Norwegian University
of Life Sciences

Master's Thesis 2023 30 ECTS
Faculty of Chemistry, Biotechnology and Food science

Synthetic Studies Towards the Alkamide Avenalumamide AF8

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Acknowledgements

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

I would like to express my gratitude towards my supervisors Marius Aursnes and Yngve H. Stenstrøm for their guidance, feedback and help during this master thesis.

Many thanks to my fellow lab master students for their help and support during the practical work.

Lastly, I would like to acknowledge my family and friends. Their encouragements and moral support kept my motivation high during this process.

Ås, juli 2023

Bettina Florelle Ineza

Abstract

Avenanthramides (AVAs) are a group of phenolic compounds that can be found in oat (*Avena Sativa*) and act as defence mechanism against several pathogens. Avenanthramides (AVAs) are responsible for some health benefits in oat such as antioxidant and anti-inflammatory. The aim in this thesis is to synthesize one avenanthramide called Avenalumamide AF8. Several synthetic methods were explored in the synthesis toward Avenalumamide AF8.

In the synthesis of Avenalumamide AF8, the focus was to synthesize the biggest unit of the compound and this unit of is an avenalumic acid moiety. Potassium (1*E*,3*E*)-5-oxopenta-1,3-dien-1-olate was synthesized with great yields in a pyridinium anion ring-opening reaction and served as a starting material in subsequent reactions towards the synthesis of the avenalumic acid moiety. The starting material was used with a Grignard reagent in an attempted synthesis of (2*E*,4*E*)-5-(4-hydroxyphenyl) penta-2,4-dienal. This compound was later synthesized with an organolithium instead of a Grignard reagent, but the result was a product with low yield. The synthesis attempt of methyl (2*E*,4*E*)-5-bromopenta-2,4-dienaote was another approach towards the synthesis of the avenalumic acid moiety.

The synthesis towards Avenalumamide AF8 was not completed because of the limited time frame for this master thesis. With more time, some of these reactions would have been optimized and the synthesis of the anthranilic acid moity of Avenlumamide AF8 would have been explored.

Sammendrag

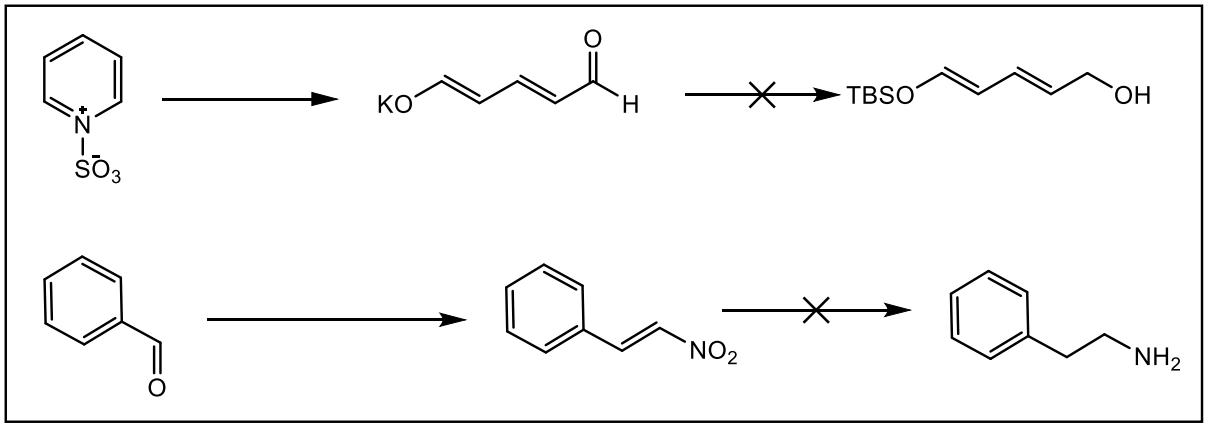
Avenantramider (AVAs) er en gruppe av fenol forbindelser som finnes i havre (*Avena Sativa*) og som forsvarer havre mot diverse patogene. Disse fenol forbindelser er ansvarlig for en del av helsefordeler til havre blant annet som å være antioksidant og anti-inflammatorisk. Målet med denne masteroppgaven, er å syntetisere forbindelsen Avenalumamid AF8 som tilhører gruppen avenantramider (AVAs). Ulike syntetiske metoder ble brukt i syntesen mot Avenalumamid AF8.

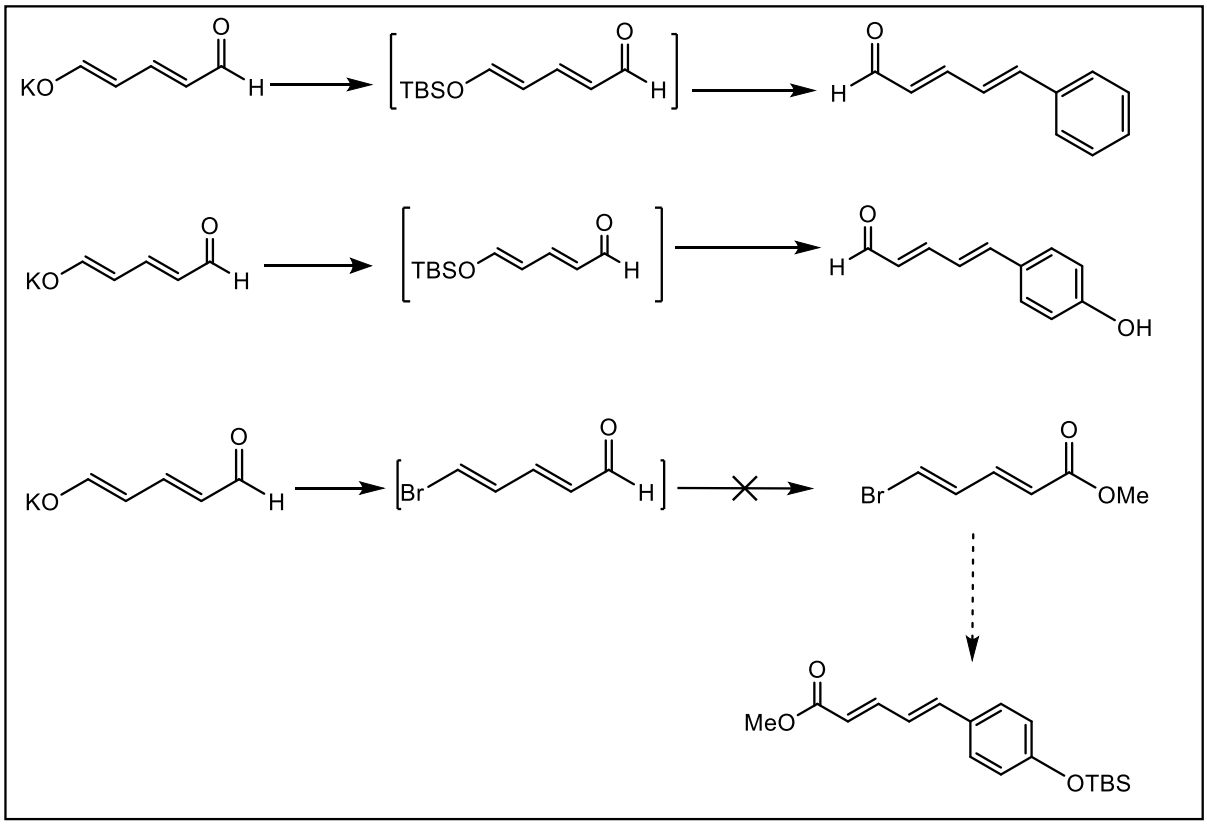
Den største delen av forbindelse Avenalumaid AF8 er en avenalum syre del og den ble satt søkelys på først i denne syntesen. Kalium (1*E*,3*E*)-5-oksopenta-1,3-dien-1-olate ble syntetisert i en pyridinium anion ring-åpning reaksjon med gode utbytter. Denne aldehyd forbindelsen ble brukt som utgangsstoff i senere reaksjoner i syntesen av avenalum syre delen. Aldehyd forbindelsen ble brukt sammen med en Grignard reagens i et forsøk på å syntetisere (2*E*,4*E*)-5-(4-hydroksyfenyl) penta-2,4-dienal. Denne syntesen ble senere utført med en organolitium istedenfor en Grignard reagens, produktet fra denne syntesen ga et lavt utbytte. Syntesen av metyl (2*E*,4*E*)-5-bromopenta-2,4-dienaote var en annen metode som ble utført i syntesen av avenlum syre delen.

Syntesen av Avenlumamid AF8 ble ikke fullført på grunn av begrensning av tid som er satt for en masteroppgave av denne størrelsen. Noen av reaksjonene ovenfor, ville ha blitt gjentatt hvis det var mer tid. Arbeidet mot anthranilic syre delen ville også ha blitt utforsket.

Graphical abstract

ChemDraw Professional 21.0 was used to draw all figures and schemes.





Abbreviations

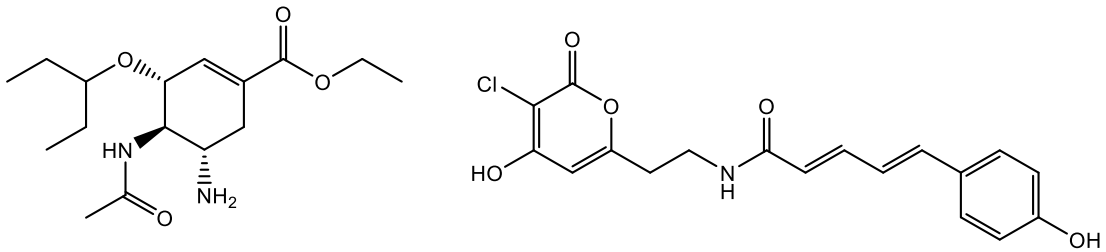
AVAs/Avns	Avenanthramides
DCM	Dichloromethane
DMAP	4-dimethylaminopyridine
EtOAc	Ethyl acetate
HHT	Hydroxycinnamoyl-CoA: 5-hydroxyanthranilate N-hydroxycinnamoyltransferase
ROS	Reactive oxygen species
TBDPS	Tert-butyldiphenylsilyl
TBS/TBDMS	Tert-butyldimethylsilyl
TBSCl	Tert-Butyldimethylsilyl chloride
THF	Tetrahydrofuran
TIPS	Triisopropylsilyl
TLC	Thin layer chromatography
TMS	Trimethylsilyl
Tranilast	N-[3’,4’-dimethoxycinnamoyl] anthranilic acid

Table of content

1. Introduction

1.1 Background and the aim of this thesis

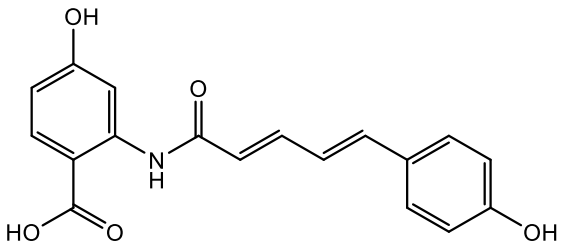
The aim of this thesis was to synthesize alkamide avenalumamide AF8 (fig.1) which belongs to a group of natural products called avenanthramides (AVAs). Initially, the plan for this master project was to use the pyridinium anionic ring-opening reaction[1] and Diels-Alder reactions to synthesize Tamiflu (oseltamivir). After experiencing some difficulties with some reactions, the aim of the thesis was changed. The new plan was to synthesize a compound called fuligopyrone B and is found in a plasmodial slime mold called *Fuligo septica*.[2] In due course, Avenalumamide AF8 became the main target.



Tamiflu (oseltamivir) **Fuligopyrone B**

Figur 1: Structures of Tamiflu(oseltamivir) and Fuligopyrone B

Avenalumamide AF8 consist of an avenalumic acid in amide conjugation with an aminophenolic (4-hydroxy-2-aminbenzoic).[3] AVAs are low-molecular-weight and water-soluble phenolic compounds which can be found in oats especially oat groat and hulls.[3, 4] These phenolic compounds act as a defence mechanism against numerous pathogens [5] and they are produced in response to an infection or elicitors used on oat leaves.[6] Other compounds than avenanthramides in oats include β-glucan, saponins, flavonoids, phenolic acids, and vitamins.[7] Oat also consists of vitamin E and minerals such as zinc, iron, magnesium etc.[8]



Avenalumamide AF8

Figure 2: The structure of avenalumamide AF8.

Oat is an important cereal crop that has been cultivated in more than 2000 years in different parts of the world. [5] Oats can be divided in two main species which are oats with hulls (*Avena Sativa*) and oats without hulls (*Avena nuda*).[9],[10] Oat thrives best in cool and humid climate [11], and it is mostly cultivated in Northen America, European countries followed by Russia, Australia, China and Brazil .[8, 10] These countries are also the largest oat consumers (fig.2).[10] Oat cultivation is one of the low-input systems and it provides soil health especially during crop rotation where soil structure is improved, and crop pests are reduced. [11] This crop has been getting attention from scientific researchers and industries, because of its value as human food, animal feed, health care and cosmetics.[5] In contrast to other cereals, oats contain high levels of soluble fibres, more protein, more fat and less starch [4]. European Food Safety Authority (EFSA) and USA's Food & Drug Administration (FDA) have formally approved various health claims regarding oats for its positive effects on human health. These health benefits include hypocholesterolemic properties, cardiovascular advantages through positive effects on blood glucose level, and body weight and blood pressure management improvement.[11]

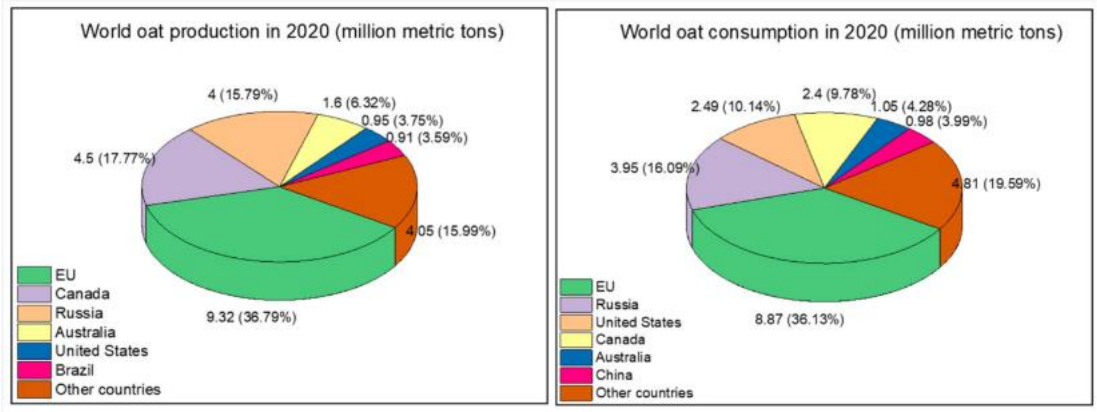


Figure 3: World oat production and consumption in 2020.[10]

Coeliac disease or celiac disease is an autoimmune disease that is caused by gluten. Many studies conducted, showed that celiac disease patients could consume oats without signs of intestinal inflammation. There is of course exception to these studies.[11] In a Norwegian study carried out *in vitro,* implied that oat intolerance may exist.[12] However, oat products are still a better choice for celiac disease persons in view of the health benefits, because present gluten-free products contain less vitamins and minerals, fibres are high in starch and salt, and consist of more saturated fats and many additives compared to gluten-based products. People with celiac disease cannot use regular commercial oats and oat products, because they are often

contaminated with gluten from wheat, barley and rye.[11] Therefore, gluten-free oats have to be produced, prepared and processed in a way to avoid contamination.[13]

Oat groat is softer than other grains and it is usually processed as a whole grain. Because of oat components and its unique physiological structure, oats are processed differently from other grains. To convert the mostly non-digestible oat kernel into food, milling and other processing steps are required.[14] These processing techniques make sure that the consumer requirements are met, the shelf life is expanded and that the product diversity is increased.[10] In Norway, oats are mostly used in animal feed production, but human consumption has increased in recent years, and some oat products can be found in stores. Some of these products are cereal, porridge, bread flour, oat milk and oat ice cream.[15]

1.2 Natural products

Organic compounds that all organisms need to live, grow, and reproduce are carbohydrates, proteins, fats, and nucleic acids. The pathway for synthesizing and modifying these compounds are generally the same in all organisms and can be described as primary metabolism. There is another pathway that produces compounds for specific organisms or a group of organisms. This pathway is called secondary metabolism and compounds in this metabolism are often produced for their benefits for the organism.[16] Primary metabolites er responsible for normal growth, development, and reproduction of organisms while secondary metabolites have ecological function. The lack of primary metabolites leads to instant death. On the other hand, the lack of secondary metabolites results in long term impairment of organism's survivability or aesthetics, and in same case, no critical change is observed. Plants secondary metabolites can be divided into three main groups which are terpenoids, nitrogen containing compounds and phenolic compounds.[17]

Throughout history, natural products have been used as traditional medicines, remedies, potions, spices, perfumes, oils, and drugs without identifying the bioactive compounds in these natural products.[18, 19] The main source of knowledge of natural products is from indigenous people around the world. They experimented on natural products for hundreds of centuries through palatability trials to find food and treatment of diseases.[18] The academic studies of natural products started in the 1850s. These studies encouraged development of separation methods, spectroscopic approaches to structure elucidation, and synthetic methods that are used in current organic chemistry.[19]

In drug discovery, natural products have played an important role, particularly for cancer and infectious diseases. Natural products contain diverse and complex bioactive compounds, and in drug discovery process, natural products present exclusive features compared to conventional synthetic molecules. [20] Acetylsalicylic acid and morphine are two well-known drugs that are synthesized from natural products.[18] Various disadvantages of natural products have led pharmaceutical companies to reduce drug discovery programmes based on natural products.[20]

Alkamides are secondary metabolites involving over 200 compounds scattered in plants and many of these plant species have been used in traditional medicine of different civilizations. [21] The general structure of alkamides was shown from condensation of an unsaturated fatty acid and an amine. An interest in these alkamides has increased because of their widely variety of biological activities.[21]

1.2.1 Avenanthramides

Avenanthramides are alkamides, and the basis chemical structure of all AVAs consists of an amide of an anthranilic acid moiety and a cinnamic or avenalumic acid residue.[7, 22] Avns have many naming methods but originally, they were known as avenalumin I, II, and III. Nowadays, Collins and Dimberg nomenclatures [22] are most widely accepted. When Collins extracted a mixture of avenalumin-like compounds in oat species, the Avns were characterized alphabetically (A to Z) while Dimberg established a new system where anthranilate derivates were assigned a number and corresponding phenylpropionates were assigned a letter. The letters assigned to the phenylpropionates are c for caffeic acid, f for ferulic acid, and p for p-coumaric acid (fig.2). The phenylpropionates that consist of avenalumic acid derivates (fig.2), the letter d is added to the Avns when using Dimberg's nomenclature method. There are currently 40 types of Avns that have been identified, but some of these Avns have not been purified and isolated because of their low natural concentrations.[22]

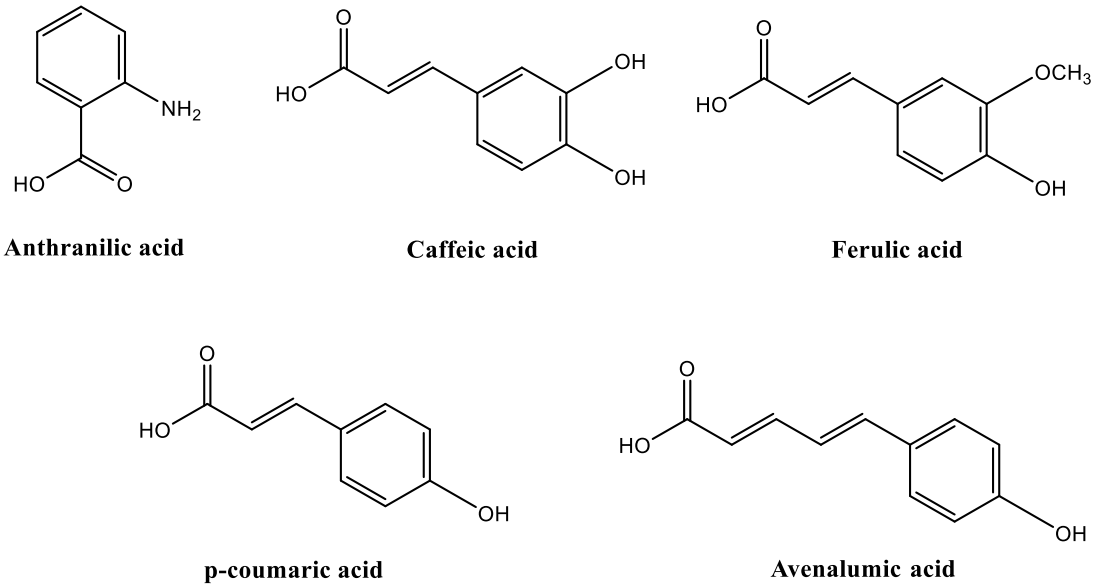


Figure 4: The structures of the building blocks of Avenanthramides.

Avns have several bioactivities such as antioxidation, anti- inflammation, anti-cancer and many more. Avns are bioavailable to the blood circulation, and the bioavailability has been tested and observed in humans and golden hamsters. [6]The bioavailability of Avns observed in plasma in golden hamsters were very low. The reason for this could possibly be that the Avns were allocated in other tissues. The bioavailability of Avns observed in human plasma were however higher than in hamsters.[9]

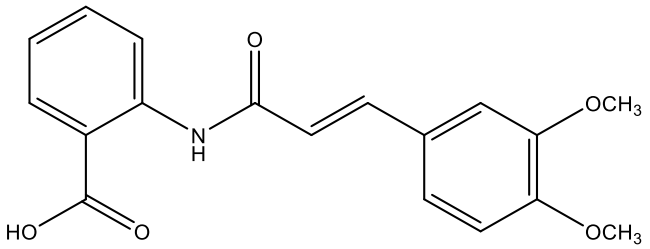
Originally, the antioxidants properties of oat components were observed in oat flour. Oat flour was used as a food preservative to prevent oxidative deterioration by slowing down the initial peroxide formation and rancidity.[9] *In vitro* and *in vivo* experiments of Avns extracted from oat and synthetically prepared Avns showed great antioxidant properties. [4] The first two Avns isolated, reported greater antioxidant properties than other phenolic antioxidants such as vanillin and caffeic acid. [4, 6] Avns exhibit cooperative interaction with vitamin C, indicating that Avns may act cooperatively with other antioxidative compounds.[6]

Oxidative stress can be caused when exuberance reducing reactive oxygen species (ROS) are produced in cells where the normal antioxidant capacity is overwhelmed or the impairment of antioxidant defence mechanism. It is documented that oxidative stress plays a large role in the pathogenesis and progression of major human diseases. Antioxidants have capacity to remove these excess ROS and inhibit oxidative stress. Antioxidants are therefore used as therapeutic options for prevention and treatment of several oxidative stress-related diseases.[9]

Avns prevent pro-inflammatory processes in different diseases. The anti-inflammatory activity of Avns has been tested on human aortic endothelial cells. The study reported that Avns was found to be mediated via modulation of the cellular and molecular processes that play an important role in the inflammation of arteries and the development of atherosclerosis. Avns utilize their anti-inflammatory activity in the skin also. Oats has been used for centuries as a remedy for different types of dermatologic disorders and complications such as atopic dermatitis, eczema, psoriasis, etc. The anti-inflammatory activity of colloidal oatmeal extract containing Avns have been disclosed in many in vitro and in vivo studies aimed to enlighten the different mechanisms of action of naturally derived colloidal oatmeal. Avns can also inhibit the chances of secondary inflammation due to the disrupted barrier function of the skin by controlling the sensory nerve response to the site of itching and the following scratching. [4, 6]

Natural products or their analogues are of great interest for chemoprevention and are seen as a promising, inexpensive, and easily relevant approach to the cancer control and management. Many studies were conducted with Avns and their analogues as potential chemopreventive agents. These studies proposed that Avns can use their anticancer effects to modulate different cell signalling pathways and activate cell death signals, and by that prevent cancer development or progression. [6] Tripathi *et al.*[6] proposed that future studies should research on the synergistic effect of Avns with other compounds so that more efficient and less toxic therapy is developed. It is also suggested that Avns should be used alone or with present chemotherapeutic agents to improve therapeutic effects and to reduce toxicity from chemotherapy. The latter is suggested because of safety pharmacology of Avns.[6]

There is a synthetic drug that has similar structure to Avns. This synthetic drug is a popular antihistamine compound and is called Tranilast (N-[3',4'-dimethoxycinnamoyl]anthranilic acid) (fig.4).[6] Tranilast was initially developed in Japan and is currently utilized in Japan and South Korea to treat bronchial asthma, atopic dermatitis, keloids and hypertrophic scars and other allergic dissorders.[4, 9] In clinical trials and cell culture studies, it was observed that tranilast and Avns respectively exhibited antiproliferative effect on vascular smooth muscle cells (VSMCs).[4]



Tranilast

Figure 5: The structure of Tranilast (N-[3',4'-dimethoxycinnamoyl] anthranilic acid).

The main enzyme in the biosynthesis of Avns is hydroxycinnamoyl-CoA: hydroxyanthranilate N-hydroxycinnamoyltransferase (HHT EC 2.3.1.302, 1-4 genes). The biosynthesis starts with the production of *trans*-cinnamic acids from phenylalanine in the presence of phenylalanine ammonia-lyase (PAL). Cinnamate is converted to p-coumaric acid by hydroxylation in the presence of cinnamic acid 4-hydroxylase (C4'H). P-coumaric acid is then transformed into its activated CoA thioester analogue by 4-coumarate-CoA ligase and resulting in p-coumaroyl-CoA (donor molecule). The p-coumaroyl-CoA (donor molecule) is conjugated to 5-hydroxyanthranilic acid (acceptor molecule) and catalysed by HHT to generate Avn A. This is followed by many reactions that are generated by different enzymes.[23]

Formerly, it was believed that the biosynthesis of this Avns compounds (Avn-A, Avn-B, Avn-C or 2p, 2f, 2c) were catalysed by a single HHT enzyme, able to accept all the substituted cinnamoyl-CoA thioesters. It was later demonstrated that two HHT enzymes catalysed the N-acylation of 5-hydroxyanthranilic acid with p-courmaroyl-CoA or caffeoyl-CoA, but not with feruloyl-CoA. This means that oat HHT are only involved in the biosynthesis of Avn-A (p-coumaroyl acid derivative) and Avn C (caffeoyl acid derivatives), but not Avn-B (ferulic acid derivative). in this scenario, Avn-C was methylated by the enzyme caffeoyl-CoA *O*-methyltransferase (CCoAOMT) to produce Avn-B. To produce avenalumic acid derivatives, a proposed biosynthesis began with the condensation of p-coumaroyl-CoA and malonyl-CoA, to form 5-(4-hydroxyphenyl)-3-oxo-4-pentenoyl-CoA. This intermediate was then reduced and dehydrated to avenalumoyl-CoA. In the presence of 5-hydroxyanthranilic acid, avenalumoyl-CoA was converted to Avn-L (avenalumic acid derivative).[23]

1.3 Chemical background

1.3.1 Protection groups

Organic compounds with more than one functional group require protection groups when the functional groups can react under specific conditions and a less reactive group is in the presence of a more reactive group. A good protection group is a group that can be introduced and removed in high yield without affecting the rest of the molecule. It should also survive a wide range of conditions during the synthesis. Silyl ethers are one of the protection groups and they are versatile especially for alcohols. Silyl ethers are attacked by hydroxide ion, water, or fluoride ion, because they have a strong affinity for electronegative atoms such as oxygen, fluor. These silyl ethers are removed by nucleophilic displacement with fluoride or oxygen nucleophiles, and the steric bulk of the silyl group often decides the rate of removal.[24]

The least complex of the silyl ethers is trimethylsilyl (TMS) and it is also easily removed by water in trace of base or acid. If one of methyl groups is replaced with tertiary butyl group, it gives another silyl ether called *tert*-butyldimethylsilyl (TBS or TBDMS). TBS has become a very popular protection group in organic synthesis, because it is stable in reactions and survives aqueous work-up or column chromatography on silica gel. In a synthesis, TBS is resistance to base, and it is introduced as *tert*-butyldimetylsilyl chloride (TBSCl) by a substitution reaction. Two other common silyl protection groups are triisopropylsilyl (TIPS) and *tert*-butyldiphenylsilyl (TBDPS). TBDPS is effective for chemoselective protection on a compound with several functional groups because of its steric bulk. [24]

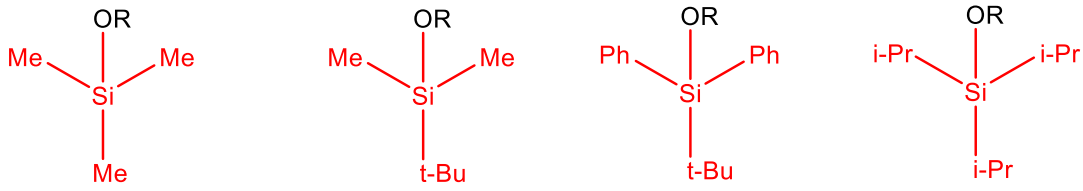


Figure 6: Four protection groups of silyl ethers.

1.3.2 Organometallic compounds

Forming new C-C bonds is one of the most important topics in organic chemistry. One way to make these C-C bonds, is using organometallic compounds in combination with carbonyl compounds where these organometallic compounds act as nucleophiles towards an electrophilic carbonyl group. The carbon atoms of the carbonyl groups are electrophilic because of the polarization of carbon-oxygen bond towards the more electronegative oxygen and that makes the carbon a site for nucleophilic attack. In organometallic reagents the key bond is polarized towards carbon which makes carbon a nucleophilic centre. Organometallic compounds are often reactive towards water and oxygen to produce alkanes, and they are therefore handled under an atmosphere of nitrogen or argon.[25]

Two of the most important organometallic compounds are organolithiums and organomagnesium halides (Grignard reagents).[25] Organomagnesium compounds were discovered by Victor Grignard and were given his name in 1900.[26, 27] Many methods can be used to prepare Grignard reagents and some of them are, insertion, metalation or halogen-metal exchange. For preparation of organolithiums, methods like reduction, halogen-metal exchange or metalation/deprotonation can be used.[27] Several organometallic reagents are commercially available, and in synthesis, either self-made or purchased organometallic reagents may be used.[25]

The metal insertion of magnesium is often used to make a Grignard reagent. It requires reacting magnesium chips with alkyl, allyl, or aryl halides in a non-protic solvent like ether forming a solution of alkylmagnesium halide. Alkyl or aryl halides that can be used, are iodides, bromides, and chlorides. The reaction of making Grignard reagent is called oxidative insertion or oxidative addition where magnesium is inserted into the carbon-halogen bond and magnesium is oxidated from Mg(0) to Mg(II). The polarity of the carbon in the carbon-halogen bond is reversed and this is umpolung meaning “inverse polarity”. This reaction is driven by the fact that magnesium is easily oxidized. The mechanism for this reaction is complicated and has not been completely understood. Maybe true product is a complex between the Grignard reagent and two molecules of the ether solvent since Mg(II) prefers a tetrahedral structure (fig.4).[25]

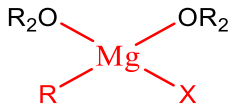
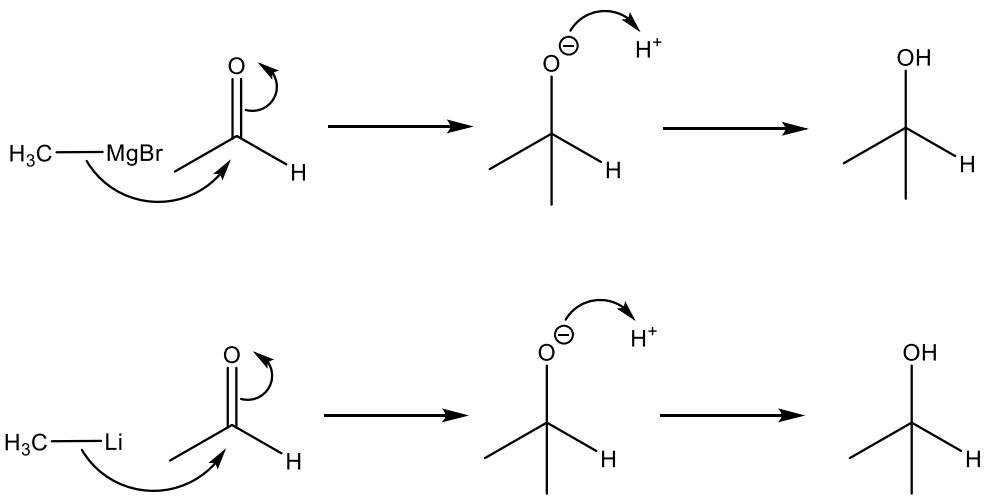


Figure 7: Complex of ether with Grignard reagent.

The halogen-metal exchange is the most common used method to prepare organolithium.[27] In this method, organolithiums remove halogen atoms from alkyl or aryl halides and they swap places.[25] Halogen-metal exchange reactions are usually very fast and the organolithiums formed are often stable only at very low temperature. This causes difficulties when used in very large scale because of the need of cryogenic temperatures.[27]

Organolithiums can also be made by a similar oxidative insertion reaction as Grignard reagents from lithium metal and alkyl or aryl halides. This reaction requires two atoms of lithium and generates one equivalent of lithium halide salt. Lithium is oxidated from Li(0) to Li(I) and no halide is attached to lithium. Hydrocarbon solvents such as pentane or hexane are most often used as solvent, but ether solvent is common for methyl lithium.[25]

The mechanism for Grignard addition and organolithium addition is shown in Scheme 1, and Grignard reagents and organolithium reagents react similarly. In this example, methyl magnesium bromide and methyllithium reacts with ethanal to form an alkoxide first. Then, the negatively charged oxygen is protonated with water or acid.[28]



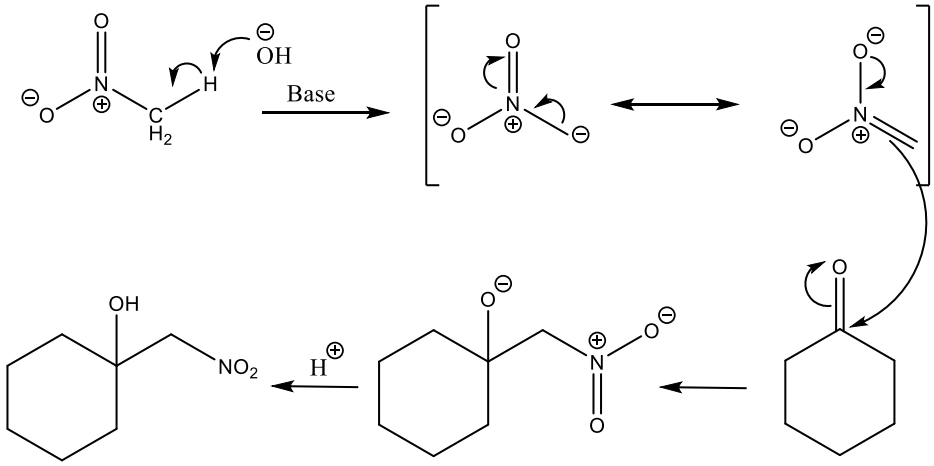
Scheme 1: The mechanism for Grignard addition and organolithium addition.

1.3.3 Henry reaction

Henry reaction is a reaction where C-C bond is formed between nitroalkane and aldehydes or ketones [29]. The Henry reaction is also commonly known as a nitroaldol reaction. It includes a reactive nitronate compound that is generated as a part of a base-catalytic cycle through the action of an appropriate catalyst and resulting in a nitro alcohol.[30] Soluble bases that have be used in Henry reactions are alkali metal hydroxide, carbonates, bicarbonates, alkoxides, alkaline earth metal hydroxide, aluminium ethoxides, complexes and organic bases.[29]

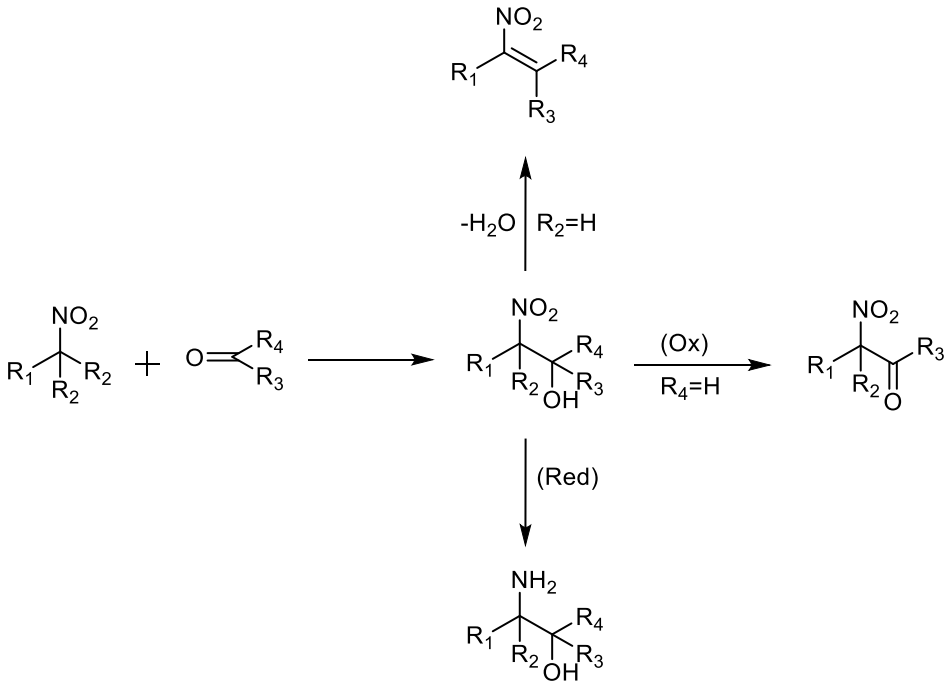
This reaction has numerous aspects that are similar to an aldol reaction [30] and some bases reagents used in this reaction can be also used in aldol reaction[31]. Therefore, it is crucial to choose experimental conditions that can suppress these competitive reactions when aldehyde is used as carbonyl sources. It is also essential to control the basicity of the reaction medium to acquire great nitro alcohols yields. To avoid aldol reaction, the concentration of the carbonyl sources has to be kept to a minimum for an adequate rate of reaction.[31]

The mechanism for Henry reaction is shown in Scheme 2. The reaction starts with the deprotonation of a primary nitroalkane by a base to give a resonance stabilize anionic intermediate.[32] The nitro group stabilize a negative charge on the neighbouring carbon. The nitro and *aci*-nitro forms relate by the nitro-*aci*-nitro tautomerism and share a common anion.[31] The anion intermediate then attacks the carbonyl compound to form an alkoxide. The conjugate acid of the base donates a proton to the alkoxide to produce a nitro alcohol.[32, 33]



Scheme 2: The Henry reaction depicting the nitroaldol reaction mechanism.

Henry reaction is one of the classical name reactions in organic synthesis [34] and is important, because products from this reaction can transform into other compound families.[30] Nitro alcohol compounds can be transformed into nitroalkenes which are important building blocks in synthesis through dehydration reactions. Other compound families the CH-NO_2 moiety can be converted into, are ketones, aldehydes or carboxylic acids through Nef oxidation, amine compounds through reduction or other derivatives through substitution of the nitro group by nucleophiles.[30]



Scheme 3: Transformation of nitro alcohol into some other compound families.[34]

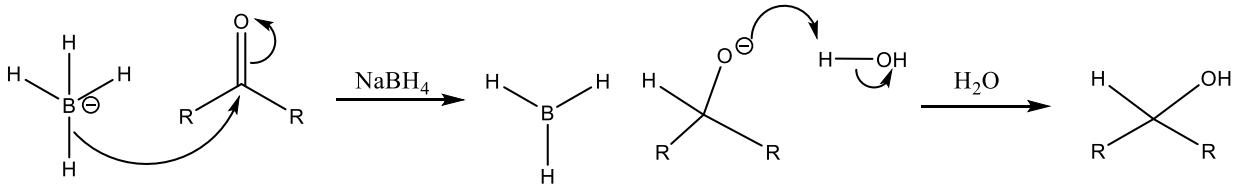
The dehydration of nitro alcohol into nitroalkenes with several reagents is an important reaction. Nitroalkenes are intermediates in several effective methods for their transformations into other functionalities. The Nef oxidation was originally performed with strong oxidizing agents in strong acidic media. Subsequent, numerous very mild and effective procedures were created to achieve this transformation. Linear α-nitro ketones have been utilized in some direct and indirect methods to carry out the replacement of the nitro group by hydrogen.[31]

Nitro alcohols can be converted to amino alcohols by reduction with several reducing agents. This reaction leads to production of useful compounds and different synthetic methods. The importance of vicinal amino alcohol is broad in organic chemistry and their biological relevance is observed in the structures of mediators of the sympathetic nervous system, in the

chemistry and biochemistry of sphingolipids, and in the structures of some carbohydrates components of a group of biologically important anthracycline antibiotics.[31]

1.3.4 Reduction of carbonyl groups

Sodium borohydride ($NaBH_4$) is a water-soluble salt that contains a tetrahedral BH^-_4 anion. Sodium borohydride is used in reduction of aldehydes and ketones into alcohols. In this reaction, a hydrogen atom from BH^-_4 anion together with a pair of electrons in the boron-hydrogen bond will be transferred to the carbon atom of the carbonyl group. The mechanism of the reduction is shown in Scheme 4. The electron deficient BH_3 molecule can be stabilized by the help of the alkoxide and to form an alkoxyborohydride anion. This by-product is a reducing reagent, and can be used in reaction so that all four hydrogen atoms could be transferred to molecules of carbonyl group.[35]



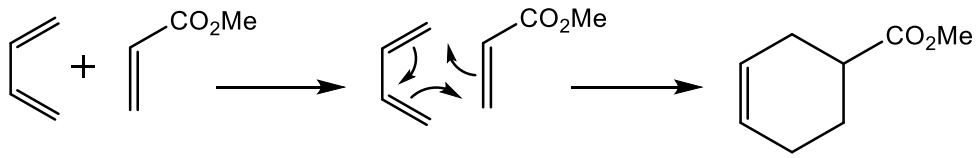
Scheme 4: Mechanism of reduction of a carbonyl group with sodium borohydride.

Normally, aldehydes and ketones are reduced in good yield to the corresponding alcohol by sodium borohydride in water or alcohol solvents. The solvents give the proton needed to form the alcohol from the alkoxide. However, the reduction of ketones is slower that the reduction of aldehydes. Less reactive carbonyl compounds such as esters and amides cannot be reduced by sodium borohydride.[35]

Sodium borohydride is a weak hydride donor since it can be used in water. Lithium aluminium hydride ($LiAlH_4$) is stronger hydride donor, and it reacts dangerously with water in an exothermic reaction that produces highly flammable hydrogen. Lithium aluminium hydride is stronger that sodium borohydride, because aluminium is more metallic than boron and it is prepared to give up a hydrogen atom whether to a carbonyl group or water. [35]

1.3.5 Diels-Alder reaction

Diels-Alder reaction is a reaction between a conjugated diene and an alkene called dienophile. This type of reaction is called pericyclic and electrons in this type of reactions move around a circle. Electrons move easily out of π orbitals into σ orbitals where two π bonds disappear and two σ bonds take their place. There are neither positive nor negative charges on intermediates and there are no intermediates at all. The mechanism for Dies-Alder reaction is shown in Scheme 5. The arrows are connect and each arrow leads directly to the next. [36]



Scheme 5: A mechanism for Diels-Alder reaction

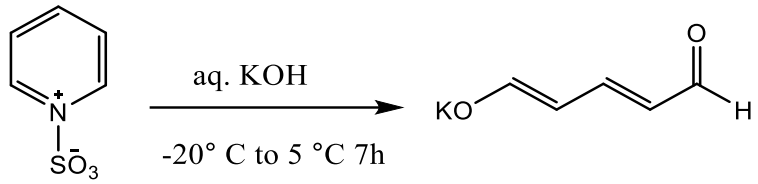
In Diels-Alder reaction the diene component can be open-chain or cyclic with various substituents. However, the diene component must be able to take up s-*cis* conformation. All the dienophiles in Diels-Alder reactions have an electron-withdrawing group conjugated to the alkene. This is not exclusive for feature of Diels-Alder dienophiles, but some extra conjugation must be provided for the addition to occur.[36]

1.3.6 Amide bond formation

The amide bond is a fundamental chemical bond that broadly occurs in natural and industrial products and the formation of an amide bond is one of the most important organic reactions. Currently, the synthetic methods for the amide bond are variations of dehydration of carboxylic acid and amine, and they are performed with either a catalyst or through the prior conversion of carboxylic acid into an activated carboxylate. The conversion is necessary, because of the requirement of rough reaction conditions and long reaction times for immediate amidation of carboxylic acid. For this reason, the amidation of esters with amines are highly preferable for the formation of amides.[37] However, direct amide bond formation between amines and inactivated esters are rarely used because of the need to deprotonate the amine with an aggressive organometallic reagent.[38]

2. Results and discussion

2.1 Synthesis of potassium (1*E*,3*E*)-5-oxopenta-1,3-dien-1-olate (**2**)



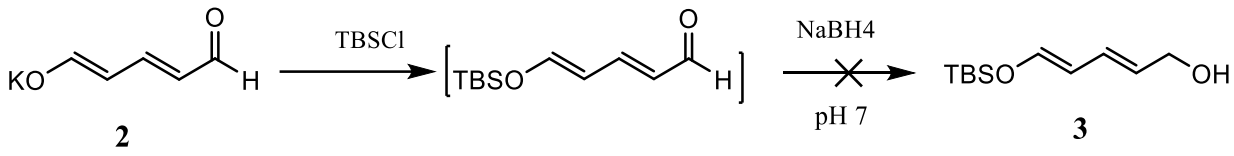
1 **2**

*Scheme 6: Synthesis of potassium (1E,3E)-5-oxopenta-1,3-dien-1-olate (**2**)*

Glutaconaldehyde potassium salt (**2**) was synthesized by using pyridinium-1-sulfate (**1**) and potassium hydroxide according to a procedure from Primdahl *et al.*[1] and the salt became a starting material in some of the subsequent reactions. This reaction was performed twice on the same scale and both reactions were successful. The first time, the product weighted ca.10 g giving 23 % yield while the second time the product weighted almost triple the mass of the first time (28.7 g) giving 67% yield. The latter came close to 68% yield reported in Primdahl *et al.*[1]. The reason for less amount of product in the first reaction is due to a funnel that was used under hot filtration that led to a slow filtration. The second time performing this reaction, excessive bumping arose under evaporating methanol and most of the mixture was transferred to the solvent flask on rotary evaporator. The mixture was then transferred back into the original flask and that may have caused impurities in the final product.

Spectral data from ^{1}H and ^{13}C NMR spectra (fig.8&9) shows that glutaconaldehyde potassium salt was successfully synthesised. On the ^{1}H NMR spectra, the two peaks at 8.7 ppm (2H) indicate one proton that is characteristic for an aldehyde and the other proton indicate the neighbouring proton to the oxygen with a potassium metal. The carbons coupled with these two protons give a signal at 184.9 ppm on ^{13}C NMR spectra. The chemical shift for aldehyde is usually from 9-10 ppm on ^{1}H NMR spectra and 190-220 ppm on ^{13}C NMR spectra. The cause for lower chemical shift for the aldehyde is due to resonance which is why the signal gives two protons.

2.2 Synthesis of (2*E*, 4*E*)-5-(tert-butyldimethylsilyl) oxy) penta-2,4-dien-1-ol (**3**)



2 **3**

*Scheme 7: Synthesis of (2E, 4E)-5-(tert-butyldimethylsilyl) oxy) penta-2,4-dien-1-ol (**3**).*

One oxygen on glutaconaldehyde potassium salt **2** was first protected with a silyl ether and then the salt was expected to be reduced with sodium borohydride to a primary alcohol (**3**) using procedures from Lewis *et al* [39] and Lubineau *et al* [40] **.** The diene in compound **3** was intended to be used in Diels-Alder for the synthesis of Tamiflu. Unfortunately, this reaction failed and the data from both ^{1}H and ^{13}C NMR were difficult to interpret. Few peaks were present on the ^{1}H NMR spectra while there were no peaks on ^{13}C NMR spectra. The peaks on ^{1}H NMR spectra were very weak, and it was difficult to identify what was made.

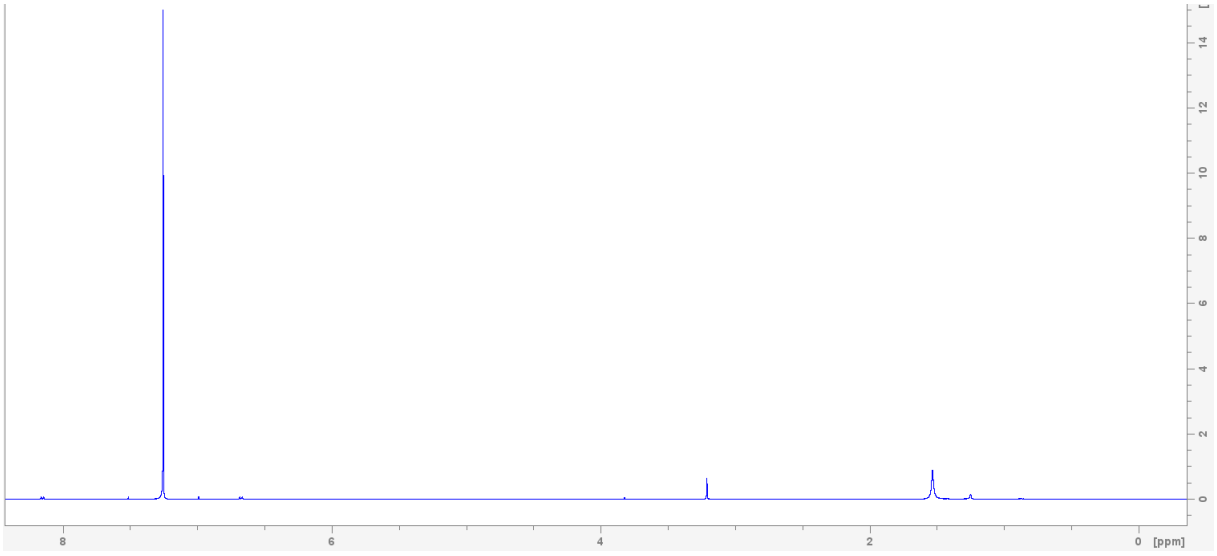
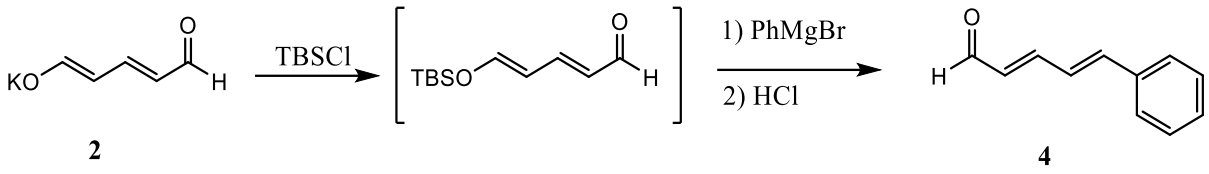


*Figure 8: ^{1}H NMR spectra of the result from the synthesis of (2E, 4E)-5-(tert-butyldimethylsilyl) oxy) penta-2,4-dien-1-ol (**3**).*

The first part of this reaction was performed again to see if the TBS-protection was successful. The theory was that this would explain why the reduction failed. Two ^{1}H NMR with chloroform and dimethyl sulfoxide as solvents were taken, and the spectra did not contain peaks that are neither characteristic for TBS nor characteristic for an aldehyde.

2.3 Synthesis of (2*E*, 4*E*)-5-phenylpenta-2,4-dienal (**4**)



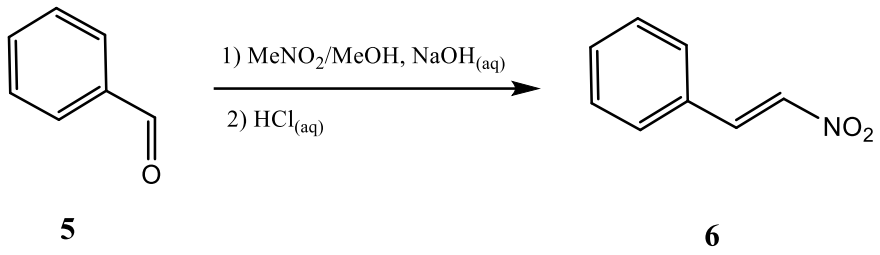
2 **4**

*Scheme 8: Synthesis of (2E, 4E)-5-phenylpenta-2,4-dienal (**4**).*

In this method from Lewis *et al* [39], one oxygen in glutaconaldehylde potassium salt **2** was first protected with TBSCl before a Grignard reagent was used to form C-C bond to synthesize compound **4**. This reaction was successful, and ^{1}H, ^{13}C and COSY NMR (fig.10,11&12) confirmed that the result was a pure compound. This reaction was performed on 15% scale of the scale from Lewis *et al.* [39] The product obtained was so little and this made it challenging to calculate the yield.

The spectral data from ^{1}H and ^{13}C NMR spectra showed peaks that are characteristic for compound **4**. The signals at 9.6 ppm (^{1}H NMR) and 193.7 ppm (^{13}C NMR) are characteristic for aldehyde, and the chemical shifts at 7.4, 7.2 and 6.9 ppm (^{1}H NMR) are characteristic for protons in an aromatic ring.

2.4 Synthesis of [(*E*)-(2-nitroethenyl)] benzene (**6**)



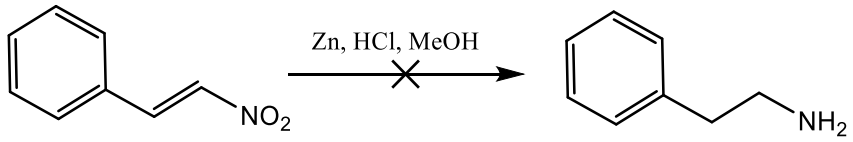
5 **6**

*Scheme 9: Synthesis of [(E)-(2-nitrotehyl)] benzene (**6**).*

Benzaldehyde (**5**), nitromethane and sodium hydroxide in methanol was used to synthesize compound **6** according to a procedure from Yang *et al.*[41]. This reaction was performed twice, because the reaction failed the first time, but it was successful the second time. The reason for the success the second time may be the upscaling of reagents. ^{1}H NMR was not taken the second time to confirm the structure of compound **6**, but the result was expected to be solid material and this solid material was recrystallised successfully.

The product from the first reaction was not much and this caused poor quality spectral data from ^{1}H NMR spectra. It was impossible to identify what was made. The reason that might have caused the failed reaction the first time, may have been that the dehydration reaction with hydrochloric acid to get the nitroalkene was unsuccessful and that instead a nitro alcohol was the synthesized.

2.5 Synthesis of 2-phenylethan-1-amine (**7**)



6 **7**

*Scheme 10: Synthesis of 2-phenylathan-1-amine (**6**).*

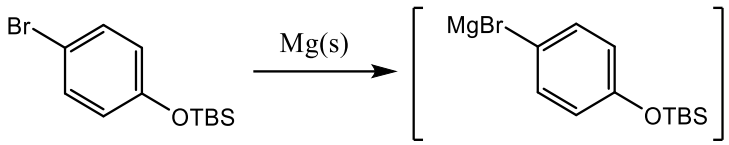
The attempted synthesis of compound **7** from compound **6**, zinc powder and concentrated hydrochloride acid in methanol was performed according to a method from Scharf *et al* [42]. The intention of this reaction was to convert the nitroalkene into the amine for the synthesis of fuligopyrone B, but the reaction was unsuccessful. The crude product was a white solid

substance, and the crude product was expected to be a yellow oil according to Scharf *et al.*[42]. ^{1}H NMR was taken twice with chloroform and acetonitrile, and the data from both spectra contained few peaks. This made it difficult to interpret the spectral data and identify the crude product.

2.6 Synthesis of (2*E*,4*E*)-5-(4-hydroxyphenyl) penta-2,4-dienal (**11**)

2.6.1 First attempt

Step 1:

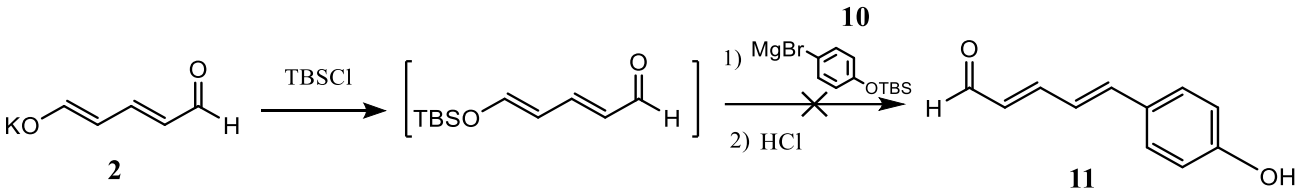


9 **10**

*Scheme 11: Formation of Grignard reagent **10.***

The synthesis of compound **11** started with the protection of 4-bromofenol (**8**) with TBSCl with a procedure from Garlets *et al.* [43] The protection was a success and ^{1}H NMR was taken to confirm. The spectroscopic data showed to double doublet peaks at 7.13ppm (2H) and 6.5ppm (2H), respectively, which are characteristic for protons in an aromatic ring. Two single peaks at 0.79ppm (9H) and 0.002 ppm (6H) represent the protons in TBS. Compound **9** was then used to make a Grignard reagent **10**. The Grignard reagent was made two times and the first time a mistake was made which caused the reaction to fail. The mistake was that the solution encountered air and ^{1}H NMR was taken to verify that the Grignard reagent was made. The Grignard reagent was supposed to be drawn out with a syringe and transferred to the reaction in step 2.

Step 2:



*Scheme 12: First synthesis attempt of (2E,4E)-5-(4-hydroxyphenyl) penta-2,4-dienal (**11**).*

Glutaconaldehylde potassium salt **2** was protected with TBSCl in THF and combined with the solution from the reaction in step 1 to synthesize compound **11** in a method from Lewis *et al.* [39] As previously stated, the mistake made in the reaction in step 1 caused failure performing this reaction the first time. This reaction still failed the second time despite making no mistake. The ^{1}H NMR spectra of the crude product showed some trace of chemical shifts that are characteristic for TBS and aldehyde. The peaks for protons in TBS were very high while the peaks for protons for carbonyl group were almost non existing. The Grignard reagent used in this reaction was fresh and self-made, and the reaction was unsuccessful. This reaction is similar to the synthesis of (2*E*, 4*E*)-5-phenylpenta-2,4-dienal (**4**) where the Grignard reagent used in that reaction was commercially purchased.

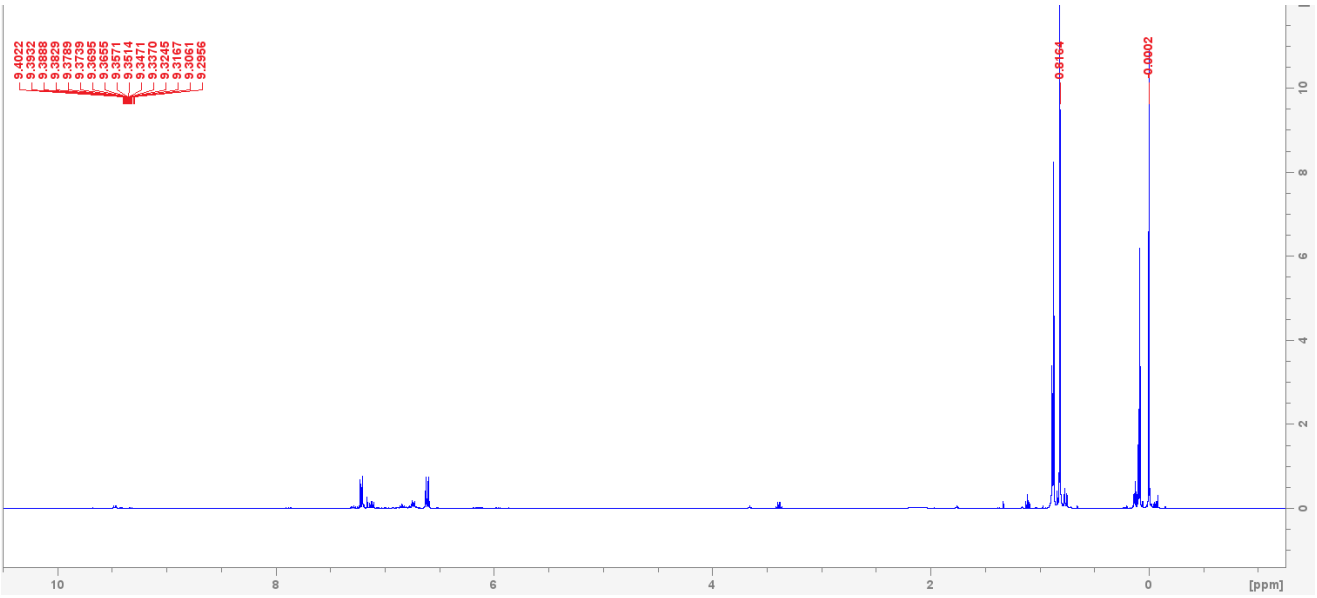
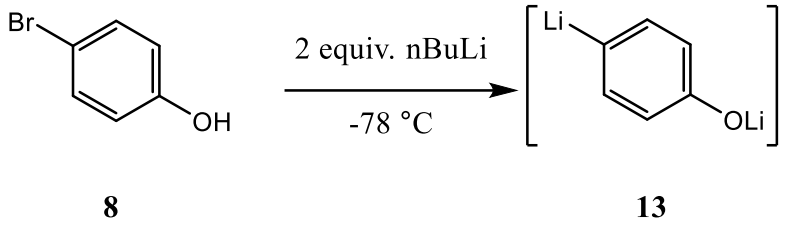


*Figure 9: ^{1}H NMR spectra of the crude product in the first synthesis attempt of (2E,4E)-5-(4-hydroxyphenyl) penta-2,4-dienal (**11**).*

2.6.2 Third attempt

Step 1:

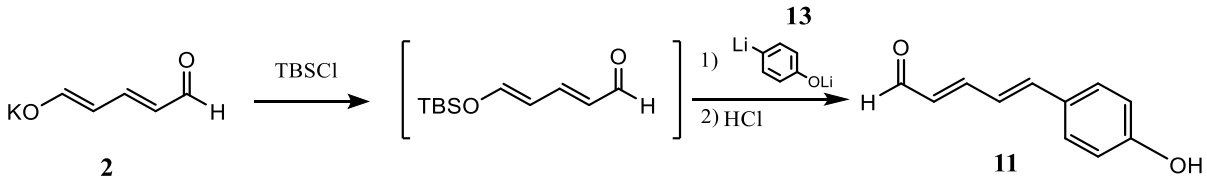


8 **13**

Scheme 13: Replacement of bromine with lithium.

Bromine was replaced with lithium on 4-bromofenol (**8**) by using n-butyllithium in hexane at -78 ˚C according to a method from Chakraborty *et al*.[44] This reaction was first performed on TBS-protected 4-bromofenol (**9**) in the second synthesis attempt of (2*E*,4*E*)-5-(4-hydroxyphenyl) penta-2,4-dienal (**11**). The preparation for this reaction was easier compared to the preparation for the second attempt.

Step 2:



2 **11**

*Scheme 14: synthesis of (2E,4E)-5-(4-hydroxyphenyl) penta-2,4-dienal (**11**).*

The solution from the reaction in step 1 and protected glutaconaldehyde potassium salt (**2**) was used to synthesize compound **11**. The reaction was successful, but it was challenging to get good results. This resulted in low yield even when the reaction was upscaled. Compound **11** is volatile, and this caused difficulties getting good spectroscopic data. The data from ^{1}H NMR spectra (fig.14) looked promising while the data from ^{13}C NMR spectra contained only two weak chemical shifts. ^{1}H NMR spectra showed one peak at 9.6 ppm (1H) which confirms a proton in a carbonyl group and two peaks at 7.4 ppm (2H) and at 6.9 ppm (2H) which are characteristic for protons in an aromatic ring. The ^{1}H NMR spectra of the crude product in the second attempt contained chemical shifts that are characteristic for compound **11**, but the ^{1}H NMR spectra of the crude product in the third attempt had more and higher signals.

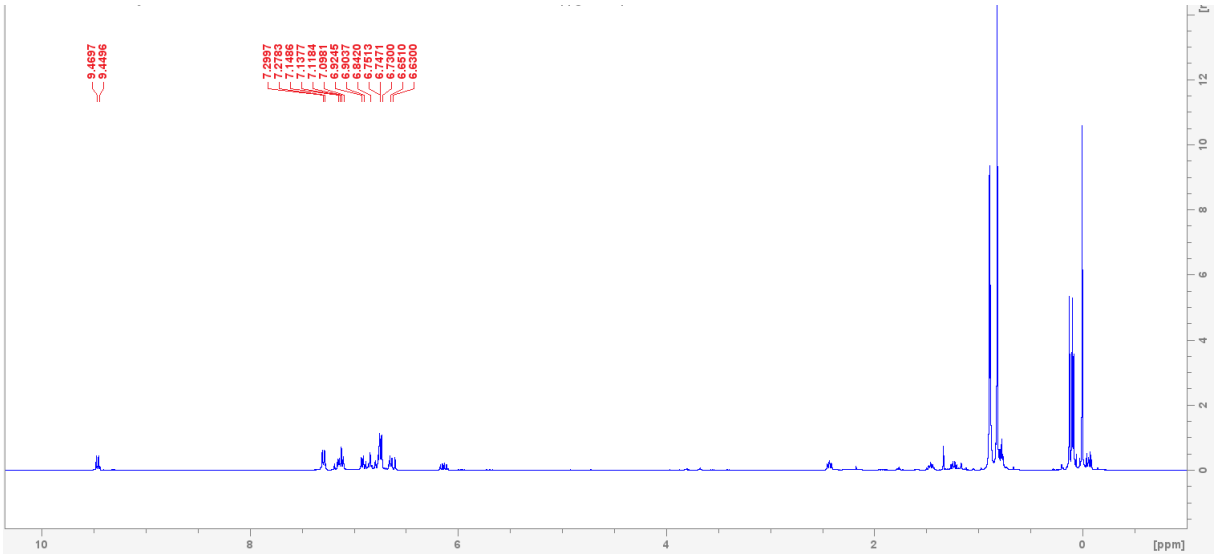
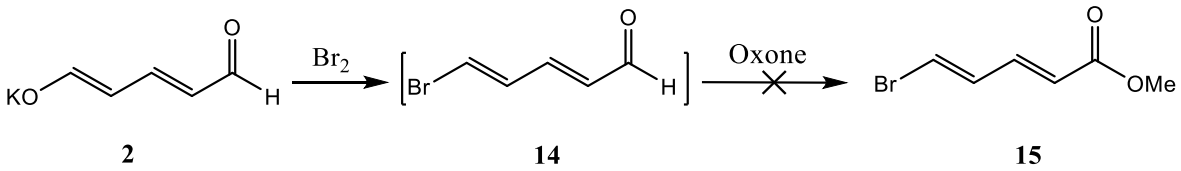


*Figure 10: [1]H NMR spectra of the crude product in the second synthesis attempt of (2E,4E)-5-(4-hydroxyphenyl) penta-2,4-dienal (**11**).*

2.7 Synthesis of methyl (2*E*,4*E*)-5-bromopenta-2,4-dienoate (**15**)



2 **14** **15**

*Scheme 15: Synthesis of methyl (2E,4E)-5-bromopenta-2,4-dienoate (**15**).*

Glutaconaldehyde potassium salt (**2**) was added in solution of bromine dissolved in dichloromethane and later oxone was added to synthesize compound **15**. According to Primdahl *et al.*[1] this reaction creates two isomers, methyl (*2E,4E*)- and (*2E,4Z*)-5-bromopenta-2,4-dienoate. Pure (*2E,4E*) isomer is colourless crystals while (*2E,4Z*) is a colourless oil. The product for this reaction gave a colourless oil, but TLC of this colourless oil and pure (*2E,4E*) isomer showed that the product was a mixture of the isomers although there were no crystals in the product.

3. Conclusion and future work

This thesis aimed to synthesize alkamide Avenalumamide AF8. The synthesis was cut short because of time, but some necessary reactions for the synthesis were successful. The focus was to first synthesize the avenalumic acid moiety since it is the biggest unit and then synthesis the anthranilic acid moiety. Therefore, most of the reactions performed were of the synthesis of the avenalumic acid moiety to find the right pathway that would provide the compound with great spectroscopic data and yield.

The synthesis of glutaconaldehyde potassium salt **2** was successful both times it was performed, and great yields were achieved. Glutaconaldehyde potassium salt was then used as a starting material in other reactions. One of these reactions is the synthesis of compound **4**. This synthesis with a Grignard reagent was successful and a pure compound was obtained. This reaction was just a test reaction, so an upscaling of the reaction to achieve a good yield was not necessary.

The reduction of glutaconaldehyde potassium with sodium borohydride to a primary alcohol was unsuccessful. The TBS-protection reaction failed, and this could be the reason for the failed reduction. Other attempts of the whole synthesis were not performed and the diene in compound **3** would have been useful in Diels-Alder reactions planned for the synthesis of Tamiflu. The aim of this master thesis was therefore changed.

The Henry reaction and dehydration reaction performed to synthesize compound **6** was successful in the second attempt. The crystals of compound **6** achieved should have been purified, but it was unnecessary since the nitroalkene was intended to be transformed into compound **7**. Sadly, the transformation failed. If this reaction was successful, it would have been beneficial for the synthesis of fuligopyrone B.

The synthesis of compound **11** took a while to perfect, but the last attempt was successful although it was challenging to achieve good yield. It was then difficult to use the product in the following reaction. Therefore, another pathway was used to get to the same product, and this pathway is the synthesis of compound **15**. Unfortunately, this reaction failed and because the lack of time this synthesis could not be executed again.

The future work in the synthesis of avenalumamide AF8 is firstly synthesizing compound **15**, and then using the compound with an organoboronic acid in a Suzuki coupling to form a methyl ester. In the end, an amide bond between an anthranilic acid and the methyl ester must

be formed to obtain the fully synthesized avenalumamide AF8. If there were more time, some of the reactions performed in this thesis would have been optimized and the synthesis of Avenalumamide AF8 would have been completed.

4. Experimental

4.1 General information

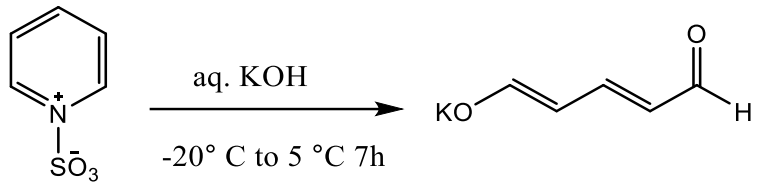
All reagents used, were of great quality. In reactions where anhydrous solvents were required, septum sealed flasks were used, and the solvents were drawn out by syringes.

Thin layer chromatography was performed on TLC silica gel 60 F_{254} plates from Merck. Long-wave UV-light and $KMnO_4$-solution were used for visualization.

NMR spectra were recorded on an instrument called Bruker Ascend 400 at 25˚C. ^{1}H NMR spectra were recorded at 400 MHz while ^{13}C NMR spectra were recorded at 100 MHz and deuterated chloroform ($CDCl_3$) was used as solvent. The reference peaks were calibrated at 7.26 ppm for ^{1}H NMR and 77.16 ppm for ^{13}C NMR. Chemical shifts (δ) are reported in parts per million and coupling constants (*J*) in hertz (Hz).

Purifications were achieved by flash column chromatography with silica gel 60 (40-63µm) in heptane/hexane.

4.2 Synthesis of potassium (1E,3E)-5-oxopenta-1,3-dien-1-olate (**2**)



1 **2**

*Scheme 16: Synthesis of potassium (1E,3E)-5-oxopenta-1,3-dien-1-olate (**2**).*

This synthesis was performed according to Primdahl *et al.*[1]

Potassium hydroxide (71.8g, 1.28 mol, 4.08 equiv.) was disolved in 175 mL water and the solution was put on a cooling bath at -20 ˚C while been stirred. Pyridinium-1-sulfonate (**1**) (ca.50g, 0.314 mol, 1.0 equiv.) was added portion wise over 5 minutes and the mixture were stirred for 1 hour before the cooling bath was removed. The mixture was stirred in room temperature in 4h 15 min until a dark brown colour occurred. The flask with the mixture was cooled down to 0˚C and was filtered through a Büchner funnel. The crude was washed with acetone (4 x 40 ml) and was air dried overnight. The crude was collected in a round flask and activated charcoal (5 g) and methanol (700 ml) were added, and 15 minutes refluxing was executed. The mixture in the round flask was filtered while still hot and the solid material in the funnel was washed with boiling methanol (2 x 200 ml). The solvent in the filtrate was evaluated on a rotavapor and the solid material was washed with acetone (5 x 20 ml) and filtered through a Büchner funnel. The solid in the funnel was collected in a round flask and dried on rotavapor to get an orange glutaconaldehyde potassium salt (**2**).

Yield: 28.7 g (67%)

Data:

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.7 (d, *J* = 9.2 Hz, 2H), 7.05 (t, *J* = 13.04 Hz, 1H), 5.13 (q, *J* = 9.16, 3.88 Hz, 2H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 184.9, 160.4, 106.8

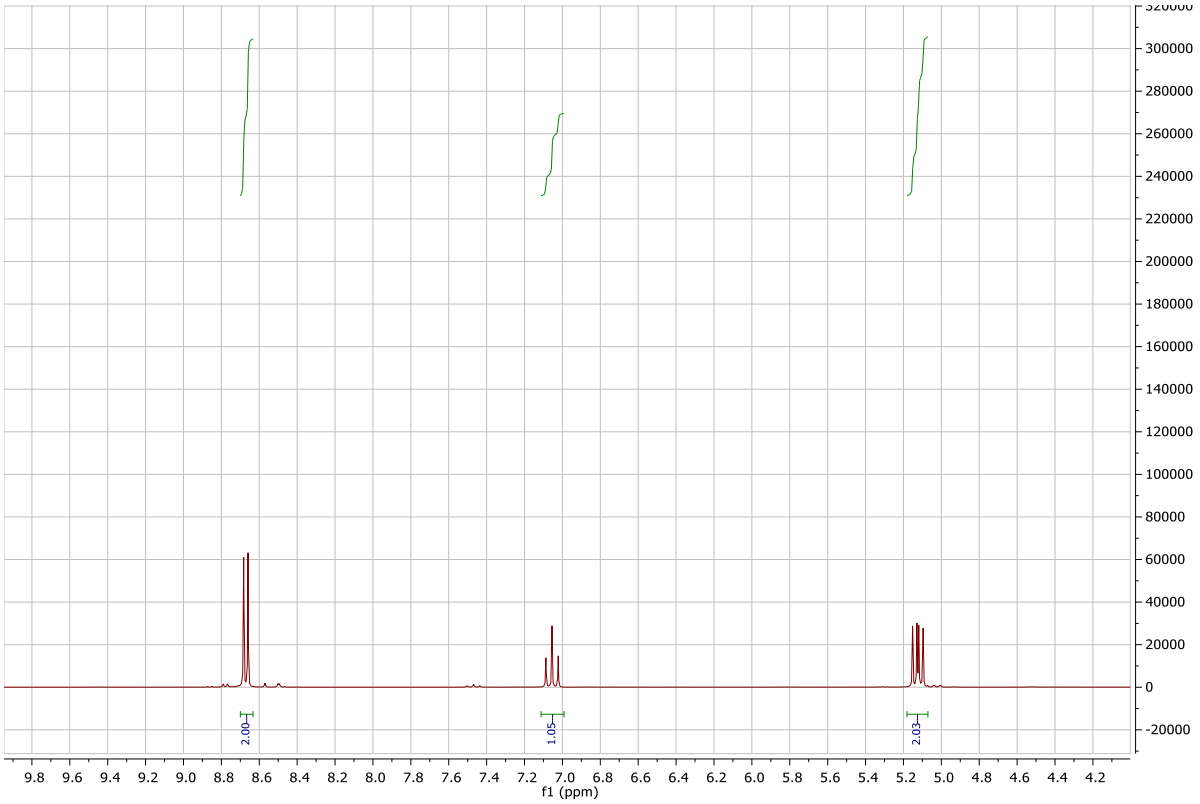


*Figure 11: [1]H NMR spectra of potassium (1E,3E)-5-oxopenta-1,3-dien-1-olate (**2**).*

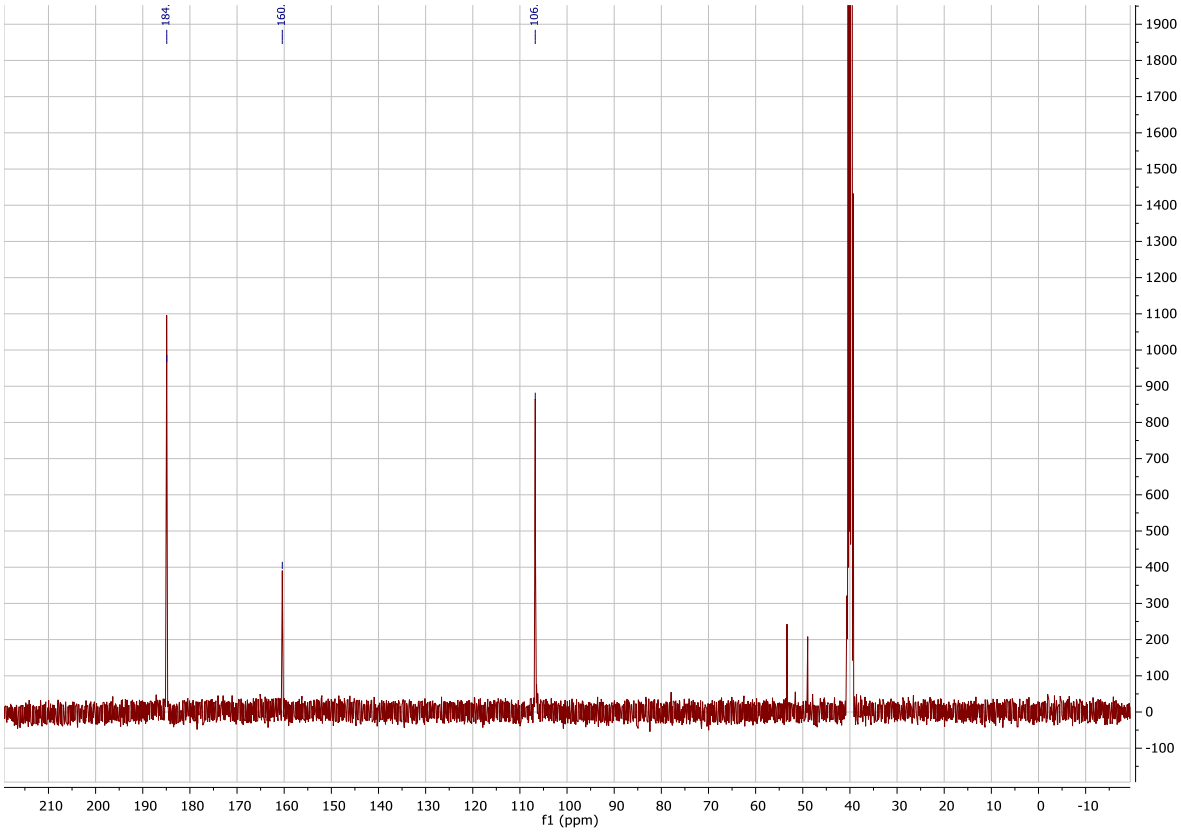
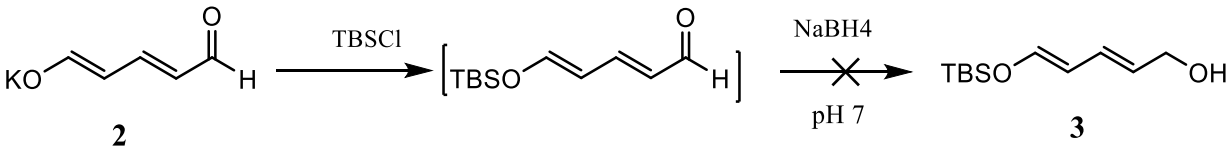


*Figure 12: [13]C NMR spectra of potassium (1E,3E)-5-oxopenta-1,3-dien-1-olate (**2**).*

4.3 Synthesis of (2*E*, 4*E*)-5-(tert-butyldimethylsilyl) oxy) penta-2,4-dien-1-ol (**3**)



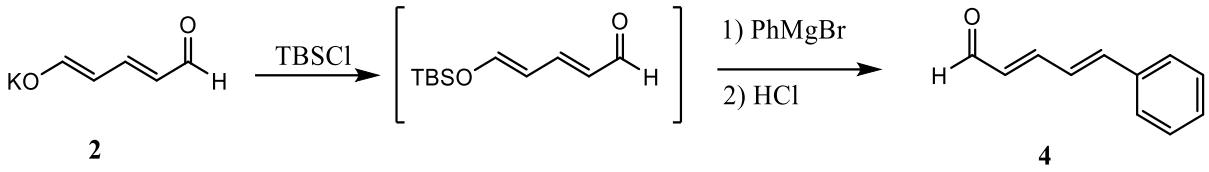
2 **3**

*Scheme 17: Synthesis of tert-butyldimetyl-5-oxopenta-1,3-dien-1-ol (**3**).*

This method was performed after the procedure from Lewis *et al* [39] and Lubineau *et al.*[40]

Glutaconaldehyde potassium salt (**2**) (150 mg, 1.10 mmol, 1.0 equiv.), DMAP (ca.15 mg) and triethylamine (ca.5 drops) were added in a flame-dried flask and was dissolved in THF (3 ml) under nitrogen. The reaction was stirred and TBSCl (166.7 mg, 1.11 mmol, 1.0 equiv.) was added. The mixture was stirred overnight and the following day, the solvent was evaporated. Ethanol (1ml), water (0.80 ml) and phosphate buffer (0.20 ml) were then added, and the flask was put on an ice bath at 0˚C before sodium borohydride (ca.42 mg, 1.12 mmol, 1.02 equiv.) was added dropwise. The mixture was stirred for 1 hour at 0˚C before the reaction was quenched with acetone (0.12 ml). The solution was diluted with water (2 x 60 ml) and was extracted with DCM (3 x 6 ml). The combined organic layer was washed with brine (ca. 6ml), dried with $MgSO_4$ and DCM was evaporated on rotavapor. ^{1}H NMR and ^{13}C NMR were taken to see if the reduction was successful.

4.4 Synthesis of (2*E*, 4*E*)-5-phenylpenta-2,4-dienal (**4**)



2 **4**

*Scheme 18: Synthesis of (2E, 4E)-5-phenylpenta-2,4-dienal (**4**).*

This synthesis was performed according to Lewis *et al.*[39]

Glutaconaldehyde potassium salt (**2**) (150 mg, 1.10 mmol, 1.0 equiv.), DMAP (ca.15 mg) and triethylamine (ca. 5 drops) were added in a flame-dried flask and was dissolved in THF (3 ml) under nitrogen. The reaction was stirred and TBSCl (166.5 mg, 1.11 mmol, 1.0 equiv.) was added. The mixture was stirred for 2 hours. After 2 hours the mixture was cooled down to 0 ˚ C and phenyl magnesium bromide (0.4 ml, 1.20 mmol, 1.09 equiv.) in THF (3 ml) was added. The reaction was allowed to warm itself to room temperature over 2 hours. Diluted hydrochloric acid (1.5 ml, 3M) was then added, and the reaction was stirred overnight. The solution was extracted with diethyl ether (3 x 7,5 ml), and the combined organic layers were washed with brine (3.75 ml), dried with magnesium sulphate and the solvent was evaporated. The crude product was an orange oil. TLC was taken before the oil was purified with 10 % diethyl ether in heptane as eluent. The purified product was a dark orange oil.

Data:

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.6 (d, *J* = 7.92 Hz, 1H), 7.4 (m, 2H), 7.3 (m, 3H), 7.2 (m, 1H), 6.9 (t, *J* = 7.08 Hz, 2H), 6.2 (q, *J* = 7.92, 7.24 Hz, 1H)

^{13}C NMR (100 MHz, CDCl3): δ 193.71, 152.16, 142.57, 135.73, 131.78, 129.83, 129.08, 127.67, 126.33

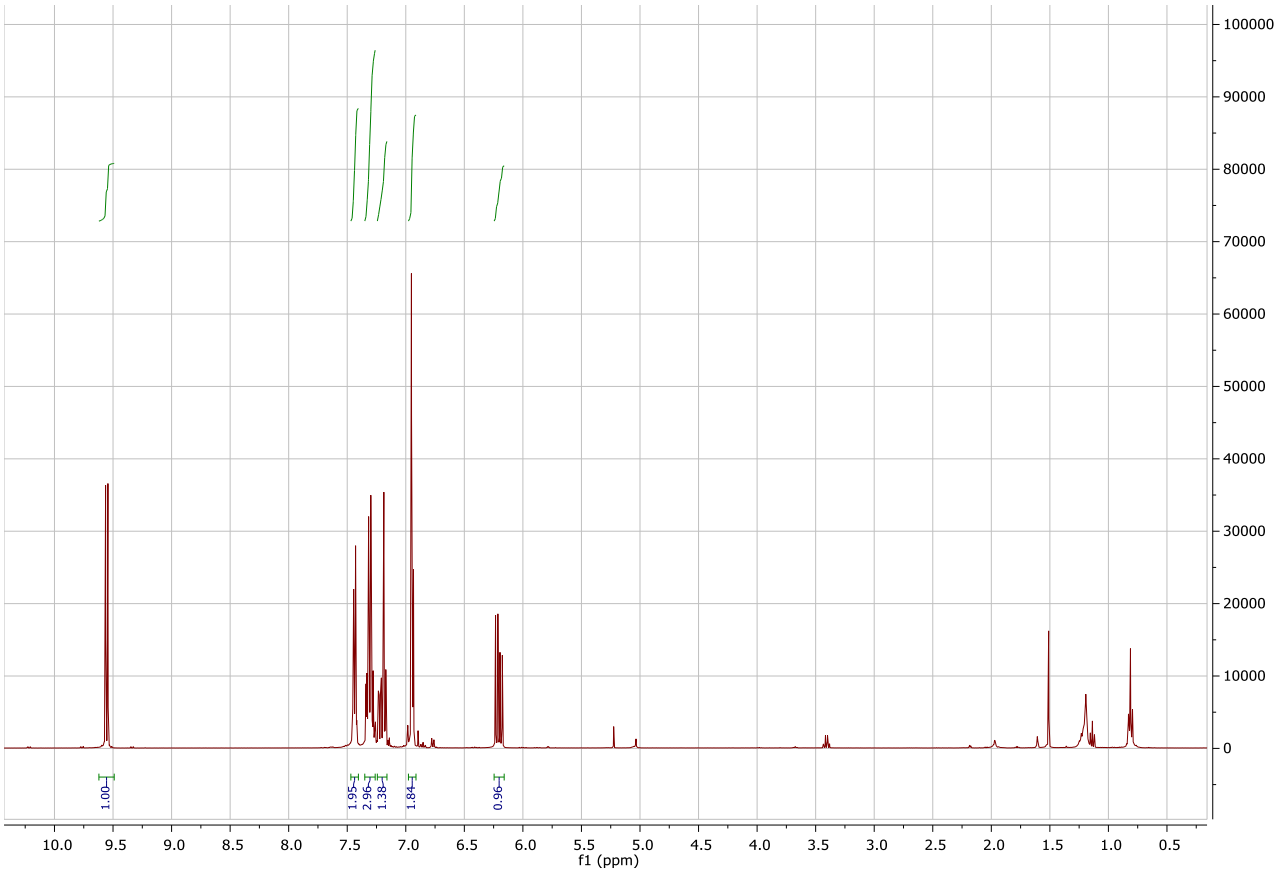


*Figure 13: ^{1}H NMR-spectra of (2E, 4E)-5-phenylpenta-2,4-dienal (**4**).*

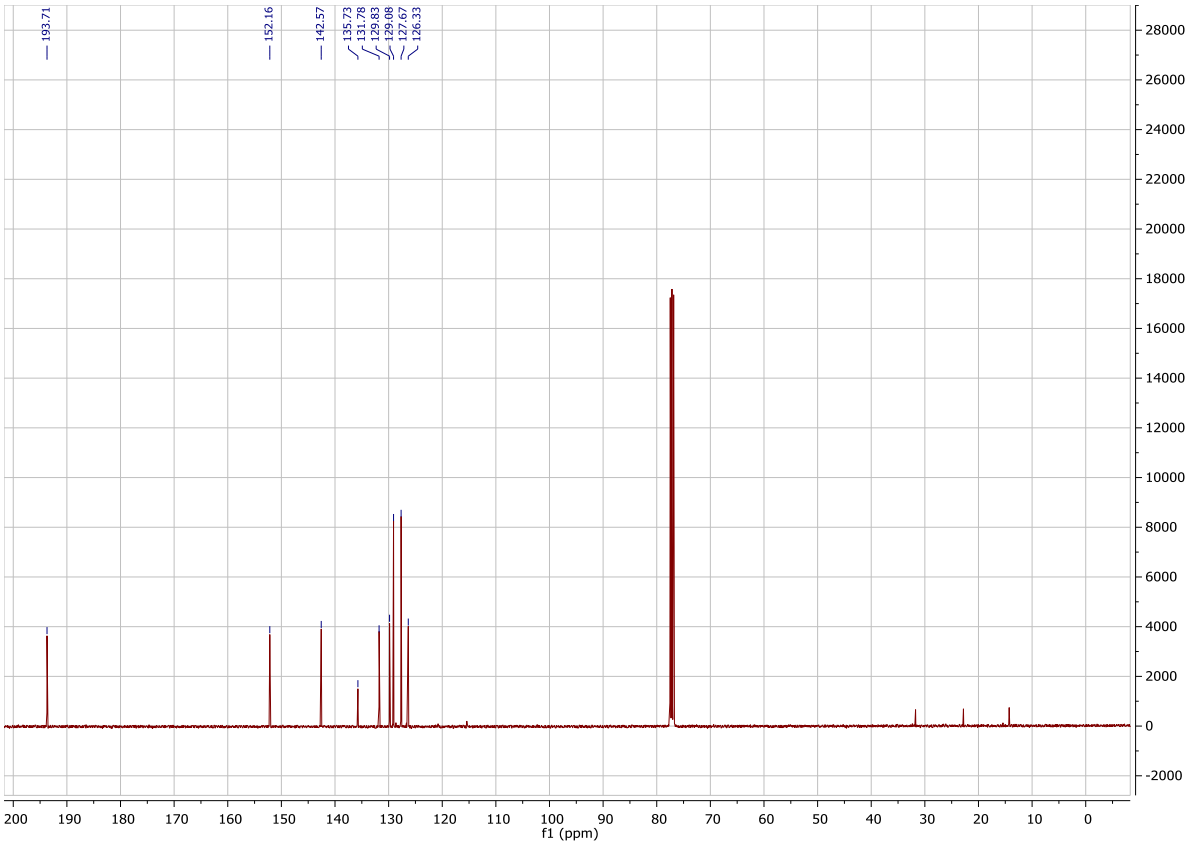


*Figure 14: ^{13}C NMR-spectra of (2E, 4E)-5-phenylpenta-2,4-dienal (**4**).*

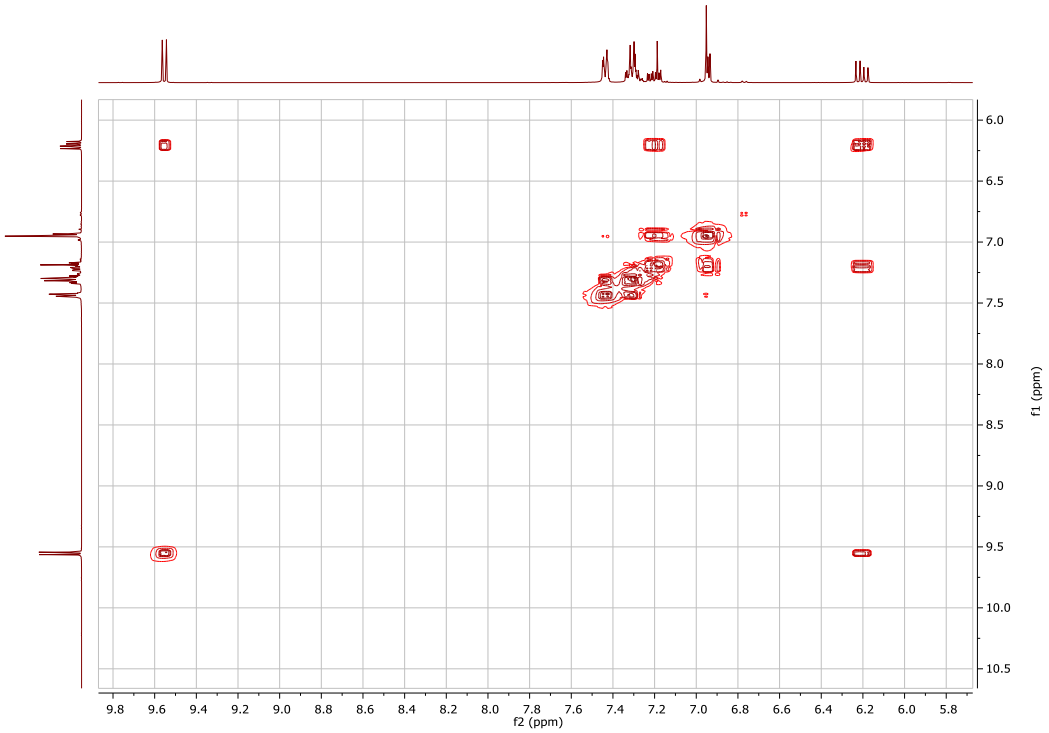
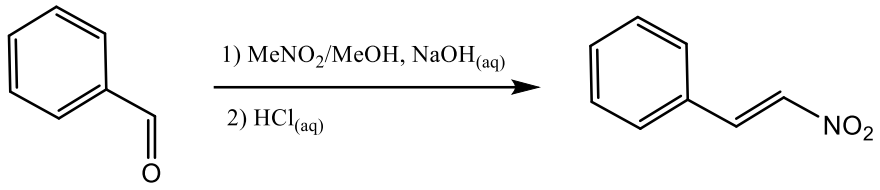


*Figure 15: Cosy spectra of (2E, 4E)-5-phenylpenta-2,4-dienal (**4**).*

4.5 Synthesis of [(*E*)-(2-nitroethenyl)] benzene (**6**)



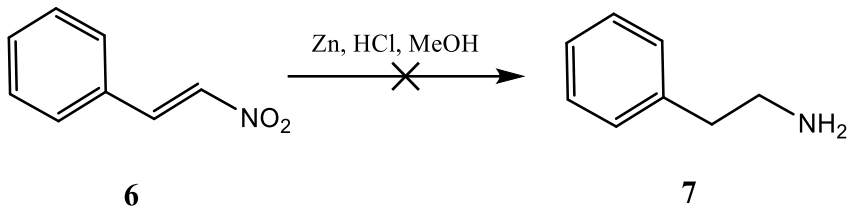
5 **6**

*Scheme 19: Synthesis of [(E)-(2-nitroethenyl)] benzene (**6**).*

This procedure was performed according to Yang *et al*.[41]

Nitromethane (0.54 ml, 10 mmol) was dissolved in methanol (10 ml) and was transferred into a round-bottom flask with benzaldehyde (**5**) (1 ml, 10 mmol, 1.0 equiv.) at 0˚C. A solution of NaOH (0.44 g, 11 mmol, 1.1 equiv. in 2 ml water) was then added dropwise and more methanol (4 ml) was added. The mixture was stirred for 1 hour at 0˚ before water (10 ml) was added. The mixture was then transferred into a new round-bottom flask with dissolved hydrochloric acid (4.7 ml) in water (10 ml) and was stirred for 15 minutes. The mixture was extracted with DCM (3 x 15 ml) and combined organic layers were dried with magnesium sulphate. TLC was taken with 10 % diethyl ether in heptane as eluent. The crude product was a light-yellow solid. The solid material was melted down in a warm bath and heptane was added as a solvent. To phases occurred and a little bit of ethyl acetate was added to achieve one phase. The solution was left in room temperature for some minutes before it was put in a freezer for ca.10 minutes. After ca.10 minutes light yellow crystals occurred and supernatant was removed. The recrystallization was repeated with the supernatant and a small number of light-yellow crystals was observed.

4.6 Synthesis of 2-phenylethan-1-amine (**7**)



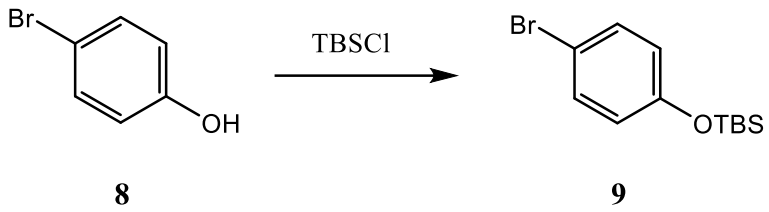
6 **7**

Scheme 20: Synthesis of 2-phenylethan-1-amine (7).

This reaction was performed according to a procedure from Scharf *et al.*[42]

In round-bottom flask with a stir bar, methanol (2.8 ml) was added and cooled down to – 10 ˚C (acetone/ice). Compound **6** (200 mg, 1.34 mmol, 1.0 equiv.), zinc powder (1.5 g, 22.9 mmol, 17.1 equiv.) and concentrated hydrochloric acid (4 ml) were added in small portions over the course of 30 minutes. The mixture was stirred for 15 minutes at -10 ˚C before the mixture was warmed to 0 ˚C and was stirred overnight. The solution was filtered through a filter paper while filtrate was cooled down to 0 ˚C. The filtrate was basified with solid sodium hydroxide and was dried with magnesium sulphate. The solvent was evaporated, and the crude product was a white solid substance. To ^{1}H NMR were taken to identify the white solid substance. In the first ^{1}H NMR, chloroform was used as a solvent and the white solid substance would not dissolve. In the second ^{1}H NMR, acetonitrile was then used as a solvent.

4.7 TBSCl protection on 4-bromophenol (**8**)



8 **9**

*Scheme 21: TBSCl protection on 4-bromophenol (**8**).*

This method was performed according to Kunák *et al.*[45]

TBSCl (156 mg, 1.03 mmol) and imidazole (70 mg, 1.03 mmol) were added into a solution of 4-bromophenol (**8**) (150 mg, 0.867 mmol) in DCM (0.67 ml) at 0 ˚C. The mixture was left to warm itself to room temperature for over 16 hours. TLC was taken with heptane as eluent. The solution was filtered through a pad of silica gel and washed with heptane (3.7 ml). The solvent was evaporated, and the product was a colourless oil.

Data:

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.14-7.12 (dd, *J* = 4.48, 2.2 Hz, 2H), 6.5 (dd, *J* = 4.48, 2.2 Hz 2H), 0.79 (s, 9H), 0.002 (s, 6H)

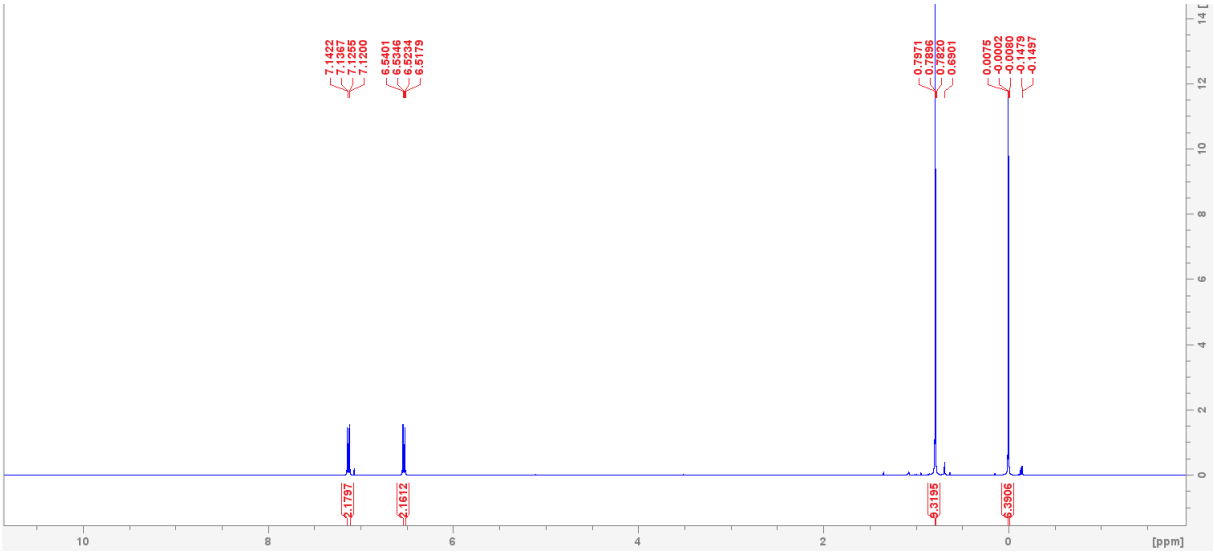
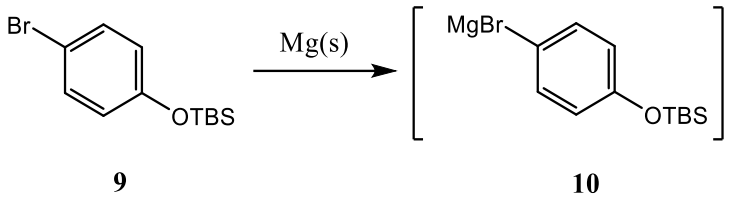


*Figure 16: ^{1}H NMR-spectra of compound (**9**).*

4.8 Synthesis of (2*E*,4*E*)-5-(4-hydroxyphenyl) penta-2,4-dienal (**11**) first attempt

Step 1:

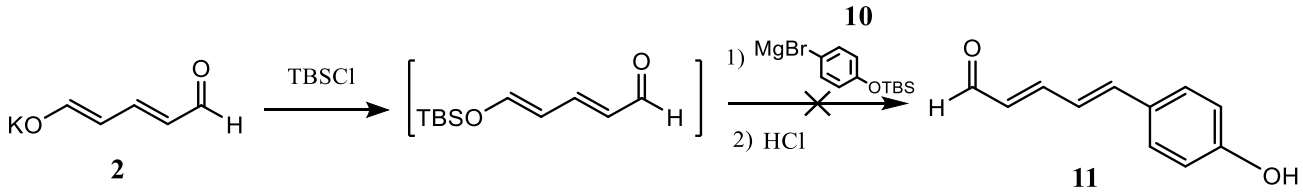


9 **10**

*Scheme 22: Formation of Grignard reagent **10**.*

This method was performed according to procedures from Garlets *et al.*[43]In a flame-dried flask magnesium (93.2 mg, 3.84 mmol), a small crystal of iodine and THF (0.26 ml) were added under nitrogen. Then a solution of compound **9** (660 mg, 2.31 mmol) dissolved in THF (27 ml) was added dropwise and the mixture was stirred while refluxing. After reflux, the mixture was cooled down in room temperature.

Step 2:



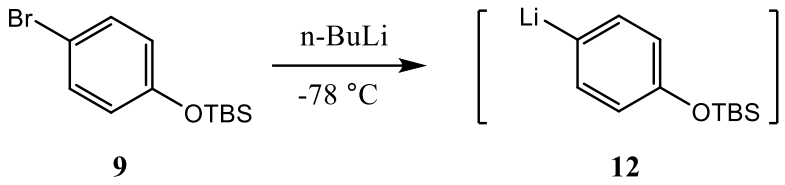
2 **11**

*Scheme 23: First synthesis attempt of (2E,4E)-5-(4-hydroxyphenyl) penta-2,4-dienal (**11**).*

This synthesis was performed according to procedures from Lewis *et al.*[39] Glutaconaldehyde potassium salt (**2**) (150 mg, 1.10 mmol, 1.0 equiv.), DMAP (ca.15 mg) and triethylamine (ca. 5 drops) were added in a flame-dried flask and was dissolved in THF (3 ml) under nitrogen. The reaction was stirred and TBSCl (166.5 mg, 1.11 mmol, 1.0 equiv.) was added. The mixture was stirred for 2 hours before it was cooled down to 0 ˚ C and Grignard reagent **10** was added. The reaction was left to warm itself to room temperature over 2 hours. Diluted hydrochloric acid (3 ml, 3M) was added, and the reaction was stirred overnight. The solution was extracted with EtOAc (3 x 15 ml), and the combined organic layers were washed with brine (7.5 ml), dried with magnesium sulphate and the solvent was evaporated. The crude product was dark brown slurry. TLC was taken with heptane as eluent and ^{1}H NMR was taken.

4.9 Synthesis of (2*E*,4*E*)-5-(4-hydroxyphenyl) penta-2,4-dienal (**11**) second attempt

Step 1:



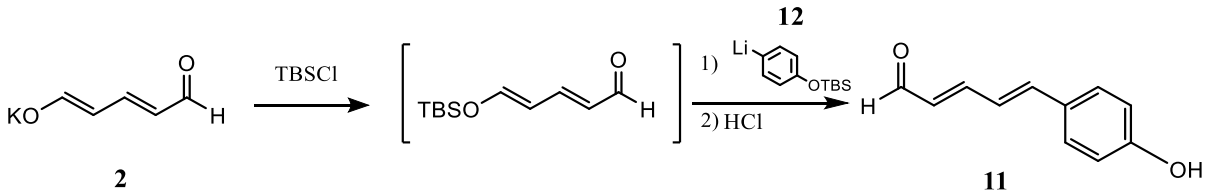
9 **12**

Scheme 24: Replacement of bromine with lithium.

This method is performed according to the procedure from Chakraborty *et al.*[44]

Compound **9** (971.8 mg, 3.36 mmol) was dissolved in THF (33.6 ml) in a flame-dried flask under nitrogen, and the solution was cooled down to -78 ˚C. A solution of n-butyllithium (2.1 ml, 3.36 mmol, 1.6 M in hexanes) was added dropwise and the mixture was stirred for 45 minutes at -78 ˚C.

Step 2:



2 **11**

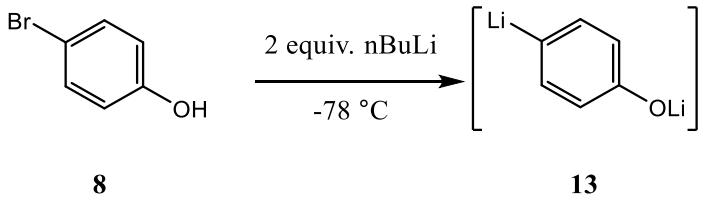
*Scheme 25: Second attempt of synthesis of (2E,4E)-5-(4-hydroxyphenyl) penta-2,4-dienal (**11**).*

This synthesis was performed according to procedures from Lewis *et al.*[39]

Glutaconaldehyde potassium salt (**2**) (150 mg, 1.10 mmol, 1.0 equiv.), DMAP (ca.15 mg) and triethylamine (ca. 5 drops) were added in a flame-dried flask and was dissolved in THF (3 ml) under nitrogen. The reaction was stirred and TBSCl (166.5 mg, 1.11 mmol, 1.0 equiv.) was added. The mixture was stirred for 2 hours before it was cooled down to 0 ˚C and compound **12** was added. The reaction was left to warm itself to room temperature over 2 hours. Diluted hydrochloric acid (3 ml, 3M) was added, and the reaction was stirred overnight. The solution was extracted with diethyl ether (3 x 15 ml), and the combined organic layers were washed with brine (7.5 ml), dried with magnesium sulphate and the solvent was evaporated. The crude product was a dark brown oil. TLC was taken with 10 % diethyl ether in heptane as eluent and ^{1}H NMR was taken.

4.10 Synthesis of (2E,4E)-5-(4-hydroxyphenyl) penta-2,4-dienal (**11**) third attempt

Step 1:



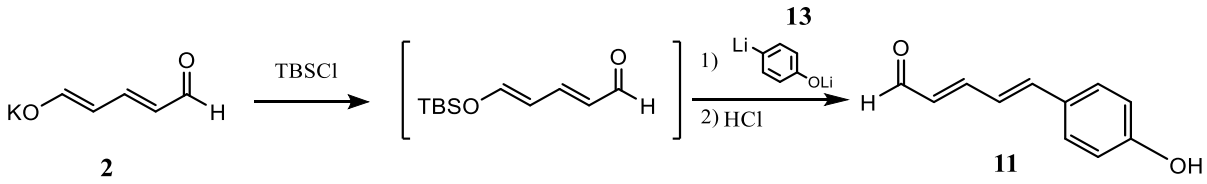
8 **13**

Scheme 26: Replacement of bromine with lithium.

This method is performed according to the procedure from Chakraborty *et al.*[44]

4-bromophenol **8** (406.5 mg, 2.35 mmol, 1.1 equiv.) was dissolved in THF (23.5 ml) in a flame-dried flask under nitrogen, and the solution was cooled down to -78 ˚C. A solution of n-butyllithium (3.23 ml, 5.17 mmol, 2.2 equiv., 1.6 M in hexanes) was added dropwise and the mixture was stirred for 45 minutes at -78 ˚C.

Step 2:



2 **11**

*Scheme 27: Third synthesis attempt of (2E,4E)-5-(4-hydroxyphenyl) penta-2,4-dienal (**11**).*

This synthesis was performed according to procedures from Lewis *et al.*[39]

Potassium glutaconaldehyde (**2**) (150 mg, 1.10 mmol, 1.0 equiv.), DMAP (ca.15 mg) and triethylamine (ca. 5 drops) were added in a flame-dried flask and was dissolved in THF (3 ml) under nitrogen. The reaction was stirred and TBSCl (166.5 mg, 1.11 mmol, 1.0 equiv.) was added. The mixture was stirred for 2 hours before it was cooled down to 0 ˚C and compound **13** was added. The reaction was left to warm itself to room temperature over 2 hours. Diluted hydrochloric acid (3 ml, 3M) was added, and the reaction was stirred for 2 hours. The solution was extracted with diethyl ether (3 x 15 ml), and the combined organic layers were washed

with brine (7.5 ml), dried with magnesium sulphate and the solvent was evaporated. TLC was taken with 30 % EtOAc in heptane as eluent. The crude product was purified by chromatography with 20 % EtOAc in heptane as eluent and pure product was an orange oil. Both ^{1}H and ^{13}C NMR were taken.

Yield: 10.5 mg (5.5%)

Data:

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.6 (d, *J* = 7.97 Hz, 1H), 7.4 (dd, *J* = 4.72, 1.88 Hz, 2H), 6.9 (m, 2H), 6.85 (m, 2H), 6.23 (q, *J* = 8, 7.16 Hz, 1H), 5.0 (s, 1H)

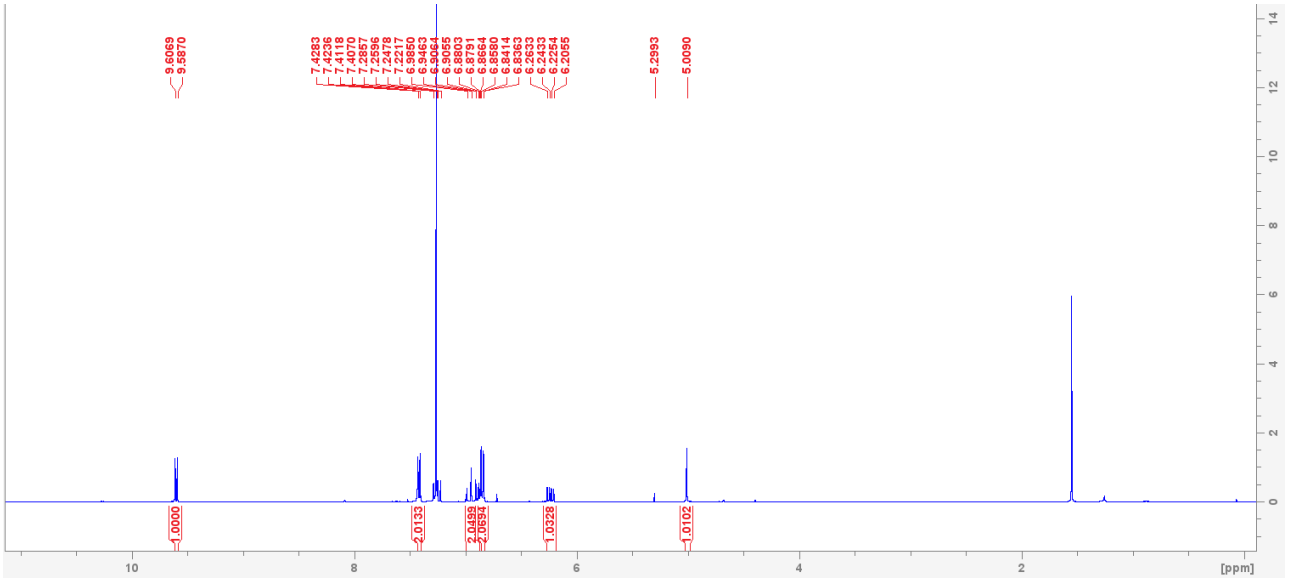
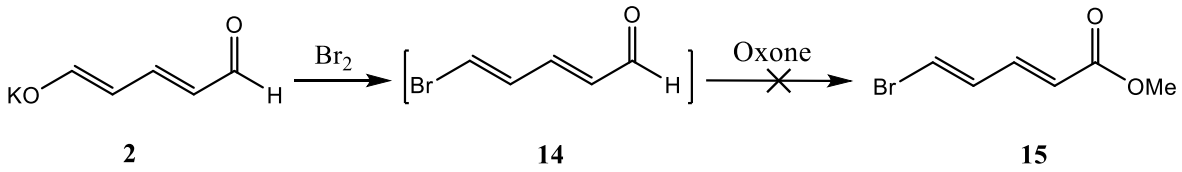


*Figure 17: ^{1}H NMR-spectra of (2E,4E)-5-(4-hydroxyphenyl) penta-2,4-dienal (**11**).*

4.11 Synthesis of methyl (2*E*,4*E*)-5-bromopenta-2,4-dienoate (**15**)



2 **14** **15**

*Scheme 28: Synthesis of methyl (2E,4E)-5-bromopenta-2,4-dienoate (**15**).*

This synthesis was performed according to procedure from Primdahl *et al.*[1]

Triphenylphosphine (11.2 g, 42.6 mmol, 1.35 equiv.) was dissolved in dichloromethane (150 ml) and was cooled down to 0˚C. A solution of bromine (2.10 ml, 41.0 mmol, 1.3 equiv.) in dichloromethane (38 ml) was added dropwise and the reaction was stirred. A red-brown colour appeared after adding the bromine solution and more triphenylphosphine was added until the colour disappeared. Potassium glutaconaldehyde (**2**) (4.3 g, 31.6 mmol, 1 equiv.) was added and the reaction stirred overnight. The mixture was filtered through a pad of silica gel and was washed with dichloromethane. The filtrate was put on rotavapor to evaporate dichloromethane and give compound **14**. Compound **14** was then dissolved in methanol (200 ml) and potassium peroxymonosulfate (9.45 g, 30.7 mmol) was added. The flask was flushed with nitrogen and the reaction was stirred for 24 hours. The flask was concentrated in vacuo to remove almost all methanol until a slurry was attained. Ethyl acetate (50 ml) was added, and the reaction was stirred while an aqueous solution of HCl (1M) was carefully added until all the salt was dissolved. The mixture was extracted with ethyl acetate (5x50 ml), combined organic layers were dried with magnesium sulphate and ethyl acetate was evaporated on rotavapor. The crude was purified with 10 % diethyl ether in heptane as eluent. The fractions were combined, concentrated in vacuo to remove 80 % of the solvent and the flask was put in a freezer (-20˚C).

5. References

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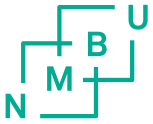

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