), 34.41 (**C-9**), 36.95 (**C-2**), 37.85 (**C-13**), 41.57 (**C-11**), 43.70 (**CH$_2$NH**), 56.10 (**CH$_3$O**), 61.19 (**CHN$_3$**), 70.26 (**CHOH**), 110.85 (**C-2'**), 114.52 (**C-5'**), 120.96 (**C-6'**), 130.54 (**C-1'**), 145.27 (**C-4'**), 146.83 (**C-3'**), 172.98 (**NHCO**).

Second diastereoisomer:

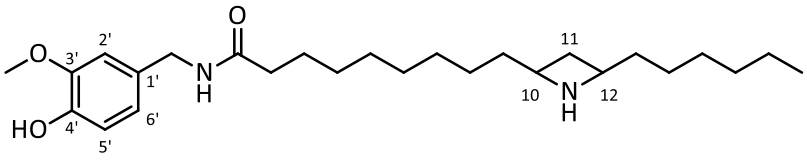
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.25 – 1.34 (m, 17H, **CH_2**, **H-11a**), 1.43 – 1.47 (m, 3H, **CH_2**, **H-11b**), 1.51 – 1.66 (m, 6H, **CH_2**), 2.19 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.81 (s, 1H, **CHO*H***), 3.55 – 3.63 (m, 1H, **CHN_3**), 3.78 – 3.85 (m, 1H, **C*H*OH**), 3.88 (s, 3H, **CH_3O**), 4.35 (d, 2H, *J* = 5.7 Hz, **C*H_2*NH**), 5.66 (br s, 1H, **OH**), 6.80 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, **H_{Ar}**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.22 (**CH_3**), 22.75 (**CH_2**), 25.71 (**CH_2**), 25.83 (**CH_2**), 26.18 (**CH_2**), 29.29 (**CH_2**), 29.32 (**CH_2**), 29.35 (**CH_2**), 29.40 (**CH_2**), 29.42 (**CH_2**), 31.95 (**CH_2**), 35.07 (**C-9**), 36.95 (**C-2**), 38.30 (**C-13**), 41.96 (**C-11**), 43.70 (**CH_2NH**), 56.10 (**CH_3O**), 59.95 (**CHN_3**), 68.81 (**CHOH**), 110.85 (**C-2'**), 114.52 (**C-5'**), 120.96 (**C-6'**), 130.54 (**C-1'**), 145.27 (**C-4'**), 146.83 (**C-3'**), 172.98 (**NHCO**).

IR (ATR) ν=3299, 3087, 2926, 2854, 2098, 1644, 1514, 1272, 1034, 722 cm^{-1}.

HR-MS (ESI^+): *m/z*: $[M+Na]^+$ Calcd. for $C_{26}H_{44}N_4O_4Na$ 458.3377; Found 458.3376.

9-(4-Hexylazetidin-2-yl)-*N*-(4-hydroxy-3-methoxybenzyl)nonanamide (**90**)



Chemical Formula: $C_{26}H_{44}N_2O_3$
MW=432.64 g·mol^{-1}

PPh_3 (80 mg, 0.30 mmol) was added to a solution of 10-azido-12-hydroxy-*N*-(4-hydroxy-3-methoxybenzyl)octadecanamide **137** (145 mg, 0.30 mmol) in dry MeCN (2 mL) under argon atmosphere. The reaction mixture was stirred at reflux for 24 h. The solvent was evaporated under reduced pressure to yield the azetidine **90** (20 mg, 15%) as a yellowish solid after purification by silica gel flash column chromatography (EtOAc/MeOH 95:5). R_f=0.45 (EtOAc/MeOH 9:1). mp=95-98 °C.

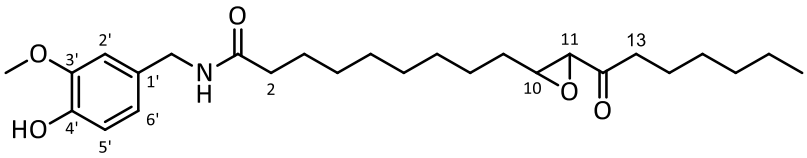
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.13 – 1.34 (m, 18H, **CH_2**), 1.51 – 1.69 (m, 6H, **CH_2**), 2.01 (t, 2H, *J* = 6.9 Hz, **H-11**), 2.18 (t, 2H, *J* = 6.9 Hz, **H-2**), 3.62 – 3.72 (m, 2H, **H-10**, **H-12**), 3.85 (s, 3H, **CH_3O**), 4.28 – 4.40 (m, 2H, **C*H_2*NH**), 4.51 (br s, 1H, **CH_2N*H*CH_2**), 5.74 – 5.82 (m, 1H, **CH_2N*H***), 6.77 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, **H_{Ar}**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.22 (**CH_3**), 22.74 (**CH_2**), 25.73 (**CH_2**), 25.78 (**CH_2**), 25.93 (**CH_2**), 29.18 (**CH_2**), 29.30 (**CH_2**), 29.32 (**CH_2**), 29.39 (**CH_2**), 29.57 (**CH_2**), 31.99 (**C-11**), 32.27 (**CH_2**), 36.91 (**C-2**), 37.82 (**CH_2**), 37.93 (**CH_2**), 43.62 (**CH_2NH**), 55.67 (**C-10**, **C-12**), 56.01 (**CH_3O**), 111.18 (**C-2'**), 115.21 (**C-5'**), 120.86 (**C-6'**), 130.32 (**C-1'**), 145.85 (**C-4'**), 147.47 (**C-3'**), 172.96 (**NHCO**).

IR (ATR) v=3303, 2919, 2850, 1637, 15742, 1464, 1262, 1155, 1126, 1034, 721 cm^{-1}.

HR-MS (ESI^+): *m/z*: $[M+H]^+$ Calcd. for $C_{26}H_{45}N_2O_3$ 433.3425; Found 433.3459.

9-(3-Heptanoyloxiran-2-yl)-*N*-(4-hydroxy-3-methoxybenzyl)nonanamide (**91**)



Chemical Formula: $C_{26}H_{41}NO_5$
MW=447.61 g·mol^{-1}

30% aqueous solution of H_2O_2 (170 µL, 2.16 mol) was added to a solution of (*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-12-oxooctadec-10-enamide **75** (120 mg, 0.29 mmol) and 1 M NaOH (280 µL, 0.28 mmol) in MeOH (1.2 mL) cooled at 0 °C. The reaction mixture was stirred at room temperature for 4 h. Saturated aqueous solution of $Na_2S_2O_4$ was added to destroy any remaining peroxide while the temperature was kept below 40 °C. H_2O was added and the aqueous solution was extracted with diethyl ether. The organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated under reduced pressure to yield the epoxyketone **91** (80 mg, 64%) as a white solid after purification by silica gel column chromatography ($CHCl_3$/EtOAc 8:2). R_f=0.38 ($CHCl_3$/EtOAc 8:2). mp=54-55 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.25 – 1.33 (m, 16H, **CH_2**), 1.40 – 1.47 (m, 2H, **CH_2**), 1.61 – 1.68 (m, 4H, **CH_2**), 2.19 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.27 (ddd, 1H, *J* = 17.1, 8.5, 6.3 Hz, **H-13a**), 2.42 (ddd, 1H, *J* = 17.1, 8.5, 6.3 Hz, **H-13b**), 3.00 – 3.04 (m, 1H, **H-10**), 3.19 (d, 1H, *J* = 1.8 Hz, **H-11**), 3.88 (s, 3H, **CH_3O**), 4.35 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 5.60 (s, 1H, **CH_2N*H***), 5.65 (br s, 1H, **OH**), 6.80 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, **H_{Ar}**).

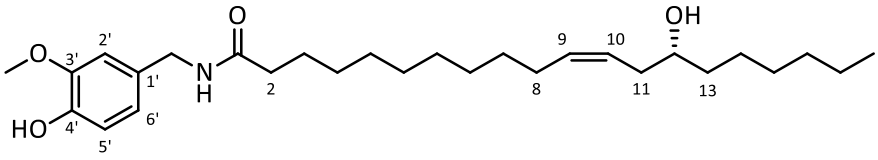
^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.15 (**CH_3**), 22.61 (**CH_2**), 23.24 (**CH_2**), 25.85 (**CH_2**), 25.88 (**CH_2**), 28.97 (**CH_2**), 29.29 (**CH_2**), 29.34 (**CH_2**), 29.37 (**CH_2**), 29.85 (**CH_2**), 31.68 (**CH_2**), 31.94 (**CH_2**), 36.96 (**C-2**), 37.35 (**C-13**), 43.70 (**CH_2NH**), 56.11 (**CH_3O**), 58.48 (**C-10**), 59.80 (**C-11**), 110.87 (**C-2'**), 114.52 (**C-5'**), 120.98 (**C-6'**), 130.58 (**C-1'**), 145.28 (**C-4'**), 146.83 (**C-3'**), 172.94 (**NHCO**), 208.21 (***C*OCH$_2$**).

IR (ATR) v=3507, 3294, 2923, 2851, 1712, 1637, 1517, 1238, 1024, 857, 706 cm^{-1}.

HR-MS (ESI^+): *m/z*: $[M+Na]^+$ Calcd. for $C_{26}H_{41}NO_5Na$ 470.2877; Found 470.286.

$[\alpha]_D^{20}$<1° (c 0.5, DCM)

(*R,Z*)-14-Hydroxy-*N*-(4-hydroxy-3-methoxybenzyl)eicos-11-enamide (**92**)



Chemical Formula: $C_{28}H_{47}NO_4$
MW=461.68 g·mol^{-1}

Amidation of lesquerolic acid **12a** (160 mg, 0.49 mmol) was performed according to the general procedure **5.2.5.** for amide coupling using HATU (279 mg, 0.73 mmol), 4-hydroxy-3-methoxybenzylamine hydrochloride **99** (102 mg, 0.54 mmol) and DIPEA (256 µL, 1.47 mmol) in anhydrous DMF (7 mL) to yield the amide **92** (48 mg, 21%) as a yellow oil after purification by silica gel flash column chromatography (DCM/EtOAc 95:5). R_f=0.48 (DCM/EtOAc 9:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.23 – 1.36 (m, 20H, **CH_2**), 1.42 – 1.49 (m, 2H, **CH_2**), 1.62 – 1.68 (m, 2H, **CH_2**), 2.04 (q, 2H, *J* = 6.4 Hz, **H-8**), 2.15 – 2.24 (m, 4H, **H-2**, **H-11**), 3.57 – 3.64 (m, 1H, **C*H*OH**), 3.88 (s, 3H, **CH_3O**), 4.35 (d, *J* = 5.7 Hz, 2H, **C*H*$_2$NH**), 5.35 – 5.45 (m, 1H, **H-10**), 5.50 – 5.60 (m, 1H, **H-9**), 5.60 – 5.71 (m, 2H, **CH_2N*H*, OH**), 6.78 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, **H_{Ar}**).

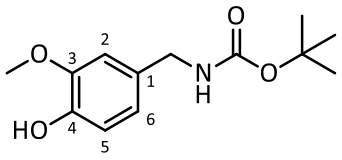
^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.21 (**CH_3**), 22.74 (**CH_2**), 25.83 (**CH_2**), 25.88 (**CH_2**), 27.51 (**CH_2**), 29.36 (**CH_2**), 29.37 (**CH_2**), 29.39 (**CH_2**), 29.47 (**CH_2**), 29.51 (**CH_2**), 29.55 (**CH_2**), 29.73 (**CH_2**), 31.95 (**C-8**), 35.46 (**C-11**), 36.93 (**C-2**), 36.94 (**C-13**), 43.63 (**CH_2NH**), 56.04 (**CH_3O**), 71.64 (**CHOH**), 110.85 (**C-2'**), 114.54 (**C-5'**), 120.88 (**C-6'**), 125.28 (**C-10**), 130.48 (**C-1'**), 133.54 (**C-9**), 145.26 (**C-4'**), 146.86 (**C-3'**), 173.10 (**NHCO**).

IR (ATR) ν=3289, 2923, 2853, 1644, 1514, 1273, 1123, 1035, 722 cm^{-1}.

HR-MS (ESI$^+$): *m/z*: $[M+H]^+$ Calcd. for $C_{28}H_{48}NO_4$ 462.3578; Found 462.361.

$[\alpha]_D^{20}$=+13.18° (c 1.77, DCM)

tert-Butyl 4-hydroxy-3-methoxybenzylcarbamate (**138**)



Chemical Formula: $C_{13}H_{19}NO_4$
MW=253.29 g·mol^{-1}
[130972-89-3]

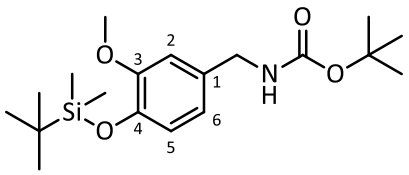
Di-*tert*-butyl dicarbonate (2.30 g, 10 mmol) was slowly added to a solution of 4-hydroxy-3-methoxybenzylamine hydrochloride **99** (2 g, 10 mmol) and TEA (4.8 mL, 35 mmol) in DCM (60 mL) previously cooled at 0 °C (ice-H_2O bath). The reaction mixture was stirred at room temperature for 24 h. The mixture was acidified until pH 1 with 1 M aqueous solution of HCl and organic layer was recovered, washed again with 1 M HCl and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to yield the *N*-Boc amine **138** (1.96 g, 74%) as a colorless oil after purification by silica gel flash column chromatography (petroleum ether/EtOAc 9:1). R_f=0.47 (petroleum ether/EtOAc 9:1). The spectral data were in accordance with the literature:[21]

^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.46 (s, 9H, **C(CH$_3$)$_3$**), 3.88 (s, 3H, **CH$_3$O**), 4.22 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 4.78 (s, 1H, **CH$_2$N*H***), 5.58 (s, 1H, **OH**), 6.74 – 6.87 (m, 3H, **H$_{Ar}$**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 28.56 (**C(*C*H$_3$)$_3$**), 44.80 (**CH$_2$NH**), 56.05 (**CH$_3$O**), 79.6 (**C**(CH$_3$)$_3$), 110.47 (**C-2**), 114.42 (**C-5**), 120.61 (**C-6**), 131.05 (**C-1**), 145.09 (**C-4**), 146.75 (**C-3**), 156.02 (**NHCO**).

IR (ATR) ν=3357, 2927, 2934, 1686, 1512, 1157, 1032, 860 cm^{-1}.

tert-Butyl-4-[(*tert*-butyldimethylsilyl)oxy]-3-methoxybenzylcarbamate (**139**)



Chemical Formula: $C_{19}H_{33}NO_4Si$
MW=367.56 g·mol^{-1}
[944256-04-6]

tert-butyldimethylsilyl chloride (1.81 g, 12 mmol) was slowly added to a solution of *tert*-butyl 4-hydroxy-3-methoxybenzylcarbamate **138** (1.96 g, 7.74 mmol), DMAP (38 mg, 0.31 mmol) and TEA (2.7 mL, 19 mmol) in DCM (65 mL). The reaction was stirred at room temperature for 24 h. The

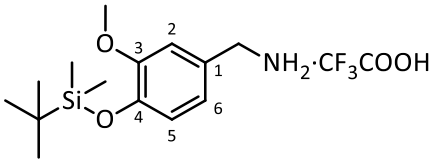
mixture was acidified until pH 1 with 1 M aqueous solution of HCl and organic layer was recovered, washed again with 1 M HCl and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to yield the silylated phenol **139** (2 g, 70%) as a white solid after purification by silica gel flash column chromatography (petroleum ether/EtOAc 9:1). R_f=0.5 (petroleum ether/EtOAc 9:1). mp=66-68 °C. The spectral data were in accordance with the literature:[22]

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.14 (s, 6H, **Si(CH$_3$)$_2$**), 0.99 (s, 9H, **SiC(CH$_3$)$_3$**), 1.46 (s, 9H, **OC(CH$_3$)$_3$**), 3.79 (s, 3H, **CH$_3$O**), 4.22 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 4.79 (br s, 1H, **CH$_2$N*H***), 6.68 – 6.80 (m, 3H, **H$_{Ar}$**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = -4.51 (**Si(CH$_3$)$_2$**), 18.58 (**Si*C*(CH$_3$)$_3$**), 25.86 (**SiC(*C*H$_3$)$_3$**), 28.56 (**OC(*C*H$_3$)$_3$**), 44.77 (**CH$_2$NH**), 55.59 (**CH$_3$O**), 79.53 (**O*C*(CH$_3$)$_3$**), 111.78 (**C-2**), 120.00 (**C-6**), 120.93 (**C-5**), 132.46 (**C-1**), 144.46 (**C-4**), 151.14 (**C-3**), 156.02 (**NHCO**).

IR (ATR) ν=3320, 2931, 1678, 1509, 1275, 1159, 802 cm^{-1}.

(4-{[*tert*-Butyl(dimethyl)silyl]oxy}-3-methoxyphenyl)methanamine trifluoroacetic salt (**140**)



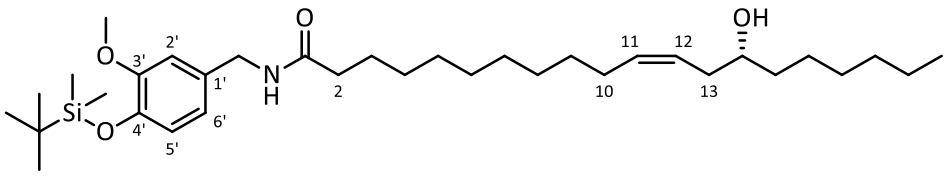
Chemical Formula: $C_{16}H_{26}F_3NO_4Si$
MW=381.46 g·mol^{-1}

Trifluoroacetic acid (1.6 mL, 20 mmol) was added to a solution of *tert*-butyl-4-[(*tert*-butyldimethylsilyl)oxy]-3-methoxybenzylcarbamate **139** (500 mg, 1.36 mmol) in DCM (6 mL). The reaction mixture was stirred at room temperature for 3 h. The solvent was evaporated under reduced pressure to yield the salt **140** (363 mg, 70%) as a white-off solid. mp=70-73 °C.

^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 0.12 (s, 6H, **Si(CH$_3$)$_2$**), 0.95 (s, 9H, **SiC(CH$_3$)$_3$**), 3.77 (s, 3H, **CH$_3$O**), 3.87– 3.99 (m, 2H, **C*H*$_2$NH$_2$**), 6.76 – 7.15 (m, 3H, **H$_{Ar}$**), 8.15 (br s, 3H, **CH$_2$N*H*$_2$**, **CF$_3$COO*H***).

IR (ATR) ν=3153, 2957, 2931, 1774, 1676, 1602, 1511, 1159, 1034, 781, 704 cm^{-1}.

(*R,Z*)-*N*-(3-{[*tert*-Butyl(dimethyl)silyl]oxy}-4-methoxybenzyl)-14-hydroxyeicos-11-enamide (**141**)



Chemical Formula: $C_{34}H_{61}NO_4Si$
MW=575.94 g·mol^{-1}

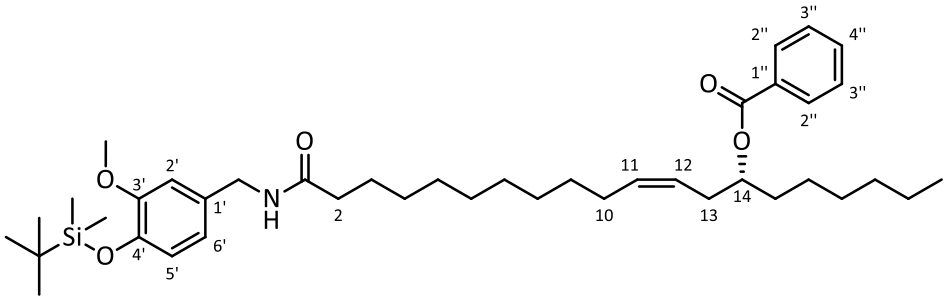
Amidation of lesquerolic acid **12a** (85 mg, 0.26 mmol) was performed according to the general procedure **5.2.5.** for amide coupling using HATU (148 mg, 0.39 mmol), 4-{[*tert*-butyl(dimethyl)silyl]oxy}-3-methoxyphenyl) methanamine trifluoroacetic salt **140** (83 mg, 0.31 mmol) and DIPEA (68 µL, 0.39 mmol) in anhydrous DMF (3.7 mL) to yield the amide **141** (70 mg, 48%) as a yellow oil after purification by silica gel flash column chromatography (petroleum ether/EtOAc 8:2). R_f=0.47 (petroleum ether/EtOAc 7:3).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.14 (s, 6H, **Si(CH$_3$)$_2$**), 0.88 (t, 3H, *J* = 6.9 Hz, **CH$_3$**), 0.98 (s, 9H, **SiC(CH$_3$)$_3$**), 1.24 – 1.34 (m, 20H, **CH$_2$**), 1.42 – 1.47 (m, 2H, **CH$_2$**), 1.60 – 1.70 (m, 2H, **CH$_2$**), 2.04 (q, 2H, *J* = 6.4, **H-10**), 2.16 – 2.23 (m, 4H, **H-2**, **H-13**), 3.56 – 3.64 (m, 1H, **C*H*OH**), 3.78 (s, 3H, **CH$_3$O**), 4.35 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 5.34 – 5.44 (m, 1H, **H-12**), 5.50 – 5.60 (m, 1H, **H-11**), 5.71 (br s, 1H, **CH$_2$N*H***), 6.74 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, **H$_{Ar}$**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = -4.50 (**Si(CH$_3$)$_2$**), 14.23 (**CH$_3$**), 18.58 (**Si*C*(CH$_3$)$_3$**), 22.76 (**CH$_2$**), 25.84 (**CH$_2$**, **SiC(*C*H$_3$)$_3$**), 25.92 (**CH$_2$**), 27.53 (**CH$_2$**), 29.37 (**CH$_2$**), 29.42 (**CH$_2$**), 29.43 (**CH$_2$**), 29.49 (**CH$_2$**), 29.53 (**CH$_2$**), 29.55 (**CH$_2$**), 29.76 (**CH$_2$**), 30.46 (**CH$_2$**), 31.98 (**CH$_2$**), 35.50 (**CH$_2$**), 36.98 (**C-2**), 43.62 (**CH$_2$NH**), 55.61 (**CH$_3$O**), 71.63 (**CHOH**), 112.07 (**C-2'**), 120.29 (**C-6'**), 120.98 (**C-5'**), 125.29 (**C-12**), 131.97 (**C-1'**), 133.57 (**C-11**), 144.61 (**C-4'**), 151.21 (**C-3'**), 173.01 (**NHCO**).

IR (ATR) ν=3288, 2925, 2854, 1645, 1511, 1282, 1039, 838, 723 cm^{-1}.

(*R,Z*)-20-[(4-{[*tert*-Butyl(dimethyl)silyl]oxy}-3-methoxybenzyl)amino]-20-oxoeicos-9-en-7-yl benzoate (**142**)



Chemical Formula: $C_{41}H_{65}NO_5Si$
MW=680.04 g·mol^{-1}

Esterification of (*R,Z*)-*N*-(3-{[*tert*-butyl(dimethyl)silyl]oxy}-4-methoxybenzyl)-14-hydroxyeicos-11-enamide **141** (27 mg, 0.05 mmol) was performed according to the general procedure **5.2.9.** for DCC-mediated esterification using DCC (27 mg, 0.13 mmol), DMAP (10 mg, 0.08 mmol) and benzoic acid (8 mg, 0.06 mmol) in anhydrous toluene (1 mL) to yield the ester **142** (19 mg, 54%) as a yellow oil after purification by silica gel column chromatography (petroleum ether/EtOAc 8:2). R_f=0.6 (petroleum ether/EtOAc 7:3).

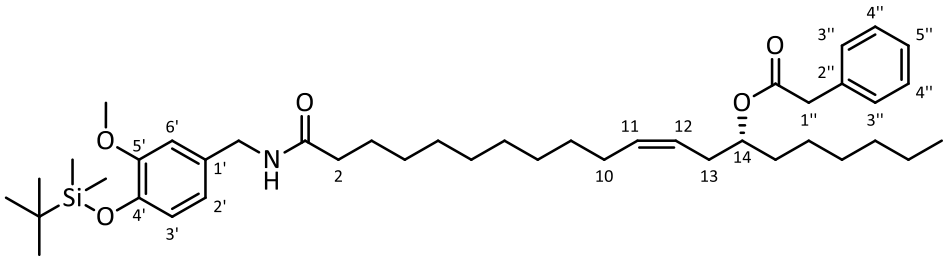
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.14 (s, 6H, **Si(CH$_3$)$_2$**), 0.86 (t, 3H, *J* = 6.9 Hz, **CH$_3$**), 0.99 (s, 9H, **SiC(CH$_3$)$_3$**), 1.22 – 1.33 (m, 20H, **CH$_2$**), 1.62 – 1.71 (m, 4H, **CH$_2$**), 2.03 (q, 2H, *J* = 6.4 Hz, **H-10**), 2.20 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.35 – 2.52 (m, 2H, **H-13**), 3.78 (s, 3H, **CH$_3$O**), 4.36 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 5.07 – 5.18 (m, 1H, **H-14**), 5.34 – 5.52 (m, 2H, **H-11**, **H-12**), 5.64 (br s, 1H, **CH$_2$N*H***), 6.74 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, **H$_{Ar}$**), 7.38 – 7.46 (m, 2H, **H-3''**), 7.51 – 7.57 (m, 1H, **H-4''**), 8.00 – 8.07 (m, 2H, **H-2''**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = -4.48 (**Si(CH$_3$)$_2$**), 14.21 (**CH$_3$**), 18.60 (**Si*C*(CH$_3$)$_3$**), 22.74 (**CH$_2$**), 25.55 (**CH$_2$**), 25.86 (**SiC(*C*H$_3$)$_3$**), 25.95 (**CH$_2$**), 27.54 (**CH$_2$**), 29.35 (**CH$_2$**), 29.41 (**CH$_2$**), 29.47 (**CH$_2$**), 29.49 (**CH$_2$**), 29.58 (**CH$_2$**), 29.62 (**CH$_2$**), 29.71 (**CH$_2$**), 31.88 (**CH$_2$**), 32.17 (**CH$_2$**), 33.87 (**CH$_2$**), 37.03 (**C-2**), 43.65 (**CH$_2$NH**), 55.64 (**CH$_3$O**), 74.85 (**C-14**), 112.10 (**C-2'**), 120.32 (**C-6'**), 121.01 (**C-5'**), 124.25 (**C-12**), 128.41 (2x**C-3''**), 129.69 (2x**C-2''**), 130.95 (**C-1''**), 131.96 (**C-1'**), 132.84 (**C-11**), 132.96 (**C-4''**), 144.66 (**C-4'**), 151.24 (**C-3'**), 166.41 (**COOPh**), 172.98 (**NHCO**).

IR (ATR) ν=3288, 2925, 2854, 1717, 1511, 1270, 1110, 899, 838, 710 cm^{-1}.

HR-MS (ESI$^+$): *m/z*: $[M+H]^+$ Calcd. for $C_{41}H_{66}NO_5Si$ 680.4705; Found 680.4702.

(*R,Z*)-14-[(3-{[*tert*-Butyl(dimethyl)silyl]oxy}-4-methoxybenzyl)amino]-1-hexyl-14-oxotetradec-3-enyl phenylacetate (**143**)



Chemical Formula: $C_{42}H_{67}NO_5Si$
MW=694.07 g·mol^{-1}

Esterification of (*R,Z*)-*N*-(3-{[*tert*-butyl(dimethyl)silyl]oxy}-4-methoxybenzyl)-14-hydroxyeicos-11-enamide **141** (68 mg, 0.12 mmol) was performed according to the general procedure **5.2.9.** for DCC-mediated esterification using DCC (61 mg, 0.29 mmol), DMAP (22 mg, 0.18 mmol) and phenylacetic acid (24 mg, 0.18 mmol) in anhydrous toluene (2.4 mL) to yield the ester **143** (70 mg, 86%) as a yellowish oil after purification by silica gel column chromatography (petroleum ether/EtOAc 8:2).

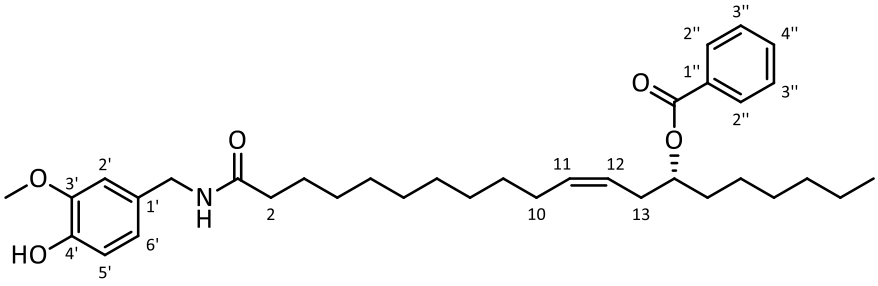
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.14 (s, 6H, **Si(CH$_3$)$_2$**), 0.86 (t, 3H, *J* = 6.9 Hz, **CH$_3$**), 0.99 (s, 9H, **SiC(CH$_3$)$_3$**), 1.23 – 1.30 (m, 20H, **CH$_2$**), 1.48 – 1.54 (m, 2H, **CH$_2$**), 1.60 – 1.69 (m, 2H, **CH$_2$**), 1.98 (q, 2H, *J* = 6.4 Hz, **H-10**), 2.19 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.24 – 2.30 (m, 2H, **H-13**), 3.58 (s, 2H, **H-1'**), 3.78 (s, 3H, **CH$_3$O**), 4.35 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 4.83 – 2.91 (m, 1H, **H-14**), 5.23 – 5.32 (m, 1H, **H-12**), 5.39 – 5.48 (m, 1H, **H-11**), 5.66 (br s, 1H, **CH$_2$N*H***), 6.74 (ddd, 3H, *J* = 12,5, 9.9, 5.0 Hz, **H$_{Ar}$**), 7.21 – 7.33 (m, 5H, **H$_{Phenyl}$**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = -4.49 (**Si(CH$_3$)$_2$**), 14.21 (**CH$_3$**), 18.59 (**Si*C*(CH$_3$)$_3$**), 22.67 (**CH$_2$**), 25.34 (**CH$_2$**), 25.86 (**SiC(*C*H$_3$)$_3$**), 25.94 (**CH$_2$**), 27.47 (**CH$_2$**), 29.21 (**CH$_2$**), 29.42 (**CH$_2$**), 29.49 (**CH$_2$**), 29.60 (**CH$_2$**), 29.64 (**CH$_2$**), 29.72 (**CH$_2$**), 30.47 (**CH$_2$**), 31.83 (**CH$_2$**), 32.05 (**CH$_2$**), 34.37 (**CH$_2$**), 37.01 (**C-2**), 41.91 (**C-1''**), 43.63 (**CH$_2$NH**), 55.63 (**CH$_3$O**), 74.64 (**C-14**), 112.09 (**C-2'**), 120.30 (**C-6'**), 121.00 (**C-5'**), 124.23 (**C-12**), 127.08 (**C-5''**), 128.61 (2x**C-4''**), 129.36 (2x**C-3''**), 131.97 (**C-1'**), 132.84 (**C-11**), 134.47 (**C-2''**), 144.64 (**C-4'**), 151.22 (**C-3'**), 171.46 (**COOCH$_2$**), 172.98 (**NHCO**).

IR (ATR) ν=3289, 2925, 2854, 1732, 1644, 1512, 1281, 1038, 838, 781 cm^{-1}.

HR-MS (ESI$^+$): *m/z*: $[M+Na]^+$ Calcd. for $C_{42}H_{67}NO_5SiNa$ 716.4681; Found 716.467.

(*R,Z*)-20-[(4-Hydroxy-3-methoxybenzyl)amino]-20-oxoeicos-9-en-7-yl benzoate (**93**)



Chemical Formula: $C_{35}H_{51}NO_5$
MW=565.78 g·mol^{-1}

Deprotection of (*R,Z*)-20-[(4-{[*tert*-butyl(dimethyl)silyl]oxy}-3-methoxybenzyl)amino]-20-oxoeicos-9-en-7-yl benzoate **142** (8 mg, 0.01 mmol) was performed according to the general procedure **5.2.10.** for TBDMS deprotection using TBAF (18 µL of a commercial 1 M solution in THF, 0.02 mmol) in anhydrous THF (350 µL) to yield the deprotected phenol **93** (5 mg, 74%) as a colorless oil after purification by silica gel flash column chromatography (petroleum ether/EtOAc 4:6). R_f=0.48 (petroleum ether/EtOAc 1:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.86 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.20 – 1.35 (m, 18H, **CH_2**), 1.59 – 1.75 (m, 6H, **CH_2**), 2.02 (q, 2H, *J* = 6.4 Hz, **H-10**), 2.19 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.33 – 2.51 (m, 2H, **H-13**), 3.87 (s, 3H, **CH_3O**), 4.36 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 5.09 – 5.17 (m, 1H, **H-14**), 5.36 – 5.51 (m, 2H, **H-11**, **H-12**), 5.55 – 5.62 (b s, 1H, **CH_2N*H***), 5.65 (br s, 1H, **OH**), 6.80 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, **H_{Ar}**), 7.43 (t, 2H, *J* = 6.9 Hz, **H-2''**), 7.52 – 7.57 (m, 1H, **H-4''**), 8.01 – 8.05 (m, 2H, **H-3''**).

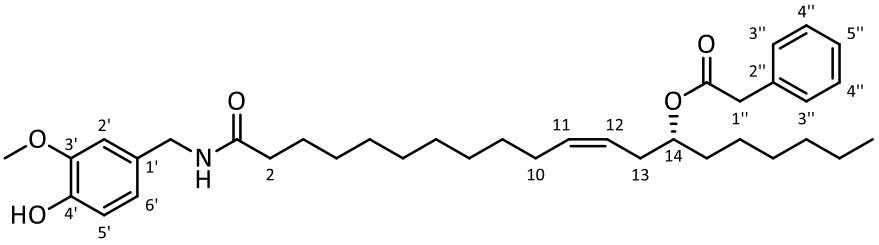
^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.21 (**CH_3**), 22.74 (**CH_2**), 25.55 (**CH_2**), 25.93 (**CH_2**), 27.54 (**CH_2**), 29.36 (**CH_2**), 29.40 (**CH_2**), 29.45 (**CH_2**), 29.47 (**CH_2**), 29.57 (**CH_2**), 29.60 (**CH_2**), 29.70 (**CH_2**), 31.88 (**CH_2**), 32.16 (**CH_2**), 33.86 (**C-13**), 37.02 (**C-2**), 43.69 (**CH_2NH**), 56.09 (**CH_3O**), 74.86 (**C-14**), 110.83 (**C-2'**), 114.49 (**C-5'**), 120.97 (**C-6'**), 124.26 (**C-12**), 128.42 (2x**C-3''**), 129.69 (2x**C-2''**), 130.57 (**C-1'**), 130.94 (**C-1''**), 132.85 (**C-11**), 132.97 (**C-4''**), 145.26 (**C-4'**), 146.81 (**C-3'**), 166.42 (**COOPh**), 172.99 (**NHCO**).

IR (NaCl cell, CS_2) v=3544, 3442, 3005, 2949, 2922, 2851, 1716, 1681, 1270, 709 cm^{-1}.

HR-MS (ESI$^+$): *m/z*: $[M+H]^+$ Calcd. for $C_{35}H_{52}NO_5$ 566.384; Found 566.388.

$[\alpha]_D^{20}$<+1° (c 0.4, DCM)

(*R,Z*)-20-[(4-Hydroxy-3-methoxybenzyl)amino]-20-oxoeicos-9-en-7-yl 2-phenylacetate (**94**)



Chemical Formula: $C_{36}H_{53}NO_5$
MW=579.81 g·mol^{-1}

Deprotection of (*R,Z*)-14-[(3-{[*tert*-butyl(dimethyl)silyl]oxy}-4-methoxybenzyl)amino]-1-hexyl-14-oxotetradec-3-enyl phenylacetate **143** (70 mg, 0.10 mmol) was performed according to the general procedure **5.2.10.** for TBDMS deprotection using TBAF (152 µL of a commercial 1 M solution in THF, 0.15 mmol) in anhydrous THF (3 mL) to yield the compound **94** (45 mg, 77%) as a yellowish oil after purification by silica gel flash column chromatography (petroleum ether/EtOAc 6:4). R_f=0.4 (petroleum ether/EtOAc 1:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.19 – 1.33 (m, 20H, **CH_2**), 1.47 – 1.56 (m, 2H, **CH_2**), 1.60 – 1.70 (m, 2H, **CH_2**), 1.99 (q, 2H, *J* = 6.4 Hz, **H-10**), 2.19 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.23 – 2.36 (m, 2H, **H-13**), 3.59 (s, 2H, **H-1''**), 3.88 (s, 3H, **CH_3O**), 4.35 (d, 2H, *J* = 5.7 Hz, **C*H*$_2$NH**), 4.88 (p, 1H, *J* = 6.2 Hz, **H-14**), 5.24 – 5.33 (m, 1H, **H-12**), 5.40 – 5.49 (m, 1H, **H-11**), 5.64 – 5.76 (m, 2H, **CH_2N*H*, OH**), 6.80 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, **H_{Ar}**), 7.23 – 7.34 (m, 5H, **H_{Phenyl}**).

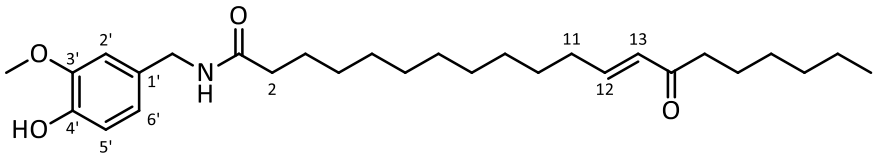
^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.20 (**CH_3**), 22.66 (**CH_2**), 25.32 (**CH_2**), 25.91 (**CH_2**), 27.46 (**CH_2**), 29.20 (**CH_2**), 29.40 (**CH_2**), 29.44 (**CH_2**), 29.46 (**CH_2**), 29.58 (**CH_2**), 29.61 (**CH_2**), 29.70 (**CH_2**), 31.82 (**CH_2**), 32.03 (**CH_2**), 33.68 (**C-13**), 36.97 (**C-2**), 41.90 (**C-1''**), 43.65 (**CH_2NH**), 56.06 (**CH_3O**), 74.64 (**C-14**), 110.82 (**C-2'**), 114.49 (**C-5'**), 120.91 (**C-6'**), 124.22 (**C-12**), 127.08 (**C-5''**), 128.60 (2x**C-4''**), 129.35 (2x**C-3''**), 130.54 (**C-1'**), 132.83 (**C-11**), 134.45 (**C-2''**), 145.25 (**C-4'**), 146.82 (**C-3'**), 171.47 (**$COOCH_2$**), 173.01 (**NHCO**).

IR (ATR) ν=3289, 2924, 2853, 1729, 1643, 1514, 1271, 1033, 719 cm^{-1}.

HR-MS (ESI$^+$): m/z: $[M+H]^+$ Calcd. for $C_{36}H_{54}NO_5$ 580.4002; Found 580.4000.

$[\alpha]_D^{20}$=+18.74° (c 1.1, DCM)

(*E*)-*N*-(4-Hydroxy-3-methoxybenzyl)-14-oxoeicos-12-enamide (**95**)



Chemical Formula: $C_{28}H_{45}NO_4$
MW=459.66 g·mol^{-1}

Amidation of (*E*)-14-oxo-12-eicosenoic acid **12d** (50 mg, 0.15 mmol) was performed according to the general procedure **5.2.5.** for amide coupling using HATU (88 mg, 0.23 mmol), 4-hydroxy-3-methoxybenzylamine hydrochloride **99** (32 mg, 0.17 mmol) and DIPEA (80 µL, 0.46 mmol) in anhydrous DMF (2 mL) to yield the amide **95** (20 mg, 28%) as a white sticky solid after purification by silica gel flash column chromatography (petroleum ether/EtOAc 7:3). R_f=0.36 (petroleum ether/EtOAc 7:3). mp=52-55 °C.

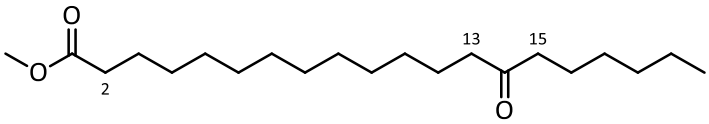
^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.24 – 1.32 (m, 18H, **CH_2**), 1.40 – 1.49 (m, 2H, **CH_2**), 1.58 – 1.65 (m, 4H, **CH_2**), 2.16 – 2.23 (m, 4H, **H-2**, **H-11**), 2.52 (t, *J* = 6.9 Hz, 2H, **$COCH_2$**), 3.87 (s, 3H, **CH_3O**), 4.35 (d, 2H, *J* = 5.7 Hz, **CH_2NH**), 5.63 (s, 1H, **CH_2NH**), 5.68 (br s, 1H, **OH**), 6.08 (dt, 1H, *J* = 15.9, 1.5 Hz, **H-13**), 6.73 – 6.88 (m, 4H, **H_{Ar}**, **H-12**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.19 (**CH_3**), 22.66 (**CH_2**), 24.47 (**CH_2**), 25.91 (**CH_2**), 28.24 (**CH_2**), 29.15 (**CH_2**), 29.28 (**CH_2**), 29.43 (**CH_2**), 29.45 (**CH_2**), 29.47 (**CH_2**), 29.55 (**CH_2**), 29.56 (**CH_2**), 31.78 (**C-11**), 32.57 (**CH_2**), 37.00 (**C-2**), 40.27 (**$CO\underline{C}H_2$**), 43.67 (**CH_2NH**), 56.08 (**CH_3O**), 110.84 (**C-2'**), 114.50 (**C-5'**), 120.94 (**C-6'**), 130.47 (**C-1'**), 130.56 (**C-13**), 145.26 (**C-4'**), 146.82 (**C-3'**), 147.49 (**C-12**), 173.00 (**NHCO**), 201.25 (**$\underline{C}OCH_2$**).

IR (ATR) ν=3304, 2918, 2849, 1634, 1518, 1463, 1275, 978, 799, 720 cm^{-1}.

HR-MS (ESI$^+$): *m/z*: $[M+H]^+$ Calcd. for $C_{28}H_{46}NO_4$ 460.3421; Found 460.3453.

Methyl 14-oxoeicosanoate (**13e**)



Chemical Formula: $C_{21}H_{40}O_3$
MW=340.54 g·mol^{-1}
[10411-44-6]

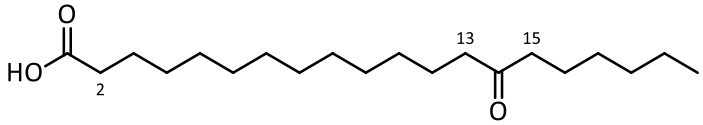
Pd/C (10 wt. % loading) (134 mg) was added to a solution of methyl (*E*)-14-oxo-12-eicosanoate **13d** (134 mg, 0.40 mmol) in MeOH (1.3 mL). Hydrogen pressure was applied (1 atm, room temperature) and the mixture was stirred at room temperature for 2.5 h. The reaction mixture was filtered over a bed of Celite® and the solvent was evaporated under reduced pressure to yield the saturated ketone **13e** (126 mg, 93%) as a white-off solid. mp=52-55 °C. The spectral data were in accordance with the literature:[23]

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.86 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.20 – 1.30 (m, 22H, **CH_2**), 1.49 – 1.65 (m, 6H, **CH_2**), 2.28 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.37 (t, 4H, *J* = 6.9 Hz, **H-13**, **H-15**), 3.65 (s, 3H, **CH_3CO**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.16 (**CH_3**), 22.62 (**CH_2**), 23.97 (**CH_2**), 24.00 (**CH_2**), 25.07 (**CH_2**), 29.06 (**CH_2**), 29.26 (**CH_2**), 29.37 (**CH_2**), 29.38 (**CH_2**), 29.54 (2x**CH_2**), 29.57, 29.67 (2x**CH_2**), 31.74 (**CH_2**), 34.23 (**C-2**), 42.94 (**C-13**, **C-15**), 51.56 (***C*H$_3$CO**), 174.46 (***C*OOCH$_3$**), 211.89 (***C*OCH$_2$**).

IR (ATR) ν=2954, 2915, 2848, 1732, 1706, 1461, 1179, 995, 971, 719 cm^{-1}.

14-Oxoeicosanoic acid (**12e**)



Chemical Formula: $C_{20}H_{38}O_3$
MW=326.51 g·mol^{-1}
[18312-06-6]

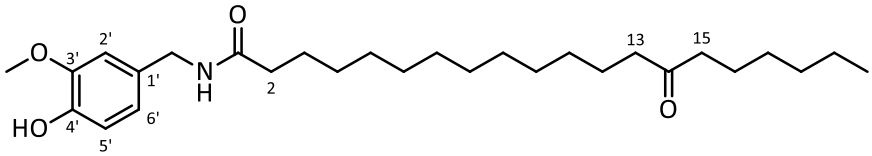
The hydrolysis of methyl 14-oxoeicosanoate **13e** (115 mg, 0.34 mmol) was performed according to the general procedure **5.2.6.** for chemical ester hydrolysis using LiOH·H_2O (42 mg, 1.01 mmol) in THF/H_2O 1:1 (1.6 mL) to yield the fatty acid **12e** (75 mg, 68%) as a white solid. The spectral data were in accordance with the literature:[23]

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.23 – 1.33 (m, 22H, **CH_2**), 1.49 – 1.69 (m, 6H, **CH_2**), 2.30 – 2.42 (m, 6H, **H-2**, **H-13**, **H-15**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.18 (**CH_3**), 22.65 (**CH_2**), 24.02 (**CH_2**), 24.05 (**CH_2**), 24.84 (**CH_2**), 29.10 (**CH_2**), 29.18 (**CH_2**), 29.34 (**CH_2**), 29.41 (**CH_2**), 29.53 (**CH_2**), 29.54 (**CH_2**), 29.58 (**CH_2**), 29.65 (**CH_2**), 29.66 (**CH_2**), 31.77 (**CH_2**), 33.94 (**C-2**), 42.98 (**C-13**, **C-15**), 178.69 (**COOH**), 212.04 (**$\underline{C}$OCH$_2$**).

IR (ATR) ν=3059, 2954, 2914, 2848, 1698, 1380, 1257, 1128, 877, 719, 686 cm^{-1}.

N-(4-Hydroxy-3-methoxybenzyl)-14-oxoeicosanamide (**96**)



Chemical Formula: $C_{28}H_{47}NO_4$
MW=461.68 g·mol^{-1}

Amidation of 12-oxooctadecanoic acid **12e** (71 mg, 0.22 mmol) was performed according to the general procedure **5.2.5.** for amide coupling using HATU (124 mg, 0.32 mmol), 4-hydroxy-3-methoxybenzylamine hydrochloride **99** (45 mg, 0.24 mmol) and DIPEA (115 µL, 0.65 mmol) in anhydrous DMF (3 mL) to yield the amide **96** (75 mg, 75%) as a white solid after purification by silica gel flash column chromatography (petroleum ether/EtOAc 6:4). R_f=0.37 (petroleum ether/EtOAc 6:4). mp=90-91 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, **CH_3**), 1.21 – 1.33 (m, 22H, **CH_2**), 1.51 – 1.68 (m, 6H, **CH_2**), 2.19 (t, 2H, *J* = 6.9 Hz, **H-2**), 2.38 (t, 4H, *J* = 6.9 Hz, **H-13**, **H-15**), 3.88 (s, 3H, **CH_3O**), 4.35 (d, 2H, *J* = 5.7 Hz, **C$\underline{H_2}$NH**), 5.61 (s, 1H, **CH$_2$N$\underline{H}$**), 5.65 (br s, 1H, **OH**), 6.80 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, **H_{Ar}**).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.18 (**CH_3**), 22.65 (**CH_2**), 24.01 (**CH_2**), 24.03 (**CH_2**), 25.93 (**CH_2**), 29.09 (**CH_2**), 29.40 (**CH_2**), 29.45 (**CH_2**), 29.47 (**CH_2**), 29.55 (**CH_2**), 29.57 (**CH_2**), 29.60 (**CH_2**), 29.68 (2x**CH_2**), 31.76 (**CH_2**), 37.03 (**C-2**), 42.97 (**C-15**), 42.99 (**C-13**), 43.68 (**CH$_2$NH**), 56.09 (**CH_3O**), 110.82 (**C-2'**), 114.49 (**C-5'**), 120.95 (**C-6'**), 130.57 (**C-1'**), 145.25 (**C-4'**), 146.82 (**C-3'**), 173.00 (**NHCO**), 211.96 (**$\underline{C}$OCH$_2$**).

IR (ATR) ν=3396, 3316, 2914, 2849, 1702, 1637, 1509, 1243, 1125, 1040, 800, 720 cm^{-1}.

HR-MS (ESI^+): *m/z*: $[M+H]^+$ Calcd. for $C_{28}H_{48}NO_4$ 462.3578; Found 462.3594.

5.4. BIOLOGICAL METHODS

5.4.1. TRP calcium assays

HEK-293 cells stably over-expressing recombinant human TRPV1, rat TRPV2 or rat TRPA1 were selected by G-418 (Geneticin; 600 μg·mL-1), grown on 100 mm diameter Petri dishes as monolayers in minimum essential medium supplemented with non-essential amino acids, 10% fetal bovine serum and 2 mM glutamine, and maintained under 5% CO_2 at 37 °C. Cells were transfected at approximately 80% confluence with Lipofectamine 2000 (Invitrogen, Carlsbad, CA) by using a plasmid pcDNA3 (Invitrogen) containing the human TRPV1-cDNA, or the rat TRPA1-cDNA, according to the manufacture's protocol. Stable expression of each channel was checked by quantitative-PCR. On the day of the experiment, the cells (50000-60000 per well) were loaded for 1h at 25 °C with the cytoplasmic calcium indicator Fluo-4-AM (Invitrogen) 4 μM in dimethyl sulphoxide containing 0.02% Pluronic F-217 (Invitrogen, Carlsbad, CA, USA). After loading, cells were washed twice in Tyrode's buffer (145 mM NaCl, 2.5 mM KCl, 1.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM D-glucose and 10 mM HEPES, pH 7.4), resuspended in the same buffer, and transferred to the quartz cuvette of the espectrofluorimeter (λ_{EX}=488 nm; λ_{EM}=516 nm) (Perkin-Elmer Life and Analytical Sciences, Waltham, MA, USA) under continuous stirring. Experiments were carried by measuring cell fluorescence before and after the addition of various concentrations of test compounds. The values of the effect on $[Ca^{2+}]_i$ in wild-type (i.e. not transfected with any construct) HEK-293 cells were taken as baselines and subtracted from the values obtained from transfected cells. The potency of the test compounds (EC_{50} values) were determined as the concentration of test substances required to produce half-maximal increases in $[Ca^{2+}]_i$ the efficacy of the agonists was determined by comparing their effect with the analogous effect observed with 4μM ionomycin. The effects of the test compounds on TRPA1 are expressed as a percentage of the effect obtained with 100 μM allylisothiocyanate (AITC). Antagonist/desensitizing behaviour was evaluated against capsaicin (0.1 μM) for TRPV1, lysophosphatidylcholine (LPC) (3μM) for TRPV2 or allyl isothiocyanate (AITC) (100 μM) for TRPA1 by adding the compounds in the quartz cuvette 5 min before stimulation of cells with agonists. Data are expressed as the concentration exerting a half-maximal inhibition of agonist effect (IC_{50}). The effect on $[Ca^{2+}]_i$ exerted by the agonist alone was taken as 100%. Dose response curves were fitted by a sigmoidal regression with variable slope. Curve fitting and parameter stimulation were performed with GraphPad Prism® (GraphPad Software Inc., San Diego, CA, USA). All determinations were performed at least in triplicate.

5.4.2. Fatty Acid Amide Hydrolase Assay

The effect of increasing concentrations of the new synthetic compounds on the enzymatic hydrolysis of [^{14}C]anandamide was measuring by using membranes prepared from rat brain.[24] In brief, the entire rat brain was homogenized at 4 °C in 50 mM Tris-HCl buffer, pH 7.0, by using an Ultraturrax and a Dounce homogenizer. Homogenates were first centrifuged at 800*g* to rid the debris, and the supernatant was centrifuged at 10000*g*. The pellet from this latter centrifugation was used for the assay. Membranes (70-100 μg) were incubated and [^{14}C]anandamide (10000 cpm, 1.8 μM) in 50 mM Tris-HCl, pH 9, for 30 min at 37 °C. [^{14}C]ethanolamine produce from [^{14}C]anandamide hydrolysis was used to calculate FAAH activity and was measured by scintillation counting of the aqueous phase after extraction of the incubation mixture with 2 volumes of $CHCl_3$/MeOH (1:1 by volume). Data are expressed as the concentration exerting a half-maximal inhibition (IC_{50}). The efficacy was calculated as a percentage of the effect obtained with URB597, which is an inhibitor of FAAH (0.1 μM).

5.5. REFERENCES

(1) Leonard, J.; Lygo, B.; Procter, G. *Advanced Practical Organic Chemistry*; Third ed.; CRC Press: Boca Raton, 2013.

(2) Kyobe, J. W.; Mubofu, E. B.; Makame, Y. M. M.; Mlowe, S.; Revaprasadu, N. *New J. Chem.* **2015**, *39*, 7251-7259.

(3) King, G. *J. Chem. Soc.* **1951**, 1980-1984.

(4) Miao, X.; Malacea, R.; Fischmeister, C.; Bruneau, C.; Dixneuf, P. H. *Green Chem.* **2011**, *13*, 2911-2919.

(5) Maletz, R.; Schäfer, H. J.; Quermann, R. *Lipid / Fett* **1996**, *98*, 370-379.

(6) Frykman, H. B.; Isbell, T. A. *J. Am. Oil Chem. Soc.* **1997**, *74*, 699-702.

(7) Sonnet, P. E.; Hayes, D. *J. Am. Oil Chem. Soc.* **1995**, *72*, 1069-1071.

(8) Canadian Institutes of Health Research, Alberta Innovates-Health Solutions, The Metabolomics Innovation Centre, The Human Metabolome Database. www.hmdb.ca (Accessed March 24, 2016).

(9) Gannett, P. M.; Nagel, D. L.; Reilly, P. J.; Lawson, T.; Sharpe, J.; Toth, B. *J. Org. Chem.* **1988**, *53*, 1064-1071.

(10) Winkler, M.; Moraux, T.; Khairy, H. A.; Scott, R. H.; Slawin, A. M. Z.; O'Hagan, D. *ChemBioChem* **2009**, *10*, 823-828.

(11) Lie Ken Jie, M. S. F.; Wong, K. P. *Chem. Phys. Lipids* **1992**, *62*, 177-183.

(12) Nichols, J.; Schipper, E. *J. Am. Chem. Soc.* **1958**, *80*, 5705-5710.

(13) Mhaskar, S. Y.; Mani, V. V. S. *J. Am. Oil Chem. Soc.* **1994**, *71*, 543-544.

(14) Thalmann, A.; Oertle, K.; Gerlach, H. *Org. Synth.* **1985**, *63*, 192-197.

(15) Pasha, M. K.; Ahmad, F. *Lipids* **1993**, *28*, 1027-1031.

(16) Lie Ken Jie, M. S. F.; Lau, M. M. L. *Chem. Phys. Lipids* **1999**, *101*, 237-242.

(17) Stein, R. A.; Haack, K. B. *J. Org. Chem.* **1968**, *33*, 3784-3786.

(18) Foglia, T. A.; Sonnet, P. E.; Nunez, A.; Dudley, R. L. *J. Am. Oil Chem. Soc.* **1998**, *75*, 601-607.

(19) Lie Ken Jie, M. S. F.; Mustafa, J.; Pasha, M. K. *Lipids* **1998**, *33*, 941-945.

(20) Yurawecz, M. P.; Hood, J. K.; Mossoba, M. M.; Roach, J. A. G.; Ku, Y. *Lipids* **1995**, *30*, 595-598.

(21) Lee, J.; Lee, J.; Kang, M. S.; Kim, K. P.; Chung, S. J.; Blumberg, P. M.; Yi, J. B.; Park, Y. H. *Bioorg. Med. Chem.* **2002**, *10*, 1171-1179.

(22) Shyamali, G.; Maryanoff, B. E.; Kinney, W. A.; Lawson, E. C.; Luci, D. K.; Marchenko, N.; Sviridov, S. Isoindoline derivatives as urotensin II receptor antagonists and their preparation,

pharmaceutical compositions and use in the treatment of urotensin II mediated diseases. WO2007081995A2. July 19, 2007.

(23) Nelson, J. S.; Applewhite, T. H. *J. Am. Oil Chem. Soc.* **1967**, *44*, 305-306.

(24) Maurelli, S.; Bisogno, T.; De Petrocellis, L.; Di Luccia, A.; Marino, G.; Di Marzo, V. *FEBS Lett.* **1995**, *377*, 82-86.

General Discussion

Nature annually generates about 170 billion metric tons of biomass, of which only 3-4% is currently used for food purposes, for the production of fuels or for chemicals manufacturing.[1] Oleochemicals are chemicals derived from oils and fats in a way similar to petrochemicals which are derived from petroleum oils.[2] As far as renewable chemicals production is concerned, the oleochemical industry serves as a longstanding example of a biobased industry, one that over the last century has developed in parallel with the petrochemical industry.[3] Vegetable oils constitute the main fraction of the renewable oil and fat production (i.e. 80%, the remaining 20% being animal fats). Especially remarkable vegetable oils are the homoallyl hydroxy fatty acids, which have a hydroxy group in the homoallylic position. Castor and lesquerella oil are the most representative oils of this group. The presence of the double bond and the hydroxy group provides wide possibilities for chemical modifications and therefor, the synthesis of derivatives.

α,β-Unsaturated carbonyl fatty acids can be prepared by oxidation and subsequent isomerization both in basic or acid media of homoallyl hydroxy fatty acids.[4-10] In view of synthetic interests, alternative methods of oxidation are necessary to prepare α,β-unsaturated carbonyl fatty acids in a selective and in one-step manner. The oxidation of ricinoleic acid, the main component of castor oil, was firstly carried out by transition metal-free oxidants. Within this group, the hydroperoxides such as aqueous hydrogen peroxide and *tert*-butyl hydroperoxide (TBHP) have an especial interest because of their numerous advantages (stability, low price, readily available, etc.).[11,12] One of the first experiments was the use of these hydroperoxides with hydrobromic acid, which would allow to carry out the oxidation in absence of solvent.[13] However, under these conditions, the ricinoleic acid was not oxidized. In recent years the substitution of hydroperoxides by solid peroxy compounds, such as sodium perborate (SPB) and sodium percarbonate (SPC) has gained considerable attention.[14-16] The combination of these solid peroxy compounds with the same co-oxidant (HBr) was not successful neither. To complete the non-transition metal oxidation studies, other oxidant systems based on bismuth(III) and hypervalent iodine compounds in presence of TBHP or Oxone™ were evaluated.[17-19] Unfortunately, both Bi_2O_3/*tert*-BuOOH and IBA/Oxone™ systems did not lead to the formation of the desired α,β-unsaturated carbonyl fatty acid. Then, oxidations based on the use of transition metals were studied. Several heterogeneous catalysts were tested and none of them led to the oxidation of ricinoleic acid. Finally, the ruthenium-catalyzed Oppenauer-type oxidation proved to be an excellent reaction to achieve this sort of compounds. For this, it was necessary to ascertain which hydrogen acceptor was most suitable. Using the Shvo's catalyst as oxidant, several hydrogen acceptors such aldehydes, ketones or unsaturated esters were screened.

Acrolein was found to be the most suitable hydrogen acceptor allowing to achieve the α,β-unsaturated carbonyl fatty acid in a fast, selective and in one-step synthetic reaction.

Sebacic acid, 10-carbon dicarboxylic acid, is an industrially important building block due to its potential in the production of various intermediates. Sebacic acid is manufactured by the alkaline oxidative cleavage of castor oil to high temperatures (about 250 °C) with excess of alkali.[20-24] This treatment results in saponification of the castor oil to ricinoleic acid that is then cleaved to give 2-octanol and sebacic acid. This process requires the use of a high amount of oxidant and energy and the presence of catalysts to split the ricinoleic acid in either a batch or continuous process. These drawbacks make it necessary to find synthetic routes that are more energetically favorable to the synthesis of this appreciated industrial product. In this work, microwave and ultrasounds technologies were used as environmental friendly heating systems in order to reduce temperatures and reaction time. α,β-Unsaturated carbonyl fatty acids are very suitable intermediates to synthesize dicarboxylic acids due to the high reactivity of the conjugated double bond. Thus, (*E*)-12-oxo-10-octadecenoic acid or its methyl ester derivative obtained in the previous step were used as starting material of the next step. First, the alkaline oxidative cleavage of (*E*)-12-oxo-10-octadecenoic acid was carried out using the microwave technology due to the advantages of speed, selectivity, precise control and improved economics owing to consumption of less energy.[25] The effect of the temperature and the concentration of alkali of the reaction were assessed in order to achieve the optimal reaction conditions. These studies allowed that the reaction temperature was reduced from 250 to 150 °C and the concentration of alkali from 50% to 6%. Unfortunately, the good yields obtained could not to be reproduced when the reaction was scaling up. Other environmental friendly oxidants such bleach or hydrogen peroxide in basic media were tested. However, they gave poor results. Finally, sebacic acid was achieved using the Oxone™/$NaIO_4$ system in a good yield.[26] This reaction was carried out using greener solvents and was compatible with the ultrasound technology, which decreased the reaction time and avoided the external heating.

TRPV1 has become the model receptor for pain signal integration. This protein is structurally related to members of the transient receptor potential (TRP) and TRP-like (TRPL) family of cation channels, is expressed uniquely in sensory ganglia, and is activated by noxious heat and protons and probably functions as a transducer for painful thermal stimuli.[27] *N*-vanillyl-acylamides (NVAs) are a group of compounds known as capsaicinoids, which are natural ingredients specifically derived from *Capsicum* fruits. Capsaicin, the most abundant NVA in capsaicinoids, causes a pungent sensation and possesses various physiological and biological activities.[28] It has been revealed that several

physiological activities caused by capsaicin are also related to the activation of TRPV1.[29] NVAs with a longer or shorter acyl chain than capsaicin have less pungency, and NVAs with a chain length of more than 18 carbons chain length do not generate any stimulus. Long chain *N*-vanillyl acyl amides (LCNVAs) have been developed as synthetic capsaicin analogues with capsaicin-like physiological activities and with no, or less, harmful stimuli.[30] Among the LCNVAs developed as synthetic capsaicinoids, olvanil, which contains an oleic (C18:1) acyl moiety, has received much attention because of its high capsaicin-like potency.[30] Other LCNVAs having acyl moieties of naturally occurring type have been developed [31] An analogue of olvanil is the rinvanil.[32] This compound is easily available from commercial and cheap ricinoleic acid its potency (EC_{50}=6.0 nM) is comparable to capsaicin and olvanil. On the other hand, fatty acid amide hydrolase (FAAH) is increasingly being considered a relevant therapeutic target, especially in models of inflammatory pain.[33] Anandamide has been shown to be an activator of TRPV1 in vitro and is mainly hydrolysed by FAAH.[34,35] The inhibition of FAAH causes high levels of anandamide inside of the neuron bounding to the TRPV1 receptor and producing the analgesia effect.[36] Based on SAR studies of TRPV1 agonists and FAAH inhibitors, modifications at the lipophilic region of the homoallyl hydroxy fatty acids were carried out to synthetize 22 novel long chain *N*-vanillyl acylamides with “dual mechanism” of action against pain. Concerning to the biological evaluation, the compounds derived from lesquerolic acid showed better agonist behaviour in terms of potency in TRPV1 receptor. The presence of polar groups such hydroxy, amines or carboxylic groups led to a decrease of the activity. On the contrary, the introduction of a furan ring in the alkyl chain gave the best results of the whole series. Respecting the FAAH enzyme, it seems that the presence of a heterocycle ring in the side-chain brought activity to the molecules. Although the furan derivative was not the best in its series, it also had an inhibitory character and, therefore, this type of compound could be the starting point to discover new derivatives with “dual” activity.

REFERENCES

(1) Corma, A.; Iborra, S.; Velty, A. *Chem. Rev.* **2007**, *107*, 2411-2502.

(2) Haupt, D. E.; Drinkard, G.; Pierce, H. F. *J. Am. Oil Chem. Soc.* **1984**, *61*, 276-281.

(3) Hill, K. *Pure Appl. Chem.* **2000**, *72*, 1255-1264.

(4) Ellis, G. W. *J. Chem. Soc.* **1950**, 9-12.

(5) Maletz, R.; Schäfer, H. J.; Quermann, R. *Fett/Lipid* **1996**, *98*, 370-379.

(6) Nichols, J.; Schipper, E. *J. Am. Oil Chem. Soc.* **1958**, *80*, 5705-5710.

(7) Davletbakova, A. M.; Baibulatova, N. Z.; Dokichev, V. A.; Yunusova, S. G.; Yunusov, M. S. *Russ. J. Organ. Chem.* **2001**, *37*, 781-783.

(8) Lie Ken Jie, M. S. F.; Lam, C. N. W. *Lipids* **2004**, *39*, 583-587.

(9) aus dem Kahmen, M.; Schäfer, H. J. *Fett/Lipid* **1998**, *100*, 227-235.

(10) Conacher, H. B. S.; Gunstone, F. D. *Chem. Phys. Lipids* **1969**, *3*, 203-220.

(11) Sheldon, R. A. In *Organic Peroxygen Chemistry*; Herrmann, Wolfgang A., Ed.; Topics in Current Chemistry 162; Springer Berlin Heidelberg: Heidelberg, 1993, p 21-43.

(12) Sanderson, R. W. *Pure Appl. Chem.* **2000**, *72*, 1289-1304.

(13) Sharma, V. B.; Jain, S. L.; Sain, B. *Synlett* **2005**, *2005*, 173-175.

(14) McKillop, A.; Sanderson, W. R. *Tetrahedron* **1995**, *51*, 6145-6166.

(15) Gomez, M. V.; Caballero, R.; Vazquez, E.; Moreno, A.; de la Hoz, A.; Diaz-Ortiz, A. *Green Chem.* **2007**, *9*, 331-336.

(16) Jain, S. L.; Sharma, V. B.; Sain, B. *Tetrahedron* **2006**, *62*, 6841-6847.

(17) Malik, P.; Chakraborty, D. *Synthesis* **2010**, *2010*, 3736-3740.

(18) Malik, P.; Chakraborty, D. *Tetrahedron Lett.* **2010**, *51*, 3521-3523.

(19) Thottumkara, A. P.; Bowsher, M. S.; Vinod, T. K. *Org. Lett.* **2005**, *7*, 2933-2936.

(20) Anderson, D. In *Bailey's Industrial Oil and Fat Products. Edible Oil and Fat Products: Processing Technologies.*; Shahidi, F., Ed.; John Wiley & Sons: New Jersey, 2005, p 1–56.

(21) Hargreaves, G. H.; Owen, L. N. *J. Chem. Soc.* **1947**, 753-756.

(22) Naughton, F. C. *J. Am. Oil Chem. Soc.* **1974**, *51*, 65-71.

(23) McKetta, J. J.; Dekker, M. In *Encyclopedia of Chemical Processing and Design: Volume 6 - Calcination Equipment to Catalysis*; McKetta, J.J., Dekker, M., Eds.; Taylor & Francis: New York, 1978, p 401–420.

(24) Dytham, R. A.; Weedon, B. C. L. *Tetrahedron* **1960**, *8*, 246-260.

(25) Tierney, J.; Lidström, P. *Microwave Assisted Organic Synthesis*; Tierney, J., Lidström, P., Eds.; Blackwell Publishing: Oxford, 2009.

(26) Spannring, P.; Bruijnincx, P. C. A.; Weckhuysen, B. M.; Gebbink, R. J. M. K. *RSC Adv.* **2013**, *3*, 6606-6613.

(27) Caterina, M. J.; Schumacher, M. A.; Tominaga, M.; Rosen, T. A.; Levine, J. D.; Julius, D. *Nature* **1997**, *389*, 816-824.

(28) Govindarajan, V. S.; Sathyanarayana, M. N. *Crit. Rev. Food Sci. Nutr.* **1991**, *29*, 435-474.

(29) Tominaga, M.; Caterina, M. J.; Malmberg, A. B.; Rosen, T. A.; Gilbert, H.; Skinner, K.; Raumann, B. E.; Basbaum, A. I.; Julius, D. *Neuron* **1998**, *21*, 531-543.

(30) Szallasi, A.; Di Marzo, V. *Trends Neurosci.* **2000**, *23*, 491-497.

(31) Melck, D.; Bisogno, T.; De Petrocellis, L.; Chuang, H.-H.; Julius, D.; Bifulco, M.; Di Marzo, V. *Biochem. Biophys. Res. Commun.* **1999**, *262*, 275-284.

(32) Appendino, G.; Minassi, A.; Morello, A. S.; De Petrocellis, L.; Di Marzo, V. *J. Med. Chem.* **2002**, *45*, 3739-3745.

(33) Otrubova, K.; Ezzili, C.; Boger, D. L. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4674-4685.

(34) Cravatt, B. F.; Lichtman, A. H. *Curr. Opin. Chem. Biol.* **2003**, *7*, 469-475.

(35) Sharkey, K. A.; Cristino, L.; Oland, L. D.; Van Sickle, M. D.; Starowicz, K.; Pittman, Q. J.; Guglielmotti, V.; Davison, J. S.; Di Marzo, V. *Eur. J. Neurosci.* **2007**, *25*, 2773-2782.

(36) Petrosino, S.; Di Marzo, V. *Curr. Opin. Chem. Biol.* **2010**, *11*, 51-62.

General Conclusions

GENERAL CONCLUSIONS

1.- This PhD work allowed the exploration of homoallyl hydroxy fatty acid chemistry, focused on ricinoleic and lesquerolic acids, which are important industrial raw materials. Several publications and patents about the synthesis and industrial applications of their derivatives are found in literature.

2.- The preparation of α,β-unsaturated carbonyl fatty acids was carried out by the ruthenium-catalyzed Oppenauer-type oxidation of homoallyl hydroxy fatty acids using the Shvo's catalyst as oxidant and acrolein as hydrogen acceptor. This procedure favors the formation of the α,β-unsaturated carbonyl fatty acid over the expected saturated fatty acid.

3.- The acids, their methyl ester and even the triacylglycerides were easily oxidized with this new synthetic route. Except in the case of the triacylglycerides, the α,β-unsaturated carbonyl compounds were isolated in good yields (range 50% to 70%).

4.- The alkaline oxidative cleavage, the conventional method used to obtain sebacic acid, was subjected to an optimization of the conditions due to the use of microwave technology. The optimization allowed to reduce the temperature (40%) and the amount of oxidant (88%) of the reaction on a small scale.

5.- The Oxone™/periodate system led to the formation of sebacic acid by an energetically benign oxidative cleavage. Sebacic acid was isolated in 41% yield. This system did not require toxic reagents and efficient heating systems such as ultrasound technology could be used.

6.- Chemical modifications of the homoallyl hydroxy moiety of ricinoleic and lesquerolic acid were carried out to synthetize 22 new long-chain *N*-vanillyl acylamides (LCNVAs) with overall yields ranging from 7% to 65%.

7.- Biological tests of the new synthetized long-chain *N*-vanillyl acylamides showed the "dual" biological response in TRPV1 and FAAH enzyme of the compound **89** (EC_{50}=0.24 nM and IC_{50}=4.49 μM). In addition, the compound **89** resulted selective against other receptors such as TRPV2 (not active) and TRPA1 (EC_{50}=10.5 μM) that are also expressed in nociceptive neurons.

RECOMMENDATIONS FOR FUTURE WORKS

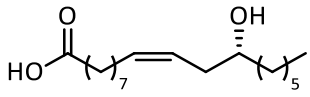
The following are suggestions which would further extend the work presented in this thesis:

- The ruthenium-catalyzed Oppenauer-type oxidation of methyl ricinoleate carried out in the continuous flow reactor did not give as good results as the batch tests. However, the conversion of the starting material was good. Optimization of the parameters should be further explored in order to improve the oxidation yield.

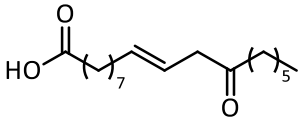
- As stated in literature, a large number of organic reactions can be carried out in higher yields, shorter reaction times, or milder conditions under ultrasound irradiation. This can be considered as a processing aid in terms of energy conservation and waste minimization compared to conventional heating. Further experiments may be carried out ultrasound with the reactor to achieve the best results for the oxidative cleavage of the α,β-unsaturated carbonyl with the Oxone™/periodate system.

- The biological results of the new long chain *N*-vanillyl acylamides opens a new line of study for the development of analgesics with "dual" action in pain receptors. The existence of a furan ring in the lipophilic chain led to both activation of TRPV1 and inhibition of FAAH enzyme. Substituents in the furan ring should be introduced to complete the structure-activity relationships study of this moiety.

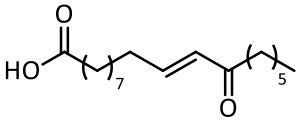
Molecule Index



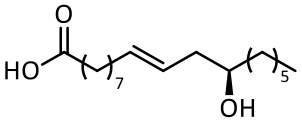
1a



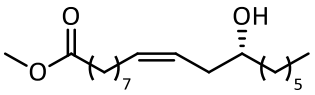
1c



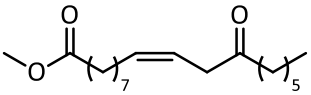
1d



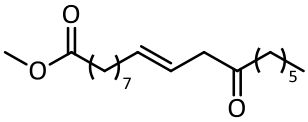
2



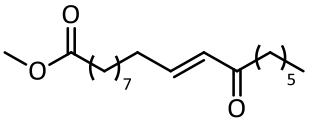
10a



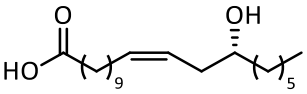
10b



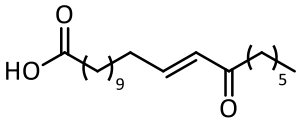
10c



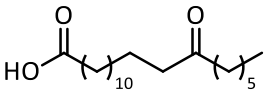
10d



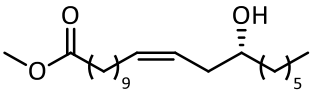
12a



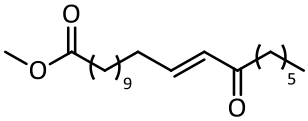
12d



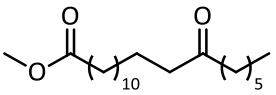
12e



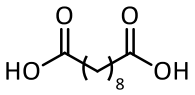
13a



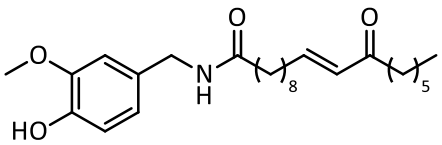
13d



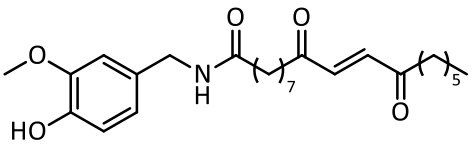
13e



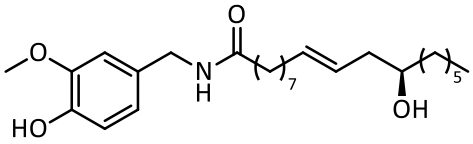
14



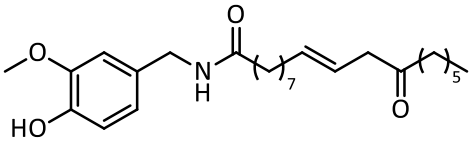
75



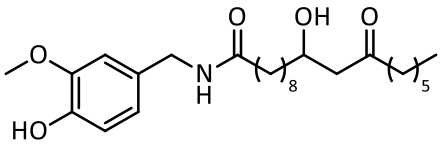
76



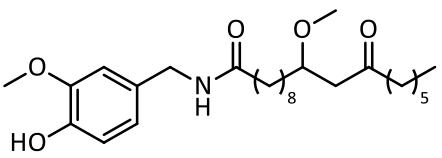
77



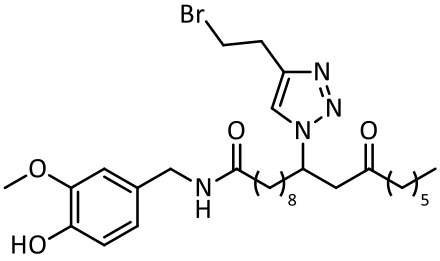
78



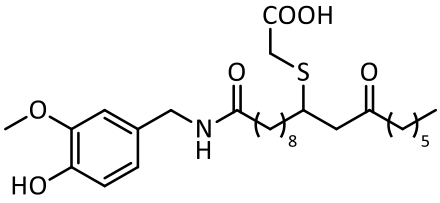
79



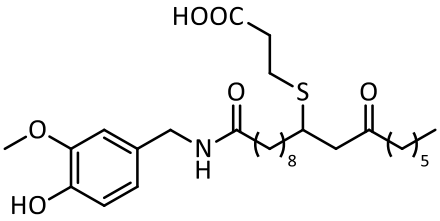
80



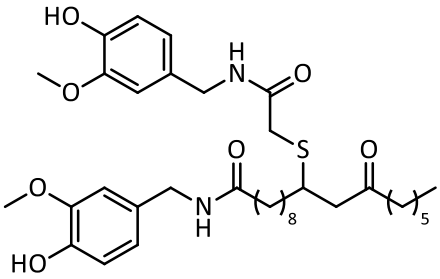
81



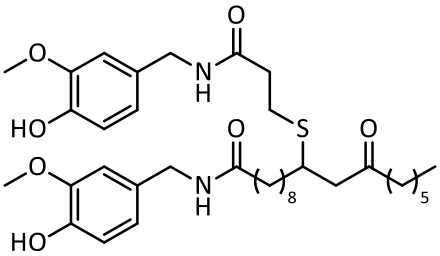
82



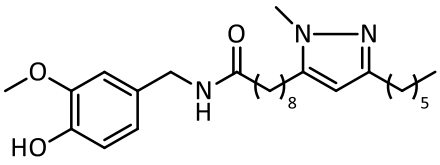
83



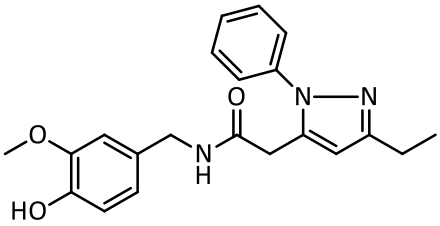
84



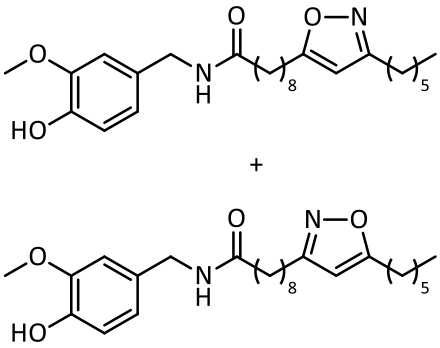
85



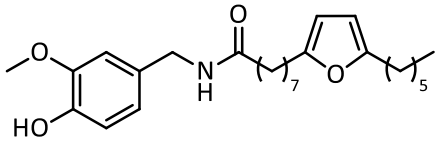
86



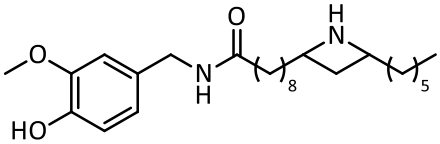
87



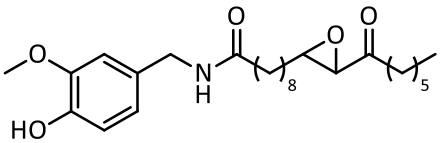
88



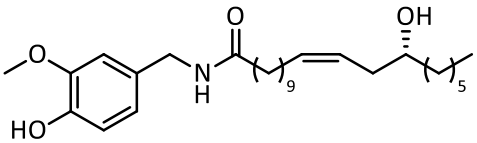
89



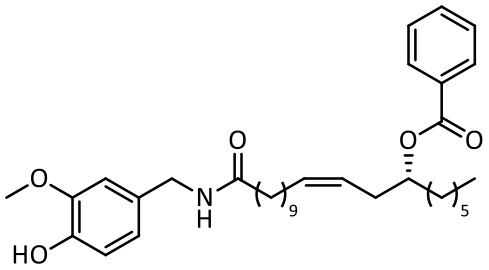
90



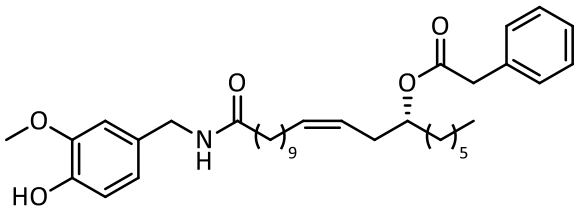
91



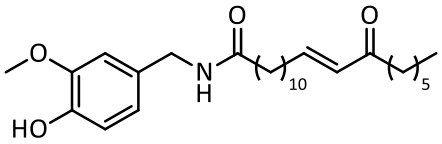
92



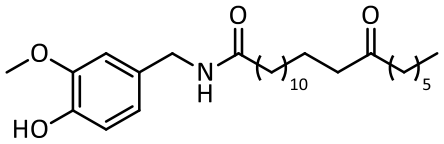
93



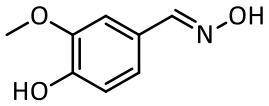
94



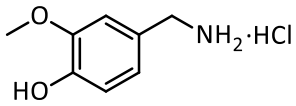
95



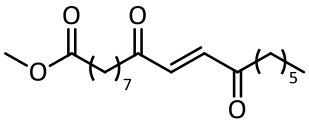
96



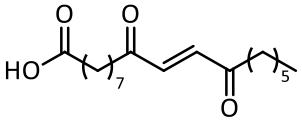
98



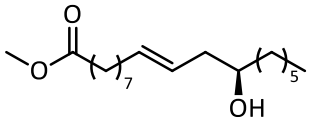
99



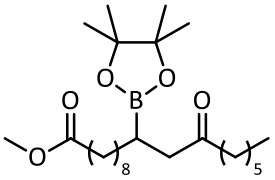
112



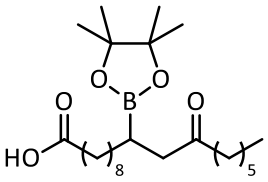
113



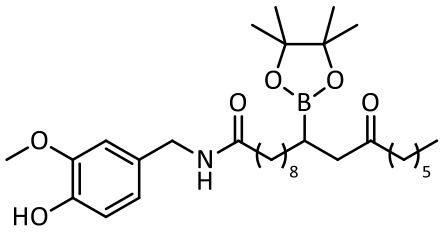
114



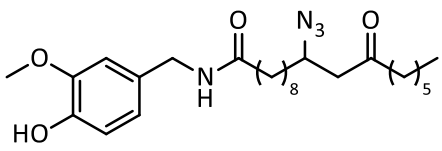
115



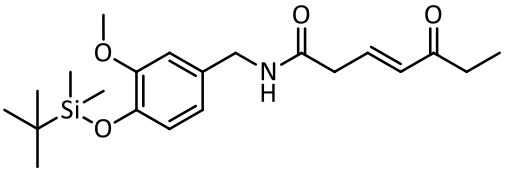
116



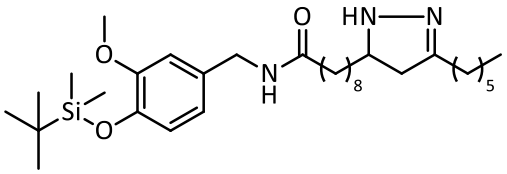
117



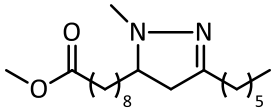
118



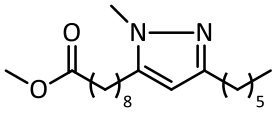
123



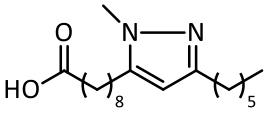
124



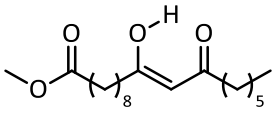
126



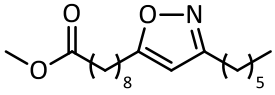
127



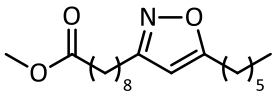
128



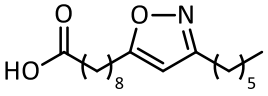
129



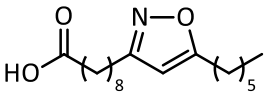
+



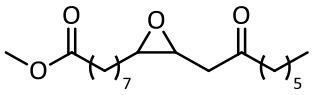
130



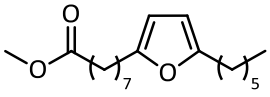
+



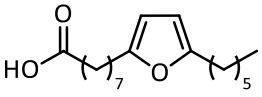
131



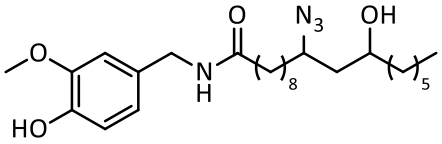
134



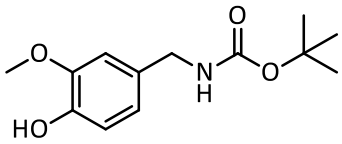
135



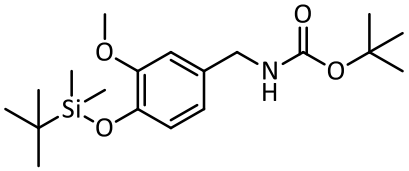
136



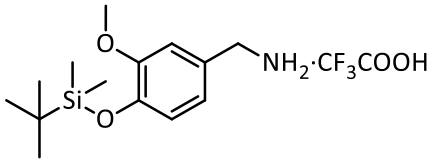
137



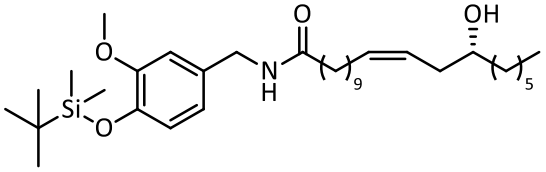
138



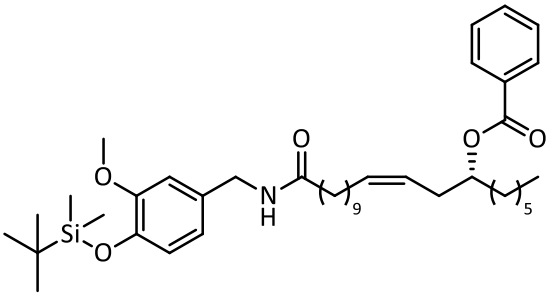
139



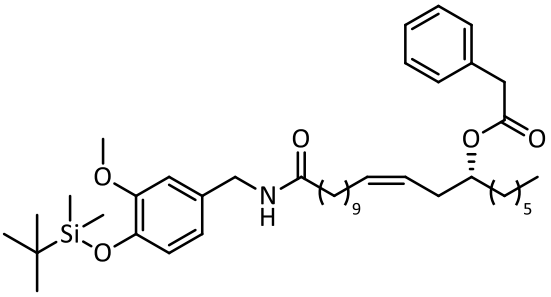
140



141



142



143