A general synthetic strategy and biological activity of B_1-Phytoprostanes

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Abbreviations

α	optical rotation in degrees
[α]	specific rotation
Ac	acetyl
AGP	arabinogalactan-protein
Aux/IAA	auxin-responsive protein
aq	aqueous
t-Bu	*tert*-butyl
°C	degrees Celsius
calcd	calculated
cDNA	complementary deoxyribonucleic acid
CI	chemical ionisation
cm	centimeter(s)
cm^{-1}	wavenumber(s)
concd	concentrated
Cp	cyclopentadienyl
Cyt P-450	cytochrome P-450
d	day(s); doublet (spectral); deci
d	density
DIBALH	diisobutylaluminum hydride
DIP	dinor isoprostane(s)
DMAP	4-(*N,N*-dimethylamino)-pyridine
DMF	dimethylformamide
DMNT	4,8-dimethylnona-1,3,7-triene
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EI	electron impact
equiv	equivalent/s
ESI	electrospray ionization
Et	ethyl
g	gram(s)
GC	gas chromatography
GST	glutathione-*S*-transferase
h	hour(s)
HR	hypersensitive response
HRMS	high-resolution mass spectrometry
HSP	heat shock protein
Hz	hertz
IR	infrared
J	coupling constant (in NMR spectrometry)
JA	jasmonic acid
k	kilo
KOTE	ketotrienoic acid
l	liter(s)
LDA	lithium diisopropylamide
lit.	literature
LTP4	lipid transfer protein 4
μ	micro
m	multiplet (spectral); meter(s); milli
M	molar (moles per liter); mega
max	maximum
Me	methyl
MeJA	methyl jasmonic acid
MeSA	methyl salicylate
MHz	megahertz
min	minute(s); minimum
mM	millimolar (millimoles per liter)
mol	mole(s); molecular mp
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MW	molecular weight
m/*z*	mass-to-charge ratio
nm	nanometer(s)
NMR	nuclear magnetic resonance
Nu	nucleophile
OD	optical density
OPDA	12-oxophytodienoic acid
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
PG	prostaglandin(s)
Ph	phenyl
pm	picometer(s)
PP	phytoprostane(s)
ppm	part(s) per million
Pr	propyl
PR	pathogenesis-related protein
i-Pr	isopropyl
q	quartet (spectral)
rel	relative
RNS1	ribonuclease 1
ROS	reactive oxygen species
rRNA	ribosomal ribonucleic acid
rt	room temperature
s	singlet (spectral);

sec	second(s)
SPME	solid phase micro extraction
t	triplet (spectral)
TBDMS	*tert*-butyldimethylsilyl
temp	temperature
TFA	trifluoroacetic acid
THF	tetrahydrofuran
THP	tetrahydropyran-2-yl
TLC	thin-layer chromatography
TMS	trimethylsilyl; tetramethylsilane
TMTT	4,8,12-trimethyltrideca-1,3,7,11-tetraene
tRNA	transfer ribonucleic acid
UV	ultraviolet
vol	volume
VSP1,2	vegetative storage protein

1 Introduction

During the last century, researchers have focused on the influence of certain chemicals involved in interactions between living organisms. The production or biosynthesis of such organic compounds and their distribution between the producers or between other organisms are important questions in chemical ecology. The response to signalling molecules, the effects of certain toxins and especially the interactions between different kingdoms, such as insect-plant interactions, fungi-plant interactions or plant-plant interactions, represent major research topics of this field.

Over their entire lifetime, plants are exposed to a variety of stress factors. Such stress factors can be either abiotic or biotic. Abiotic stress is caused by environmental factors, such as water deficit, extreme temperature or light conditions. Biotic stress is caused by different living organisms, such as herbivores, pathogens or fungi. Stress affects plants in their development and reproduction processes. Since they are unable to move, they have evolved efficient strategies to cope with or to adapt to such stresses.

Plants have developed a wide range of defensive mechanisms to survive and fight against attacking enemies. Recognition of enemies and the triggered answer cascade is part of the study of chemical ecology as well.

1.1 Plant defence reactions

During evolution, plants have developed efficient strategies against different natural stresses. Their defence can either be constitutively, providing general protection against infestion, since it is inherent to a specific host, or it is induced by certain effectors, such as pathogens or herbivore attack. The induced defence has certain advantages because natural resources are conserved and resistance is not minimised. Mechanical barriers, such as thorns, spikes or wax layers can be formed to protect the plant against first attacks. Beside this mechanical defence, the chemically induced defence is more interesting to researchers. By producing toxic substrates (direct chemical defence), e.g. nicotine from the tobacco plant

Nicotiana attenuata[1] or morphine from the poppy plant *Papaver somniferum*[2], plants protect themself against enemies, because the attacking enemies are either deterred by the smell of the released substances or they are killed directly. Plants are also able to defend themselves indirectly, as it can be found in the plant *Acacia farnesiana*.[3] With the formation and secretion of extrafloral nectaries (EFN), this acacia is able to attract ants. Secretory nectar glands are found in flowers, but sometimes similar organs are also located in other vegetative parts of the plant and are therefore called extrafloral. EFN is mainly composed of a mixture of sugars and minor amino acids attracting certain ants that provide protection to the plant by stinging, spraying or swarming over insects landing on the plant. This effect is called mutualism, where the plant serves as a food source for ants, and the ants in turn defend their host against predators.

Another phenomenon of indirect defence is the release of volatile organic compounds[4] as an answer to mechanically wounding of the plant, herbivore attack or as a result of exposing plants to certain stimuli, such as heavy metal or hydroperoxide treatment. Plants emit a specific blend of volatiles, e.g. a mixture of methyl salicylate, different terpenoids, or green leaf alcohols. This so-called “cry for help” is often a first characteristic for indirect defence including higher trophic levels, which means that plants release such volatiles for attracting carnivorous insects or parasitoids, that reduce the feeding pressure by predation of the herbivore.[5] This simple theory includes complicated recognition mechanisms within the plant. According to the nature of the attack, a specific response cascade is triggered (**Figure 1**). Recognition of stress factors allows plants to act in a specific way to defend themselves. A variety of signalling pathways are involved in the initiation of defence responses. After identificitaion *via* specific receptors, recognition triggers subsequent activation of intracellular signal transduction cascades, ranging from second messenger stimulation, such as calcium influx,[6,7] to induction of protein phosphorylation, including mitogen-activated protein kinases (MAP-kinases)[8] or synthesis of reactive oxygen species (ROS),[9] which in turn results in a characteristic expression of defensive genes, leading to phytoalexin accumulation or to the release of specific volatile blends. Of special importance in such early responses are certain signal molecules, the so-called plant- or phytohormones that are major players in plant defence regulation.

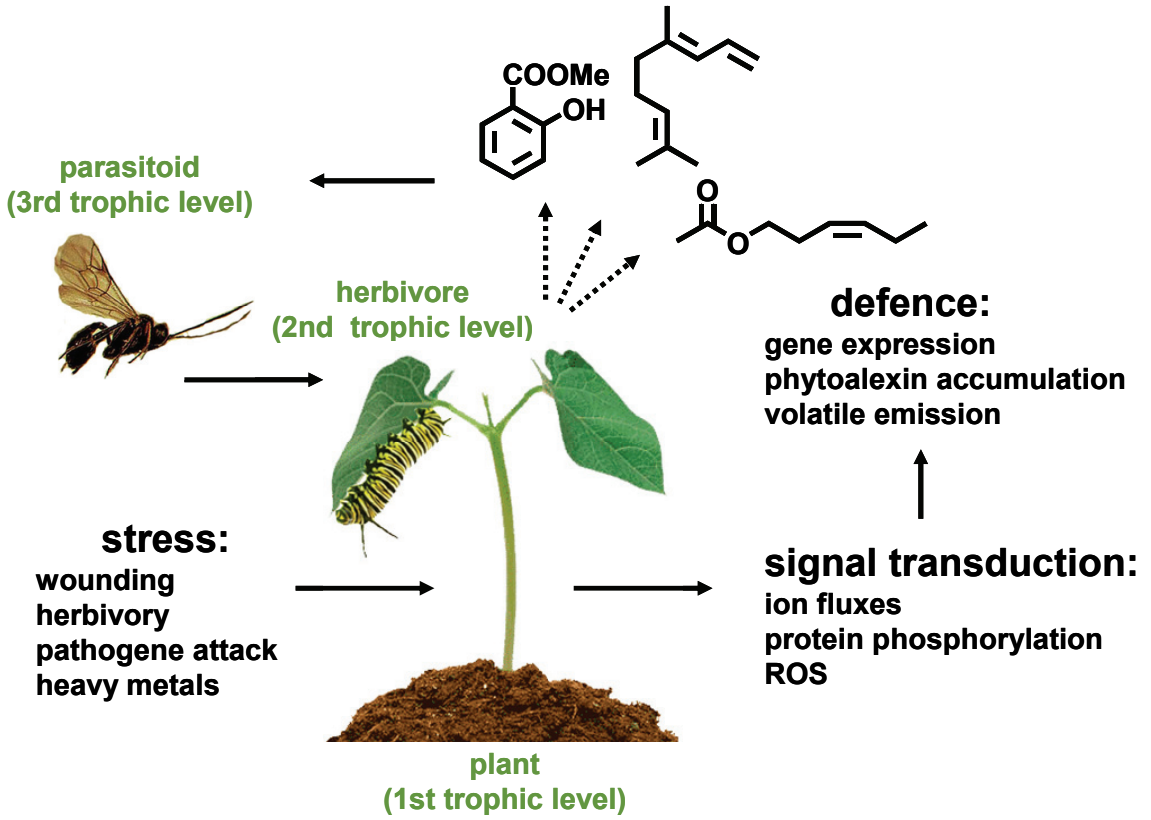


Figure 1. Interaction between plants, herbivores and parasitoids. Caused by certain stress factors, plants answer through a complex cascade of signal transduction pathways leading to defensive gene expression, accumulation of phytoalexins and the release of special volatiles that attract e.g. parasitic wasps to their prey.

1.2 Phytohormones as regulators of plant development and defence

Plants adapt to various environmental stimuli by using sophisticated defence strategies, according to the nature of the attack. A central role in such defensive mechanisms refers to plant- or phytohormones. They are not only regulators of plant development and reproduction, but are also involved in many other physiological processes of primary and secondary metabolism.[10] In contrast to animal hormones, plant hormones are not produced in specific glandular cells, but in cells located all over the tissue. Such signal molecules, e.g. auxins, cytokinins, gibberellins, brassinosteroids, abscisic acid, ethylene and jasmonates, regulate plants growth, differentiation and the communication between different plant cells. Therefore, the name phyto- or plant hormone is applied for these regulating molecules. Phytohormones can interact synergistically or antagonistically (cross-talk) to allow differentiated responses to various threats. Often not a single

hormone is responsible for a special response reaction, but the interaction between two or more hormones is involved.

Plant hormones are devided into two groups, one for promoting and one for inhibiting plant growth.[10]

Plant growth hormones are auxins, e.g. indol-3-acetic acid (**1**), cytokinins, e.g. kinetin (**2**), gibberellins, e.g. gibberellic acid (**3**) and brassinosteroids, e.g. castasteron (**4**) (**Figure 2**).

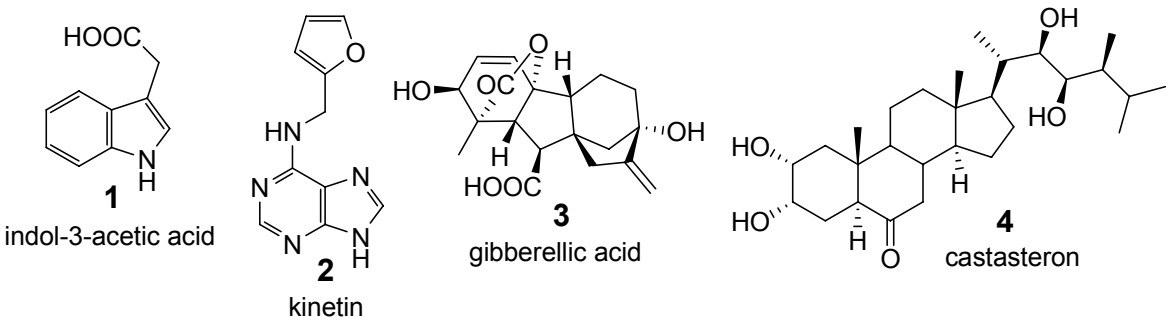


Figure 2. Auxins, cytokinins, gibberellins and brassinosteroids are plant growth hormones.

Auxins can be found in all higher plants, especially in high doses in the developing seedling. In lower concentrations they induce root and shoot elongation, cell division in the cambium and root formation. They also influence the dropping of leaves, flowers and fruits. The most important auxin is indol-3-acetic acid (**1**). Navarro *et al.*[11] demonstrated a link between auxin signalling in plants and resistance to bacterial pathogens. These results indicate that decreasing plant auxin signalling can increase resistance to bacterial pathogens. Some microorganisms, like *E.coli*, even use auxins as signalling molecules, although they are not able to produce them.[12] According to Bianco *et al*., treatment with **1** triggered an increased tolerance to several stress conditions (heat and cold shock, UV-irradiation, osmotic and acid shock and oxidative stress) and to different toxic compounds (antibiotics, detergents and dyes).

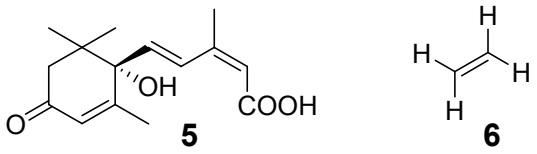
Cytokinins are plant hormones that stimulate cell division and other growth- or differentiation processes. They reduce senescence and stimulate dark-germination of light-dependent seeds. Cytokinins, such as kinetin (**2**), are amino-substituted derivatives of adenine or adenosin. It has been found that cytokinins are able to inhibit the hypersensitive response and induce susceptibility in potato plants.[13]

Gibberellins are another group of plant growth promoters that are found in young unripe fruits and seeds, in root tips and shoots. They are responsible for the

elongation of internodies, enhance seed germination and flower development. Gibberellins are pentacyclic diterpenoic acids. It is suggested that gibberellic acid (**3**) inhibits leaf senescence of Pelargonium by reducing levels of reactive oxygen species (ROS).[14]

The first brassinosteroid isolated from the rape plant led to enhanced stem elongation. More than 40 different natural brassinosteroids could be identified, but castasteron (**4**) and brassinolide are the most abundant ones. They differ in the B-ring of the steroid skeleton and the 2α- and 3α- hydroxy-groups seem to be essential for the hormone effects.

Plant hormones inhibiting growth are abscisic acid (**5**) and ethylene (**6**) (**Figure 3**).



5 abscisic acid **6** ethylene

Figure 3. Abscisic acid (**5**) and ethylene (**6**) are plant growth inhibitors.

Abscisic acid (**5**) occurs in high concentrations in dormant seeds. As a natural growth inhibitor it maintains the dormancy of buds by inhibiting cell division. Abscisic acid (**5**) regulates transpiration, stress responses and embryogenesis. Most effects seem to be related to water availability, where it acts as a signal of water loss by regulation of stomatal behaviour.[15] **5** is a mono-cyclic sesquiterpenoid that is involved in rapid opening processes of ion channels and its biosynthesis is correlated with coordination of stress.[16]

Ethylene (**6**) is a gaseous effector, participating in many physiological processes; it influences plant growth, acts as a stress hormone and exhibits various morphogenic effects. **6** triggers senescence, accelerates fruit ripening, fading of flowers and abscission of leaves and petals.[17] The ethylene-signalling pathway coordinates together with other phytohormones diverse responses to all kinds of stresses. It slows down plant growth until the stress situation is solved. On the genetic level, **6** induces the expression and transcription of several genes involved in defensive responses.[18]

The number of physiological processes controlled by such phytohormones is enormous and it is difficult to differentiate functions of each hormone from the others. Besides such classical plant hormones, another class of hormone-like

substances was identified as being ubiquitous in higher plants, the oxylipins. Jasmonic acid (JA) (**7**), its methyl ester (MeJA) (**8**) and its biosynthetic precursor 12-oxophytodienoic acid (OPDA) (**9**) are the predominant oxylipins researches are focussing their interest on.

As a consequence of oxidative stress, caused by mechanical wounding, pathogen or herbivore attack, the plant's surface is damaged and an oxidation of free or esterified lipids is initiated either by enzymes (lipoxygenases) or by reactive oxygen species (ROS). These peroxidation processes lead to a highly diverse class of oxylipins (**Figure 4**). Derived from linolenic (**10**), linoleic acid or other poly-unsaturated fatty acids (PUFAs) located in the plant's membrane, oxylipins of different chain length or different degrees of functionalisation are formed. The pathway leading to OPDA (**9**) and jasmonates is also called the octadecanoid pathway (**Figure 5**). α-Linolenic acid (**10**) is released from the membrane lipids by phospholipases and is oxidised to the (13*S*)-hydroperoxyperoxide (**11**) by a lipoxygenase. **11** is then converted to an unstable allene oxide intermediate (**12**) by the allene oxide synthase and undergoes cyclisation catalysed by the allene oxide cyclase leading to the JA (**7**) precursor OPDA (**9**).

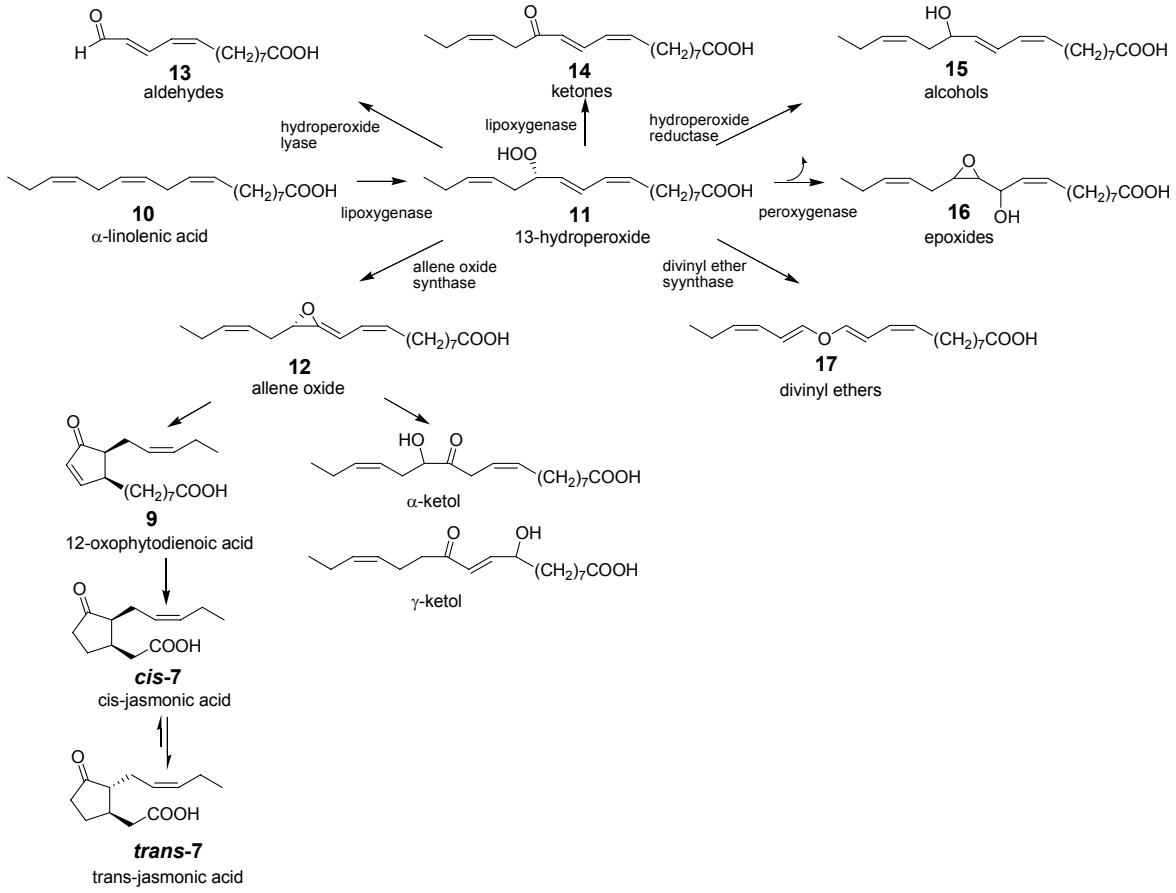


Figure 4. Enzymatically formed oxylipins derived from the α-linolenic acid (13*S*)-hydroperoxide (**11**).

Activation and cyclisation of linolenic acid (**10**) (**Figure 5**) take place in the chloroplast, whereas the steps downstream of OPDA (**9**) take place in peroxisomes and in the cytoplasm, where OPDA (**9**) or its CoA ester is transported to. In the cytoplasm, the cyclopentenone ring double bond of **9** is reduced by a 12-oxophytodienoate reductase. In the peroxysomes, stepwise β-oxidation finally leads to the plant hormone *cis*-JA (**7**).[19] The highly active *cis*-**7** rapidly undergoes epimerisation to the thermodynamically more stable but less active *trans*-isomer.[20] **7** is biologically active and has hormone properties. It plays a key role in regulating plant growth and development by influencing seed germination,[21] flower formation[22] and fertility[23] and seems to participate in leaf senescence and in defence mechanisms against wounding and herbivory.[24-26]

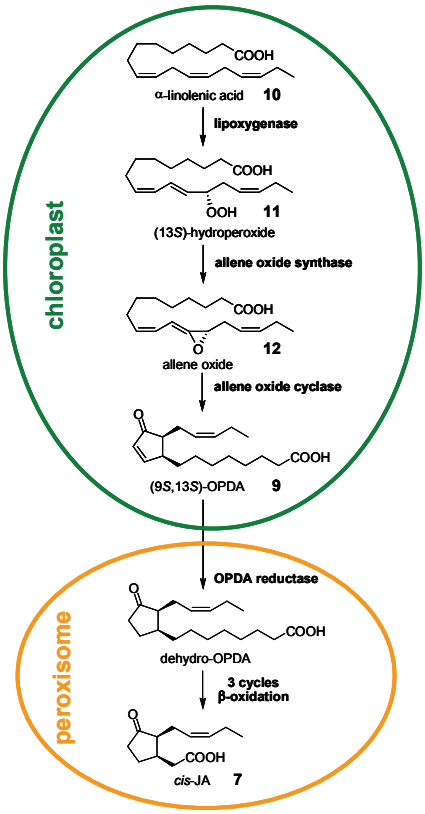


Figure 5. The octadecanoid pathway in plants.

Similar to other plant hormones, jasmonates have both activating and inhibiting effects. The early precursor of the JA-biosynthesis OPDA (**9**), is also biologically active as a signal transducer independent from the JA (**7**) signalling pathway.[27] Besides **7** and **9**, other fatty acid derived oxylipins recently came into focus of researchers. All are derived from 13- or 9-fatty acid hydroperoxides, that are further converted by different enzymes to aldehydes (**13**), ketones (**14**), alcohols (**15**), epoxides (**16**) or ethers (**17**) (**Figure 4**). But plant oxylipins are not only formed enzymatically, they can also be produced without the action of enzymes. Phytoprostanes are a recently characterised class of such non-enzymatically formed oxylipins.[28] The effects of oxylipins are widespread and in many cases not fully understood. However, there is increasing evidence, that not only jasmonates, but also other oxylipins play an important role and/or are even the major signalling compounds in plant defensive strategies.[29]

1.3 Phytoprostanes and dinor isoprostanes

The octadecanoid pathway in higher plants leading to jasmonates is relatively well-studied.[27] Many enzymes are involved in the oxidation and transformation of linolenic acid (**10**) leading to a defined stereochemistry of the resulting oxylipin metabolites. But more and more information is arising, that there are other fatty acid derived oxylipins independently from JA (**7**) that also have biological importance.[29] Especially on the fungal and pathogen level, a direct protection of the plant can be mediated by OPDA (**9**). **9** and other molecules containing an α,β-unsaturated carbonyl group have been postulated as potent gene regulators in diseased plant tissues,[30] as a result of their activity as Michael-acceptors. A new substance class belonging to this JA-independent group is the so-called dinor Isoprostanes (DIP) or phytoprostanes (PP), which are formed non-enzymatically from α-linolenic acid (**10**).

1.3.1 Occurrence, biosynthesis and diversity of phytoprostanes

In the early 1990s, a series of prostaglandin like compounds was identified to be produced in mammals independently of the cyclooxygenase pathway from arachidonic acid.[31] These compounds were termed isoprostanes, because their cyclopentenoid structure is closely related to that of the prostaglandins; however they are completely racemic.

Since higher plants generally do not possess arachidonic acid (C20:4), neither prostaglandins nor isoprostanes can be produced. The major poly-unsaturated fatty acid in higher plants is α-linolenic acid (18:3ω3) (**10**). Similar to the origin of mammalian isoprostanes, a new substance class was identified to be produced in higher plants, termed phytoprostanes (PPs). PPs are generated from α-linolenic acid (**10**) in a non-enzymatical pathway initiated by enhanced formation of free-radicals.[32] They are denominated analogous to the prostaglandin nomenclature.[33]

γ-Linolenic acid (18:3ω6) (**18**) is only a minor fatty acid in higher plants; it occurs in high levels (10-24 % of dry weight) in borage seeds (*Borago officinalis*), black currant (*Ribes nigrum*) or evening primerose (*Oenotherae biennis*) and is a trace

compound in animal lipids. Analogous to the oxidation of α-linolenic acid (**10**) that forms phytoprostanes (PPs), γ-linolenic acid (**18**) can be transformed to dinor isoprostanes (DIPs), that only differ from the PPs by the length of their side chains attached to the cyclopentenone ring system.[34]

As shown in **Figure 6**, the cyclopentenone skeleton is a result of the free-radical oxidation of α-linolenic acid (**10**) *via* its 12- or 13-hydroperoxy radical (**19**) initiated by reactive oxygen species (ROS). Oxidation of γ-linolenic acid (**18**) occurs *via* the 9- or 10-hydroperoxy radical (**20**). Two different regioisomeric products are formed in each case. The resulting bicyclic endoperoxides (PPG_1) (**21**) are instable intermediates that are prone to undergo reduction, rearrangements or elimination of water leading to the high diversity of different PP structures. **Figure 6** focuses on the biosynthesis of the B_1-PP skeleton (**22**) that is synthesised within this work; other metabolites of the PP pathway can be found in **Figure 7**.

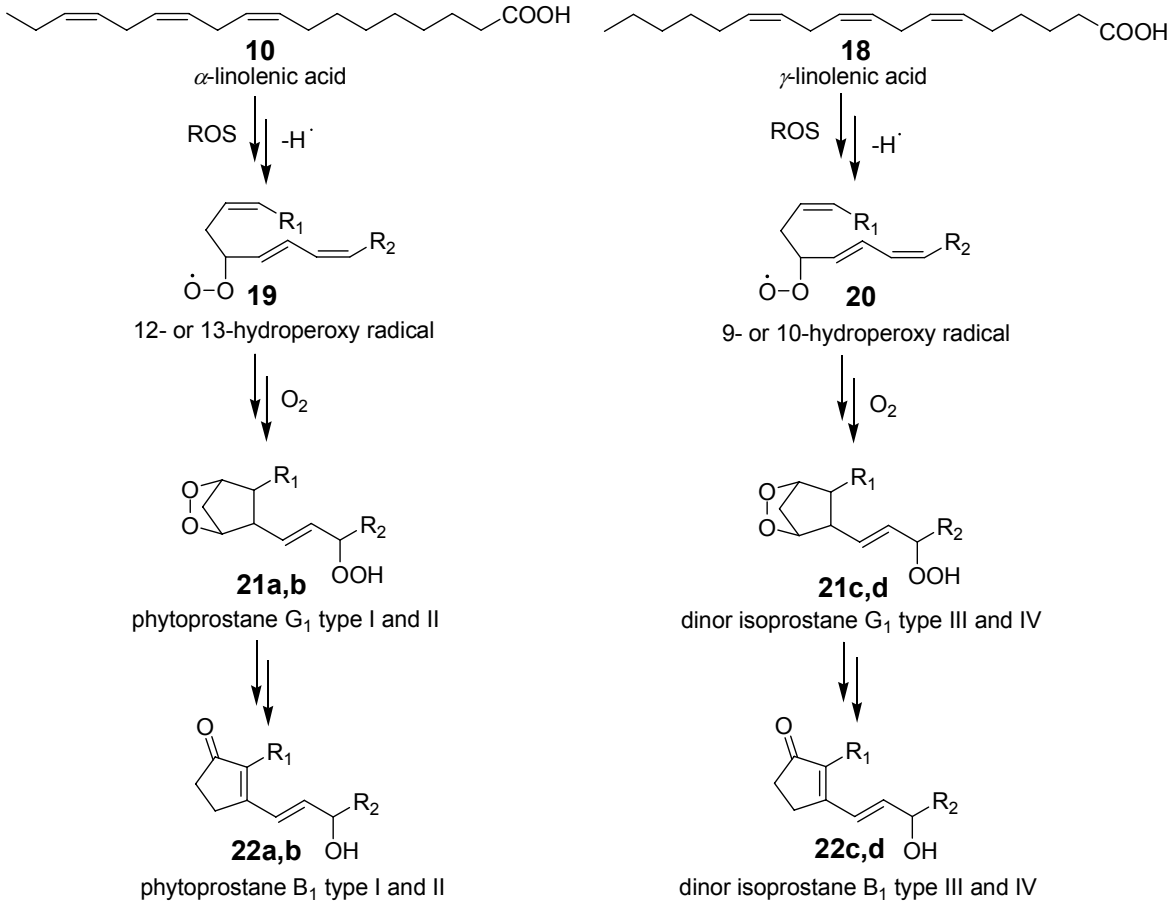


Figure 6. Biosynthesis of phytoprostanes (PP) B_1 type I and II and of dinor isoprostanes (DIP) B_1 type III and IV derived from free-radical oxidation of α- and γ-linolenic acid.

	PPB_1 type I (22a)	PPB_1 type II (22b)	$DIPB_1$ type III (22c)	$DIPB_1$ type IV (22d)
R_1	$(CH_2)_7COOH$	CH_2CH_3	$(CH_2)_4COOH$	$(CH_2)_4CH_3$
R_2	CH_2CH_3	$(CH_2)_7COOH$	$(CH_2)_4CH_3$	$(CH_2)_4COOH$

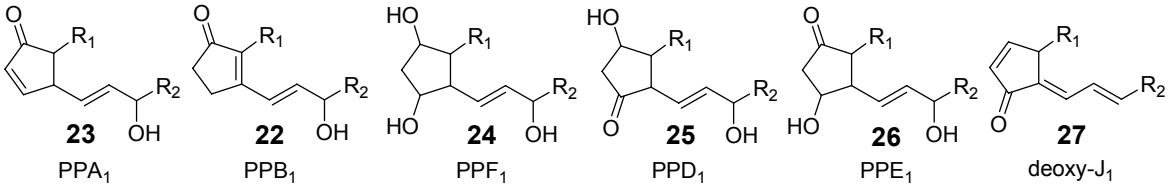


Figure 7. Structural diversity of PPs denominated according to the prostaglandin nomenclature. (R_1, R_2 see **Figure 6**)

PPs are formed ubiquitously in higher plants at a low background level, but their concentration dramatically increases in response to oxidative stress caused by leaf damage or pathogen infection.[35]

1.3.2 Biological importance of B_1-phytoprostanes

Phytoprostanes (PP) are generated by free radical processes during aerobic metabolism. PPs are therefore considered as reliable markers of oxidative stress in higher plants.[35] Oxidative stress on the leaf surfaces can be caused by different factors, such as mechanical wounding, herbivore or pathogen attack. High levels of PPs were observed after *in vivo* treatment with fungal pathogens (*Botrytis cinerea*) or peroxides and after addition of heavy metal ions.[34] PP accumulation is a transient process, suggesting that these biomarkers are rapidly generated and metabolised *in vivo*.[32] Despite other PP classes, the B_1-types (**22a,b**) seem to be particularly active in certain plant species. A cDNA microarray of *Arabidopsis thaliana* callus cultures showed after treatment with both enantiomeric PPB_1 types (**22a,b**) a strong detoxification and defence response.[36] The expression levels of several glutathione-*S*-transferases, glycosyl transferases and a putative ATP-binding cassette were increased. This enhances the plant's ability to detoxify and sequestrate reactive and therefore dangerous products of lipid peroxidation. PPs

might thus be regarded as sensitive biomarkers that occur in response to oxidative stress.

The induction of secondary metabolism is another important factor in plant defence reactions. Both PPB_1 (**22a,b**) stimulated the accumulation of secondary metabolites (**Figure 8**) in different plant species.[36] In *Eschscholzia californica* cell cultures, the PPB_1 type II isomer (**22b**) induced the accumulation of benzophenanthridine alkaloids similar to methyl jasmonate (MeJA) (**8**). *Crotalaria cobalticola* cell cultures accumulated the chalcone isobavachalcone (**29**) in response to PPB_1 type II (**22b**) and MeJA (**8**) treatment, whereas type I PPB_1 (**22a**) was less active. Also in tobacco cell cultures, **22b** showed a comparable activity to **8** by inducing the accumulation of scopoletin (**30**). Interestingly, there was no difference in the activity of the corresponding *R*- and *S*-enantiomer of both PPB_1 species (**22a,b**), suggesting that the postulated biosynthetic pathway, based on racemic products, is correct. The biosynthesis of the phytoalexin camalexin (**31**) in *Arabidopsis* cell cultures was similarly induced by both PPB_1 regioisomers (**22a,b**).

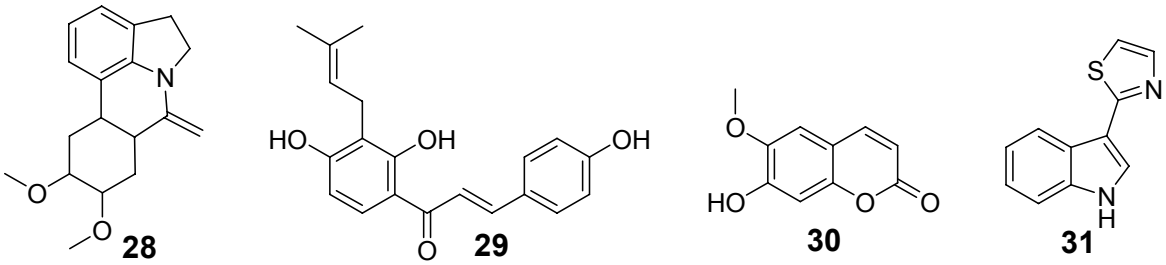


Figure 8. Secondary metabolites that are produced after treatment with PPB_1s (**22a,b**) or MeJA (**8**): a) benzophenanthridine alkaloids; b) chalcones; c) scopoletin; d) camalexin play a role in the plant's defence against bacteria or fungi.

Currently, interest is rising in the activity of PPs in humans. Four classes of PPs (F_1, E_1, A_1, B_1) were found in quite high concentrations up to 99 mg/l in several fresh cooking oils and after oral consumption of the oils in the human gastro-intestinal tract.[37] Thus, humans might be exposed to high levels of PPs and their effects should be studied in detail. Results of this study indicate that PPs are novel, biologically active lipids in plant nutrition. It is further suggested that PPs do not only modulate plant defence but also have an impact on human immune cells similar to their arachidonic acid derived congeners of the prostaglandin family. PPs might be involved in reducing the risk of several cardiovascular diseases, such as atherosclerosis or infarction. It has been shown, that pollen-derived PPE_1 (**26**) can

modulate dendritic cell function similar to prostaglandins E_2.[38] Especially in fresh birch pollen, high concentrations of PPF_1 (**24**) were found, suggesting that certain PPs are involved in allergic reactions similar to the mammalian isoprostanes. Therefore, it is indicated that the influence of PPs is not limited to the plant kingdom.

1.3.3 Relationship between prostaglandins, isoprostanes and phytoprostanes

Formerly, the understanding of animal and plant signalling mechanisms developed independently. In recent years, more work has been done to show that different stimuli are similar across these two kingdoms. The responses to certain stress factors, such as wounding or pathogen attack, overlap in the prostaglandin and octadecanoid signalling processes.[39] The structural relation between prostaglandins, isoprostanes and phytoprostanes is obvious because of the mechanistically related biosynthesis from polyunsaturated fatty acids. Although prostaglandins are formed by enzymes, there is no evidence, that iso- or phytoprostanes do require enzymes for their formation. In contrast to a defined stereochemistry in prostaglandins, iso- and phytoprostanes are biosynthesised as racemic mixtures. All three natural product classes contain a cyclopentanoid ring structure, and differ only in the substitution and length of the side chains derived from arachidonic or α-linolenic acid (**Figure 9**).

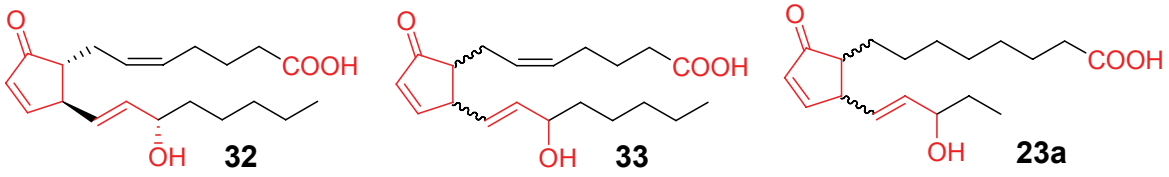


Figure 9. Comparison between prostaglandin A_2 (**32**), isoprostane A_2 (**33**) and phytoprostane A_1 (**23a**).

The unsubstituted cyclopentenone-system is a highly active unit being able to act as a Michael-acceptor and thus might bind to electrophilic substrates (**Figure 10**). A covalent bond between this α,β-unsaturated oxo moiety of the cyclopentenones and a thiol-group of proteins and glutathione (**34**) is possible. Prostaglandin

derivatives and PPs are able to induce enzymes, such as glutathione-*S*-transferases, that catalyse the conjugation of toxic products of lipid peroxidation. This indicates that these cyclopentenone metabolites might also act as scavengers of ROS from the oxidative burst.

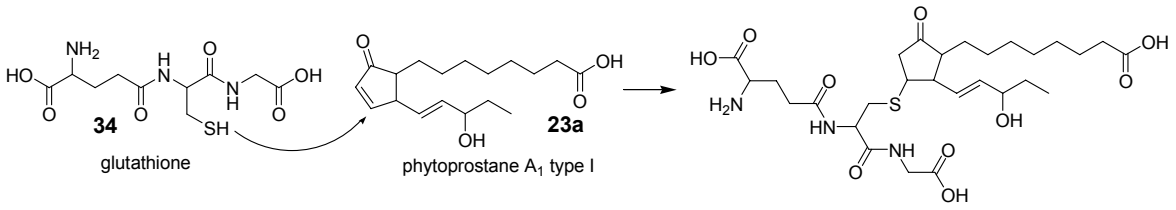


Figure 10. Nucleophilic attack of glutathione (**34**) to the "soft" electrophile, the α,β-unsaturated carbonyl group of PPs.

The side chains of prostaglandins are mostly oriented *trans*, whereas the ones from iso- and phytoprostanes mainly exist in *cis*-configuration. A carboxyl-moiety is located at the end of one side chain and the C3-side chain contains an allylic hydroxyfunction, which is important for the biological activity of the cyclopentanoid metabolites.

Prostaglandins have been found in nearly all mammalian organs in concentrations ranging from 0.01 to 1 μg/g bodyweight.[10] Higher concentrations were isolated from the mammalian seminal fluid.[40] Derived from phospholipids, containing arachidonic acid, the prostaglandins, prostacyclins, or thromboxanes can be generated. There are many different physiological effects caused by prostaglandins. They act as local hormones on a variety of cells such as vascular smooth muscle cells causing constriction or dilation. Therefore prostaglandins are involved in reproductive processes. PGE_2 causes uterine contractions and has been used to induce childbirth or abortion. Prostaglandins activate the inflammatory response, cause pain and fever. After tissue damage, the level of prostaglandins is increased. Certain prostaglandins are found in several other organs of the gastrointestinal tract (inhibit acid synthesis and increase secretion of protective mucus) and increase blood flow in kidneys. Recently, it has been shown, that prostaglandins could also be detected in plants and microorganisms. Several prostaglandins were found in plant species, such as onion,[41] red alga,[42] poplar and larch[43], in prokaryotic organisms, such as cyanobacteria or *Pseudomonas*,[44] or in yeasts.[45] Similar to their physiological importance in animals, there should be analogous or different effects in higher and lower plants.

In mammals, prostaglandins regulate reproduction, and in certain plant species, e.g. the short day plant *Pharbitis nil*,[46] they are possibly involved in flower induction. They regulate cell membrane permeability in mammals and in plants.[47] It was found, that certain prostaglandins promoted the effect of a phosphatase enzyme in barley similar to the plant hormone gibberellic acid (**3**).[48]

Isoprostanes have been found in many animal tissues and in most biological fluids, such as plasma and urine.[49] Their occurrence depends on the extent of oxidative stress. Isoprostanes are linked to an excessive production of lipid peroxidation products, eventually leading to the development of cancer, cardiovascular or neurological diseases. They have been associated with an excessive generation of free radicals and with various disease states of the lung or oxidative damage of the liver. An increased concentration of isoprostanes has also been found in patients with Alzheimer's or Parkinson's diseases. Indeed, the extent of their biological importance *in vivo* is still unclear and controversial. But they are regarded as indicators of oxidative stress in mammals. Perhaps they act in a similar way to their enzymatically formed analogues, the prostaglandins, by regulating the vascular smooth muscle cells. It has been shown, that many, but not all, isoprostanes are able to bind to and activate prostaglandin receptors.

Phytoprostanes (PPs) are somehow different from their mammalian counterparts, consisting only of an 18-carbon skeleton. The functional groups around the active centre are similar, suggesting that there is also similar physiological importance. Although the occurrence is limited to the plant kingdom, it is suggested that PPs are also active in animals.[37-39] In plants PPs are mediators of oxidative stress, being connected to the enhanced formation of free radicals causing an oxidation of α-linolenic acid (**10**).[32] As discussed in chapter 1.3.2, PPs act in stimulating defensive and detoxification genes and increase the amount of secondary metabolites, involved in plant defence and detoxification processes. It might also be possible, that PPs are able to bind to certain prostaglandin receptors and cause prostaglandin-type reactions in animals similar to the isoprostanes.[50]

2 Objectives for this work

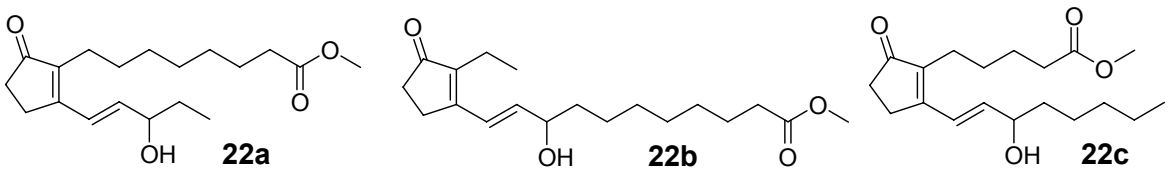
Prostaglandins were discovered in the human seminal plasma by von Euler *et al*. in the 30s of the 20th century.[40] More interest on this specific substance group arose about 20 years later, as they were identified as hormone-like substances in animals derived from arachidonic acid. Until today more than 77.000 publications exist dealing with these compounds. The biological relevance of prostaglandins was first studied by extracting the active compounds from the seminal fluids and performing different activity tests with. Later, also chemical syntheses were designed to obtain these metabolites in higher concentrations than by extraction of biological tissues.

Isoprostanes were first identified and characterised in the early 90s as prostaglandins-like compounds.[31] Similar to their enzymatically-formed analogues, they were extracted from mammalian tissue material and then used for specific tests for investigating their biological importance. But the lack of satisfactory natural sources has prompted scientists to focus on chemical synthesis. According to databases, more than 2.000 publications deal with the topic of isoprostanes including many synthetic approaches.

The field of phytoprostanes was opened in 2000, when Müller *et al*. detected these metabolites by non-enzymatic oxidation of α-linolenic acid (**10**).[32] Phytoprostanes were then regarded as sensitive markers of oxidative stress in higher plants similar to the isoprostanes formed in mammals. Currently, there exist only about 20 publications dealing with this topic, showing that there can be done a lot to contribute to this interesting and new field of science. Until 2003, phytoprostane activity was only studied by using the compounds derived from autoxidation processes. The first synthetic approach was published in 2003 for PPF_1 (**24**).[51] Later, syntheses for PPE_1 (**26**), PPB_1 (**22**) and deoxy- PPJ_1 (**27**) were developed, but most of them are quite long and ineffective.[52-54]

The aim of this work was to develop a short and effective synthetic access to the phytoprostane (PP) B_1 series (**22**) (**Figure 11**). Despite the types I (**22a**) and II (**22b**) derived from α-linolenic acid (**10**), the dinor isoprostanes (DIP) B_1 type III (**22c**) derived from γ-linolenate (**18**) should be generated using this synthetic

approach. Different structural analogues should give more insight into structure-function relationships of these prostaglandin-type metabolites.



PPB_1 - I methyl ester PPB_1 - II methyl ester $DIPB_1$ - III methyl ester

Figure 11. Structures of PPB_1-I methyl ester (**22a**), PPB_1-II methyl ester (**22b**) and $DIPB_1$-III methyl ester (**22c**).

In order to better understand the biological importance of phytoprostanes, more bio-assays dealing with different topics must be performed. It is known, that phytoprostanes accumulate with enhanced free radical formation and are considered as markers of oxidative burst. However, their ultimate function in higher plants is still unknown. It has to be determined, in which defensive mechanisms due to oxidative stresses, phytoprostanes are involved. Is it defence against herbivores (induction of volatile emission), against pathogens (growth inhibition) or just an answer to mechanical wounding? Are phytoprostanes just artefacts, that are formed incidentally by linolenate oxidation? Are they just by-products or really important metabolites for plant defence? Do they have an impact on the induction of the accumulation of defensive genes similar to jasmonic acid (**7**) treatment, wounding or pathogen attack? These are some of the questions, that will be addressed within this work.

Intentions

- Synthesis of the methyl esters of phytoprostane B_1 types I (**22a**) and II (**22b**) and dinor isoprostane B_1 type III (**22c**)
- Synthesis of structural analogues to the natural occurring phytoprostanes
- Studies with DNA microarrays of extracted total RNA from phytoprostane-treated *Arabidopsis thaliana* plants
- Antimicrobial tests on different bacterial strains (agar-diffusion test)
- Volatile emission tests
- Intracellular Ca^{2+}-measurements

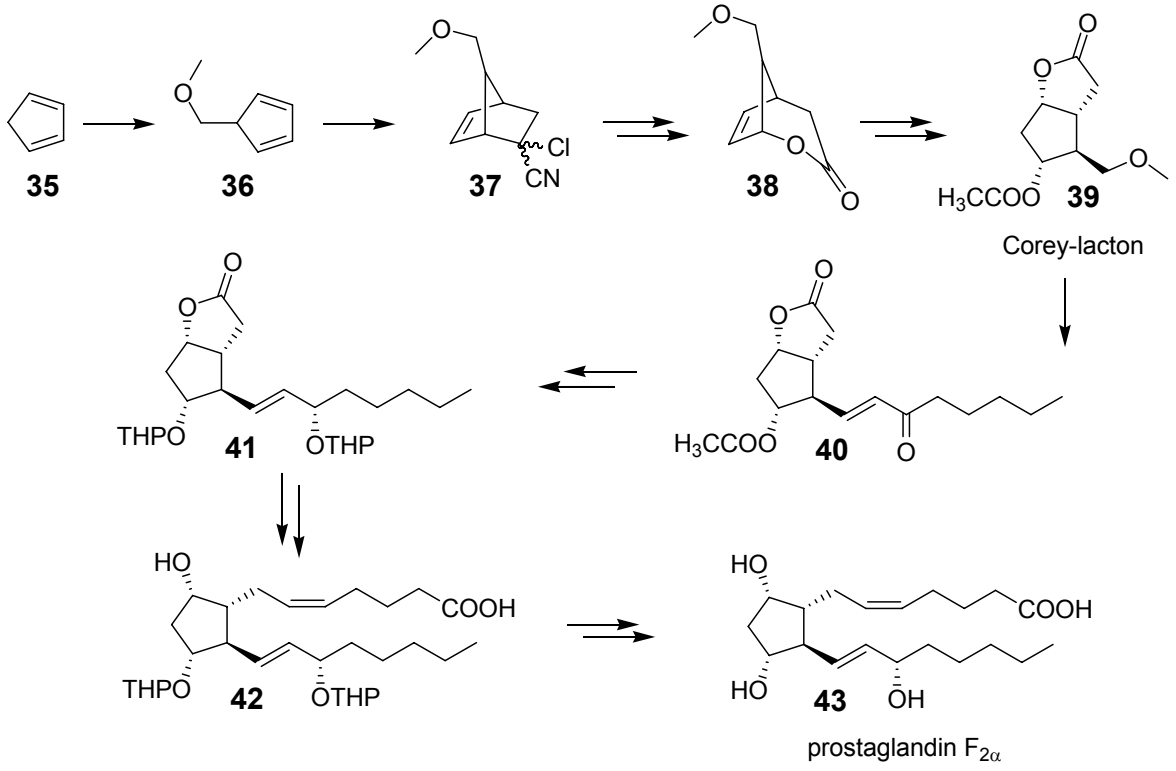
3 Results and discussion

3.1 Synthetic strategies

3.1.1 Known phytoprostane syntheses

Phytoprostanes (PPs) can be generated by autoxidation of α-linolenic acid (**10**)[55] or by following established prostaglandin- or isoprostane syntheses. Autoxidation processes are always time consuming and mostly lead to a complicated product mixture from which the desired compounds have to be isolated using complex cleaning steps.

The first total synthesis of prostaglandins, the enzymatically formed mammalian congeners of PPs, was published by Corey *et al.* in 1968 (**Scheme 1**).[56]

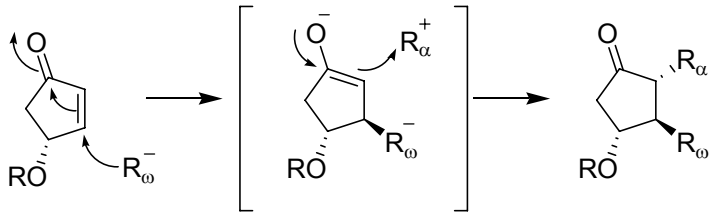


Scheme 1. Corey's first total synthesis of prostaglandin $F_{2\alpha}$(**43**) starting from cyclopentadiene (**35**).

In this synthetic approach cyclopentadiene (**35**) was alkylated to **36** and a Diels-Alder addition with 2-chloroacrylnitrile to **37** was performed, yielding after alkaline

saponification two enantiomeric ketones, that were oxidised to give two enantiomeric seven-ring lactones **38**. Further acetylation and oxidation steps generated the so-called Corey-lactone (**39**), a versatile synthetic building block. In a Wittig-reaction, the C3-side chain was introduced into **39** to form **40** and after reduction and protection of the hydroxy-groups with THP to **41**, a mild reduction with isobutyl aluminium hydride gave a hemiacetal in which the C2-side chain could then be introduced *via* Wittig-reaction to form **42**. After deprotection, the pure prostaglandin $F_{2\alpha}$ (**43**) was obtained. This first total synthesis of prostaglandins included many steps, yielding only 2 % of the pure prostaglandin $F_{2\alpha}$(**43**).

Later, more efficient syntheses for prostaglandins were introduced using three-component-coupling processes (**Scheme 2**) by attaching the α- and ω-side chain to a five-membered ring.[57] For these reactions, organic copper compounds served as donor synthons (R_ω^-), whereas aldehydes were used as acceptors (R_α^+). Thus, the nucleophilic attack on the β-C-atom and the electrophilic attack on the α-C-atom of the α,β-unsaturated cyclopentenone were achieved.

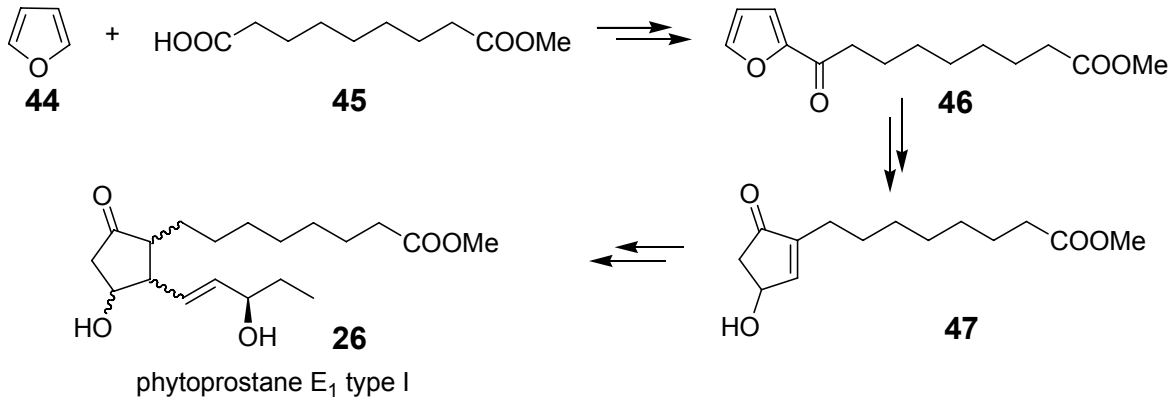


Scheme 2. General mechanism of the three-component coupling reaction to introduce various α- and ω-side chains into five membered rings of prostaglandins or analogues.

In the last few years, also phytoprostane (PP) syntheses along this concept have been developed.

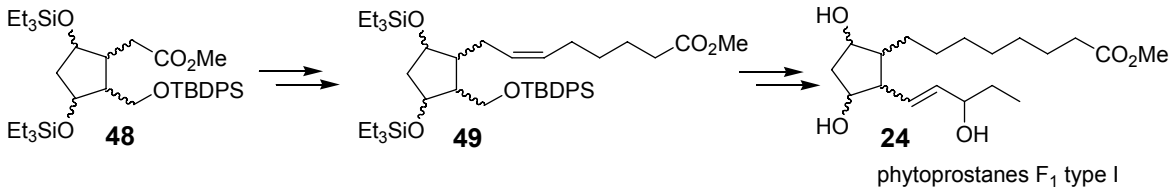
Rodriguez *et al.*[58] reported a two-component coupling process for isoprostanes combined with a diastereoselective protonation with chelating proton donors under reaction control, that was later adapted for a synthesis of PPE_1 (**26**) (**Scheme 3**).[52] Furan (**44**) was acylated with azelaic acid monomethyl ester (**45**) to give the ketoester **46**, which was then reduced and rearranged to the racemic hydroxycyclopentenone **47** in H_2O using a catalytic amount of chloral in the presence of triethylamine. The racemic precursor **47** could be converted into its chiral intermediate by lipase-catalysis. The product was further coupled with the

optically pure iodovinyl side chain followed by a diastereoselective protonation. After deprotection, the PPE_1 (**26**) could be generated within 10 steps. Overall yields were not given, but might have been up to 15 %.



Scheme 3. Essential steps for the synthesis of PPE_1 (**26**) starting from furan (**44**).

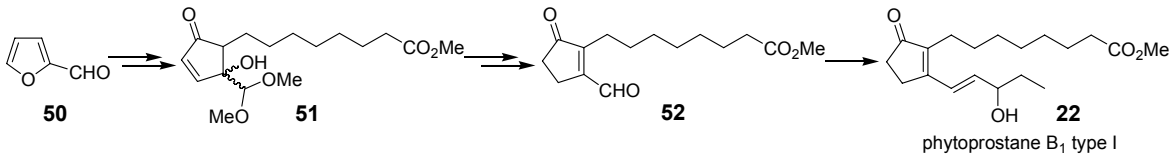
A French group excelled at working on efficient synthetic strategies on phytoprostanes (PPs). They first synthesised PPF_1 type I and its 16-epimer (**24**)[51] by introducing the C2-chain *via* Wittig reaction into the ester **48** to form **49** followed by certain reduction, protection and oxidation steps and a Horner-Wittig-Emmons-condensation with the C3-side chain. Final reduction with *R*- or *S*- BINAL-H gave the desired compound **24** in about 11 % yield over 8 steps (**Scheme 4**). This route required enhanced synthetic effort, since all precursors had to be generated first. In 2004, this group published a similar synthetic approach, but now covering all diastereomers of PPF_1 types I and II (**24**) [59] Again, the synthetic effort was high with respect to all diastereomeric isoforms.



Scheme 4. Essential steps for the synthesis of all diastereomers of PPF_1 type I (**24**).

In 2005, the same group found a way to synthesise PPB_1 type I and II (**22**) (**Scheme 5**).[53] Therefore, they modified their original method and used furfural (**50**) as starting material. First, **50** had to be transformed to a 3-hydroxy-4-cyclopentenone precursor **51** in 6 steps, including a Wittig reaction, hydrogenation

of the additional double bond, followed by a formylation step, protection, oxidation and an acidic hydrolysis of the resulting bis-acetal. After further steps of hydrogenation, dehydration and acidic hydrolysis, the resulting aldehyde **52** underwent reaction with chiral phosphonium ylides to introduce the corresponding C3-chains. This synthetic route required 10 synthetically demanding steps with an overall yield of only 5 % and much presynthetic work had to be done for generating the particular chiral phosphonium salts as coupling reagents.



Scheme 5. Essential steps for the synthesis of *R/S*-PPB$_1$ type I (**22**).

In 2005, an approach was published[54] using a disclosed conjugate addition-olefination process resulting in racemic deoxy-J$_1$-PP type I and II (dPPJ$_1$) (**27**). This synthesis is also lengthy and consequently not useful for larger scale production. Since the dPPJ$_1$ (**27**) are the major metabolites of the PP pathway, shorter chemical syntheses are required to generate these metabolites in larger amounts. Interestingly, these PPJ$_1$s are structurally closely related to 12-oxo-phytodienoic acid (**9**), an important enzymatically formed plant defence mediator.

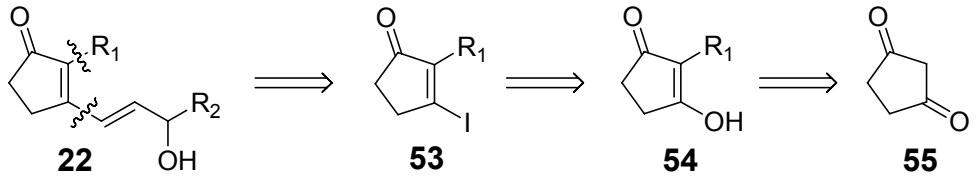
Considering the enormous synthetic effort and the low yields of published synthetic approaches to PPs, the attempt for this work was to develop a shorter and more efficient synthesis that should also be suitable for synthesising structural analogues of PPs.

3.1.2 Synthetic concepts

Due to the increasing interest in the biological activity of phytoprostanes (PPs), many total syntheses have been developed.[51-54,59,60] Most of them include many reaction steps, are time-consuming and ineffective. Therefore, the aim of this thesis was to design a short, effective and high yielding synthetic route to obtain the PPs of the B$_1$-family (**22**). Based on the previously published protocol for the

synthesis of tetrahydrodicranenone B,[61] a flexible approach to the B_1-PPs (**22**) and several structural analogues was developed (**Scheme 6**).

PPs of the B_1-series (**22**), that represent 2-alkyl-3-alkenyl-substituted cyclopentenones, can be generated as follows. By transition metal catalysis following Heck- or Sonogashira protocols, the C3-substituent could be introduced into the cyclopentenone molecule by substitution of the alkenyl iodide **53**. **53** could be generated from the mono-alkylated hydroxycyclopentenone **54** *via* standard iodination reactions. For PPB_1 type II (**22b**), the mono-alkylated hydroxycyclopentenone **54b** was commercially available, whereas for type I (**22a**) and III (**22c**), the C2-chain had to be attached to 1,3-cyclopentanedione (**55**) first.



phytoprostane B_1 type I, II methyl ester, dinor isoprostane B_1 type III methyl ester | alkenyl iodide | mono-alkylated hydroxycyclo-pentenone | 1,3-cyclopentane-dione

Scheme 6. Retrosynthetic approach to PPs of the B_1-series (**22**).

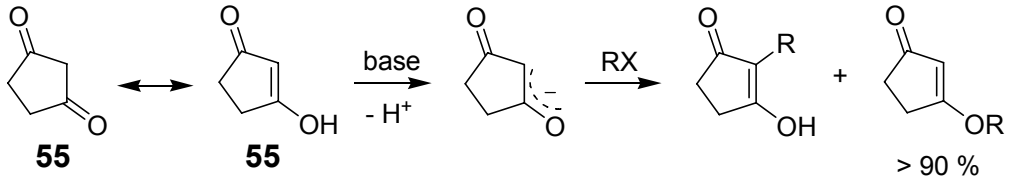
	PPB_1 type I (22a)	**PPB_1 type II (22b)**	**$DIPB_1$ type III (22c)**
R_1	$(CH_2)_7COOMe$	CH_2CH_3	$(CH_2)_4COOMe$
R_2	CH_2CH_3	$(CH_2)_7COOMe$	$(CH_2)_4CH_3$

The central alkenyl-iodide **53** turned out to be an excellent and versatile synthon for coupling of various side chains to the cyclopentenone molecule. It cannot only be coupled with allylic alcohols to yield PPs, but also with non-functionalised side chains or with diverse side chains containing an oxo- or alkynyl-moiety etc. Side chains with different degrees of functionalisation and lengths can be attached to **53**. Thus, it is possible to generate many analogous of PPs as well as substances of the isoprostane or prostaglandin family along the same protocol.

In the following subchapters, the synthetic approach is described, allowing a simple and highly efficient synthesis of PPs of the B_1-series (**22**). This synthetic method turned out to be a useful approach for generating a variety of structural analogues to the natural occurring PPs.

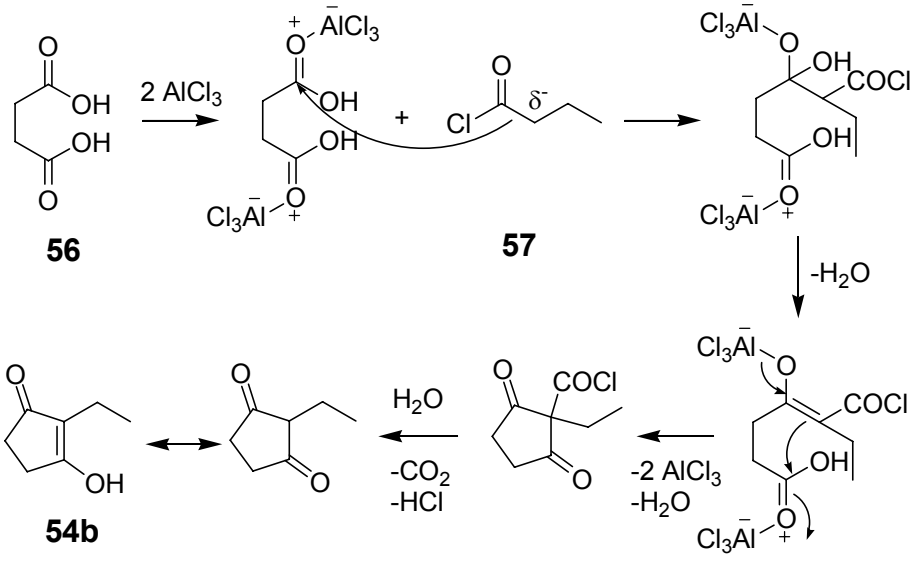
3.1.2.1 Attachment of the C2-substituent to the five-membered ring

Phytoprostanes (PPs) belong to a large group of natural products that comprise a substituted cyclopentanoid ring system. The C2- and C3-substituents can be attached to the molecule by different coupling reactions. For PPB_1 (**22**) syntheses, 1,3-cyclopentanedione (**55**) appeared to be a useful precursor. Analysing the structure of **55**, it can easily be seen, that this precursor is prone to undergo keto-enol-tautomerism; it predominantly occurs in its enol-from. As known from literature,[62] the percentage of the enol-form is high with two 1,3-carbonyl groups. The enol-form contains a system of conjugated double bonds and can build out a hydrogen bond for stabilisation. Both effects lower the energy of the enol-form. The resulting enolate anion as an ambident nucleophile can either be *C*- or *O*-alkylated. The favoured reaction product here was always the *O*-alkylated cyclopentenone, whatever bases have been applied (**Scheme 7**).[63]



Scheme 7. The problem of keto-enol-tautomerism of **55** in the synthesis of *C*-alkyated cyclopentenones**.**

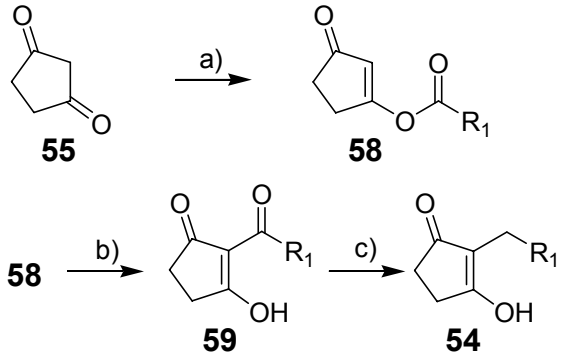
Therefore, an alternative route had to be developed. Chemists working on steroid synthesis found a method to overcome the problem of *O*-alkylation.[64] For the synthesis of 2-ethylcyclopentane-1,3-dione or better 2-ethyl-3-hydroxy-cyclopent-2-enone (**54b**), succinic acid (**56**) could be cross-coupled in a Friedel-Crafts-analogous acylation with butyryl chloride (**57**) in the presence of 2 equiv aluminium trichloride (**Scheme 8**). The mechanism for this alkylation is not clearly understood. Probably, the acid chloride attacks the positively charged C-atom of the keto-function as a nucleophile. After elimination of water, the resulting double bond could attack the second positively charged carbonylcarbon. The ring is closed and after decarboxylation and elimination of the chloride and the aluminium chloride, the desired product **54b** can be generated.



Scheme 8. Proposed mechanism for the generation of 2-ethyl-3-hydroxycyclopent-2-enone (**54b**).

Yields for this procedure were generally good, but with respect to the handling of butyryl chloride and the not well elaborated cleaning steps, commercially available 2-ethyl-3-hydroxycyclopent-2-enone (**54b**) was used for synthesis of PPB_1 type II (**22b**). Furthermore, this method is limited to short alkyl chains. For introduction of the C2-substituent of PPB_1 type I (**22a**) and $DIPB_1$ type III (**22c**), having longer C-chains and a carboxyl function, the method has limitations, because of competing side reactions and formation of by-products. Therefore, an alternative alkylation method was used.

Following a Russian three step procedure, the alkylation in C2-position of the cyclopentanedione molecule was finally achieved (**Scheme 9**).



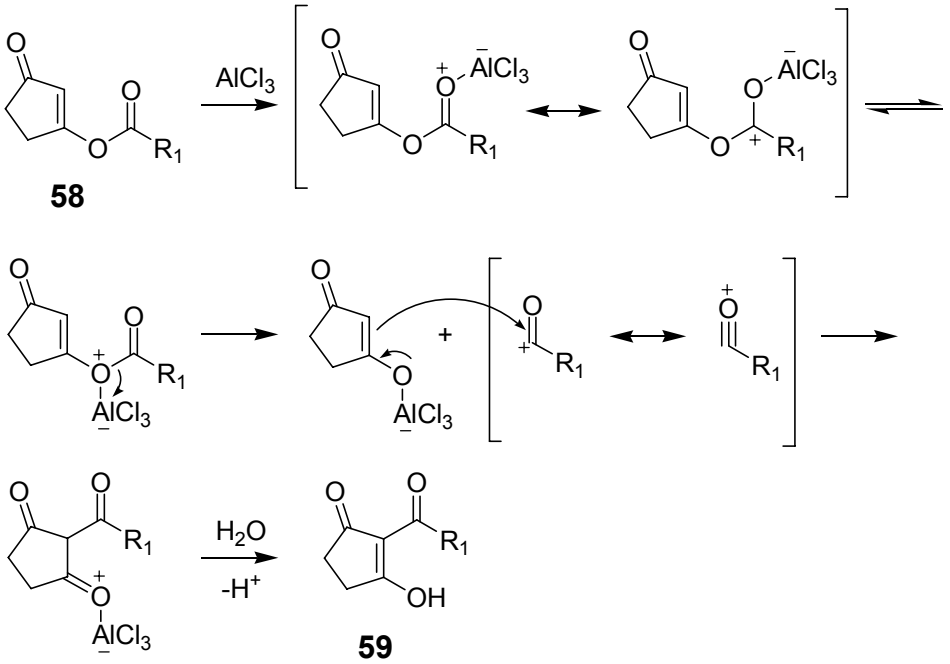
Reagents and conditions: a) 1.1 equiv of $ClOCR_1$, 1 equiv of pyridine, $C_2H_4Cl_2$, 20 h; b) 0.4 equiv of acetone cyanohydrine, 1.4 equiv of Et_3N, CH_3CN, 24 h; c) 3 equiv Et_3SiH, CF_3COOH with 1 % $LiClO_4$, 24 h.

Scheme 9. Synthesis of *C*-alkyl-hydroxycyclopentenones **54a,c**.

	R_1	**Compound** (yield in %)
PPB_1 type I	$(CH_2)_6COOMe$	**58a** (98), **59a** (92), **54a** (89)
$DIPB_1$ type III	$(CH_2)_3COOMe$	**58b** (98), **59b** (85), **54c** (83)

The first step was an *O*-acylation of the enolate of cyclopentane-1,3-dione (**55**) with bifunctional acylhalides, e.g. methyl 7-(chlorocarbonyl)heptanoate, in the presence of pyridine in dry dichloroethane. This yielded **58a,b** nearly quantitatively.[65] Subsequent treatment of the resulting enol esters **58a,b** with acetone cyanohydrine or, less efficiently, with Lewis acids such as $AlCl_3$, $ZnCl_2$ or with 4,4-dimethylaminopyridine,[66] promoted the rearrangement of the side chain from the *O*- to the *C*-position to form **59a,b**. Despite the toxicity of cyano compounds, acetone cyanohydrine was the reagent of choice, since it generated the rearranged products **59a,b** in high yields. $AlCl_3$ caused problems due to required anhydrous conditions. Final reduction of the oxo-moiety in the side chain of **59a,b** gave the mono-alkylated precursors **54a,b** in good yields.

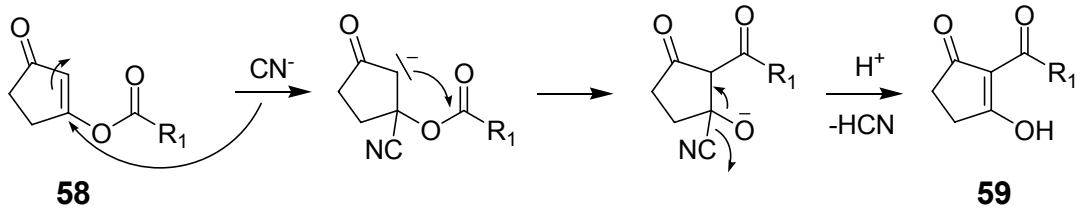
The mechanism of such an *O*-*C*-rearrangement of **58** under Lewis-acid catalysis is not well understood. It might proceed similar to the Fries-rearrangement (**Scheme 10**).



Scheme 10. Proposed mechanism for the rearrangement of *O*-acylated cyclopentenones **58** to *C*-acylated isomers **59** according to Fries-rearrangement.

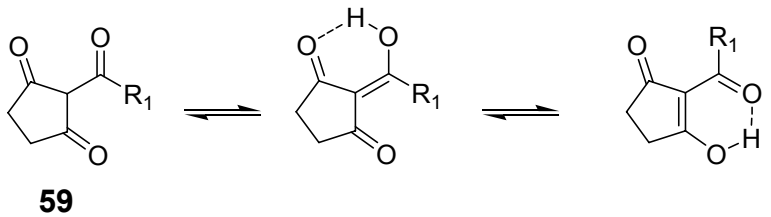
The reaction is catalysed by Lewis acids that complex the *O*-acylated educt **58** and generate an acylium ion. The acylium ion then reacts with the double bond and after hydrolysis the product **59** is released.

Using acetone cyanohydrine, another mechanism is most likely (**Scheme 11**). The cyanide ion might be released into the medium and can attack the *O*-alkylated ring C3-atom of **58**. A carbanion is generated with localisation of the negative charge at the C2-atom. Now, an intra molecular nucleophilic attack on the carbonyl-C-atom of the neighboured ester group can happen. After elimination of the cyanide ion, the desired product **59** is obtained.



Scheme 11. Proposed mechanism for the *O*-*C*-rearrangement of **58** to **59** under cyanide catalysis.

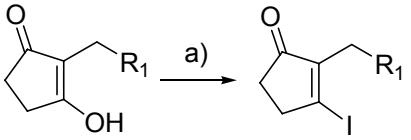
Reduction of the oxofunction in the side chain of **59a,b** forming **54a,c** was best achieved using triethylsilane as hydride donor in trifluoroacetic acid containing 1 % of lithium perchlorate.[67] The selectivity of this reduction was surprising, because normally the ring oxo moiety and the side chain ketone are chemically identical. Perhaps the differentiated behaviour might be explained by the resonance stability (**Scheme 12**), whereby the preferred position of the enolate is in the ring. The acid media seems to play a role in stabilising the hydride donor and the lithium additive coordinates the oxygen. But so far nothing is known from literature, why this side chain ketone of **59** was selectively reduced.



Scheme 12. Resonance stability of the enon-system in **59**.

Reduction with sodium cyano boron hydride in acidic media turned out to be inconvenient because of difficulties in the isolation of products from the reaction mixtures.[67]

The 2-alkyl-3-hydroxycyclopentenones **54a-c** were easily converted to their corresponding alkenyl iodides **53a-c** by heating with an *in situ* generated mixture of PPh_3 and I_2 in dry acetonitrile in the presence of triethylamine (**Scheme 13**).[61] Other standard iodination reactions, for example using imidazole as a base and a solvent mixture of diethyl ether and acetonitrile, resulted in lower yields.[68]



54 **53**

Reagents and conditions: a) 1.125 equiv of PPh_3, 1.125 equiv of I_2, 1.1 equiv of Et_3N, CH_3CN, 24 h.

Scheme 13. Iodination of 2-alkyl-3-hydroxycyclopentenones **53a-c**.

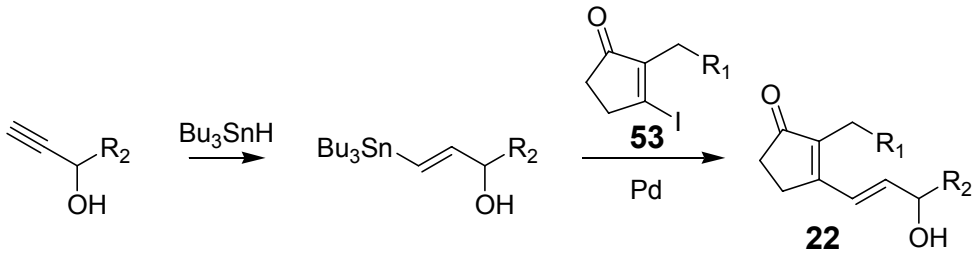
	R_1	**Compound** (yield in %)
PPB_1 type I	$(CH_2)_6COOMe$	**53a** (68)
PPB_1 type II	CH_3	**53b** (77)
$DIPB_1$ type III	$(CH_2)_3COOMe$	**53c** (82)

With the sequence of *O*-acylation, rearrangement and reduction, the C2-side chain could be introduced into the cyclopentanedione molecule in three steps with overall yields between 69 and 80 %. The following iodination reaction worked well for all three types of PPB_1. With the alkenyl iodides **53a-c** as synthons, useful precursors for the introduction of the second C3-side chains by organometallic coupling reactions were generated.

3.1.2.2 Attachment of the C3-substituent to the five-membered ring

The aim was to generate phytoprostanes and a variety of structural analogues based on one simple and uniform synthetic route. Initial retrosynthetic approaches

were to couple the alkenyl iodides **53a-c** in a Stille-type reaction with the corresponding allylic alcohol containing C2-substituents (**Scheme 14**). The first step for this alkylation could be a hydrostannylation of the corresponding acetylenic alcohols **60a-c** using tributylstannane in the presence of azobis(isobutyronitrile (AIBN),[69] followed by palladium catalysed coupling of the cyclopentenone electrophiles **53a-c**.[70] An advantage for this coupling is the alcohol function, which does not need to be protected; however, the toxicity of tin reagents limits their use.



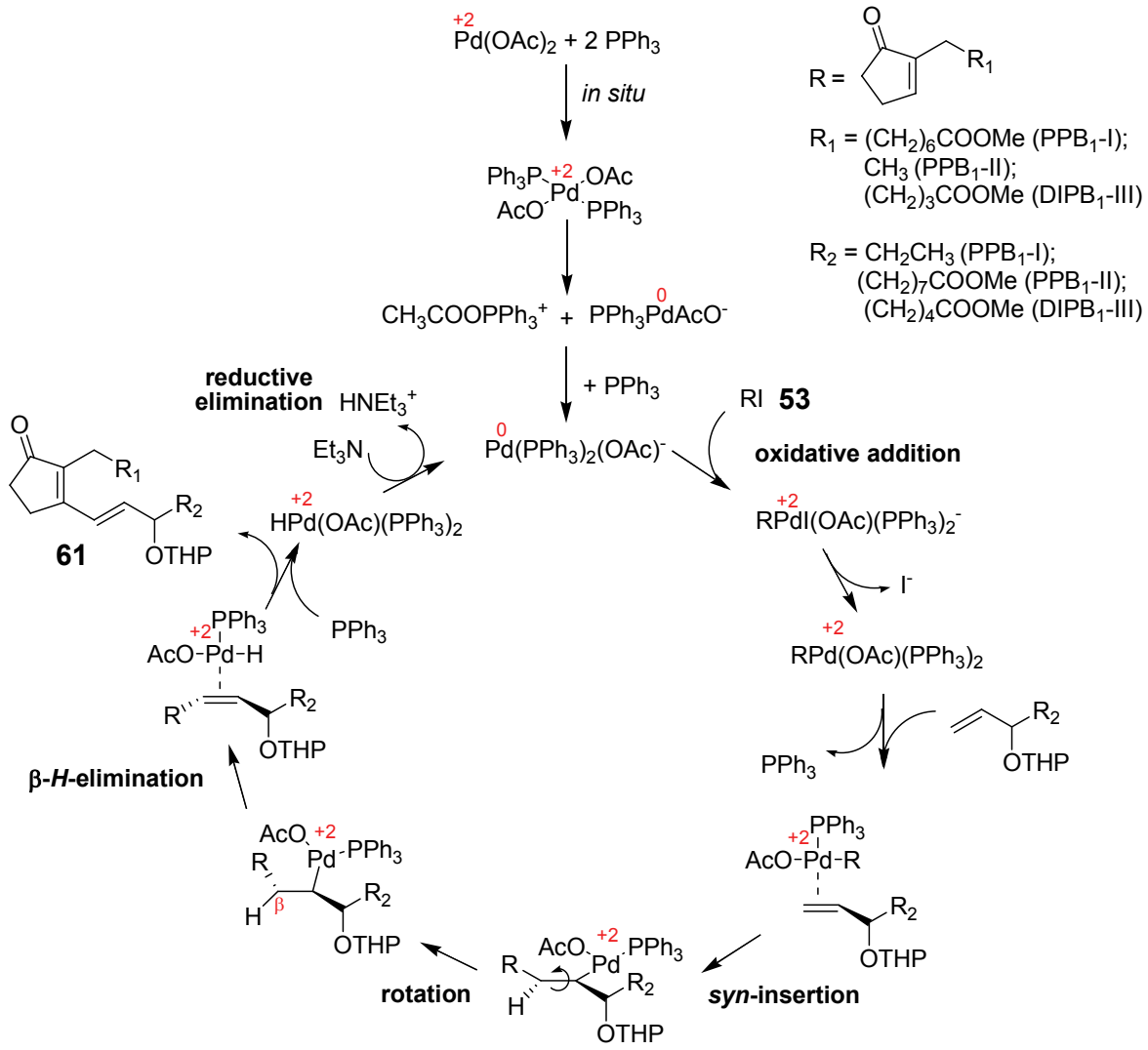
Scheme 14. Stille coupling reaction to B_1-phytoprostanes (**22**).

	PPB_1 type I (22a)	**PPB_1 type II (22b)**	**$DIPB_1$ type III (22c)**
R_1	$(CH_2)_6COOH$	CH_3	$(CH_2)_3COOH$
R_2	CH_2CH_3	$(CH_2)_7COOH$	$(CH_2)_4CH_3$

The alternative use of Grignard reagents for the C-C-coupling is not applicable for these types of molecules, because the carbonyl moiety at the terminus of the side chains can interfere.

Instead, the Heck-type alkylation of the alkenyl iodides **53a-c** with protected terminal 1-alken-3-ols was chosen, using a 2:1 mixture of PPh_3 and $Pd(OAc)_2$ as catalyst and triethylamine for trapping hydrogen iodide. The catalytic cycle of the Heck-reaction according to Brückner[71] is shown in **Scheme 15**. The Pd^0 compound required in this cycle is prepared *in situ* from a Pd^{2+} precursor. In the first step, the oxidative addition, Pd^0 inserts into the alkenyl-iodide bond of **53**, whereby the oxidation level of Pd increases to +2. Afterwards, Pd forms a Π–complex with the protected allylalkenol and the alkenol inserts into the Pd-carbon bond. After rotation around the central bond, a β-hydride elimination step occurs with the formation of a new Pd-alkene Π–complex. This complex is

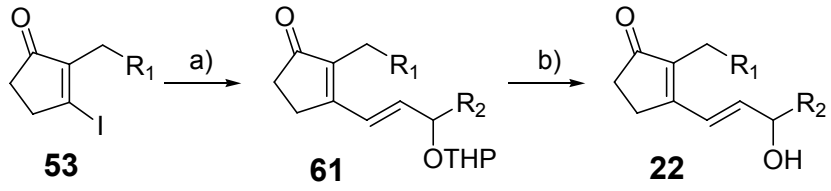
destroyed and the product **61** is released. The Pd^0 species is regenerated by reductive elimination of Pd^{2+} by Et_3N.



Scheme 15. Mechanism of the Heck-coupling reaction to generate PPB_1s.

First attempts for this reaction with *tert*-butyldimethylsilyl-protected alcohols according to the synthesis of prostaglandin B_1[72] were promising (yields of around 30 %), but problems occurred upon removal of the protective group. According to literature, tetrabutylammonium fluoride should easily cleave off the silyl-group, but it failed completely in case of the phytoprostane molecules. Even an aqueous solution of HF did not cleave off the protecting group; the *tert*-butyldimethylsilyl-group turned out to be resistant to deprotection. Therefore, trimethylsilyl-protected alcohols were used instead, because of their enhanced sensitivity to cleaving reagents. Heck-coupling worked quite well according to GC-MS, and deprotection in the presence of HCl also succeeded, but the yields over these two steps were

not satisfying (13-29 %). The reason why silylated alcohols gave such low yields for the phytoprostane compounds is unclear, because in prostaglandin syntheses this protection group has often been used. Best results were obtained in the case of PPB_1 (**22**) syntheses using the tetrahydropyranylethers (THP) of the 1-alken-3-ols.[73] The alkenols were either commercially available or synthesised by allylic oxidation with selenium dioxide.[74] After Heck-coupling according to Naora *et al.*,[72] the THP-group in **61a-c** could be easily removed by a mixture of CH_3COOH / THF / H_2O. The free alcohols **22a-c** could be generated (**Scheme 16**) in good yields.



53 **61** **22**

Reagents and conditions: a) 1.67 equiv of $CH_2CHCH(OTHP)R_2$, 2 equiv Et_3N, $Pd(OAc)_2$*$2PPh_3$, 24 h, 100 °C; b) CH_3COOH / THF / H_2O, 45 °C, 20 h

Scheme 16. Heck reaction of alkenyl iodides **53a-c** with THP-protected allylic alcohols.

	R_1	**R_2**	**Compound** (yield in %)
PPB_1 type I	$(CH_2)_6COOMe$	CH_2CH_3	**22a** (65)
PPB_1 type II	CH_3	$(CH_2)_7COOMe$	**22b** (69)
$DIPB_1$ type III	$(CH_2)_3COOMe$	$(CH_2)_4CH_3$	**22c** (52)

Even though there should be no difference in the biological activities of the *R*- or *S*-enantiomer of the PPs, the synthetic route is also applicable for the synthesis of chiral products. To demonstrate the general applicability, commercially available (*R*)-(-)-oct-1-en-3-ol was protected and subjected to a Heck coupling and yielded the chiral $DIPB_1$ type III ***(R)*-(-)-22c** in overall 57 % along the same protocol.

3.1.2.3 Syntheses of structural analogues of phytoprostanes

Cyclopentanoid natural products, such as prostaglandins or jasmonic acid (**7**), are diverse and represent biologically significant classes of compounds. Cyclopentanones are useful precursors for the synthesis of various cyclopentyl

compounds due to the versatility of their functionality. The introduction of the cyclopentenone moiety into different target molecules may enhance their anticancer potential.[75] It has been shown, that the ability of binding of the α,β-unsaturated carbonyl function of cyclopentenones to specific nucleophiles, such as free sulfhydryl groups of reduced glutathione or cysteine residues in proteins located in damaged cells might lead to an inactivation of these proteins. This fact might be useful for developing anticancer drugs containing a cyclopentenyl group as an essential phamacophore.

Coronalon (**62**) is a synthetic 6-ethyl indanoyl isoleucine conjugate that shows high activity in mimicking octadecanoid phytohormones, such as OPDA (**9**) or JA (**7**). It is derived from the natural product coronatine (**63**), first isolated from *P. syringae* or *X. campestris*. Lauchli *et al*.[76] developed a synthetic strategy for the analogue **62**, being of comparable activity like coronatine (**63**) itself or like JA (**7**) in various biological assays.

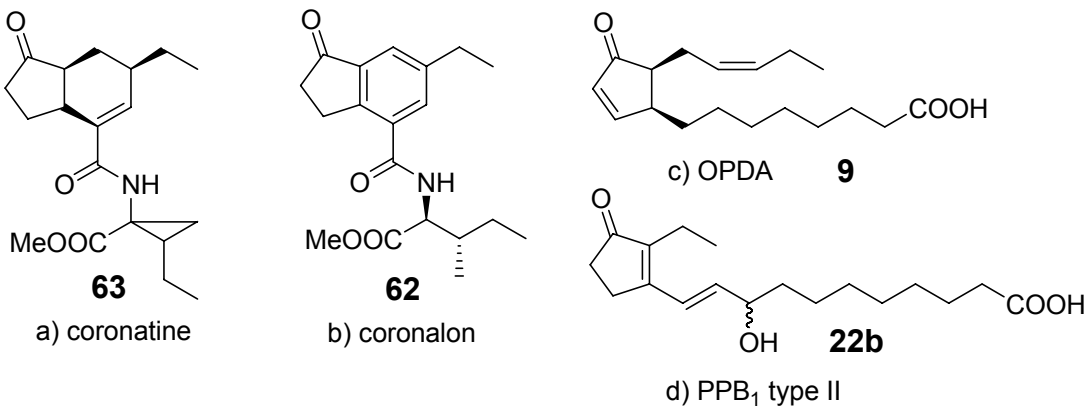
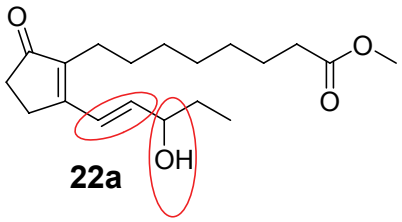


Figure 12. Structures of a) coronatine (**63**), b) coronalon (**62**), c) OPDA (**9**) and d) PPB_1 type II (**22b**) are comparable to each other, perhaps leading to a similar biological activity in higher plants.

62 and **63** appeared to be the most effective elicitors of plant responses, such as volatile emission in lima bean, induction of secondary metabolites, such as certain benzophenanthridine alkaloids or flavanoids.[77] A comparison of the biological activity of coronalon (**62**) with that of PPs suggests similarities, for example the induction of the accumulation of secondary metabolites, e.g. benzophenanthridine alkaloids.

Eventually, the analogues structural elements of coronalon (**62**), OPDA (**9**) and PPs, may account for the comparable biological importance in higher plants (**Figure 12**).

Due to such interesting structural relationships between different classes of natural products, it seems promising to develop structural analogues of the natural occurring PPs. This might help to better understand their mode of action and to study structure-function-relationships. Modifications of the parent structure of the PPB_1s (**Figure 13**) can be performed at the allylic alcohol, first by removing the oxygen atom or secondly by oxidising the OH-group further to its oxo-analogue. Moreover, the double bond can be substituted by a triple bond or by the electron lone pair of an ethereal oxygen atom.



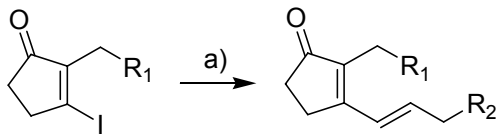
22a

Figure 13. Positions for structural modifications of the PPB_1 type I (**22a**) parent structure.

Synthesis of non-functionalised analogues of PPB_1 types

Having developed a short synthetic access to the PPB_1-series (**22a-c**), a variety of analogues can be generated using this method. Based on the Heck protocol (see chapter 3.1.2.2), alkenylation with non-functionalised alkenes giving **64a-c** proceeded in high yields and without formation of side products (**Scheme 17**).

For the PPB_1 type I analogue **64a**, pent-1-ene had to be coupled with the alkenyl iodide **53a**. Due to its low boiling point the reaction had to be carried out at only 40°C under reflux, and at each of the 4 days of reaction time, fresh pentene was added to the reaction mixture to give the PP analogue **64a** in nearly quantitative yield (97 %).



53 **64**

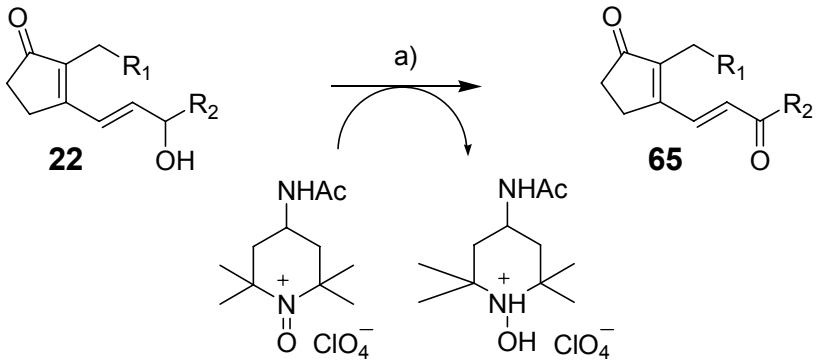
Reagents and conditions: a) 1.67 equiv of $CH_2CHCH_2R_2$, 2 equiv of Et_3N, $Pd(OAc)_2$*$2PPh_3$, 24 h, 4d, 100 °C (40 °C)

Scheme 17. Synthesis of PPB_1 analogues **64a-c** bearing a non-functionalised side chain.

	R_1	R_2	**Compound** (yield in %)
PPB_1 type I	$(CH_2)_6COOMe$	CH_2CH_3	**64a** (88)
PPB_1 type II	CH_3	$(CH_2)_7COOMe$	**64b** (91)
$DIPB_1$ type III	$(CH_2)_3COOMe$	$(CH_2)_4CH_3$	**64c** (86)

Synthesis of oxo-analogues of the PPB_1 types

During oxidative stress the allylic hydroxy group of the C3-side chain of the PPs could be further oxidised to the corresponding diketones **65**. Such diketones **65** might have interesting biological activity owing to the presence of two Michael-acceptor systems that might be able to react with different biomolecules, e.g. proteins. Since the direct coupling with terminally unsaturated alken-3-ones failed due to reductive elimination of the side chain double bond, the PPs **22a-c** were oxidised to the diketones **65a-c**. Oxidation with mild reagents, such as MnO_2,[78] required long reaction times and resulted in low yields (15-23 %). Chromium reagents are typically quite successful, but work-up and disposal problems limit their utility. Bobbitt's reagent (4-acetylamino-2,2,6,6-tetramethylpiperidine-1-oxoammonium per-chlorate) in the presence of silica gel was used instead for oxidation of the hydroxy group (**Scheme 18**).[79,80] During oxidation, the bright yellow Bobbitt's reagent was reduced to a white hydroxylamine salt allowing visual control of the progress of the reaction.



Reagents and conditions: a) 1.05 equiv of Bobbitt's reagent (4-acetylamino-2,2,6,6-tetramethyl-piperidine-1-oxoammonium perchlorate), silica gel, CH_2Cl_2, 5 h.

Scheme 18. Oxidation of PPs **22a-c** to their corresponding diketones **65a-c** using Bobbitt's reagent.

	R_1	R_2	**Compound** (yield in %)
PPB_1 type I	$(CH_2)_6COOMe$	CH_2CH_3	**65a** (81)
PPB_1 type II	CH_3	$(CH_2)_7COOMe$	**65b** (86)
$DIPB_1$ type III	$(CH_2)_3COOMe$	$(CH_2)_4CH_3$	**65c** (78)

Synthesis of acetylenic analogues of the PPB_1 types

The introduction of an acetylenic moiety instead of the (*E*)-allyl alcohol into the PPs changes the electronic and steric properties of the molecules (**Figure 14**) and may result in altered biological activities. The triple bond makes the molecule more rigid and inflexible, which may lead to a binding to different target structures. Also, the affinity of binding to a JA (**7**) receptor might be altered.

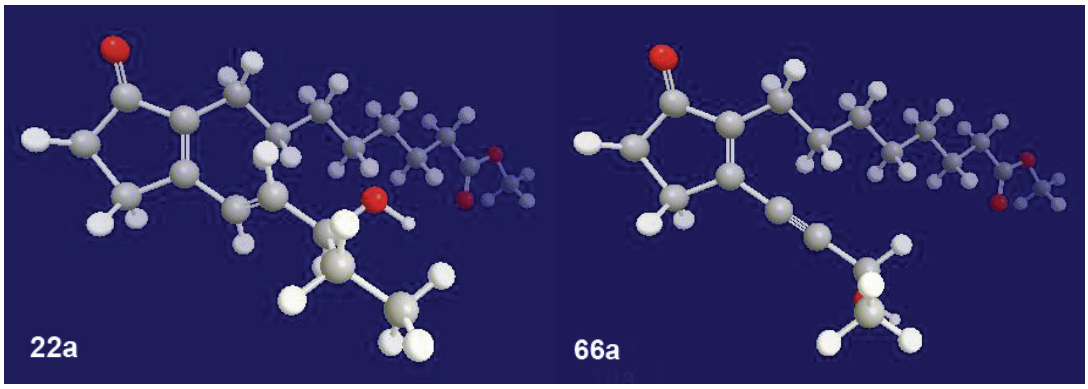
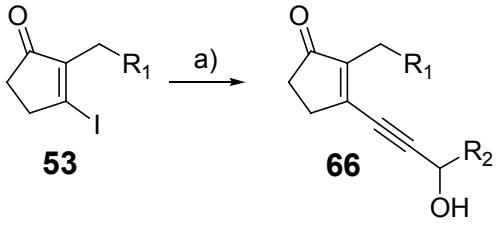


Figure 14. 3D structures of the PPB_1 type I (**22a**) and its acetylenic analogue **66a**.

Precursors to introduce triple bond containing side chains were either commercially available or, in case of PPB_1 type II (**22b**), synthesised from methyl undec-10-ynoate using a procedure similar to the allylic oxidation with SeO_2.[74] The alkenyl iodides **53a-c** proved to be excellent substrates for a Sonogashira-type alkylation with terminal acetylenes (**Scheme 19**).[81] The iodides **53a-c** were coupled with the acetylenic side chains by reaction with $PdCl_2(PPh_3)_2$ and CuI as catalyst in dry DMF. After stirring the mixture for 24 h at room temperature, higher yields (91-96 %) than those for the Heck-type alkylation were obtained. The same reaction could also be carried out with commercially available (*R*)-(+)-oct-1-yn-3-ol, yielding the chiral PP analogue **(*R*)-(+)-66c** in 70 %.

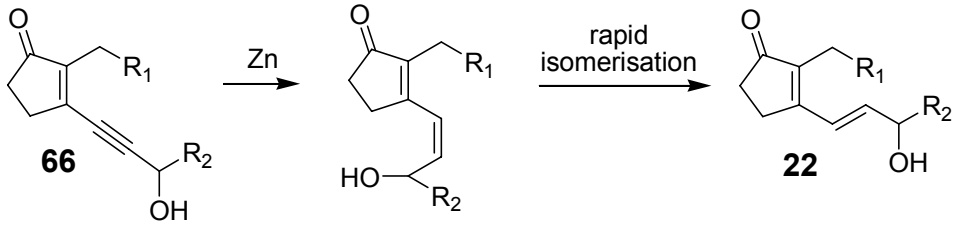


Reagents and conditions: a) 1.5 equiv of $CHCC(OH)R_2$, 0.07 equiv of $PdCl_2(PPh_3)_2$, 0.15 equiv of CuI, 1.2 equiv of Et_3N, DMF, 24 h.

Scheme 19. Synthesis of acetylenic PPB_1 analogues **66a-c**.

	R_1	**R_2**	**Compound** (yield in %)
PPB_1 type I	$(CH_2)_6COOMe$	CH_2CH_3	**66a** (88)
PPB_1 type II	CH_3	$(CH_2)_7COOMe$	**66b** (91)
$DIPB_1$ type III	$(CH_2)_3COOMe$	$(CH_2)_4CH_3$	**66c** (86)

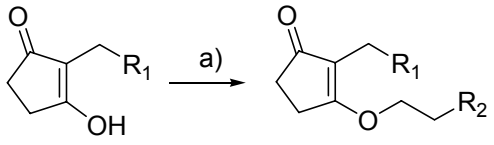
Attempts to reduce the triple bond with activated zinc[82] were not successful (**Scheme 20**) since the resulting (*Z*)-alkenes were unstable and readily underwent isomerisation to (*E*)-alkenes **22** under the action of light or elevated temperatures.



Scheme 20. The reduced (*Z*)-alkenes readily underwent isomerisation to the more stable (*E*)-alkenes **22**.

Synthesis of ether analogues of the PPB_1 types

To examine structure-function-relationships, compounds **67a-c** were synthesised. The analogues contain an ether oxygen instead of an allylic hydroxy group in the C3-side chain. The electron lone pairs of the oxygen could mimic the function of the double bond of the natural PPs. Such *O*-alkylated substances **67a-c** could easily be generated by reaction between the 2-alkyl-3-hydroxy-cyclopent-2-enones **54a-c** and the corresponding haloesters. Sodium hydride served as the base for deprotonation of the alcohol function of **54a-c** in DMSO (**Scheme 21**).



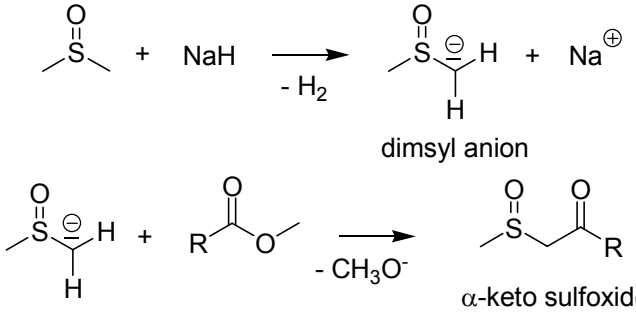
54 **67**

Reagents and conditions: a) 1 equiv of $X(CH_2)_2R_2$, 1.5 equiv of NaH, DMSO, 24 h.

Scheme 21. *O*-alkylation of the alkylhydroxycyclopentenones **54a-c**.

	R_1	R_2	**Compound** (yield in %)
PPB_1 type I	$(CH_2)_6COOMe$	CH_2CH_3	**67a** (42)
PPB_1 type II	CH_3	$(CH_2)_7COOMe$	**67b** (74)
$DIPB_1$ type III	$(CH_2)_3COOMe$	$(CH_2)_4CH_3$	**67c** (48)

Yields for this *O*-alkylation were generally not very high, perhaps due to the presence of the dimsyl-anion that might react with the ester moiety to form α-keto sulfoxides (**Scheme 22**).



Scheme 22. Side reaction of the *O*-alkylation leading to α-keto-sulfoxides.

The developed synthetic strategy of first *C*-alkylating cyclopentanedione (**55**), followed by iodination and organo metallic coupling reactions served as an elegant and short method for generating PPs of the B_1-series (**22a-c**) and for a variety of different structural analogues (**64, 65, 66, 67**) (**Figure 15**). The route includes fewer steps and yields were generally higher than those of previously published PP syntheses.[51-54,59]

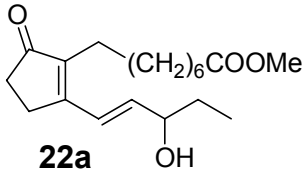
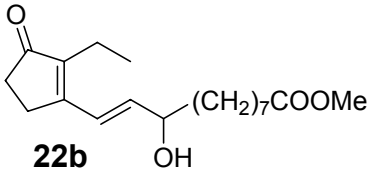
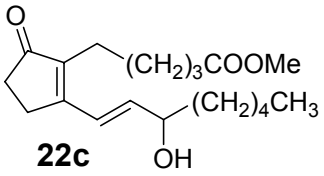
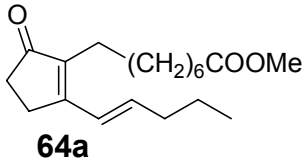
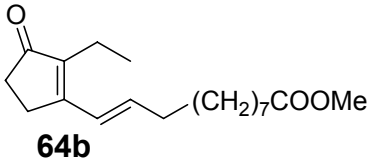
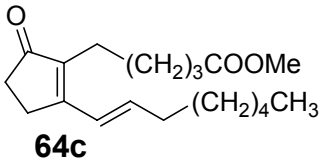
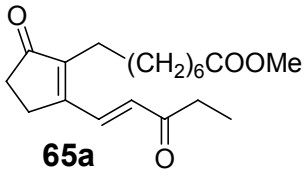
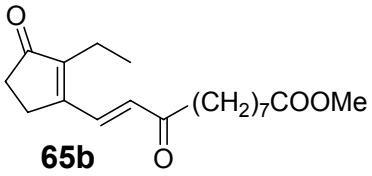
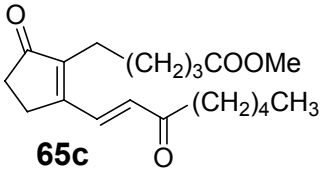
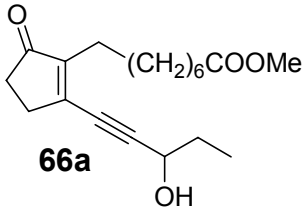
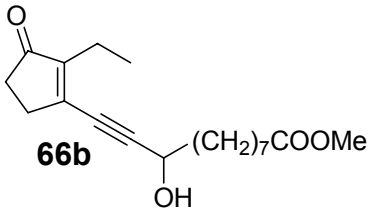
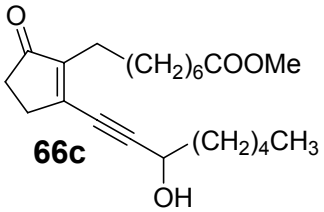
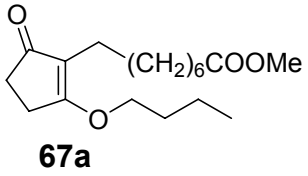
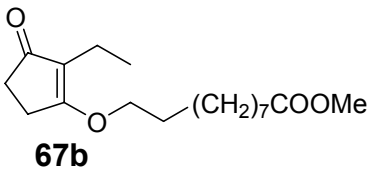
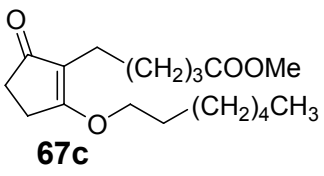
PPB_1 type I	**PPB_1 type II**	**$DIPB_1$ type III**
steps (overall yield)	steps (overall yield)	steps (overall yield)
 **22a**	 **22b**	 **22c**
6 (35 %)	3 (53 %)	6 (29 %)
 **64a**	 **64b**	 **64c**
5 (53 %)	2 (45 %)	5 (40 %)
 **65a**	 **65b**	 **65c**
7 (28 %)	4 (46 %)	7 (23 %)
 **66a**	 **66b**	 **66c**
5 (48 %)	2 (70 %)	5 (49 %)
 **67a**	 **67b**	 **67c**
4 (34 %)	1 (74 %)	4 (33 %)

Figure 15. Overview of synthesised PPs and analogues. Required synthetic steps and overall yields are shown.

With all the substrates synthesised so far, several biological activity tests could be performed. First results of altered gene expression levels after phytoprostane treatment using the *Arabidopsis thaliana* whole genome microarray are presented and discussed in the following chapters. The effect of PPB_1s and analogues on the growth of several plant pathogens, the influence of certain PPB_1s on lima bean volatile emission and the effect on intracellular calcium levels after PPB_1 treatment in tobacco suspension cells are shown.

3.2 Bioactivity tests

3.2.1 DNA microarrays

3.2.1.1 Introduction to the microarray technology[83]

A microarray, or DNA chip, is a collection of microscopic DNA spots attached to a solid surface, such as glass, plastic or silicon. It enables researchers to monitor the entire genome, or parts of it, on a single chip. With it, researchers can have a better picture of the interactions among thousands of genes simultaneously (**Figure 16**).

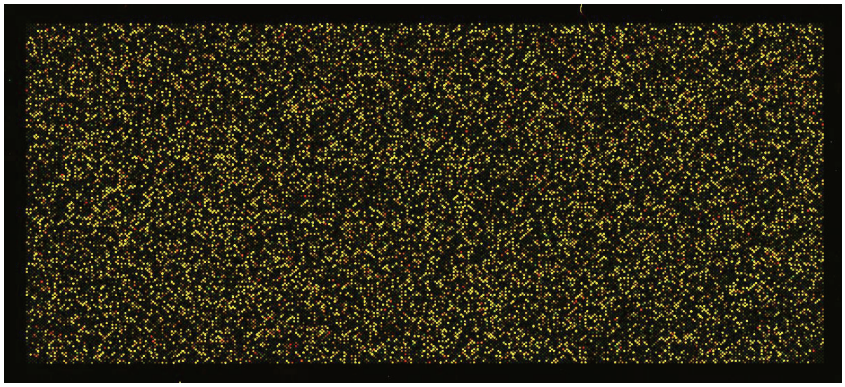


Figure 16. Representative scanner-picture (of a phytoprostane hybridisation) of the Agilent ATH3 *Arabidopsis* array (44.000 spots).

The microarray technology evolved from southern blotting, whereby fragmented DNA is attached to a substrate and then probed with a known gene or fragment. Measuring gene expression by using microarrays is relevant in many research fields of biology and medicine. Differences in transcription patterns of different tissues and differences on the genomic level can be monitored. For example, microarrays can be used to identify disease genes by comparing gene expression in diseased and normal cells.

There exists a difference between protein- and nucleic acid microarrays. Proteins (mostly antibodies) must be cleaned first or directly synthesised before they can be coupled to a biochip. Aminosilane-biochips belong to the class of planar biochips, useful for nucleic acids or proteins. The aminosilane surface has a positive charge and allows an unspecific, non-covalent coupling with a negatively

charged amino acid end of a peptide or protein. This electrostatic interaction is called unspecific adsorption and the spatial distribution of the bound macromolecules is unpredictable. A covalent alternative is achieved by using planar aldehyde surfaces. The reactive aldehyde group is coupled onto the chip surface and primary amines of peptides or proteins can bind to form covalent C=N double bonds (Schiff's bases) with elimination of water. The spatial distribution is again random. Besides such planar biochips, two and three dimensional structures exist, where the glass is coated with thin layers of polymer membranes. Protein biochips have several disadvantages compared to nucleic acid chips. They require complex cleaning and amplification steps and are not very stable in isolated forms.

On so-called nucleic acid microarrays, normally DNA or sometimes RNA is bound to the surface. The DNA is the target for hybridisation partners. Nucleic acid mircoarrays are subdivided into two large groups; the oligonucleotide and the "classical" DNA- or PCR-microarrays. On oligonucleotide arrays, short, single stranded DNA fragments are bound, whereas on the classical ones, longer nucleic acid chains are fixed.

Today, different methods exist to produce DNA-microarrays. Arrays are being distinguished between the so-called on-chip or *in situ* synthesis of oligonucleotides directly on the glass surface and the deposition arrays, where cDNA or oligonucleotides are presynthesised and then deposited onto the surface of the array. There exist commercially available designs from companies, such as GE Healthcare, Affymetrix, Ocimum Biosolution or Agilent, covering complete genomes.

Such DNA microarrays can be used to detect RNAs that may or may not be translated into active proteins. Scientists refer to this kind of analysis as expression analysis or expression profiling. Since there can be tens of thousands of distinct probes on an array, each microarray experiment can accomplish the equivalent number of genetic tests in parallel.

The principle of microarray detection is mostly based on the measurement of fluorescence intensities, but also radioactive markers are applied. Fluorophores, such as cyanine dyes, are bound to the free nucleotides, and after interaction of both partners a specific fluorescence signal can be detected. The detection with a microarray scanner allows the visualisation of up-regulated and down-regulated

genes. The method has the drawback that the absolute levels of gene expression cannot be observed, but only one chip is needed per experiment.

In this work, an Agilent microarray design was applied, which uses an *in situ* synthesised 60-mer oligonucleotide technology representing the full *Arabidopsis thaliana* genome (all predicted and verified coding regions and small mRNAs). The extracted total RNA was amplified and fluorescently labelled. Therefore, one sample from phytoprostane treated plants was labelled with cyanine 5 (excited by a 633 nm laser) and a second sample, the water negative control, labelled with cyanine 3 (excited by a 532 nm laser). A primer containing a polymerase promoter was annealed to the RNA and a reverse transcriptase synthesised the first and second strands of cDNA. Then, cRNA was synthesised from the double-stranded cDNA using a RNA polymerase. When labelling was complete, samples were hybridised to the microarray, which means the cRNA was attached to the complementary strands of the fixed oligonucleotides on the array by hydrogen bonds. Genes whose expression differs between the two samples were detected and identified by laser scanning. Afterwards, the data were analysed using the GeneSifter software (**Figure 17**).

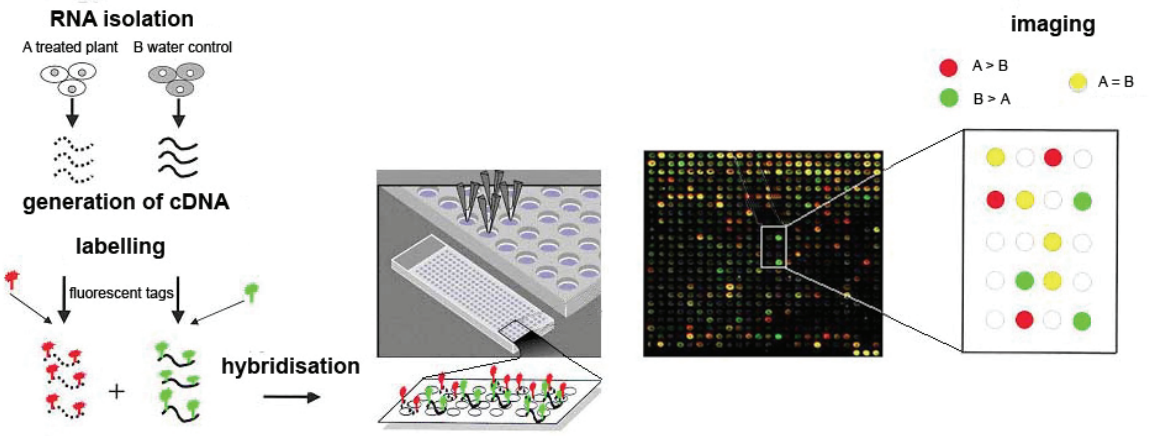


Figure 17. Principle of the amplification, labelling and hybridisation of extracted RNA.

3.2.1.2 First results of the Agilent *Arabidopsis* whole genome array

The application of microarrays for studying biological activities of certain chemicals on the genomic level becomes more interesting for researchers. Within

this work, first details of the effect of selected phytoprostanes (PPs) on gene expression in *Arabidopsis thaliana* plants are given and partly analysed.

Leaves of this model plant were either treated with 1 mM, 100 μM or 10 μM aqueous solutions of synthesised PPB_1s or their analogues. After 9 h, leaves were harvested, immediately frozen in liquid nitrogen and the total RNA was extracted. Due to the time-consuming and highly expensive microarray technology, only three compounds out of the variety of synthesised PPs and analogues, with promising biological activity, were selected. The extracted total RNA was amplified, labelled, hybridised on the microarray and scanned. Samples of treated leaves after application of 100 μM PPB_1 type I (**22a**) and II (**22b**) and the type I ketone (**65a**) in comparison to a water control were used on the array in two replicates. A 1 mM concentration of the ketone (**65a**) was used to study the effect of higher concentrations on gene expression. The whole *Arabidopsis* genome contains about 29.000 annotated genes. This fact makes a complete data analysis of the array rather complex and therefore just the 100 most up- or down-regulated genes were selected for analysis. Lists of those genes are attached in the supplementary material.

Looking at the performed microarray data analysis in total, a general stress induced answer can be seen. The *Arabidopsis* plants reacted strongly under the influence of the PPs. The 1 mM treatment resulted in an up-regulation of about 1800 genes, whereas about 2000 genes were down-regulated. In response to the applied 100 μM concentrations, still about 800 genes were up- and about 1500 were down-regulated. Genes with relative expression levels higher than 1.8 fold for up-regulation and more than 0.6 fold for down-regulation compared to the untreated controls were considered. It turned out to be difficult to distinguish between the PP-induced answers, because several answers overlapped and at a certain point also toxic effects of too high concentrations might have occurred, a fact that is also common for other phytohormones. Reactive oxygen species (ROS) are known as highly toxic molecules depending on their nature and concentration, but are also involved as important signalling molecules that control various processes in higher plants, including pathogen defence, programmed cell death and stomatal behaviour.[84] Similar to this phenomenon, PPs might also be toxic for plants in higher concentrations. Additionally, it is not possible to judge

how much of the externally applied PPs were actually transported into the tissue. Although elicitor-specific concentrations of 1 mM or 100 μM were applied in this bio-assay, it might be possible that only 10 percent were actually taken up by the plant. To statistically support expression differences of individual genes, at least four replicates have to be employed. With only two replicates used in this assay, there might be a large variation in response to the PPs.

Application of 1 mM PPB_1 type I ketone 65a

It remains unclear, whether the strong answer after application of 1 mM **65a** is due to the activity of the PP itself or is just a general stress-induced answer of the plant because of the toxicity of this high concentration. A number of up-regulated genes (see **Figure 18**) indicate a more general stress answer. Heat shock proteins and glutathione *S*-transferases were highly up-regulated after application of a 1 mM concentration of **65a**. These genes are often connected with and also up-regulated in response to increased environmental stress.

If plants are exposed to elevated temperatures, the expression of so-called heat shock proteins (HSP) is increased.[85] Production of high levels of heat shock proteins can also be triggered by exposure to different kinds of environmental stress conditions, such as infection, inflammation, starvation, water deprivation or exposure of the cell to certain toxins. Consequently, heat shock proteins are implicated with or even referred to as stress proteins and their upregulation is sometimes described more generally as part of the plant's response to environmental stress. Many of these heat shock proteins function as molecular chaperones. They play an important role in protein-protein interactions such as folding and assisting in the establishment of proper protein conformation. By helping to stabilise partially unfolded proteins, HSPs aid in transporting proteins across membranes within the cell.

Glutathione *S*-transferases (GSTs) are enzymes that catalyse the nucleophilic attack of the tripeptide glutathione on lipophilic cytotoxins with electrophilic centres. Thus, their fundamental function in plants is the detoxification of endogenous and xenobiotic compounds.[86] GSTs have been intensively studied for their role in herbicide metabolism and selectivity.[87] Besides the formation of glutathione-conjugates, GSTs may also act as peroxidases by catalysing the reduction of hydroperoxy fatty acids into less toxic hydroxy fatty acids, thus

protecting cells from oxygen toxicity. GSTs may be implicated in many plant physiological processes. They are differentially regulated in response to certain biotic or abiotic stresses and can be induced upon pathogen attack or fungal elicitors,[88] in response to treatment with hydroperoxides,[89] mechanical wounding and insect feeding,[90] heavy metals,[86] heat schock and plant hormones, such as ethylene,[91] auxin,[92] methyl jasmonate and salicylic acid,[93] and abscisic acid.[94] Consequently, GSTs play an important role in plant growth and development and are linked to many phytohormone activities.

Applying **65a** at 1 mM, the expression levels of certain pathogenesis- or defence-related genes and several enzymes belonging to the class of cytochrome P-450 (**Figure 18**) were also affected. But again, there is no evidence that PPs are involved in specific plant defence reactions, although some genes linked to defence were up-regulated.

Pathogenesis related (PR) proteins have been defined as proteins induced by various types of pathogens, such as viruses, bacteria, fungi or by the application of certain chemicals mimicking the effect of pathogen infection.[95] The induction of PR gene expression during pathogen infection is mediated by various signalling molecules. Salicylic acid and reactive oxygen species (ROS) mediate the expression of acidic PR genes,[96] whereas ethylene and methyl jasmonate mediate basic PR genes.[97] PR proteins are known to have antifungal activity and some of them are glucanases or chitinases degrading fungal cell wall components. Other PR proteins are referred to as thaumatin- or osmotin- like proteins, leading to lysis of spores, inhibition of hyphal growth or reduction of spore germination.[98,99] Resistance to a pathogen is often correlated with a hypersensitive response (HR), a localised induced cell death at the site of infection. Pathogenesis related proteins are involved in systemically acquired resistance and stress response in plants, although their precise role is mostly unknown.

Regulation	Category	Description	Gene identifier
up	environmental stress	heat shock proteins (HSP)	At1g53540, At5g51440, At5g59720, At5g52640, At5g12020, At1g16030, At1g74310, At2g26150, At3g09440
up	oxidative stress / detoxification	glutathione *S*-transferases (GST)	At2g29490, At1g02920, At1g02930, At2g29460, At5g62480, At3g09270
up	defence / resistance	pathogenesis-related proteins (PR) / defence-related proteins	At5g48657, At1g65390, At3g24954, At5g48657, At5g20480, At2g40330, At5g44420, At4g36010
up	electron transport / oxygen binding	cytochrome P-450 proteins (Cyt P-450)	At3g26200, At2g27690, At3g28740, At4g37370, At3g26290
down	hormone-inducible genes	auxin-responsive proteins	At5g18050, At3g03820, At2g21210, At5g18060, At5g18010, At5g18020, At5g18030, At5g18080, At4g14550, At1g29500, At1g29510, At1g29450, At1g28130, At4g34760, At1g29460, At4g32280, At1g29440, At1g52830

Figure 18. Several up- or down-regulated *Arabidopsis* genes in response to **65a** treatment in 1 mM concentration and their proposed metabolic function. The 100 most up- or down-regulated genes are listed in the supplementary material.

Cytochrome P-450 (Cyt P-450) enzymes are a superfamily of haem-containing mono-oxygenases that are found in all kingdoms of life, and which show extraordinary diversity in their reaction chemistry.[100] They are regarded as soluble or membrane-bound oxygenases that are able to perform hydroxylation reactions. These enzymes play an important role in many essential biotransformations in mammals, such as the biosynthesis of steroids, the arachidonic acid metabolism or the transformation of xenobiotics. In plants, these proteins are important for the biosynthesis of several compounds such as hormones, defensive compounds and fatty acids. Cyt P-450 enzymes are involved in the plant's secondary metabolism and have been implicated in several biosynthetic pathways leading to the

synthesis of lignin phenolics, membrane sterols, phytoalexins and terpenoids.[101] It has been shown that plants have evolved highly specific Cyt P-450-linked secondary pathways to produce defence-related phytoalexins. Many Cyt P-450 enzymes are expressed in response to pathogenesis and are involved in detoxification of herbicides.

Many auxin-responsive proteins (Aux/IAA) were down-regulated after treatment with 1 mM PP-analogue **65a**. Aux/IAA genes were identified as members of a family of genes that were rapidly induced in response to auxin.[102] They can regulate many of the developmental processes governed by auxin. Recent findings indicate that repression of auxin signalling contributes to antibacterial disease resistance.[11] Thus, down-regulation of auxin-responsive proteins by PPs might contribute to the plant's defence against pathogens.

The performed microarray showed that certain disease-resistant proteins were up-regulated, whereas many auxin-responsive proteins were down-regulated, suggesting more or less regulatory functions for PPs during disease resistance of higher plants. It is likely to propose a role of PP compounds in the stress-induced answering cascade of higher plants; their exact function, however, remains uncertain. It is also unknown whether these compounds are mainly positively or negatively involved in plant stress responses. Interestingly, there was a substantial amount of overlap of PPB_1-induced gene expression compared with the microarray performed by Müller *et al*.[36] Several GSTs, HSPs and Cyt P-450s were identified to be up-regulated in response to PPB_1 treatment, similar to the present results. However, a direct comparison is not possible, since Müller used *Arabidopsis* callus cultures and a custom-designed mini microarray, encoding for only 626 defence- and pathogenesis-related proteins. It is expected, that the applied PP concentrations to the callus cultures might have also been too high and thus were toxic for the plant. Performing such microarray analyses should always include preliminary tests to determine the correct concentration barriers for application and it is necessary to known how much of the applied substances will really reach the plant's inside.

Application of 100 μM PPB_1 type I (22a), type I ketone (65a) and type II (22b)

The response to 100 μM PP concentrations was not a clear general stress answer as for the 1 mM concentration. Several prominent genes were up- or down-regulated and even opposed regulation as compared to the 1 mM treatment occurred. No large differences were obvious between the PPB_1 type I (**22a**), the corresponding ketone **65a** and the type II (**22b**). Looking solely at the quantity, type II (**22b**) was perhaps a bit more active than type I (**22a**) and its ketone **65a**. Qualitatively no substantial differences were visible. Therefore the expression rates of all three treatments were combined and analysed together.

The dominant gene families, up-regulated in response to 1 mM PP concentration, such as heat shock proteins, glutathione *S*-transferases, pathogenesis-related and cytochrome P-450 proteins, only played a minor role in response to lower elicitor concentrations. This suggests that the gene expression levels after PP treatment are extremely dependent on the applied concentration. It could not be tested whether the 100 μM PP-concentrations might have been too low for a specific answer, or if PPs represented only by-products of lipid metabolism without important defence-related function.

Some prominent genes regulated by lower PP concentrations should be mentioned here. The ribonuclease 1 (RNS1) was strongly up-regulated in response to 100 μM PP concentrations, whereas in response to 1 mM application there was only a minor induction visible. RNases have been postulated to accumulate in response to wounding or pathogen attack in tomato and tobacco,[103,104] thus contributing to the control of fungal invasion. In *Arabidopsis*, the RNS1 transcript accumulates in both wounded and systemic but unwounded leaves.[105] The regulation of these activities by wounding was independent of JA (**7**) and oligosaccharides, indicating a novel, not yet identified wound signalling pathway. PPs might probably be involved in such a JA-independent pathway. PPF_1 (**24**) compounds have been shown to occur after wounding in peppermint leaves,[28] and all PP classes have been connected with oxidative stress. It has to be further studied, if those PPs are minor or even major components involved in oxidative stress reactions caused by wounding.

Other highly up-regulated genes after PP application to *Arabidopsis* leaves were β-amylases. These are the key enzymes for starch degradation[106] and are regulated as a consequence of stress, caused by phytohormone activity[107] or

abiotic factors.[108] In contrast to PPs, gibberellic acid (**3**) did not enhance β-amylase activity, and abscisic acid (**5**) even inhibits its activity. Interestingly, after 1 mM PP application, the amylase activity was also down-regulated.

Another prominently up-regulated gene in this array was the *Arabidopsis* Touch 4 gene. Its gene expression is regulated by auxin (**1**) and brassinosteroids, by environmental stimuli, and during development.[109] It encodes a xyloglucan endotransglucosylase/hydrolase that affects cell wall-modifications in response to environmental stress and growth. After 1 mM PP application, this enzyme was down-regulated.

Lipid transfer protein 4 (LTP4) was up-regulated by lower, and down-regulated by higher applied PP concentrations. LTPs are small, basic and abundant proteins in higher plants.[110] They are capable of binding fatty acids and of transferring phospholipids between membranes *in vitro*. Their biological function is presently unknown, but they have been suggested to participate in the defence of plants against pathogens.

Arabinogalactan-proteins (AGP) were also up-regulated in response to 100 μM PP concentration, but down-regulated after 1 mM concentration. AGPs are widely distributed in plants and are involved in growth and developmental processes, in cell signalling and programmed cell death.[111] It was found, that these enzymes allow Agrobacterium to rapidly reduce the systemic acquired resistance response during infection.[112]

In response to the applied low PP concentrations, vegetative storage protein (VSP) 1 and 2 were also strongly up-regulated, whereas in response to 1 mM **65a** both were down-regulated, indicating that there is high concentration dependence for expression of these types of genes. VSPs are synthesised and accumulated in cell vacuoles, which are well supplied with carbon and nitrogen to be used for growth and development of organs.[113] Their mRNAs are inducible in developed leaves by various environmental stresses, such as water deficiency.[114] Additionally, VSP genes in *Arabidopsis thaliana* can be regulated by wounding, treatment with MeJA (**8**) or coronatine (**63**).[115]

Based on the collected data, it becomes obvious that PP treatment leads to down-regulation of certain proteins involved in flowering. MAF5, MAF4 and FLC genes were significantly down-regulated. The *Arabidopsis* FLOWERING LOCUS

C (FLC) gene is a key floral repressor in the maintenance of a vernalisation (induction of flowering in response to a long cold period) response.[116] But MAF5 is upregulated by vernalisation and could therefore play an opposite role to FLC. Overexpression of MAF3 or MAF4 produces alterations in flowering time that suggest that these genes also act as floral repressors and might contribute to the maintenance of a vernalisation requirement.

Several proteins involved in chlorophyll binding were also down-regulated. These proteins bind non-covalently to chlorophyll and harvest light energy for photosynthesis.[117] Similar to the PP activity in *Arabidopsis*, abscisic acid (**5**) repressed chlorophyll binding protein expression in soybean.[118]

Whether PPs are involved in plant defence reactions, are metabolites of JA-independent pathways or play a role in detoxification responses, cannot be concluded from the microarray data. It could just be demonstrated, that PPs might be involved in several wounding processes and might somehow be related to phytohormones and phytohormone activities. Using the GeneSifter software, functional classifications of the expressed genes can be made by means of statistical analysis. **Figure 19** shows a diagram of regulated genes involved in response to a stimulus (47 %), physiological processes (20 %), cellular processes (19 %) and other biological and developmental processes.

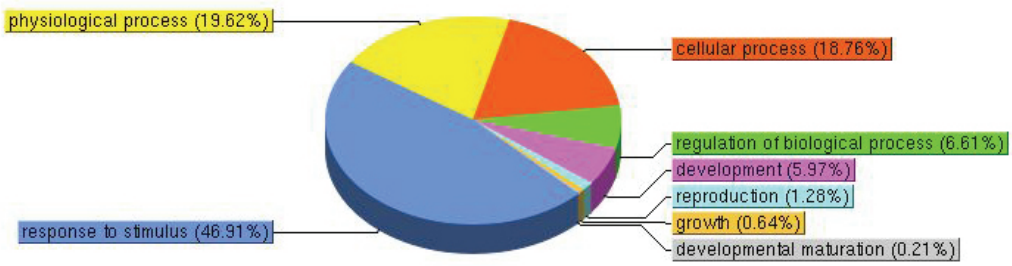


Figure 19. Functional classification of phytoprostane-regulated genes.

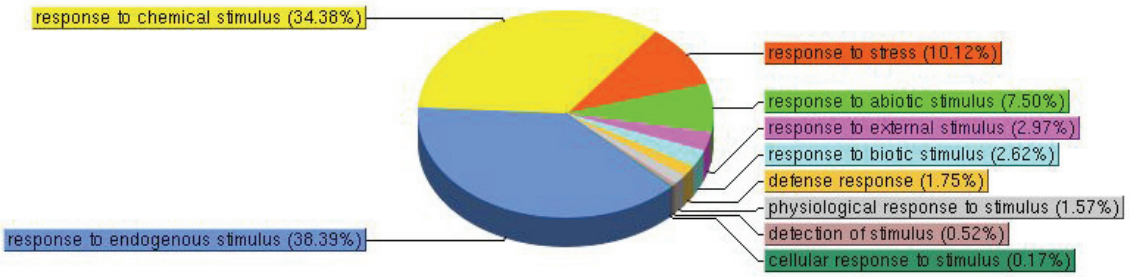


Figure 20. Classification of regulated genes in response to a stimulus.

The response to a stimulus can be further separated into endogenous stimulus (38 %), chemical stimulus (34 %) and other abiotic or biotic stimuli and stress responses (**Figure 20**).

Such endogenous or chemical responses can be further split into defined stimuli, belonging to responses of hormones (71 %), salicylic or jasmonic acid (**7**) (10 % and 9 %) and other chemical substances (**Figure 21**). These results suggest that the genes induced as a plant response to PPs and JA (**7**) are not closely related. The correlation of PPs to the response of other phytohormones is much higher, as it can be seen in **Figure 22**. Especially auxin-responsive genes seem to be affected by PPs.

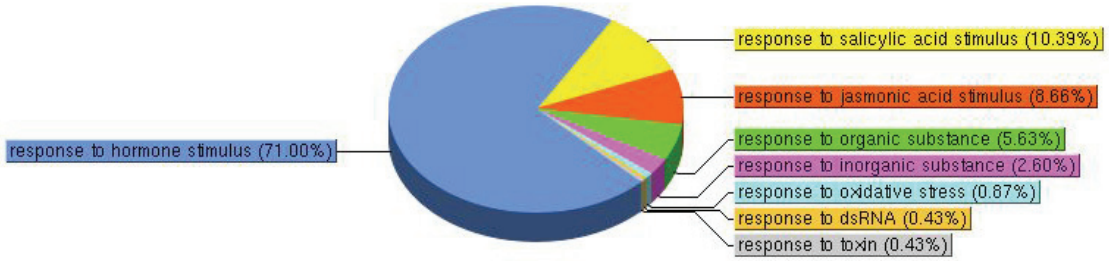


Figure 21. Classification of regulated genes in response to a chemical stimulus.

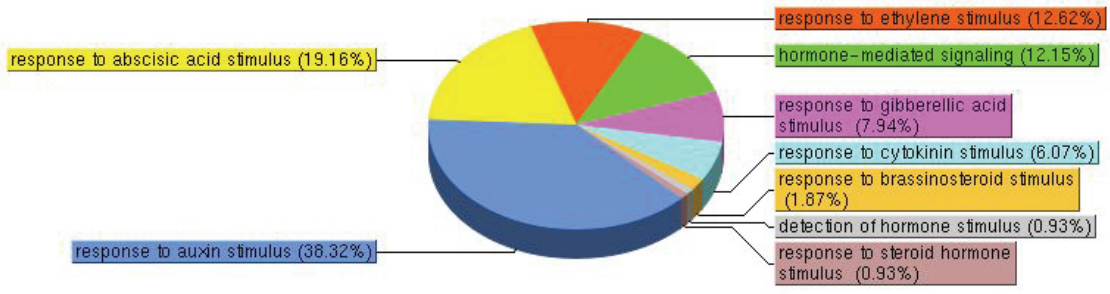


Figure 22. Classification of regulated genes in response to phytohormone stimulus.

Generally, PPs might be able to mimic the expression of certain genes being connected with phytohormone activities or to those being regulated by wounding of the plant or pathogenesis. However, due to the limited number of biological replicates in this bioassay, final conclusions on the absolute effect of PPs on plant cells could not be drawn.

3.2.2 Phytoprostanes and antimicrobial tests

Antibiotic activity is known from several oxylipins, such as 13-ketotrienoic acid or 12-oxo-phytodienoic acid (**9**).[119] Many of these oxylipins were active against several pathogens, including bacteria, fungi or oomycetes by inhibiting *in vitro* growth. Since phytoprostanes occurred in high levels after infection with the fungus *Botrytis cinerea*[34], it needs to be tested, if they are able to act directly against such microorganisms. Recent results showed,[120] that levels of PPF_1s (**24**) and several other non-enzymatically formed oxylipins increased after infection with *Pseudomonas syringae*, indicating that there exists a non-enzymatic lipid oxidation component in the plant response to pathogens.

In this chapter, first results of an agar diffusion test[121] are presented, by applying the synthesised test substances to different bacterial plant pathogens. The influence on fungi or oomycete growth was not tested.

To test growth inhibition of synthetic PPs, the following plant pathogens were selected: *Pseudomonas syringae, Ervinia amylovora, Erwinia carotovora* and *Xanthomonas campestris.* The pathogen-induced plant diseases are shown in **Figure 23.**

bacterial strain	disease
Pseudomonas syringae	soybean blight, producer of coronatine
Erwinia amylovora	fire blight of Rosaceae
Erwinia carotovora	soft rot of many vegetables
Xanthomonas campestris	black rot of crucifers

Figure 23. Diseases of higher plants caused by certain pathogens.

For the agar diffusion test, PPB_1s and analogues were applied at concentrations of 20 mM. These concentrations are very high but give first hints for the inhibitory activity. Synthetic substrates were used as ethanolic solutions. They were put into punched 8 mm wells of agar minimal medium plates, where the bacteria were growing on. After 48 h incubation time, the diameters around the wells were measured (**Figure 24**). Based on these results, further dilutions of the PPs were applied to the bacterial strains.

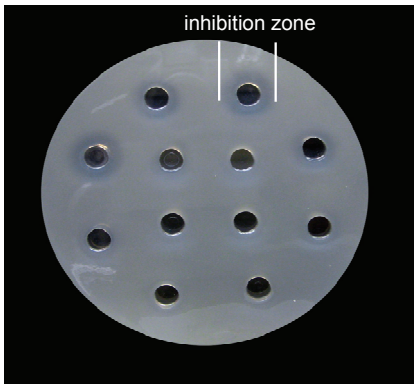


Figure 24. Agar diffusion test of applied PPs, using different bacterial strains. Inhibition zones around the wells were compared.

Two unsaturated ketotrienoic acids, 9-,13-KOTE, served as positive, pure ethanol as a negative control. First measurements with high concentrations (20 mM) of synthesised PPs and their analogues showed no influence on growth of *P. syringae, E. carotovora* and *X. campestris.* At this concentration, the substrates only inhibited the growth of *E. amylovora*. Therefore, this plant pathogen, the so-called fire-blight bacteria, was used in further experiments. Fire blight is a bacterial disease of apples and pears, killing blossoms, shoots, limbs and sometimes, even whole trees. Especially in warm und humid regions, the bacterium spreads immediately by building out drops of bacterial ooze (**Figure 25**). First step to overcome and manage the incidence of fire blight is the removal of the source of infection. Chemical control is achieved by applying copper salts on the outer surfaces of infected trees or by antibiotic treatment at blooms. But since this disease is relatively widespread and some strains of the pathogen are already resistant against certain antibiotics, more effective biological weapons have to be developed.

Figure 25. Symptoms of rosaceae plants after infection with the fire-blight bacteria *Erwinia amylovora*.

For investigating the influence of synthesised PPs and analogues on the growth of *E. amylovora*, higher dilutions of the test substances were applied. Based on these results, a prediction of structure-function relationships seems to be possible. **Figure 26** shows the applied substances at different concentrations and the resulting inhibition zone diameters.

The inhibition zones after applying both ketotrienoic acids were generally about one third higher than those of the synthesised compounds (data not shown). Except for the synthesised analogues with non-functionalised side chains **64a-c**, all tested substrates were active in a concentration of about 10 mM. The non-functionalised analogues **64a-c** just showed small inhibition zones at concentrations higher than 10 mM (data not shown). After applying lower concentrations of 2 and 1 mM, a trend was visible, showing that only the ketones **65a-c**, the acetylenic analogues **66a-c** and the *O*-alkyl ethers **67a-c** of all three types were still active. Interestingly, all compounds of type I and III were less active than those of type II, a fact that corresponds with the bio-activity of PPB_1 type II (**22b**) on secondary metabolite induction.[36] The oxygen atom in the ω-side chain seems to be important for the antimicrobial activity of certain PPs. Analogues containing no oxygen **64a-c** were not active at all. The highest activity was found for type I alkyne **66a** and for type III *O*-alkyl ether **67c**.

	10 mM	2 mM	1 mM	0.5 mM	0.4 mM
Phytoprostanes					
type I **22a**	15	0	0	0	0
type II **22b**	16	12	15	0	0
type III **22c**	15	0	0	0	0
Non-functionalised					
type I **64a**	0	0	0	0	0
type II **64b**	0	0	0	0	0
type III **64c**	0	0	0	0	0
Ketones					
type I **65a**	16	16	15	0	0
type II **65b**	15	15	15	0	0
type III **65c**	15	15	12	0	0
Acetylenes					
type I **66a**	19	24	20	14	13
type II **66b**	19	20	18	0	0
type III **66c**	15	20	17	0	0
O-alkyl ethers					
type I **67a**	16	16	0	0	0
type II **67b**	20	16	11	0	0
type III **67c**	18	18	12	12	0

Figure 26. Resulting diameters of inhibitions zones [mm] after treatment of *Erwinia amylovora* strains with different concentrations of PPs and analogues.

PPs were produced after treatment of plants with heavy metal solutions.[28] Treatment of tobacco cell suspensions with high concentrations of copper salts lead to a high amount of dead cells.[36] Preincubation of the cells with B_1-PPs (**22**) extremely reduced the mortality. This indicates that PPs increase the plant's capability to detoxify lipid peroxidation products. Interestingly, the fire-blight disease is chemically treated with copper salts, suggesting that there might be a connection between the copper treatment and induced PP accumulation leading to defined defence or repair mechanisms of the infested plants. How and to which extend PPs are able to act against such microorganisms, is still not known. Perhaps they induce the production of signal molecules subsequently inducing

defence gene expression or regulating localised cell death, known as hypersensitive response. This is a powerful tool for plant defence mechanisms against pathogens.[122] It causes necrosis and cell death to restrict the growth of the infesting pathogen. Cells undergoing the hypersensitive response produce reactive oxygen species, such as peroxides or radicals.[123] As known from literature, PPs are formed as a result of free radical formation or as consequence of enhanced oxidative stress caused by peroxides and induce the synthesis of antimicrobial secondary metabolites.[35] Copper as a heavy metal itself also stimulates ROS[124] and PP formation.[28] But the reason why synthesised B_1-PPs (**22**) were only active against *E. amylovora*, is still not clear. Moreover, why the corresponding diketones **65a-c** were more active than the natural occurring PPs **22a-c**, is also not known. Perhaps during oxidative stress, the hydroxy function could be further oxidised to an oxo group, creating a perfect Michael-acceptor system that might then be responsible for the enhanced activity. To test the acetylenic analogues **66a-c** on their inhibitory effect on the growth of *E. amylovora* appeared to be interesting, since these metabolites were the most active ones. It is not surprising, that these acetylenic metabolites **66a-c** play a role in acting against microorganisms. Previously, it has been reported, that several hexadecadiynoic or nonadecadiynoic acids display potent antifungal activities.[125] Acetylenic compounds are found in plants, fungi, microorganisms and marine invertebrates. Natural occurring aquatic acetylenes are of particular interest, since some display important biological activities and have antitumor, anticancer, antibacterial and antimicrobial properties.[126] Most include an α-acetylenic hydroxy or keto group, similar to the synthesised compounds **66a-c**. Whether the antimicrobial activity is based on the electrophilic nature of those compounds or on the interaction with specific cellular targets, is not known. Previously it was been shown, that different oxylipins, including an electrophilic unit, like OPDA (**9**) and KOTE, have antimicrobial activity, whereas JA (**8**) did not affect bacterial growth.[119] It is also not clear, why the synthesised *O*-alkyl ethers **67a-c** do inhibit bacterial growth better than their corresponding natural occurring PPs **22a-c**.

Such agar diffusion tests are good methods for giving first hints of the inhibitory effects of synthetic substrates on microbial growth, but are not reliable for investigating exact functions of those chemicals. Other methods have to be taken into account.

3.2.3 Phytoprostanes and volatile emission (indirect defence)

Higher plants have developed efficient strategies for indirect defence. The emission of volatiles is one of the most interesting strategies to cope with a variety of environmental stresses the plant is confronted with each day. The plant reacts with the emission of a specified volatile pattern, which might serve a a signals for long distance interactions with neighboured uninfected plants or to guide different predatory wasps or flies to their prey.[5]

There exist different methods for measuring plant emitted volatiles.[127]

The zNose (**Figure 27**) is a portable miniaturised GC, including a short column for separation. It is equipped with a small Helium bottle and can be used easily for field experiments. With the zNose, collection of kinetic data on volatile emission is possible, even over long time periods.

Figure 27. zNose instrument during volatile collection.

The method of closed-loop stripping (**Figure 28**) is limited to laboratory work. It serves as the routine method for measuring volatile patterns due to treatments of different plants with different elicitors. Plant leaves are placed into water solutions of the elicitor. With small pumps, volatiles are circulated and absorbed onto charcoal traps. After a certain time period, the filters are eluted with dichloromethane and the volatiles measured with GC/MS. Compounds are identified *via* their retention times and mass spectra according to standards.

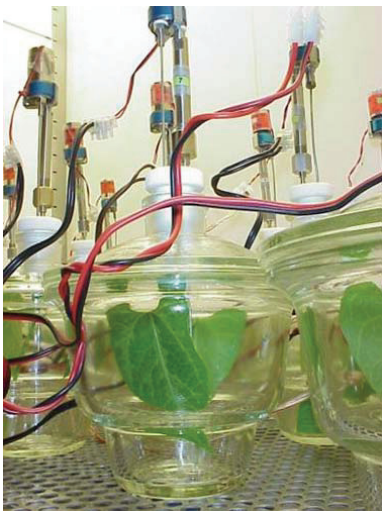
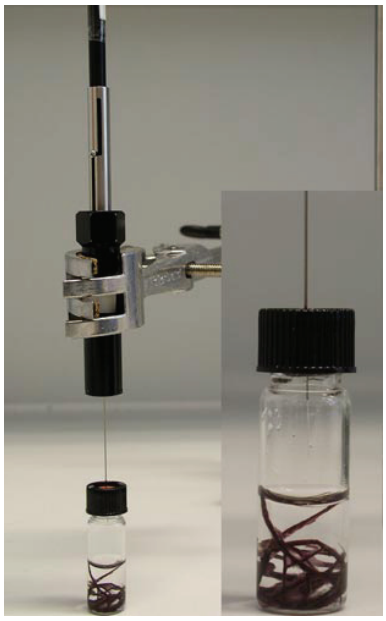


Figure 28. Closed-loop stripping method on lima bean plants.



Solid phase micro extraction (SPME) (**Figure 29**) uses a silica fibre with a non-volatile polymer, such as polydimethylsiloxane. The SPME fibre is directly inserted to the headspace of a sample and afterwards into the injector of the GC/MS, where the volatiles can be evaporised and analysed.

Figure 29. Volatile collection using SPME.

To test the influence of the synthesised PPs and analogues as elicitors for volatile emission from lima bean leaves (*Phaseolus lunatus*), the closed-loop stripping method was applied. But the results proved to be less satisfactory. In some cases there was an induction of volatile emission upon treatment and in some none. This fact made it hard to correctly evaluate the results. The free acids of substrates **22a,b, 64a,b, 65a,b, 67a,b** (after hydrolysis using porcine pancreas lipase) were tested for their ability to induce specific volatile biosynthesis in lima bean plants. 1 mM aqueous solutions of the synthesised PPs and analogues were applied to the plants over a time period of 48 h. But due to the low amount of replicates, there were no conclusive results. **Figure 30** shows a volatile pattern that was most often emitted after treatment with the test substances. No specific differences in the emitted volatile patterns, which could be listed to different structural properties of the applied PPs, could be detected. The peaks at around 6:56 and 12:62 minutes represent the two homoterpenoids 4,8-dimethylnona-1,3,7-triene (DMNT) (**68**) and 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) (**69**) and the peak at 7:64 is the methyl ester of salicylic acid (MeSa) (**70**). DMNT (**68**) and TMTT (**69**) originate from degradation products of the tertiary terpenoid alcohols nerolidol and geranyllinalool.

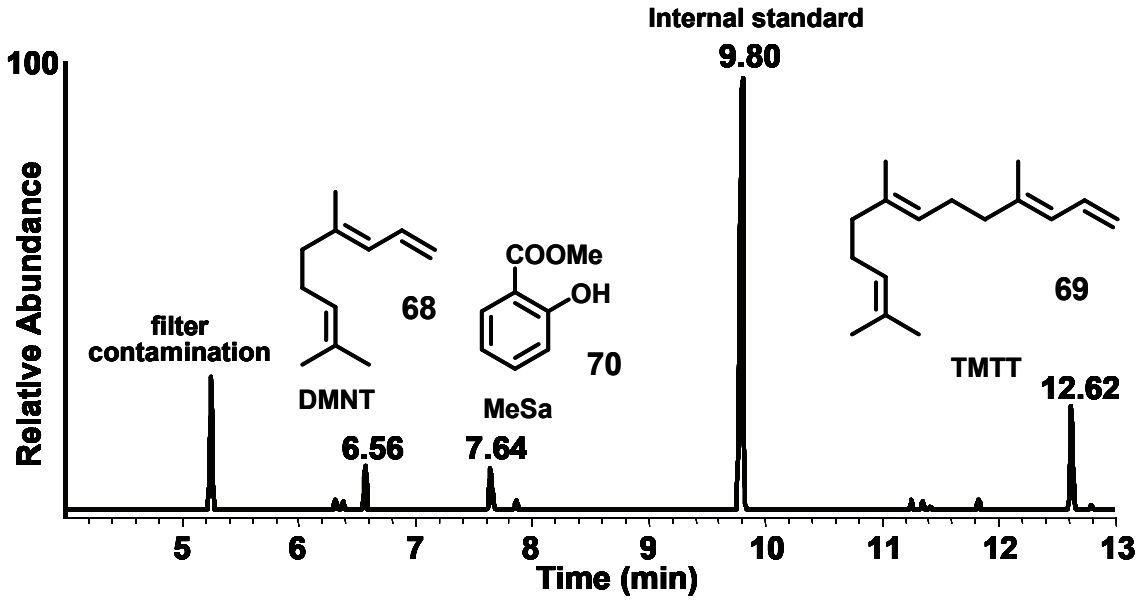


Figure 30. General volatile emission pattern after treatment of lima bean leaves with different synthesised PPs.

Both PPB_1 types showed a rather weak activity when compared to the released volatile patterns after treatment with JA (**8**), OPDA (**9**) or coronalon (**62**).[76,128] The volatile pattern largely corresponded to the volatiles observed upon treatment of lima bean leaves with several microbial elicitors. The channel-forming peptaibol alamethicin is an antibiotic, which forms voltage-dependent three-dimensional structures in membranes that act as ion channels. Alamethicin is also able to induce the emission of ethylene (**6**) and other volatiles.[129] The same volatiles, like after PP treatment, namely DMNT (**68**), TMTT (**69**) and MeSa (**70**), were emitted. JA (**8**) treatment induced a more complex mixture including other terpenoids, such as ocimene, linalool and caryophyllene.[128] OPDA (**9**) in low concentrations induced DMNT (**68**) and TMTT (**69**), whereas in higher concentrations a more complex spectrum similar to JA (**8**) was induced. Mechanical wounding, caused by treatment of the lima bean plant with MecWorm also induced a complex mixture of fatty acid derived volatiles and different terpenoids. This mode of treatment was sufficient to induce the emission of a volatile blend qualitatively similar to that as known from real herbivore feeding.[130] Taken together these results indicate, that PPs might not be relevant for plant-herbivore interactions, but appear to play some kind of role in plant-pathogen reactions. The corresponding volatile pattern suggests the ability of PPs to act similar to microbial elicitors. The plant is able to answer microbial infections with a variety of different defence mechanisms to limit the pathogen attack or to inhibit their growth. B_1-PPs were able to inhibit the

growth of the plant pathogen *E. amylovora* and induced, at least in some cases, the emission of volatiles resembling the effect of channel-forming microbial elicitors. This could be a first hint of those chemicals being involved in antimicrobial defence reactions.

3.2.4 Phytoprostanes and Ca^{2+}-dependent signalling

The importance of calcium as a “second messenger” in animal cells is known for long time, but its regulative function in plant cells has gained more attention during the last years.[131,132] The general mode of action of Ca^{2+} is through a change in its intracellular concentration. After a certain stimulus, the messenger system interacts with the membrane and opens channels that allow a transient Ca^{2+} influx from external space. Usually, the intracellular concentration of Ca^{2+} is maintained at low levels. There is an increasing number of Ca^{2+}-mediated processes in plant life. It is for instance involved in polarised cell growth, mitosis and cytokinesis.[133] Ca^{2+} also acts as a “second messenger” in plants by triggering light-stimulated responses, such as germination,[134] peroxide secretion[135] or membrane depolarisation.[136] Calcium also interacts with certain phytohormones; e.g. gibberellic acid (**3**) and abscisic acid (**5**) regulate cytoplasmatic calcium in barley.[137]

As a result of increasing interest on calcium signatures in plants, a practical approach to follow such signatures based on the non-invasive aequorin technology has been published.[138] Aequorin is a Ca^{2+}-binding photoprotein, first isolated from the luminescent jellyfish. It is composed of two distinct units, the apoprotein apoaequorin, with an approximate molecular weight of 22 kDa, and the prosthetic group coelenterazine (**71**) (molecular weight 472). It belongs to the so-called luciferin family, a class of light-emitting biological molecules (**Figure 31**).

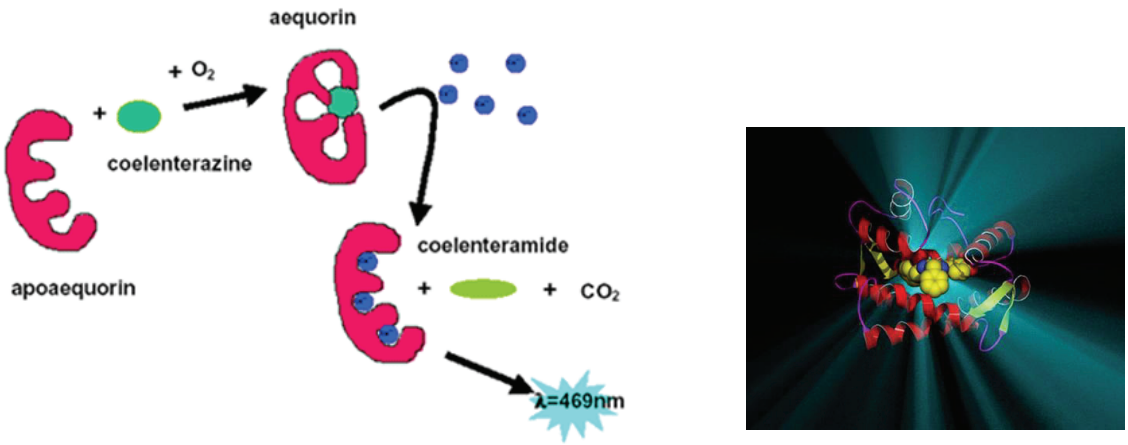
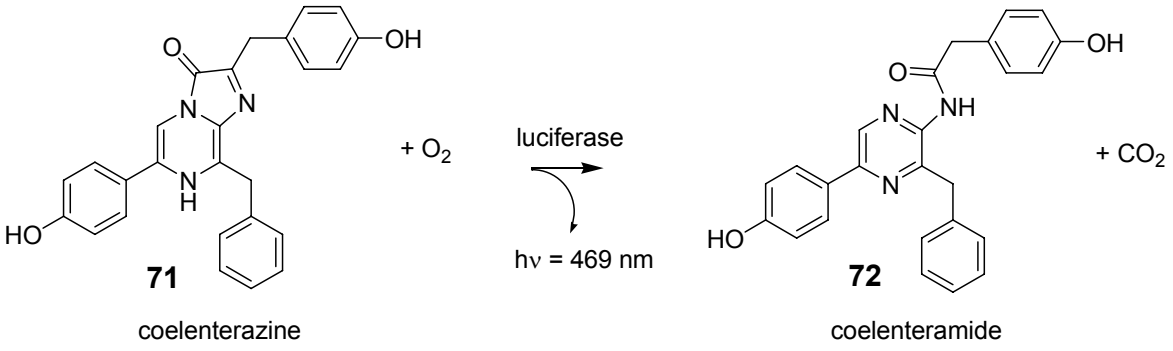


Figure 31. Aequorin as a light-emitting molecule, consisting of apoaequorin and coelenterazine (**71**).

In the presence of molecular oxygen the two components of aequorin reconstitute spontaneously. The protein contains three Ca^{2+}-binding sites. When Ca^{2+} occupies these sites, the protein undergoes a conformational change and converts through oxidation its prosthetic group, coelenterazine (**71**), into excited coelenteramide (**72**) and CO_2. As the excited **72** relaxes to the ground state, blue light with a wavelength of 469 nm is emitted (**Figure 32**). The emitted light can be detected with a luminometer.



71 coelenterazine **72** coelenteramide

Figure 32. Light emission by relaxation of coelenteramide (**72**) to the ground state.

Within this work, the cytosolic and nucleoplasmatic calcium concentrations in two different plant systems, transgenic tobacco and soybean cells, were examined. Therefore, tobacco cells were exposed to a biotic stimulus, the type II PPB_1 (**22b**), and the calcium concentration based on the described aequorin system was measured. Additionally, soybean cells were treated with both types of PPB_1 (**22a,b**), but only the cytosolic calcium concentrations were investigated. Ethanol served as negative control and 1 or 2 mM ethanolic solutions were applied. The

aequorin light emission was detected with a luminometer using a calibration curve for quantification of the Ca^{2+} concentration.

The addition of PP **22b** to tobacco cells in both concentrations resulted in a rapid increase of luminescence indicating an increase in the intracellular calcium concentration in the cytosol as well as in the nucleus (**Figure 33, 34**). The maximal concentration of intracellular calcium after the addition of 1 and 2 mM PPB_1 type II (**22b**) in the cytosol was found to be approximately 0.5 μM after about 30 sec. In the nucleus, the free calcium concentration was detected to be much higher, for 2 mM PPB_1 type II (**22b**) 2.4 μM after about 25 min and for 1 mM **22b** 1.94 μM after about 49 min.

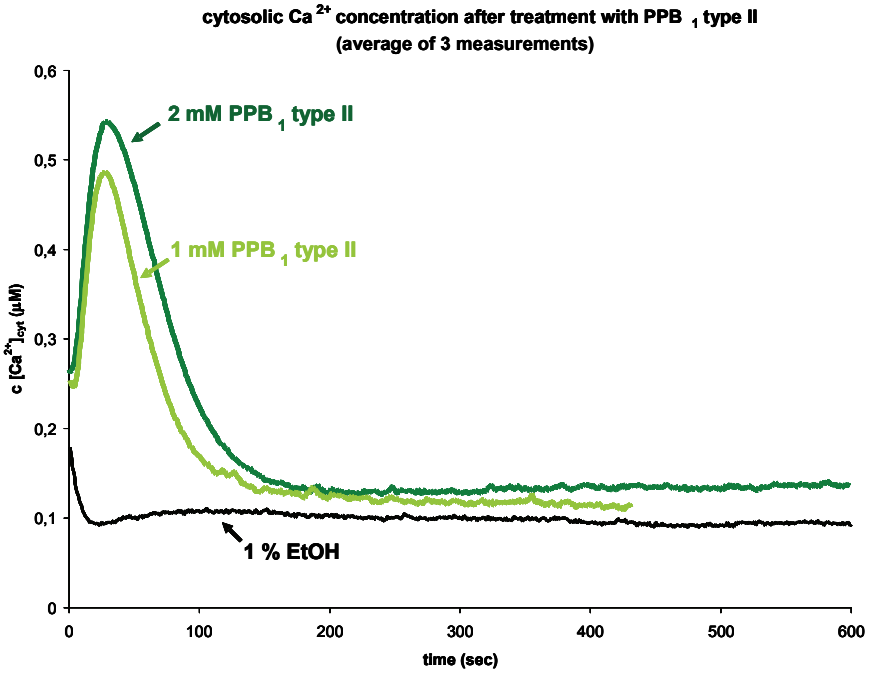


Figure 33. Intracellular cytosolic Ca^{2+}-concentration (average of 3 measure-ments) after PPB_1 type II (**22b**) treatment of tobacco cells. For 2 mM **22b** the highest Ca^{2+}-level is 0.54 μM, for 1 mM **22b** 0.49 μM, both after about 30 sec.

The Ca^{2+}-concentrations in the nucleus reached their maximum later than in the cytosol and were much higher. The time difference of the maxima in the cytosol was negligible at both concentrations, whereas in the nucleus it was more than 20 minutes. The PP treatment in the cytosol led to a sharp increase in the concentration of free calcium and also to a sharp decrease, while in the nucleus the in- and decrease were more steady over a longer period.

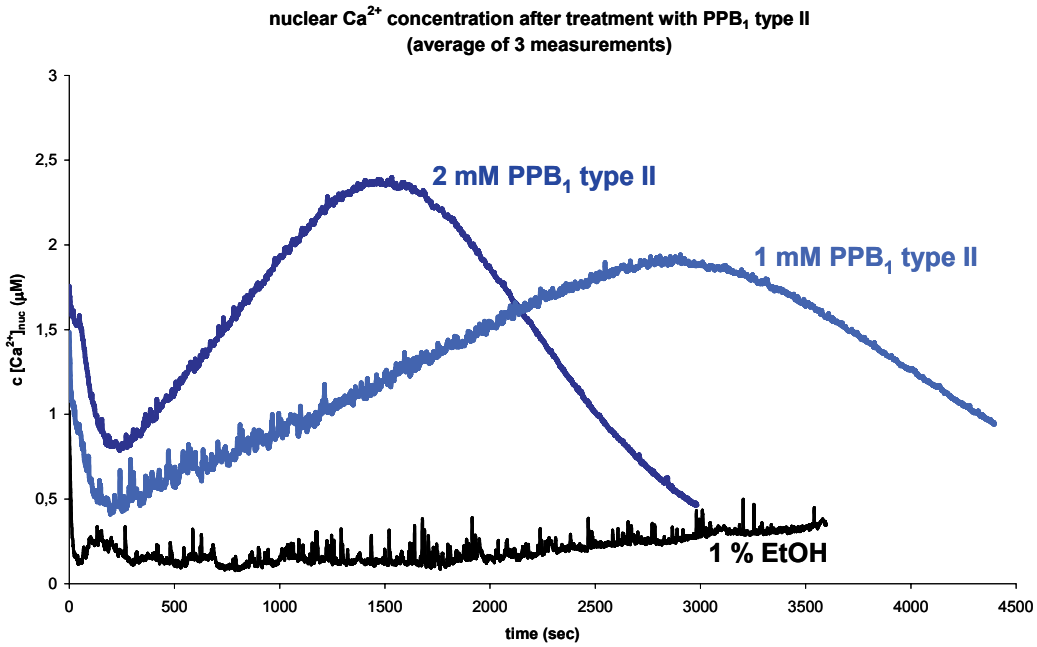


Figure 34. Intracellular nuclear Ca^{2+}-concentration (average of 3 measurements) after PPB_1 type II (**22b**) treatment of tobacco cells. For 2 mM **22b** the highest Ca^{2+}-level is 2.4 µM, for 1 mM **22b** 1.94 µM.

This resulting transient increase in the cytosolic calcium levels in tobacco could be confirmed in the tested soybean cell cultures. The curves looked similar for both types of PPB_1 (**22a,b**), although type II (**22b**) led to a slightly higher calcium concentration than type I (**22a**). In soybean, the basic level of calcium seemed to be somewhat higher than in tobacco, so the concentration maxima after addition of PPB_1s showed a slight increase in comparison to tobacco (data not shown).

Hence, treatment of transgenic tobacco or soybean cells with PPB_1 showed an increase of intracellular free calcium levels. In the cytosol, the calcium answer was faster than in the nucleus. In plants, an increase in cytosolic calcium concentrations has been reported in response to certain external stimuli, such as light,[139] oxidative stress,[140] hormones and different elicitors.[141] Thus, calcium seems to mediate many processes in plant growth and development and of course, in plant defence. The key role of Ca^{2+} in the signalling pathways received particular attention in the field of plant defence against pathogens.[142] Large and sustained calcium influx triggers numerous reactions in plant-pathogen interactions, such as the activation of membrane depolarisation, production of reactive oxygen species, MAPK activation, protein phosphorylation, nitric oxide production, and defence gene activation and cell death.[133] The way this second

messenger acts in special cases has been the focus of intense research for many years.

The increase of intracellular Ca^{2+}-levels by PPs is consistent with the results obtained from the antimicrobial and the volatile emission test. It was shown that PPB_1s could be active against the plant pathogen *Erwinia amylovora*. Additionally, synthetic PPs did not induce the emission of herbivore-specific volatiles in lima bean. It might be possible that PPs play a role in plant-pathogen interactions. In which special way PPs act, if they are perhaps mediators of pathogen-related answers or if they have a direct impact on pathogen recognition, signal transduction and defensive gene activation leading to cell death, is not known from those tests. A first microarray analysis showed that high concentrations of PPB_1s induced several genes encoding for enzymes involved in pathogen defence and wounding.

4 Summary and perspectives

During their whole lifetime, plants are exposed to a variety of stresses. Plants have either adapted to such stress or have developed effective strategies to overcome and fight against it. Phytohormones are known as key intermediates of such signalling pathways, but unfortunately, our knowledge on detailed recognition mechanisms and their mode of action is quite limited. Particularly, the influence of oxidised fatty acid-derived phytoprostanes is not fully understood.

This work was envisaged to contribute to a better understanding in the field of phytoprostane activity in higher plants. By developing efficient synthetic strategies of selected phytoprostanes and analogues thereof and by performing selected bioassays with these synthesised compounds, the presented work provides helpful suggestions for further studies in this interesting field of natural product chemistry.

Development of a short and effective synthesis to B_1-phytoprostanes

In recent years, several syntheses of phytoprostanes (PPs) have been developed,[51-54,59] requiring many steps and providing only low overall yields.

Within this work, a short and effective synthetic method was developed to generate phytoprostanes of the B_1-series with less synthetic effort and in high overall yields. Based on the previously published protocol to tetrahydrodicranenone B by Lauchli *et al.*,[61] a flexible approach was designed to allow the preparation of a whole spectrum of compounds. By using only two key operations to introduce different side chains with different lengths and degrees of functionalisation, a number of B_1-PPs types (**22a-c**) and various analogues thereof (**64-67 a-c**) became rapidly accessible.

Cyclopentanedione (**55**) served as a multi-functional precursor for a number of syntheses. **55** was first mono-alkylated at C2 with substituents of different chain lengths (**54a-c**). Followed by an iodination, several oxocyclopentenyl iodides (**53a-c**) were generated, that could be then coupled with various side chains under Heck- or Sonogashira-like reactions (**Figure 35**). Furthermore, it was shown, that this protocol is also applicable for synthesis of chiral products.

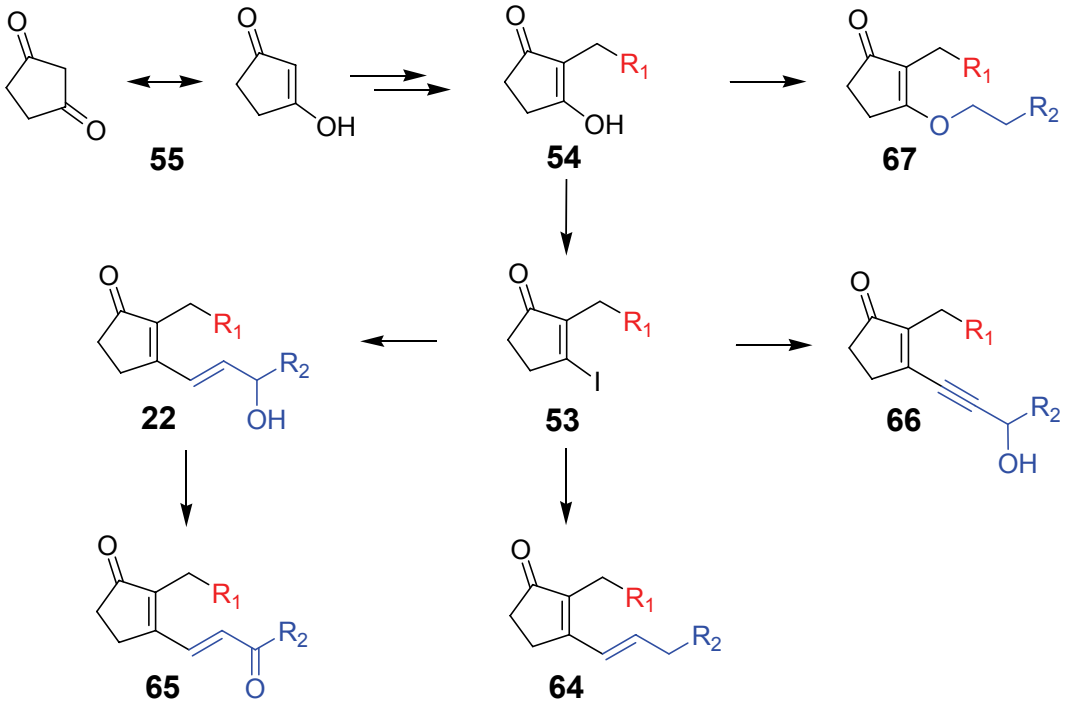


Figure 35. General protocol for the synthesis of B_1-PPs and several analogues with different C3-side chains.

	PPB_1 type I	**PPB_1 type II**	**$DIPB_1$ type III**
R_1	$(CH_2)_6COOMe$	CH_3	$(CH_2)_3COOMe$
R_2	CH_2CH_3	$(CH_2)_7COOMe$	$(CH_2)_4CH_3$

This general and flexible synthetic protocol might be useful for synthesising additional analogous compounds of PPs. Such analogues could have different degrees of functionalisation also in the C2-side chains. Also heterocyclic analogues, similar to prostaglandin heteroanalogues,[67] could be generated to examine structure-function relationships. An additional ring-oxygen might also increase the biological activity of those compounds.

Phytoprostanes induce gene expression in *Arabidopsis thaliana*

Together with Dr. H. Vogel from the Department of Entomology, the influence of selected PPs on inducing gene expression in *Arabidopsis thaliana* plants was investigated. The total RNA of treated leaves was extracted, amplified, labelled, and hybridised on the Agilent microarray, representing the whole *Arabidopsis* genome. After applying a 1 mM aqueous solution of PPB_1 type I ketone **65a** to the plant, more than 1800 genes were up- and about 2000 down-regulated. In

response to lower (100 μM) concentrations of PPB_1 type I (**22a**), II (**22b**) and type I ketone (**65a**), still about 800 genes were up- and 1500 down-regulated.

The gene expression levels in response to the 1 mM application showed a general stress induced answer, which might be in part a result of the toxicity of compound **65a** in this non-physiological concentration. Several heat shock proteins (HSPs), glutathione-*S*-transferases (GSTs) and cytochrome P-450 enzymes (Cyt P-450s) were significantly up-regulated indicating a response typical for increasing environmental stress. The up-regulation of certain pathogenesis-related (PR) proteins after **65a** treatment might suggest a more specific response to pathogens. Many auxin-responsive genes were noticeably down-regulated, supposing that PPs and auxin-related answers are differentially regulated within the plant.

Treatment of the plant with PP-compounds **22a,b** and **65a** in 100 μM concentrations led to a more selective gene expression. In comparison to the 1 mM assay several prominent genes were even regulated to the opposite. The up-regulation of many prominent genes, such as ribonuclease 1 (RNS1), lipid transfer protein 4 (LTP4), arabinogalactan-proteins (AGPs) or vegetative storage proteins (VSP1,2) by PPs suggests a role of PPs in defence responses to pathogens or wounding and supports the correlation with other phytohormones.

By statistical analyses, only a functional classification of the expressed genes was possible. Thus, PPs can be associated with a response to a certain stimulus, mainly a hormone stimulus. PPs induced the expression of genes that were also regulated by other phytohormones, especially by auxins.

For further studies it might be helpful to determine the actual concentration within the plant cells prior to assays. This could be done by measuring the non-absorbed PP material by washing it from the leave surface prior to RNA extraction.

This microarray was performed with only two replicates, which was not sufficient to allow statistical support. For this, at least four replicates are essential that will then give more concrete answers to the existing biological question.

Phytoprostanes inhibit the growth of *Erwinia amylovora*

In cooperation with Dr. B. Völksch from the Department of Microbiology of the FSU Jena, the direct antimicrobial effect of synthesised PPB_1s on several pathogens was studied. An agar diffusion test with different concentrations of the PPs was performed. The synthesised PPs were only active in inhibiting the growth of the plant pathogen *Erwinia amylovora*, whereas the growth of *Pseudomonas syringae*, *Erwinia carotovora* and *Xanthomonas campestris* was not prevented by PPs in the agar medium. The ketones **65a-c**, the acetylenes **66a-c** and the *O*-alkylethers **67a-c**, all derivatives of natural occurring B_1-phytoprostanes, showed the highest activity even in lower (1 mM) concentration ranges.

This direct antimicrobial effect, even though only found against one of the chosen bacterial strains, supports the conclusion from the microarray analysis, that PPs could be involved in certain plant responses to stress, especially against pathogens. Perhaps PPs are only mediators formed during the course of infection, but they could also have a primary function in pathogen defence reactions, such as inducing localised cell death to restrict the spread of the infecting pathogen.

Phytoprostanes do not induce herbivore-typical volatiles in lima bean

To test the effect of synthesised PPs on the induction of volatile emission in lima bean, plants were treated with 1 mM aqueous solutions of the free acids of **22a,b, 64a,b, 65a,b, 67a,b**. Using the closed-loop-stripping method, the PP-induced volatiles could be collected and analysed by GC/MS.

The results after different repeats varied; ranging from no induction to the release of volatile patterns similar to those after treatment with microbial elicitors, such as alamethicin. PPs do not induce herbivore-specific volatile patterns, indicating no significance for plant-herbivore interactions.

Phytoprostane treatment leads to an increase of intracellular calcium levels in tobacco and soybean

In cooperation with A. Walter from the Botanical Institute of the LMU München, the impact of PPB_1 type II (**22b**) on changing cytosolic and nucleoplasmatic Ca^{2+}-concentrations was studied. Therefore, transgenic tobacco or soybean cell cultures were stimulated with this elicitor and the concentration changes were measured using luminescence.[138] Treatment of tobacco cells with 1 mM or 2 mM

22b showed a transient increase of Ca^{2+}- levels in the cytosol as well as in the nucleus. The cytosolic Ca^{2+}-increase could also be monitored in soybean cultures. The increase of cytosolic Ca^{2+}- reached its maximum of about 0.5 μM within about 30 sec, whereas in the nucleus, the concentration maximum was visible after several minutes, but turned out to be much higher (~ 2.0-2.4 μM).

A similar intense and long-lasting Ca^{2+}-influx is involved in several plant-pathogen interactions.[123] The transient elevation of cytosolic calcium is necessary for stimulation of reactive oxygen species (ROS) that might act as direct toxicants to pathogens, leading to defensive gene activation and phytoalexin accumulation, which might finally induce programmed cell death.

PPs have been identified as markers of oxidative stress in plants, being connected with enhanced formation of ROS. This fact and their ability to induce strong intracellular Ca^{2+}-influx might suggest that those metabolites might be active in the signalling cascade of higher plants against pathogens.

5 Zusammenfassung

Im Verlauf ihres Lebens sind Pflanzen verschiedenen Stressfaktoren ausgesetzt, an die sie sich entweder anpassen oder gegen die sie sich durch Entwicklung effektiver Verteidigungsstrategien zur Wehr setzen müssen. Eine zentrale Aufgabe kommt dabei den Phytohormonen oder phytohormon-ähnlichen Substanzen als wichtigen Signalsubstanzen zu. Jedoch ist das Wissen auf dem Gebiet spezieller Erkennungsmechanismen derartiger Verbindungen noch unvollständig. Besonders die Rolle der Phytoprostane als neue fettsäure-abgeleitete Substanzen ist nicht im Detail geklärt.

Die vorliegende Arbeit soll zu einem besseren Verständnis der biologischen Relevanz von Phytoprostanen dienen. Durch die Entwicklung effizienter Synthesestrategien zu ausgewählten Phytoprostanen und deren Analoga, sowie durch die Durchführung gezielter biologischer Experimente mit synthetischen Substanzen, ist diese Arbeit als hilfreiche Grundlage für weitere wissenschaftliche Studien mit Phytoprostanen zu sehen.

Entwicklung einer kurzen und effektiven Synthesestrategie zu B_1-Phytoprostanen

In den letzten Jahren wurden einige Totalsynthesen zu verschiedenen Phytoprostanklassen beschrieben, welche aber meist viele Syntheseschritte beinhalteten und zu geringen Gesamtausbeuten führten.[51-54,59]

Im Rahmen dieser Doktorarbeit wurde eine kurze und effektive Synthesestrategie entwickelt, um Phytoprostane (PP) der B_1-Klasse in wenigen Schritten und guten Gesamtausbeuten zu generieren. Auf dem von Lauchli *et al*.[61] veröffentlichten Protokoll zum Tetrahydrodicranenone B basierend, wurde eine flexible Route erarbeitet, über die eine Vielzahl von Verbindungen hergestellt werden konnte. Dabei sind lediglich zwei Schlüsselschritte nötig, um Seitenketten verschiedener Länge und Funktionalität in das Cyclopentandion-Grundgerüst einzuführen. Es wurden Verbindungen vom Typ I, II und III des PPB_1 (**22a-c**) und zahlreiche strukturelle Analoga (**64-67 a-c**) hergestellt.

Cyclopentandion (**55**) erwies sich dabei als multifunktionelle synthetische Vorstufe. Zunächst wurde **55** an C2 mit unterschiedlichen Kettenlängen mono-alkyliert (**54a-c**), anschließend ins Hydroxycyclopentenyl-Iodid (**53a-c**) überführt,

um danach mit verschiedenartigen Seitenketten mittels Heck- oder Sonogashira-Reaktionen gekoppelt zu werden (**Abbildung 1**). Diese Syntheseroute konnte auch für chirale Produkte angewandt werden.

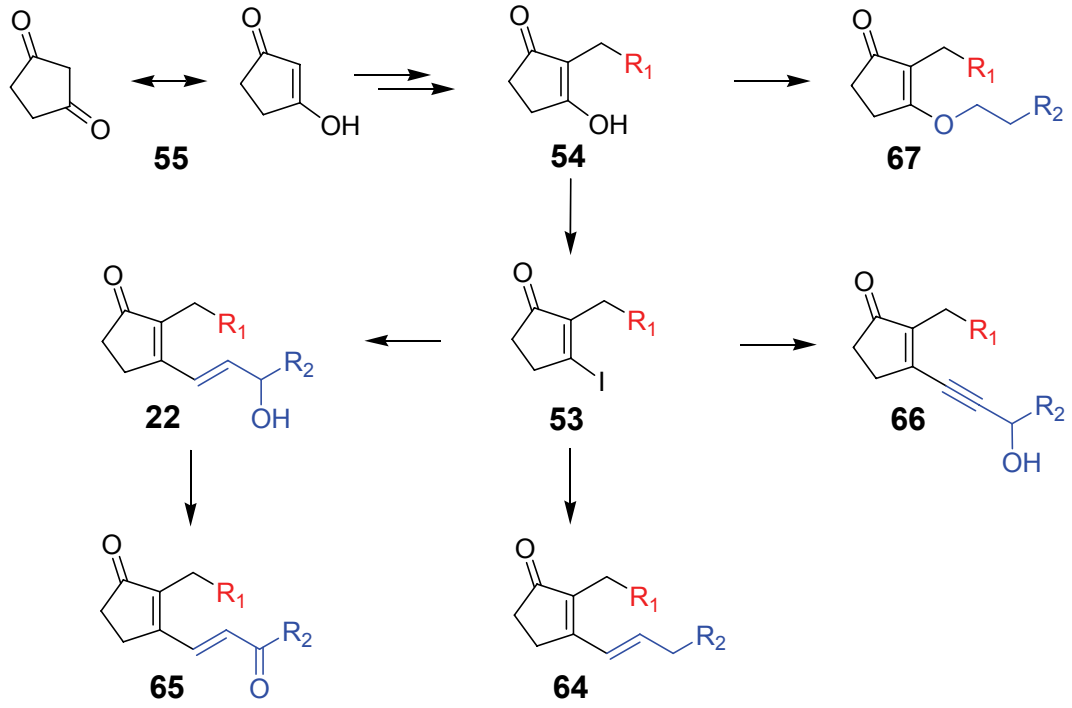


Abbildung 1. Allgemeine Syntheseübersicht für PPB_1 Verbindungen und entsprechende Strukturanaloga.

	PPB_1 Typ I	**PPB_1 Typ II**	**$DIPB_1$ Typ III**
R_1	$(CH_2)_6COOMe$	CH_3	$(CH_2)_3COOMe$
R_2	CH_2CH_3	$(CH_2)_7COOMe$	$(CH_2)_4CH_3$

Phytoprostane induzieren Genexpression in *Arabidopsis thaliana*

In Zusammenarbeit mit Dr. H. Vogel aus der Abteilung Entomologie des MPICOE wurde die Wirkung verschiedener PP auf die Genexpression in *Arabidopsis thaliana* Pflanzen untersucht. Dazu wurde die RNA von behandelten Blättern extrahiert, amplifiziert, markiert und auf einem Agilent Microarray hybridisiert. Eine Behandlung der Pflanze mit einer 1 mM wässrigen Lösung vom PPB_1-I Keton (**65a**) führte zu einer Hochregulierung von ca. 1800 Genen und zu einer Herunterregulierung von ca. 2000 Genen. Selbst geringere applizierte Konzentrationen (100 µM) von **22a,b** und **65a** zeigten noch, dass ca. 800 Gene hoch- und 1500 herunterreguliert wurden.

Als Reaktion auf die 1 mM Zugabe wurde eine generelle stress-induzierte Antwort der Pflanze provoziert, die eventuell auch auf eine zunehmende Toxizität der applizierten Substanz **65a** in derartig hohen Konzentrationen schließen lässt. Dabei wurden zahlreiche Heat-Shock Proteine (HSPs), Glutathion-*S*-Transferasen (GSTs) und verschiedene Cytochrom P-450 Enzyme (Cyt P-450) signifikant hochreguliert. Die Hochregulierung einiger Pathogenese-verwandten Gene (PRs) deutet auf eine spezifischere Antwort der Pflanze in Verbindung mit Pathogenbefall hin. Hingegen wurden einige Auxin-zugeordnete Gene durch **65a** merklich herunterreguliert.

Die Zugabe geringerer Konzentrationen von **22a,b** und **65a** führte zu selektiveren Expressionmustern; einige prominente Gene wurden sogar entgegengesetzt zur Behandlung mit 1 mM reguliert. Die Hochregulierung bestimmter Gene, welche die Ribonuclease 1, das Lipid-transfer Protein 4, verschiedene Arabinogalactan-Proteine oder vegetative Storage-Proteine codieren, deutet weiterhin auf Rolle von PP innerhalb der pflanzlichen Verteidigung gegen Verwundung oder Pathogenbefall hin and bestätigt deren Wirkungsverwandtschaft zu anderen Phytohormonen.

Da mittels der Microarray-Technologie allein keine detaillierten Informationen zur biologischen Funktion von PP erhalten werden können, wurde eine Klassifizierung vorgenommen, die auf eine Wirkungsverwandtschaft der getesteten PP zu anderen Pflanzenhormonen hindeutet. Dabei ließ sich besonders eine Verbindung zum Auxin herstellen und es kann vermutet werden, dass PP bei der pflanzlichen Verteidigung, eventuell auch gegen Pathogene, aktiv sind und eine Stressantwort der Pflanze hervorrufen.

Phytoprostane verhindern das Wachstum von *Erwinia amylovora*

In Zusammenarbeit mit Dr. B. Völksch vom Institut für Mikrobiologie der FSU Jena wurden Hemmhofmessungen nach Applikation von synthetischen PP zu verschiedenen Indikatorstämmen durchgeführt. Die untersuchten PP waren lediglich gegen den Feuerbranderreger *Erwinia amylovora* unmittelbar aktiv. Verschiedene Konzentrationen der Verbindungen zeigten deutlich, dass besonders die zu den natürlich vorkommenden PPB_1 analogen Ketone **65a-c**, die Acetylene **66a-c** und die *O*-Alkylether **67a-c** wachstumsinhibierende Wirkung aufweisen.

Dieser Test kann als Hinweis angesehen werden, dass PP eine Rolle bei der pflanzlichen Verteidigung gegen Mikroorgansimen, hier Pflanzen-pathogene, spielen.

Phytoprostane induzieren kein herbivoren-spezifisches Duftmuster in Limabohnen

Um die Wirkung von PP auf die Emission spezifischer Duftstoffe zu testen, wurden Limabohnen mit 1 mM Konzentrationen verschiedener PP (**22a,b, 64a,b, 65a,b, 67 a,b**) behandelt. Unter Anwendung der etablierten Closed-loop-stripping Methode wurden die von der Pflanze emittierten Duftstoffe gesammelt und mittels GC/MS analysiert.

Nach Applikation verschiedener PP und Analoga konnte jedoch keine genaue Aussage über die Wirkung dieser Verbindungsklasse getroffen werden. Teilweise wurde ein Duftmuster ähnlich dem nach Applikation des mikrobiellen Elizitors Alamethicin erhalten, in einigen Fällen konnte jedoch keinerlei Duft detektiert werden. Vermutlich spielen PP daher keine Rolle bei der pflanzlichen Verteidigung gegen Herbivoren.

Phytoprostane bewirken einen intrazellulären Calciumionenanstieg in Tabak und Soja

In Zusammenarbeit mit A. Walter vom Botanischen Institut der LMU München wurde die Wirkung von PPB_1-II (**22b**) auf Konzentrationsänderungen von zyosolischem und nukleoplasmatischem Calcium untersucht. Mit Hilfe von Lumineszenzmessungen[138] konnte die Wirkung in transgenen Tabak- und Soja-Zellkulturen getestet werden. In beiden Pflanzen konnte ein starker Anstieg der Calciumkonzentration verzeichnet werden. Das zytosolische Calcium erreichte seinen Maximalwert von ca. 0.5 μM nach wenigen Sekunden, wohingegen im Kern erst nach mehreren Minuten ein stärkerer, dauerhafter Anstieg (~ 2.0-2.4 μM) sichtbar wurde.

Von einem ähnlich starken, lang anhaltenden Calciumioneneinstrom wurde bei bestimmten Pflanzen-Pathogen-Interaktionen berichtet.[123] Ein erhöhter Calciumionenfluss ist notwendig, um reaktive Sauerstoffspezies (ROS) zu generieren, die dann ihrerseits direkt gegen Pathogene wirken können. Es werden bestimmte verteidigungsspezifische Gene reguliert, die zur Aktivierung

verschiedener Phytoalexine beitragen, was schlussendlich zum programmierten Zelltod und damit zur Verteidigung der Pflanze führt.

PP wurden mit erhöhten ROS-Pegeln assoziiert und als Marker von oxidativem Stress identifiziert. Dies und das Verursachen starker intrazellulärer Calciumeinströme durch PP-Applikation könnten weitere Hinweise darauf sein, dass PP im engen Zusammenhang mit der pflanzenspezifischen Verteidigung gegen Pathogene stehen.

6 Experimental part

6.1 General methods and instruments

6.1.1 Synthetic methods

Melting points

Melting points of solids were recorded on a BÜCHI Melting apparatus Point B-540.

Fourier Transform Infrared Spectroscopy (FT-IR)

IR-Spectra were recorded with a Bruker Equinox 55 FT-IR. Solids were measured as KBr plates, liquids as a film between two ZnS plates.

Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR-spectra were recorded with a Bruker Avance DRX 500 or Bruker AV 400. Chemical shifts are given in ppm and coupling constants *J* in Hz. The solvent signal of $DCCl_3$ was used for calibration: ^{1}H NMR δ = 7.26 ppm, ^{13}C NMR δ = 77.16 ppm.

Electronic Ionisation Mass Spectrometry (EI-MS)

High resolution mass spectrometry was performed by direct injection of the sample on a double focussing magnetic sector mass spectrometer. For GC-MS (70 eV) a DB5 column was used (15 m × 0.25 mm, 0.25 μm), He served as carrier gas.

Specific rotation

Specific rotations were measured on a Jasco P-1030 Polarimeter based on the equation $[\alpha] = (100 \times \alpha) / (l \times c)$, where the concentration c is in g/100 ml of methanol and the path length *l* in dm.

Chromatography

Silica gel (Si 60, 0.2-0.063 mm, VWR International GmbH) was used for flash chromatography. TLC plates (Si 60, F_{254}, Merck) were used for analytical chromatography.

Solvents and chemicals

Solvents for syntheses were purchased from Sigma-Aldrich as p.a. grade or as dried over molecular sieves. Solvents for chromatography were purchased from VWR International GmbH or KMF Laborchemie Handels GmbH. Selected chemicals were obtained from specified providers.

6.1.2 Biomolecular methods

Treatment of the *Arabidopsis thaliana* plants

Arabidopsis thaliana ecotype Columbia (Col-0) seeds were purchased from Nottingham Arabidopsis Stock Centre and cultivated in Vötsch-chambers under 11.5 h light conditions (20 °C, 50 % humidity, 80 % light) and 12.5 h under dark conditions (16 °C, 50 % humidity). After 5 weeks, one leaf each plant was treated with a 1 mmol, 100 μmol or 10μmol aqueous solution of the phytoprostanes or their analogues in two repeats. After 9 h under continuous light conditions, the leaves were immediately harvested and shock-frozen in liquid N_2. Water-treated leaves served as negative control.

RNA extraction of the *Arabidopsis thaliana* leaves

General procedure for RNA-extraction:

- Grind frozen plant material in liquid N_2, put 2 spatula-points in an Eppendorf tube
- Add 1 ml TRIzol Reagent (Invitrogen, Carlsbad, CA), collect on ice, vortex, leave 10-20 min at rt
- Add 120 μl 1-bromo-3-chloropropane, vortex for 20 s, leave 15-20 min on ice
- Centrifuge 15 min at full speed, pipette off the upper phase

- Add 500 μl propan-2-ol, vortex, leave over night at -20 °C
- Centrifuge 30 min at 4°C at full speed, discard the supernatant
- Add 0.8 ml 80 % ethanol, centrifuge 10 min at full speed, discard supernatant, centrifuge, pipette off the rest of the solvent, dry ~ 10 min
- Add 90 μl RNA storage solution, heat 30 min at 55 °C, centrifuge
- Add 10 μl TURBO DNAse buffer and 1 μl DNAse (TURBO DNAse, Ambion), incubate 30 min at 37 °C
- Use the RNeasy MinElute Cleanup Kit (Qiagen, Hilden, Germany) for further cleaning (procedure is written in the handbook)
- Determine RNA quantity spectrophotometrically
- Verify RNA integrity on an Agilent 2100 Bioanalyser using RNA Nano chips (Agilent)

Agilent Arabidopsis 3 arrays

The content on the Arabidopsis 3 microarray is derived from the ATH1 v.5 database of The Institute for Genomic Research (TIGR) and from the Arabidopsis MPSS database at the University of Delaware. Nearly 40,000 features (60-mer oligonucleotides) represent full genome coverage of *Arabidopsis thaliana* (28,500 annotated genes from TIGR) and more than 10,000 unannotated transcripts from University of Delaware (Arabidopsis MPSS website).

Amplification, Labelling and Hybridisation

Total RNA was amplified using the Agilent low input linear amplification kit according to the process outlined by the manufacturer (Agilent Technologies). 1-5 μg of amplified target cRNA was labelled with either cy5 or cy3 using the Micromax kit (Perkin Elmer). The labelled material was passed through zymo RNA Clean-up Kit-5 columns (Zymo Research Corporation, CA) to remove any surplus label and eluted in 15-20 μl of RNAse-free water (Ambion, Austin, TX). Concentration of labelled cRNA and label incorporation was determined by Nanodrop-1000 spectrophotometer analysis. All of the labelling and post labelling procedures were conducted in ozone-free enclosure to ensure the integrity of the label. Labelled material was setup for fragmentation reaction according to the Agilent protocol described in their processing manual and hybridised overnight in the rotating oven at 60° C in an ozone-free chamber. Wash conditions were as

outlined in the Agilent processing manual and arrays were scanned using the Agilent G2565BA fluorescent microarray scanner. Agilents feature extraction software (Version 7.5) was used for extracting array data. Further analysis was done using Rosetta Luminator and GeneSifter software.

6.1.3 Microbiological methods

The inhibitory activities of the toxins (compounds, substances) were evaluated by an agar plate diffusion test using following strains; *Ervinia amylovora, Xanthomonas campestris, Pseudomonas syringae, Escherichia coli, Bacillus subtilis, Erwinia carotovora.* The test plates (130 mm in diameter) contained 50 ml agar-minimal medium to which 2 ml of the bacterial suspension (A_{578nm} = 1) were added. 50 or 100 µl of the toxin solution (different dilutions) were added into wells (8 mm in diameter) in the agar plates. After 48 h incubation at 28 °C, the test plates were checked for inhibition zones around the wells.

6.1.4 Volatile emission tests

Lima bean plants

Induction experiments were performed with plantlets of the Lima bean *Phaseolus lunatus* (Ferry Morse cv. Jackson Wonder Bush). Individual plants were grown from seeds in a plastic pot (Ø = 5.5 cm) at 23 C and 80 % humidity using daylight fluorescent tubes at about 270 µE m^2 s^{-1} with a light phase of 14 h. Experiments were conducted with 14 day-old seedlings showing two fully developed leaves

Induction experiments

Test solutions of the synthetic compounds were made in tap water at 1 mM concentration. Plantlets of *P. lunatus* were cut with razor blades and immediately transferred into 5 ml vials containing the test solution. These were enclosed in small desiccators (750 ml) and maintained at room temperature for 24 h. On top of the desiccators, small pumps containing a charcoal trap were put. Air was continuously circulated through the closed system and the released volatiles were

absorbed on the trap (closed-loop stripping). Experiments were started in the afternoon and the light dark cycle completed as above. Control experiments were conducted under identical conditions by placing freshly cut plantlets into pure tap water. All experiments were carried out on 3 plants.

Collection and analysis of headspace volatiles

The volatiles emitted from the plants were collected continuously on small charcoal traps (1 mg charcoal, CLSA-Filter, Le Ruisseau de Montbrun, F-09350 Daumazan sur Arize, France) over a period of 24 h using air circulation as described. After desorption of the volatiles from the carbon trap with 2 × 20 µl of a solution of 1-bromodecane (internal standard, 200 µg/ml) in dichloromethane. The extracts were directly analysed by GC/MS instrument. The instrument was equipped with an Alltech EC-5 capillary (15 m × 0.25 mm × 0.25 µm). Helium at a constant flow (1.5 ml min^{-1}) served as carrier gas. Samples were automatically injected with a split ratio of 1:10; the injector temperature was adjusted to 220 °C. Separation of the compounds was achieved under programmed conditions (40 °C for 2 min, 10 °C min^{-1} to 200 °C, 30 °C min^{-1} to 280 °C, hold for 2 min). The transfer line was set to 270 °C. Filament emission current was 250 µA with an ion source temperature of 200 °C. Individual compounds were analysed by their retention time and by matching against reference spectra. Their peak area was measured relatively to the internal standard.

6.1.5 Ca^{2+}-measurements

Cell cultures

The transformed tobacco (*Nicotiana tabacum* L. cv BY-2) suspension cells were grown under agitation (130 rpm) at 25 °C in darkness in Linsmaier & Skoog (LS) medium supplemented with 30 g l^{-1} of sucrose and 1 mg ml^{-1} of 2,4-dichlorophenylacetic acid, pH 5.8. Subculturing was done every 2 weeks with a 2 % inoculum of a 14 days old culture. The cell suspension cultures of soybean (*Glycine max* L., line 6.6.12) expressing apoaequorin were grown at 22 °C under constant light conditions (3,000 lux) on a rotary shaker (125 rpm) in Murashige &

Skoog medium supplemented with 5 g l^{-1} sucrose, 1 mg l^{-1} α-naphthylacetic acid, and 0.2 mg l^{-1} kinetin, pH 5.8.

Construction of nucleus-targeted apoaequorin

This preparation was done according to Mithöfer *et al*.[129]

Luminescence measurements

Aequorin light emission was measured using a digital luminometer (Bio Orbit 1250, Turku, Finland). BY-2 cells were collected by filtration during the exponential growth phase, washed with fresh medium and resuspended at a 20% packed cell volume in fresh medium. *In vivo* reconstitution of the aequorin was performed by incubating an appropriate volume of washed cells with 2.5 µM of coelenterazine (Calbiochem, Bad Soden, Germany) under agitation for at least 3 hrs. A variable amount (50 µl to 100 µl) of reconstituted cells were transferred to a luminometer cuvette and luminescence was recorded every second during the experiment. Typically, the luminescence was monitored until the base-line luminescence was reached. At the end of the experiment, the remaining reconstituted aequorin was estimated by discharging by addition of an equal volume of 100 mM $CaCl_2$ containing 10 % ethanol (v/v) and 2 % Nonidet P-40(v/v). The emitted light expressed as RLUs (Relative Luminescence Units) was calibrated as Ca^{2+} concentrations by a method based on the calibration curve of Allen *et al*:

$$[Ca^{2+}] = \{(L_0/L_{max})^{1/3} + [KTR(L_0/L_{max})^{1/3}] - 1\}/\{KR - [KR(L_0/L_{max})^{1/3}]\},$$

where L_0 is the luminescence intensity per second and L_{max} is the total amount of luminescence present in the entire sample over the course of the experiment. $[Ca^{2+}]$ is the calculated Ca^{2+} concentration, KR is the dissociation constant for the first Ca^{2+} ion to bind, and KTR is the binding constant of the second Ca^{2+} ion to bind to aequorin. The luminescence data were determined using the KR and KTR values of 2 x 10^6 M^{-1} and 55 M^{-1}, respectively, calculated by van der Luit *et al*. using native coelenterazine and the specific aequorin isoform that were used in these experiments. For soybean, transgenic 6.6.12 cell lines were used to reconstitute aequorin *in vivo* with 10 mM synthetic coelenterazine on a shaker (125 rpm) in the dark for up to 24 hrs. The Ca^{2+}-specific luminescence measurements were performed at room temperature in a final volume of 100 µl containing 5-10 mg (fresh mass) of reconstituted cell suspension culture. Again,

the residual aequorin was completely discharged and the resulting luminescence was used to estimate the total amount of aequorin present in various experiments in order to determine the rate of aequorin consumption. This enabled us to calculate the cytosolic Ca^{2+} concentrations. According to Moyen *et al.* the equation was used:

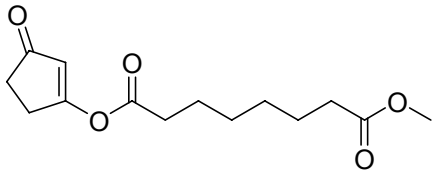
$$pCa = 0.332588(-\log k) + 5.5593$$

where *k* is a rate constant equal to luminescence at any time point divided by total remaining luminescence counts. In each experiment, the concentration of reconstituted aequorin was not limiting under any of the experimental conditions, with a maximal consumption not exceeding 10 %.

6.2 Syntheses

6.2.1 *O*-alkylation of cyclopentane-1,3-dione 55 (general procedure):

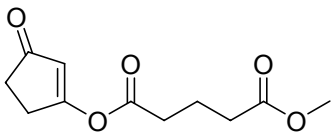
Methyl 3-oxocyclopent-1-enyl-octanedioate (58a)



To a solution of cyclopentane-1,3-dione (**55**) (3.14 g, 32 mmol, 1 equiv) and pyridine (2.6 ml, 32 mmol, 1 equiv) in 150 ml of absolute dichloroethane was added methyl 7-(chlorocarbonyl)heptanoate (5 ml, 35.2 mmol, 1.1 equiv) in 75 ml of absolute dichloroethane. The mixture was kept at rt for 20 h. It was then washed with water, diluted HCl, saturated $NaHCO_3$ and water again. After drying over Na_2SO_4, the solvent was removed on a rotary evaporator and the chromatographically pure enol ester **58a** was obtained as a light yellow solid (8.4 g, 98 %). Mp: 44-47 °C. IR ν (cm^{-1}): 1776 (C=O), 1731 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.31-1.42 (m, 4H), 1.6-1.73 (m, 4H), 2.31 (t, *J*=7.5 Hz, 2H), 2.42-2.45 (m, 2H), 2.52 (t, *J*=7.4 Hz, 2H), 2.73-2.75 (m, 2H), 3.66 (s, 3H), 6.21 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 24.3, 24.8, 28.7, 28.8, 28.9, 33.4, 34.0, 34.5, 51.6, 116.6, 169.3, 174.2, 179.7, 206.9. EI-MS: $[M+H]^{+\bullet}$ 268 (1), 171 (100), 139 (63),

125 (3), 111 (70), 99 (52), 83 (82), 74 (11), 69 (69), 59 (25), 55 (91). HR-MS calcd for $C_{14}H_{20}O_5$ ($M^{+\bullet}$) 268.131074, found: 268.130173.

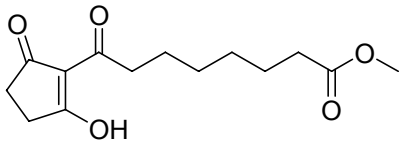
Methyl 3-oxocyclopent-1-enyl-glutarate (58b)



Synthesised from 2 g (20.4 mmol) cyclopentane-1,3-dione (**55**), 1.64 ml (20.4 mmol) pyridine, 100 ml absolute dichloroethane, 3,7 g (22.4 mmol) methyl 4-(chlorocarbonyl)butanoate in 50 ml absolute dichloroethane. Yield: 4.53 g (98 %), yellow oil. IR ν (cm^{-1}): 1782 (C=O), 1737 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ:1.98-2.04 (m, 2H), 2.41-2.45 (m, 4H), 2.62 (t, *J*=7.22 Hz, 2H), 2.73-2.75 (m, 2H), 3.68 (s, 3H), 6.21 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ:19.7, 28.9, 32.7, 33.4, 33.6, 51.9, 116.7, 168.7, 173.2, 179.7, 206.9. EI-MS: $[M-31]^{+\bullet}$ 195 (13), 167 (13), 129 (100), 101 (57), 69 (11), 59 (42), 55 (21). HR-MS calcd for $C_{10}H_{11}O_4$ ($[M-31]^{+\bullet}$) 195.065734 (-CH_3O), found: 195.065903.

6.2.2 *O-C*-isomerisation of the enol esters 58a,b (general procedure):

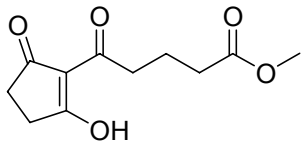
Methyl 8-(2-hydroxy-5-oxocyclopent-1-enyl)-8-oxo-octanoate (59a)



To a solution of methyl 3-oxocyclopent-1-enyl octanedioate (**58a**) (6 g, 22.4 mmol, 1 equiv) in 60 ml of absolute CH_3CN was added triethylamine (4.4 ml, 31.3 mmol, 1.4 equiv) and acetone cyanohydrine (0.8 ml, 9 mmol, 0.4 equiv). The mixture was stirred at rt for 24 h and then treated with 100 ml of diluted HCl. After extraction with 3 x $CHCl_3$, the combined organic extracts were washed with water, dried over Na_2SO_4 and evaporated (yellow solid, 5.53 g, 92 %). Mp: 51-53 °C. IR ν (cm-1): 1737 (C=O), 1703 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.28-1.4 (m, 4H), 1.57-1.66 (m, 4H), 2.29 (t, J=7.4 Hz. 2H), 2.48-2.51 (m, 2H), 2.72-2.75 (m, 2H), 2.89 (t, *J*=7.5 Hz, 2H), 3.65 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ:23.9, 24.8, 28.6, 28.9

(2C), 33.8, 34.1, 38.4, 51.6, 114.2, 174.3, 199.9, 201.8, 204.4. EI-MS: M+• 268 (29), 250 (5), 237 (23), 218 (28), 195 (23), 167 (8), 153 (92), 140 (95), 125 (100), 97 (20), 87 (17), 74 (12), 69 (32), 59 (11), 55 (32). HR-MS calcd for $C_{14}H_{20}O_5$ (M+•) 268.131074, found: 268.130988.

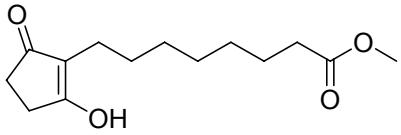
Methyl 5-(2-hydroxy-5-oxocyclopent-1-enyl)-5-oxopentanoate (59b)



Synthesised from 4 g (17.7 mmol) methyl 3-oxocyclopent-1-enyl glutarate (**58b**) in 40 ml of absolute CH_3CN, 3.5 ml (24.8 mmol) triethylamine, 0.65 ml (7.1 mmol) acetone cyanohydrine. Yield: 3.4 g (85 %), yellow solid. Mp: 35-37 °C. IR ν (cm^{-1}): 1735 (C=O), 1699 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ:1.94-2.0 (m, 2H), 2.39 (t, *J*=7.45 Hz, 2H), 2.49-2.51 (m, 2H), 2.74-2.76 (m, 2H), 2.97 (t, *J*=7.22 Hz, 2H), 3.66 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 19.0, 28.3, 33.3, 33.7, 38.0, 51.7, 114.5, 173.5, 199.9, 201.2, 203.7. EI-MS: $M^{+\bullet}$ 226 (72), 208 (10), 195 (57), 166 (74), 153 (98), 140 (25), 125 (100), 97 (22), 74 (39), 69 (58), 59 (28), 55 (60). HR-MS calcd for $C_{11}H_{14}O_5$ ($M^{+\bullet}$) 226.084124, found: 226.084606.

6.2.3 Reduction of 59a,b (general procedure):

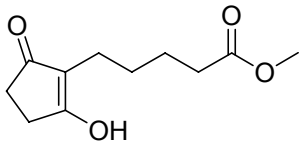
Methyl 8-(2-hydroxy-5-oxocyclopent-1-enyl)octanoate (54a)



To a solution of methyl 8-(2-hydroxy-5-oxocyclopent-1-enyl)-8-oxooctanoate (**59a**) (5 g, 18.7 mmol, 1 equiv) in 90 ml trifluoroacetic acid containing 1 % of lithium perchlorate was added in portions triethylsilane (8.9 ml, 56 mmol, 3 equiv). The mixture was left overnight at rt. The acid was distilled off and the residue was cooled and washed 3× with cold hexane. Then the residue was treated with $CHCl_3$, filtered and evaporated (white solid, 4.21 g, 89 %). Mp: 91-93 °C. IR ν (cm^{-1}): 1739 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ:1.29-1.4 (m, 4H), 1.58-1.67 (m, 6H), 2.29 (t, *J*=7.4 Hz, 2H), 2.49-2.52 (m, 2H), 2.72-2.75 (m, 2H), 2.89 (t, *J*=7.3 Hz,

2H), 3.65 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ:6.5, 6.9, 21.1, 25.0, 28.0, 29.1, 29.2, 29.5, 30.6, 34.2, 51.7, 118.6, 174.8, 198.6. EI-MS: $M^{+\bullet}$ 254 (27), 223 (27), 181 (24), 153 (17), 139 (36), 125 (61), 112 (100), 99 (12), 83 (19), 74 (7), 69 (14), 59 (10), 55 (41). HR-MS calcd for $C_{14}H_{22}O_4$ ($M^{+\bullet}$) 254.151809, found: 254.152723.

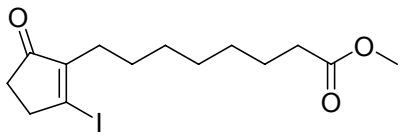
Methyl 5-(2-hydroxy-5-oxocyclopent-1-enyl)pentanoate (54c)



Synthesised from 2.5 g (11.1 mmol) methyl 5-(2-hydroxy-5-oxocyclopent-1-enyl)-5-oxopentanoate (**59c**) in 55 ml trifluoroacetic acid containing 1 % of lithium perchlorate, 5.3 ml (33.2 mmol) triethylsilane. Yield: 1.94 g (83 %), white solid. Mp: 126-128 °C. IR ν (cm^{-1}): 1737 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.43-1.48 (m, 2H), 1.58-1.64 (m, 2H), 2.18 (t, *J*=7.45 Hz, 2H), 2.34 (t, *J*=7.45 Hz, 2H), 2.55 (s, 4H), 3.66 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 22.5, 24.4, 27.6, 31.0, 33.8, 35.7, 51.7, 174.0, 177.4, 197.6, 198.7. EI-MS: $M^{+\bullet}$ 212 (46), 181 (57), 152 (36), 139 (100), 125 (53), 111 (67), 83 (22), 74 (17), 59 (29), 55 (40). HR-MS calcd for $C_{11}H_{16}O_4$ ($M^{+\bullet}$) 212.104859, found: 212.104912.

6.2.4 Iodination of *C*-alkylated cyclopentane-1,3-diones 54a-c (general procedure):

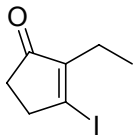
Methyl 8-(2-iodo-5-oxocyclopent-1-enyl)octanoate (53a)



To a solution of triphenylphosphine (3.5 g, 13.3 mmol, 1.125 equiv) in 120 ml of dry CH_3CN was added iodine (3.4 g, 13.3 mmol, 1.125 equiv) in one portion. This slurry was stirred for 2 h at rt and methyl 8-(2-hydroxy-5-oxocyclopent-1-enyl)octanoate (**54a**) (3 g , 11.8 mmol, 1 equiv) was added in one portion followed by immediate addition of triethylamine (1.8 ml, 13 mmol, 1.1 equiv). The resulting mixture was heated to 80 °C for 1h, stirred over night at rt and then heated again to 90 °C for 1h. Ether was added and the mixture was filtered and evaporated. The

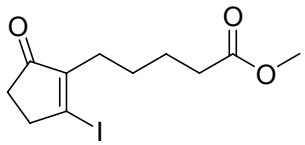
residue was loaded onto a silica column using petrol ether / diethyl ether (1:2) as eluent (light yellow oil, 2.94 g, 68 %). IR ν (cm^{-1}): 1737 (C=O), 1704 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.28-1.44 (m, 8H), 1.59-1.64 (m, 2H), 2.21-2.31 (m, 4H), 2.49-2.51 (m, 2H), 2.98-3.0 (m, 2H), 3.65 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ:25.0, 27.3, 27.5, 29.1 (2C), 29.4, 34.2, 36.9, 39.3, 51.6, 133.9, 151.5, 174.4, 202.5. EI-MS: $M^{+\bullet}$ 364 (23), 333 (35), 291 (8), 249 (19), 237 (100), 222 (34), 205 (84), 177 (11), 145 (18), 69 (13), 59 (27), 55 (43). HR-MS calcd for $C_{14}H_{21}IO_3$ ($M^{+\bullet}$) 364.053547, found: 364.054787.

2-Ethyl-3-iodocyclopent-2-enone (53b)



Synthesised from 4.5 g (17.9 mmol) triphenylphosphine in 150 ml dry CH_3CN, 4.7 g (17.9 mmol) iodine, 2 g (15.9 mmol) 2-ethylcyclopentane-1,3-dione (**54b**), 2.4 ml (17.5 mmol) triethylamine, column: petrol ether / diethyl ether (1:1). Yield: 2.9 g (77 %), yellow oil. IR ν (cm^{-1}): 1709 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.0 (t, J= 7.55Hz, 3H), 2.25-2.3 (m, 2H), 2.5-2.52 (m, 2H), 2.97-3.0 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 11.9, 21.1, 36.9, 39.3, 133.2, 152.6, 202.4. EI-MS: $M^{+\bullet}$ 236 (100), 127 (2), 109 (24), 81 (32). HR-MS calcd for $C_7H_9IO_3$ ($M^{+\bullet}$) 235.969817, found: 235.969070.

Methyl 5-(2-iodo-5-oxocyclopent-1-enyl)pentanoate (53c)

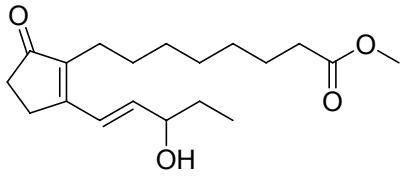


Synthesised from 2.1 g (8 mmol) triphenylphosphine in 60 ml dry CH_3CN, 2 g (8 mmol) iodine, 1.5 g (7.1 mmol) methyl 5-(2-hydroxy-5-oxocyclopent-1-enyl)pentanoate (**54c**), 1.1 ml (7.8 mmol) triethylamine. Yield: 1.82 g (82 %), yellow solid. Mp: 46-48 °C. IR ν (cm^{-1}): 1739 (C=O), 1688 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.43-1.49 (m, 2H), 1.61-1.67 (m, 2H), 2.25-2.28 (m, 2H), 2.33 (t, J=7.55 Hz, 2H), 2.5-2.52 (m, 2H), 2.98-3.0 (m, 2H), 3.66 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 24.8, 26.8, 27.2, 33.9, 36.9, 39.4, 51.7, 134.2, 151.0, 174.1, 202.4.

EI-MS: $M^{+\bullet}$ 322 (14), 290 (49), 249 (72), 222 (15), 195 (15), 163 (84), 129 (54), 115 (59), 100 (69), 87 (58), 59 (100), 55 (78). HR-MS calcd for $C_{11}H_{15}IO_3$ ($M^{+\bullet}$) 322.006597, found: 322.006295.

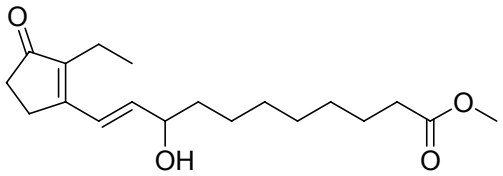
6.2.5 Alkylation of the alkenyl iodides 53a-c (general procedure):

Phytoprostane B_1 type I - Methyl 8-(2-((*E*)-3-hydroxypent-1-enyl)-5-oxocyclopent-1-enyl)octanoate (22a)



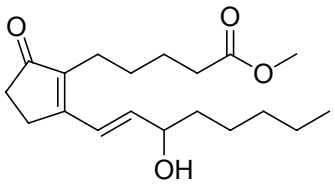
A mixture of methyl 8-(2-iodo-5-oxocyclopent-1-enyl)octanoate (**53a**) (1.7 g, 4.7 mmol, 1 equiv), tetrahydro-2-(pent-1-en-3-yloxy)-2*H*-pyran (1.3 g, 7.8 mmol, 1.67 equiv)[73] (prepared according to literature and used without chromatographic purification) and triethylamine (1.3 ml, 9.3 mmol, 2 equiv) with 120 mg of the palladium catalyst (mixture of palladium diacetate and triphenylphosphine, 1:2 mole ratio) was heated to 100 °C in a small flask for 24 h. Diethyl ether was added and the insoluble compounds were filtered off. After evaporation, the residue was loaded onto a silica gel column using diethyl ether as eluent. For the protected alcohols the THP-group was first cleaved off by dissolving the residue in 42 ml of a 4:2:1 mixture of acetic acid / THF / water and heating to 45 °C over night (white solid, 0.94 g, 65 % over two steps). Mp: 43-46 °C. IR ν (cm^{-1}): 3338 (OH), 1734 (C=O), 1673 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.98 (t, *J*=7.46 Hz, 3H), 1.25-1.28 (m, 6H), 1.35-1.4 (m, 2H), 1.56-1.68 (m, 4H), 2.02 (1H, OH), 2.24-2.29 (m, 4H), 2.4-2.42 (m, 2H), 2.63-2.65 (m, 2H), 3.65 (s, 3H), 4.24-4.28 (m, 1H), 6.25 (dd, *J*= 5.93, 15.68 Hz, 1H), 6.78 (d, *J*=15.87 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 9.8, 23.1, 24.9, 25.7, 28.8, 29.0, 29.1, 29.4, 30.3, 34.0, 34.2, 51.6, 73.7, 124.2, 140.1, 141.5, 163.3, 174.6, 209.8. EI-MS: $M^{+\bullet}$ 322 (28), 304 (12), 291 (9), 273 (16), 265 (51), 263 (50), 233 (100), 205 (28), 133 (26), 121 (29), 105 (21), 91 (32), 79 (28), 59 (21), 55 (42). HR-MS calcd for $C_{19}H_{30}O_4$ ($M^{+\bullet}$) 322.214410, found: 322.212974.

Phytoprostane B_1 type II - (10*E*)-Methyl 11-(2-ethyl-3-oxocyclopent-1-enyl)-9-hydroxyundec-10-enoate (22b)



Synthesised from 1 g (4.2 mmol) 2-ethyl-3-iodocyclopent-2-enone (**53b**), 2.1 g (7.1 mmol) methyl 9-(tetrahydro-2*H*-pyran-2-yloxy)undec-10-enoate,[73,74] (synthesised from methyl 9-hydroxyundec-10-enoate according to literature in 93 % yield; used without chromatographic purification) 1.2 ml (8.5 mmol) triethylamine, 70 mg of the palladium catalyst (mixture of palladium diacetate and triphenylphosphine, 1:2 mole ratio), dissolved in 28 ml of a 4:2:1 mixture of acetic acid/THF/water. Yield: 0.94 g (69 %), yellow oil. IR ν (cm^{-1}): 3430 (OH), 1737 (C=O), 1692 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.0 (t, *J*=7.55 Hz, 3H), 1.31-1.45 (m, 8H), 1.56-1.64 (m, 4H), 1.84 (1H, OH), 2.27-2.31 (m, 4H), 2.4-2.42 (m, 2H), 2.62-2.64 (m, 2H), 3.65 (s, 3H), 4.3-4.34 (m, 1H), 6.25 (dd, *J*=5.93, 15.68 Hz, 1H), 6.81 (d, *J*=15.68 Hz; 1H) ^{13}C NMR (125 MHz, $CDCl_3$) δ: 13.6, 16.4, 25.0, 25.4, 25.7, 29.1, 29.3, 29.4, 34.0, 34.2, 37.4, 51.6, 72.5, 123.9, 140.2, 142.9, 162.8, 174.4, 209.6. EI-MS: $M^{+\bullet}$ 322 (0.25), 304 (100), 273 (13), 189 (55), 175 (23), 161 (25), 133 (18), 119 (10), 105 (18), 91 (20), 59 (4), 55 (10). HR-MS calcd for $C_{19}H_{30}O_4$ ($M^{+\bullet}$) 322.214410, found: 322.214393.

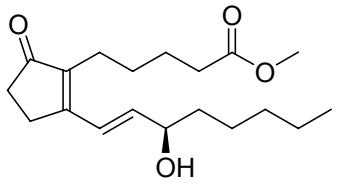
Dinor isoprostane B_1, type III - Methyl 5-(2-((*E*)-3-hydroxyoct-1-enyl)-5-oxo-cyclopent-1-enyl)-pentanoate (22c)



Synthesised from 0.5 g (1.6 mmol) methyl 5-(2-iodo-5-oxocyclopent-1-enyl) pentanoate (**53c**), 0.6 g (2.6 mmol) tetrahydro-2-(oct-1-en-3-yloxy)-2*H*-pyran,[73] 0.4 ml (3.1 mmol) triethylamine, 35 mg of the palladium catalyst (mixture of palladium diacetate and triphenylphosphine, 1:2 mole ratio), dissolved in 14 ml of a 4:2:1 mixture of acetic acid/THF/water. Yield: 0.26 g (52 %), yellow oil. IR ν (cm^{-1}): 3435 (OH), 1736 (C=O), 1689 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.9 (t, *J*=7.1 Hz, 3H), 1.29-1.38 (m, 4H), 1.4-1.47 (m, 4H), 1.57-1.64 (m, 4H), 1.91 (s, 1H, OH),

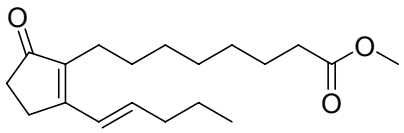
2.28-2.33 (m, 4H), 2.4-2.43 (m, 2H), 2.64-2.65 (m, 2H), 3.65 (s, 3H), 4.33-4.35 (m, 1H), 6.28 (dd, *J*=15.68 Hz, 1H), 6.81 (d, *J*=15.68 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.2, 22.6, 22.7, 24.8, 25.2, 25.8, 28.3, 31.9, 33.9 (2C), 37.3, 51.7, 72.5, 123.8, 140.7, 140.9, 163.7, 174.3, 209.6. EI-MS: $M^{+\bullet}$ 322 (0.4), 304 (100), 273 (40), 244 (70), 215 (44), 187 (64), 173 (46), 159 (33), 147 (48), 133 (70), 105 (49), 91 (54), 55 (14). HR-MS calcd for $C_{19}H_{30}O_4$ ($M^{+\bullet}$) 322.214410, found: 322.213049.

Methyl 5-(2-((*R,E*)-(-)-3-hydroxyoct-1-enyl)-5-oxocyclopent-1-enyl)-pentanoate ((*R*)-(-)-22c)



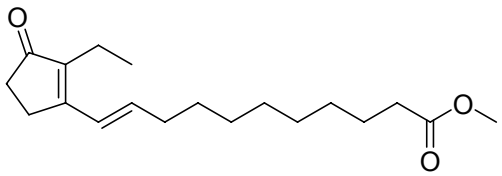
Synthesised from (*R*)-(-)-oct-1-en-3-ol-THP. Yield: 57 %. $[\alpha]^{25}_D$= -24.5° (*c* 0.51, MeOH).

Methyl 8-(5-oxo-2-((*E*)-pent-1-enyl)cyclopent-1-enyl)-octanoate (64a)



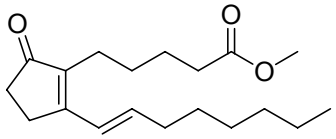
Synthesised from 0.15 g (0.41 mmol) methyl 8-(2-iodo-5-oxocyclopent-1-enyl)octanoate (**53a**), 4 × 90 µl (4 × 0.82 mmol) pent-1-ene, 115 µl (0.82 mmol) triethylamine, 12 mg of the palladium catalyst (mixture of palladium diacetate and triphenylphosphine, 1:2 mole ratio), 40 °C, 4 d. Yield: 122 mg (97 %), yellow oil. IR ν (cm^{-1}): 1738 (C=O), 1693 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.95 (t, *J*=7.35 Hz, 3H), 1.26-1.38 (m, 8H), 1.48-1.54 (m, 2H), 1.57-1.63 (m, 2H), 2.21-2.29 (m, 6H), 2.37-2.39 (m, 2H), 2.61-2.63 (m, 2H), 3.65 (s, 3H), 6.23-6.29 (m, 1H), 6.59 (d, *J*=15.7 Hz), 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 13.8, 22.3, 23.1, 25.1, 25.7, 29.0, 29.2 (2C), 29.6, 33.9, 34.2, 35.6, 51.5, 125.3, 139.4, 139.8, 164.3, 174.4, 209.7. EI-MS: $M^{+\bullet}$ 306 (32), 275 (24), 263 (65), 231 (100), 203 (20), 191 (15), 177 (9), 163 (7), 133 (20), 121 (55), 105 (16), 91 (18), 79 (15), 59 (3), 55 (10). HR-MS calcd for $C_{19}H_{30}O_3$ ($M^{+\bullet}$) 306.219495, found: 306.220961.

(10*E*)-Methyl 11-(2-ethyl-3-oxocyclopent-1-enyl)-undec-10-enoate (64b)



Synthesised from 0.2 g (0.85 mmol) 2-ethyl-3-iodocyclopent-2-enone (**53b**), 0.27 g (1.27 mmol) methyl undec-10-enoate, 0.24 ml (1.69 mmol) triethylamine, 15 mg of the palladium catalyst (mixture of palladium diacetate and triphenylphosphine, 1:2 mole ratio). Yield: 159 mg (59 %), yellow oil. IR ν (cm^{-1}): 1738 (C=O), 1704 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.0 (t, *J*=7.56 Hz, 3H), 1.31 (m, 8H), 1.43-1.49 (m, 2H), 1.59-1.63 (m, 2H), 2.23-2.31 (m, 6H), 2.39-2.4 (m, 2H), 2.61-2.63 (m, 2H), 3.66 (s, 3H), 6.24-6.3 (m, 1H), 6.61 (d, *J*=15.58 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 13.6, 16.4, 25.1, 25.7, 29.0, 29.2, 29.3 (2C), 29.4, 33.6, 34.0, 34.2, 51.6, 125.0, 139.6, 141.1, 164.0, 174.4, 209.6. EI-MS: $M^{+\bullet}$ 306 (18), 275 (8), 163 (26), 135 (100), 124 (10), 107 (8), 91 (10), 69 (1), 59 (2), 55 (6). HR-MS calcd for $C_{19}H_{30}O_3$ ($M^{+\bullet}$) 306.219495, found: 306.218033.

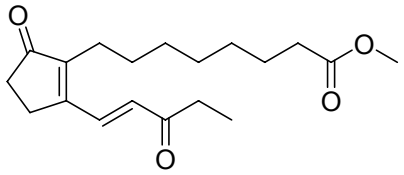
Methyl 5-(2-((*E*)-oct-1-enyl)-5-oxocyclopent-1-enyl)-pentanoate (64c)



Synthesised from 0.2 g (0.62 mmol) methyl 5-(2-iodo-5-oxocyclopent-1-enyl)pentanoate (**53c**), 0.12 g (1.04 mmol) oct-1-ene, 0.17 ml (1.24 mmol) triethylamine, 15 mg of the palladium catalyst (mixture of palladium diacetate and triphenylphosphine, 1:2 mole ratio). Yield: 132 mg (70 %), yellow oil. IR ν (cm^{-1}): 1739 (C=O), 1693 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.89 (t, *J*=6.99 Hz, 3H), 1.26-1.35 (m, 6H), 1.39-1.49 (m, 4H), 1.58-1.64 (m, 2H), 2.22-2.32 (m, 6H), 2.38-2.4 (m, 2H), 2.62-2.64 (m, 2H), 3.65 (s, 3H), 6.25-6.31 (m, 1H), 6.58 (d, *J*=15.8 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.2, 22.7, 22.8, 25.0, 25.8, 28.4, 29.0, 29.1, 31.8, 33.7, 33.9, 34.0, 51.6, 125.0, 139.1, 140.1, 164.7, 174.2, 209.6. EI-MS: $M^{+\bullet}$ 306 (38), 275 (23), 221 (97), 189 (100), 147 (29), 121 (25), 105 (17), 91 (23), 59 (4), 55 (11). HR-MS calcd for $C_{19}H_{30}O_3$ ($M^{+\bullet}$) 306.219495, found: 306.220204.

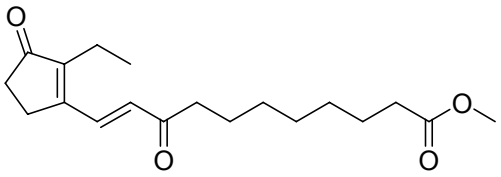
6.2.6 Oxidation of phytoprostanes 22a-c (general procedure):

Methyl 8-(5-oxo-2-((*E*)-3-oxopent-1-enyl)cyclopent-1-enyl)-octa-noate (65a)



To a stirred solution of Phytoprostane B_1 type I methyl ester (**22a**) (50 mg, 155 μmol, 1 equiv) in 3 ml of methylene chloride was added Bobbitt's reagent (4-acetylamino-2,2,6,6-tetramethylpiperidine-1-oxoammonium perchlorate) (51 mg, 163 μmol, 1.05 equiv) and 50 mg of silica gel to catalyse the oxidation. The yellow slurry was stirred until the colour changed to white (~ 5 h) and GC showed complete oxidation of the alcohol. The slurry was filtered and the filter washed with methylene chloride. After evaporation of the solvent, the residue was loaded onto a silica gel column using diethyl ether for elution (yellow oil, 40 mg, 81 %). IR ν (cm^{-1}): 1737 (C=O), 1698 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.16 (t, *J*=7.22 Hz, 3H), 1.27-1.29 (m, 6H), 1.39-1.42 (m, 2H), 1.58-1.6 (m, 2H), 2.29 (t, *J*=7.45 Hz, 2H), 2.32-2.36 (m, 2H), 2.46-2.48 (m, 2H), 2.66-2.71 (m, 4H), 3.65 (s, 3H), 6.57 (d, *J*=15.8 Hz, 1H), 7.64 (d, *J*=15.8 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 8.1, 23.6, 25.0, 25.5, 29.1 (3C), 29.5, 34.0, 34.2, 34.9, 51.6, 130.4, 134.1, 148.0, 160.2, 174.4, 200.6, 209.0. EI-MS: $M^{+\bullet}$ 320 (17), 289 (18), 263 (96), 231 (100), 203 (27), 189 (9), 171 (8), 161 (10), 147 (11), 133 (19), 121 (21), 105 (14), 91 (18), 59 (7), 55 (16). HR-MS calcd for $C_{19}H_{28}O_4$ ($M^{+\bullet}$) 320.198760, found: 320.199851.

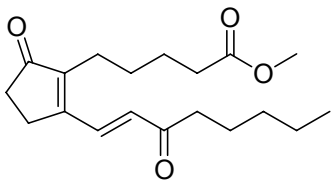
(10*E*)-Methyl 11-(2-ethyl-3-oxocyclopent-1-enyl)-9-oxoundec-10-enoate (65b)



Synthesised from 100 mg (0.31 mmol) Phytoprostane B_1 type II methyl ester (**22b**) in 5 ml methylene chloride, 102 mg (0.33 mmol) Bobbitt's reagent (4-acetylamino-2,2,6,6-tetramethylpiperidine-1-oxoammonium perchlorate), 100 mg silica gel. Yield: 85 mg (86 %), yellow oil. IR ν (cm^{-1}): 1737 (C=O), 1697 (C=O). ^{1}H NMR

(500 MHz, $CDCl_3$) δ: 1.04 (t, *J*=7.56 Hz, 3H), 1.32-1.35 (m, 6H), 1.58-1.68 (m, 4H), 2.29 (t, *J*=7.56 Hz, 2H), 2.38 (q, *J*=7.56 Hz, 2H), 2.46-2.48 (m, 2H), 2.62-2.68 (m, 4H), 3.65 (s, 3H), 6.56 (d, *J*=16.04 Hz, 1H), 7.65 (d, *J*=16.04 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 13.8, 16.9, 24.1, 25.0, 25.5, 29.1 (2C), 29.2, 34.0, 34.2, 41.6, 51.6, 130.6, 134.1, 149.4, 159.7, 174.3, 200.2, 208.9. EI-MS: $M^{+\bullet}$ 320 (26), 289 (11), 178 (11), 163 (43), 135 (100), 121 (11), 107 (9), 91 (11), 59 (3), 55 (13). HR-MS calcd for $C_{19}H_{28}O_4$ ($M^{+\bullet}$) 320.198760, found: 320.199601.

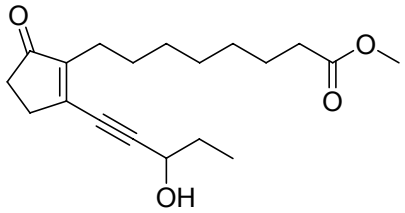
Methyl 5-(5-oxo-2-((*E*)-3-oxooct-1-enyl)cyclopent-1-enyl)-penta-noate (65c)



Synthesised from 50 mg (155 μmol) Phytoprostane B_1 type III methyl ester (**22c**) in 3 ml methylene chloride, 51 mg (163 μmol) Bobbitt's reagent (4-acetylamino-2,2,6,6-tetramethylpiperidine-1-oxoammonium perchlorate), 50 mg silica gel. Yield: 39 mg (78 %), yellow oil. IR ν (cm^{-1}): 1737 (C=O), 1697 (C=O). ^1H NMR (500 MHz, $CDCl_3$) δ: 0.9 (t, *J*=7 Hz, 3H), 1.31-1.37 (m, 4H), 1.43-1.49 (m, 2H), 1.6-1.7 (m, 4H), 2.32 (t, *J*=7.45 Hz, 2H), 2.38 (t, *J*=7.68 Hz, 2H), 2.46-2.48 (m, 2H), 2.64-2.69 (m, 4H), 3.65 (s, 3H), 6.57 (d, *J*=15.81 Hz, 1H), 7.59 (d, *J*=16.04 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.1, 22.6, 23.3, 23.9, 24.9, 25.6, 28.5, 31.5, 33.9, 34.0, 41.7, 51.7, 130.9, 134.0, 147.4, 160.6, 174.0, 200.4, 209.0. EI-MS: $M^{+\bullet}$ 320 (100), 289 (20), 221 (71), 189 (92), 161 (36), 147 (46), 133 (23), 105 (17), 91 (24), 59 (5), 55 (11). HR-MS calcd for $C_{19}H_{28}O_4$ ($M^{+\bullet}$) 320.198760, found: 320.197166.

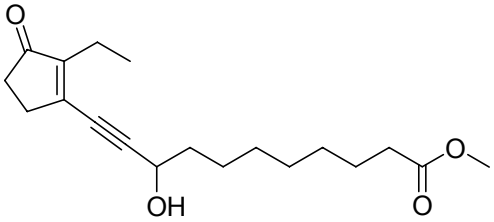
6.2.7 Alkynylation of alkenyl iodides 53a-c (general procedure):

Methyl 8-(2-(3-hydroxypent-1-ynyl)-5-oxocyclopent-1-enyl)-octanoate (66a)



To a stirred solution of $PdCl_2(PPh_3)_2$ (40 mg, 0.06 mmol, 0.07 equiv) and CuI (24 mg, 0.12 mmol, 0.15 equiv) in 1 ml of anhydrous DMF was added successively pent-1-yn-3-ol (0.1 g, 1.24 mmol, 1.5 equiv), a solution of methyl 8-(2-iodo-5-oxocyclopent-1-enyl)octanoate (**53a**) (0.3 g, 0.82 mmol, 1 equiv) in 4 ml of anhydrous DMF and triethylamine (0.14 ml, 0.99 mmol, 1.2 equiv). The mixture was stirred for 24 h at rt and saturated NH_4Cl was added and extracted with diethyl ether. After washing with saturated NaCl and water and drying over Na_2SO_4, the ether was evaporated and the crude residue loaded onto a silica gel column using diethyl ether as eluent (yellow oil, 0.23 g, 88 %). IR ν (cm^{-1}): 3424 (OH), 1738 (C=O), 1699 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.06 (t, *J*=7.45 Hz, 3H), 1.26-1.31 (m, 6H), 1.42-1.48 (m, 2H), 1.57-1.63 (m, 2H), 1.77-1.85 (m, 2H), 2.27-2.31 (m, 4H), 2.4-2.42 (m, 2H), 2.61-2.63 (m, 2H), 3.66 (s, 3H), 4.6 (t, *J*=6.5 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 9.6, 24.6, 25.0, 27.7, 29.0, 29.1, 29.3, 30.3, 30.9, 34.2, 34.3, 51.7, 64.4, 80.7, 106.4, 149.1, 149.8, 174.6, 209.0. EI-MS: $M^{+\bullet}$ 320 (5), 302 (20), 271 (28), 261 (100), 231 (30), 203 (31), 191 (37), 187 (33), 161 (27), 147 (26), 133 (46), 121 (30), 105 (29), 91 (54), 59 (14), 55 (34). HR-MS calcd for $C_{19}H_{28}O_4$ ($M^{+\bullet}$) 320.198760, found: 320.198096.

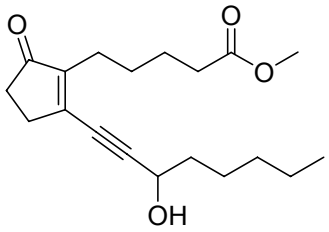
Methyl 11-(2-ethyl-3-oxocyclopent-1-enyl)-9-hydroxyundec-10-ynoate (66b)



Synthesised from 31 mg (0.04 mmol) $PdCl_2(PPh_3)_2$, 18 mg (0.09 mmol) CuI in 1 ml anhydrous DMF, 0.2 g (0.95 mmol) methyl 9-hydroxy-undec-10-ynoate,[74] (synthesized from methyl undec-10-ynoate according to literature in 22 % yield)

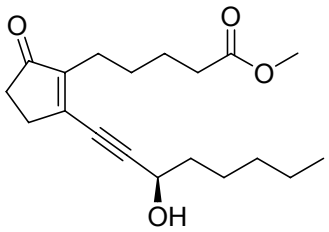
0.15 g (0.64 mmol) 2-ethyl-3-iodocyclopent-2-enone (**53b**) in 3 ml anhydrous DMF, 0.11 ml (0.76 mmol) triethylamine. Yield: 185 mg (91 %), brown oil. IR ν (cm^{-1}): 3423 (OH), 1738 (C=O), 1699 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.05 (t, *J*=7.59 Hz, 3H), 1.31-1.38 (m, 6H), 1.46-1.52 (m, 2H), 1.59-1.65 (m, 2H), 1.74-1.83 (m, 2H), 2.08 (s, 1H, OH), 2.28-2.33 (m, 4H). 2.4-2.42 (m, 2H), 2.6-2.62 (m, 2H), 3.66 (s, 3H), 4.64 (t, *J*=6.6 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 12.5, 18.0, 25.0, 25.2, 29.1 (2C), 29.3, 30.2, 34.2, 34.3, 37.7, 51.6, 63.1, 80.6, 106.5, 149.2, 150.4, 174.4, 208.9. EI-MS: $M^{+\bullet}$ 320 (3), 302 (49), 289 (10), 187 (24), 163 (54), 135 (100), 91 (56), 74 (36), 59 (17), 55 (53). HR-MS calcd for $C_{19}H_{28}O_4$ ($M^{+\bullet}$) 320.198760, found: 320.199075.

Methyl 5-(2-(3-hydroxyoct-1-ynyl)-5-oxocyclopent-1-enyl)-pentanoate (66c)



Synthesised from 31 mg (0.04 mmol) $PdCl_2(PPh_3)_2$, 18 mg (0.09 mmol) CuI in 1 ml anhydrous DMF, 0.12 g (0.93 mmol) oct-1-yn-3-ol, 0.2 g (0.62 mmol) methyl 5-(2-iodo-5-oxocyclopent-1-enyl)pentanoate (**53c**) in 3 ml anhydrous DMF, 0.1 ml (0.75 mmol) triethylamine. Yield: 171 mg (86 %), brown oil. IR ν (cm^{-1}): 3423 (OH), 1739 (C=O), 1699 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.9 (t, *J*=7.1 Hz, 3H), 1.32-1.36 (m, 4H), 1.46-1.55 (m, 4H), 1.6-1.66 (m, 2H), 1.73-1.85 (m, 2H), 2.3-2.36 (m, 4H), 2.4-2.42 (m, 2H), 2.61-2.63 (m, 2H), 3.66 (s, 3H), 4.63 (q, *J*=6.11 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.1, 22.7, 23.9, 24.7, 25.0, 27.2, 30.2, 31.6, 33.8, 34.2, 37.6, 51.8, 63.0, 80.5, 107.5, 148.5, 150.4, 174.6, 208.8. EI-MS: $M^{+\bullet}$ 320 (1), 302 (100), 271 (22), 245 (16), 215 (56), 189 (34), 171 (37), 147 (31), 133 (27), 105 (19), 91 (39), 59 (8), 55 (22). HR-MS calcd for $C_{19}H_{28}O_4$ ($M^{+\bullet}$) 320.198760, found: 320.197846.

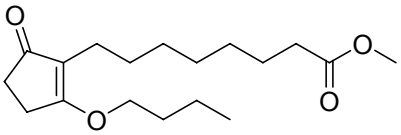
(*R*)-(+)-Methyl 5-(2-(3-hydroxyoct-1-ynyl)-5-oxocyclopent-1-enyl)pentanoate ((*R*)-(+)-66c)



Synthesised from (*R*)-(+)-oct-1-yn-3-ol. Yield: 70 %. $[\alpha]^{25}_{D}$= 6.2° (*c* 0.50, MeOH).

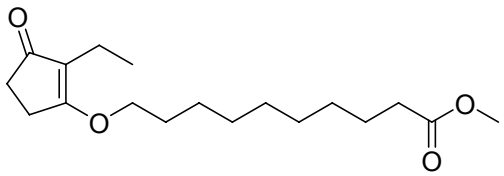
6.2.8 Preparation of the *O*-alkyl ethers from 54a-c (general procedure):

Methyl 8-(2-butoxy-5-oxocyclopent-1-enyl)-octanoate (67a)



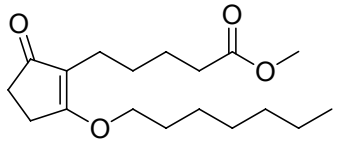
To a stirring solution of methyl 8-(2-hydroxy-5-oxocyclopent-1-enyl)octanoate (**54a**) (0.5 g, 2 mmol, 1 equiv) in 10 ml DMSO was added 1-iodobutane (0.36 g, 2 mmol, 1 equiv). Sodium hydride (0.12 g, 3 mmol, 1.5 equiv) was added in portions and the mixture was stirred for 24 h at rt. Water was added and the product extracted with diethyl ether. After washing with saturated $NaHCO_3$ and water, the organic phase was dried over Na_2SO_4 and evaporated. The residue was loaded onto a silica gel column using petrol ether / ethyl acetate (1:1) for elution (yellow oil, 254 mg, 42 %). IR ν (cm^{-1}): 1738 (C=O), 1687 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.96 (t. *J*=7.5 Hz, 3H), 1.22-1.49 (m, 10H), 1.54-1.63 (m, 2H), 1.69-1.74 (m, 2H), 2.08-2.11 (m, 2H), 2.27 (t, *J*=7.5 Hz, 2H), 2.41-2.42 (m, 2H), 2.61-2.63 (m, 2H), 3.64 (s, 3H), 4.12 (t, *J*=6.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 13.8, 19.1, 21.3, 25.0, 25.1, 27.9, 29.1, 29.2, 29.4, 31.8, 33.6, 34.2, 51.5, 69.2, 120.9, 174.4, 184.6, 205.1. EI-MS: $M^{+\bullet}$ 310 (20), 279 (15), 237 (28), 223 (7), 195 (13), 181 (21), 168 (41), 153 (8), 139 (38), 125 (31), 112 (100), 96 (10), 55 (18). HR-MS calcd for $C_{18}H_{30}O_4$ ($M^{+\bullet}$) 310.214410, found: 310.215523.

Methyl 10-(2-ethyl-3-oxocyclopent-1-enyloxy)-decanoate (67b)



Synthesised from 0.5 g (4 mmol) 2-ethylcyclopentane-1.3-dione (**54b**) in 10 ml DMSO, 1.1 g (4 mmol) methyl 10-bromodecanoate, 0.24 g (6 mmol) sodium hydride. Yield: 0.91 g (74 %), white solid. Mp: 43-44 °C. IR ν (cm^{-1}): 1739 (C=O), 1679 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.97 (t, *J*=7.45 Hz, 3H), 1.3-1.43 (m, 10H), 1.58-1.62 (m, 2H), 1.7-1.75 (m, 2H), 2.14 (q, *J*=7.48 Hz, 2H), 2.29 (t, *J*=7.56 Hz, 2H), 2.39-2.41 (m, 2H), 2.6-2.62 (m, 2H), 3.65 (s, 3H), 4.12 (t, *J*=6.53 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 12.7, 14.8, 25.0 (2C), 25.9, 29.2, 29.3 (2C), 29.4, 29.7, 33.7, 34.2, 51.5, 69.4, 122.2, 174.4, 184.1, 204.9. EI-MS: $M^{+\bullet}$ 310 (29), 279 (23), 237 (9), 185 (24), 153 (41), 135 (25), 127 (100), 126 (99), 11 (30), 83 (26), 69 (49), 59 (10), 55 (36). HR-MS calcd for $C_{18}H_{30}O_4$ ($M^{+\bullet}$) 310.214410, found 310.214056.

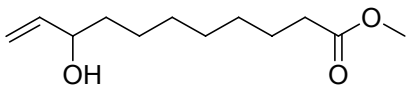
Methyl 5-(2-(heptyloxy)-5-oxocyclopent-1-enyl)-pentanoate (67c)



Synthesised from 0.2 g (0.94 mmol) methyl 5-(2-hydroxy-5-oxocyclopent-1-enyl)pentanoate (**54c**) in 5 ml DMSO, 0.21 g (0.94 mmol) 1-iodoheptane, 57 mg (1.42 mmol) sodium hydride. Yield: 0.14 g (48 %), yellow oil. IR ν (cm^{-1}): 1738 (C=O), 1688 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.88 (t, *J*=7 Hz, 3H), 1.27-1.46 (m, 10H), 1.55-1.61 (m, 2H), 1.7-1.75 (m, 2H), 2.13 (t, *J*=7.46 Hz, 2H), 2.29 (t, *J*=7.74 Hz, 2H), 2.4-2.42 (m, 2H), 2.62-2.64 (m, 2H), 3.64 (s, 3H), 4.11 (t, *J*=6.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.2, 20.9, 22.7, 24.8, 25.0, 25.8, 27.4, 29.0, 29.7, 31.8, 33.6, 34.0, 51.5, 69.5, 120.2, 174.4, 184.8, 205.0. EI-MS: $M^{+\bullet}$ 310 (32), 279 (25), 237 (66), 223 (22), 210 (19), 180 (48), 139 (100), 125 (46), 112 (86), 55 (23). HR-MS calcd for $C_{18}H_{30}O_4$ ($M^{+\bullet}$) 310.214410, found 310.213246.

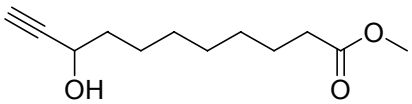
6.2.9 Allylic oxidations (general procedure):

Methyl 9-hydroxyundec-10-enoate



A mixture of selenium dioxide (1.4 g; 12.6 mmol; 0.5 equiv) and *tert*-butyl hydroperoxide (5.6 ml; 50.4 mmol; 2 equiv) in 50 ml of absolute dichloromethane was stirred at room temperature for 0.5 h. Then, methyl undec-10-enoate (5 g; 25.2 mmol; 1 equiv) in 15 ml of absolute dichloromethane was added dropwise over a period of 20 min with cooling and the mixture was stirred at room temperature for 5 d. Dichloromethane was removed, water added and extracted with benzene. The benzene layer was washed with water, saturated NaCl, dried over Na_2SO_4 and evaporated. The oily residue was loaded onto a silica gel column using petrol ether / diethyl ether (2:1) as eluent (colourless oil, 3.19 g; 59 %). IR ν (cm^{-1}): 3441 (OH), 1739 (C=O). ^{1}H-NMR (500 MHz; $CDCl_3$) δ: 1.25-1.4 (m; 8H); 1.48-1.62 (m; 4H); 2.29 (t; *J*= 7.52; 2H); 3.65 (s; 3H); 4.05-4.1 (m; 1H); 5.07-5.22 (m; 2H); 5.81-5.89 (m; 1H). ^{13}C-NMR (125 MHz; $CDCl_3$) δ: 25.03; 25.35; 29.16; 29.27; 29.43; 34.2; 37.12; 51.55; 73.31; 114.62; 141.46; 174.42. EI-MS: $[M-18]^{+\bullet 0}$ 196 (1), 158 (37). 125 (11), 115 (29), 101 (12), 87 (100), 74 (54). 59 (17), 55 (53). HR-MS calcd for $C_{12}H_{22}O_2$ ($[M-18]^{+\bullet}$) 196.146330 ($-H_2O$), found: 196.146816.

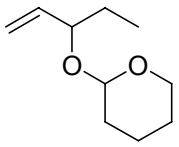
Methyl 9-hydroxyundec-10-ynoate



Synthesised from 0.28 g (2.55 mmol) selenium dioxide, 1.2 ml (1.02 mmol) tert-butyl hydroperoxide in 10 ml anhydrous dichloromethane, 1 g methyl undec-10-ynoate (5.1 mmol) in 3 ml anhydrous dichloromethane. Yield: 240 mg (22 %), colourless oil. IR ν (cm^{-1}): 3292 (OH), 1736 (C=O). ^{1}H-NMR (500 MHz; $CDCl_3$) δ: 1.3-1.35 (m; 6H); 1.42-1.48 (m; 2H); 1.58-1.74 (m; 4H); 2.29 (t; *J*=7.45 Hz; 2H); 2.45 (d; *J*=2.06 Hz; 1H); 3.66 (s; 3H); 4.34-4.37 (m; 1H). ^{13}C-NMR (125 MHz; $CDCl_3$) δ: 25.02; 25.04; 29.13 (2C); 29.21; 34.2; 37.74; 51.56; 62.4; 72.92; 85.18; 174.42. EI-MS: $[M-54]^{+\bullet}$ 158 (22), 125 (11), 115 (12), 87 (42), 74 (46), 55 (100). HR-MS calcd for $C_9H_{18}O_2$ ($[M-54]^{+\bullet}$) 158.130680 ($-C_3H_2O$); Found: 158.131166.

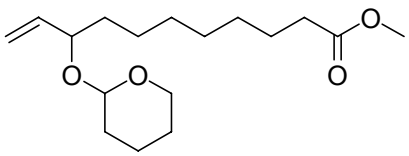
6.2.10 Preparation of the THP-ethers (general procedure):

Tetrahydro-2-(pent-1-en-3-yloxy)-2*H*-pyran



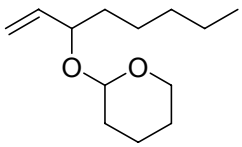
To a stirred solution of pent-1-en-3-ol (1 g; 11.6 mmol; 1 equiv) and dihydropyran (5.3 ml; 58 mmol; 5 equiv) in 50 ml methylene chloride, was added *p*-toluenesulfonic acid monohydrate (22 mg, 0.12 mmol, 0.01 equiv) and the mixture was kept at room temperature for 3 h. Then 60 ml of diethyl ether were added and the organic phase was washed with a mixture of 20 ml of saturated $NaHCO_3$, 20 ml of saturated NaCl and 40 ml of water. The water phase was extracted with ether and the combined ether phases were dried over Na_2SO_4 and evaporated (1.94 g; 96 %). The resulting oily THP-ether was used in the following Heck reaction without further purification.

Methyl 9-(tetrahydro-2*H*-pyran-2-yloxy)undec-10-enoate



Synthesised from 2 g methyl 9-hydroxyundec-10-enoate (9.3 mmol), 4.3 ml dihydropyran (46.7 mmol) in 100 ml methylene chloride, 18 mg *p*-toluenesulfonic acid monohydrate (0.1 mmol). Yield: 2.58 g (93 %). The resulting oily THP-ether was used in the following Heck reaction without further purification.

Tetrahydro-2-(oct-1-en-3-yloxy)-2*H*-pyran



Synthesised from 1g oct-1-en-3-ol (7.8 mmol), 3.6 ml dihydropyran (39 mmol) in 50 ml methylene chloride, 18 mg *p*-toluenesulfonic acid monohydrate (0.1 mmol). Yield: 1.52 g (92 %). The resulting oily THP-ether was used in the following Heck reaction without further purification.

7 References

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9 Curriculum vitae

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Marital status:	Single		

Education

1986-1998	School in Greiz; A-level: 1.5
1998-2003	Study of chemistry at the Friedrich-Schiller-University Jena, School for Chemical and Earth Sciences; Specialisation: Bioorganic Chemistry
2002-2003	Diploma thesis at the Max Planck Institute for Chemical Ecology Jena; department: Bioorganic Chemistry; Diploma: 1.8
2003-	PhD position at the Max Planck Institute for Chemical Ecology Jena; department: Bioorganic Chemistry

Work Experience

1998	two-week work placement at the City Pharmacy in Sondershausen
1999	five weeks as a student trainee at the company Höchst Marion Roussel in Frankfurt am Main
2000	six weeks of sales promotion for the Wrigley's company
2001	two month student assistant at the Friedrich-Schiller-University Jena; School for Chemical and Earth Sciences; working group Prof. Dr. D. Klemm; cellulose research

Skills and Qualification

Languages:	German: Mother tongue English: fluent, reading, writing French: basic understanding
Computing:	MS Office, Mozilla, Adobe Photoshop, Scifinder, Beilstein, ChemOffice, MassLynx, OPUS, WINNMR, Xcalibur

Publications

- Schulze, B.; Lauchli, R.; Sonwa, M. M.; Schmidt, A.; Boland, W. *Analytical Biochemistry* **2006**, 348, 269-283. Profiling of structurally labile oxylipins in plants by in situ derivatization with pentafluorobenzyl hydroxylamine.

- Schmidt, A.; Boland, W. *Journal of Organic Chemistry* **2007**. General Strategy for the Synthesis of B_1 Phytoprostanes, Dinor Isoprostanes and Analogs. *Available online:* http://pubs.acs.org/.

Public talks and posters

- Talk: "A short and efficient synthesis of Phytoprostane B_1 type I and II"; 1st IMPRS-Symposium; 19th March 2005

- Poster: "Synthesis and biological activity of phytoprostanes"; 2nd IMPRS-Symposium; 4th November 2005

- Talk: „Pflanzen contra Herbivore – Überleben mit Chemie"; Gymnasium Greiz; 21st November 2005

- Talk: "A short and efficient Synthesis of Phytoprostane B_1 type I, II, Dinor Isoprostane B_1 type III and several Analogues"; 3rd IMPRS-Symposium; 21st March 2006

- Talk: "A short and efficient synthesis of Phytoprostane B_1 type I, II and several analogues"; Naturstoffe: Chemie, Biologie und Ökologie; 31. Doktoranden-workshop in Würzburg; 8th May 2006

- Poster: „B_1-Phytoprostanes: synthesis and biological activity"; 4th IMPRS-Symposium; 10th November 2006

……..

Annika Schmidt

Selbständigkeitserklärung

Hiermit erkläre ich, Annika Schmidt, dass ich die vorliegende Arbeit selbständig und unter Verwendung der angegebenen Hilfsmittel, persönlichen Mitteilungen und Quellen angefertigt habe.

Jena, den …… ……..

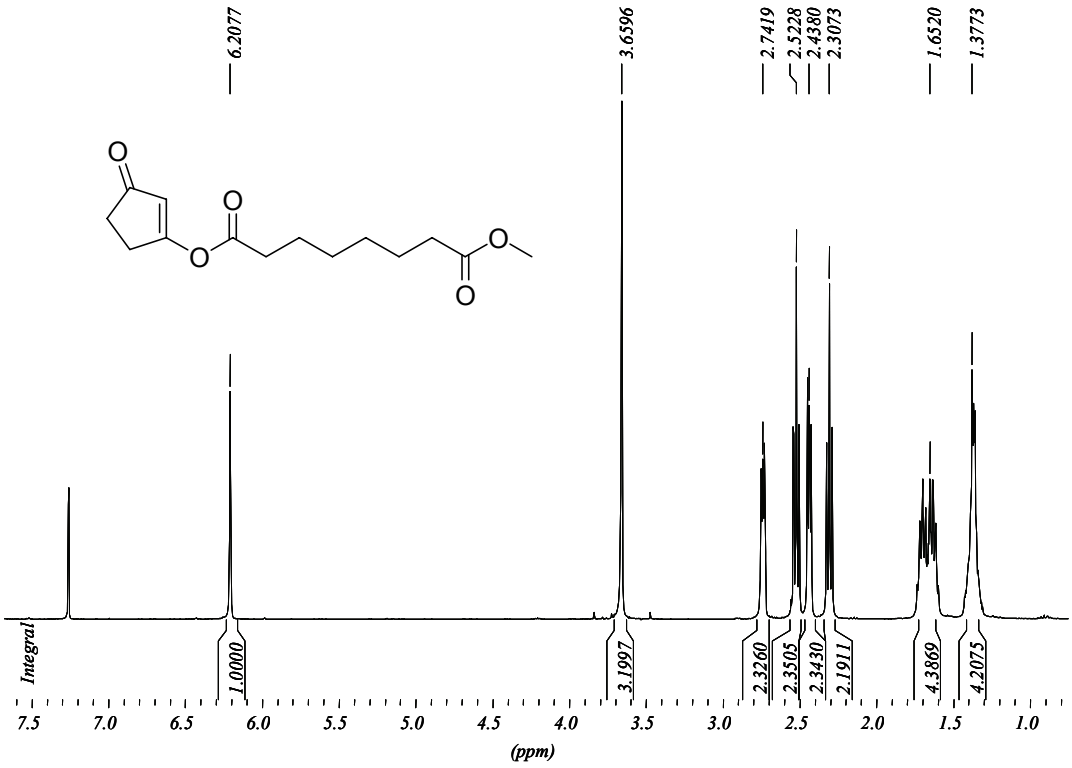
Annika Schmidt

10 Supplementary material

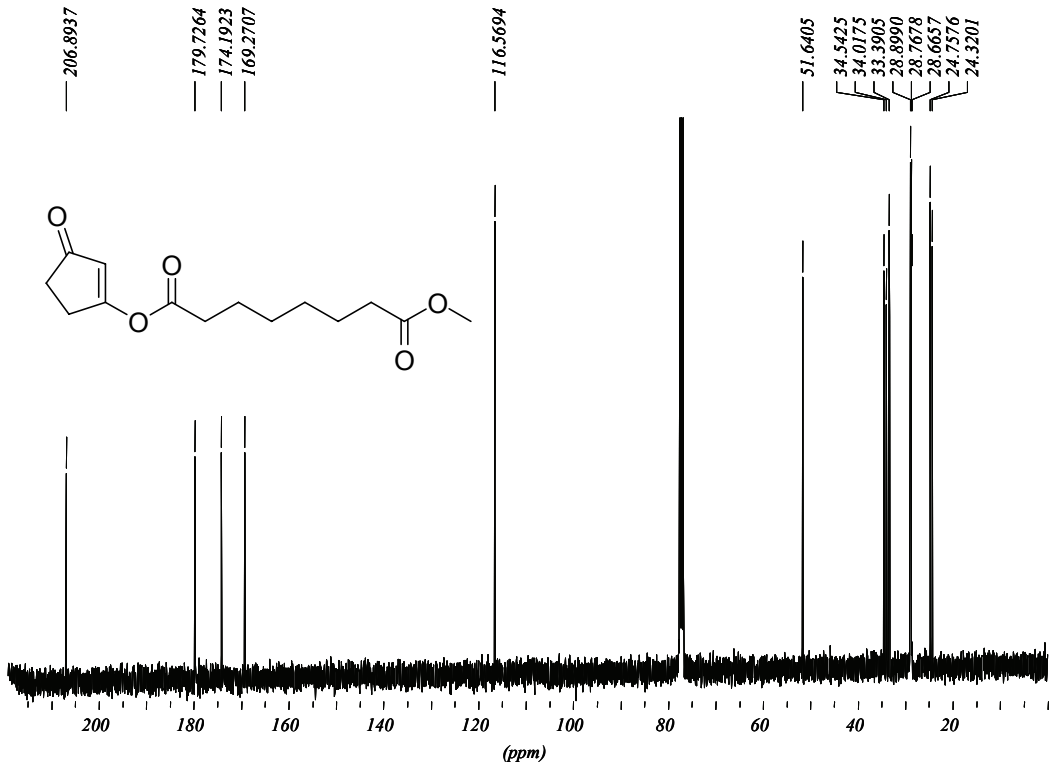
10.1 ^{1}H NMR, ^{13}C NMR, EI-MS, IR spectra of synthesised compounds

10.1.1 Methyl 3-oxocyclopent-1-enyl octanedioate (58a)

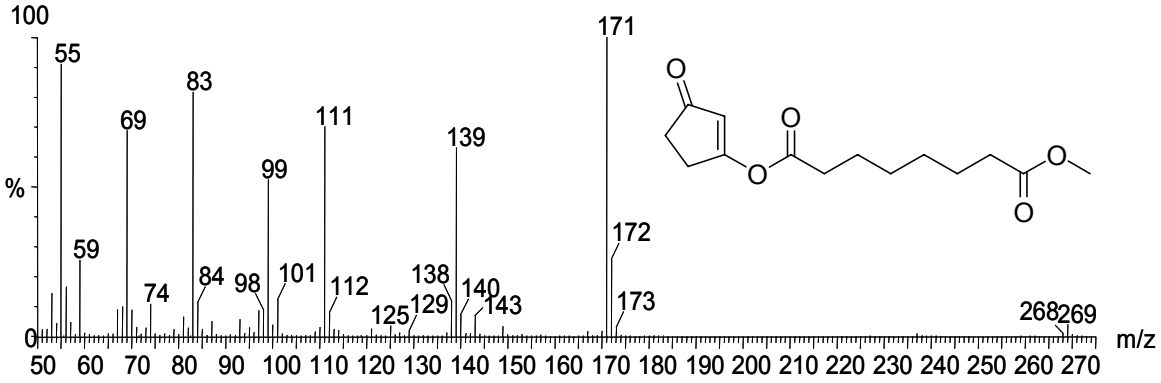
^{1}H NMR



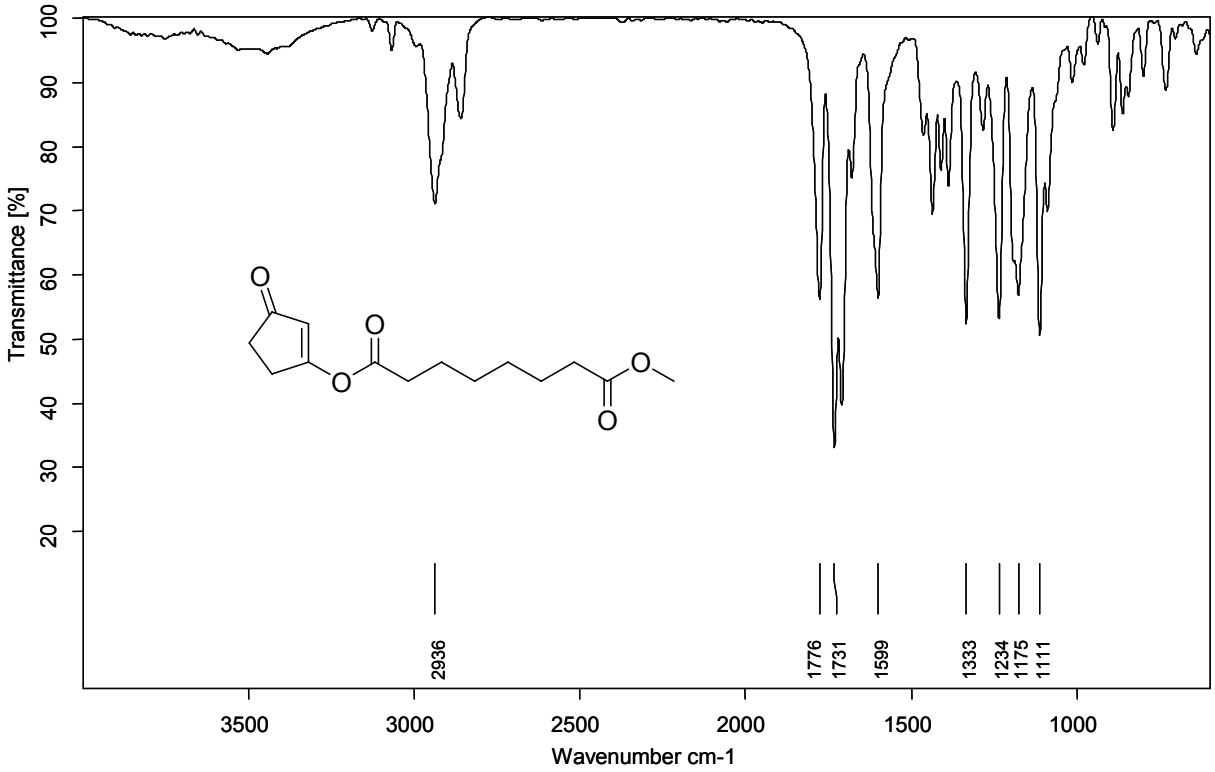
^{13}C NMR


206.8937
179.7264
174.1923
169.2707
116.5694
51.6405
34.5425
34.0175
33.3905
28.8990
28.7678
28.6657
24.7576
24.3201
200
180
160
140
120
100
80
60
40
20
(ppm)

EI-MS

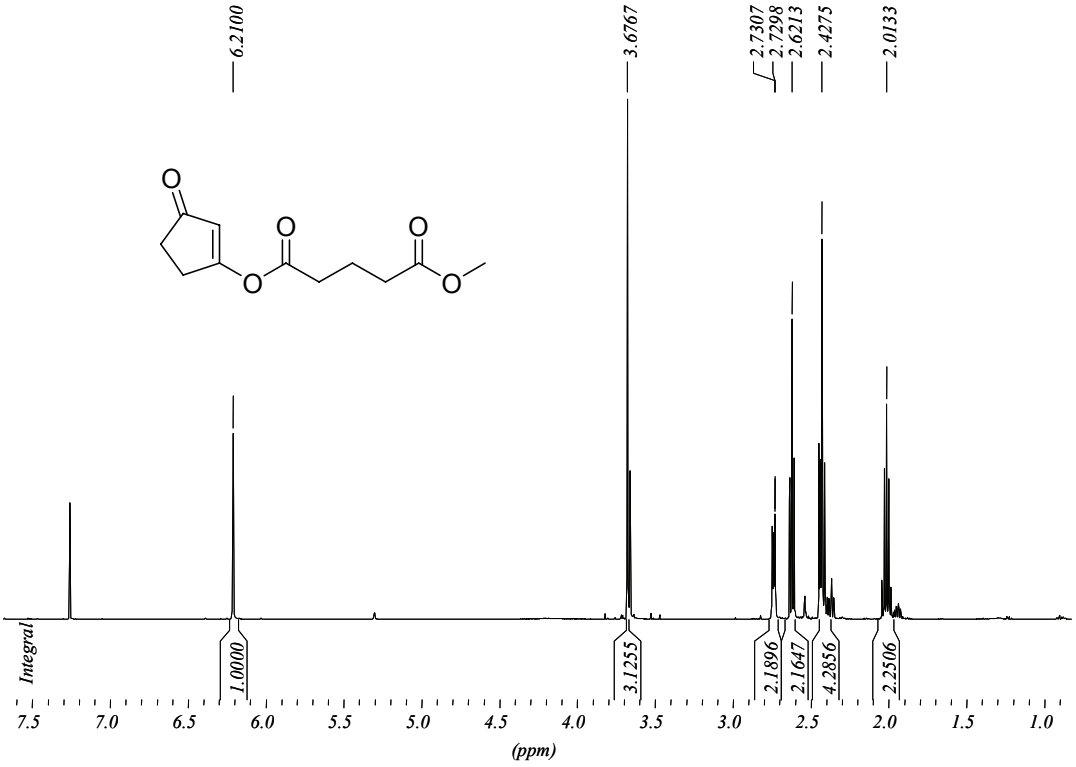

100
%
0
55
69
83
59
74
84
98
99
101
111
112
125
129
138
139
140
143
171
172
173
268
269
m/z
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270

IR

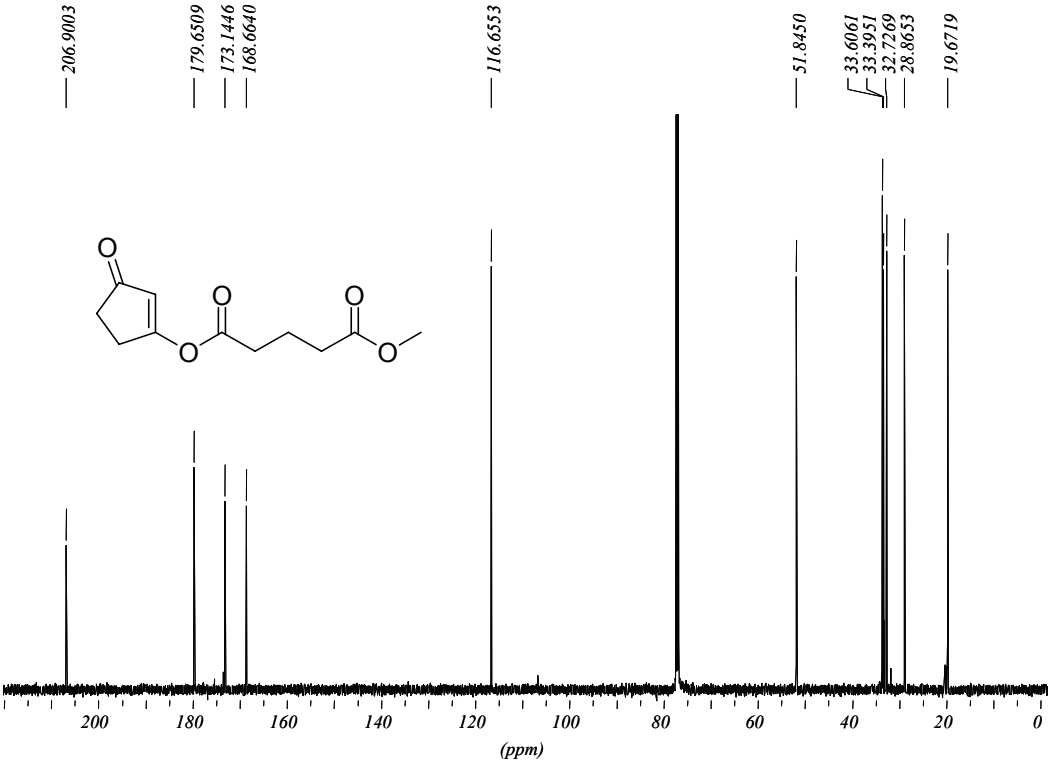

Transmittance [%]
100
90
80
70
60
50
40
30
20
2936
1776
1731
1599
1333
1234
1175
1111
3500
3000
2500
2000
1500
1000
Wavenumber cm-1
O
O
O
O
O

10.1.2 Methyl 3-oxocyclopent-1-enyl glutarate (58b)

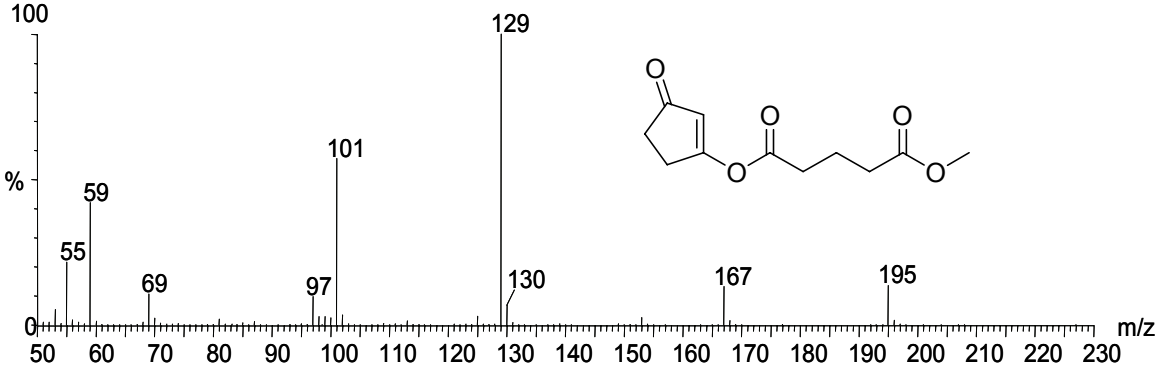
^{1}H NMR



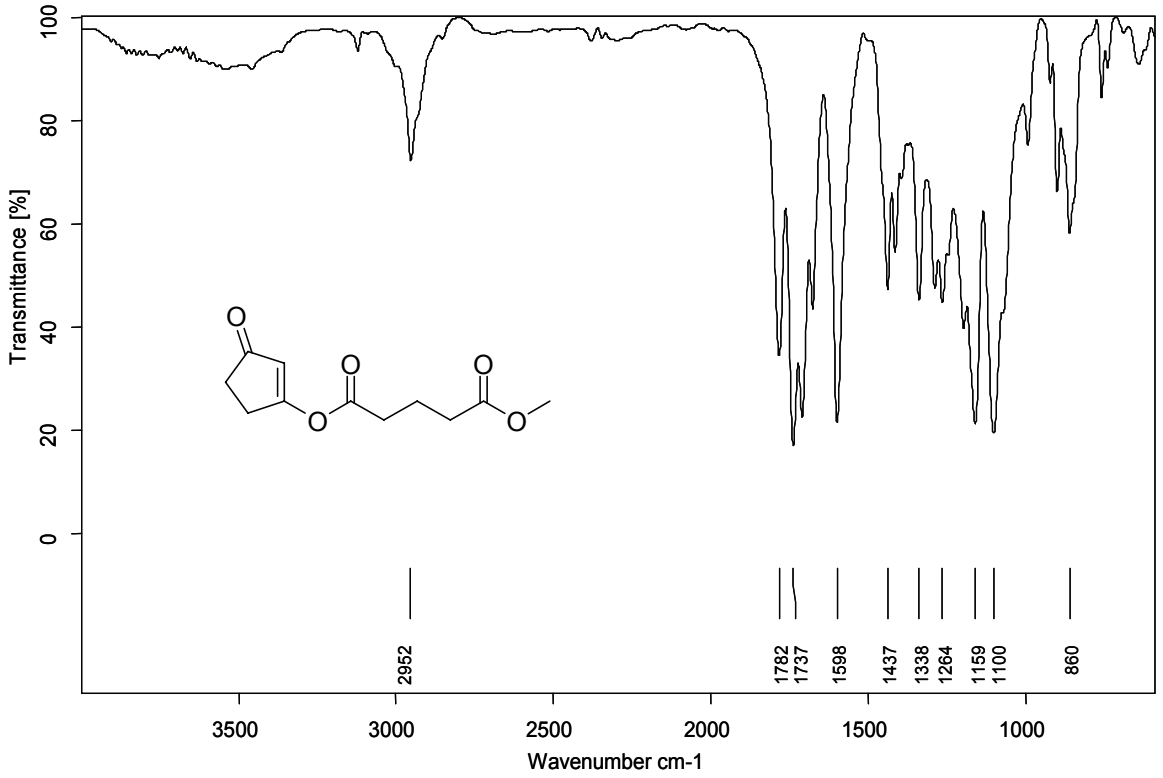
^{13}C NMR



EI-MS

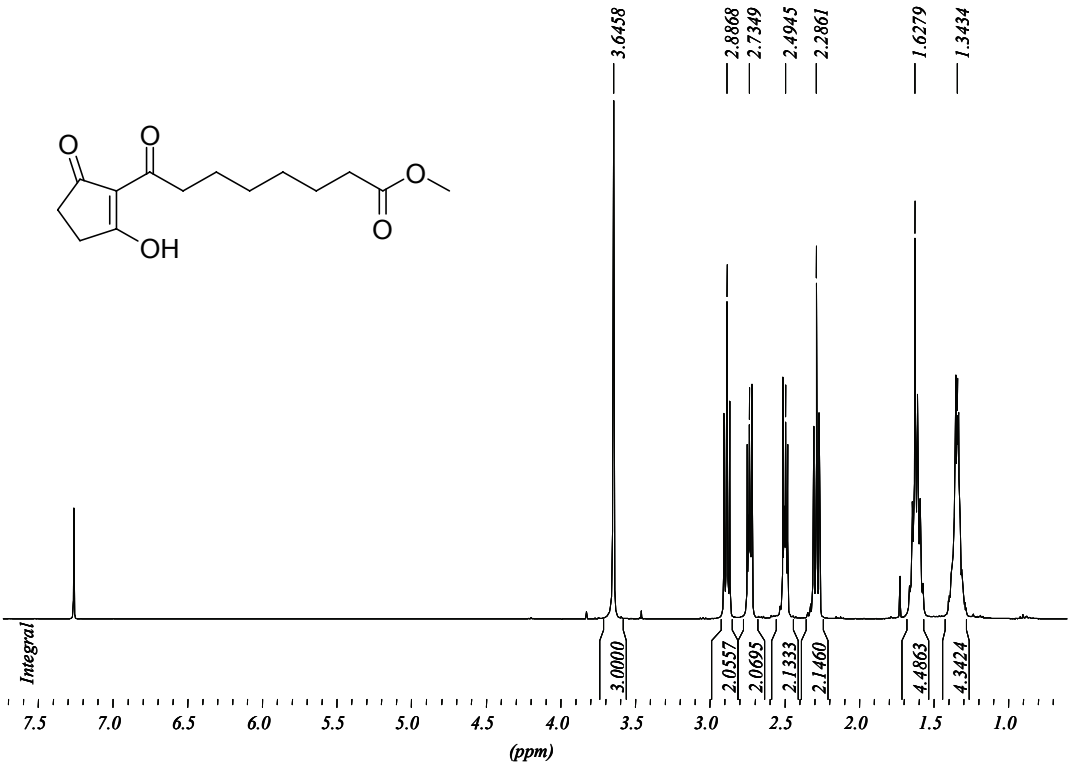

100
%
0
129
101
59
55
69
97
130
167
195
m/z
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230

IR

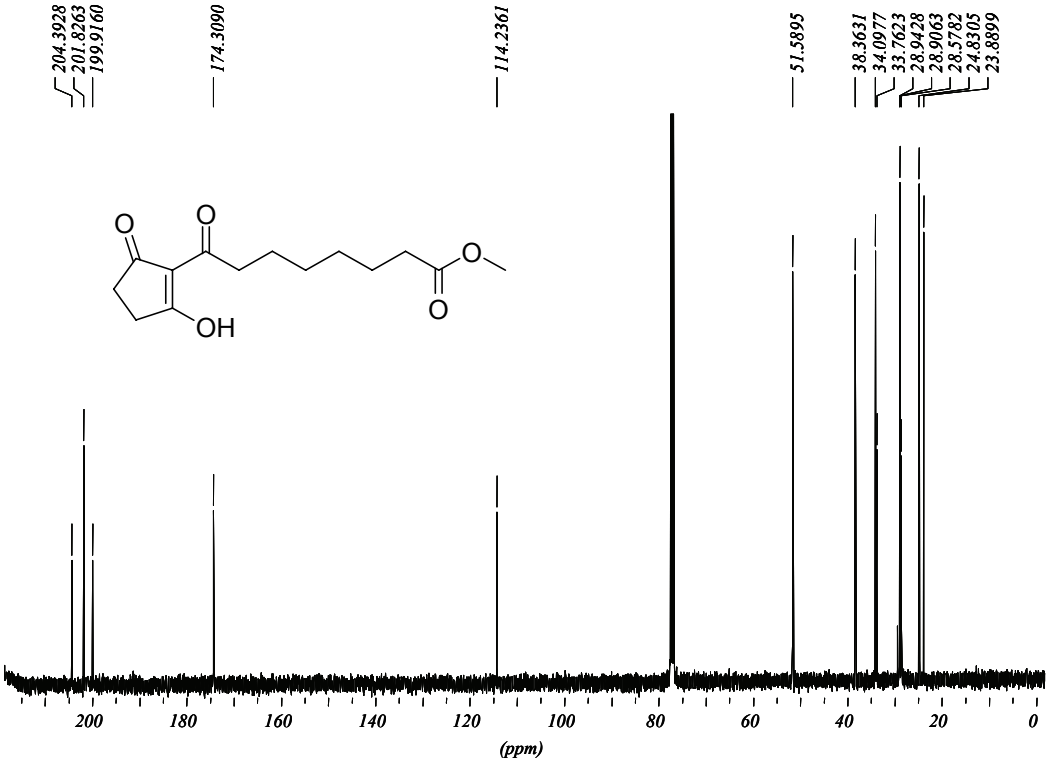

Transmittance [%]
100
80
60
40
20
0
2952
1782
1737
1598
1437
1338
1264
1159
1100
860
3500 3000 2500 2000 1500 1000
Wavenumber cm-1

10.1.3 Methyl 8-(2-hydroxy-5-oxocyclopent-1-enyl)-8-oxo-octanoate (59a)

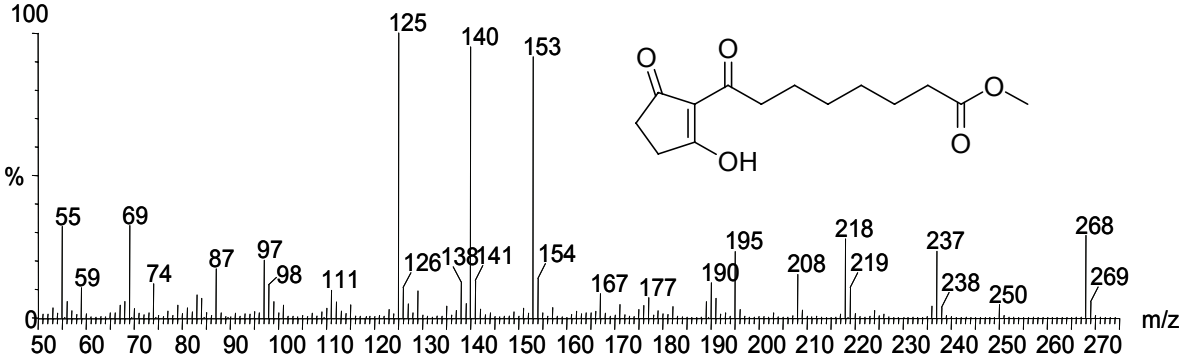
^{1}H NMR



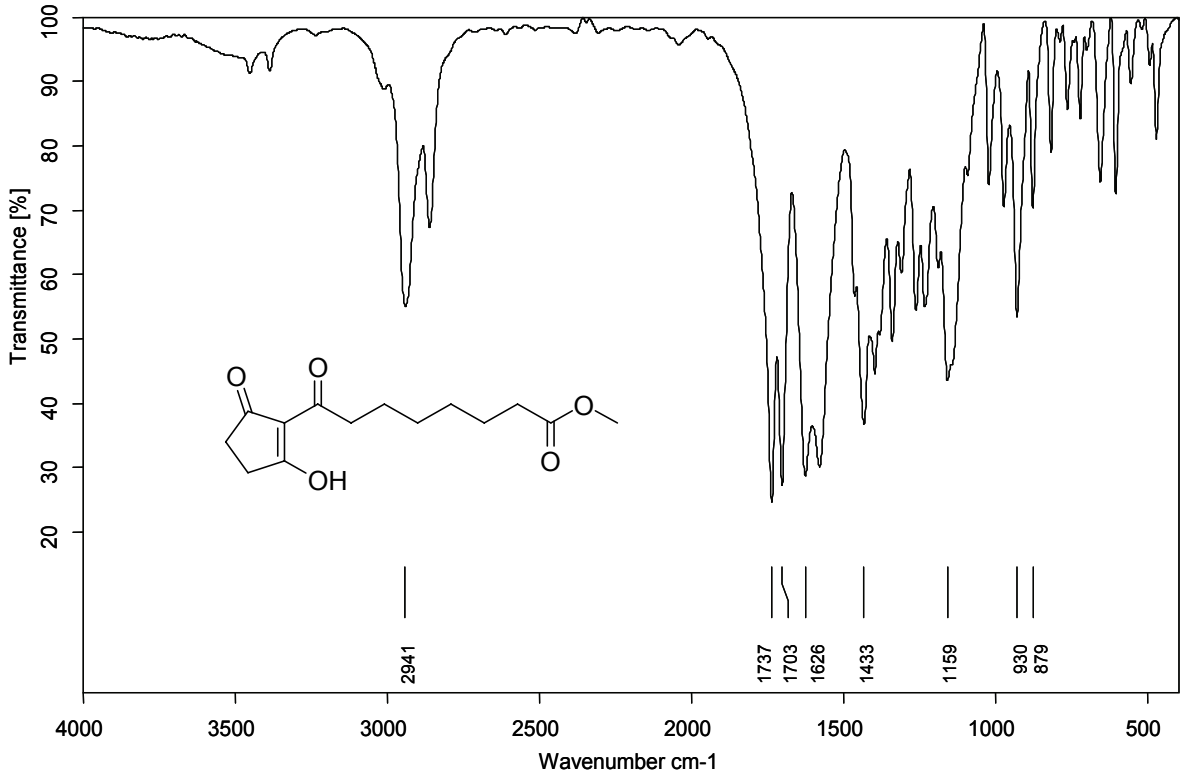
^{13}C NMR



EI-MS

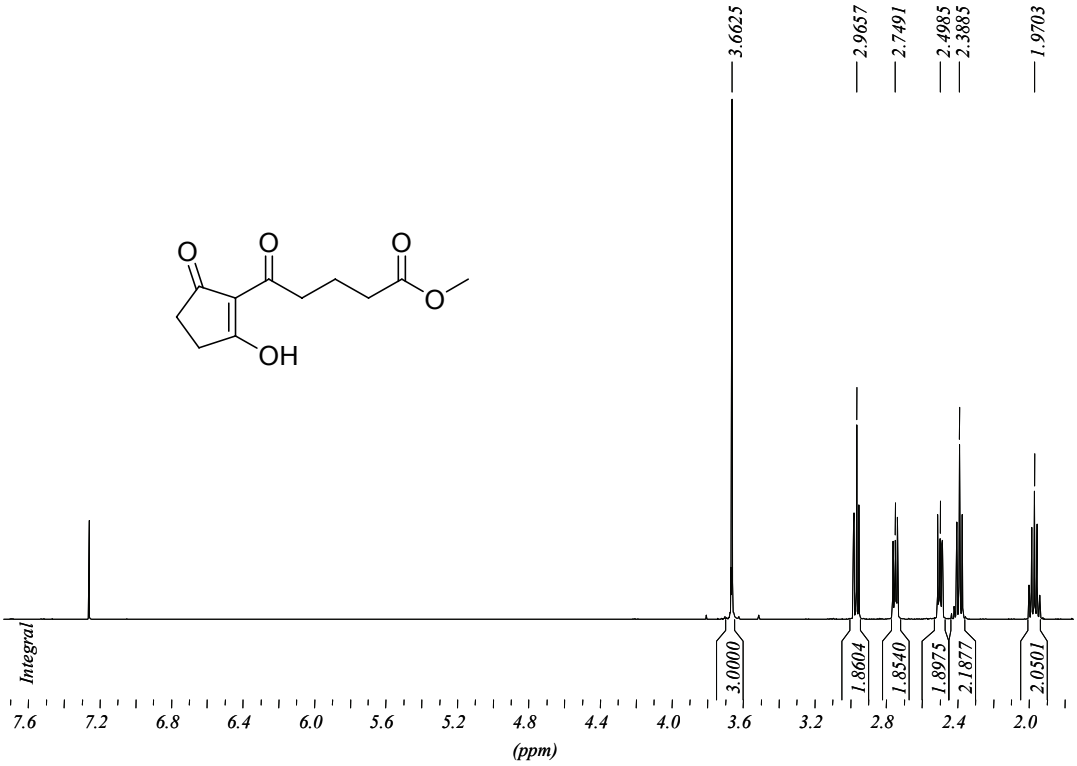

100
%
0
125
140
153
55
69
59
74
87
97
98
111
126
138
141
154
167
177
190
195
208
218
219
237
238
250
268
269
m/z
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270
O
O
O
O
OH

IR

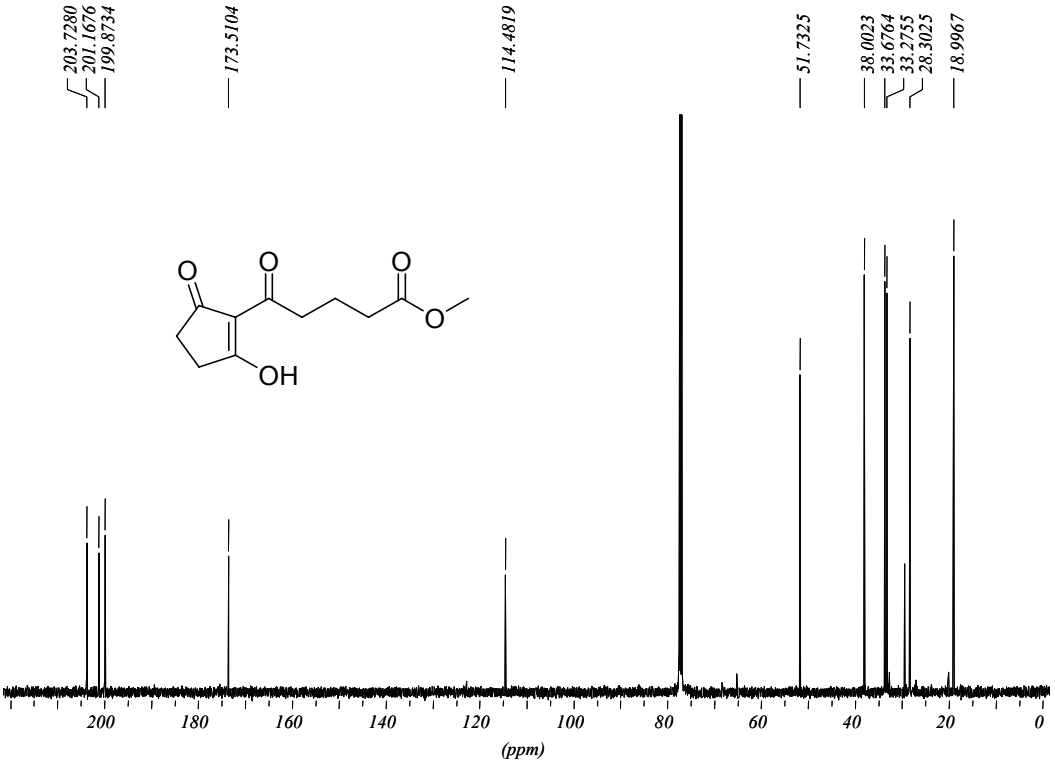

Transmittance [%]
100
90
80
70
60
50
40
30
20
2941
1737
1703
1626
1433
1159
930
879
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber cm-1
O
O
O
O
OH

10.1.4 Methyl 5-(2-hydroxy-5-oxocyclopent-1-enyl)-5-oxo-pentanoate (59b)

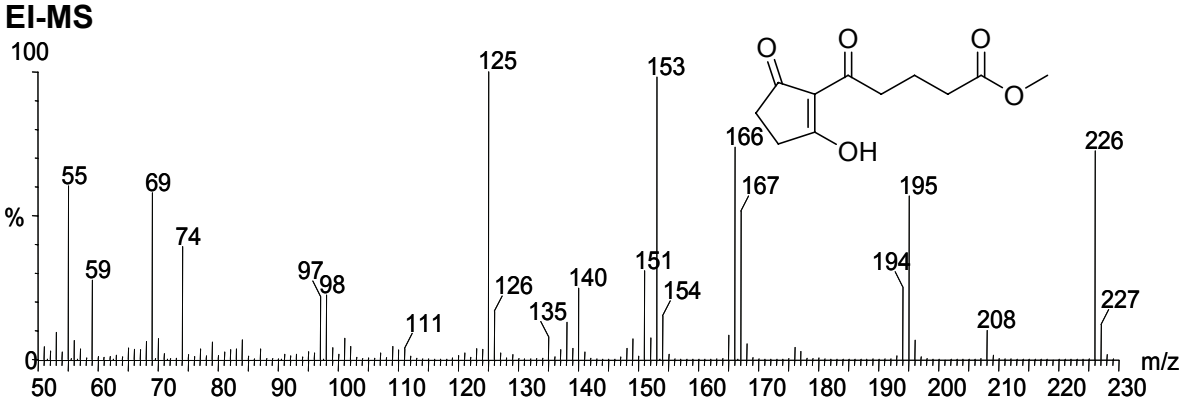
^{1}H NMR



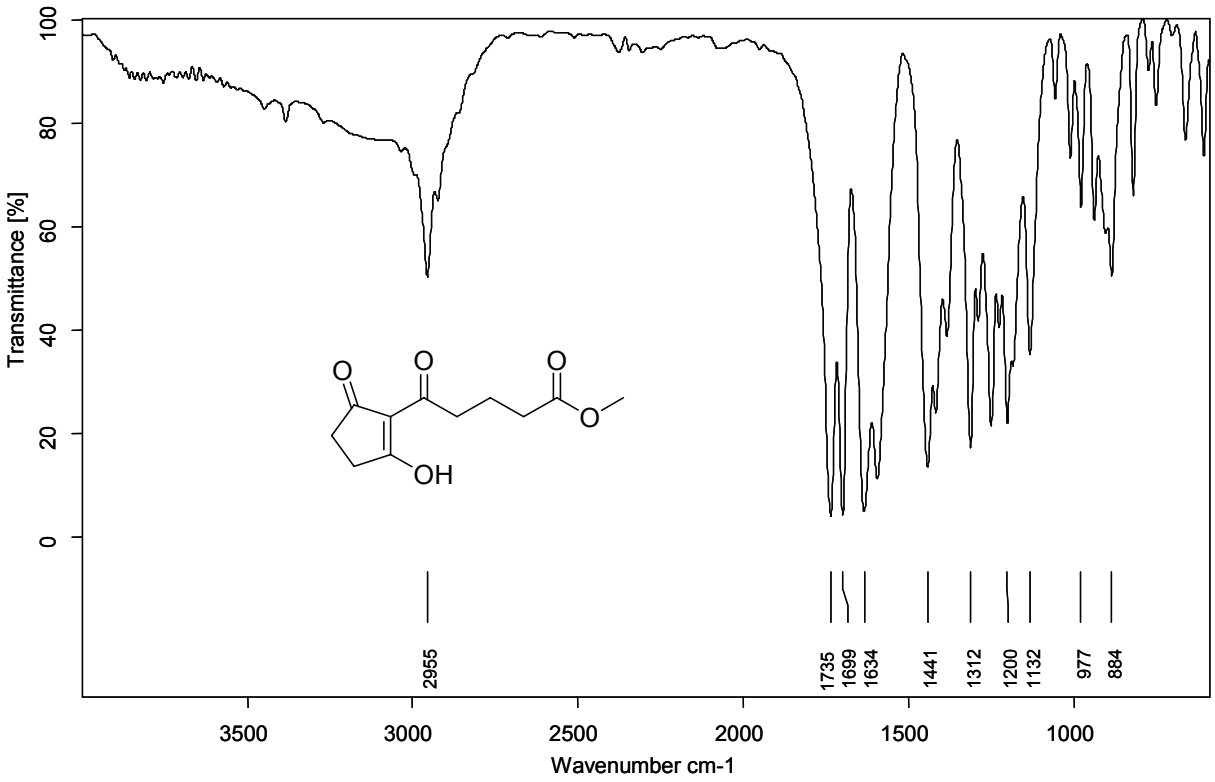
^{13}C NMR



EI-MS

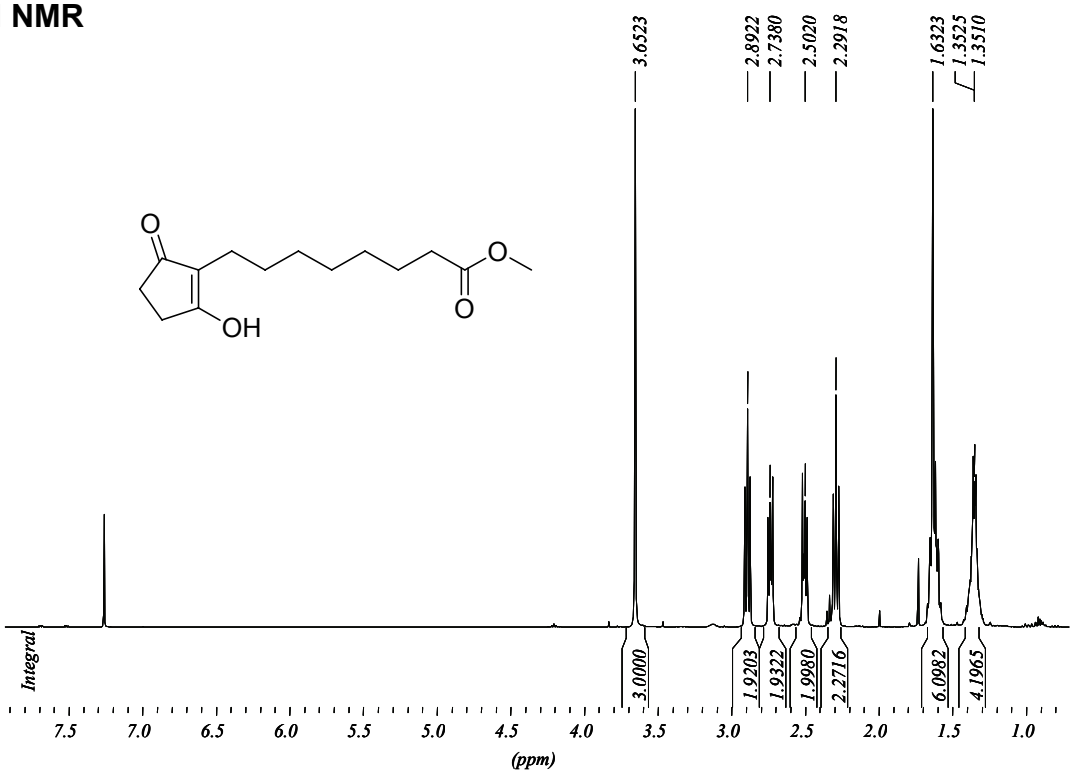

100
%
0
55
59
69
74
97
98
111
125
126
135
140
151
153
154
166
167
194
195
208
226
227
m/z
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230
O
O
O
O
OH

IR

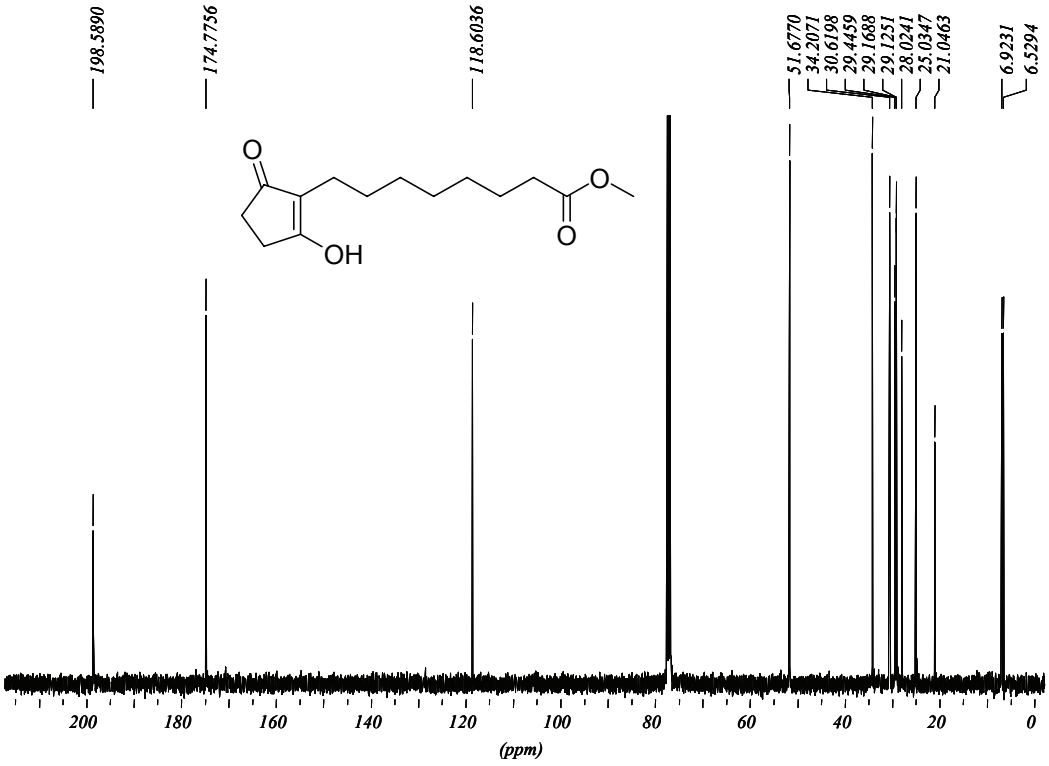

Transmittance [%]
0 20 40 60 80 100
2955
1735
1699
1634
1441
1312
1200
1132
977
884
3500 3000 2500 2000 1500 1000
Wavenumber cm-1
O
O
O
O
OH

10.1.5 Methyl 8-(2-hydroxy-5-oxocyclopent-1-enyl)octanoate (54a)

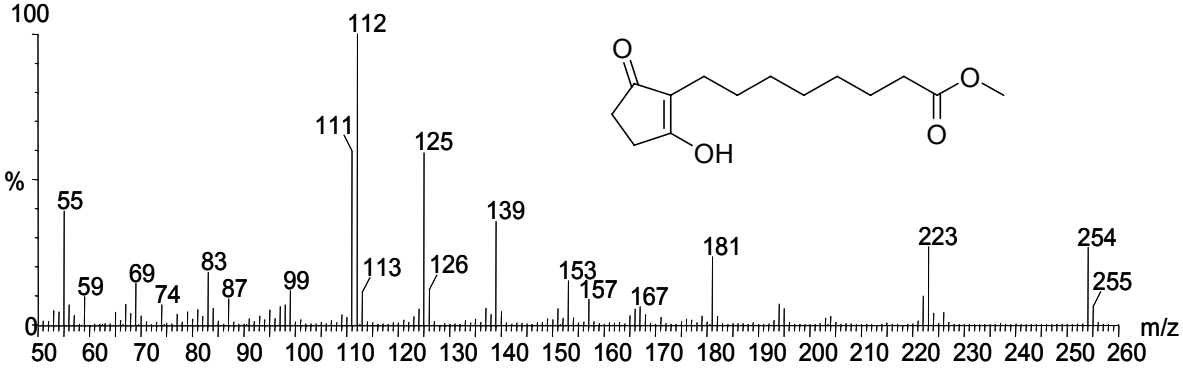
^{1}H NMR



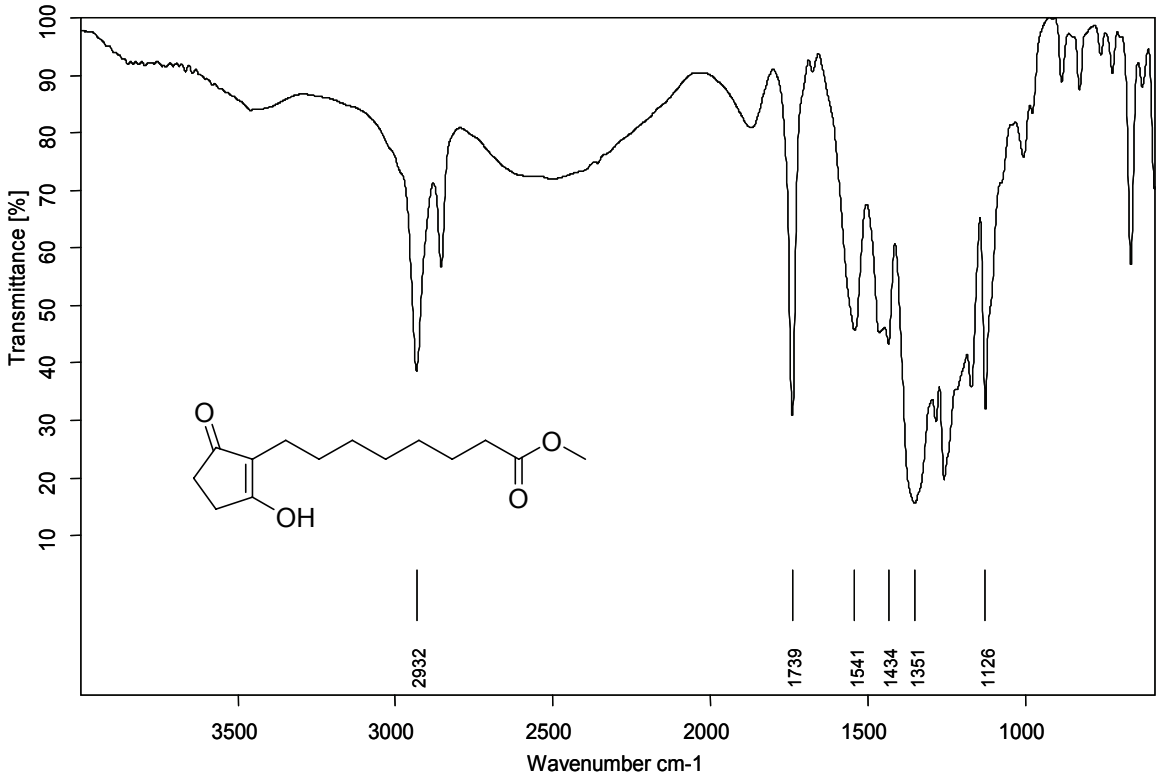
^{13}C NMR



EI-MS

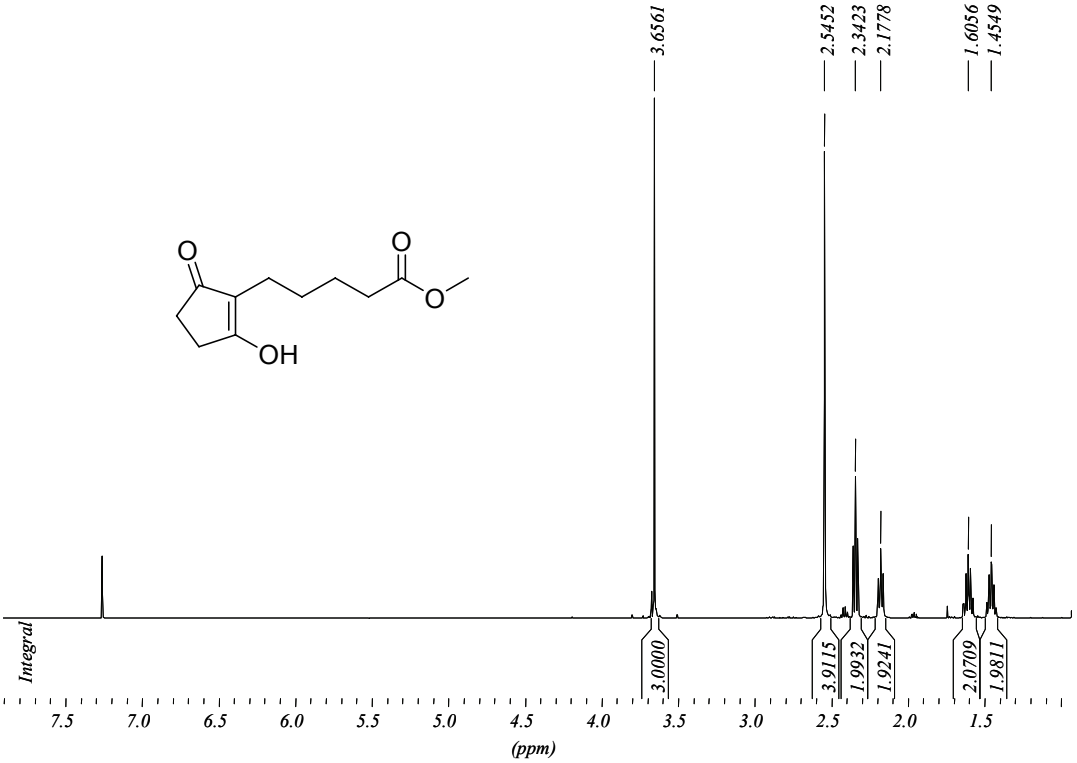

100
%
0
112
111
125
139
55
59
69
74
83
87
99
113
126
153
157
167
181
223
254
255
m/z
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260
O
O
O
OH

IR

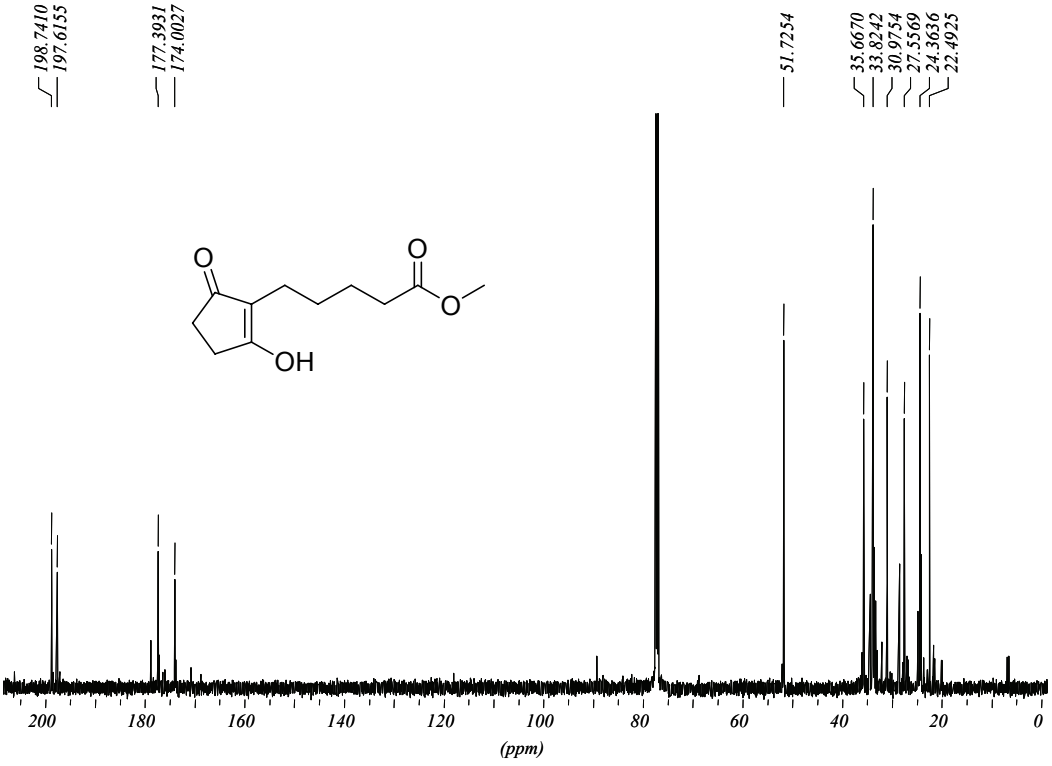

Transmittance [%]
10 20 30 40 50 60 70 80 90 100
2932
1739
1541
1434
1351
1126
3500 3000 2500 2000 1500 1000
Wavenumber cm-1
O
O
O
OH

10.1.6 Methyl 5-(2-hydroxy-5-oxocyclopent-1-enyl)pentanoate (54c)

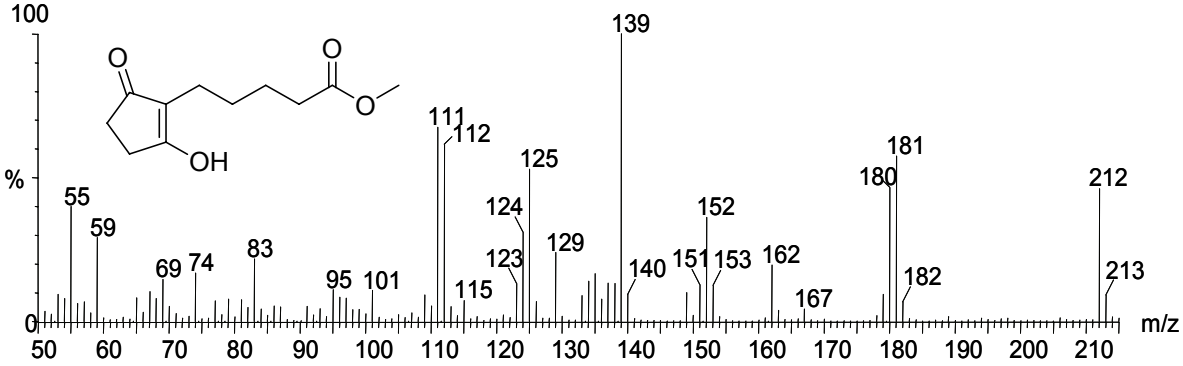
^{1}H NMR



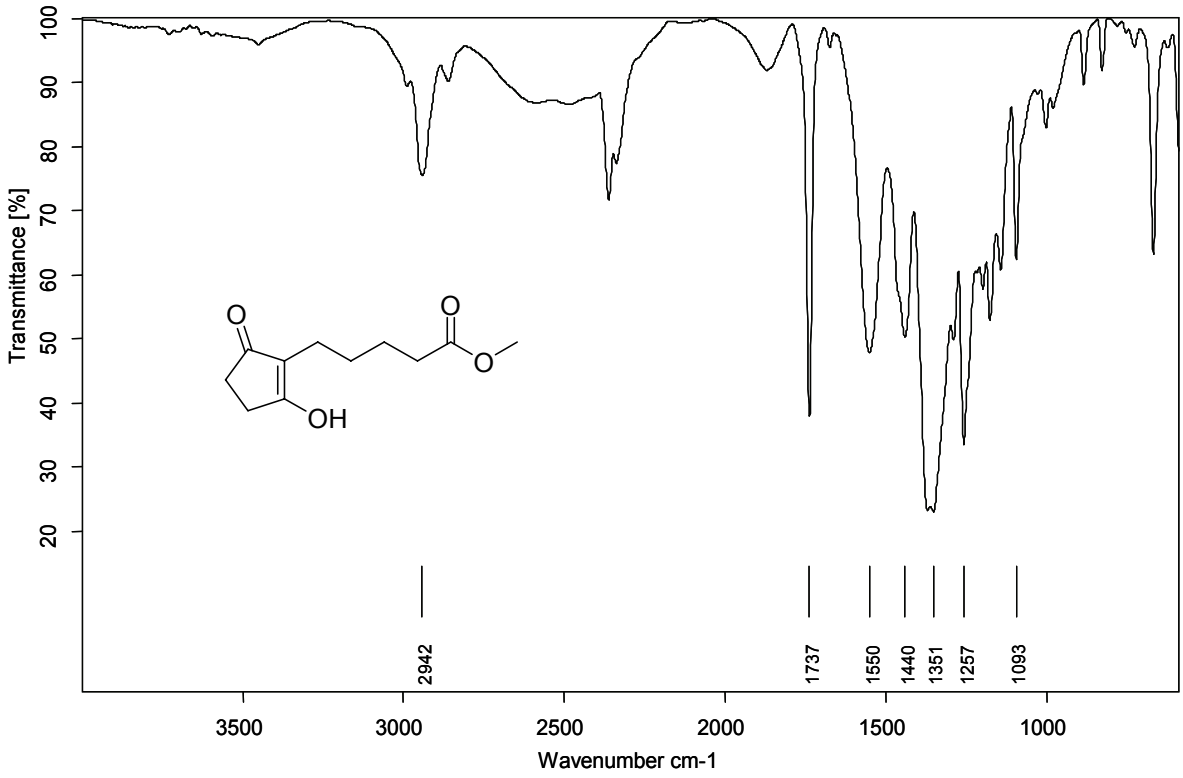
^{13}C NMR



EI-MS

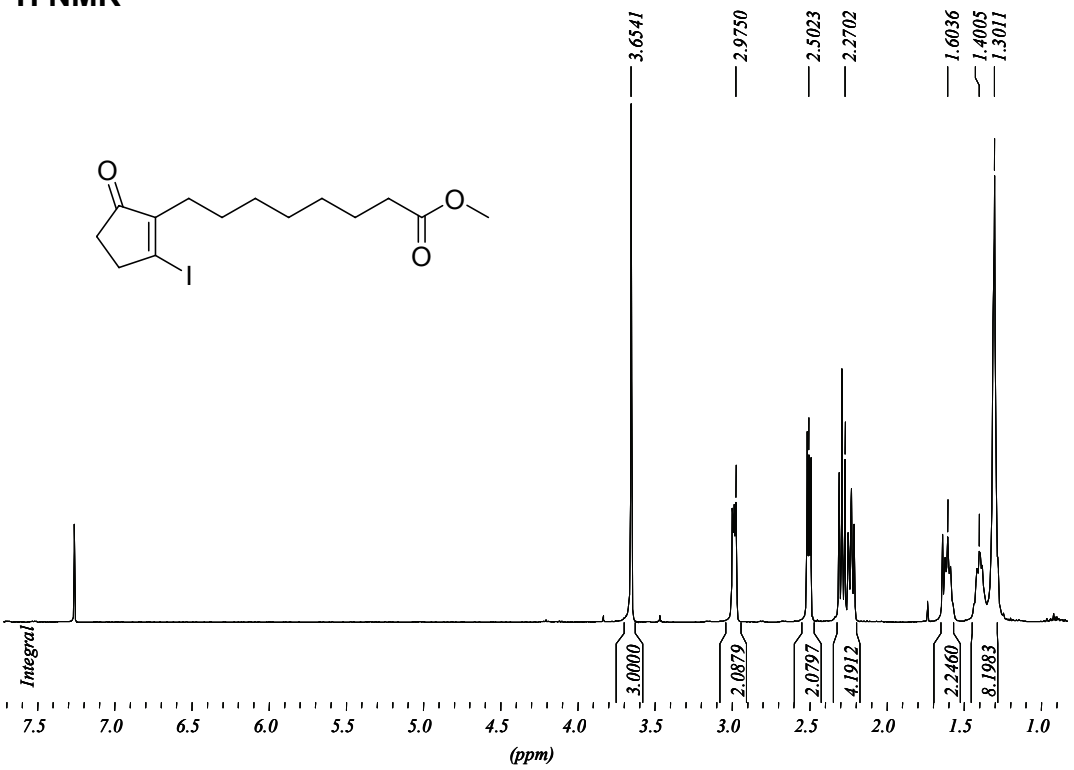

100
%
0
O
O
O
OH
139
111
112
125
181
180
212
55
59
124
152
83
129
69
74
123
140
151
153
162
182
213
95
101
115
167
m/z
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210

IR

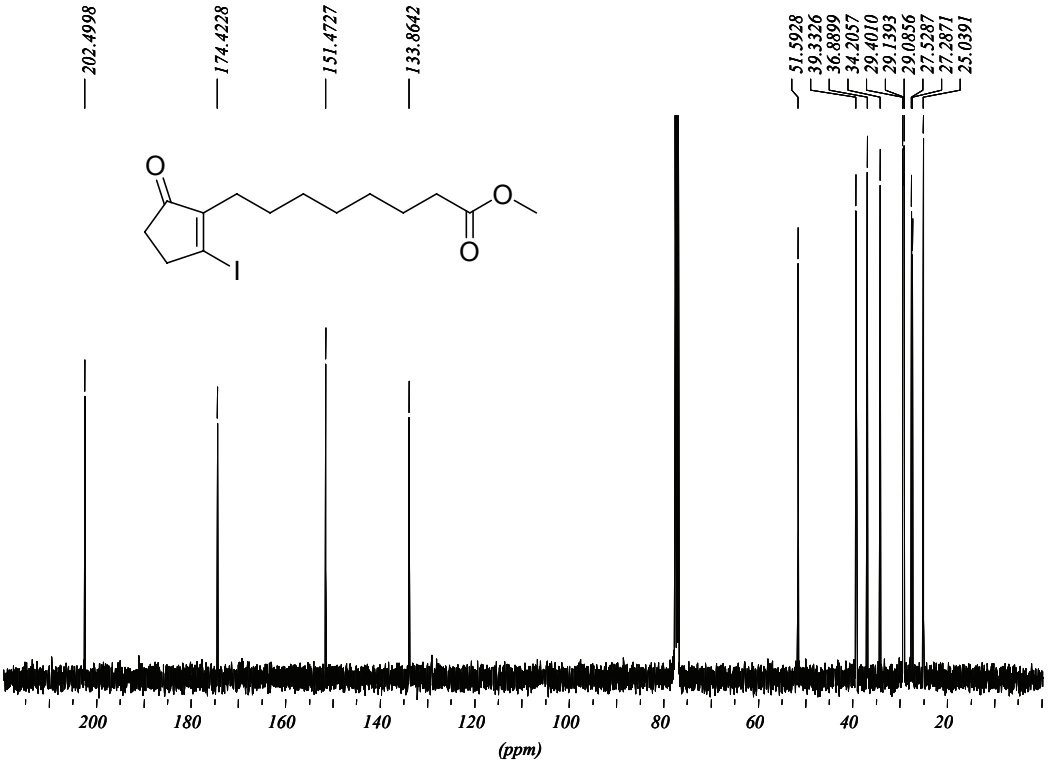

Transmittance [%]
100
90
80
70
60
50
40
30
20
O
O
O
OH
2942
1737
1550
1440
1351
1257
1093
3500
3000
2500
2000
1500
1000
Wavenumber cm-1

10.1.7 Methyl 8-(2-iodo-5-oxocyclopent-1-enyl)octanoate (53a)

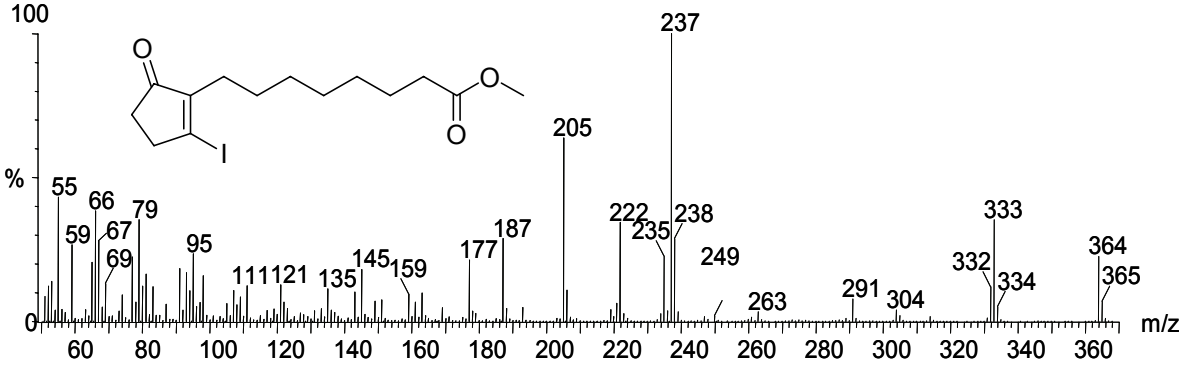
^{1}H NMR



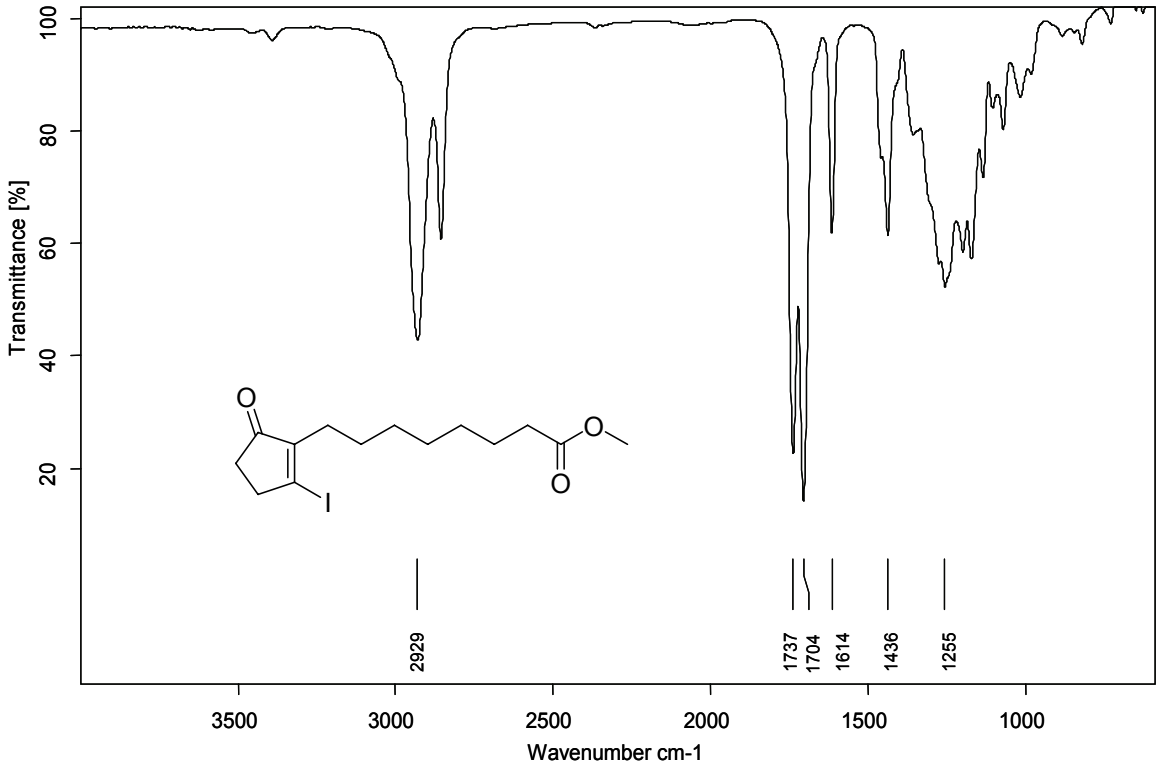
^{13}C NMR



EI-MS

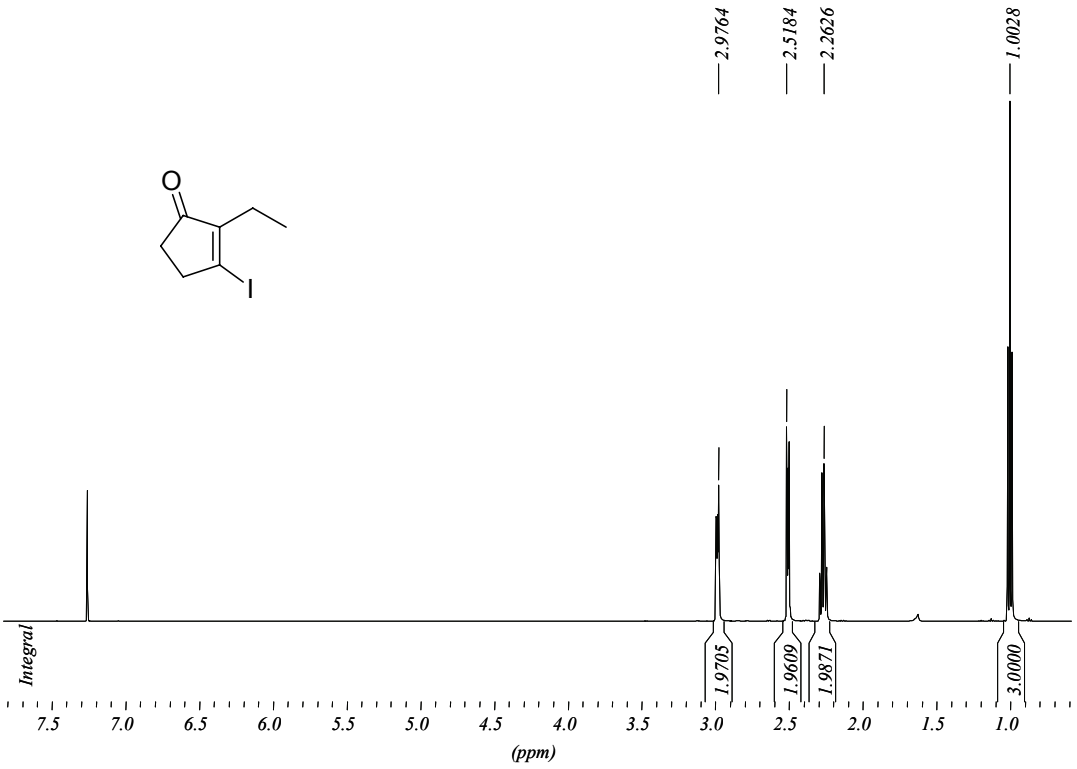

100
%
0
m/z
55
66
79
59
67
69
95
111121
135
145
159
177
187
205
222
235
237
238
249
263
291
304
332
333
334
364
365
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
O
O
O
I

IR

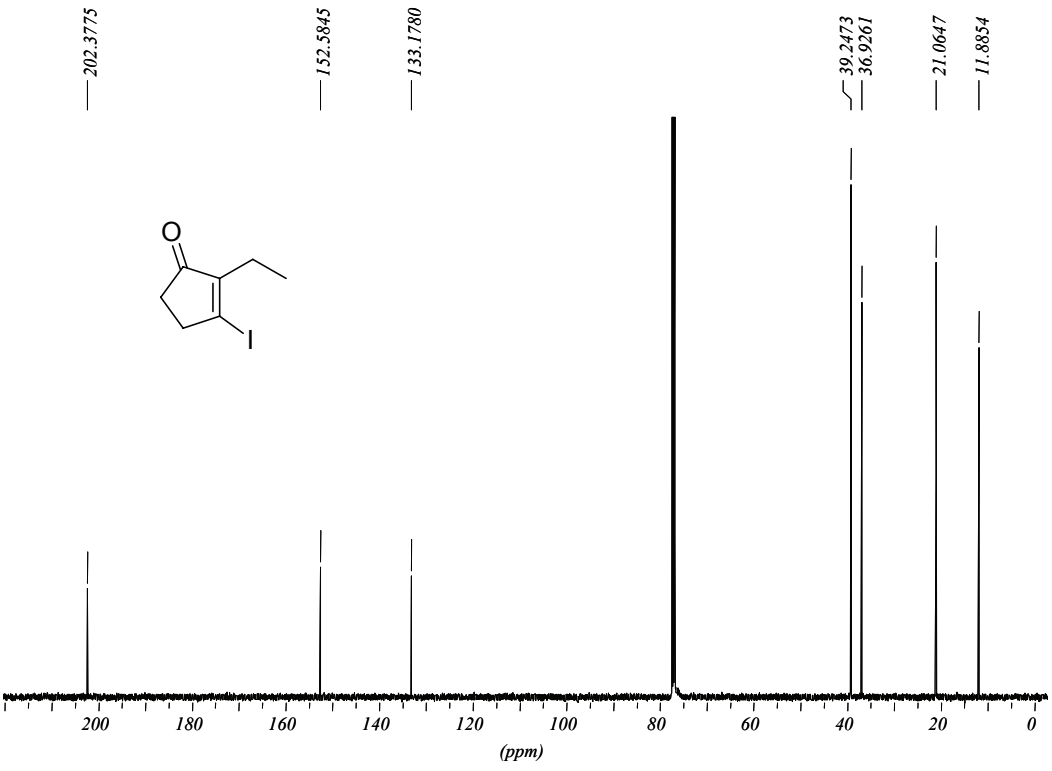

Transmittance [%]
100
80
60
40
20
2929
1737
1704
1614
1436
1255
3500
3000
2500
2000
1500
1000
Wavenumber cm-1
O
O
O
I

10.1.8 2-Ethyl-3-iodocyclopent-2-enone (53b)

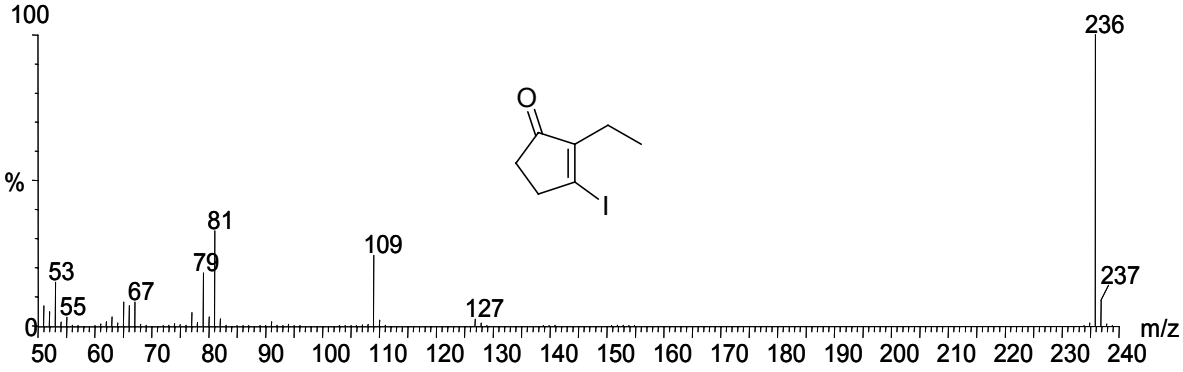
^{1}H NMR



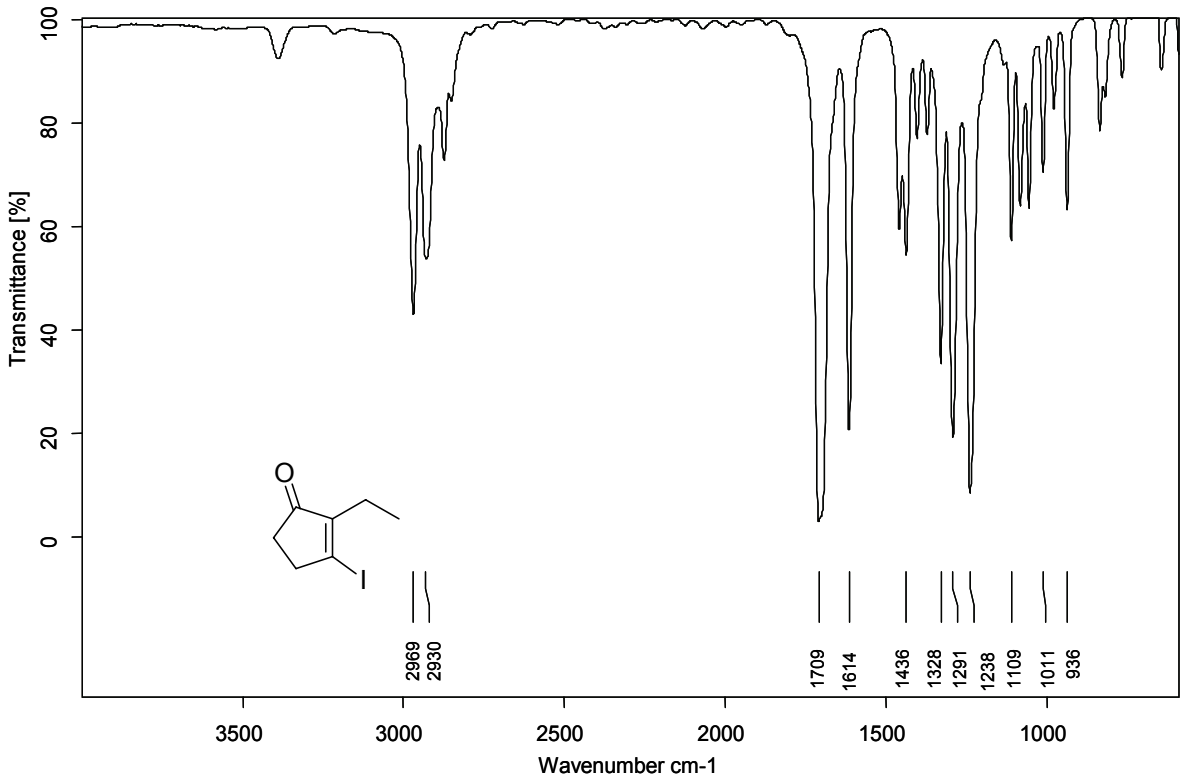
^{13}C NMR



EI-MS

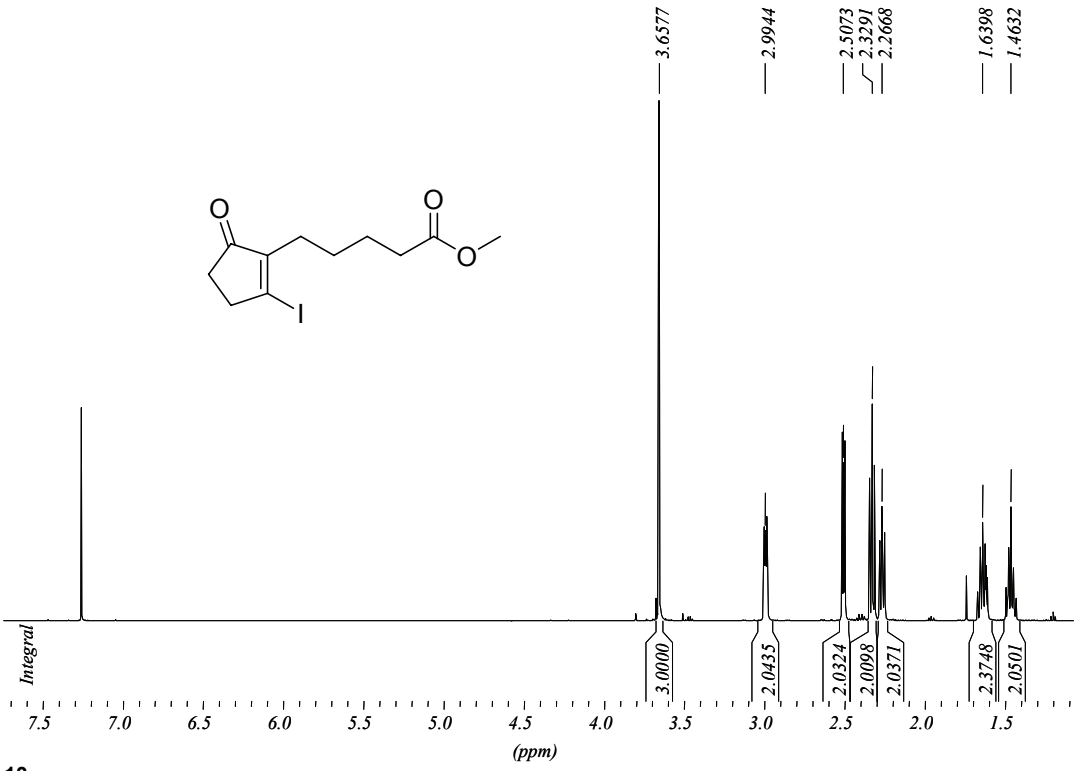

100
%
0
236
237
81
109
79
53
55
67
127
m/z
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
O
I

IR

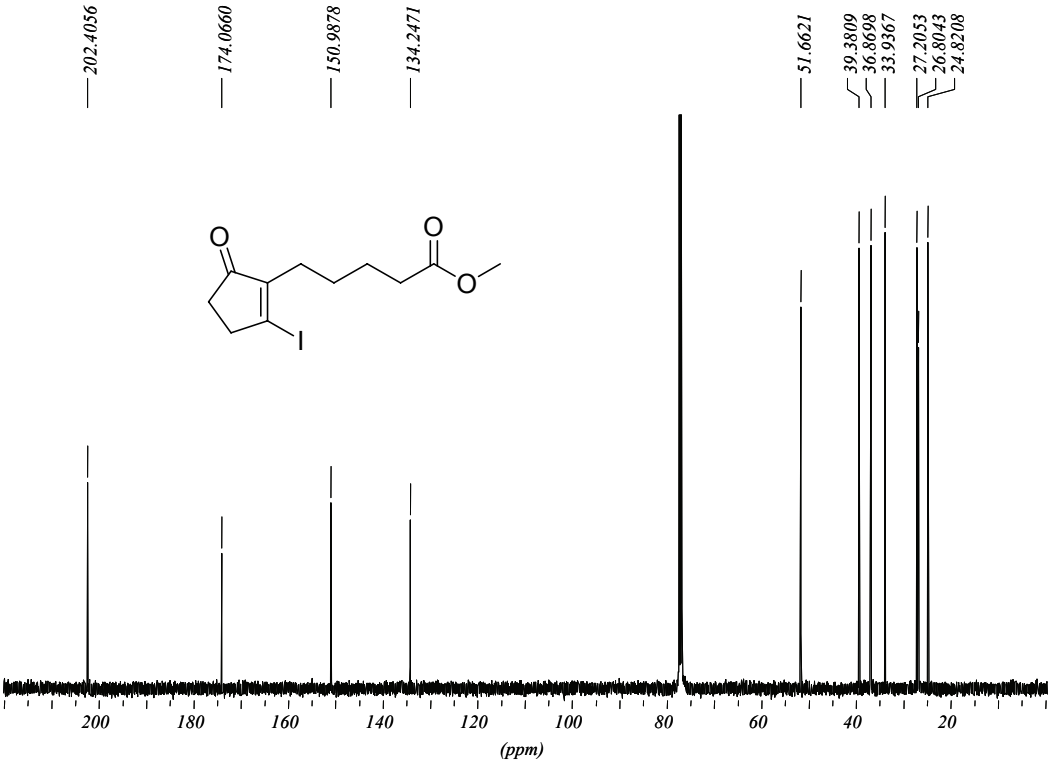

Transmittance [%]
0 20 40 60 80 100
2969
2930
1709
1614
1436
1328
1291
1238
1109
1011
936
3500 3000 2500 2000 1500 1000
Wavenumber cm-1
O
I

10.1.9 Methyl 5-(2-iodo-5-oxocyclopent-1-enyl)pentanoate (53c)

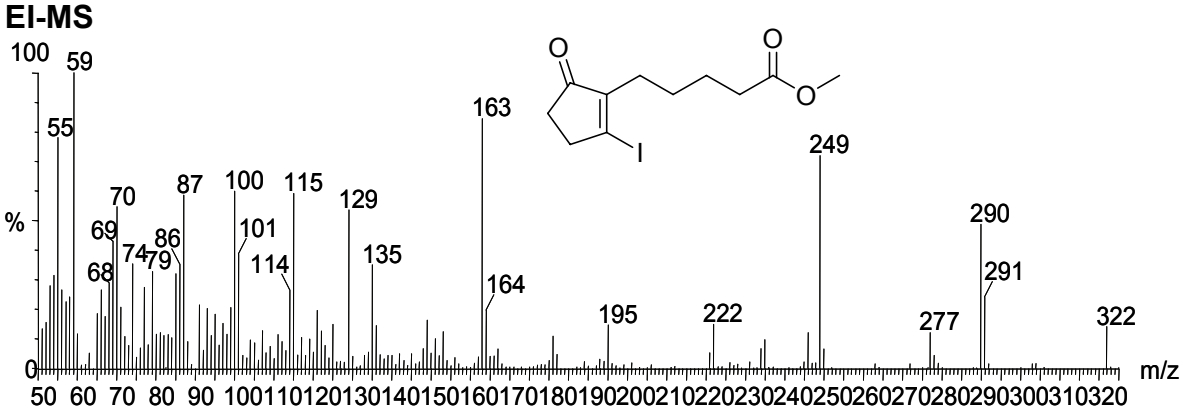
^{1}H NMR



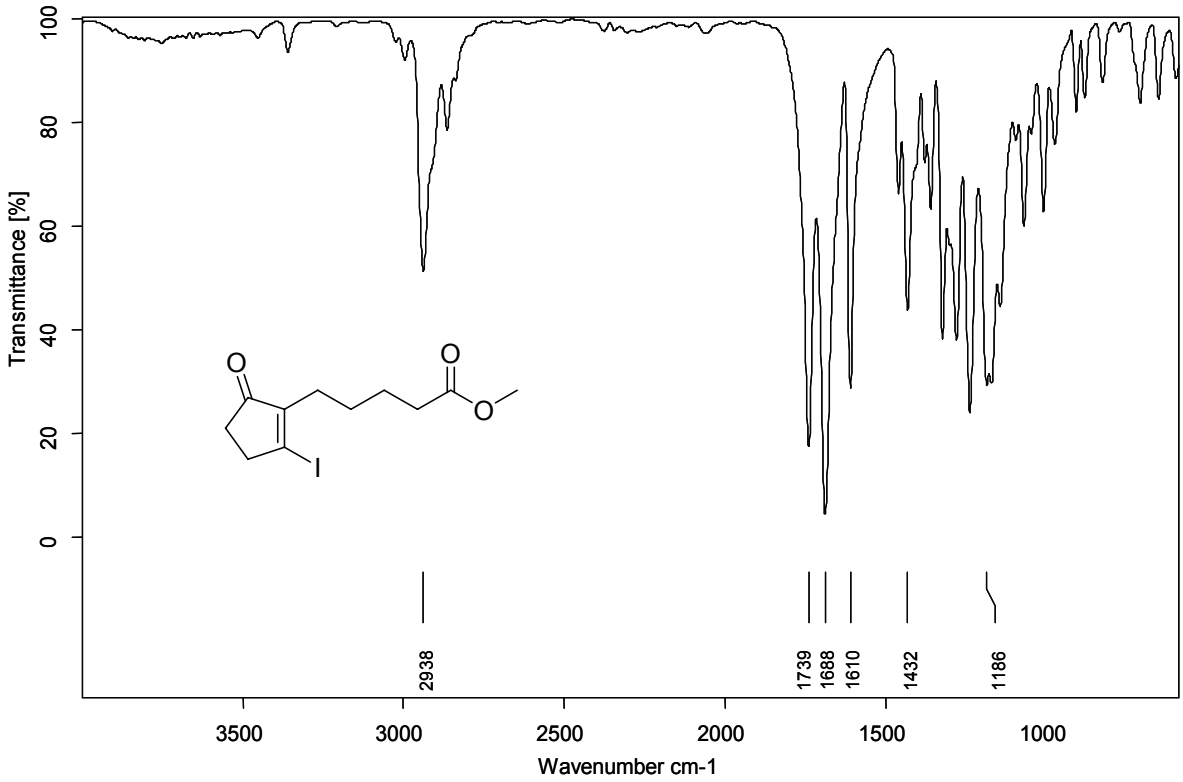
^{13}C NMR



EI-MS

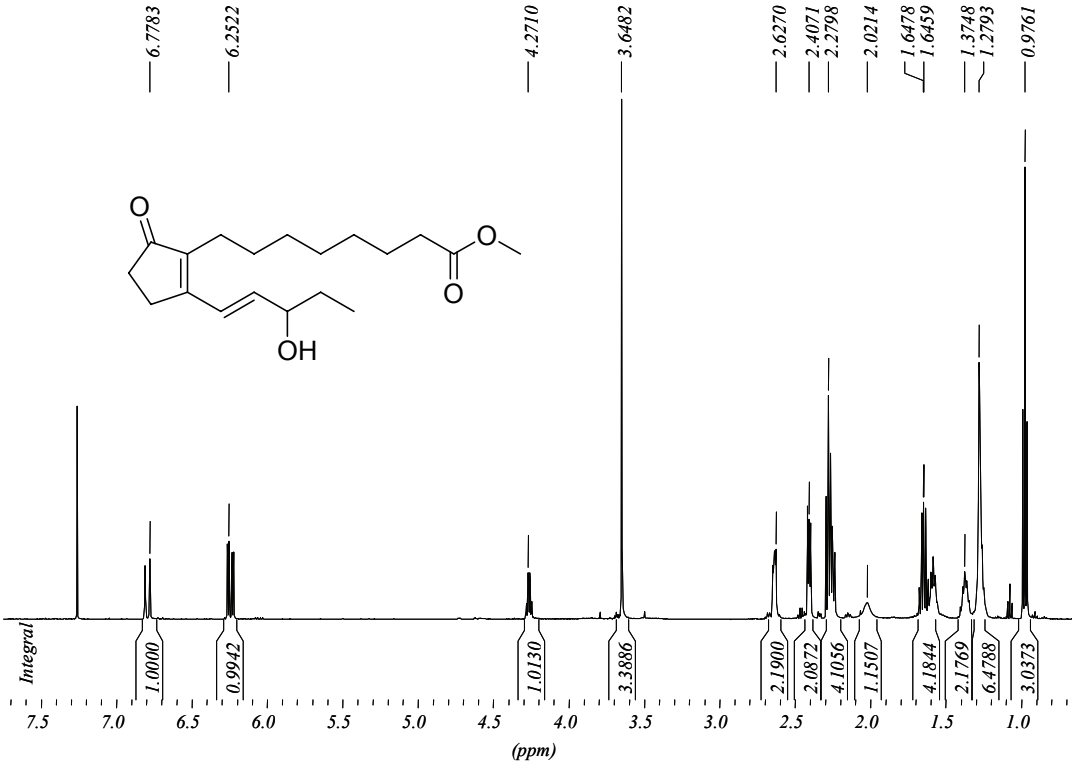

100
59
55
70
87
100
115
129
163
249
290
%
69
86
101
74
79
114
135
68
164
195
222
277
291
322
0
m/z
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320
O
O
O
I

IR

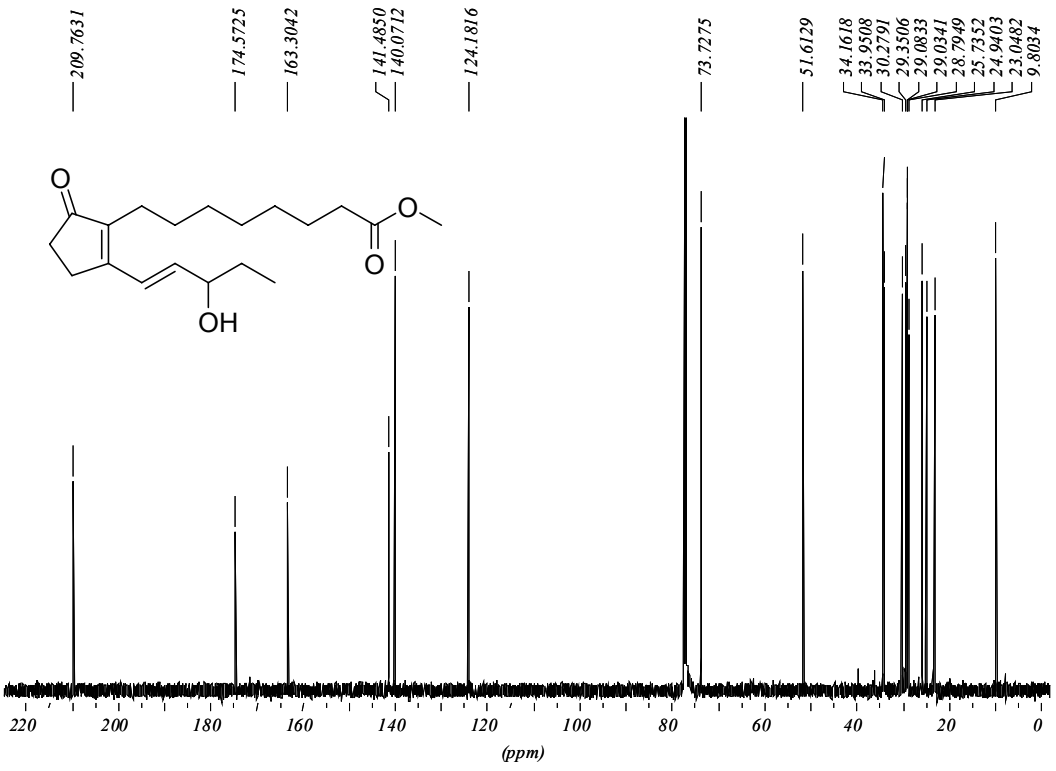

Transmittance [%]
100
80
60
40
20
0
2938
1739
1688
1610
1432
1186
3500
3000
2500
2000
1500
1000
Wavenumber cm-1
O
O
O
I

10.1.10 Phytoprostane B_1 type I methyl ester (22a)

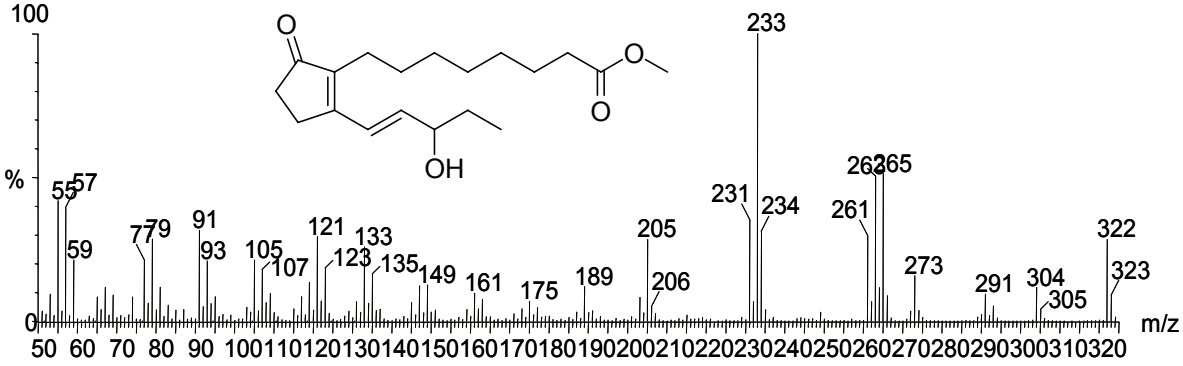
^{1}H NMR



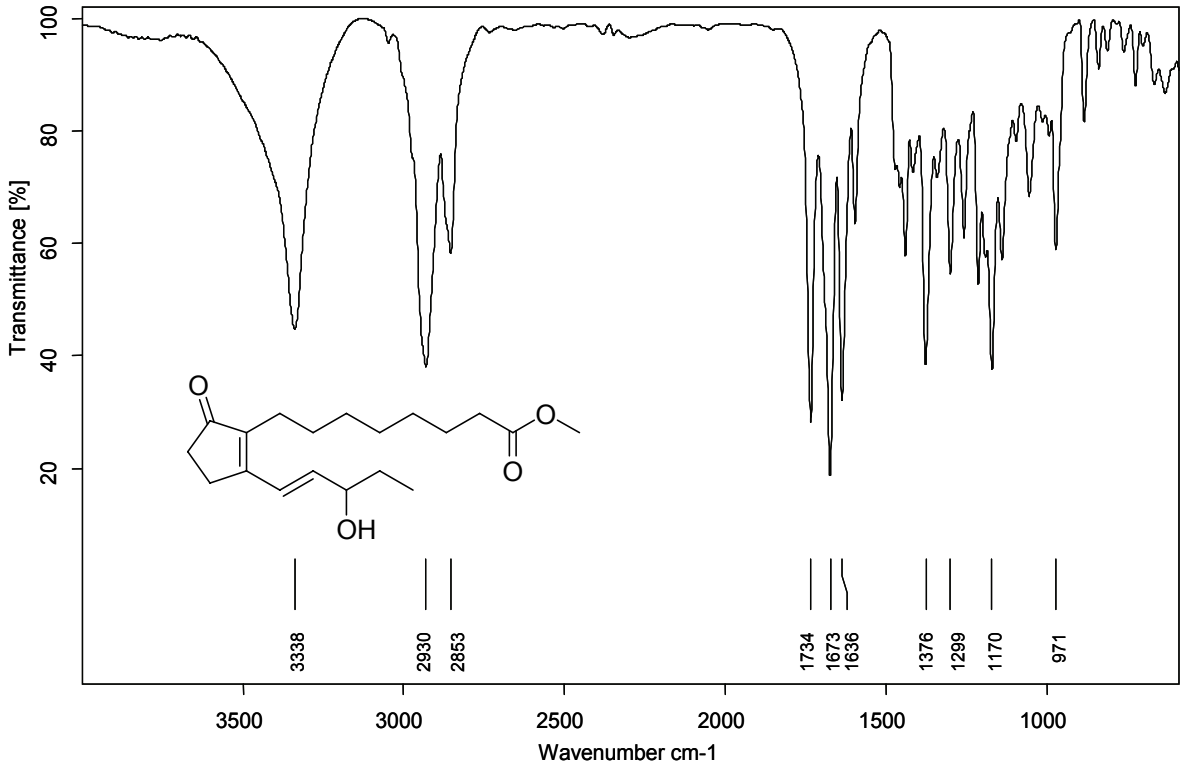
^{13}C NMR



EI-MS

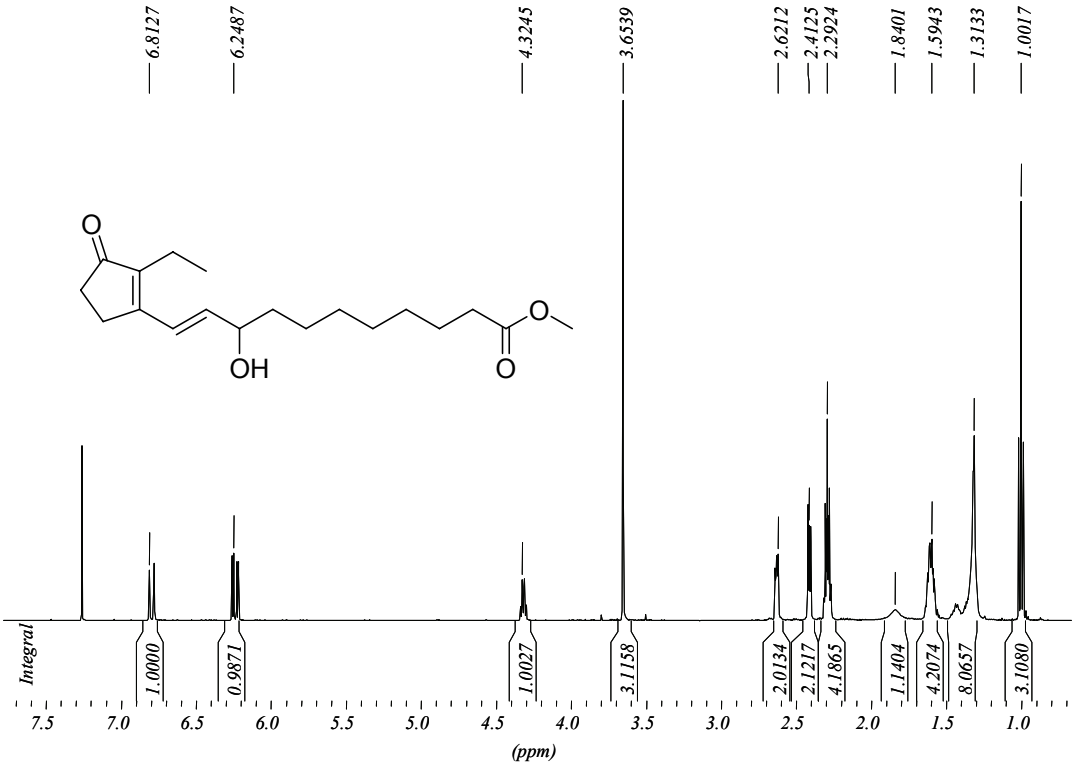

100
%
0
O
O
O
OH
233
231
234
262
265
261
205
206
55
57
59
77
79
91
93
105
107
121
123
133
135
149
161
175
189
273
291
304
305
322
323
m/z
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320

IR

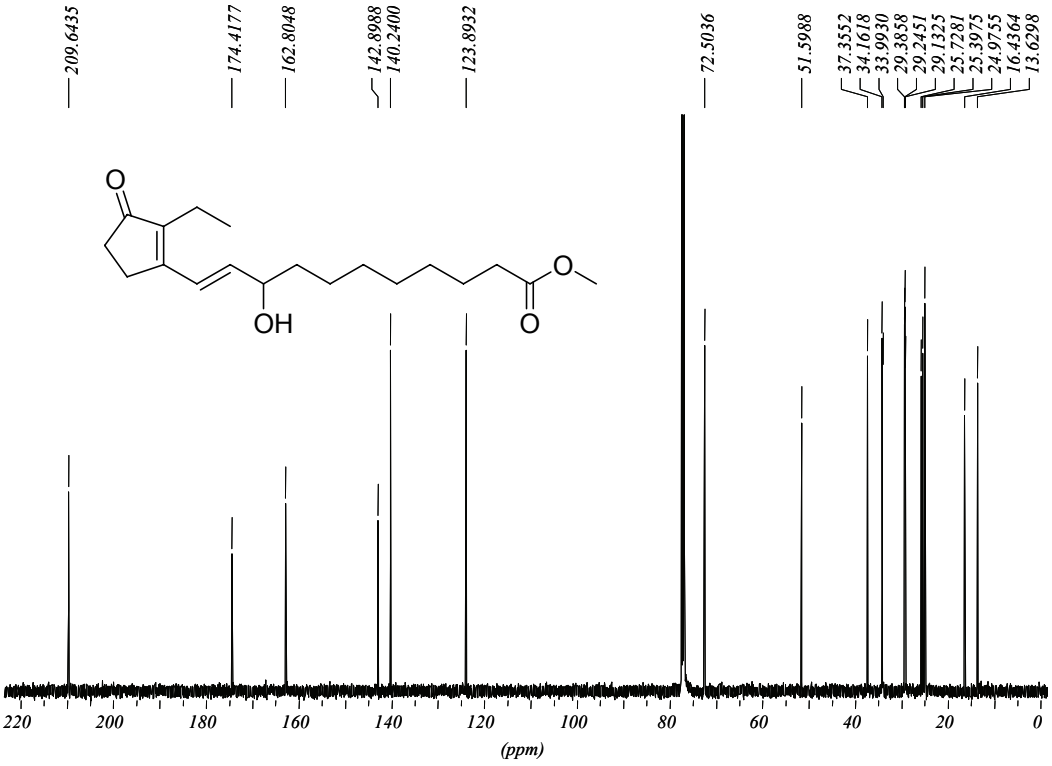

Transmittance [%]
100
80
60
40
20
O
O
O
OH
3338
2930
2853
1734
1673
1636
1376
1299
1170
971
3500
3000
2500
2000
1500
1000
Wavenumber cm-1

10.1.11 Phytoprostane B_1 type II methyl ester (22b)

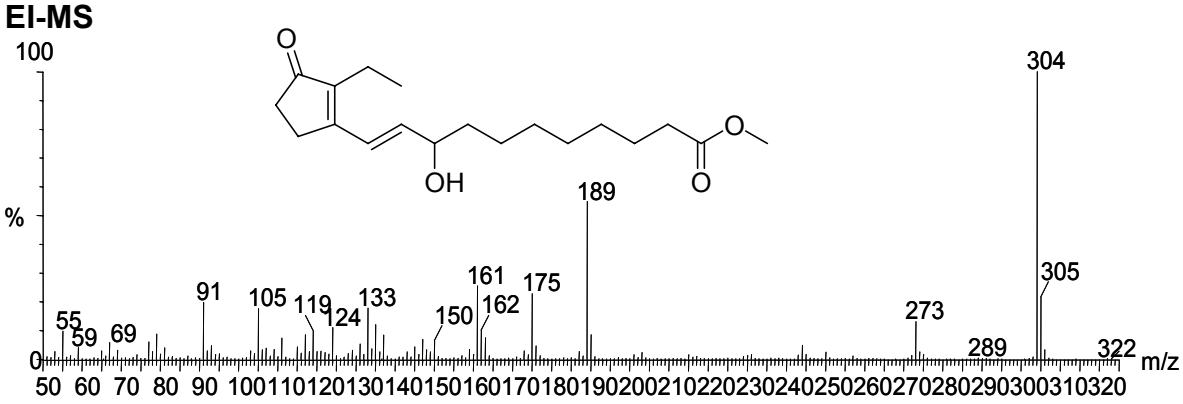
^{1}H NMR



^{13}C NMR



EI-MS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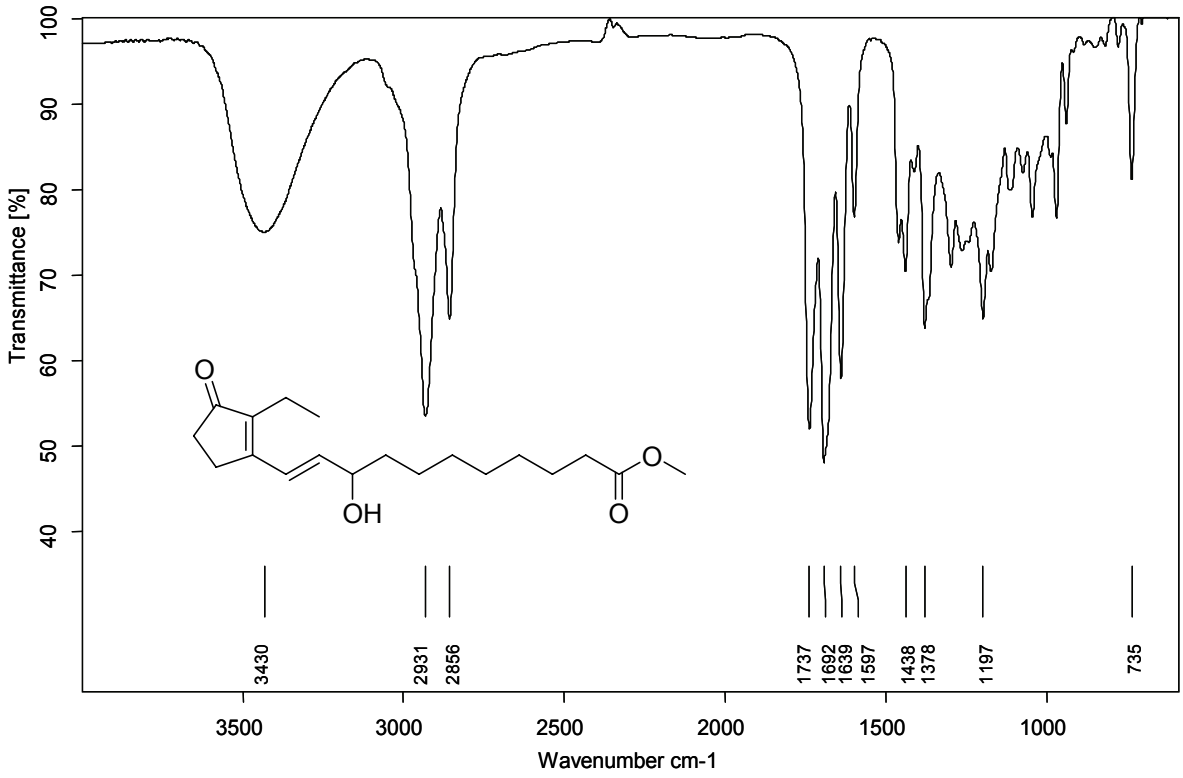
%
0
304
189
305
91
105
119
124
133
161
175
150
162
273
289
322
55
59
69
m/z
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320
O
OH
O
O

IR


Transmittance [%]
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70
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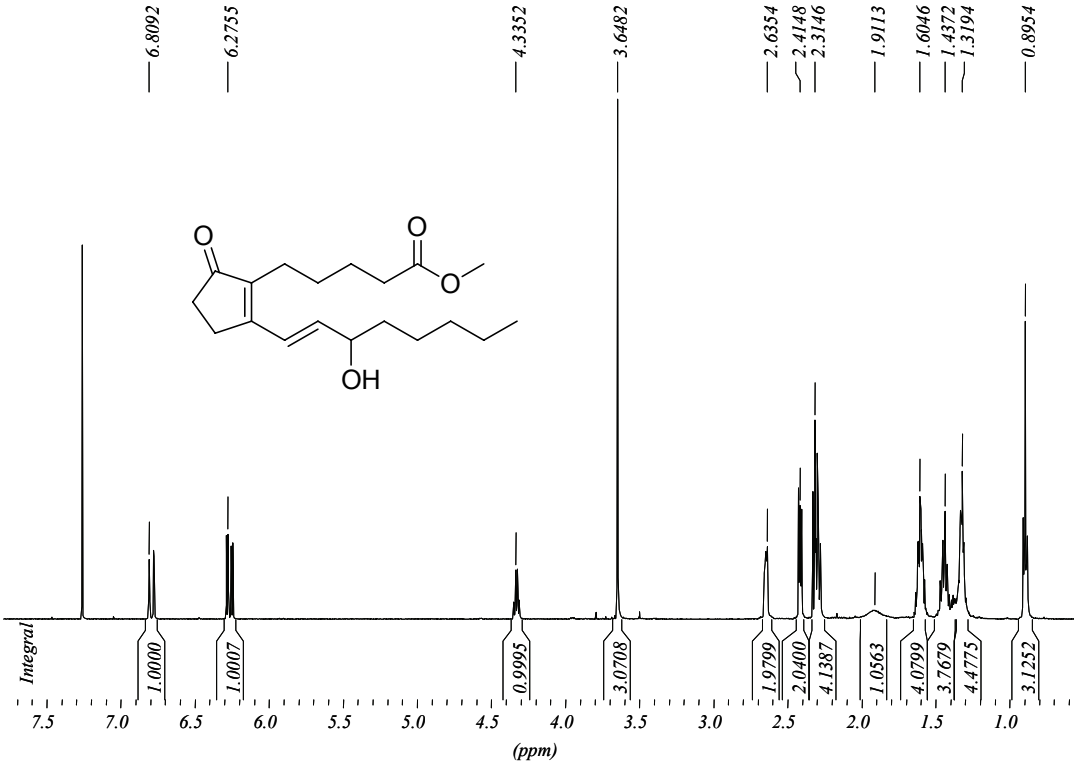
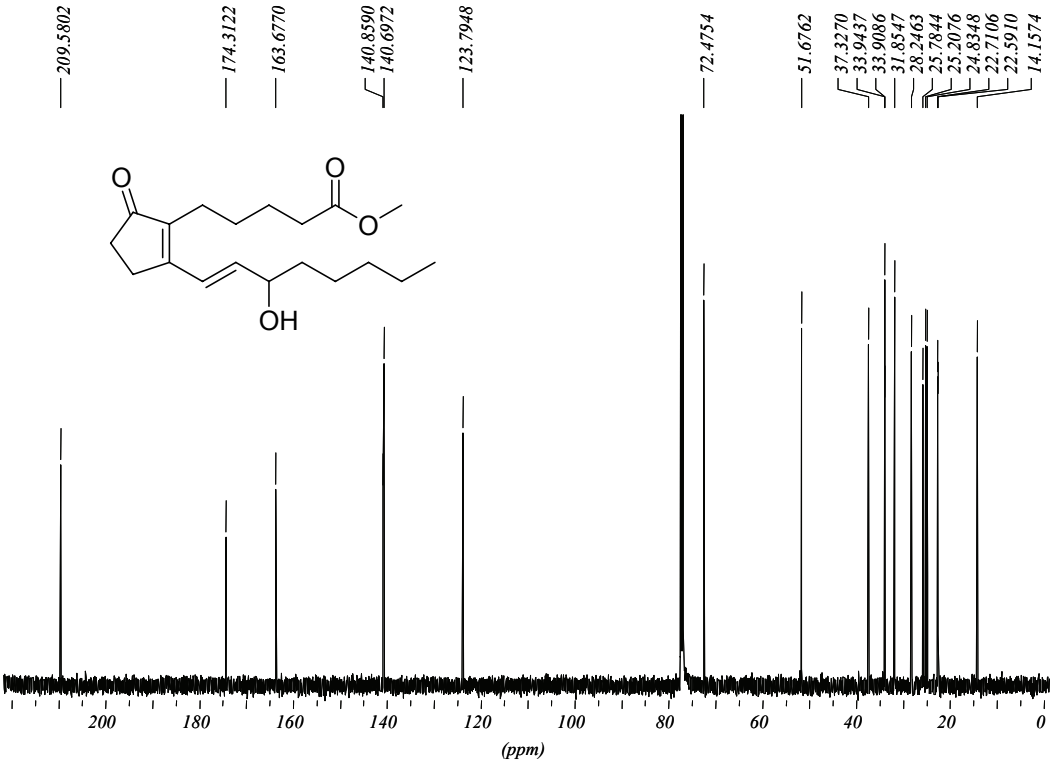
50
40
3430
2931
2856
1737
1692
1639
1597
1438
1378
1197
735
3500
3000
2500
2000
1500
1000
Wavenumber cm-1
O
OH
O
O

10.1.12 Dinor isoprostane B_1 type III methyl ester (22c)

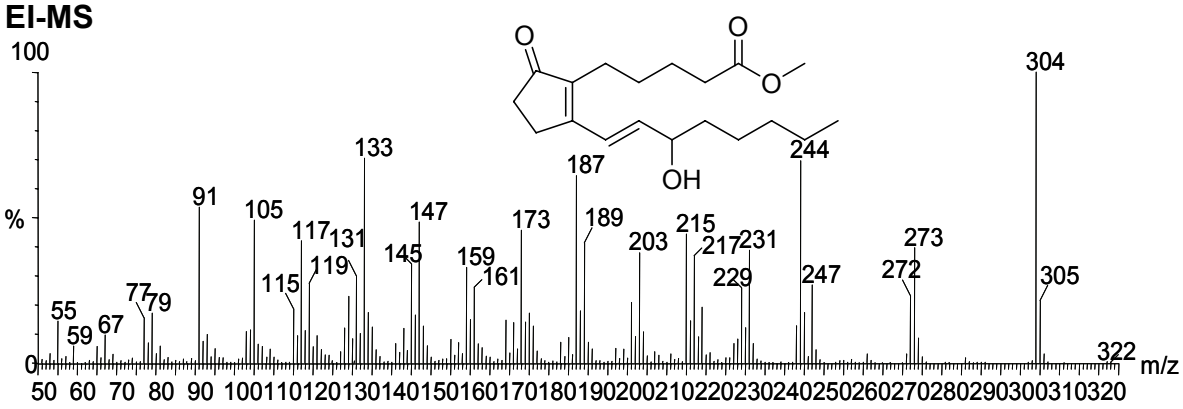
^{1}H NMR



^{13}C NMR



EI-MS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%
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O
O
OH
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133
187
244
91
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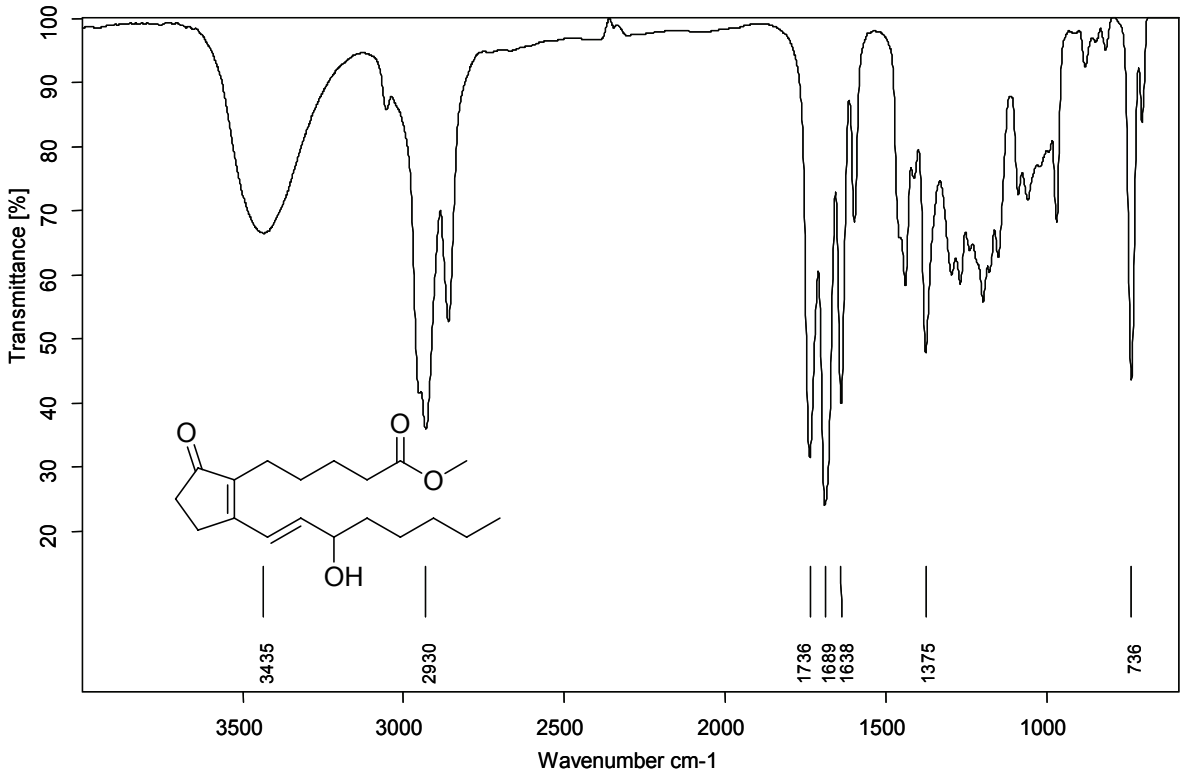
173
189
215
217
231
273
117
131
145
159
203
229
247
272
305
115
119
161
77
79
55
59
67
322
m/z
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320

IR


Transmittance [%]
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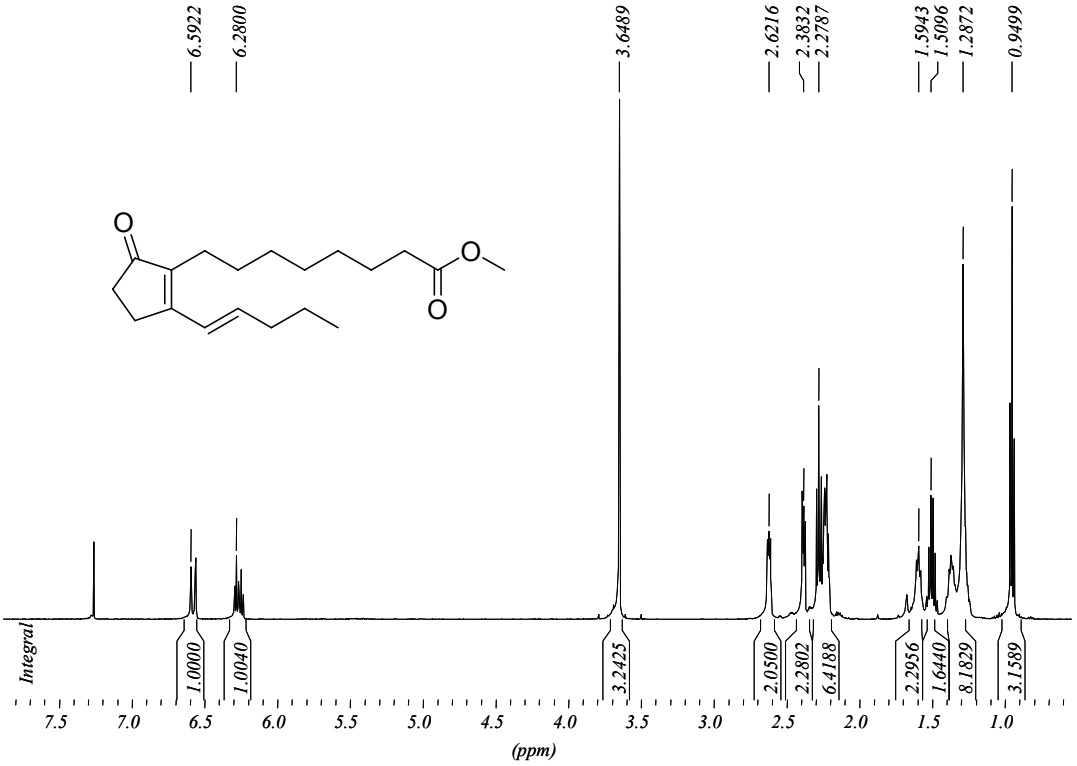
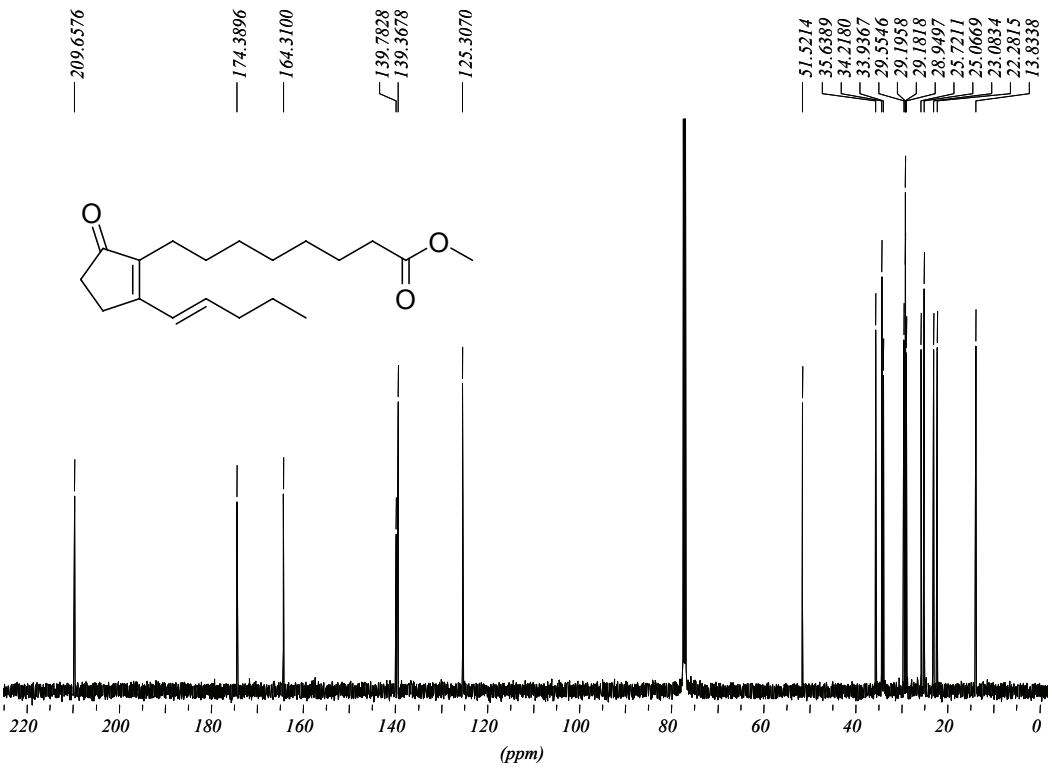
90
80
70
60
50
40
30
20
O
O
O
OH
3435
2930
1736
1689
1638
1375
736
3500
3000
2500
2000
1500
1000
Wavenumber cm-1

10.1.13 Methyl 8-(5-oxo-2-((*E*)-pent-1-enyl)cyclopent-1-enyl)-octanoate (64a)

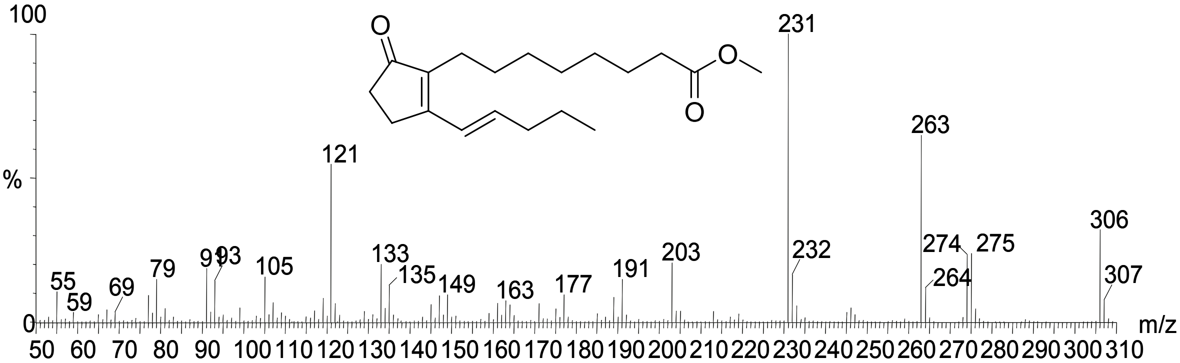
^{1}H NMR



^{13}C NMR



EI-MS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%
0
m/z
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263
121
306
55
59
69
79
91
93
105
133
135
149
163
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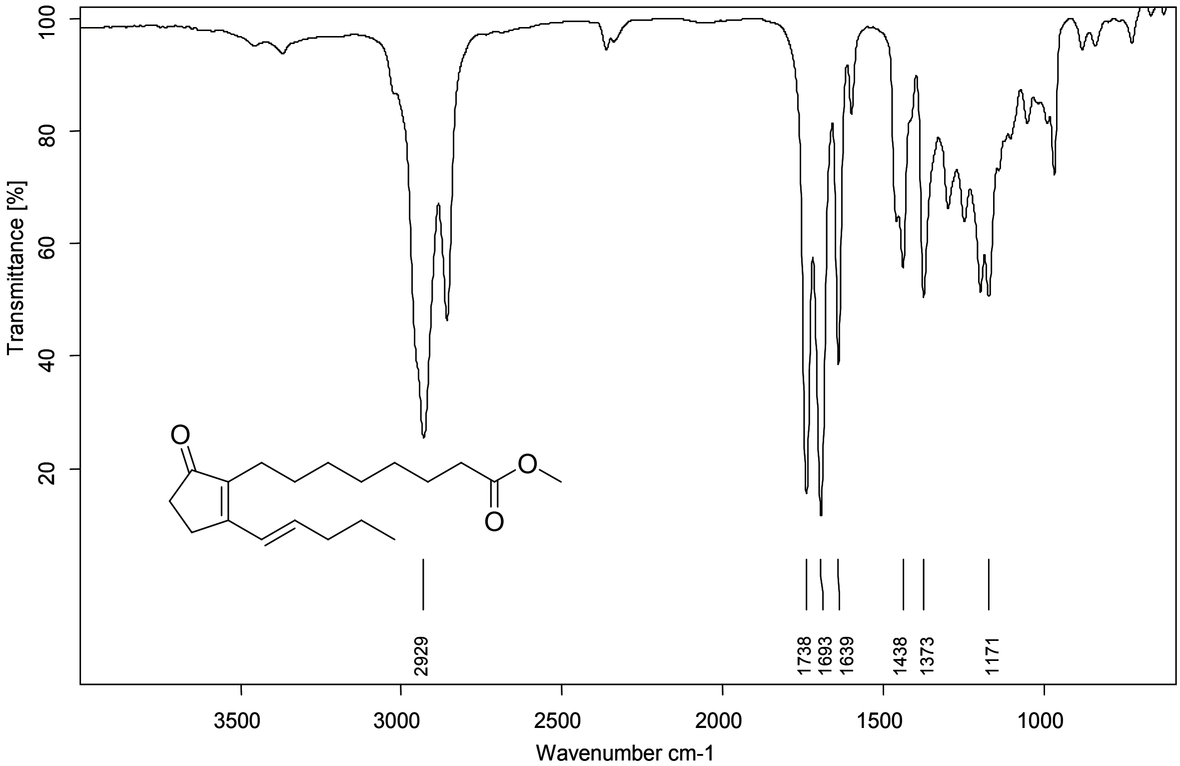
203
232
274
275
264
307
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310

IR


Transmittance [%]
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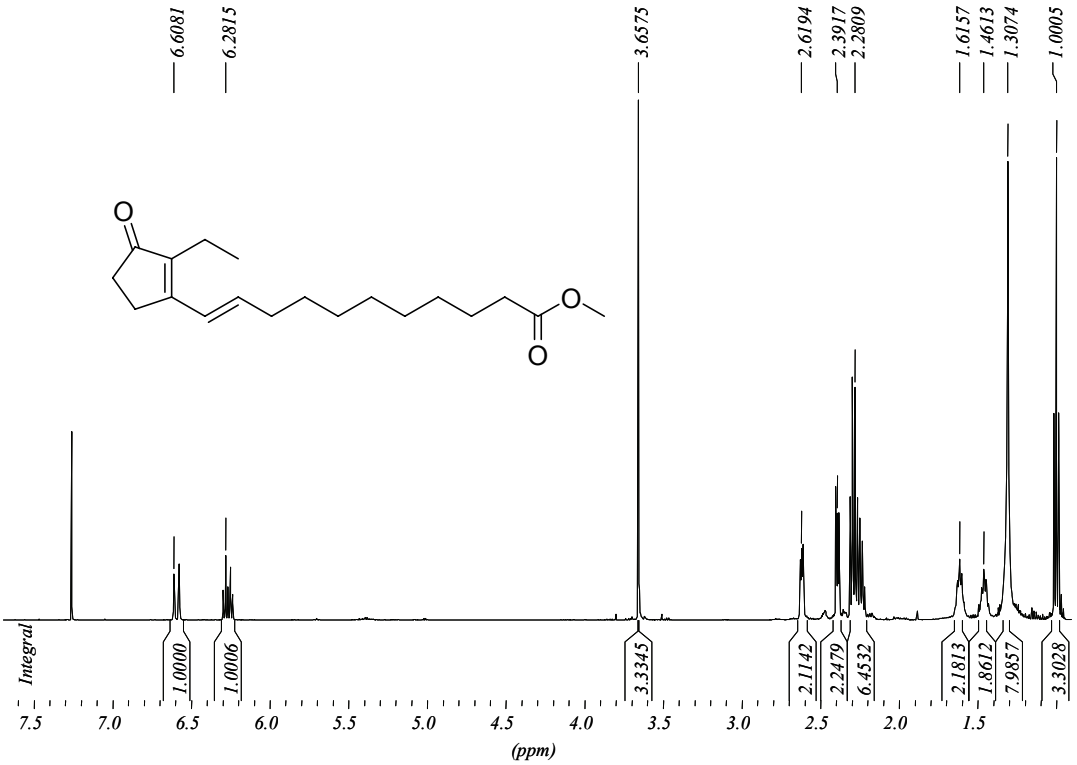
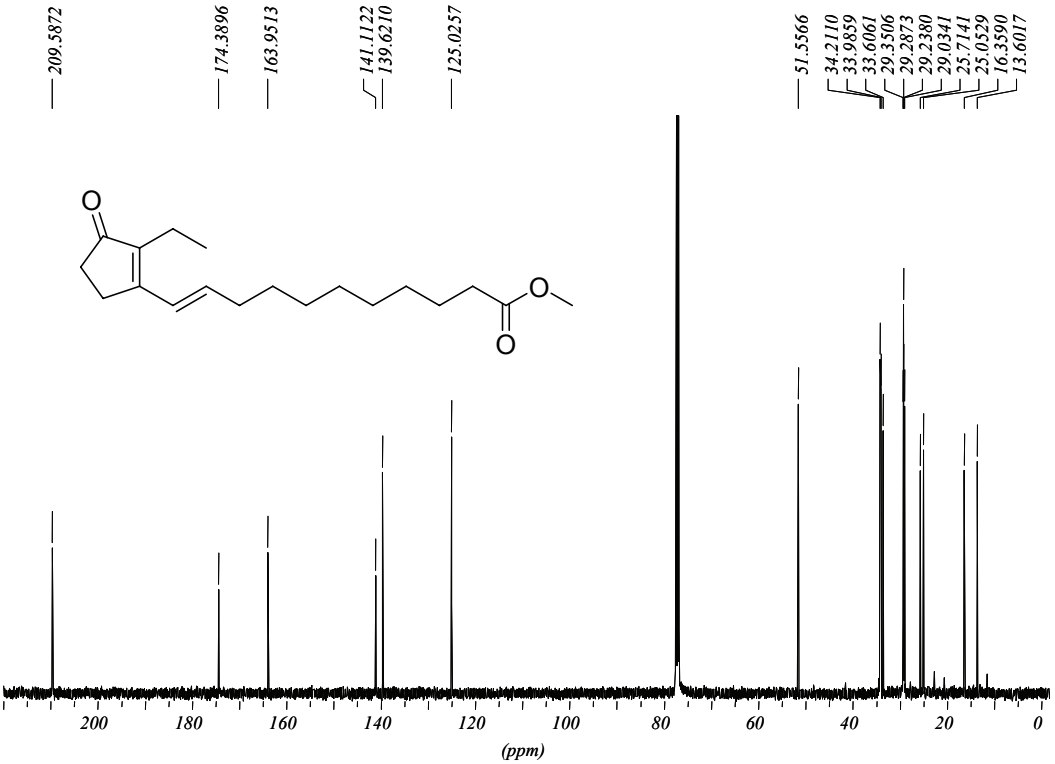
60
40
20
2929
1738
1693
1639
1438
1373
1171
3500 3000 2500 2000 1500 1000
Wavenumber cm-1

10.1.14 (10*E*)-Methyl 11-(2-ethyl-3-oxocyclopent-1-enyl)undec-10-enoate (64b)

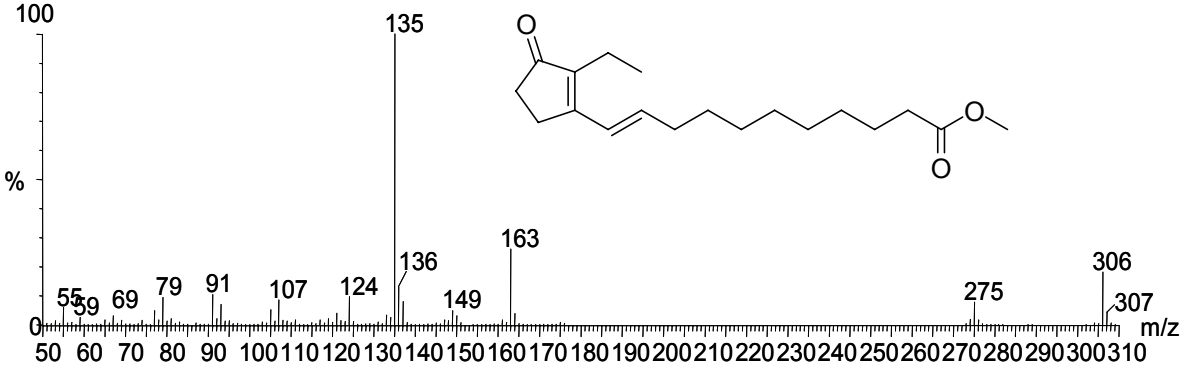
^{1}H NMR



^{13}C NMR



EI-MS


100
%
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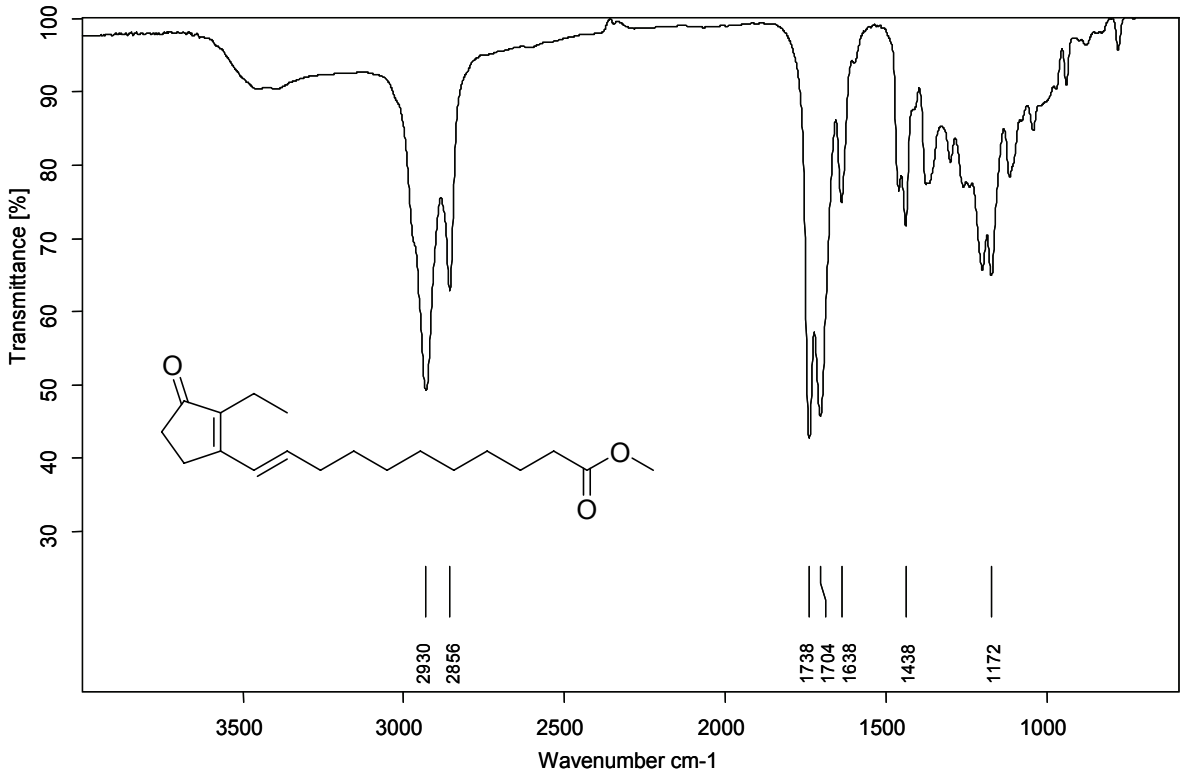
135
163
136
306
307
275
149
124
107
91
79
69
59
55
m/z
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310
O
O
O

IR


Transmittance [%]
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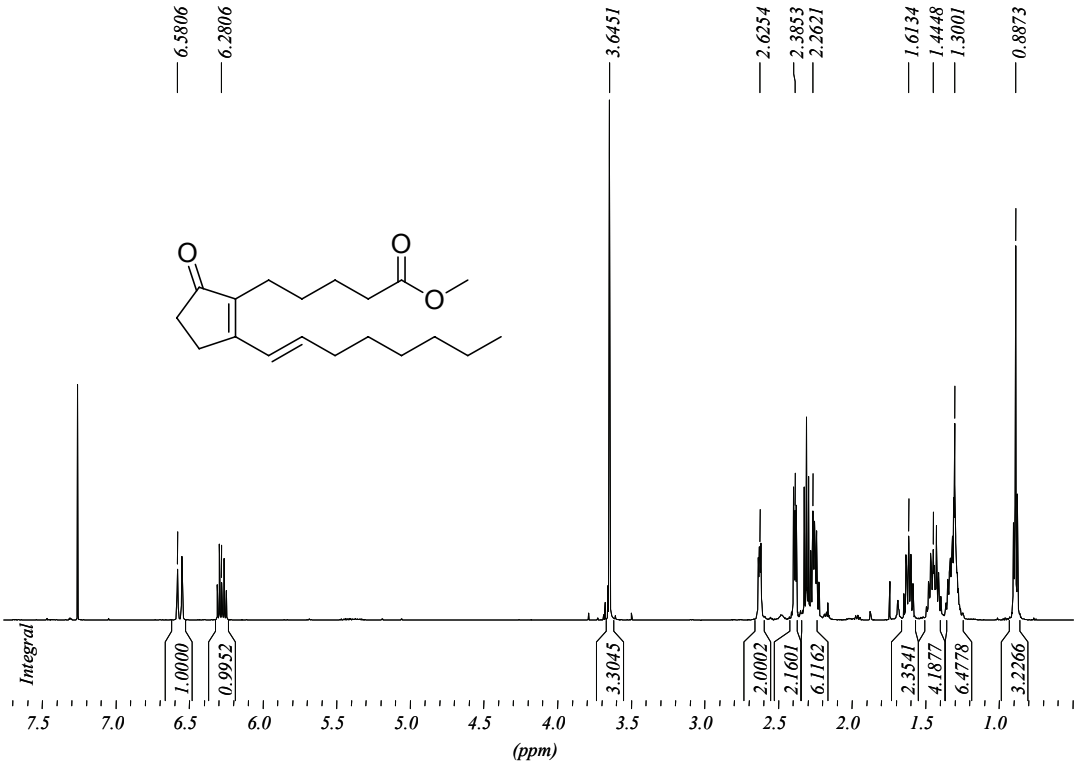
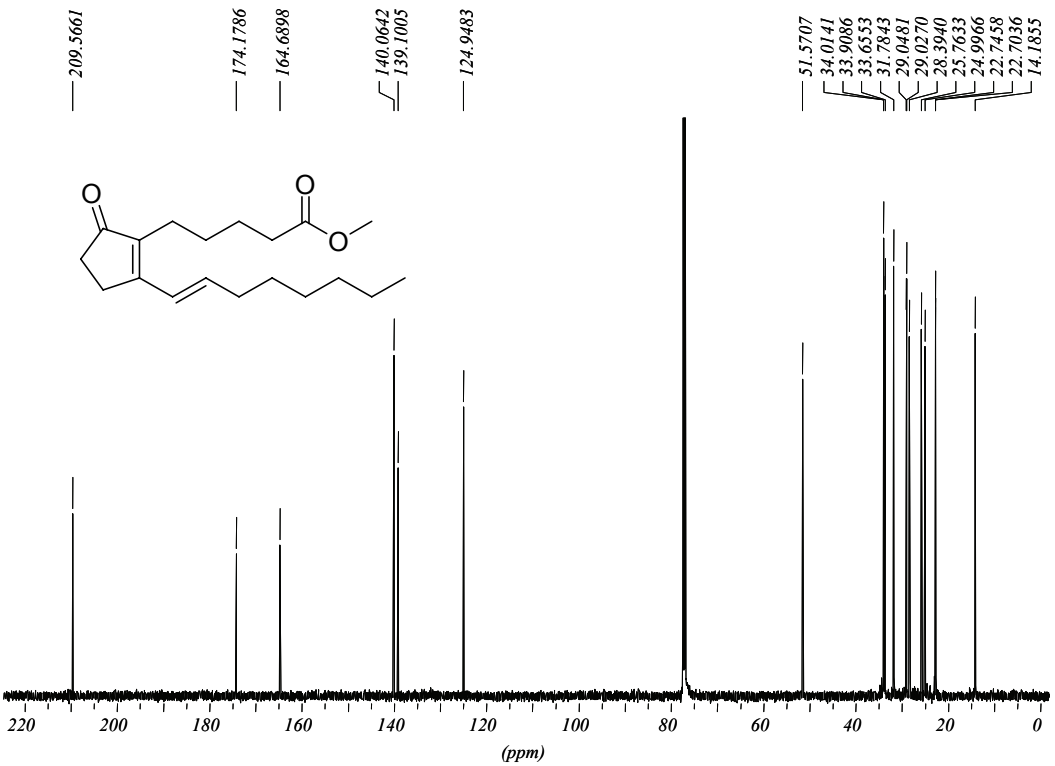
50
40
30
2930
2856
1738
1704
1638
1438
1172
3500
3000
2500
2000
1500
1000
Wavenumber cm-1
O
O
O

10.1.15 Methyl 5-(2-((*E*)-oct-1-enyl)-5-oxocyclopent-1-enyl)-pentanoate (64c)

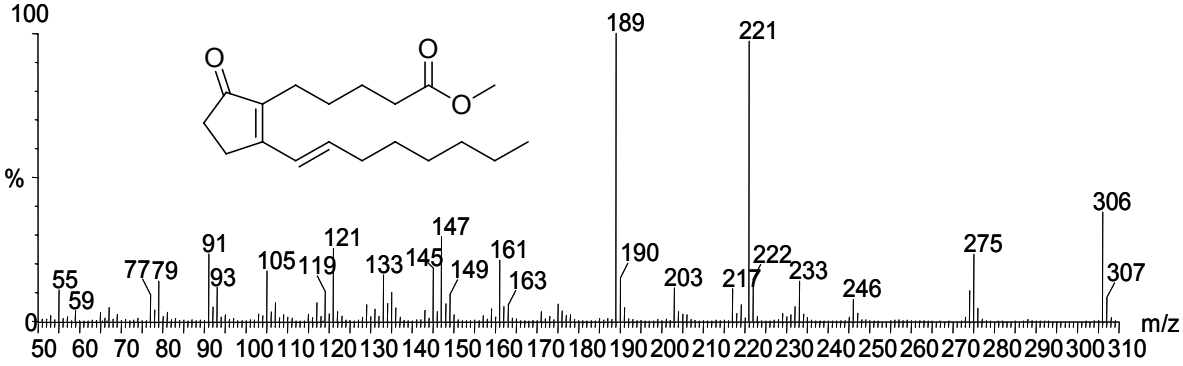
^{1}H NMR



^{13}C NMR



EI-MS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%
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221
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91
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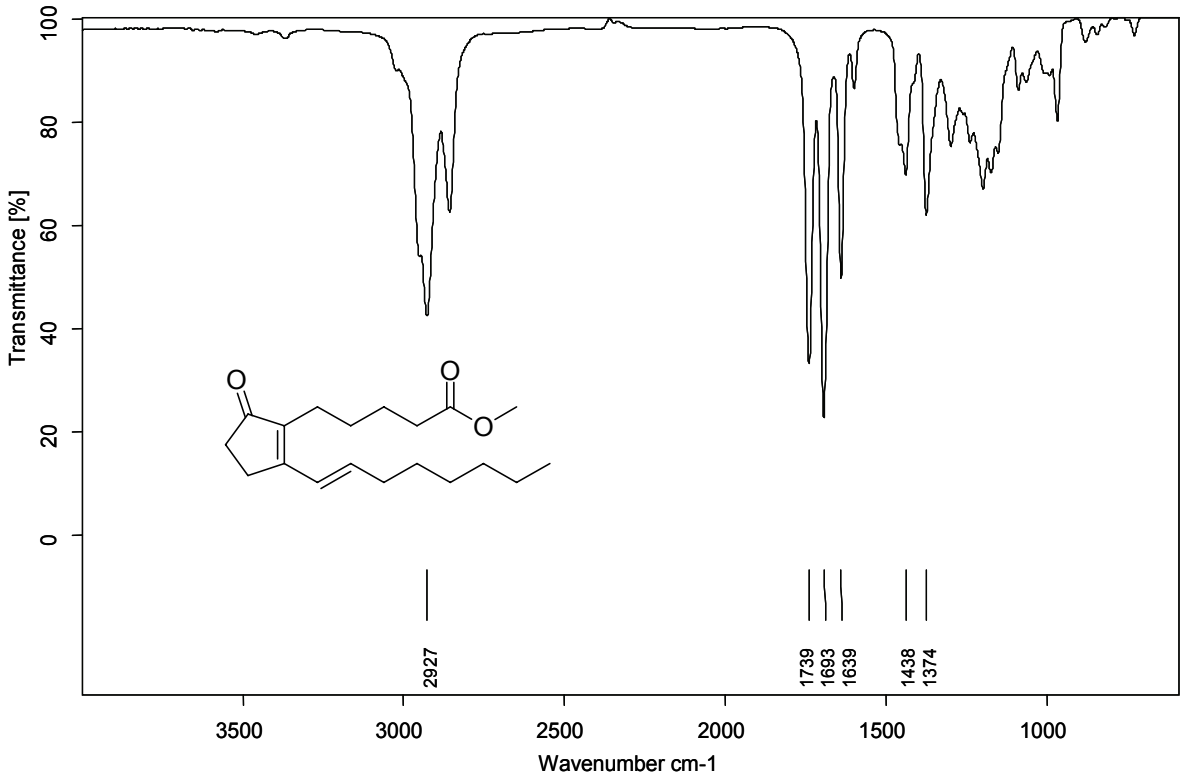
147
161
55
77
79
93
105
119
133
145
149
163
190
203
217
222
233
246
275
307
59
m/z
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310

IR


Transmittance [%]
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1639
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1374
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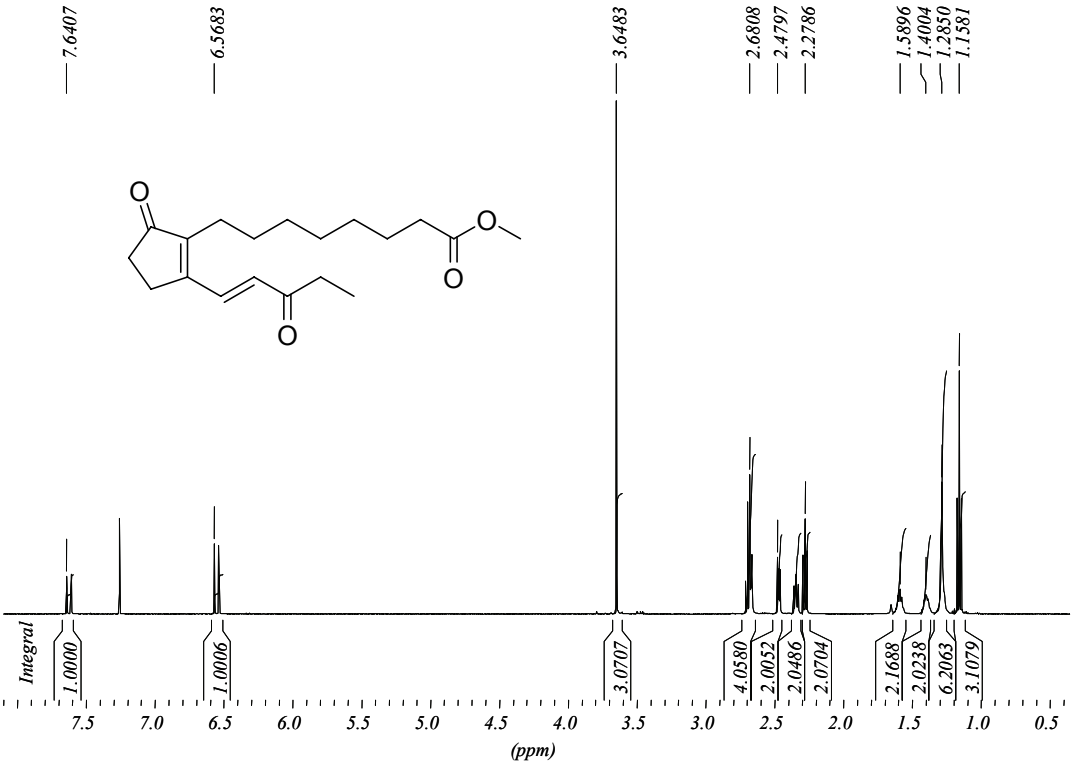
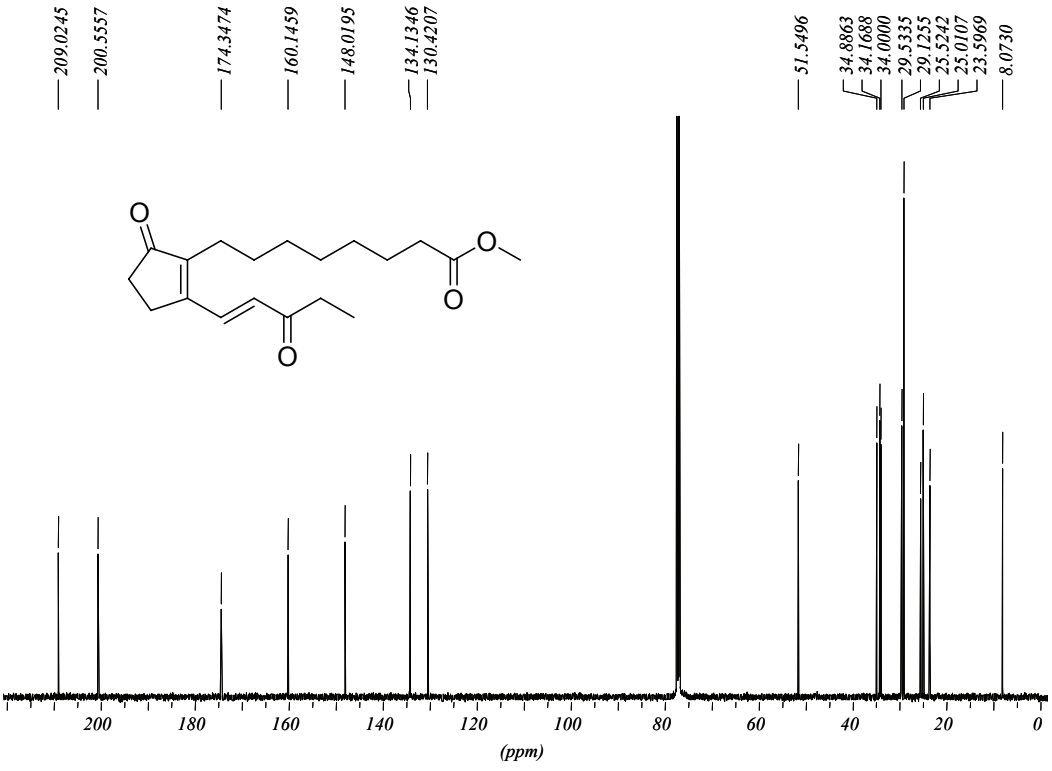
3000
2500
2000
1500
1000
Wavenumber cm-1

10.1.16 Methyl 8-(5-oxo-2-((*E*)-3-oxopent-1-enyl)cyclopent-1-enyl)-octanoate (65a)

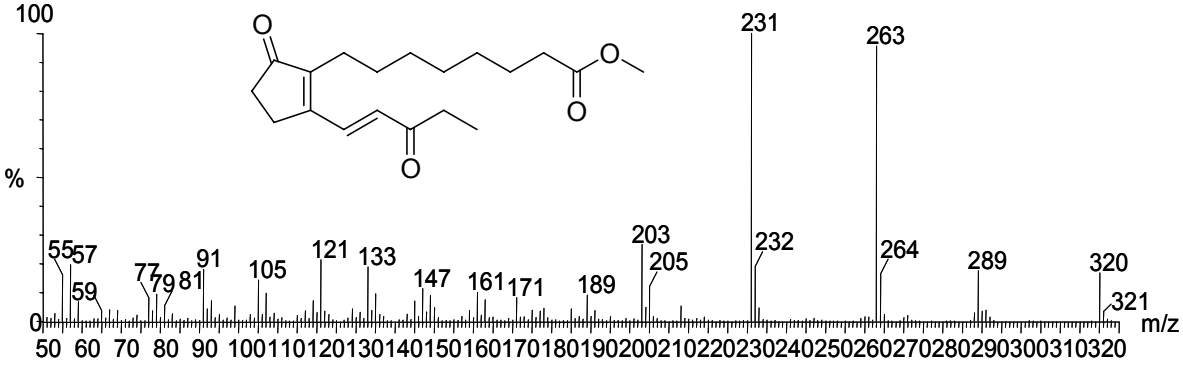
^{1}H NMR



^{13}C NMR



EI-MS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%
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O
O
O
O
231
263
203
232
264
205
289
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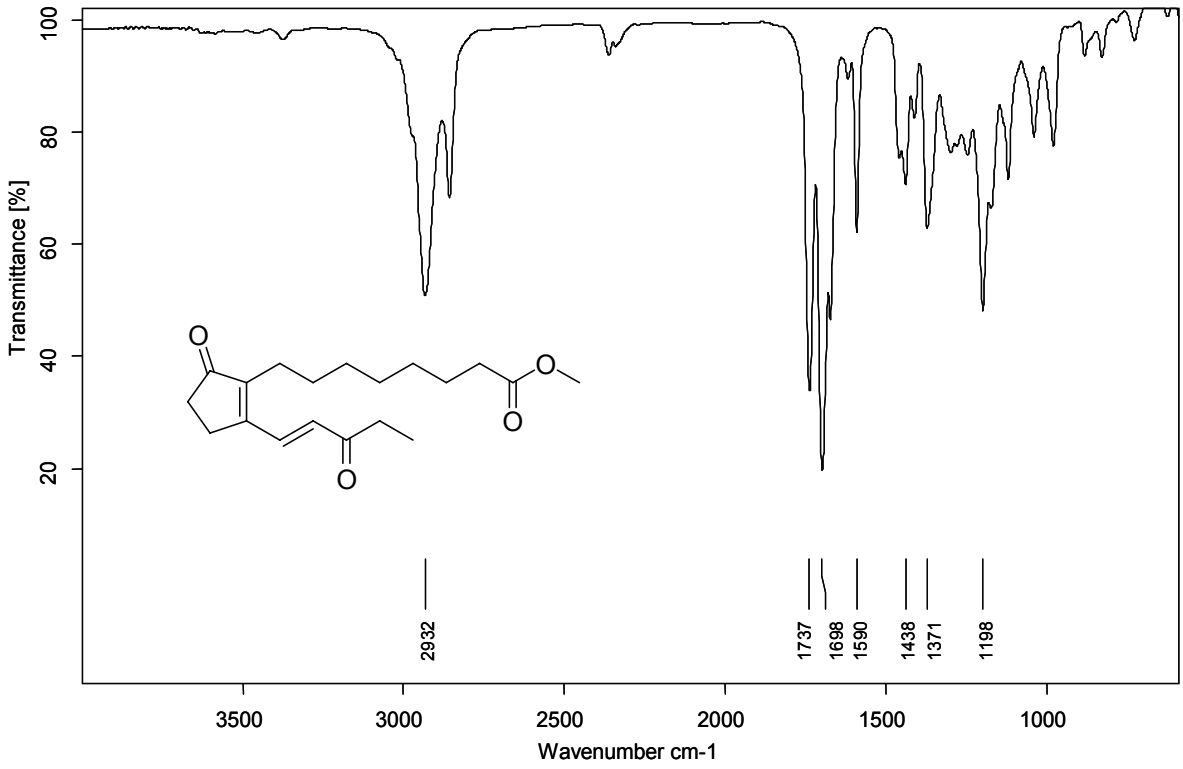
321
55
57
59
77
79
81
91
105
121
133
147
161
171
189
m/z
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320

IR


Transmittance [%]
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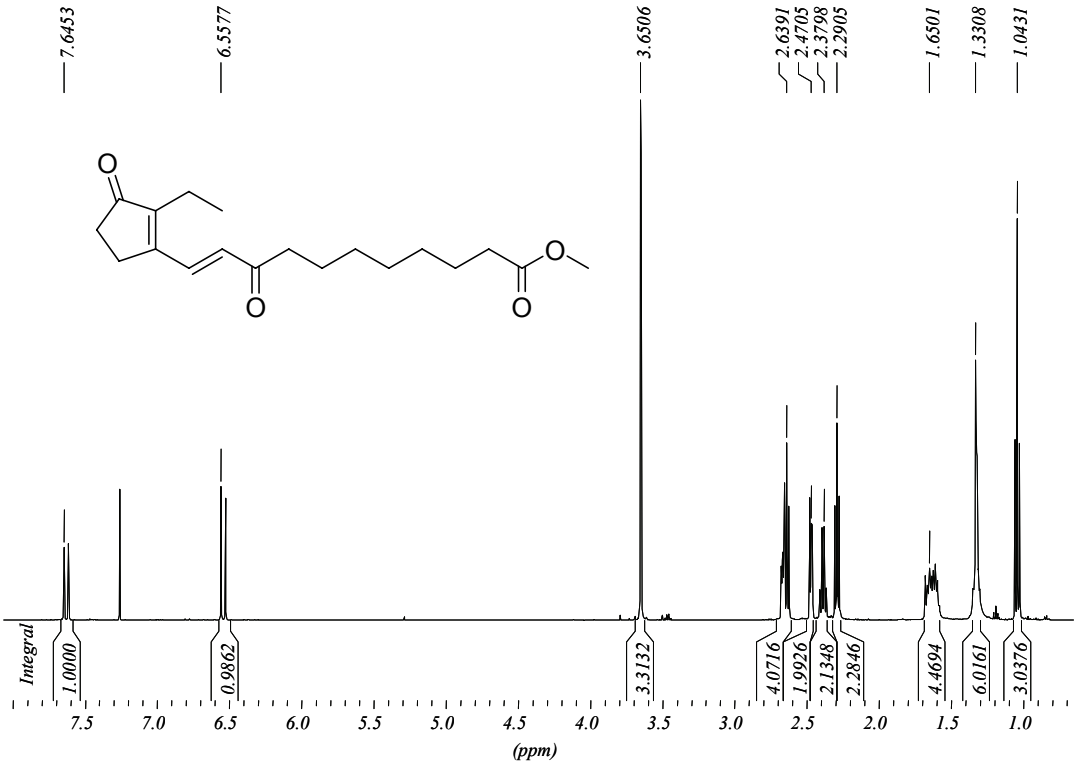
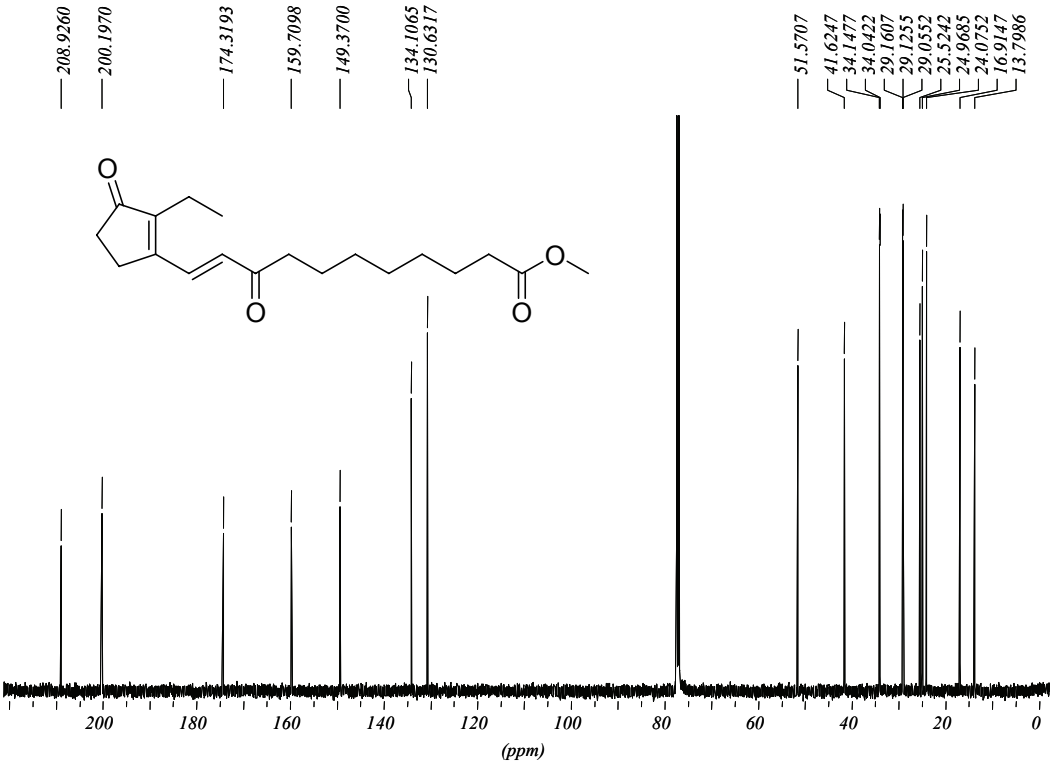
40
20
O
O
O
2932
1737
1698
1590
1438
1371
1198
3500
3000
2500
2000
1500
1000
Wavenumber cm-1

10.1.17 (10*E*)-Methyl 11-(2-ethyl-3-oxocyclopent-1-enyl)-9-oxo-undec-10-enoate (65b)

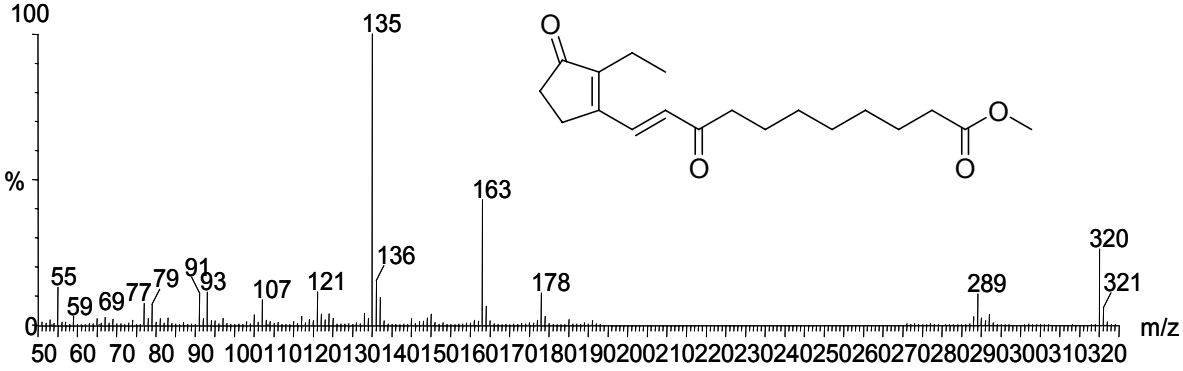
^{1}H NMR



^{13}C NMR



EI-MS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%
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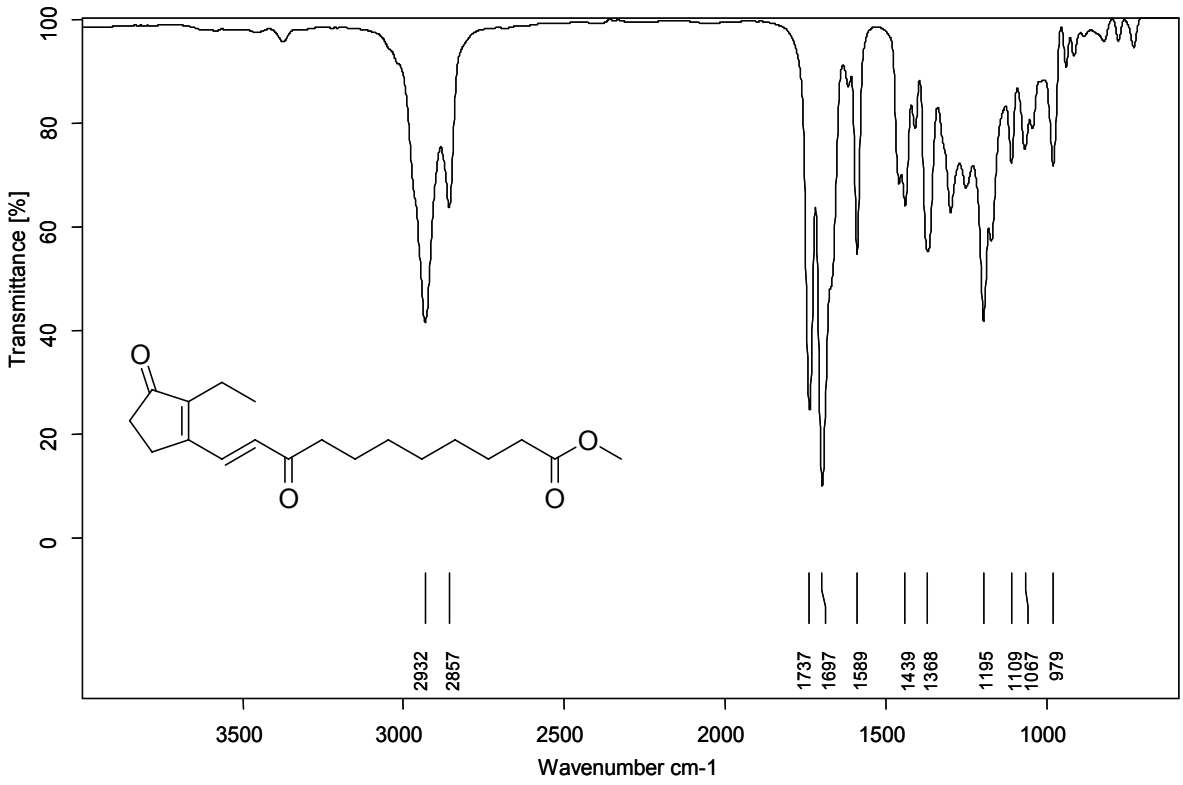
163
136
320
321
55
59
69
77
79
91
93
107
121
178
289
m/z
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320
O
O
O
O

IR


Transmittance [%]
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1697
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1439
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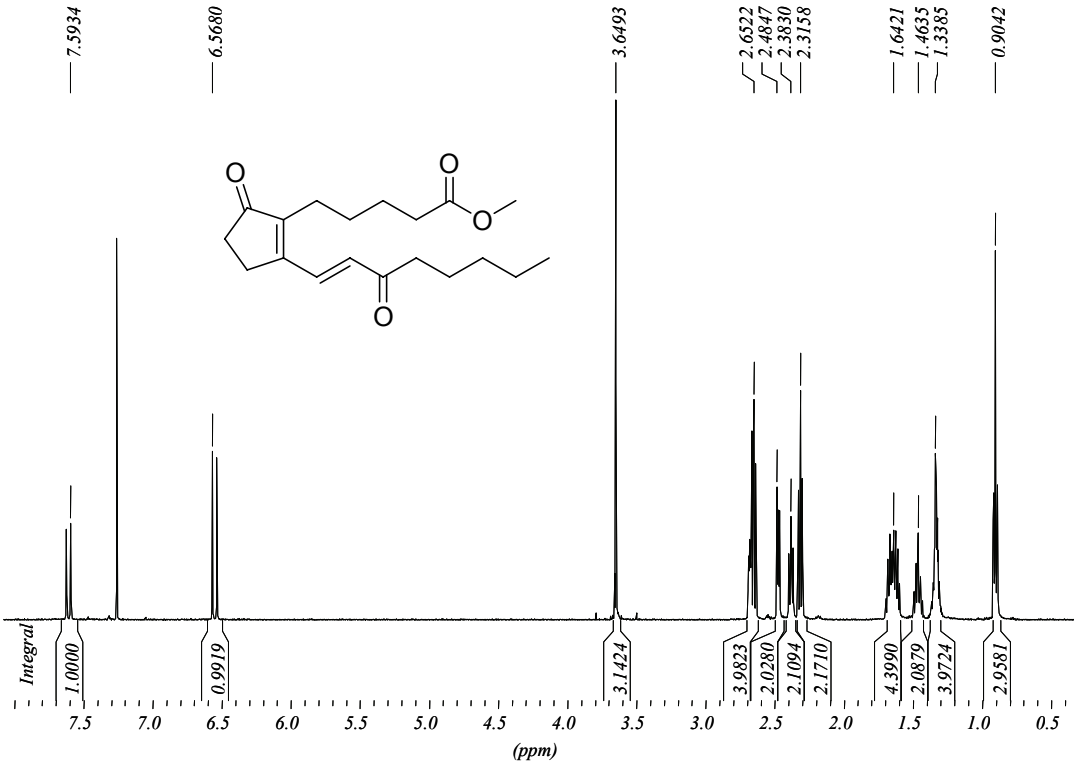
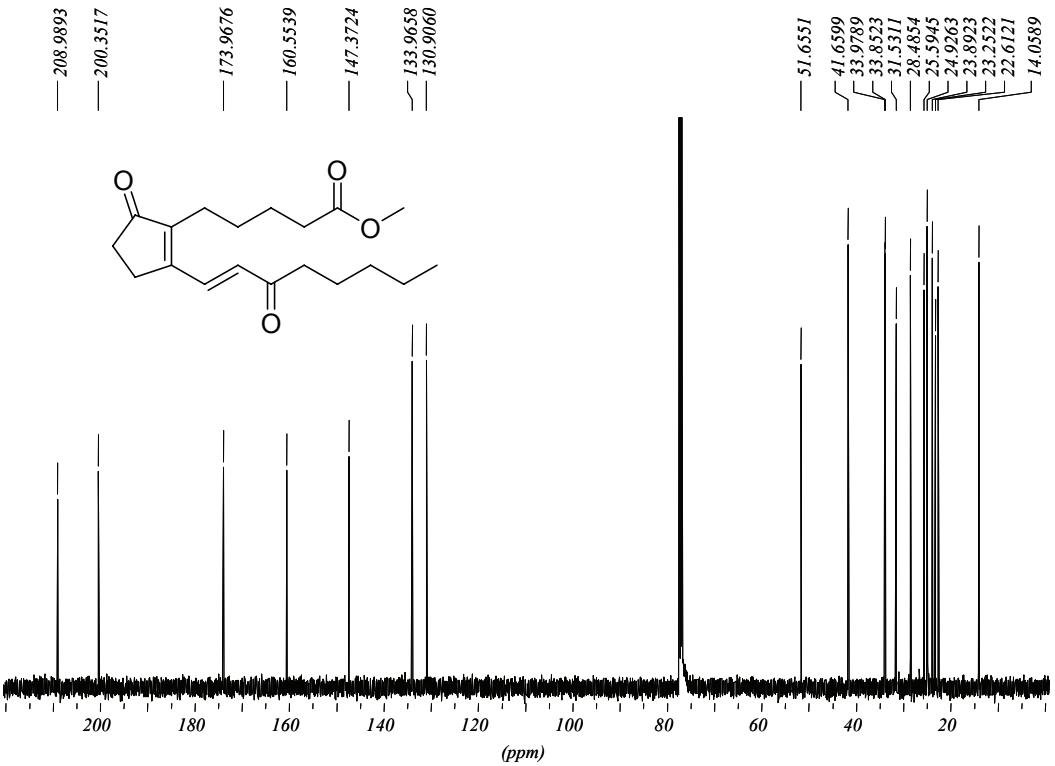
1195
1109
1067
979
3500
3000
2500
2000
1500
1000
Wavenumber cm-1
O
O
O
O

10.1.18 Methyl 5-(5-oxo-2-((*E*)-3-oxooct-1-enyl)cyclopent-1-enyl)-pentanoate (65c)

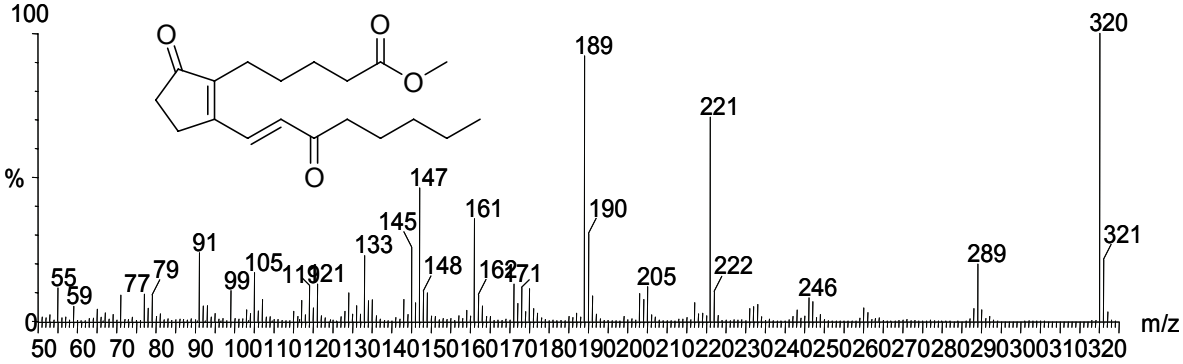
^{1}H NMR



^{13}C NMR



EI-MS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%
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m/z
320
189
221
147
161
190
145
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289
105
148
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171
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77
79
99
119
121
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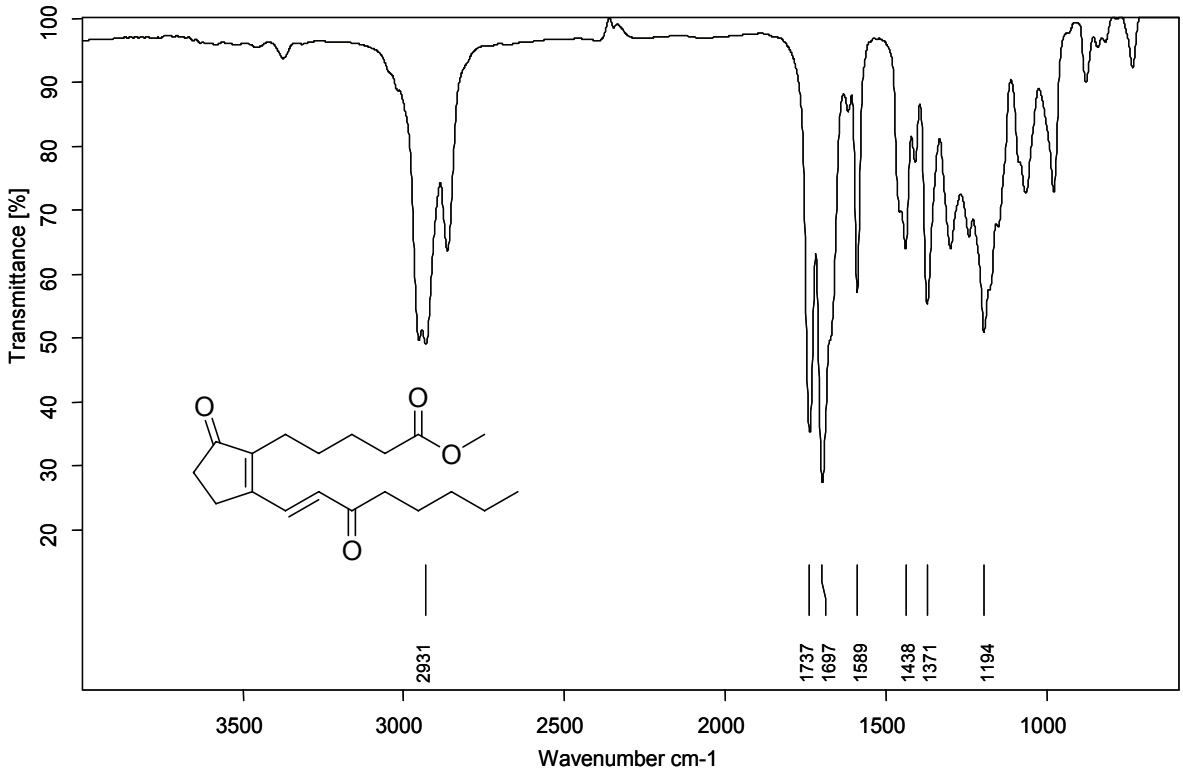
IR


Transmittance [%]
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30
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2931
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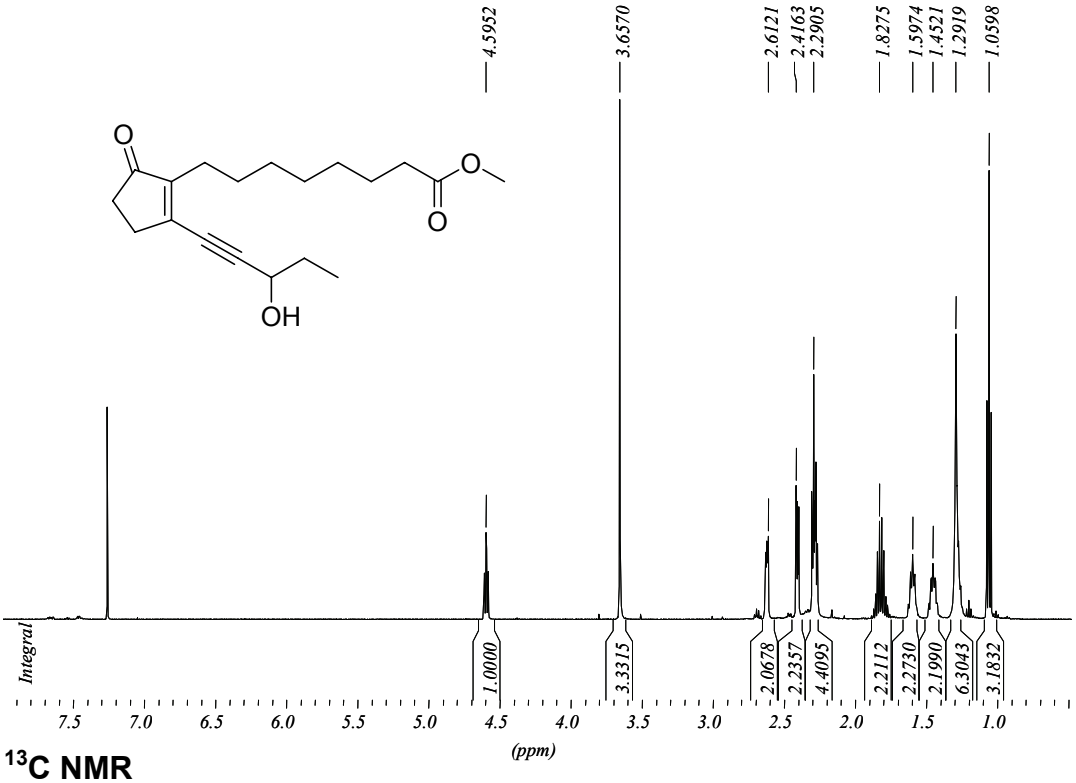
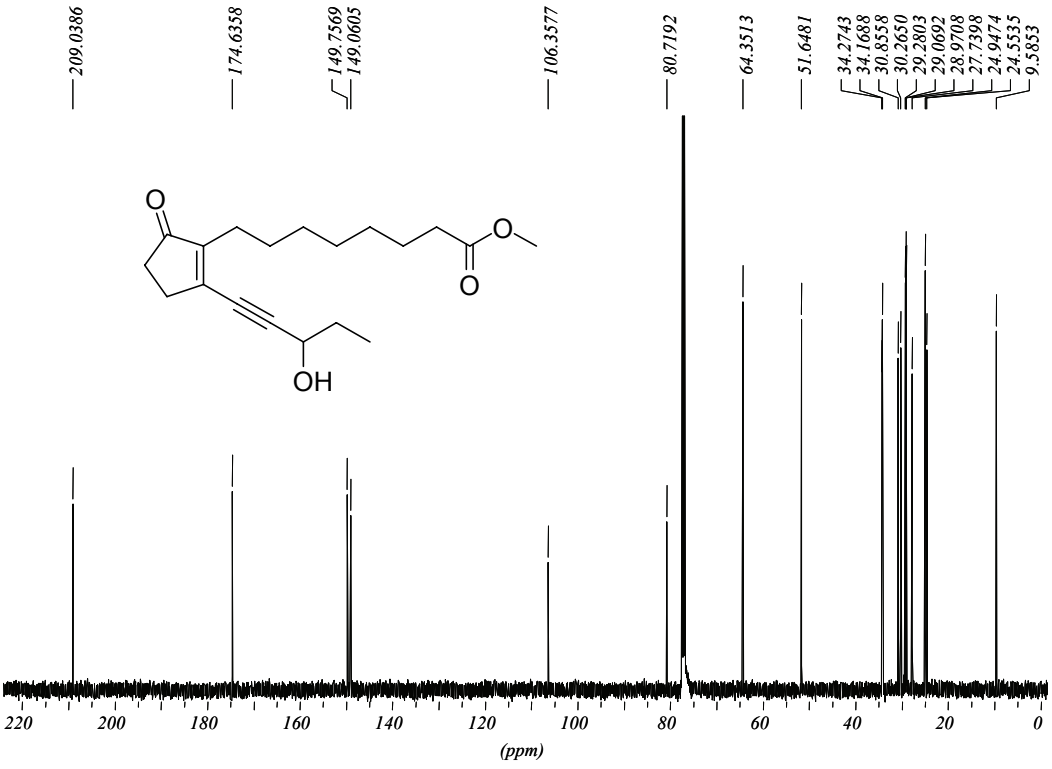
1697
1589
1438
1371
1194
3500
3000
2500
2000
1500
1000
Wavenumber cm-1

10.1.19 Methyl 8-(2-(3-hydroxypent-1-ynyl)-5-oxocyclopent-1-enyl)-octanoate (66a)

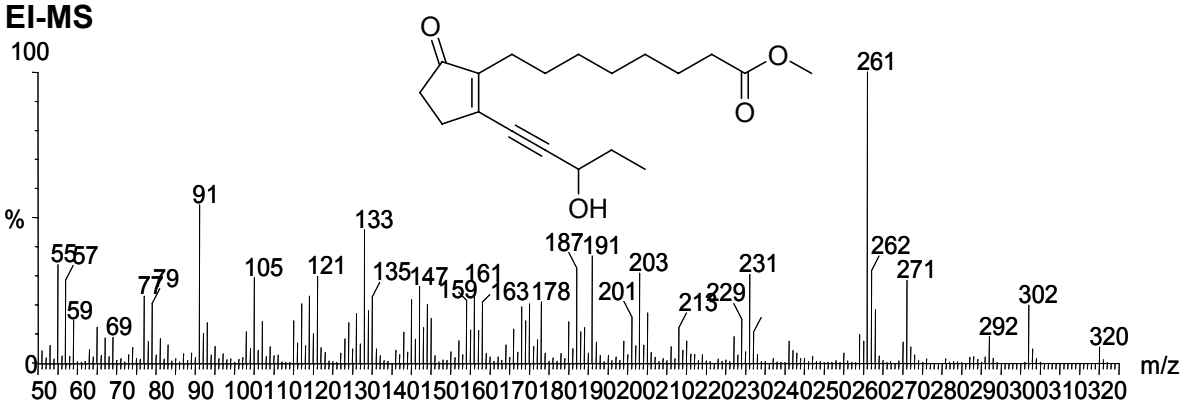
^{1}H NMR



^{13}C NMR



EI-MS


100
%
0
m/z
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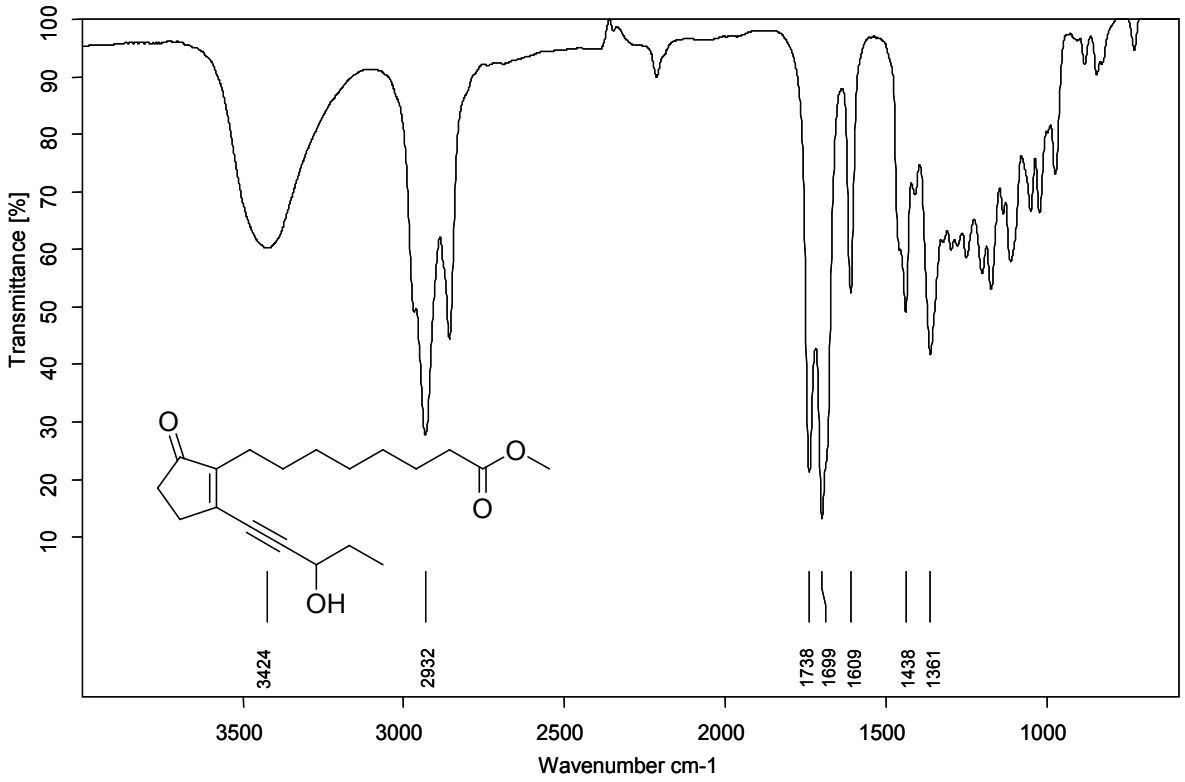
55 57 59 69 77 79 91 105 121 133 135 147 159 161 163 178 187 191 201 203 213 229 231 261 262 271 292 302 320
O
O
O
OH

IR


Transmittance [%]
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3424
2932
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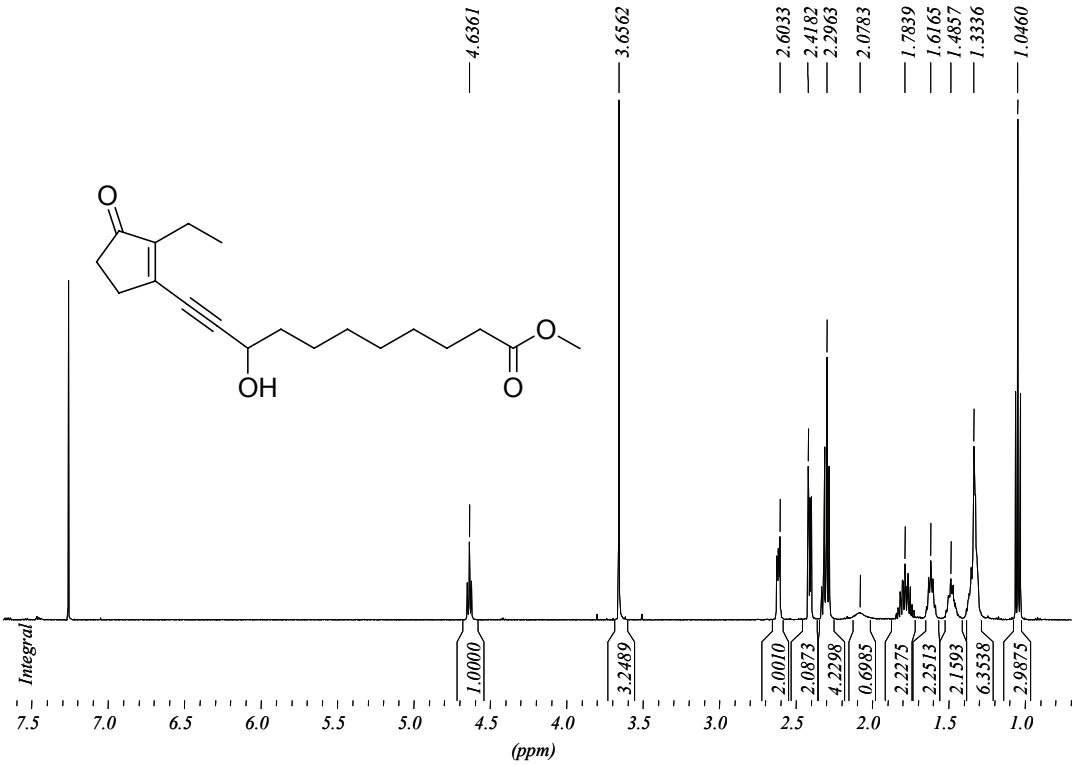
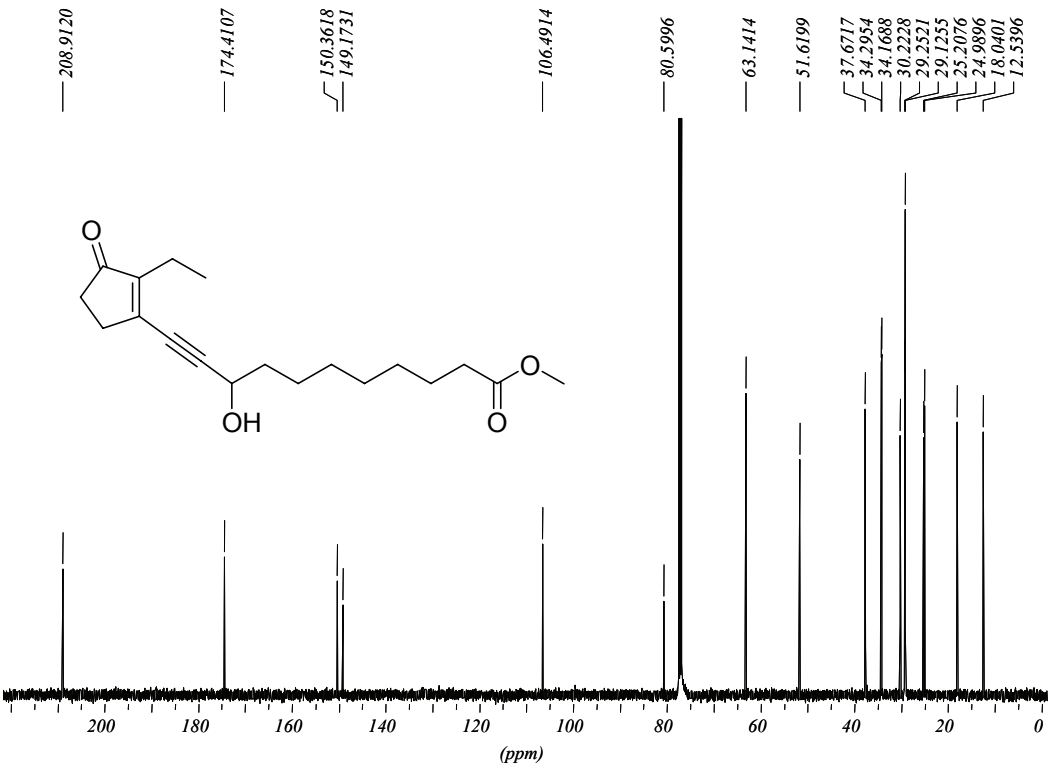
1699
1609
1438
1361
3500 3000 2500 2000 1500 1000
Wavenumber cm-1
O
O
O
OH

10.1.20 Methyl 11-(2-ethyl-3-oxocyclopent-1-enyl)-9-hydroxy-undec-10-ynoate (66b)

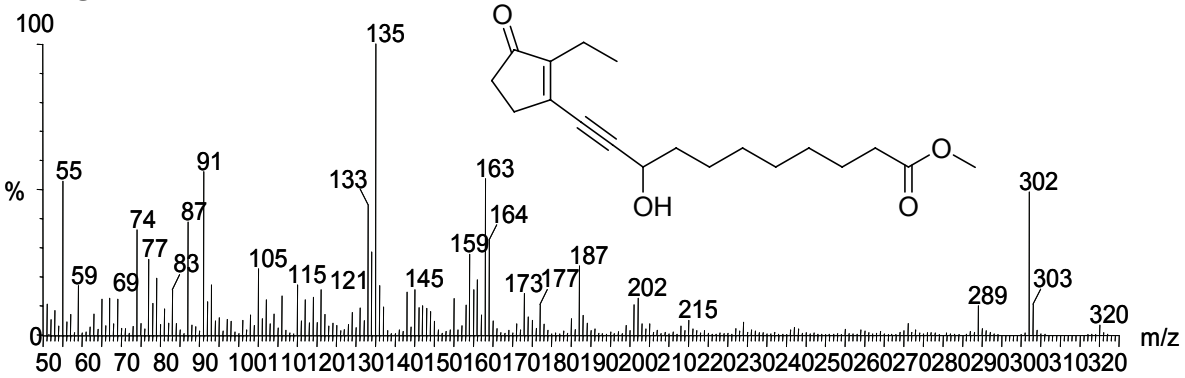
^{1}H NMR



^{13}C NMR



EI-MS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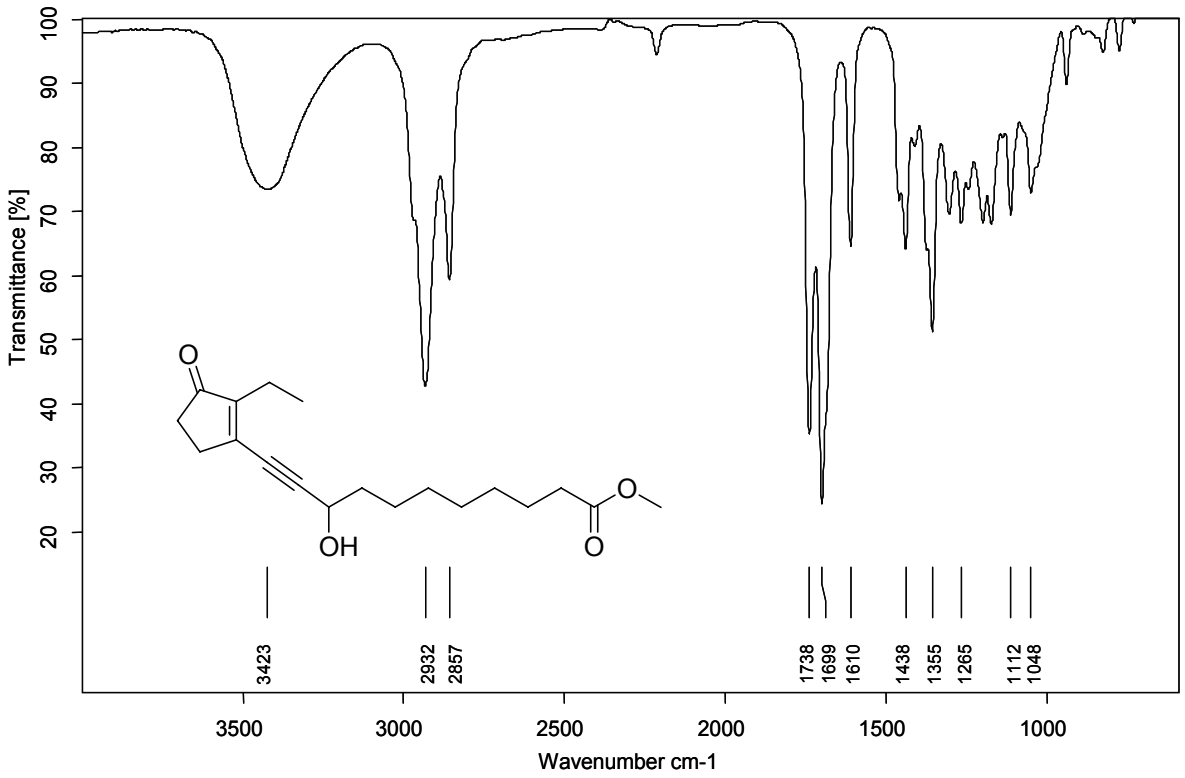
187
202
215
289
302
303
320
59
69
O
OH
O
O
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320
m/z

IR


Transmittance [%]
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50
40
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20
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O
O
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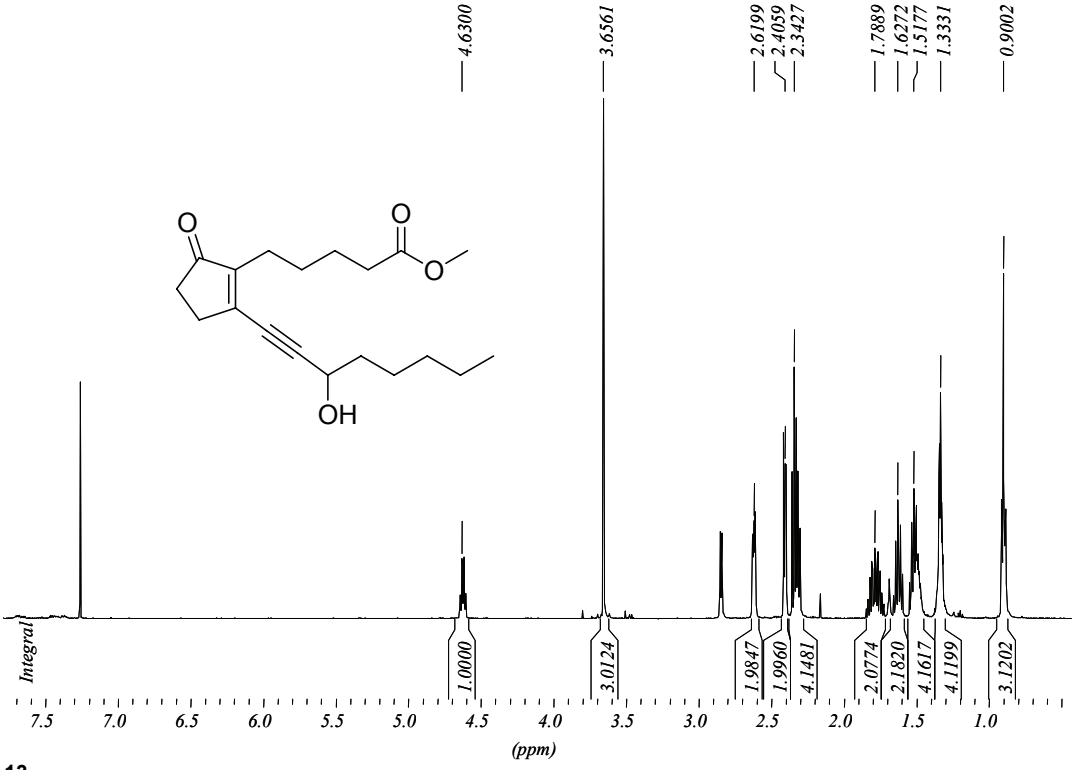
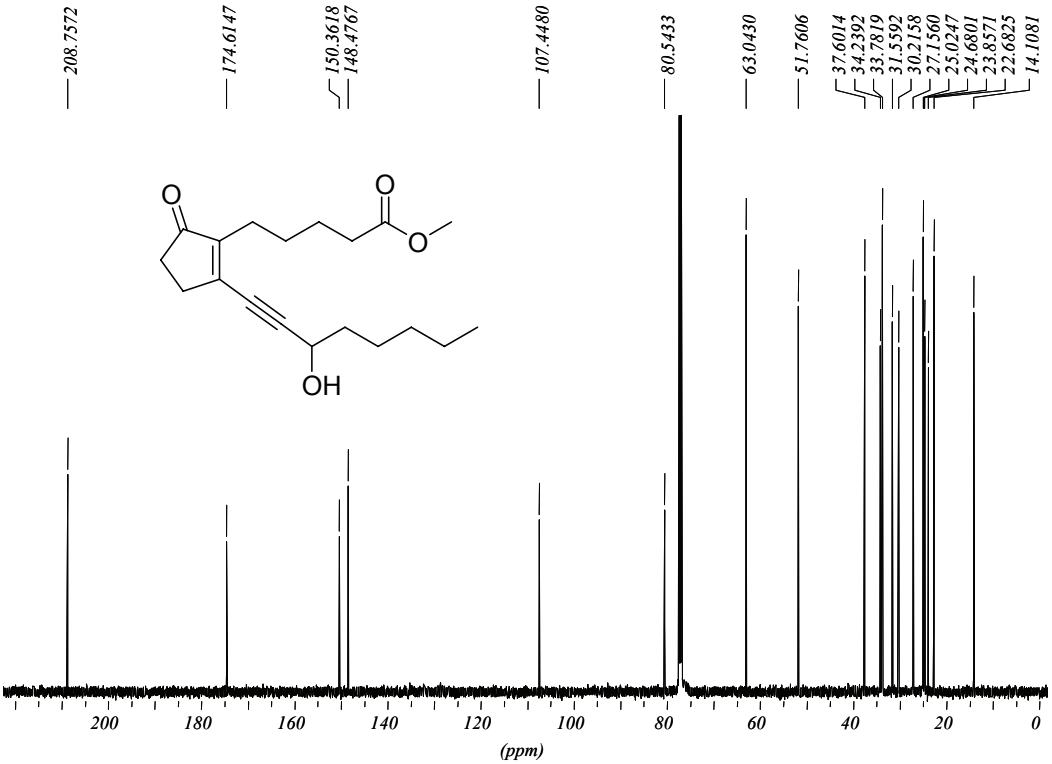
2857
1738
1699
1610
1438
1355
1265
1112
1048
3500
3000
2500
2000
1500
1000
Wavenumber cm-1

10.1.21 Methyl 5-(2-(3-hydroxyoct-1-ynyl)-5-oxocyclopent-1-enyl)-pentanoate (66c)

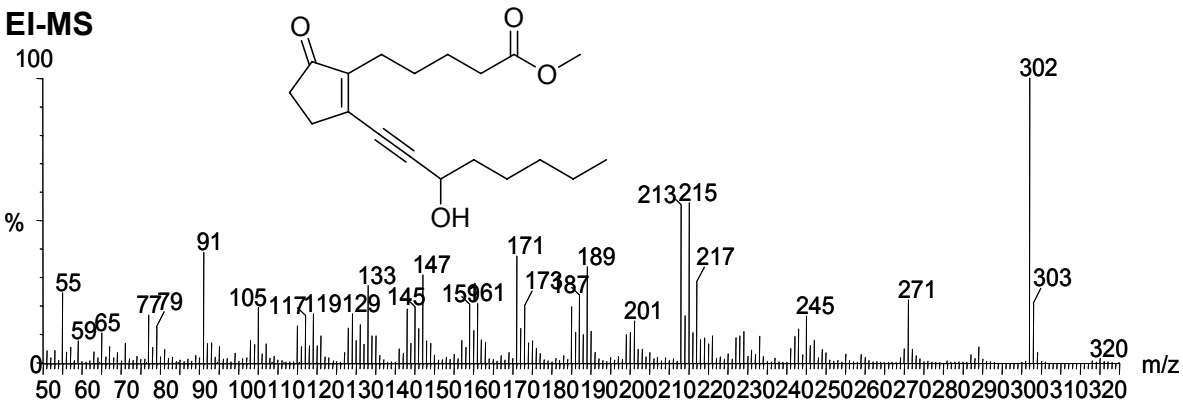
^{1}H NMR



^{13}C NMR



EI-MS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133
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79
105
117
119
129
145
159
161
173
187
201
245
59
65
320
m/z
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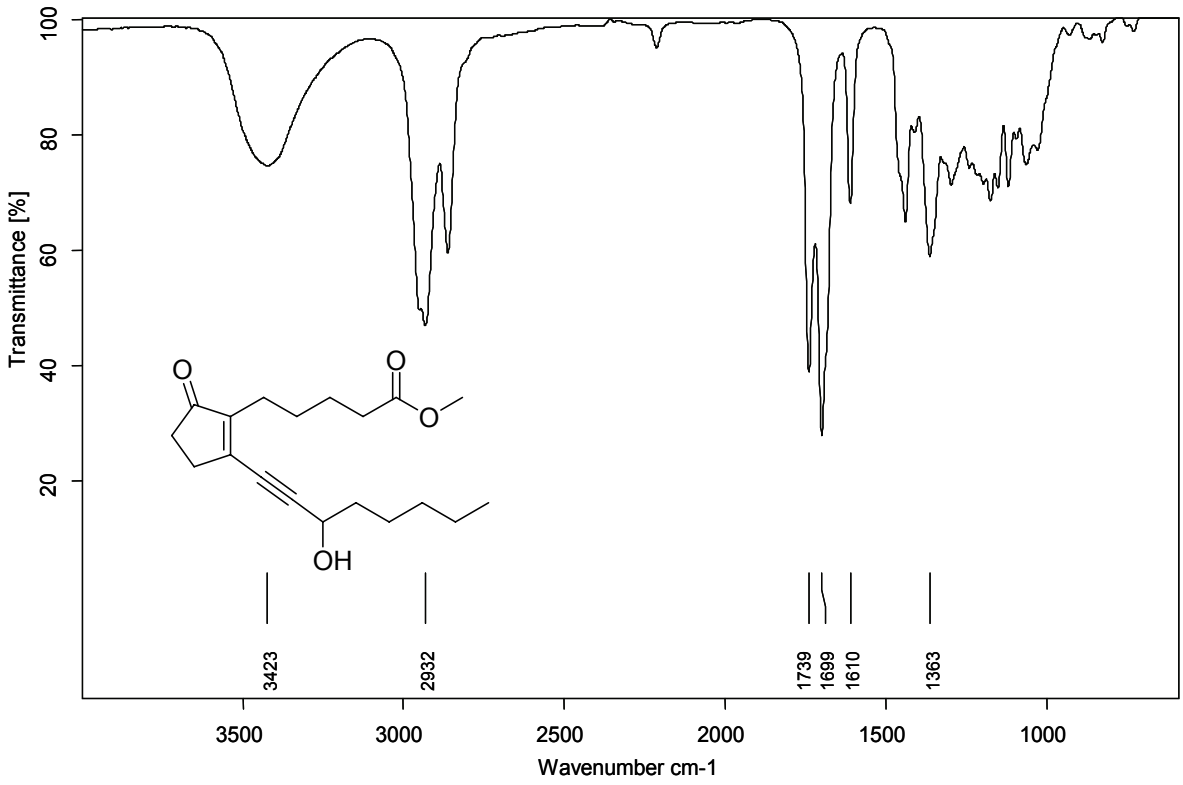
IR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OH
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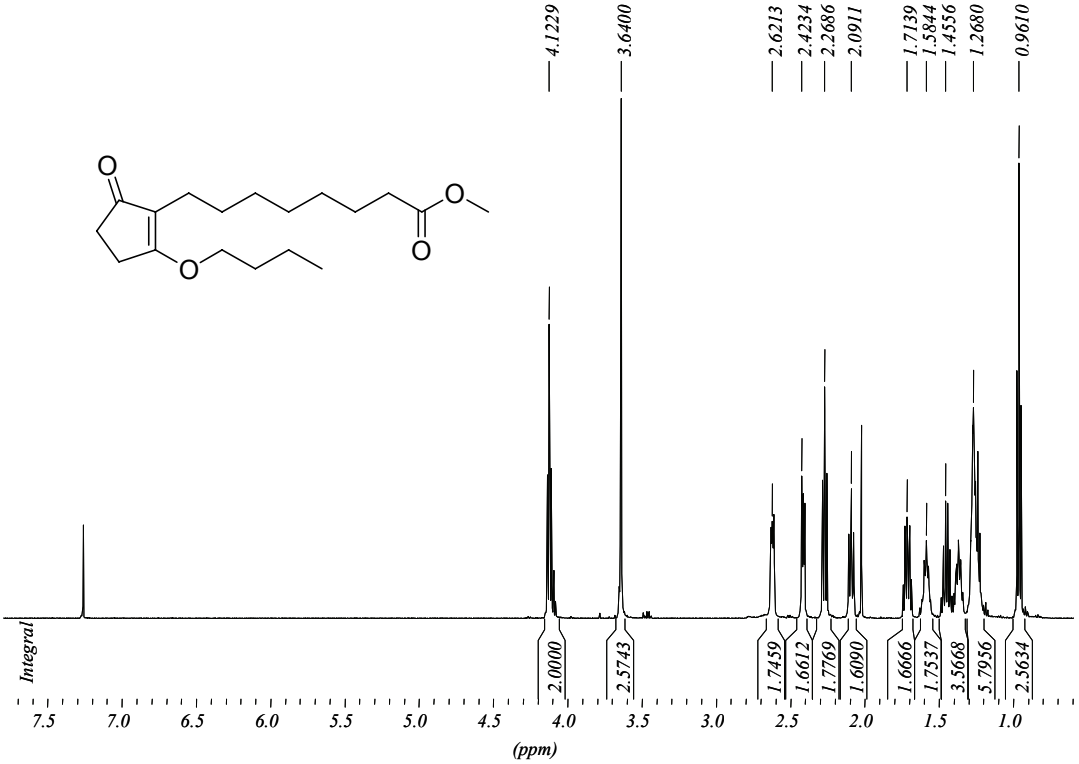
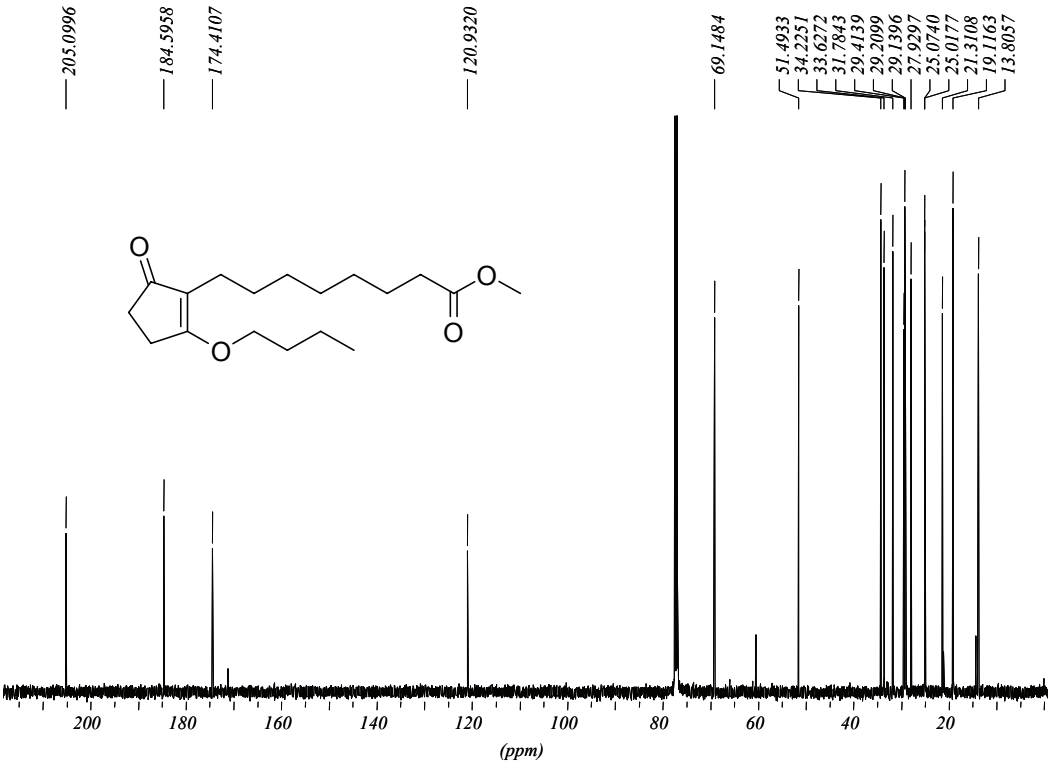
3000
2500
2000
1500
1000
Wavenumber cm-1

10.1.22 Methyl 8-(2-butoxy-5-oxocyclopent-1-enyl)octanoate (67a)

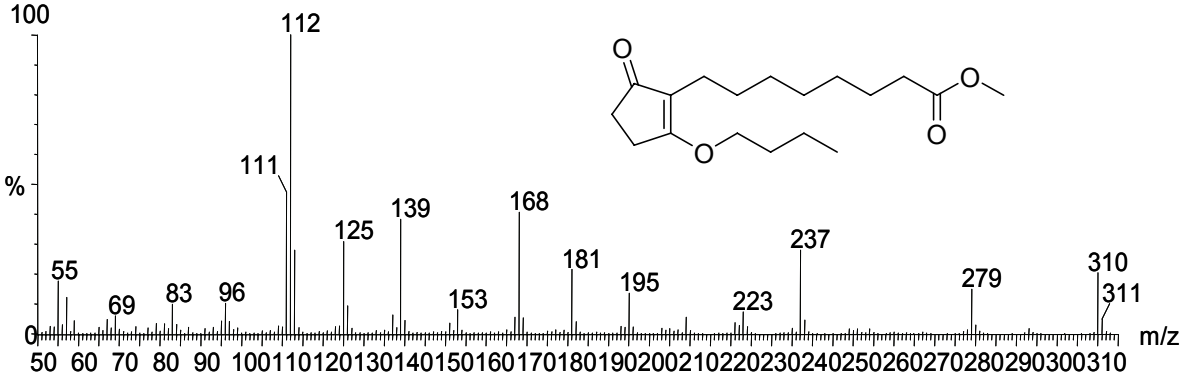
^{1}H NMR



^{13}C NMR



EI-MS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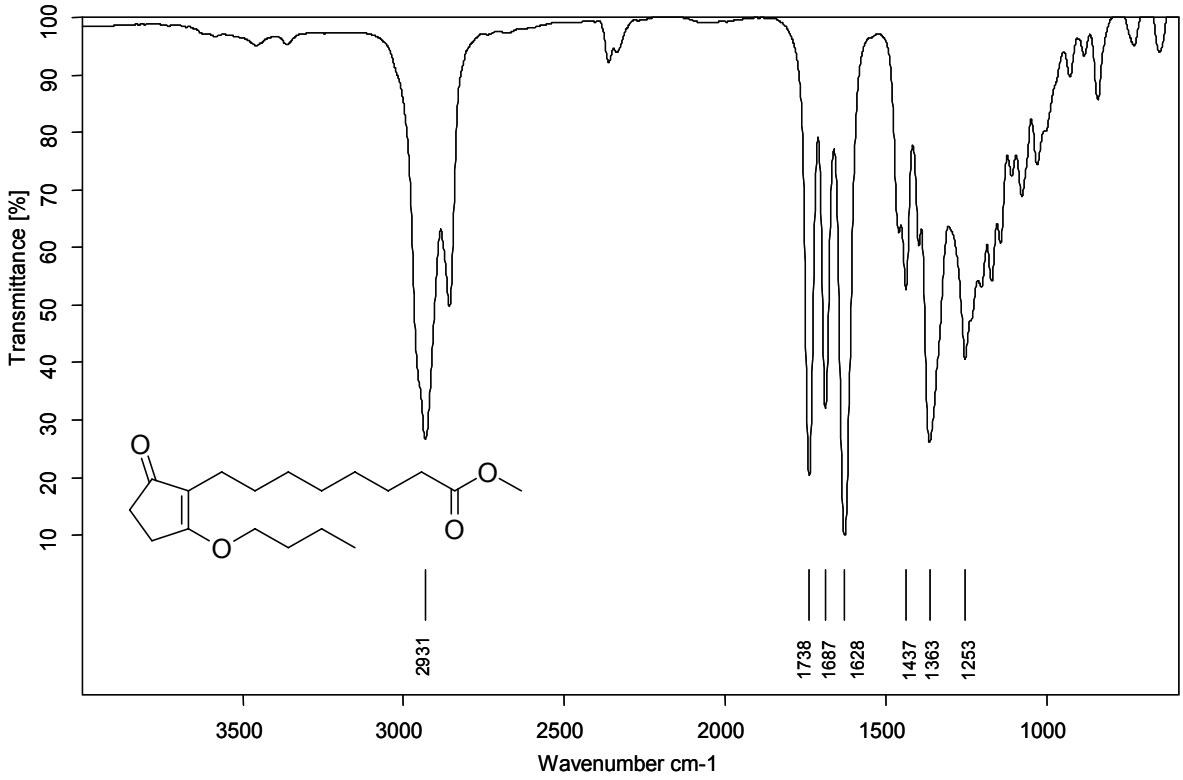
181
195
223
237
279
310
311
m/z
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310
O
O
O
O

IR


Transmittance [%]
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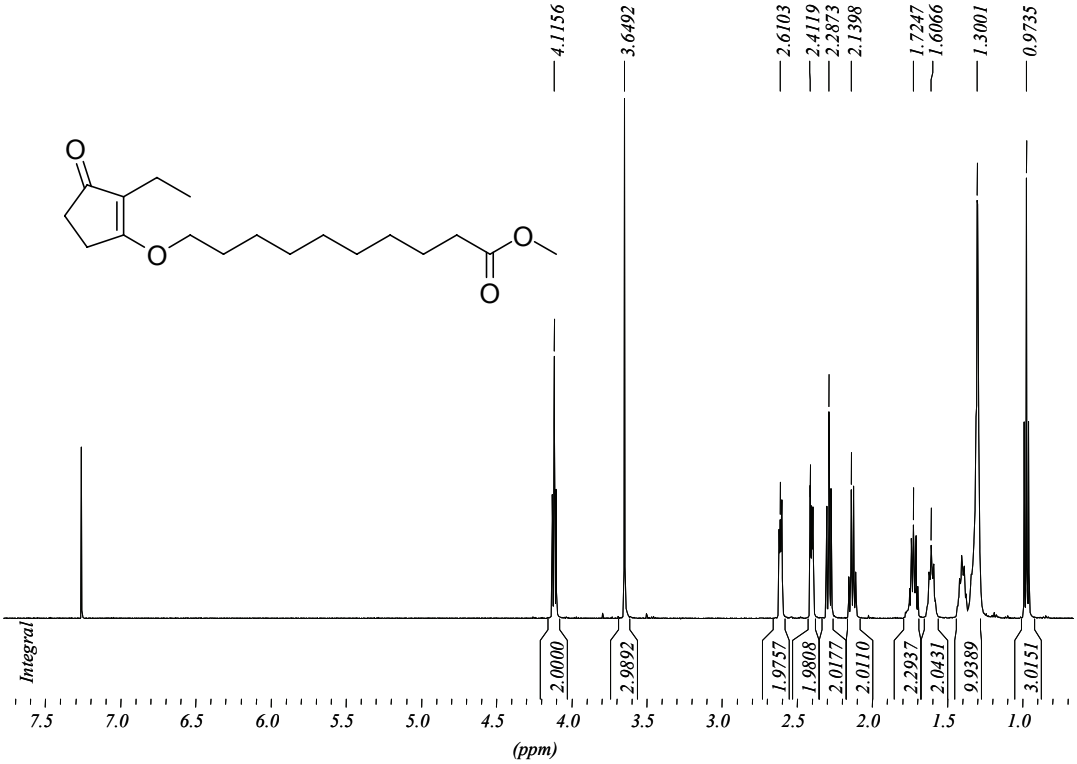
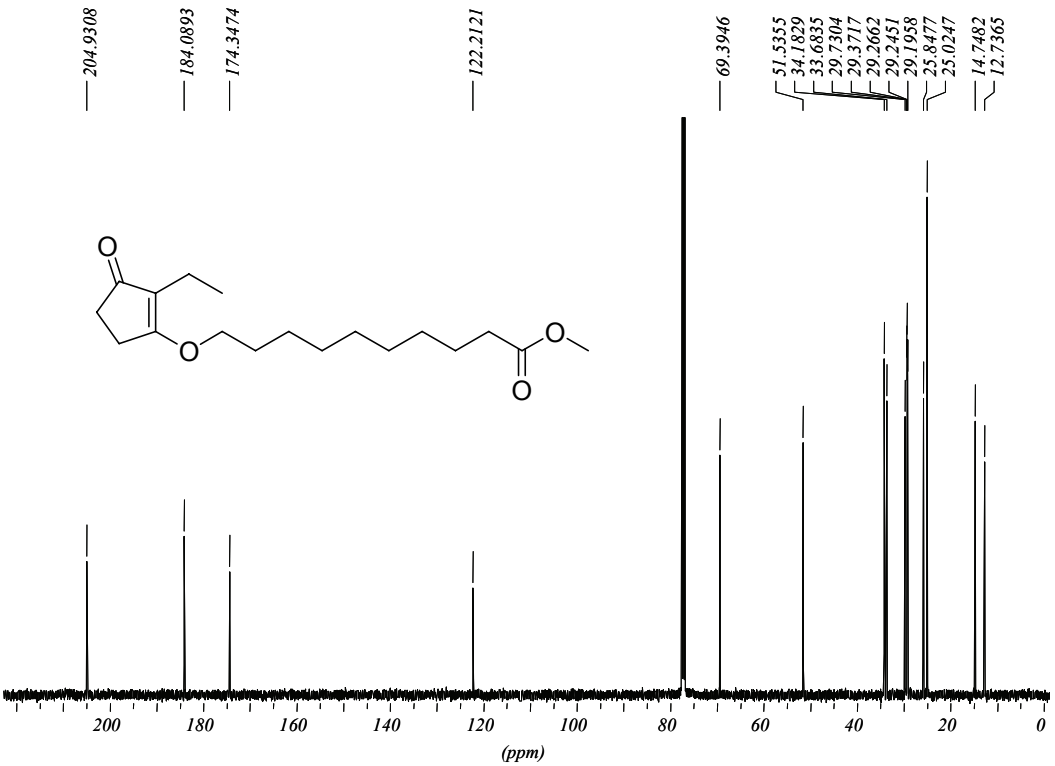
1363
1253
3500
3000
2500
2000
1500
1000
Wavenumber cm-1
O
O
O
O

10.1.23 Methyl 10-(2-ethyl-3-oxocyclopent-1-enyloxy)decanoate (67b)

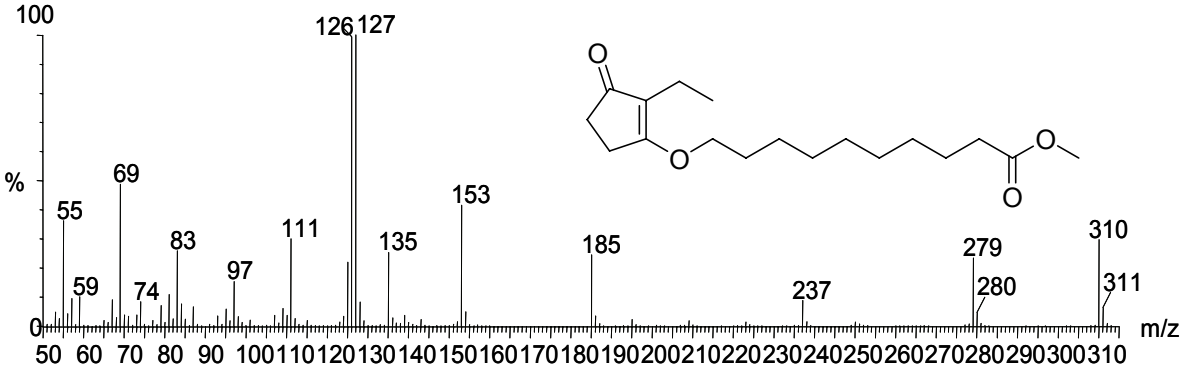
^{1}H NMR



^{13}C NMR



EI-MS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74
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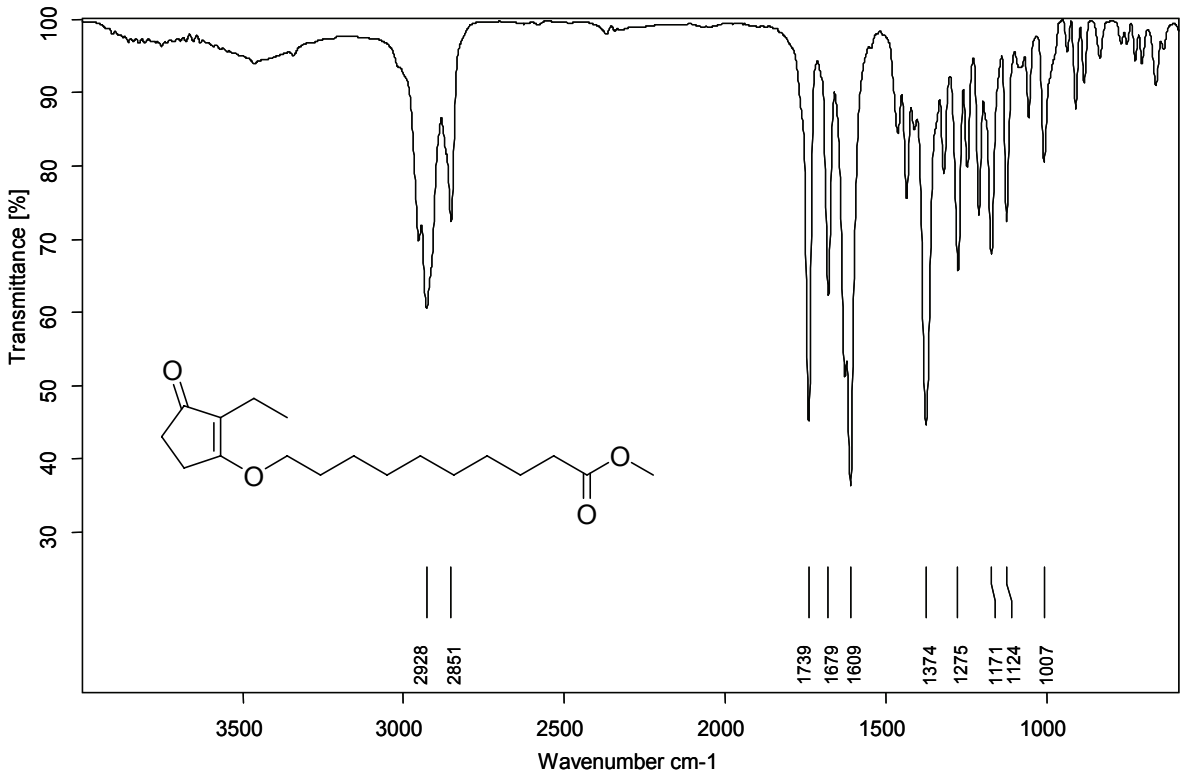
m/z
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310
O
O
O
O

IR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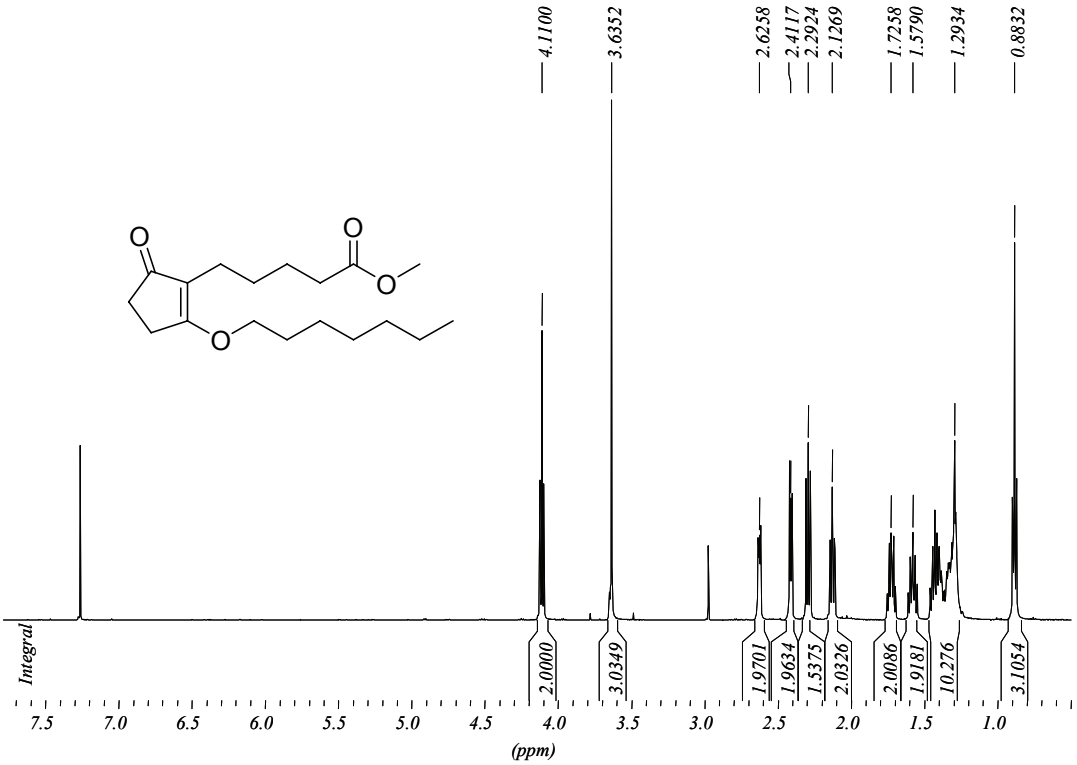
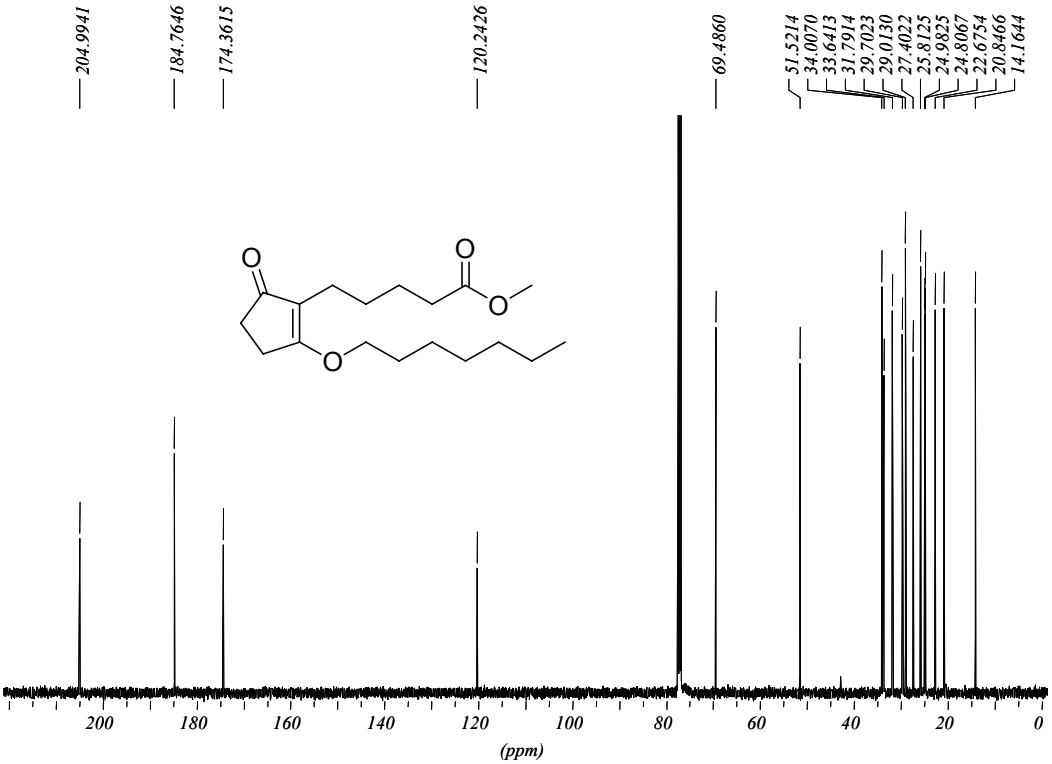
1171
1124
1007
3500
3000
2500
2000
1500
1000
Wavenumber cm-1
O
O
O
O

10.1.24 Methyl 5-(2-(heptyloxy)-5-oxocyclopent-1-enyl)-pentanoate (67c)

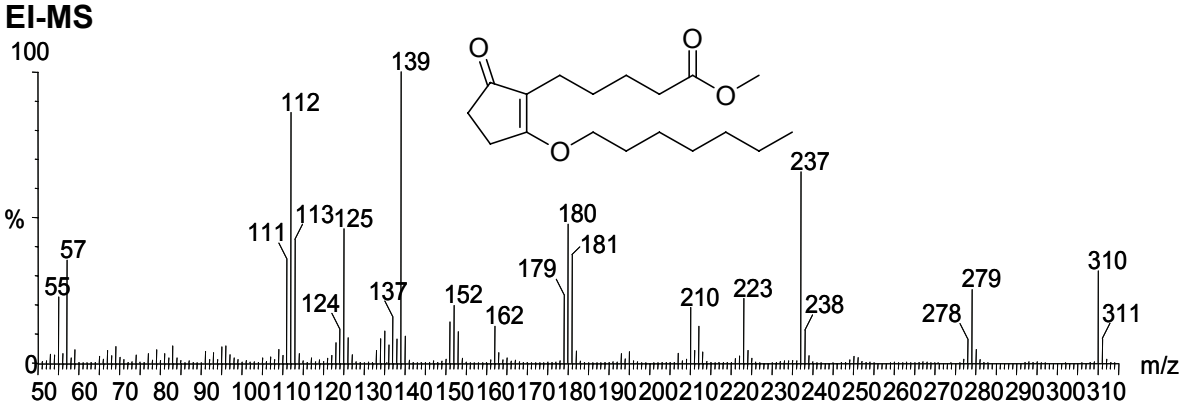
^{1}H NMR



^{13}C NMR



EI-MS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m/z
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179
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278
279
310
311
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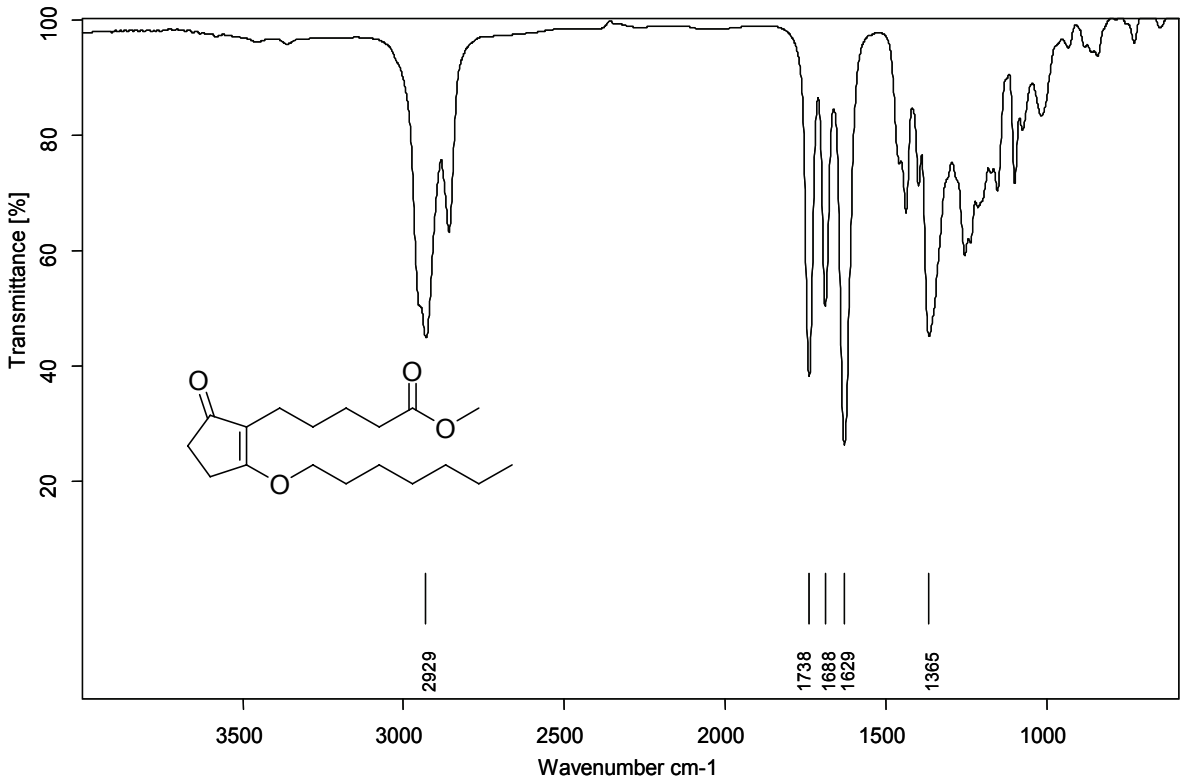
IR


Transmittance [%]
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2929
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2000
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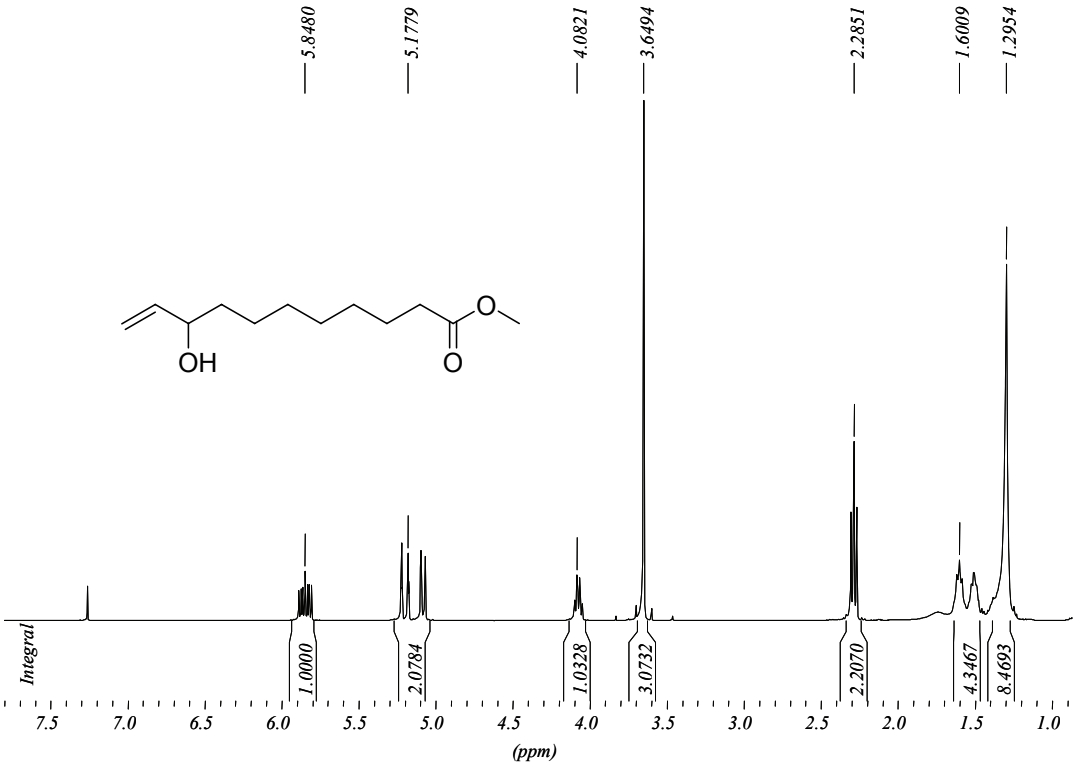
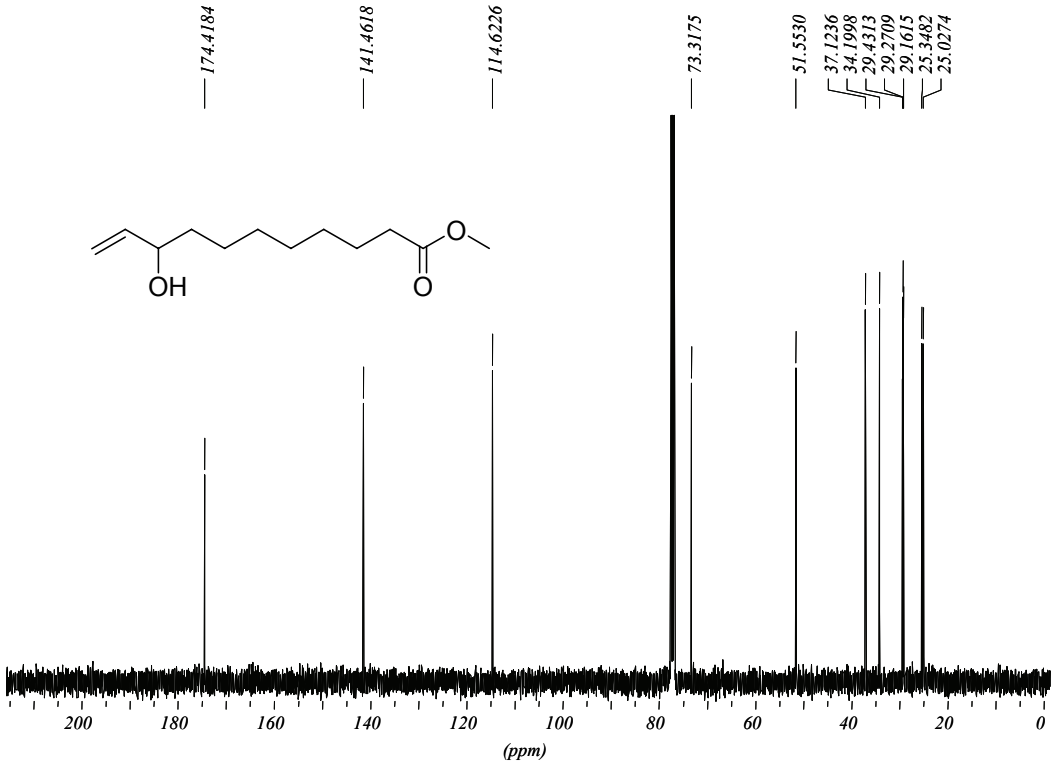
1000
Wavenumber cm-1

10.1.25 Methyl 9-hydroxyundec-10-enoate

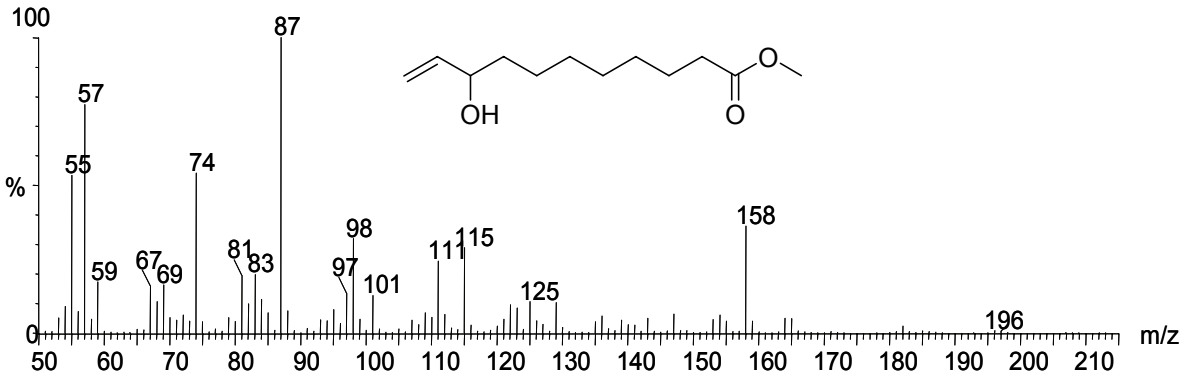
^{1}H NMR



^{13}C NMR



EI-MS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m/z
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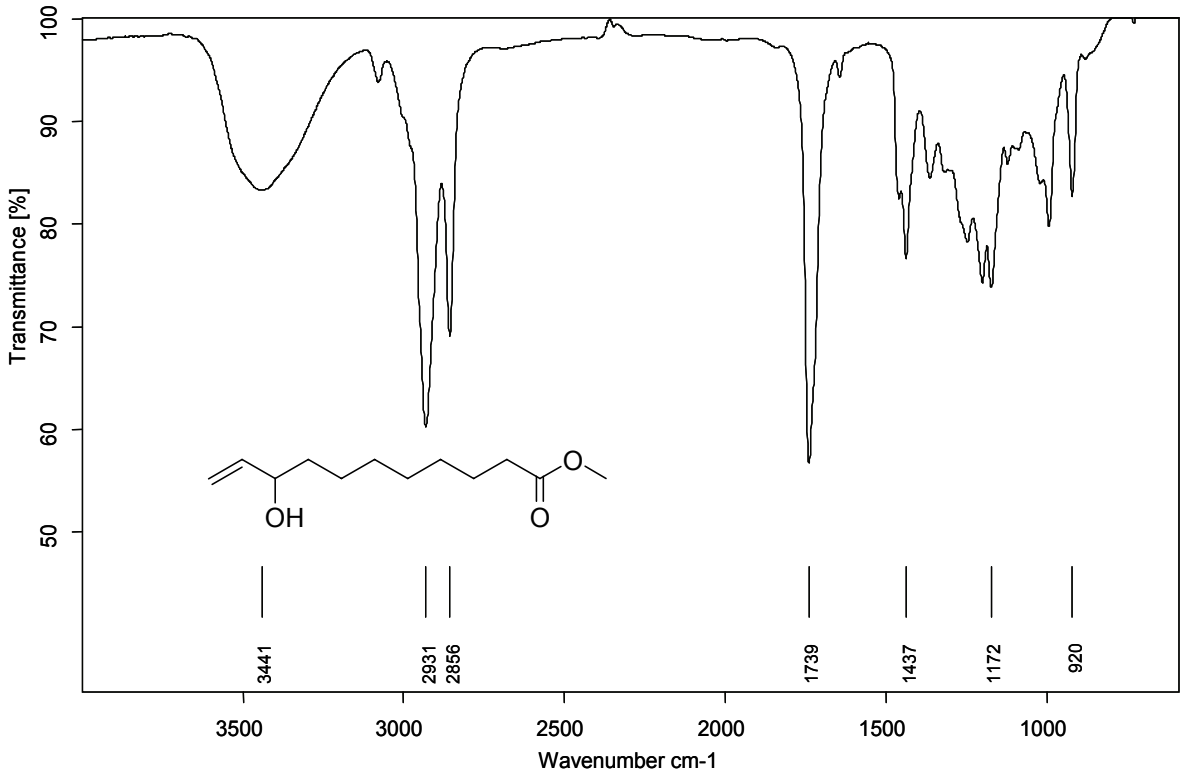
O

IR


Transmittance [%]
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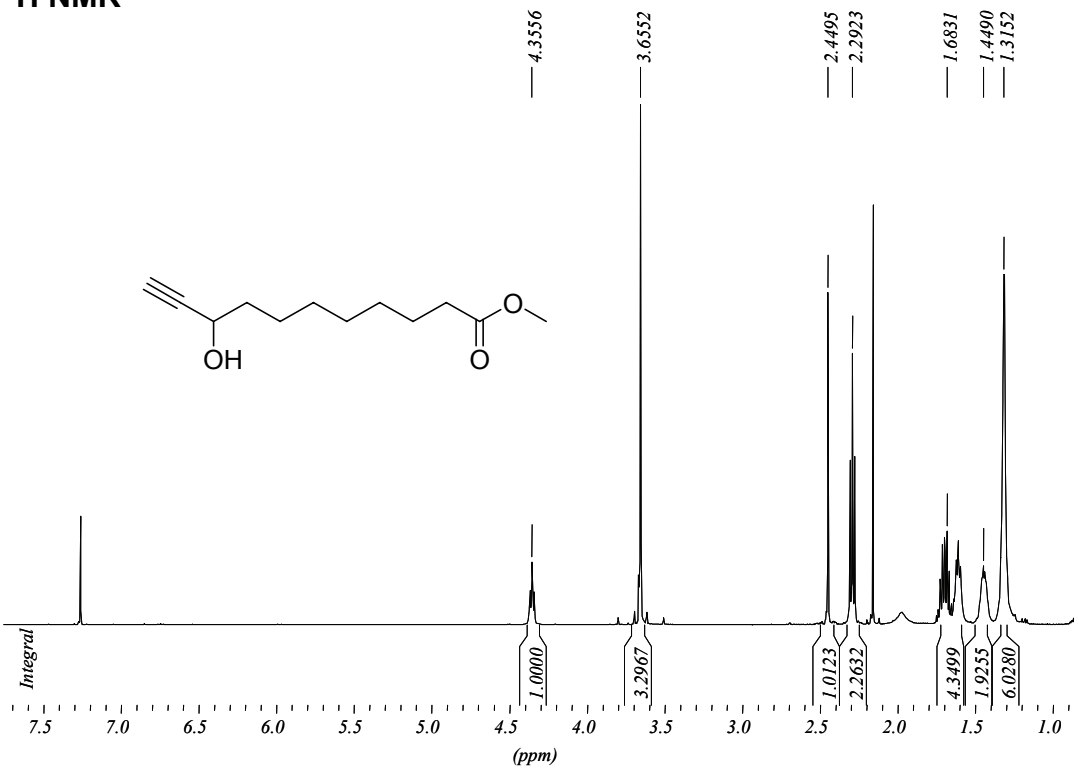
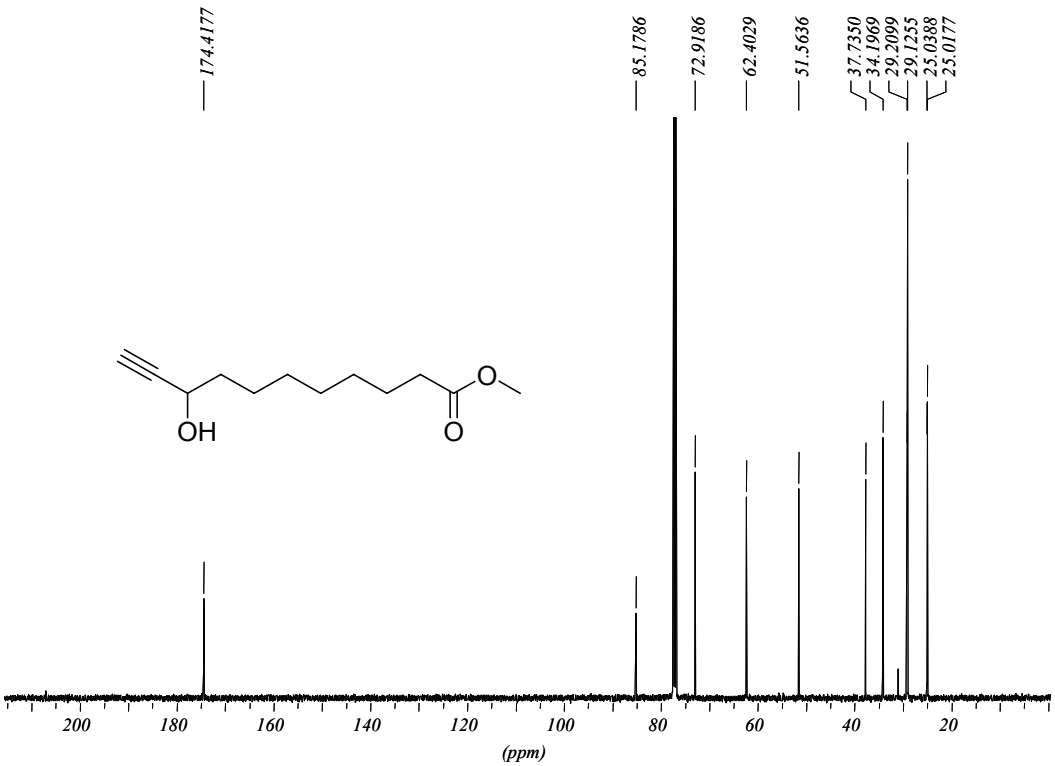
Wavenumber cm-1
OH
O
O

10.1.26 Methyl 9-hydroxyundec-10-ynoate

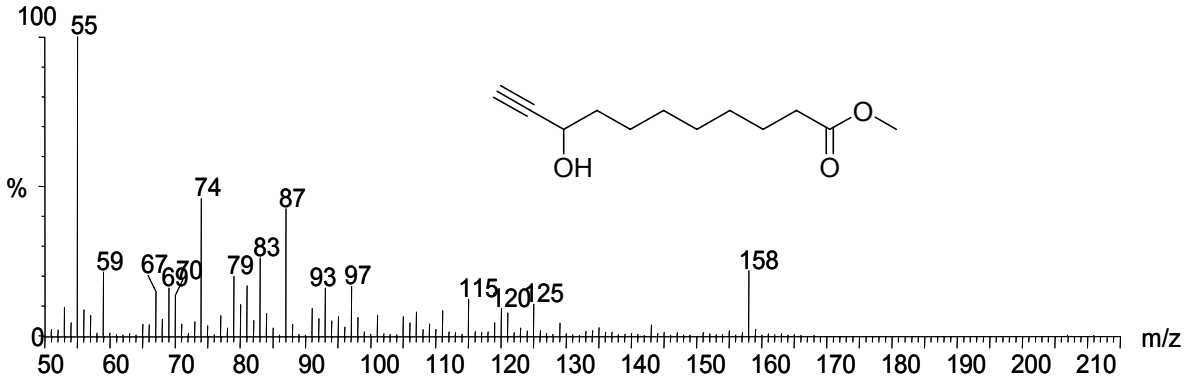
^{1}H NMR



^{13}C NMR



EI-MS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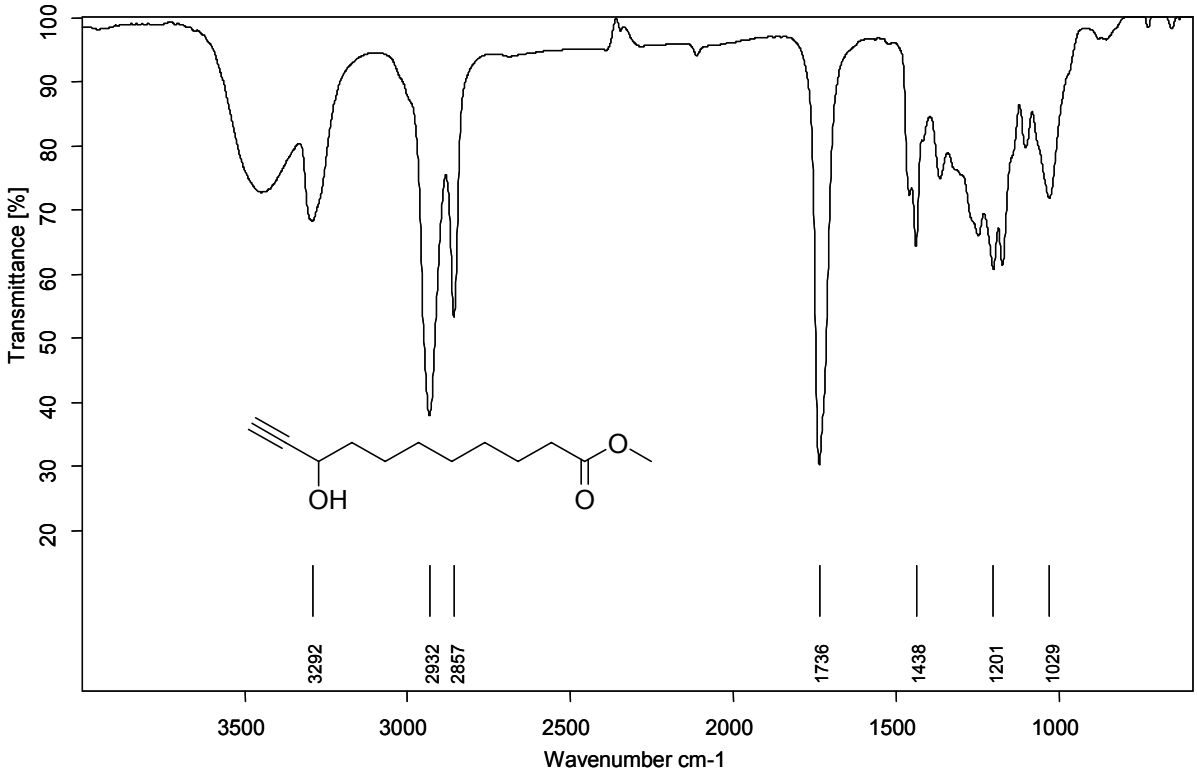
m/z
OH
O
O

IR


Transmittance [%]
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2932
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1736
1438
1201
1029
3500 3000 2500 2000 1500 1000
Wavenumber cm-1
OH
O
O

10.2 Tables of differentially expressed genes from the microarray

10.2.1 Treatment with 1 mM 65a

10.2.1.1 Up-regulated genes in response to 1 mM treatment

Gene identifier	Description
At1g53540.1	17.6 kDa class I small heat shock protein (HSP17.6C-CI) (AA 1-156) identical to (17.6 kDa class I heat shock protein (HSP 17.6) (AA 1-156)(SP:P13853) (GI:4376161) (Arabidopsis thaliana) (Nucleic Acids Res. 17 (19), 7995 (1989)) [At1g53540.1]
At2g29490.1	glutathione S-transferase, putative similar to glutathione S-transferase 103-1A [Arabidopsis thaliana] SWISS-PROT:P46421 [At2g29490.1]
At3g28210.1	zinc finger (AN1-like) family protein contains Pfam profile: PF01428 AN1-like zinc finger [At3g28210.1]
At5g48657.2	defense protein-related weak similarity to SP|Q8GYN5 RPM1-interacting protein 4 [110] [At5g48657.2]
At5g51440.1	23.5 kDa mitochondrial small heat shock protein (HSP23.5-M) similar to heat shock 22 kDa protein, mitochondrial precursor SP:Q96331 from [Arabidopsis thaliana]; identified in Scharf, K-D., et al,Cell Stress & Chaperones (2001) 6: 225-237. [At5g51440.1]
At5g59720.1	18.1 kDa class I heat shock protein (HSP18.1-CI) identical to 18.2 kDa class I heat shock protein (HSP 18.2) (SP:P19037)[Arabidopsis thaliana]; contains Pfam profile: PF00011 Hsp20/alpha crystallin family [At5g59720.1]
At5g52640.1	heat shock protein 81-1 (HSP81-1) / heat shock protein 83 (HSP83) nearly identical to SP|P27323 Heat shock protein 81-1 (HSP81-1) (Heat shock protein 83) [110]; contains Pfam profiles PF02518: ATPase, histidine kinase-, DNA gyrase B-,...
At5g11590.1	AP2 domain-containing transcription factor, putative similar to transcription factor TINY (GI:1246403) [Arabidopsis thaliana] [At5g11590.1]
At1g02920.1	glutathione S-transferase, putative similar to glutathione S-transferase GI:860955 from [Hyoscyamus muticus]; supported by cDNA GI:443697. [At1g02920.1]
At5g12020.1	17.6 kDa class II heat shock protein (HSP17.6-CII) identical to 17.6 kDa class II heat shock protein SP:P29830 from [Arabidopsis thaliana] [At5g12020.1]
At1g65390.1	disease resistance protein (TIR class), putative domain signature TIR exists, suggestive of a disease resistance protein. [At1g65390.1]
At1g51620.1	protein kinase family protein contains protein kinase domain, Pfam:PF00069 [At1g51620.1]
At3g23550.1	MATE efflux family protein similar to ripening regulated protein DDTFR18 [Lycopersicon esculentum] GI:12231296; contains Pfam profile: PF01554 uncharacterized membrane protein family [At3g23550.1]
At3g26200.1	cytochrome P450 71B22, putative (CYP71B22) Identical to cytochrome P450 71B22 (SP:Q9LTM1)[Arabidopsis thaliana];contains Pfam profile: PF00067 cytochrome P450 [At3g26200.1]
At1g16030.1	heat shock protein 70, putative / HSP70, putative similar to heat shock protein hsp70 GI:1771478 from [Pisum sativum] [At1g16030.1]
At1g74310.1	heat shock protein 101 (HSP101) identical to heat shock protein 101 GI:6715468 GB:AAF26423 from [Arabidopsis thaliana] [At1g74310.1]
At1g02930.1	glutathione S-transferase, putative similar to glutathione S-transferase GI:860955 from [Hyoscyamus muticus] [At1g02930.1]
At4g37990.1	mannitol dehydrogenase, putative (ELI3-2) identical to GI:16269 [At4g37990.1]
At1g17420.1	lipoxygenase, putative similar to lipoxygenase gi:1495804 [Solanum tuberosum], gi:1654140 [Lycopersicon esculentum] [At1g17420.1]
At4g23700.1	cation/hydrogen exchanger, putative (CHX17) similar to Na+/H+-exchanging

	protein slr1595 - Synechocystis sp., EMBL:D90902; monovalent cation:proton antiporter family 2 (CPA2) member, PMID:11500563 [At4g23700.1]
At2g47000.1	multidrug resistant (MDR) ABC transporter, putative similar to multidrug-resistant protein CjMDR1 [Coptis japonica] GI:14715462, MDR-like p-glycoprotein [Arabidopsis thaliana] GI:24324262; contains Pfam profiles PF00005: ABC transporter, PF00664: ABC...
At3g16360.1	phosphotransfer family protein similar to two-component phosphorelay mediators ATHP1 (GI:4156241), ATHP3 (GI:4156245) [Arabidopsis thaliana], histidine-containing phosphotransfer protein [Catharanthus roseus] GI:13774348 [At3g16360.1]
At2g27690.1	cytochrome P450, putative similar to Cytochrome P450 94A1 (P450-dependent fatty acid omega-hydroxylase) (SP:O81117) {Vicia sativa}; contains Pfam profile: PF00067 cytochrome P450; supported by cDNA: gi_13877668 [At2g27690.1]
At2g29460.1	glutathione S-transferase, putative [At2g29460.1]
At4g23190.1	protein kinase family protein contains Pfam PF00069: Protein kinase domain [At4g23190.1]
At1g09080.1	luminal binding protein 3 (BiP-3) (BP3) Similar to Arabidopsis luminal binding protein (gb|D89342); contains Pfam domain PF00012: dnaK protein [At1g09080.1]
At5g42380.1	calmodulin-related protein, putative similar to regulator of gene silencing calmodulin-related protein GI:12963415 from [Nicotiana tabacum] [At5g42380.1]
At2g26150.1	heat shock transcription factor family protein contains Pfam profile: PF00447 HSF-type DNA-binding domain [At2g26150.1]
At2g38340.1	AP2 domain-containing transcription factor, putative (DRE2B) Similar to DREB2A (GP:3738230) and DREB2B (GP:3738232) [Arabidopsis thaliana]; DRE binding proteins may be involved in dehydration or low temp response [At2g38340.1]
At2g21650.1	myb family transcription factor contains PFAM profile: PF00249 myb-like DNA-binding domain [At2g21650.1]
At3g22370.1	alternative oxidase 1a, mitochondrial (AOX1A) identical to GB:Q39219 [SP|Q39219] from [Arabidopsis thaliana] [At3g22370.1]
At3g15356.1	legume lectin family protein contains Pfam domain, PF00139: Legume lectins beta domain [At3g15356.1]
At3g44870.1	S-adenosyl-L-methionine:carboxyl methyltransferase family protein similar to defense-related protein cjs1 [Brassica carinata][GI:14009292][Mol Plant Pathol (2001) 2(3):159-169] [At3g44870.1]
At3g50260.1	AP2 domain-containing transcription factor, putative EREBP-3 homolog, Stylosanthes hamata, EMBL:U91982 [At3g50260.1]
At5g64750.1	AP2 domain-containing transcription factor, putative contains similarity to transcription factor [At5g64750.1]
At5g48540.1	33 kDa secretory protein-related contains Pfam PF01657: Domain of unknown function, duplicated in 33 KDa secretory proteins [At5g48540.1]
At1g10070.1	branched-chain amino acid aminotransferase 2 / branched-chain amino acid transaminase 2 (BCAT2) identical to SP|Q9M439 Branched-chain amino acid aminotransferase 2, chloroplast precursor (EC 2.6.1.42) (Atbcat-2) {Arabidopsis thaliana}; contains Pfam...
At2g40750.1	WRKY family transcription factor contains Pfam profile: PF03106 WRKY DNA - binding domain [At2g40750.1]
At3g46090.1	zinc finger (C2H2 type) family protein (ZAT7) identical to zinc finger protein ZAT7 [Arabidopsis thaliana] gi|1418341|emb|CAA67234; contains Pfam domain, PF00096: Zinc finger, C2H2 type [At3g46090.1]
At3g28740.1	cytochrome P450 family protein contains Pfam profile: PF00067 cytochrome P450 [At3g28740.1]
At2g38240.1	oxidoreductase, 2OG-Fe(II) oxygenase family protein similar to flavonol synthase [Citrus unshiu][gi:4126403], leucoanthocyanidin dioxygenase [Daucus carota][gi:5924383]; contains PF03171 2OG-Fe(II) oxygenase superfamily domain [At2g38240.1]
At1g51790.1	leucine-rich repeat protein kinase, putative smilar to light repressible receptor protein kinase [Arabidopsis thaliana] gi|1321686|emb|CAA66376; contains leucine rich repeat (LRR) domains, Pfam:PF00560; contains protein kinase domain, Pfam:PF00069...

At3g09440.1	heat shock cognate 70 kDa protein 3 (HSC70-3) (HSP70-3) identical to SP|O65719 Heat shock cognate 70 kDa protein 3 (Hsc70.3) {Arabidopsis thaliana} [At3g09440.1]
At3g04010.1	glycosyl hydrolase family 17 protein similar to beta-1,3-glucanase GB:S12402 [Nicotiana sp], GB:CAA03908 [Citrus sinensis], GB:S44364 [Lycopersicon esculentum] [At3g04010.1]
At5g43450.1	2-oxoglutarate-dependent dioxygenase, putative similar to 2A6 (GI:599622) and tomato ethylene synthesis regulatory protein E8 (SP|P10967) [At5g43450.1]
At3g44300.1	nitrilase 2 (NIT2) identical to SP|P32962 Nitrilase 2 (EC 3.5.5.1) {Arabidopsis thaliana} [At3g44300.1]
At4g37370.1	cytochrome P450, putative similar to Cytochrome P450 91A1 (SP:Q9FG65)[Arabidopsis thaliana]; cytochrome P450, Glycyrrhiza echinata, AB001379 [At4g37370.1]
At5g62480.1	glutathione S-transferase, putative [At5g62480.1]
At2g18193.1	AAA-type ATPase family protein contains Pfam profile: ATPase family PF00004 [At2g18193.1]
At5g48570.1	peptidyl-prolyl cis-trans isomerase, putative / FK506-binding protein, putative similar to rof1 [Arabidopsis thaliana] GI:1373396 [At5g48570.1]
At1g02850.1	glycosyl hydrolase family 1 protein contains Pfam PF00232 : Glycosyl hydrolase family 1 domain; TIGRFAM TIGR01233: 6-phospho-beta-galactosidase; similar to hydroxyisourate hydrolase (GI:19569603) [Glycine max] [At1g02850.1]
At1g02400.1	gibberellin 2-oxidase, putative / GA2-oxidase, putative similar to GA2ox2 [GI:4678368]; similar to dioxygenase GI:1666096 from [Marah macrocarpus]; contains PF03171 2OG-Fe(II) oxygenase superfamily domain [At1g02400.1]
At3g16530.1	legume lectin family protein contains Pfam domain, PF00139: Legume lectins beta domain [At3g16530.1]
At2g03760.1	steroid sulfotransferase, putative strong similarity to steroid sulfotransferases from [Brassica napus] GI:3420008, GI:3420004, GI:3420006; contains Pfam profile PF00685: Sulfotransferase domain [At2g03760.1]
At1g65970.1	peroxiredoxin type 2, putative strong similarity to type 2 peroxiredoxin [Brassica rapa subsp. pekinensis] GI:4928472; contains Pfam profile: PF00578 AhpC/TSA (alkyl hydroperoxide reductase and thiol-specific antioxidant) family [At1g65970.1]
At3g24954.1	leucine-rich repeat family protein contains leucine rich-repeat domains Pfam:PF00560, INTERPRO:IPR001611 [At3g24954.1]
At5g48657.1	defense protein-related weak similarity to SP|Q8GYN5 RPM1-interacting protein 4 {Arabidopsis thaliana} [At5g48657.1]
At3g25882.1	NPR1/NIM1-interacting protein 2 (NIMIN-2) identical to cDNA NIMIN-2 protein (nimin-2 gene)GI:12057155 [At3g25882.1]
At5g41080.1	glycerophosphoryl diester phosphodiesterase family protein weak similarity to SP|P37965 Glycerophosphoryl diester phosphodiesterase (EC 3.1.4.46) {Bacillus subtilis}; contains Pfam profile PF03009: Glycerophosphoryl diester phosphodiesterase family...
At5g01600.1	ferritin 1 (FER1) identical to ferritin [Arabidopsis thaliana] GI:1246401, GI:8163920 [At5g01600.1]
At5g20480.1	leucine-rich repeat transmembrane protein kinase, putative protein kinase Xa21, Oryza sativa, PIR:A57676 [At5g20480.1]
At4g01700.1	chitinase, putative similar to peanut type II chitinase GI:1237025 from [Arachis hypogaea] [At4g01700.1]
At4g01870.1	tolB protein-related contains weak similarity to TolB protein precursor (Swiss-Prot:P44677) [Haemophilus influenzae] [At4g01870.1]
At2g40670.1	two-component responsive regulator / response regulator 16 (ARR16) identical to response regulator 16 GI:11870067 from [Arabidopsis thaliana] [At2g40670.1]
At5g17860.1	cation exchanger, putative (CAX7) contains similarity to SWISS-PROT:Q9HC58 NKX3_HUMAN Sodium/potassium/calcium exchanger 3 precursor {Homo sapiens}; Ca2+:Cation Antiporter (CaCA) Family member PMID:11500563 [At5g17860.1]
At3g26290.1	cytochrome P450 71B26, putative (CYP71B26) identical to cytochrome P450 71B26 (SP:Q9LTL0) [Arabidopsis thaliana]; contains Pfam profile: PF00067 cytochrome P450 [At3g26290.1]

At1g67810.1	Fe-S metabolism associated domain-containing protein contains Pfam PF02657: Fe-S metabolism associated domain [At1g67810.1]
At1g06160.1	ethylene-responsive factor, putative similar to ethylene response factor 1 GB:AAD03544 GI:4128208 from [Arabidopsis thaliana] [At1g06160.1]
At2g40330.1	Bet v I allergen family protein contains Pfam profile PF00407: Pathogenesis-related protein Bet v I family [At2g40330.1]
At4g12290.1	copper amine oxidase, putative similar to copper amine oxidase [Cicer arietinum] gi|3819099|emb|CAA08855 [At4g12290.1]
At5g47330.1	palmitoyl protein thioesterase family protein [At5g47330.1]
At4g22590.1	trehalose-6-phosphate phosphatase, putative similar to trehalose-6-phosphate phosphatase (AtTPPA) GI:2944178; contains Pfam profile PF02358: Trehalose-phosphatase [At4g22590.1]
At1g23090.1	sulfate transporter, putative similar to sulfate transporter [Arabidopsis thaliana] GI:2285885; contains Pfam profiles PF00916: Sulfate transporter family, PF01740: STAS domain [At1g23090.1]
At5g18470.1	curculin-like (mannose-binding) lectin family protein contains Pfam profile: PF01453 lectin (probable mannose binding) [At5g18470.1]
At2g39210.1	nodulin family protein similar to nodulin-like protein [Arabidopsis thaliana] GI:3329368, nodule-specific protein Nlj70 [Lotus japonicus] GI:3329366 [At2g39210.1]
At5g59820.1	zinc finger (C2H2 type) family protein (ZAT12) identical to zinc finger protein ZAT12 [Arabidopsis thaliana] gi|1418325|emb|CAA67232 [At5g59820.1]
At5g62070.1	calmodulin-binding family protein contains Pfam profile PF00612: IQ calmodulin-binding motif [At5g62070.1]
At1g32940.1	subtilase family protein contains similarity to subtilase; SP1 GI:9957714 from [Oryza sativa] [At1g32940.1]
At1g32870.1	no apical meristem (NAM) family protein similar to to NAC2 (GI:645671) [Arabidopsis thaliana]; contains Pfam PF02365: No apical meristem (NAM) protein [At1g32870.1]
At3g28007.1	nodulin MtN3 family protein contains Pfam PF03083 MtN3/saliva family; similar to LIM7 GI:431154 (induced in meiotic prophase in lily microsporocytes) from [Lilium longiflorum] [At3g28007.1]
At5g44420.1	plant defensin protein, putative (PDF1.2a) plant defensin protein family member, personal communication, Bart Thomma (Bart.Thomma@agr.kuleuven.ac.be); similar to antifungal protein 1 preprotein [Raphanus sativus] gi|609322|gb|AAA69541 [At5g44420.1]
At3g61630.1	AP2 domain-containing transcription factor, putative transcription factor Pti6 - Lycopersicon esculentum, PIR:T07728 [At3g61630.1]
At2g26560.1	patatin, putative similar to patatin-like latex allergen [Hevea brasiliensis][PMID:10589016]; contains patatin domain PF01734 [At2g26560.1]
At1g07180.1	pyridine nucleotide-disulphide oxidoreductase family protein contains similarity to alternative NADH-dehydrogenase GI:3718005 from [Yarrowia lipolytica], SP|P32340 Rotenone-insensitive NADH-ubiquinone oxidoreductase, mitochondrial precursor (EC...
At4g03320.1	chloroplast protein import component-related similar to P. sativum Tic20 chloroplast protein import component (GI:3769673) [At4g03320.1]
At3g50930.1	AAA-type ATPase family protein contains Pfam profile: ATPase family PF00004 [At3g50930.1]
At2g26010.1	plant defensin-fusion protein, putative (PDF1.3) plant defensin protein family member, personal communication, Bart Thomma (Bart.Thomma@agr.kuleuven.ac.be); similar to antifungal protein 1 preprotein [Raphanus sativus] gi|609322|gb|AAA69541 [At2g26010.1]
At4g05020.1	NADH dehydrogenase-related similar to alternative NADH-dehydrogenase [Yarrowia lipolytica] GI:3718005, 64 kDa mitochondrial NADH dehydrogenase [Neurospora crassa] GI:4753821; contains Pfam profile PF00070: Pyridine nucleotide-disulphide oxidoreductase...
At1g08630.1	L-allo-threonine aldolase-related similar to L-allo-threonine aldolase (EC 4.1.2.-) (L-allo-TA) (L-allo-threonine acetaldehyde-lyase) (SP:O07051) {Aeromonas jandaei}; similar to ESTs gb|R30517, gb|T42772, gb|R90493, and gb|R90493 [At1g08630.1]
At1g06620.1	2-oxoglutarate-dependent dioxygenase, putative similar to 2A6 (GI:599622) and

	tomato ethylene synthesis regulatory protein E8 (SP|P10967); contains Pfam profile: PF00671 Iron/Ascorbate oxidoreductase family [At1g06620.1]
At2g14560.1	expressed protein contains Pfam profile PF04525: Protein of unknown function (DUF567) [At2g14560.1]
At3g09270.1	glutathione S-transferase, putative similar to glutathione transferase GB:CAA71784 [Glycine max] [At3g09270.1]
At2g40100.1	chlorophyll A-B binding protein (LHCB4.3) identical to Lhcb4:3 protein [Arabidopsis thaliana] GI:4741956; contains Pfam profile: PF00504 chlorophyll A-B binding protein [At2g40100.1]
At4g15490.1	UDP-glucoronosyl/UDP-glucosyl transferase family protein contains Pfam profile: PF00201 UDP-glucoronosyl and UDP-glucosyl transferase ;simalr to UDP-glucose:sinapate glucosyltransferase GI:9794913 from [Brassica napus] [At4g15490.1]
At3g22060.1	receptor protein kinase-related contains Pfam profile: PF01657 Domain of unknown function that is usually associated with protein kinase domain Pfam:PF00069 [At3g22060.1]
At1g76680.1	12-oxophytodienoate reductase (OPR1) identical to 12-oxophytodienoate reductase OPR1 GB:AAC78440 [Arabidopsis thaliana] [At1g76680.1]
At1g66580.1	60S ribosomal protein L10 (RPL10C) contains Pfam profile: PF00826: Ribosomal L10 [At1g66580.1]
At4g36010.1	pathogenesis-related thaumatin family protein similar to receptor serine/threonine kinase PR5K [Arabidopsis thaliana] GI:1235680; contains Pfam profile PF00314: Thaumatin family [At4g36010.1]
At2g44840.1	ethylene-responsive element-binding protein, putative [At2g44840.1]
At4g17090.1	beta-amylase (CT-BMY) / 1,4-alpha-D-glucan maltohydrolase identical to beta-amylase enzyme GI:6065749 from [Arabidopsis thaliana] [At4g17090.1]
	Heat Schock protein
	Glutathione S-transferase
	Cytochrome P-450
	Pathogenesis related protein

10.2.1.2 Down-regulated genes in response to 1 mM treatment

Gene number	Description
At1g77760.1	nitrate reductase 1 (NR1) identical to SP|P11832 Nitrate reductase 1 (formerly EC 1.6.6.1) (NR1){Arabidopsis thaliana} [At1g77760.1]
At5g04150.1	basic helix-loop-helix (bHLH) family protein contains Pfam profile: PF00010 helix-loop-helix DNA-binding domain; PMID: 12679534 [At5g04150.1]
At5g65010.1	asparagine synthetase 2 (ASN2) identical to asparagine synthetase (ASN2) [Arabidopsis thaliana] GI:3859536 [At5g65010.1]
At2g26980.4	CBL-interacting protein kinase 3 (CIPK3) identical to CBL-interacting protein kinase 3 [Arabidopsis thaliana] gi|9280638|gb|AAF86507 [At2g26980.4]
At2g26980.1	CBL-interacting protein kinase 3 (CIPK3) identical to CBL-interacting protein kinase 3 [Arabidopsis thaliana] gi|9280638|gb|AAF86507 [At2g26980.1]
At5g15500.1	ankyrin repeat family protein contains Pfam domain, PF00023: Ankyrin repeat [At5g15500.1]
At5g25190.1	ethylene-responsive element-binding protein, putative ethylene responsive element binding protein homolog, Stylosanthes hamata, EMBL:U91857 [At5g25190.1]
At1g10550.1	xyloglucan:xyloglucosyl transferase, putative / xyloglucan endotransglycosylase, putative / endo-xyloglucan transferase, putative similar to endoxyloglucan transferase related protein EXGT-A3 GI:2154609 from [Arabidopsis thaliana] [At1g10550.1]
At1g22990.1	heavy-metal-associated domain-containing protein / copper chaperone (CCH)-related low similarity to copper homeostasis factor [GI:3168840][PMID:9701579]; strong similarity to farnesylated protein ATFP7 [GI:4097555]; contains heavy-metal-associated...

At4g37540.1	LOB domain protein 39 / lateral organ boundaries domain protein 39 (LBD39) identical to SP|Q9SZE8 LOB domain protein 39 {Arabidopsis thaliana} [At4g37540.1]
At5g26200.1	mitochondrial substrate carrier family protein contains Pfam profile: PF00153 mitochondrial carrier protein [At5g26200.1]
At4g15690.1	glutaredoxin family protein contains INTERPRO Domain IPR002109, Glutaredoxin (thioltransferase) [At4g15690.1]
At3g14440.1	9-cis-epoxycarotenoid dioxygenase, putative / neoxanthin cleavage enzyme, putative / carotenoid cleavage dioxygenase, putative similar to 9-cis-epoxycarotenoid dioxygenase GB:AAF26356 [GI:6715257][Phaseolus vulgaris] [At3g14440.1]
At1g70920.1	homeobox-leucine zipper protein, putative / HD-ZIP transcription factor, putative similar to homeodomain leucine zipper protein GI:5006851 from [Oryza sativa] [At1g70920.1]
At2g18480.1	mannitol transporter, putative similar to mannitol transporter [Apium graveolens var. dulce] GI:12004316; contains Pfam profile PF00083: major facilitator superfamily protein [At2g18480.1]
At5g18050.1	auxin-responsive protein, putative similar to auxin-inducible SAUR (Small Auxin Up RNAs) (GI:3043536) [Raphanus sativus] [At5g18050.1]
At3g03820.1	auxin-responsive protein, putative similar to auxin-inducible SAUR (Small Auxin Up RNAs) (GI:3043536) [Raphanus sativus] [At3g03820.1]
At1g53830.1	pectinesterase family protein identical to pectinesterase 2 (PME2/ PE 2) SP:Q42534 from [Arabidopsis thaliana];contains Pfam profiles: PF01095 pectinesterase, PF04043 plant invertase/pectin methylesterase inhibitor [At1g53830.1]
At2g21210.1	auxin-responsive protein, putative similar to small auxin-up regulated protein SAUR (GI:3043536) [Raphanus sativus] [At2g21210.1]
At5g02760.1	protein phosphatase 2C family protein / PP2C family protein similar to Ser/Thr protein phosphatase 2C (PP2C6) (GI:15020818) [Arabidopsis thaliana]; similar to protein phosphatase 2C (GI:3608412) [Mesembryanthemum crystallinum]; contains Pfam PF00481 :...
At5g18060.1	auxin-responsive protein, putative similar to auxin-inducible SAUR (Small Auxin Up RNAs) GI:3043536 from radish [Raphanus sativus] [At5g18060.1]
At4g11320.1	cysteine proteinase, putative contains similarity to cysteine proteinase RD21A (thiol protease) GI:435619, SP:P43297 from [Arabidopsis thaliana] [At4g11320.1]
At5g18010.1	auxin-responsive protein, putative similar to auxin-inducible SAUR (Small Auxin Up RNAs) (GI:3043536) [Raphanus sativus] [At5g18010.1]
At3g54830.1	amino acid transporter family protein belongs to INTERPRO:IPR002422 amino acid/polyamine transporter, family II [At3g54830.1]
At5g50740.1	copper chaperone (CCH)-related low similarity to copper homeostasis factor [GI:3168840][PMID: 9701579]; similar to farnesylated protein ATFP3 [GI:4097547]; contains Pfam profile PF00403: Heavy-metal-associated domain [At5g50740.1]
At5g52910.1	timeless family protein contains Pfam domains PF05029: Timeless protein C terminal region and PF04821: Timeless protein [At5g52910.1]
At3g48360.1	speckle-type POZ protein-related contains Pfam PF00651 : BTB/POZ domain; similar to Speckle-type POZ protein (SP:O43791) [Homo sapiens] [At3g48360.1]
At1g69790.1	protein kinase, putative similar to protein kinase APK1A [Arabidopsis thaliana] SWISS-PROT:Q06548 [At1g69790.1]
At5g19600.1	sulfate transporter, putative similar to sulfate transporter [Arabidopsis thaliana] GI:2285885; contains Pfam profiles PF00916: Sulfate transporter family, PF01740: STAS domain; supporting cDNA gi|14141683|dbj|AB061739.1| [At5g19600.1]
At1g07610.1	metallothionein-like protein 1C (MT-1C) identical to Metallothionein-like protein 1C (MT-1C). (SP:Q38804) (Arabidopsis thaliana) [At1g07610.1]
At1g32560.1	late embryogenesis abundant group 1 domain-containing protein / LEA group 1 domain-containing protein contains Pfam domain, PF03760: Late embryogenesis abundant (LEA) group 1 [At1g32560.1]
At5g18020.1	auxin-responsive protein, putative similar to auxin-inducible SAUR (Small Auxin Up RNAs) (GI:3043536) [Raphanus sativus] [At5g18020.1]

At2g38170.1	calcium exchanger (CAX1) identical to high affinity calcium antiporter CAX1 [Arabidopsis thaliana] gi|9256741|gb|AAB05913, except a possible frameshift at base 58008. Sequence has been confirmed with 5 sequencing reads.; Ca2+:Cation Antiporter (CaCA)...
At1g74670.1	gibberellin-responsive protein, putative similar to SP|P46690 Gibberellin-regulated protein 4 precursor {Arabidopsis thaliana} GASA4; contains Pfam profile PF02704: Gibberellin regulated protein [At1g74670.1]
At5g22940.1	exostosin family protein contains Pfam profile: PF03016 exostosin family [At5g22940.1]
At1g73330.1	protease inhibitor, putative (DR4) identical to Dr4 GI:469114 from [Arabidopsis thaliana]; contains Pfam profile PF00197: Trypsin and protease inhibitor [At1g73330.1]
At5g18030.1	auxin-responsive protein, putative similar to auxin-inducible SAUR (Small Auxin Up RNAs) (GI:3043536) [Raphanus sativus] [At5g18030.1]
At2g32100.1	ovate protein-related contains TIGRFAM TIGR01568 : uncharacterized plant-specific domain TIGR01568 [At2g32100.1]
At4g11310.1	cysteine proteinase, putative contains similarity to cysteine proteinase RD21A (thiol protease) GI:435619, SP:P43297 from [Arabidopsis thaliana] [At4g11310.1]
At2g37870.1	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein contains Pfam protease inhibitor/seed storage/LTP family domain PF00234 [At2g37870.1]
At5g18080.1	auxin-responsive protein, putative similar to GP:3043536 SAUR {Raphanus sativus} [At5g18080.1]
At4g14550.1	auxin-responsive AUX/IAA family protein identical to IAA14 (GI:972931) [Arabidopsis thaliana]; similar to SP|Q38825 Auxin-responsive protein IAA7 (Indoleacetic acid-induced protein 7) {Arabidopsis thaliana} [At4g14550.1]
At2g42380.1	bZIP transcription factor family protein [At2g42380.1]
At5g57560.1	xyloglucan:xyloglucosyl transferase / xyloglucan endotransglycosylase / endo-xyloglucan transferase (TCH4) identical to xyloglucan endotransglycosylase TCH4 protein GI:886116 [At5g57560.1]
At5g67420.1	LOB domain protein 37 / lateral organ boundaries domain protein 37 (LBD37) identical to LOB DOMAIN 37 [Arabidopsis thaliana] GI:17227170 [At5g67420.1]
At1g02205.1	CER1 protein identical to maize gl1 homolog (glossy1 locus) GI:1209703 and CER1 GI:1199467 from [Arabidopsis thaliana] [At1g02205.1]
At1g58300.1	heme oxygenase, putative similar to heme oxygenase 4 GI:14485565 from [Arabidopsis thaliana] [At1g58300.1]
At1g03470.1	kinase interacting family protein similar to kinase interacting protein 1 (GI:13936326) [Petunia integrifolia] [At1g03470.1]
At3g19380.1	U-box domain-containing protein contains similarity to immediate-early fungal elicitor protein CMPG1 GI:14582200 [Petroselinum crispum]; contains Pfam profile PF04564: U-box domain [At3g19380.1]
At3g06770.1	glycoside hydrolase family 28 protein / polygalacturonase (pectinase) family protein weak similarity to polygalacturonase [Persea americana] GI:166951; contains PF00295: Glycosyl hydrolases family 28 (polygalacturonases) [At3g06770.1]
At5g46690.1	basic helix-loop-helix (bHLH) family protein contains similarity to bHLH DNA-binding protein [At5g46690.1]
At1g29500.1	auxin-responsive protein, putative similar to auxin-induced protein 6B (SP:P33083) [Glycine max] [At1g29500.1]
At1g20190.1	expansin, putative (EXP11) similar to GB:U30460 from [Cucumis sativus]; alpha-expansin gene family, PMID:11641069 [At1g20190.1]
At5g42200.1	zinc finger (C3HC4-type RING finger) family protein contains Pfam profile: PF00097 zinc finger, C3HC4 type (RING finger) [At5g42200.1]
At5g20110.1	dynein light chain, putative similar to SP|O02414 Dynein light chain LC6, flagellar outer arm {Anthocidaris crassispina}; contains Pfam profile PF01221: Dynein light chain type 1 [At5g20110.1]
At3g25670.1	leucine-rich repeat family protein contains leucine rich-repeat (LRR) domains Pfam:PF00560, INTERPRO:IPR001611; + [At3g25670.1]
At1g50280.1	phototropic-responsive NPH3 family protein contains NPH3 family domain,

	Pfam:PF03000 [At1g50280.1]
At5g01740.1	expressed protein wound-inducible protein wun1 protein - Solanum tuberosum, PIR:JQ0398 [At5g01740.1]
At1g29510.1	auxin-responsive protein, putative similar to auxin-induced protein 6B (SP:P33083) [Glycine max] [At1g29510.1]
At3g07340.1	basic helix-loop-helix (bHLH) family protein contains Pfam profile: PF00010 helix-loop-helix DNA-binding domain [At3g07340.1]
At1g29450.1	auxin-responsive protein, putative similar to auxin-induced protein 6B (SP:P33083) [Glycine max] [At1g29450.1]
At5g62100.1	BAG domain-containing protein similar to BAG domain containing proteins (At5g07220, At5g52060) [At5g62100.1]
At4g26790.1	GDSL-motif lipase/hydrolase family protein similar to family II lipase EXL3 (GI:15054386), EXL1 (GI:15054382), EXL2 (GI:15054384) [Arabidopsis thaliana]; contains Pfam profile PF00657: Lipase/Acylhydrolase with GDSL-like motif [At4g26790.1]
At5g62040.1	brother of FT and TFL1 protein (BFT) identical to SP|Q9FIT4 BROTHER of FT and TFL1 protein {Arabidopsis thaliana}; contains Pfam profile PF01161: Phosphatidylethanolamine-binding protein [At5g62040.1]
At4g02630.1	protein kinase family protein contains protein kinase domain, Pfam:PF00069; contains serine/threonine protein kinase domain, INTERPRO:IPR002290 [At4g02630.1]
At2g05070.1	chlorophyll A-B binding protein / LHCII type II (LHCB2.2) identical to Lhcb2 protein [Arabidopsis thaliana] GI:4741946; contains Pfam profile PF00504: Chlorophyll A-B binding protein [At2g05070.1]
At3g58120.1	bZIP transcription factor family protein contains Pfam profile: PF00170 bZIP transcription factor ;supported by cDNA gi|15100054|gb|AF401300.1|AF401300 [At3g58120.1]
At5g24770.1	vegetative storage protein 2 (VSP2) identical to SP|O82122 Vegetative storage protein 2 precursor {Arabidopsis thaliana}; contains Pfam profile PF03767: HAD superfamily (subfamily IIIB) phosphatase [At5g24770.1]
At2g18050.1	histone H1-3 (HIS1-3) similar to histone H1 [Lycopersicon pennellii] SWISS-PROT:P40267; identical to cDNA histone H1-3 (His1-3) GI:1809314, histone H1-3 [Arabidopsis thaliana] GI:1809305 [At2g18050.1]
At1g28130.1	auxin-responsive GH3 family protein similar to auxin-responsive GH3 product [Glycine max] GI:18591; contains Pfam profile PF03321: GH3 auxin-responsive promoter [At1g28130.1]
At1g49780.1	U-box domain-containing protein similar to immediate-early fungal elicitor protein CMPG1 [Petroselinum crispum] GI:14582200; contains Pfam profile PF04564: U-box domain [At1g49780.1]
At4g34760.1	auxin-responsive family protein auxin-induced protein X15, Glycine max, PIR2:JQ1097 [At4g34760.1]
At4g17460.1	homeobox-leucine zipper protein 1 (HAT1) / HD-ZIP protein 1 identical to Homeobox-leucine zipper protein HAT1 (SP:P46600) [Arabidopsis thaliana] [At4g17460.1]
At1g33340.1	epsin N-terminal homology (ENTH) domain-containing protein / clathrin assembly protein-related contains Pfam PF01417: ENTH domain. ENTH (Epsin N-terminal homology) domain; similar to clathrin assembly protein AP180 (GI:6492344) [Xenopus laevis]...
At1g29460.1	auxin-responsive protein, putative similar to auxin-induced protein 6B (SP:P33083) [Glycine max] [At1g29460.1]
At3g63450.1	RNA recognition motif (RRM)-containing protein contains InterPro entry IPR000504: RNA-binding region RNP-1 (RNA recognition motif) (RRM) [At3g63450.1]
At4g32280.1	auxin-responsive AUX/IAA family protein contains Pfam profile: PF02309: AUX/IAA family [At4g32280.1]
At5g43270.1	squamosa promoter-binding protein-like 2 (SPL2) identical to squamosa promoter binding protein-like 2 [Arabidopsis thaliana] GI:5931645; contains Pfam profile PF03110: SBP domain [At5g43270.1]
At5g57660.1	zinc finger (B-box type) family protein contains Pfam domain, PF00643: B-box zinc finger [At5g57660.1]
At5g29000.1	myb family transcription factor contains Pfam profile: PF00249 myb-like DNA-

	binding domain [At5g29000.1]
At4g03070.1	2-oxoglutarate-dependent dioxygenase (AOP1.2) identical to GI:16118887; contains PF03171: 2OG-Fe(II) oxygenase superfamily domain [At4g03070.1]
At1g29440.1	auxin-responsive family protein similar to auxin-induced protein 6B (SP:P33083) [Glycine max] [At1g29440.1]
At4g39770.1	trehalose-6-phosphate phosphatase, putative similar to trehalose-6-phosphate phosphatase (AtTPPB) [Arabidopsis thaliana] GI:2944180; contains Pfam profile PF02358: Trehalose-phosphatase [At4g39770.1]
At3g28860.1	multidrug resistance P-glycoprotein, putative similar to mdr-like P-glycoprotein GI:3849833 from [Arabidopsis thaliana]; contains Pfam profiles PF00005: ABC transporter and PF00664: ABC transporter transmembrane region; identical to cDNA MDR-like...
At4g26100.3	casein kinase, putative similar to casein kinase I, delta isoform [Arabidopsis thaliana] SWISS-PROT:P42158; contains protein kinase domain, Pfam:PF00069 [At4g26100.3]
At4g31890.1	armadillo/beta-catenin repeat family protein contains Pfam profile: PF00514 armadillo/beta-catenin-like repeat [At4g31890.1]
At5g65920.1	U-box domain-containing protein low similarity to immediate-early fungal elicitor protein CMPG1 [Petroselinum crispum] GI:14582200; contains Pfam profile PF04564: U-box domain [At5g65920.1]
At4g20820.1	FAD-binding domain-containing protein similar to SP|P30986 reticuline oxidase precursor (Berberine-bridge-forming enzyme) (BBE) (Tetrahydroprotoberberine synthase) [Eschscholzia californica]; contains PF01565 FAD binding domain [At4g20820.1]
At1g52830.1	auxin-responsive protein / indoleacetic acid-induced protein 6 (IAA6) nearly identical to SP|Q38824 Auxin-responsive protein IAA6 (Indoleacetic acid-induced protein 6) {Arabidopsis thaliana} [At1g52830.1]
At4g13560.1	late embryogenesis abundant domain-containing protein / LEA domain-containing protein similar to LEA protein [Cicer arietinum] GI:2909420; contains Pfam profile PF02987: Late embryogenesis abundant protein [At4g13560.1]
At3g27690.1	chlorophyll A-B binding protein (LHCB2:4) nearly identical to Lhcb2 protein [Arabidopsis thaliana] GI:4741950; similar to chlorophyll A-B binding protein 151 precursor (LHCP) GB:P27518 from [Gossypium hirsutum]; contains Pfam PF00504: Chlorophyll A-B...
At5g24380.1	transporter, putative similar to iron-phytosiderophore transporter protein yellow stripe 1 [Zea mays] GI:10770865; contains Pfam profile PF03169: OPT oligopeptide transporter protein [At5g24380.1]
At1g03300.1	agenet domain-containing protein contains Pfam PF05641: Agenet domain [At1g03300.1]
At4g39510.1	cytochrome P450 family protein contains Pfam PF00067: Cytochrome P450; similar to Cytochrome P450 86A2 (SP:O23066) [Arabidopsis thaliana] [At4g39510.1]
At1g51940.1	protein kinase family protein / peptidoglycan-binding LysM domain-containing protein contains protein kinases ATP-binding region signature, PROSITE:PS00107 [At1g51940.1]
At1g56600.1	galactinol synthase, putative similar to galactinol synthase, isoform GolS-1 GI:5608497 from [Ajuga reptans] [At1g56600.1]
At5g44260.1	zinc finger (CCCH-type) family protein contains Pfam domain, PF00642: Zinc finger C-x8-C-x5-C-x3-H type (and similar) [At5g44260.1]
At5g24155.1	squalene monooxygenase, putative / squalene epoxidase, putative similar to SP|O65404 (SE 1,1), SP|O65402 (SE 1,2) [At5g24155.1]
At3g49620.1	2-oxoacid-dependent oxidase, putative (DIN11) identical to partial cds of 2-oxoacid-dependent oxidase (din11) from GI:10834554 [Arabidopsis thaliana]; identical to cDNA 2-oxoacid-dependent oxidase (din11) GI:10834553; contains Pfam profile PF03171:...
At3g59060.1	basic helix-loop-helix (bHLH) family protein contains Pfam profile: PF00010 helix-loop-helix DNA-binding domain [At3g59060.1]
	Auxin-responsive protein

10.2.2 Treatment with 100 μM 22a,b, 65a

10.2.2.1 Up-regulated genes in response to 100 μM treatments

Gene identifier	Description
At4g15210.2	ATBETA-AMY (BETA-AMYLASE); beta-amylase
At2g39350.1	ATPase, coupled to transmembrane movement of substances
At4g15210.1	ATBETA-AMY (BETA-AMYLASE); beta-amylase
At2g02990.1	RNS1 (RIBONUCLEASE 1); endoribonuclease
At1g76930.1	ATEXT4 (extensin 4)
At5g57560.1	TCH4 (TOUCH 4); hydrolase, acting on glycosyl bonds / hydrolase, hydrolyzing O-glycosyl compounds
At1g70920.1	DNA binding / transcription factor
At5g59310.1	LTP4 (LIPID TRANSFER PROTEIN 4); lipid binding
At1g19610.1	LCR78/PDF1.4
At2g23130.1	AGP17 (ARABINOGALACTAN PROTEIN 17)
At2g16440.1	ATP binding / DNA binding / DNA-dependent ATPase/ nucleoside-triphosphatase/ nucleotide binding
At2g23130.2	AGP17 (ARABINOGALACTAN PROTEIN 17)
At4g38400.1	ATEXLA2 (ARABIDOPSIS THALIANA EXPANSIN-LIKE A2)
At1g35140.1	PHI-1 (PHOSPHATE-INDUCED 1)
At5g39550.1	protein binding / ubiquitin-protein ligase/ zinc ion binding
At3g59220.1	PRN (PIRIN); calmodulin binding
At3g23730.1	hydrolase, acting on glycosyl bonds / hydrolase, hydrolyzing O-glycosyl compounds
At5g24080.1	ATP binding / kinase/ protein kinase/ protein serine/threonine kinase/ protein-tyrosine kinase
At5g24770.1	VSP2 (VEGETATIVE STORAGE PROTEIN 2); acid phosphatase
At5g24780.1	VSP1 (VEGETATIVE STORAGE PROTEIN 1); acid phosphatase
At2g23290.1	DNA binding / transcription factor
At4g34250.1	acyltransferase
At4g02850.1	catalytic
At4g02330.1	pectinesterase
At1g73325.1	endopeptidase inhibitor
At5g23530.1	catalytic
At3g14740.1	protein binding / zinc ion binding
At5g41040.1	transferase
AI996914	CORI3 (CORONATINE INDUCED 1); transaminase
At2g25820.1	transcription factor
At3g54810.1	transcription factor
At1g10585.1	transcription factor
At3g50060.1	DNA binding / transcription factor
At4g00180.1	YAB3; transcription factor
At1g12210.1	RFL1 (RPS5-LIKE 1); ATP binding
At2g43510.1	ATTI1
At5g43420.1	protein binding / ubiquitin-protein ligase/ zinc ion binding
At1g71000.1	heat shock protein binding / unfolded protein binding
At4g16590.1	ATCSLA01; glucosyltransferase/ transferase, transferring glycosyl groups
At1g76650.1	calcium ion binding
At4g11280.1	ACS6 (1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID (ACC) SYNTHASE 6)
At3g05890.1	RCI2B (RARE-COLD-INDUCIBLE 2B)
At5g46050.1	transporter
At5g26010.1	catalytic/ protein phosphatase type 2C
At5g61390.1	exonuclease
At1g65450.1	transferase
At2g43840.1	UDP-glycosyltransferase/ transferase, transferring hexosyl groups

At2g42800.1	protein binding
At5g05810.1	protein binding / ubiquitin-protein ligase/ zinc ion binding
At5g41120.1	catalytic
At1g15165.1	ubiquitin-protein ligase
At3g48350.1	cysteine-type endopeptidase/ cysteine-type peptidase
At5g42100.2	hydrolase, hydrolyzing O-glycosyl compounds
At3g48570.1	protein translocase
At1g33770.1	ATP binding / kinase/ protein kinase/ protein serine/threonine kinase/ protein-tyrosine kinase
At1g04760.1	ATVAMP726
At4g18170.1	WRKY28; transcription factor
At1g17250.1	protein binding / protein kinase
At4g29900.1	ACA10; calcium-transporting ATPase/ calmodulin binding
At4g19520.1	ATP binding / transmembrane receptor
At1g53140.1	ATP binding / GTP binding / GTPase
At4g01575.1	serine-type endopeptidase inhibitor
At4g26100.3	CK1; casein kinase I/ kinase
At5g18860.1	hydrolase
At4g11320.1	cysteine-type endopeptidase/ cysteine-type peptidase
At2g22730.1	carbohydrate transporter/ sugar porter/ transporter
At1g44318.1	porphobilinogen synthase
At1g47510.1	hydrolase/ inositol or phosphatidylinositol phosphatase
At5g45820.1	CIPK20 (CBL-INTERACTING PROTEIN KINASE 20); kinase
At4g30980.1	DNA binding / transcription factor
At1g68795.1	CLE12 (CLAVATA3/ESR-RELATED 12); receptor binding
At4g37260.1	MYB73; DNA binding / transcription factor
At5g07660.1	ATP binding
At5g10080.1	pepsin A
At2g30560.1	ATP binding
At5g67300.1	DNA binding / transcription factor
At5g39840.1	ATP binding / ATP-dependent RNA helicase/ ATP-dependent helicase/ helicase/ nucleic acid binding
At1g19940.1	hydrolase, hydrolyzing O-glycosyl compounds
At4g19230.1	CYP707A1; heme binding / iron ion binding / monooxygenase/ oxygen binding
At5g64520.1	XRCC2
At5g47435.2	formyltetrahydrofolate deformylase/ hydroxymethyl-, formyl- and related transferase
BP575077	VHA-A; ATP binding / hydrogen-transporting ATP synthase, rotational mechanism / hydrogen-transporting ATPase, rotational mech
At1g52030.1	MBP2 (MYROSINASE-BINDING PROTEIN 2)
At3g62590.1	triacylglycerol lipase
At1g26770.1	ATEXPA10 (ARABIDOPSIS THALIANA EXPANSIN A10)
At5g61890.1	DNA binding / transcription factor
BP667723	ELP (ECTOPIC DEPOSITION OF LIGNIN IN PITH); chitinase
At3g10910.1	protein binding / ubiquitin-protein ligase/ zinc ion binding
At1g57980.1	purine transporter
At4g22753.1	SMO1-3 (STEROL 4-ALPHA METHYL OXIDASE); catalytic
BX822209	JKHR07A9 snoRNA, complete sequence
At2g19990.1	PR-1-LIKE (PATHOGENESIS-RELATED PROTEIN-1-LIKE)
At1g25510.1	aspartic-type endopeptidase/ pepsin A
At5g40942.1	ribonucleoside-diphosphate reductase
At1g70090.1	transferase, transferring glycosyl groups / transferase, transferring hexosyl groups
At4g01500.1	transcription factor
At1g16410.2	CYP79F1 (SUPERSHOOT 1)
At3g50970.1	XERO2
At3g49160.1	pyruvate kinase
At1g58300.1	oxidoreductase

10.2.2.2 Down-regulated genes in response to 100 μM treatments

Gene identifier	Description
At5g65080.1	MAF5 (MADS AFFECTING FLOWERING 5 VARIANT I); transcription factor
At2g41240.1	DNA binding / transcription factor
At2g45220.1	enzyme inhibitor/ pectinesterase
At5g22890.1	transcription factor
At3g04280.1	ARR22 (ARABIDOPSIS RESPONSE REGULATOR 22); transcription regulator/ two-component response regulator
At5g07450.1	cyclin-dependent protein kinase
At5g36150.1	ATPEN3; catalytic
At3g56970.1	ORG2; DNA binding / transcription factor
AV540967	CPK5 (CALMODULIN-DOMAIN PROTEIN KINASE 5); ATP binding / calcium ion binding / calcium- and calmodulin-dependent protein kinase
At5g15220.1	structural constituent of ribosome
At2g23830.1	structural molecule
At2g31083.1	CLE7 (CLAVATA3/ESR-RELATED 7); receptor binding
At1g23700.1	ATP binding / kinase/ protein kinase/ protein serine/threonine kinase/ protein-tyrosine kinase
At1g68940.1	ubiquitin-protein ligase
At3g09930.1	carboxylic ester hydrolase/ hydrolase, acting on ester bonds / lipase
At5g17750.1	ATP binding / ATPase/ nucleoside-triphosphatase/ nucleotide binding
At5g23020.1	MAM-L (METHYLTHIOALKYMALATE SYNTHASE-LIKE)
At3g22231.1	PCC1 (PATHOGEN AND CIRCADIAN CONTROLLED 1)
At1g16980.1	ATTPS2; transferase, transferring glycosyl groups
At1g67090.1	ribulose-bisphosphate carboxylase
At1g10480.1	ZFP5 (ZINC FINGER PROTEIN 5); nucleic acid binding / transcription factor/ zinc ion binding
At2g25810.1	water channel
At2g28960.1	ATP binding / kinase/ protein kinase/ protein serine/threonine kinase/ protein-tyrosine kinase
At1g15820.1	LHCB6; chlorophyll binding
At3g17150.1	enzyme inhibitor/ pectinesterase/ pectinesterase inhibitor
At1g69930.1	ATGSTU11; glutathione transferase
At3g49760.1	DNA binding / transcription factor
At5g34870.1	nucleic acid binding
At5g58010.1	DNA binding / transcription factor
At5g38000.1	oxidoreductase/ zinc ion binding
At5g10140.1	FLC (FLOWERING LOCUS C); transcription factor
At5g38420.1	ribulose-bisphosphate carboxylase
At1g12805.1	nucleotide binding
At4g34380.1	nucleotide binding
At3g45690.1	transporter
At3g26120.1	RNA binding / nucleic acid binding
At2g03160.1	ASK19; ubiquitin-protein ligase
At1g59780.1	ATP binding
At1g29910.1	CAB3 (CHLOROPHYLL A/B BINDING PROTEIN 3); chlorophyll binding
At2g26490.1	nucleotide binding
At5g65100.1	transcription factor
At5g04150.1	DNA binding / transcription factor
At2g34420.1	LHB1B2
At5g38410.1	ribulose-bisphosphate carboxylase
BX836788	CLV1 (CLAVATA 1); ATP binding / kinase/ protein serine/threonine kinase
At5g24530.1	oxidoreductase, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors
At1g51790.1	kinase
At2g41090.1	calcium ion binding
At2g34430.1	LHB1B1; chlorophyll binding

At4g23515.1	transmembrane receptor
At1g64720.1	CP5
AV523348	EMB2734; lyase
At2g02850.1	ARPN (PLANTACYANIN); copper ion binding / electron transporter
At1g61520.1	LHCA3*1; chlorophyll binding
At3g54890.1	LHCA1; chlorophyll binding
At5g45380.1	solute:sodium symporter/ urea transporter
At1g21250.1	WAK1 (CELL WALL-ASSOCIATED KINASE); kinase
At2g39730.2	RCA (RUBISCO ACTIVASE)
At3g45540.1	ubiquitin-protein ligase/ zinc ion binding
At5g54610.1	protein binding
At2g47130.1	oxidoreductase
At2g30730.1	ATP binding / kinase/ protein kinase/ protein serine/threonine kinase/ protein-tyrosine kinase
At5g60830.1	DNA binding / transcription factor
At1g52940.1	hydrolase/ protein serine/threonine phosphatase
At5g52170.1	DNA binding / transcription factor
At1g23020.1	ferric-chelate reductase/ oxidoreductase
At5g10760.1	pepsin A
At4g14400.1	ACD6 (ACCELERATED CELL DEATH 6); protein binding
At5g11920.1	hydrolase, hydrolyzing O-glycosyl compounds
At2g31500.1	CPK24; ATP binding / calcium ion binding / calcium- and calmodulin-dependent protein kinase/ kinase/ protein kinase/ protein serine/threonine kinase/ protein-tyrosine kinase
At3g04530.1	PPCK2 (PHOSPHOENOLPYRUVATE CARBOXYLASE KINASE 2); kinase
At1g03980.1	glutathione gamma-glutamylcysteinyltransferase
At1g53420.1	ATP binding / kinase/ protein serine/threonine kinase
At1g06680.1	PSBP (OXYGEN-EVOLVING ENHANCER PROTEIN 2); calcium ion binding
At1g57830.1	transmembrane receptor
AV518576	ALDH3F1; aldehyde dehydrogenase/ oxidoreductase
At3g56710.1	SIB1 (SIGMA FACTOR BINDING PROTEIN 1); binding
At5g66570.1	PSBO-1 (OXYGEN-EVOLVING ENHANCER 33)
At5g01900.1	WRKY62; transcription factor
At4g25480.1	DREB1A (DEHYDRATION RESPONSE ELEMENT B1A); DNA binding / transcription factor/ transcriptional activator
At1g74350.1	RNA binding / RNA-directed DNA polymerase
At5g55460.1	lipid binding
At2g23240.1	zinc ion binding
At1g35710.1	ATP binding / protein kinase/ protein serine/threonine kinase/ protein-tyrosine kinase
AV537449	CHS (CHALCONE SYNTHASE); naringenin-chalcone synthase
At5g65070.1	MAF4 (MADS AFFECTING FLOWERING 4); transcription factor
At2g46430.1	ATCNGC3 (CYCLIC NUCLEOTIDE GATED CHANNEL 3); calmodulin binding / cyclic nucleotide binding / ion channel
At1g10620.1	ATP binding / kinase/ protein kinase/ protein serine/threonine kinase/ protein-tyrosine kinase
At4g13480.1	DNA binding / transcription factor
At1g55060.1	UBQ12 (UBIQUITIN 12)
At5g39020.1	ATP binding / kinase/ protein kinase/ protein serine/threonine kinase/ protein-tyrosine kinase
At2g46440.1	ATCNGC11; calmodulin binding / cyclic nucleotide binding / ion channel
At3g25820.1	ATTPS-CIN; myrcene/(E)-beta-ocimene synthase
AY090988	3'-5' exonuclease/ nucleic acid binding
At3g14210.1	carboxylic ester hydrolase/ hydrolase, acting on ester bonds
At1g49860.1	ATGSTF14; glutathione transferase
At3g46070.1	nucleic acid binding / transcription factor/ zinc ion binding
At5g38430.1	ribulose-bisphosphate carboxylase
At5g54770.1	THI1 (THIAZOLE REQUIRING)
At3g63480.1	ATP binding / microtubule motor