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Titel der Masterarbeit

Synthesis and characterization of metal complexes of polyhydroxychalcones

Verfasser

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1.1 Abstract

Chalcones are part of the group of polyphenolic natural products named flavonoids. They have received a lot of attention in recent research due to their promising biological activities such as antiviral, antibacterial, anticancer, and anti-oxidative properties. In this paper a subclass of chalcones, the polyhydroxychalcones, have been synthesized, characterized and further used for metal complexation reactions.

For the synthesis of polyhydroxychalcones different synthetic approaches have been studied. The classical three-step-synthesis with methoxymethyl as protection group and potassium hydroxide as coupling agent was compared to direct synthetic routes in acidic conditions and alternative three-step-synthesis with tBDMS as protection group and LiHMDS as coupling agent. To analyze in detail the structural characteristics of chalcones, X-ray analysis on mono-crystals of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone was conducted.

Metal complexation reactions of the polyhydroxychalcones obtained were performed with transition metals such as copper, cobalt, nickel, zinc and iron. The compounds obtained were characterized via mass spectrometry, infrared spectroscopy, UV/Vis spectroscopy and ^{1}H-NMR, ^{13}C-NMR and HSQC-NMR spectroscopy.

1.2 Kurzfassung

Chalkone gehören zur Naturstoffklasse der Flavonoide, eine Gruppe von Polyphenolen. Chalkone haben in den letzten Jahren große Aufmerksamkeit in der pharmazeutischen Forschung erhalten, da sie unter anderem antibakterielle, antivirale, antikarzinogene sowie antioxidative Eigenschaften aufweisen. In dieser Masterarbeit wurde eine Untergruppe der Chalkone, die Polyhydroxychalkone, synthetisiert, charakterisiert und im Weiteren für Metall-Komplexierungs-Reaktionen verwendet.

Zur Synthese der Polyhydroxychalkone wurden unterschiedliche synthetische Ansätzen untersucht. Zum einen wurde die klassische 3-Stufen-Synthese mit Methoxymethyl Schutzgruppen und Kaliumhydroxid als Kopplungsbase angewandt, zum anderen wurden Alternativsynthesewege wie eine einstufige Direktsynthese im sauren Milieu sowie eine 3-stufige Synthese mit tBDMS als Schutzgruppe und LiHMDS als Kopplungsreagenz untersucht. Zum besseren Verständnis der Strukturmerkmale von Chalkonen wurde eine Kristallstrukturanalyse an Einkristallen von 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalkon durchgeführt.

Die Komplexierungsreaktionen der Polyhydroxychalkone wurden mit Übergangsmetallen wie Kupfer, Kobalt, Nickel, Zink und Eisen durchgeführt. Die erhaltenden Verbindungen wurden

via Massenspektrometrie, Infrarotspektroskopie, UV/Vis-Spektroskopie und ^{1}H-NMR, ^{13}C-NMR und HSQC-NMR Spektroskopie charakterisiert.

2 Theory

2.1 General Attributes of Chalcones

2.1.1 Flavonoids

Chalcones are part of the large group of flavonoidic compounds. Flavonoids are low-weight polyphenolic compounds based on a C_6-C_3-C_6 skeleton in which the C6 subunits are aromatic rings and the C3 unit is a heterocycle containing oxygen. Flavonoids are secondary plant metabolites, which means that they are not directly involved in the plant cells' growth, reproduction or development process. However, flavonoids are crucial to the plants' long-term survival as they are recognized by pollinators due to their color and odor[1]. Moreover, flavonoidic compounds are indirectly involved in cell development[1] and inhibit or kill many plant pathogenic strains of bacteria and fungi[2]. Thus, flavonoids are present in almost all plants, they can be found in fruits, vegetables and tea, for example. They constitute the largest group of naturally occuring polyphenolic with almost 9000 different flavonoids[2]. Flavonoids are often found in glycosylated form in nature, which increases their water solubility. Their ability to inhibit or kill bacterial, fungal or viral organisms as well as their anti-cancer activities have made the group of flavonoidic compounds highly interesting for pharmaceutical research and applications.

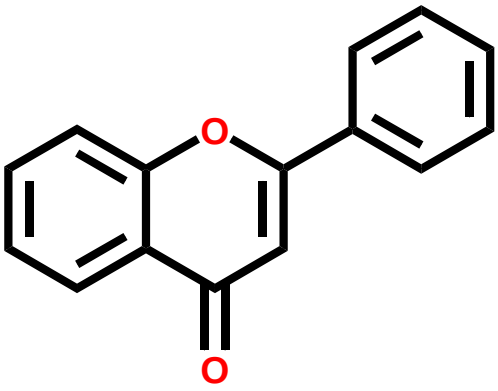


Fig. 1: Structural backbone of flavones

Flavonoids are divided into a high number of diverse subunits. The first group possesses a 2-phenyl-1,4-benzopyrone structure, as for example flavones (fig. 1), flavonols, flavans, and flavanons (fig. 2).

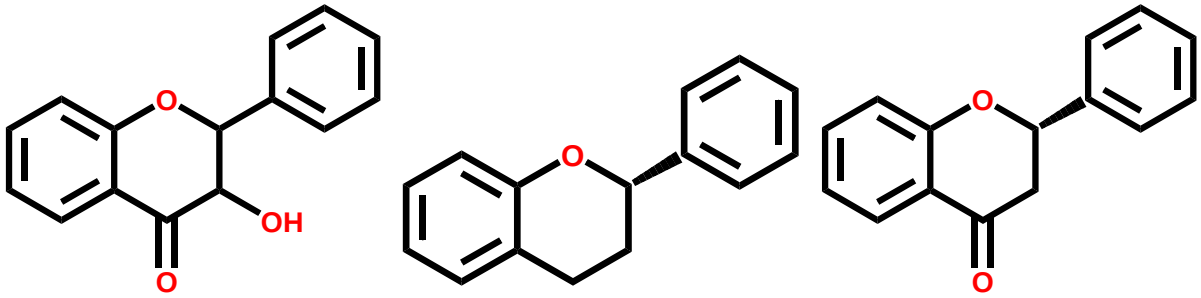


Fig. 2: Structural backbone of flavonols (left), flavans (middle), and flavanons (right)

The second group is named isoflavonoids and possesses a 3-phenyl-1,4-benzopyran structure. Isoflavonoids are little distributed in plants as the enzyme isoflavone synthase is only found in few plants. Isoflavone synthase is necessary for the aryl migration that forms isoflavonoids[3]. The group includes e.g. isoflavones, isoflavans, and rotenoids (fig. 3).

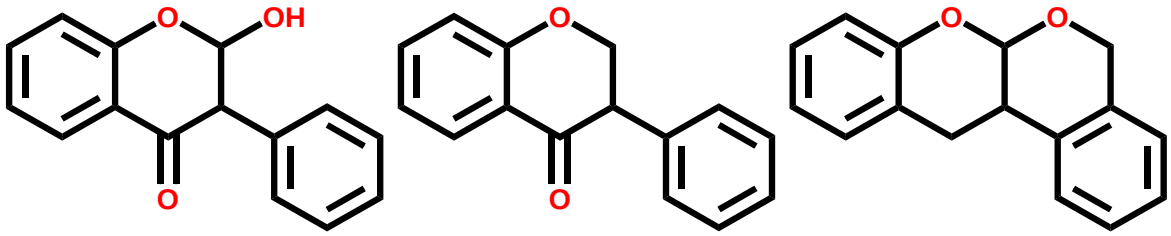


Fig. 3: Structural backbone of isoflavones (left), isoflavans (center), and rotenoids (right)

The third group of flavonoidic compounds is the group of neoflavonoids, their structure is very similar to those of flavonoids and isoflavonoids. They constitute a 4-phenyl-1,2-benzopyrone structure and comprise 4-arylcoumarins, 3,4-dihydroarylcoumarins, and neoflavenes.

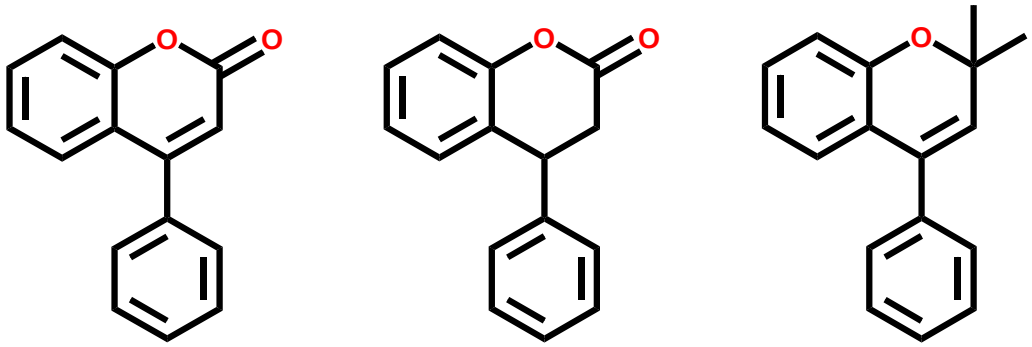


Fig. 4: Structural backbone of 4-arylcoumarins (left), 3,4-dihydroarylcoumarins (center) and neoflavenes (right)

The fourth and last group of flavonoidic compounds is the group of minor flavonoids. This is the group which includes chalcones, especially 2'-hydroxychalcones. Futhermore, chalcone-based natural products like 2'-OH-dihydrochalcones and aurones are comprised in the minor flavonoids group (fig. 5).

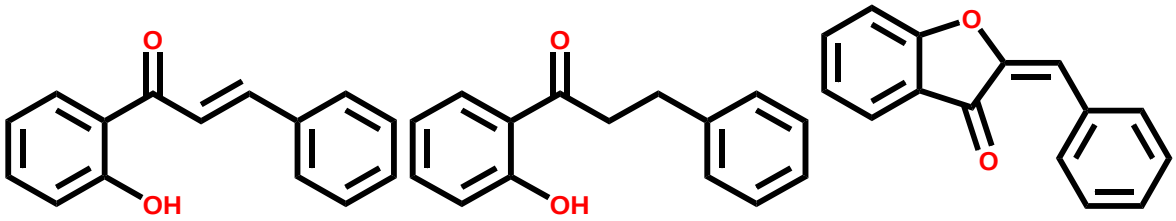


Fig. 5: Structural backbone of 2'-OH-chalcones (left), 2'-OH-dihydrochalcone (center), and aurones (right)

Flavonoids are biosynthesized (fig. 6) via the phenylpropanoid metabolic pathway in which cinnamic acid is used as a product from the shikimic acid metabolic pathway based on phenylalanine (a). This step is catalyzed by the phenylalanine ammonia lyase (PAL) to lead in cinnamic acid. Cinnamic acid is first hydroxylated (c) in para-position by 4-hydroxylase (C4H) and subsequently converted into 4-coumaryl-CoA (d) by 4-coumarate:CoA ligase (4CL). 4-Coumarate-CoA is then converted into the corresponding chalcone (f) by using three units of malonyl CoA. The cyclization and enolization in this process are performed by chalcone synthase (CS). The chalcone is further processed by either aureusidin synthase (AS) to result in the corresponding aurone (here: aureusidin (g)) or by chalcone isomerase (CHI) to result in the corresponding flavones (here: naringenin (h)).

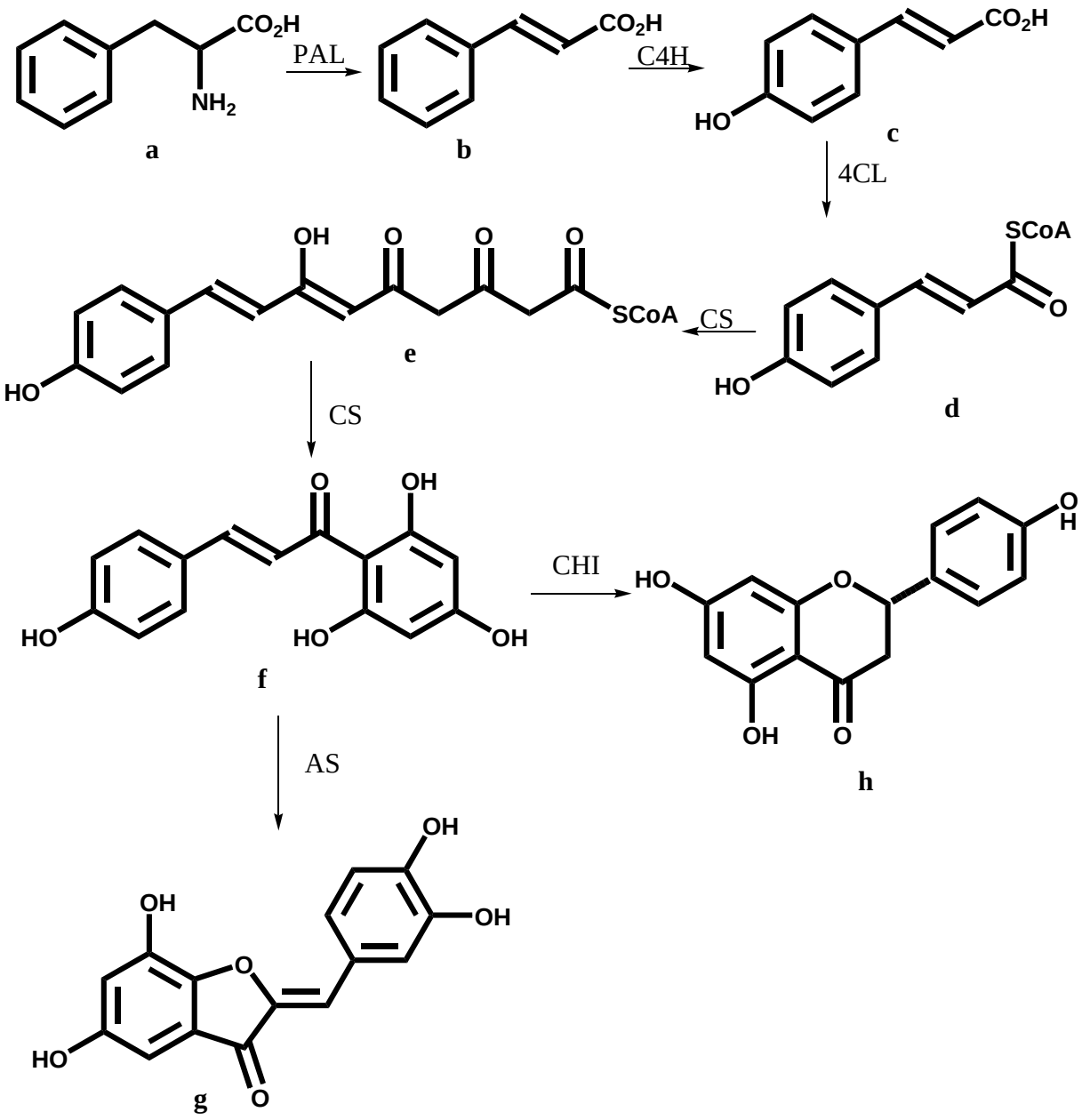


Fig. 6: Biosynthetic pathway of flavonoids

Next, a specific group of minor flavonoids, the chalcones, will be treated. The focus will be on their synthesis, their biochemistry, as well as their biological and pharmaceutical activities.

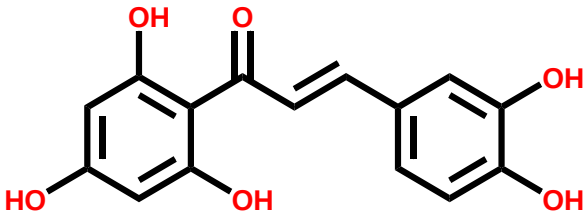
2.1.2 Chalcones

2.1.2.1 Chalcones – General Introduction

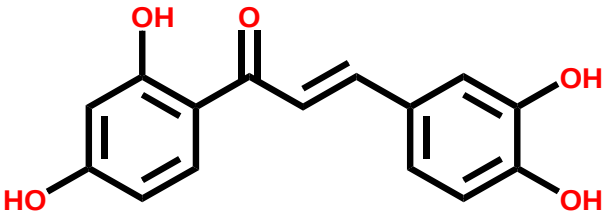
Chalcones are minor flavonoids bearing a 1,3-diphenylprop-2-en-1-one framework. For most chalcones the *trans*-conformation of the double bond is thermodynamically preferred. The conjugated π-electron-system leads to a planar and rigid structure for chalcones[4].

Chalcones occur in high diversity in nature due to diverse substitution patterns on their phenolic systems. In this paper, the focus is on polyhydroxychalcones with at least four

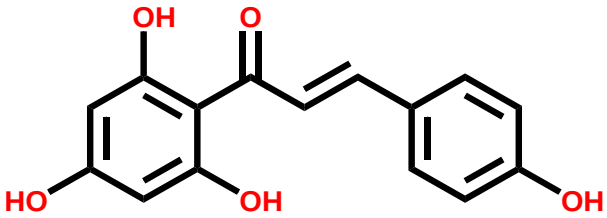
hydroxyl groups in their aromatic substitution patterns. Polyhydroxychalcones like 2',3,4,4',6'-pentahydroxychalcone (PHC), 2',3,4,4'-tetrahydroxychalcone (Butein), and 2',4,4',6'-tetrahydroxychalcone (THC) are substrates of copper type III proteins like catechol oxidases or aureusidin synthase (see chapter 2.1.2.4).



A: 2',3,4,4',6'-pentahydroxychalcone



B: 2',3,4,4'-tetrahydroxychalcone



C: 2',3,4',6'-tetrahydroxychalcone

Fig. 7: Structures of the three polyhydroxychalcones discussed in this paper

To obtain these compounds different synthetic approaches are possible.

2.1.2.2 Synthesis of chalcones

Classically, chalcones are synthesized by a Claisen-Schmidt-Condensation of an acetophenone with a corresponding benzaldehyde. This reaction is generally performed in an alkaline alcoholic solution (fig. 8). However, Claisen-Schmidt-Condensations can be performed under acidic reaction conditions as well. Nevertheless, the yields thus obtained are usually lower than those under alkaline conditions[5].

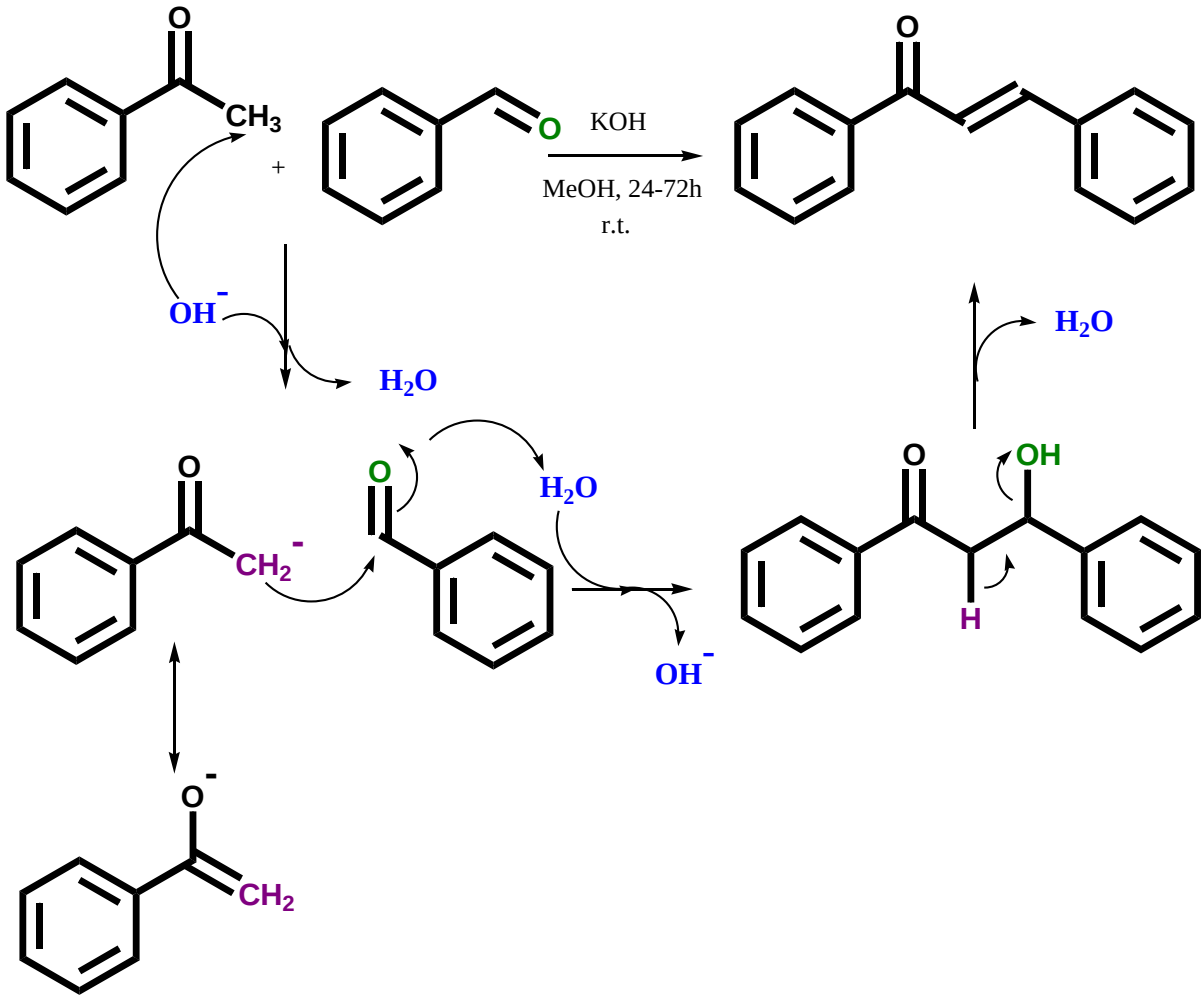


Fig. 8: Reaction mechanism of Claisen-Schmidt-Condensation of an acetophenone with a benzaldehyde in alkaline reaction conditions

However, the approach of a direct one-step reaction in alkaline solution is not possible for polyhydroxychalcones. This is due to the lower reactivity of the benzaldehyde moiety because of delocalization of the anion (fig. 9)[6]. Thus, hydroxyl protecting groups have to be introduced to perform the Claisen-Schmidt-Condensation under basic conditions.

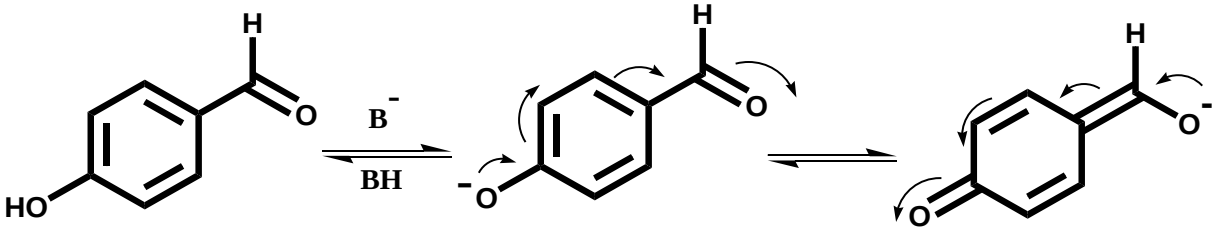


Fig. 9: Anion stabilization of p-hydroxy-benzaldehyde under basic conditions

The hydroxyl protecting groups have to satisfy some general conditions attributed to protection groups[7]:

(1) Easily and efficiently introduced with high specificity for the functional group
(2) The protection group should be cheap or readily available
(3) The protection group should be easily characterized and avoid complications such as the creation of new stereogenic centres
(4) It should be stable in column chromatography
(5) Stable under reaction conditions
(6) Selective cleavage under mild conditions
(7) The by-products of the deprotection should be easily separated from the substrate

The preferred protection group in polyhydroxychalcone synthesis is the methoxymethylether (MOM)[8], as it is stable under reaction conditions while being more easily cleaved than the methoxyether. However, neither the introduction nor the cleavage of the methoxymethyl group occur in high yields. In the literature, the yields for the condensation step vary between 74%[9] and 88%[6] (see fig. 10) for the 2',3,4,4',6'-pentahydroxychalcone and thus, the protected chalcone is obtained in satisfying yields. The reaction scheme with the yields proposed by literature[6], [7] for the 2',3,4,4',6'-pentahydroxychalcone, is shown in figure 10.

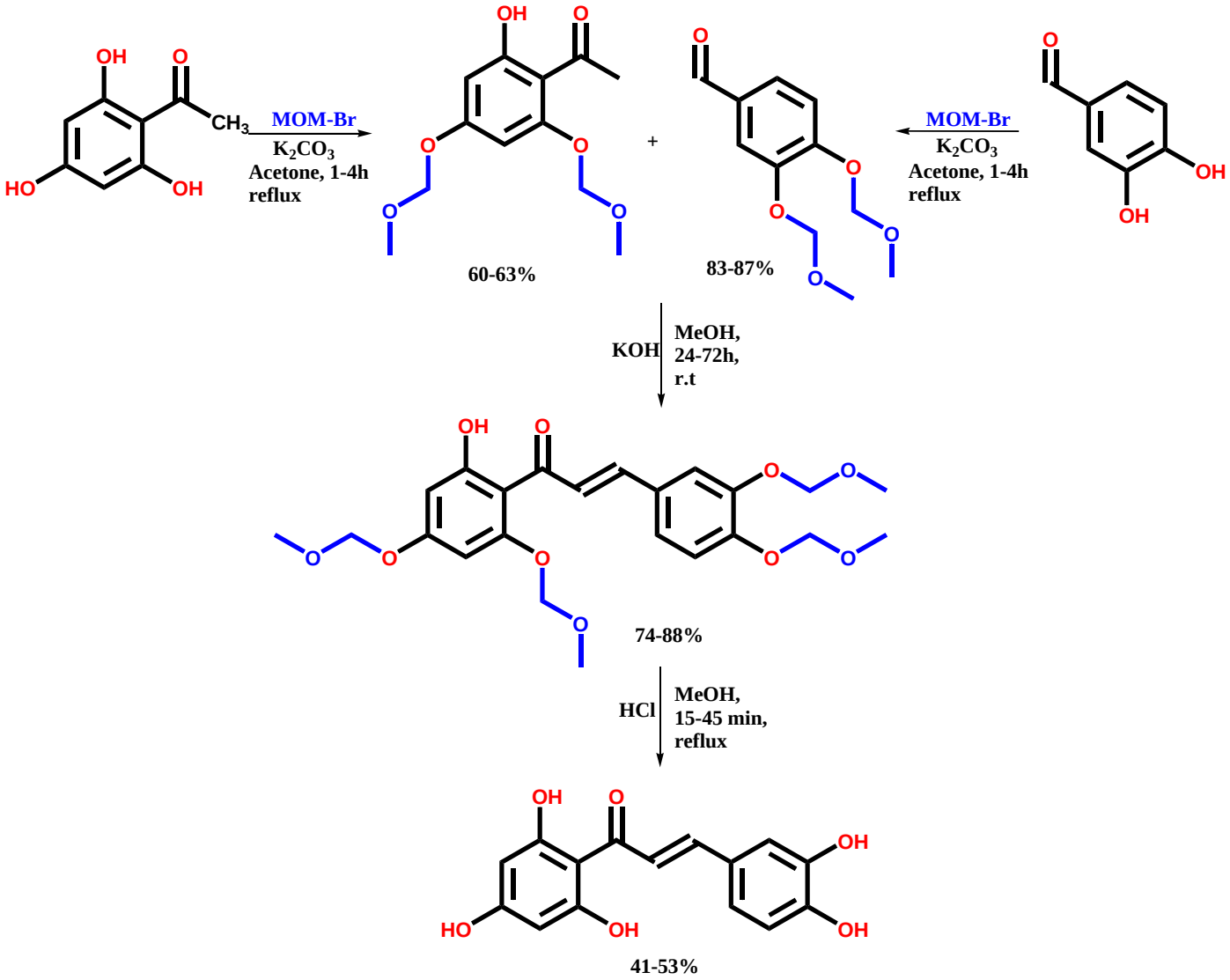


Fig. 10: Reaction scheme for the synthesis of 2',3,4,4',6'-pentahydroxychalcone with methoxymethylether protecting groups, yields from lit. [6], [7]

Interestingly, the 2'OH-group of the acetophenone is not protected because of the orthostabilization (Fig. 11) of the proton with the carbonyl moiety through a hydrogen-bridge-bond. This observation is supported by the highest pKa value for the 2'OH-group of all possible hydroxylation positions in the substitution pattern of the acetophenone. With a pKa value of 10.1 [4] the 2'OH-group is only slightly more acidic than HCO_3^- with a pKa of 10.33, which is the acidic equivalent of the CO_3^{2-} anions used as the base in the protection step[10].

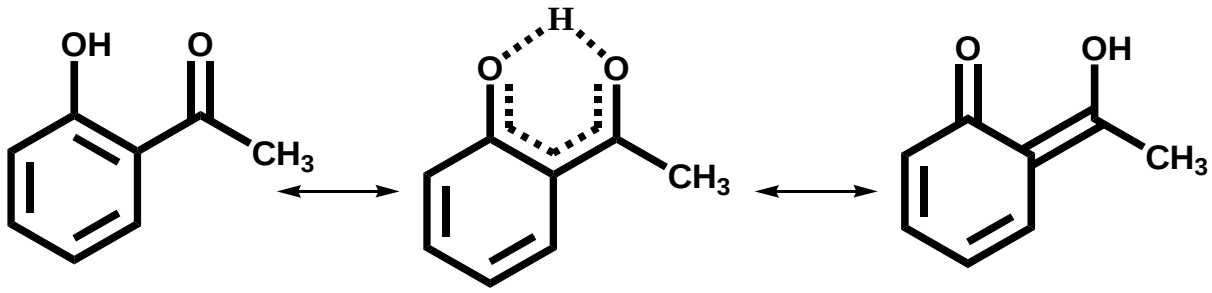


Fig. 11: Orthostabilization of 2'OH-group, the right structure is strongly disadvantageous

As can be clearly seen in figure 10, the yields of the standardized synthesis of polyhydroxychalcones are rather low, ranging in total between 15,11% - 25,56% for 2',3,4,4',6'-pentahydroxychalcone. Especially, the protection of the acetophenone and the cleavage of the methoxymethylether offer only low yields. Hence, an adaptation of the synthesis in terms of an introduction of different protecting groups or other catalytic approaches is highly recommended.

Numerous synthetic approaches for chalcones have already been proposed in literature. Acidic catalysts for the Claisen-Schmidt-condensation like $SOCl_2$/EtOH[11], dry HCl and lewis-acids like BF_3*EtOH[12] have been used in chalcone synthesis. Other catalysts that have been introduced are iodine[13], $RuCl_3$[14], calcined $NaNO_3$/natural phosphates[15], LiHMDS[16] and many others.

However, most of these approaches are not useful for the synthesis of polyhydroxychalcones as they require methoxymethyl as protecting group of the hydroxyl groups. A reaction of special interest in this context is the synthesis of chalcones with lithium bis(trimethylsilyl)amide (fig. 12 and 13) as catalyst because it allows to use more labile hydroxyl protecting groups like tBDMS (tert-butyldimethylsilyl).

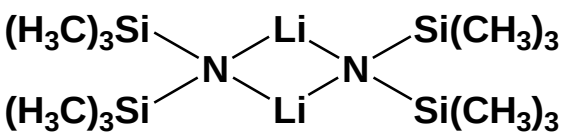


Fig. 12: Lithium bis(trimethylsilyl)amide

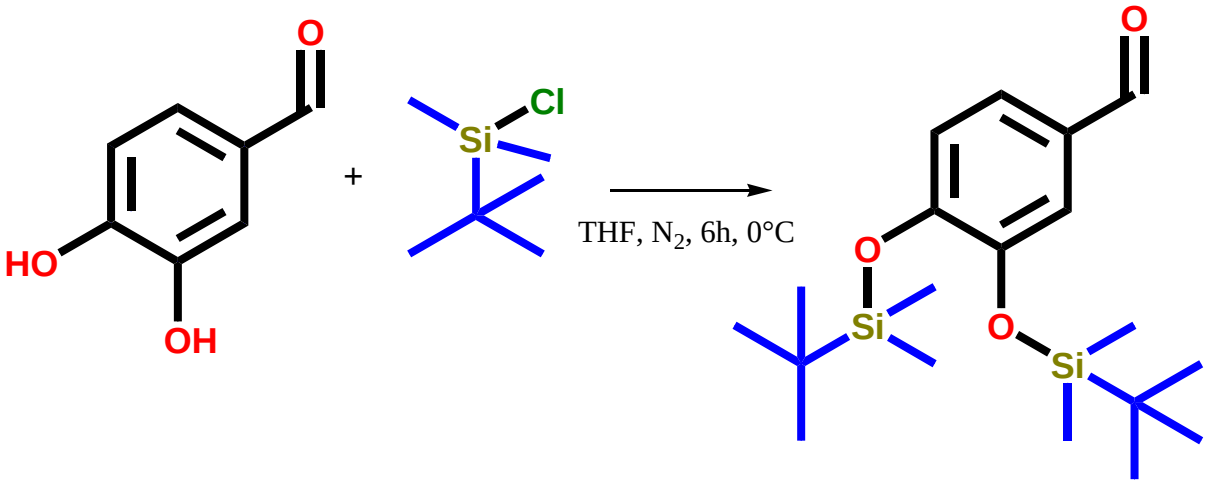


Fig. 13: Protection of 3,4-Dihydroxybenzaldehyde with tert-butyl-dimethylsilyl-chloride

Establishing an efficient one-step-synthesis for polyhydroxychalcones would overcome the difficulties that are caused by the introduction and cleavage of hydroxyl protecting groups. Possible approaches would be specific C-C-bond formations via coupling reactions like Suzuki[17]- or Heck-Coupling[18] (fig. 14).

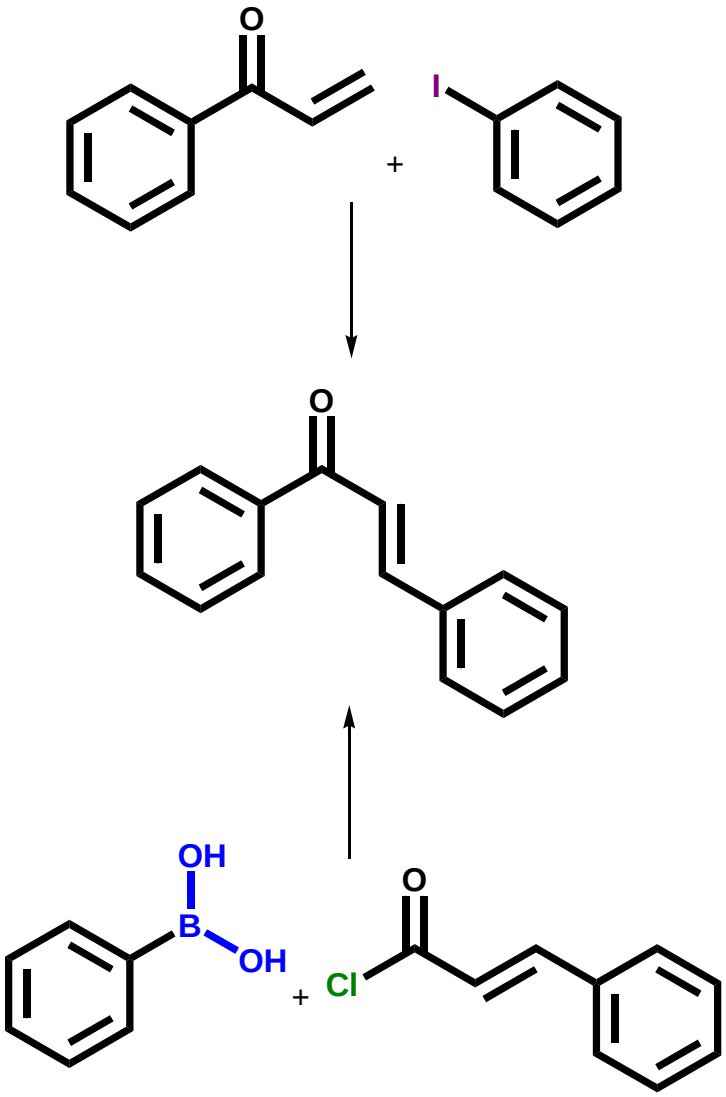


Fig. 14: Heck reaction (top) and Suzuki coupling (bottom)

However, these reactions require precursor reactions to obtain the starting materials. Moreover, neither Heck-coupling nor Suzuki-coupling has been used for direct polyhydroxychalcone synthesis yet. It may be that the reaction conditions necessitate the introduction of protection groups, making these approaches less advantageous.

2.1.2.3 Pharmaceutical potential of chalcones

The efficient synthesis of a high variety of chalcones has received a lot of attention during the past 15 years. The main reason for this high interest in chalcones is their highly promising pharmaceutical potential. Chalcones possess antioxidant[19], anticancer[20], antibacterial[21], antileishmanial[22], anti-infective[23] and anti-inflammatory[23] activities. Recently, a great number of papers dealing with structure-activity relationships (SAR) on the different biological applications have been published. A short review of the results follows.

2.1.2.3.1 Antioxidant activity

Free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are supposed to be responsible for several pathological conditions like aging and inflammation[9]. Polyphenolic compounds are well known to possess antioxidant reactivity due to their conjugated π-electron system. A key structural attribute for a high radical scavenging potential of a chalcone compound is the hydroxyl group substitution pattern[9]. A 3,4-dihydroxy structure on the B-ring can be oxidized into the ortho-catechol structure. Such a possible pathway for radical scavenging has been described e.g. by Nishida et. al. (figure 15).

As can be seen in figure 15, a 3,4-dihydroxy chalcone is first oxidized into a catechol structure on ring B and then further processed into the corresponding aurone (a) or flavone (b) with the B-ring being an ortho-catechol structure. The antioxidant activity of flavonoids in general may be one major reason for their health beneficial characteristics.

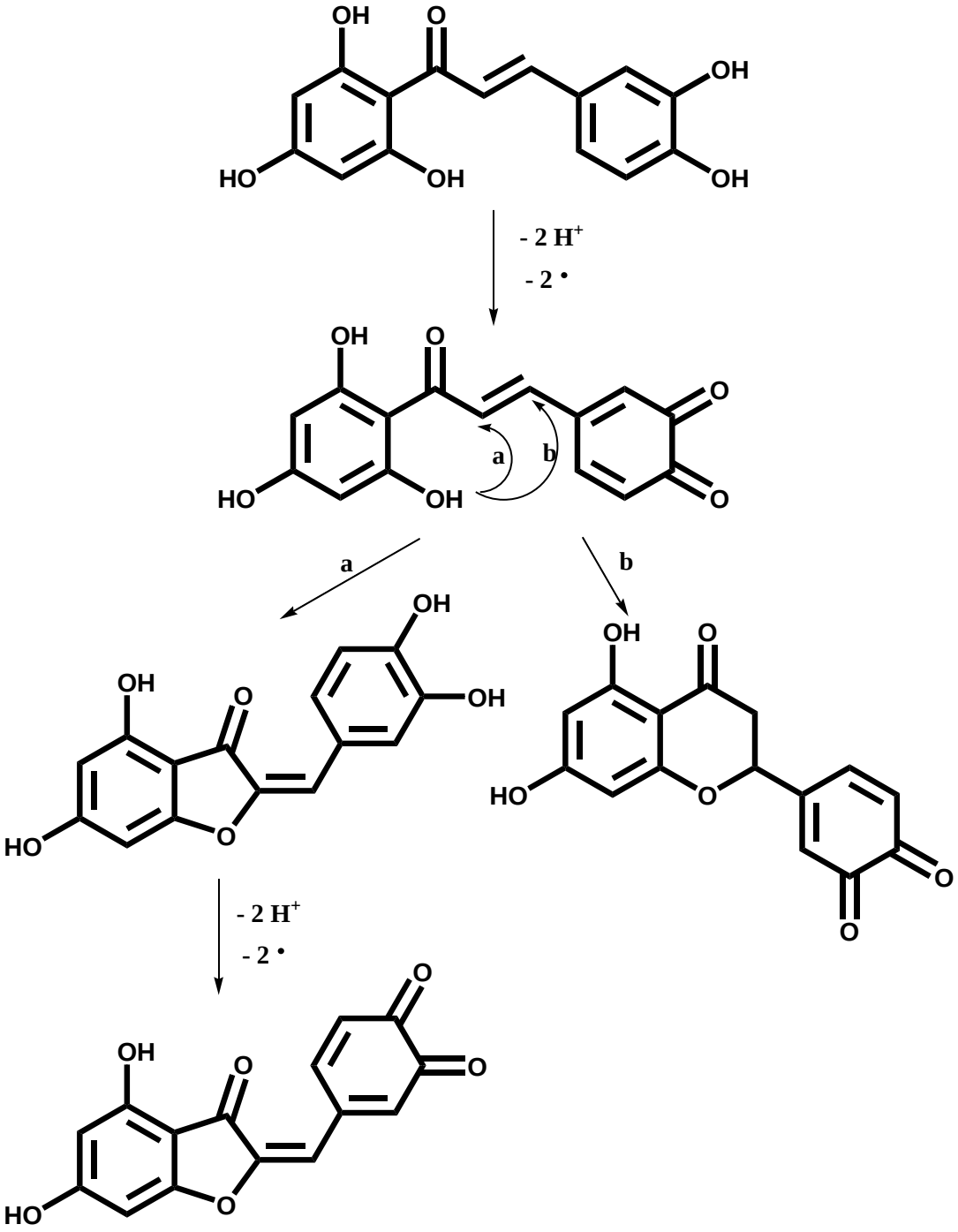


Fig. 15: Oxidation Mechanism for 2‘,3,4,4‘,6‘-Pentahydroxychalcone after Nishida et. al. [24]

2.1.2.3.2 Anticancer Activity

Interfering in tubulin assembly is a suitable target for anticancer compounds as there are already several clinically successful anticancer drugs acting as antimitotics like Paclitaxel (Taxol®, Bristol-Myers Squibb), Vinorelbine (Navelbine®, Pierre Fabre) or Combretastatin A4 (CA4; CA4P Zybrestat®, Oxigene) (figure 16). Chalcones have been found to interact in the same manner as Combretastin A4[24] by interacting with the colchicines-binding site at the interface of the αβ-dimer of tubulin protein[25].

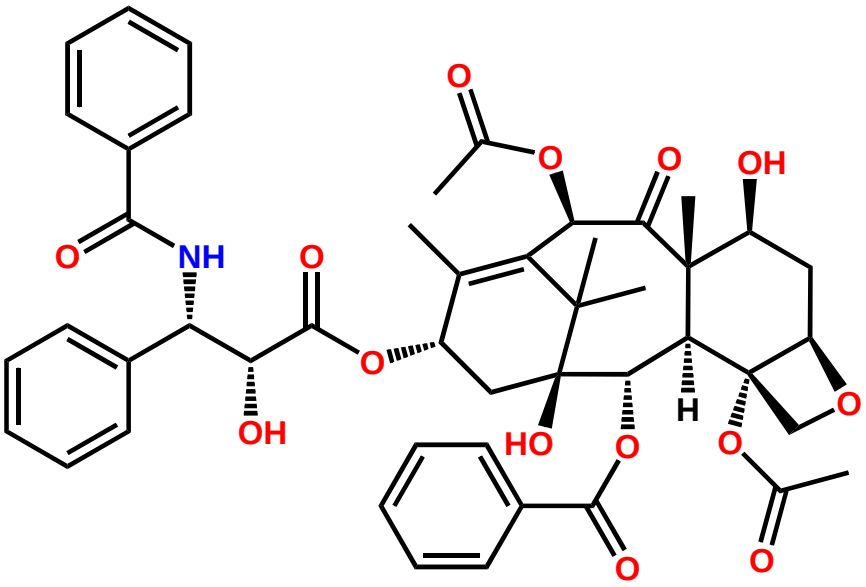


Fig. 16 a: Paclitaxel

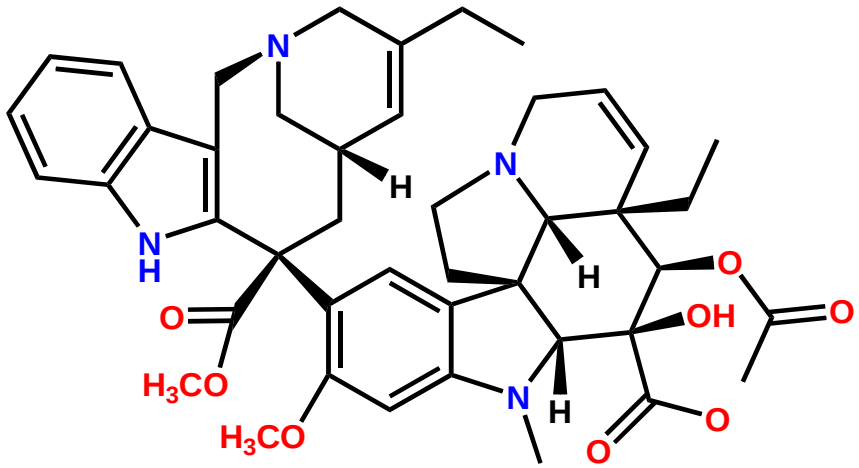


Fig. 16 b: Vinorelbine

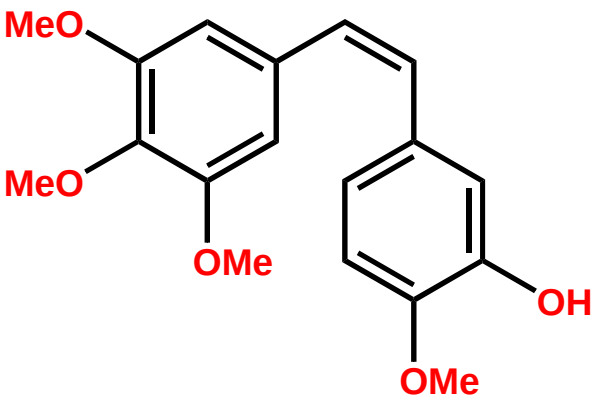


Fig. 16 c: Combretastin A4

The structural analogy of Combretastin A4 to chalcones can be seen in figure 16. Another important advantage of colchicines-binding site binding molecules compared to other

antimitotic molecules is their lower structural complexity. Thus, these anticancer compounds can be obtained in higher amounts at lower costs.

An important structural requirement for antimitotic chalcones is the methoxy-substitution pattern on ring A. Boumendjel et. al.[26] observed high anticancer activity for a 2,2',4,6,6'-pentamethoxy-chalcone (fig. 17). Furthermore, this compound showed a very low toxicity on healthy animals (mice). This demonstrates the high potential of chalcones as future anticancer drugs.

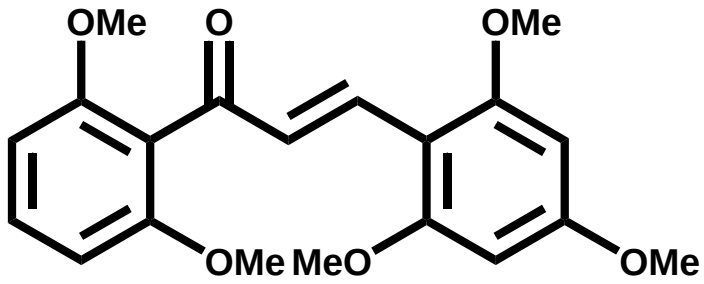


Fig. 17: 2,2',4,6,6'-pentamethoxy-chalcone

2.1.2.3.3 Antimicrobial Activity

As first potent chalcone, Licochalcone A was found to show high activity against gram positive bacteria as *Bacillus coagulans*, *B. subtilis* and *Bacillus stearothermophilus*[27]. Recently, structure activity relationships for a wide range of various antibacterial chalcones have been established. Thus, it was demonstrated that the positions of the phenolic hydroxyl groups and the isoprenyl side chains in the substitution pattern is crucial for their antibacterial activity[21]. Furthermore it was shown that the bioisosteric replacement of the 4'-hydroxy group by a carboxylic group leads to an improvement in solubility and a lower cytotoxicity against mammalian cells[5]. More complex chalcone-based compounds as for example thiazole-based chalcones[28], chalcones with basic functionalities with a high solubility and strong specific bacterial membrane binding[29], chalcone-oxazolidinone hybrids[30], and chalcone-based 1,3,5-triphenyl-2-pyrazoline derivatives[31] show promising results as anti-bacterial compounds.

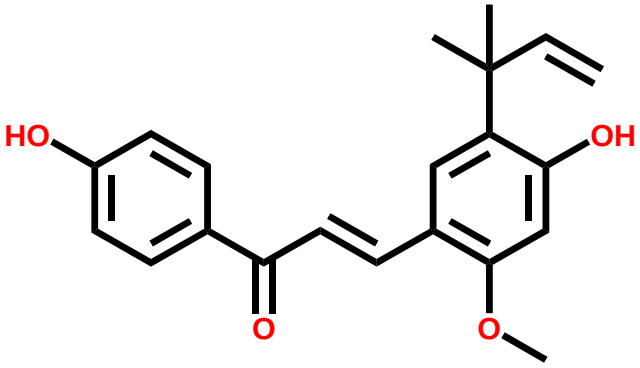


Fig. 18: Licochalcone A

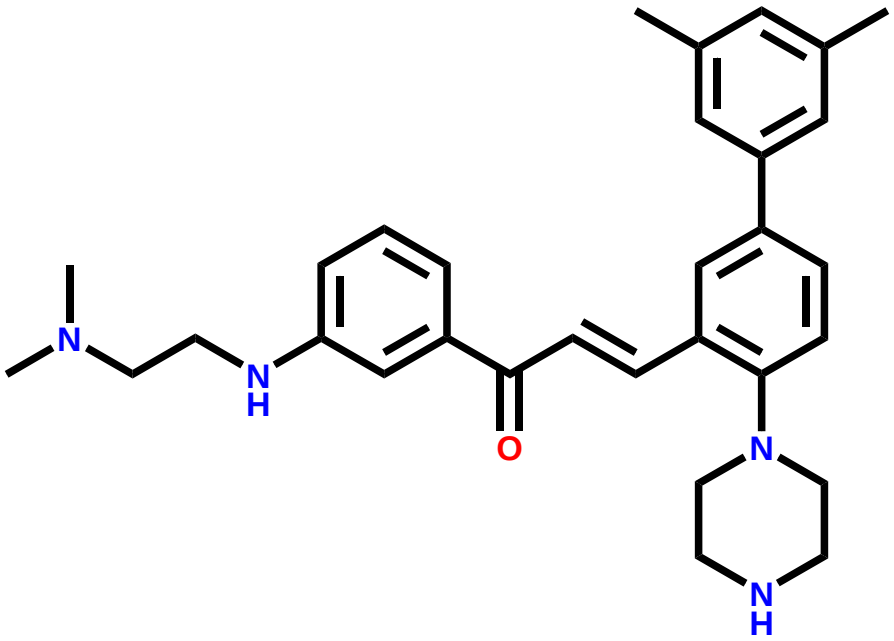


Fig. 19: Chalcone with aliphatic amine in Ring A and B by Nielsen et. al.

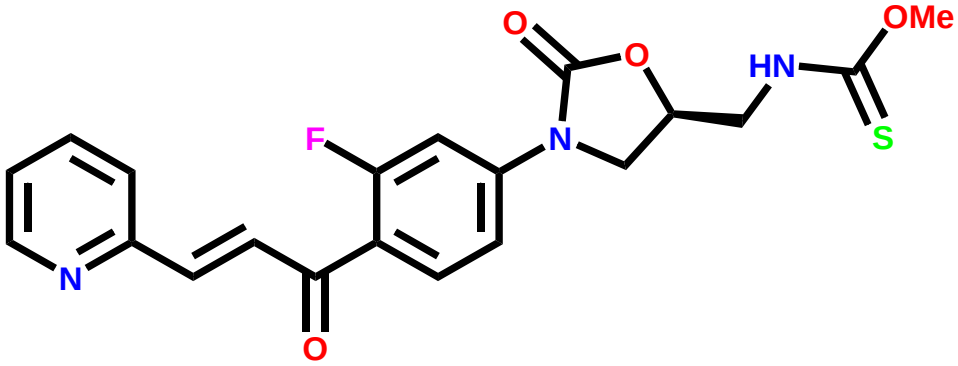


Fig. 20: Chalcone oxazolidinone hybrid by Selvakumar et. al.

Apart from their antibacterial properties, chalcones have been shown to possess antimalarial, antileishmanial, antifungal and anti-inflammatory attributes.

There are only few classes of compounds that are associated with such a diverse range of pharmaceutical application fields. However, the recent discovery that metal complexes of flavonoids and similar compounds possess an even stronger impact on biological systems than the lone ligand[32] raises the question if chalcone metal complexes could exhibit an even broader range of pharmaceutical properties than chalcones alone.

2.1.2.4 Biochemistry of polyhydroxychalcones

Chalcones are the biochemical precursors of aurones, which are responsible for the yellow color in several flowers such as snapdragon, cosmos and coreopsis. The enzyme which catalyzes the transformation of chalcones into aurones is the aureusidin synthase. Aureusidin synthase is a polyphenol oxidase homolog with the ability to form aureusidin from

2',4,4',6'-tetrahydroxychalcone (THC) and 2',3,4,4',6'-pentahydroxychalcone (PHC) as well as to form bracteatin from 2',3,4,4',6'-pentahydroxychalcone (fig. 21)[33].

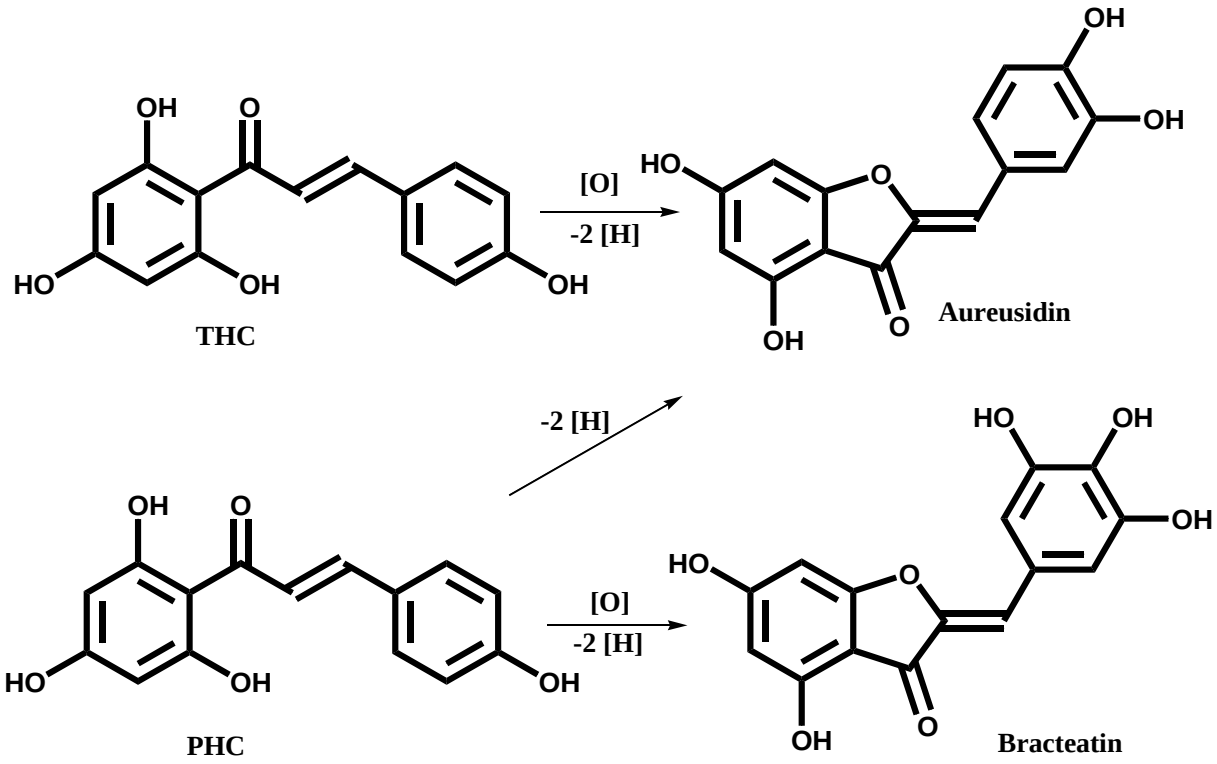


Fig. 21: Transformation of polyhydroxychalcones into aurones catalyzed via aureusidin synthase

The reaction starts with the oxidation of the B ring system and is then followed by the formation of the five-membered ring. The last step is the re-installation of the aromatic system in the B ring (fig. 22). While the first step is supposed to be catalyzed by aureusidin synthase, the second and third step may take place non-enzymatically.[34]

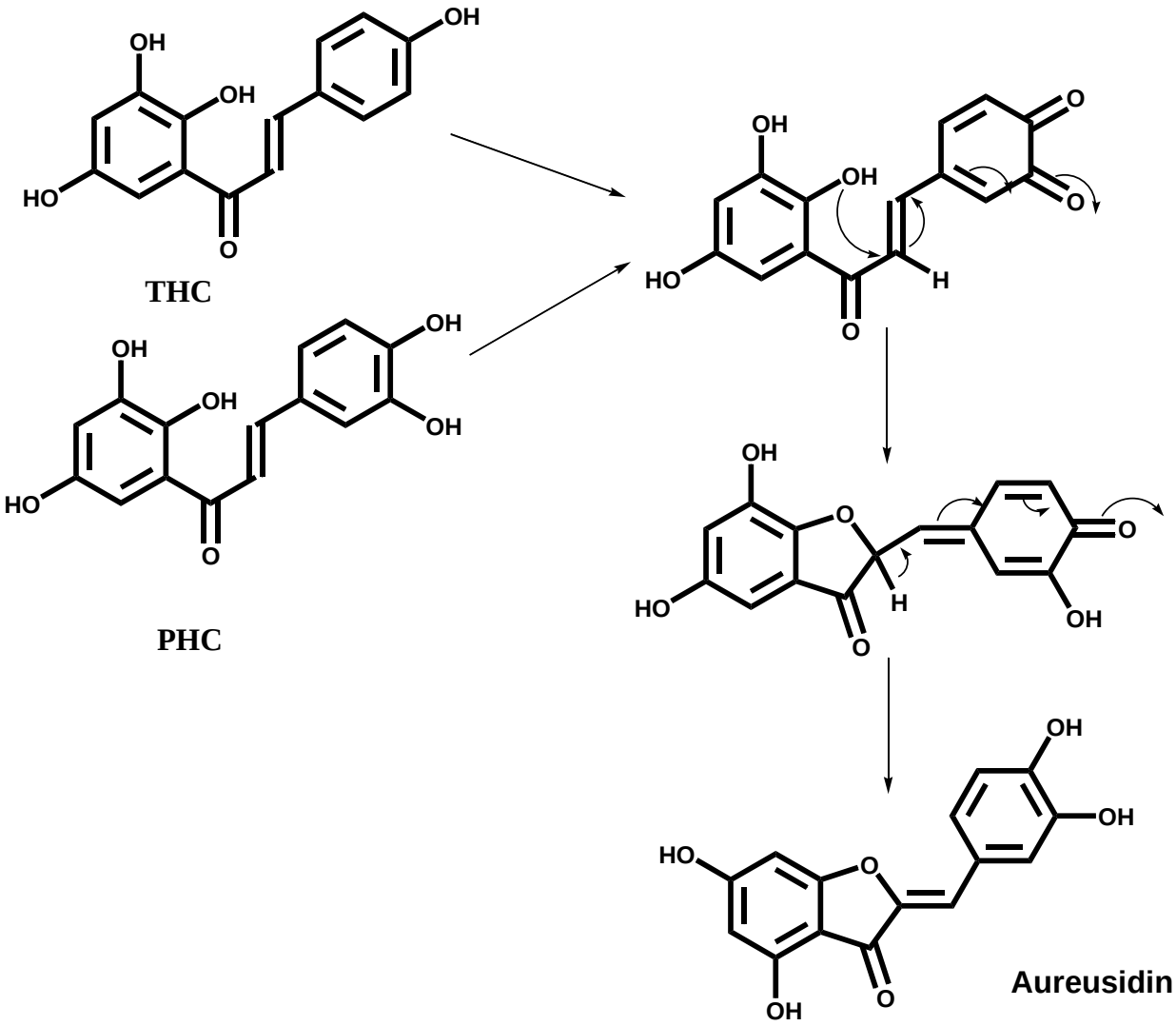


Fig. 22: Reaction mechanism of the transformation of polyhydroxychalcones into aureusidin catalyzed via aureusidin synthase

2.2 Metal ion complexes of chalcones

The field of coordination chemistry of chalcones has just been discovered as a research area. It is a branch of flavonoid-metal-complex research and thus, comparable biological properties can be expected.

2.2.1 Introduction in coordination chemistry

Metal complexes with organic molecules as ligands have already been used as pharmaceutical agents for many years. Partly, their activity can be explained by the fundamental importance of metal ions for the physiological organization of organisms. For example metal ions possess structural functions, catalytic functions (in enzymes), redox functions (electron-transport and activation) and information transfer via voltage differences through sodium and potassium ions[35].

Also, the mechanism of action of several pharmaceutical compounds is based on their metal ion. Anticancer compounds e.g. are often metal complexes in which the metal ion is released from its complex in the cell and then binds to DNA to lead to apoptosis. The most famous metal-based anticancer compound is cis-platin (fig. 23), which is widely used in the therapy of testicular cancer. Cis-platin binds to nitrogen of DNA nucleobases, especially to N7 of Guanine, which leads to an alteration of structure and properties of DNA and further to cell death. Hence, the ability of metal ions to bind organic bioligands leads to the specific function of therapeutics.

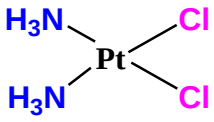


Fig. 23: cis-platin

2.2.2 Metal ion complexes of flavonoids and related compounds, and their fields of application

The complexation of metal ions by polyphenolic compounds like flavonoids has received a lot of attention in research recently. These compounds are studied as medication against all forms of diseases, e.g. cancer and neurodegenerative malady. The use of metal chelating compounds against neurodegenerative diseases can be understood by their mode of action. Epigallocatechin-3-gallate (EGCG) (fig. 24) is able to pass the hemato-encephalic barrier and thus can be transported to the brain where it can reduce the redox-active and harmful iron(III) moiety to iron(II). Hence, EGCG protects brain cells from oxidative damage which could lead to neurodegenerative diseases.

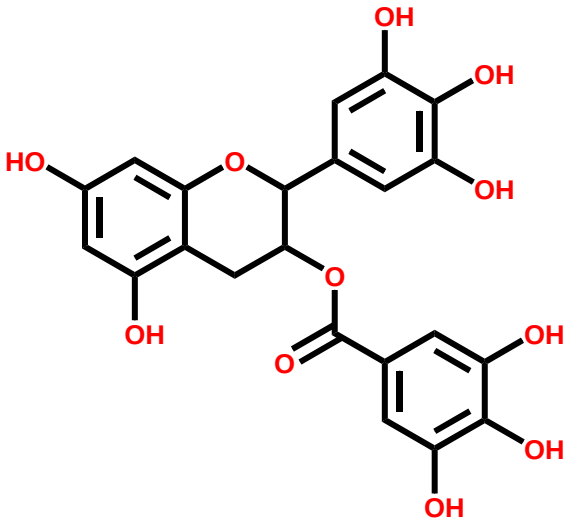


Fig. 24: Epigallocatechin-3-gallate (EGCG)

In the above mentioned mode of action, the induced pharmaceutically active compound is the flavonoid itself which binds to iron *in vivo*. However, it is possible that the complex is formed *in vitro* and then directly used as biologically active compound. A naringin-copper(II)-complex shows a higher cytotoxicity on tumor cells than naringin alone[36]. The cis-Pt(II) complex of 3-aminoflavone (fig. 25) has been investigated in regard to its activity against *leukemia cancer* and showed a high toxicity against tumor cell lines while having a lower toxicity towards normal cells. The mode of action is probably based on the induction of DNA breakage[37].

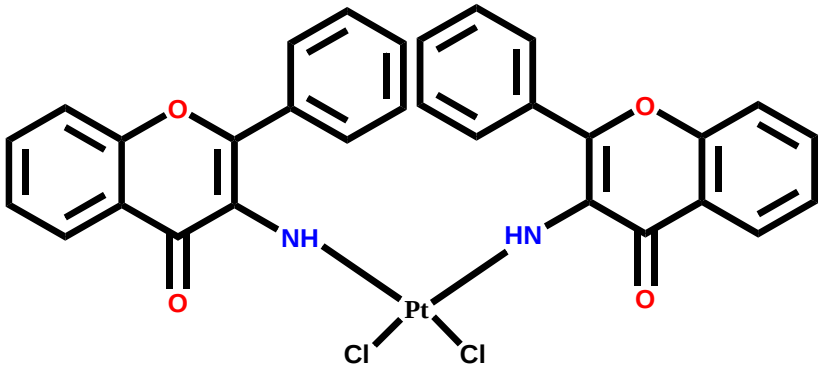


Figure 25: *cis*-[Pt(AF)$_2$Cl$_2$]

So, metal complexes of flavonoids have obtained a lot of attention in medicinal chemistry.

2.2.3 Metal ion complexes of chalcones

In contrast, metal complexes of chalcones have not been studied intensely yet. Some papers deal with the coordination of 2'-Hydroxychalcones by zinc, cadmium, mercury[38], cobalt, nickel and copper[39] (figure 26). The infrared and thermal spectroscopic properties of these compounds have been investigated. The bioactivity of some metal complexes of 2-hydroxyphenyl-3-(1H-indol-3-yl)-prop-2-en-1-one (HPIP) has been studied and the metal complexes showed a stronger antimicrobial activity than HPIP alone[40].

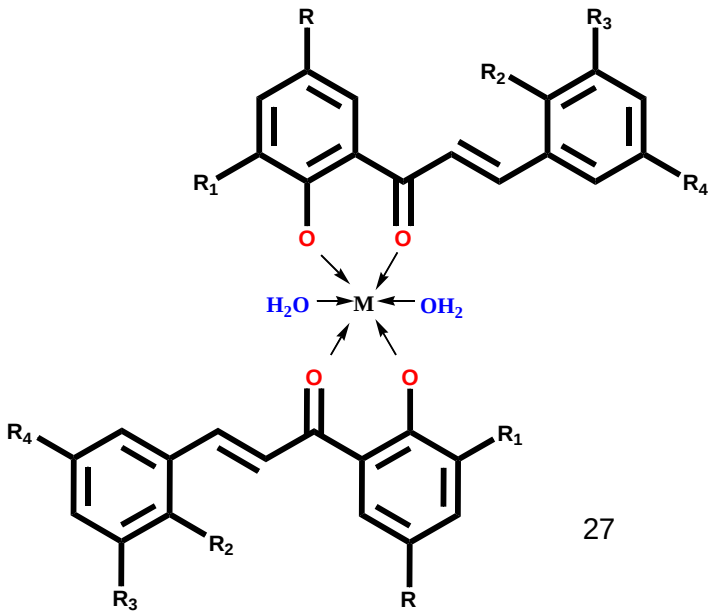


Fig. 26: Metal ion complexation via 2'-Hydroxychalcones (M = Co(II), Ni(II))

The mode of coordination is not completely understood yet. Moreover, no metal complexes of polyhydroxychalcones have been synthesized and characterized to date.

3 Objectives

The first objective of this paper is to analyze the structural characteristics of chalcones by X-ray structure analysis. For this purpose, mono-crystals of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy) chalcone were grown and measured on a diffractometer. The results of this approach can be found in section 4.1.

The second objective is to synthesize several polyhydroxychalcones (2',3,4,4',6'-pentahydroxychalcone (PHC), 2',4,4',6'-tetrahydroxychalcone (THC) and 2',3,4,4'-tetrahydroxychalcone (Butein)). For PHC and Butein, this was done via 3-step-synthesis starting with benzaldehyde and the corresponding acetophenone. The starting materials were protected with methoxymethylether (section 4.2.1) and then coupled (section 4.2.2) under alkaline reaction conditions. The deprotection was performed with hydrochloric acid (section 4.2.3). THC was formed by a ring opening reaction starting from naringenin (section 4.2.4). All compounds were characterized via ^{1}H-NMR spectroscopy. Further, the carbon NMR spectrum of PHC was solved (section 4.2.3) by using HSQC-^{1}H-^{13}C-coupling NMR spectroscopy.

The third objective is to establish alternative syntheses to the classical 3-step polyhydroxychalcone synthesis with MOM protection groups and the coupling under alkaline conditions. To this end, a different deprotection technique (section 4.3.1) was tested. A direct synthesis of polyhydroxychalcones by an acidic one-step reaction was investigated (section 4.3.2) and a different three step synthesis with tBMDS as protection group and LiHMDS as coupling reagent was analyzed (section 4.3.3)

The fourth objective of this paper is to investigate the metal chelation of polyhydroxychalcones. For this purpose, several complexation assays with metal ions such as copper, cobalt, nickel, iron, and zinc and 2'-hydroxy-3,4,4',6'-tertrakis(methoxymethoxy)chalcone were performed (section 4.4.1) and analyzed via mass spectrometry, infrared spectroscopy and UV/Vis-spectroscopy. Additional investigations were carried out with the polyhydroxychalcones PHC, THC and Butein (section 4.4.2) and analyzed via ^{1}H-NMR spectroscopy and UV/Vis-spectroscopy.

4 Results and Discussion

4.1 X-Ray structure analysis of 2'-Hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)-chalcone

2'-Hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone was obtained during the synthesis described in figure 10 and section 4.2.2. It was the major starting compound for the complexation reactions in section 4.4. To understand the physical and chemical properties of this compound and of chalcones in general, X-ray structure analysis on mono-crystals was performed. The crystals were grown in methanol/acetonitrile. The crystals were solved with a R-factor of 6,97%.

2'-Hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone crystallizes in a monoclinic crystal system. The first illustration of the molecule (fig. 27) shows the high planarity of the molecule because of the delocalized π-electron system. Interestingly, the crystal cell includes two molecules and not only one. This is due to the very short distance between the two molecules, which is only about 3,42 Å (fig. 28). The reason for this short distance is the interaction between the delocalized π-electrons of the two molecules. This process is called π-π-electron stacking and can be found in several systems like in DNA. All the protection groups are pointing in the direction away from the other molecule, so they do not hinder the π-π-electron stacking between the molecules. However, this most likely means that these dimers cannot form more complex structures because of the steric bulks of the protection groups. They prevent another molecule from interacting with one of the two molecules.

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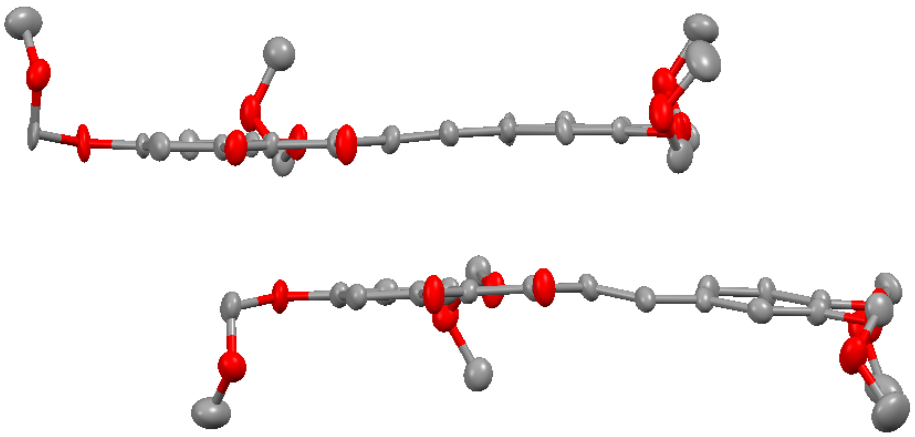


Fig. 27: Crystal structure of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone without hydrogen atoms

A totally delocalized π-electron system should lead to the same bond length for every carbon-carbon bond. This can be observed in a benzene molecule, where every bond has a length of 1,39 Å instead of 1,47 Å for a carbon-carbon single bond and 1,33 for a carbon-carbon double bond[41]. This is because every bond has a single bond character and a double bond character at the same time. So, for the α,β-unsaturated carbonyl moiety in between the two aromatic systems in a chalcone molecule, one could expect that all bonds possess similar lengths, because all carbons are sp^2 hybridized. Interestingly, this is not the case here. The bond between the A-ring and the carbonyl carbon is around 1,467-1,480 Å, the bond between the carbonyl carbon and the α carbon is 1,470-1,472 Å and the bond between ring B and the β carbon is 1,447-1,462 Å. So, all of these carbon bonds have mainly single bond character. In contrast, the α – β carbon bond has a length of 1,323 – 1,337 Å and thus, mainly a double bond character. This means, that the delocalization of the π-electrons is only partial.

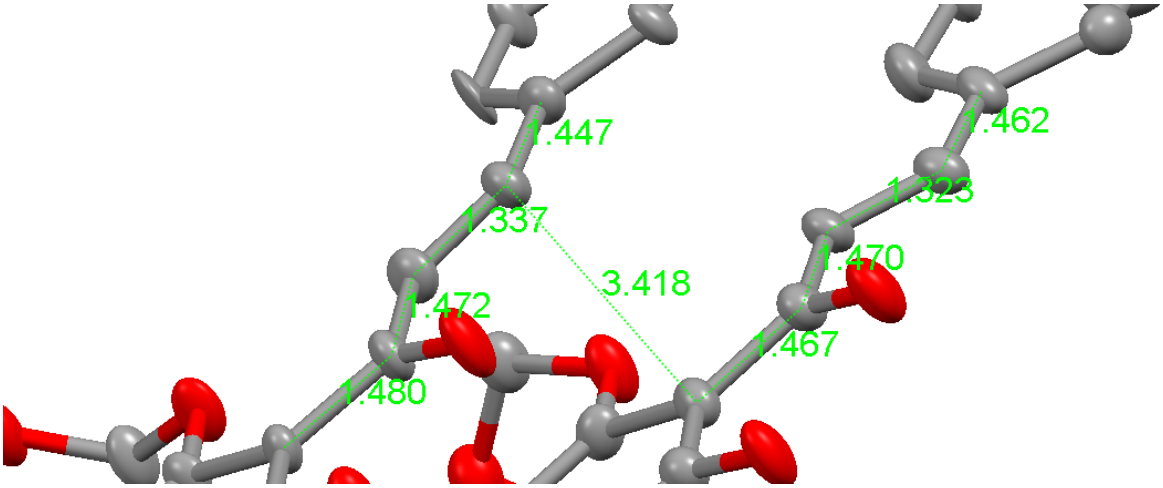


Fig. 28: Specific bond lengths in 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone

Crystallographic data:

Compound	2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone
Chemical formula	C_{23} H_{30} O_{10}
Molecular mass	464,46 g/mol
Cell lengths	a: 8.784 b: 17.523 c: 14.815
Cell angles	α: 90.00 β: 93.34 γ: 90.00
Space group	P c
Crystal system	Monocline
Crystal color, form	Yellow needle

Table 1: Crystallographic data of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone

4.2 Synthesis and characterization of polyhydroxychalcones

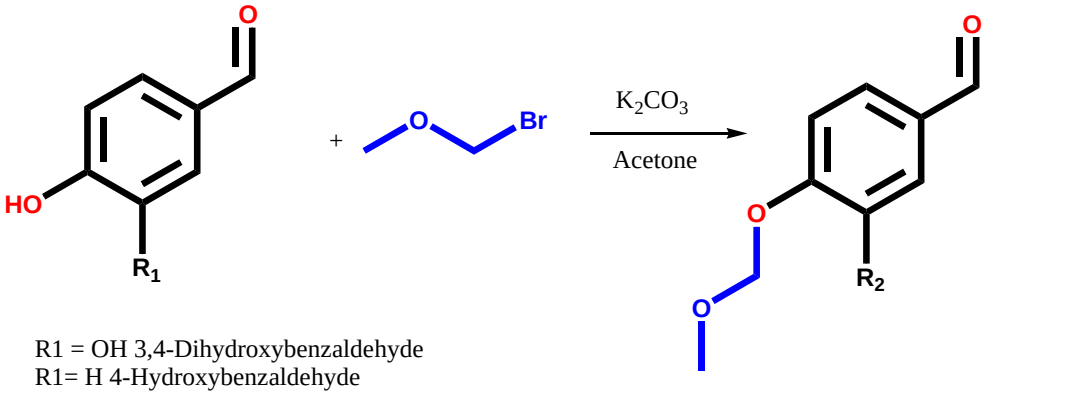
The first approach to synthesize polyhydroxychalcone was the three-step-synthesis performed by Detsi et. al.[7] and Nishida et. al.[8] as described in section 2.1.2.2.

In the first step, the two benzaldehydes (3,4-Dihydroxybenzaldehyde, 4-Hydroxybenzaldehyde) and the two acetophenones (2',4',6'-Trihydroxybenzaldehyde, 2',4'-Dihydroxybenzaldehyde) were protected by methoxymethylbromide. The second step is the condensation reaction of these benzaldehydes with the corresponding acetophenone. In the last step, the protection group is cleaved under acidic reaction conditions.

4.2.1 Spectroscopic analysis of the protection of benzaldehydes and acetophenones

4.2.1.1 Reaction scheme

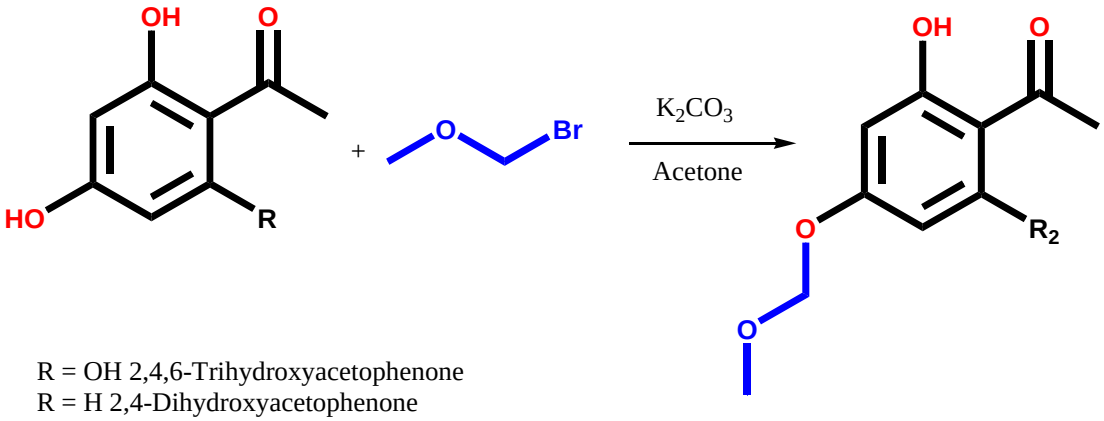
The first step of the classical synthesis of polyhydroxychalcones is their protection with methoxymethylbromide. The scheme of this step is shown in figures 29 and 30.



R1 = OH 3,4-Dihydroxybenzaldehyde
R1= H 4-Hydroxybenzaldehyde

R2 = OMOM 3,4-Bis(methoxymethoxy)benzaldehyde
R2 = H 4-Methoxymethoxybenzaldehyde

Fig. 29: Protection of benzaldehyde with methoxymethylbromide



R = OH 2,4,6-Trihydroxyacetophenone
R = H 2,4-Dihydroxyacetophenone

R = OMOM 2-Hydroxy-4,6-di(methoxymethoxy)acetophenone
R = H 2,4-Dihydroxyacetophenone

Fig. 30: Protection of acetophenone with methoxymethylbromide

The products obtained were purified via liquid column chromatography (hexane 7: 3 ethyl acetate) and characterized via ^{1}H-NMR. The ^{1}H-NMR spectra of 3,4-Bis(methoxymethoxy)benzaldehyde (figure 31), 4-Methoxymethoxybenzaldehyde (figure 32) 2'-Hydroxy-4',6'-di(methoxymethoxy)- acetophenone (fig. 34) and 2'-Hydroxy-4'-methoxymethoxyacetophenone (fig. 35) are shown below.

4.2.1.2 ^{1}H-NMR spectra

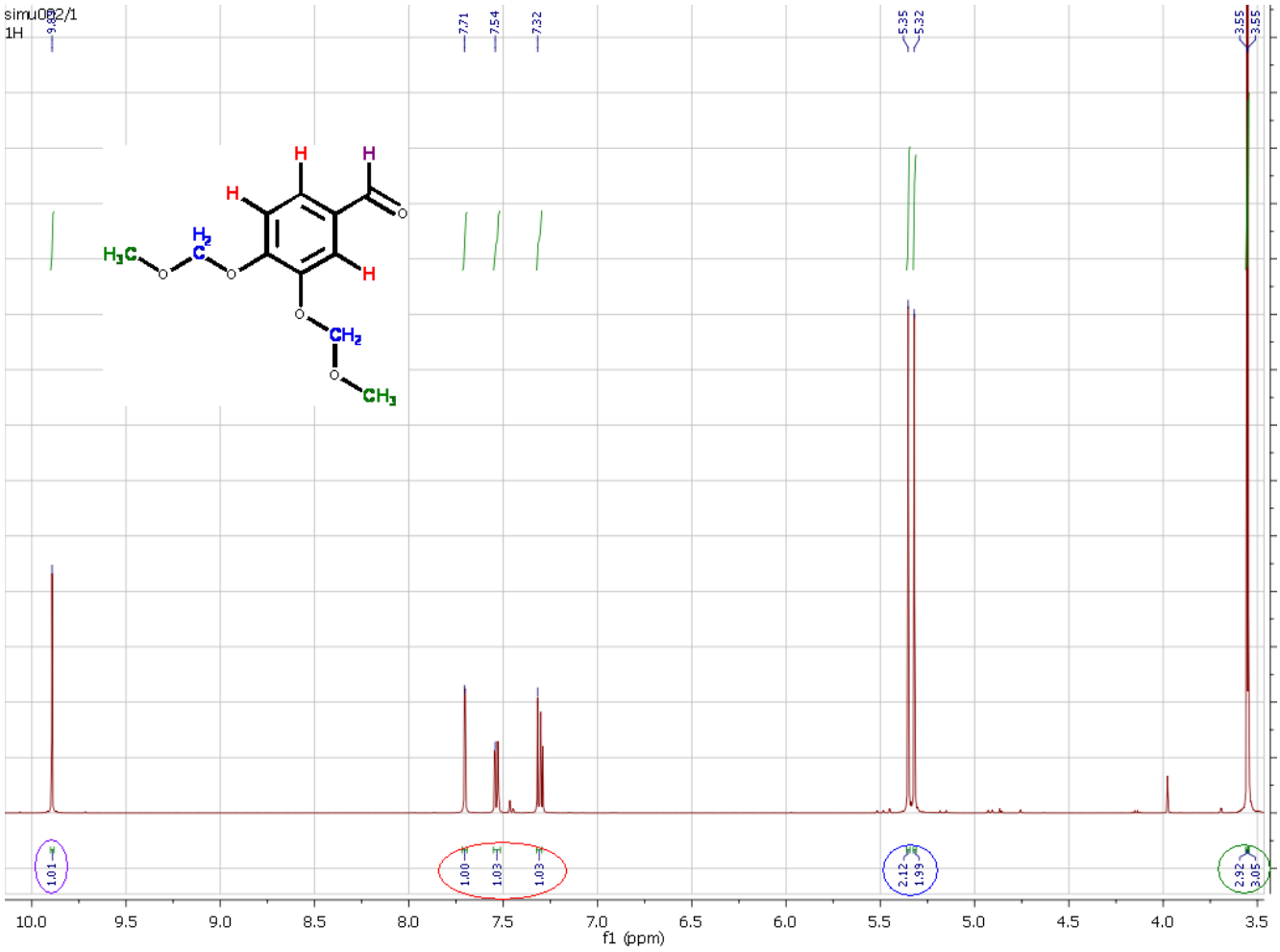


Fig. 31: ^{1}H-NMR spectra of 3,4-bis(methoxymethoxy)benzaldehyde in $CDCl_3$

^{1}H-NMR-shifts of 3,4-bis(methoxymethoxy)benzaldehyde:

δ [ppm]	Intensity	Type of signal	Coupling constant [Hz]	Type of hydrogen
9.89	1 H	Singlet	-	Aldehyde
7.71	1 H	Doublet	1.92	H-C2
7.54	1 H	Doublet of doublets	8.35 / 1.92	H-C6
7.32	1 H	Doublet	8.35	H-C5

5.35	2 H	Singlet	-	Methylene
5.32	2 H	Singlet	-	Methylene
3.55	3 H	Singlet	-	Methyl
3.55	3 H	Singlet	-	Methyl

Table 2: ^{1}H-NMR shifts and coupling constants of 3,4-bis(methoxymethoxy)benzaldehyde in $CDCl_3$

The hydrogen corresponding to the carbonyl function is placed in the downfield close to 10 ppm. The hydrogen in ortho-position to the carbonyl function on the C2 is at 7.71 ppm. This can be deduced by its small coupling constant. A longer distance of the two coupling partners leads to a lower coupling constant[42]. Thus, the coupling constant between H-C2 and H-C6 (1.92 Hz) is lower than the coupling between H-C5 and H-C6 (8.35 Hz). H-C6 is forming a doublet of doublets as it is coupling with H-C5 and on long range with H-C2, at the same time. The two protection groups have their signals at ~5.35 ppm (methylene group; integral 2 H) and ~3.55 ppm (methyl group, integral 3 H).

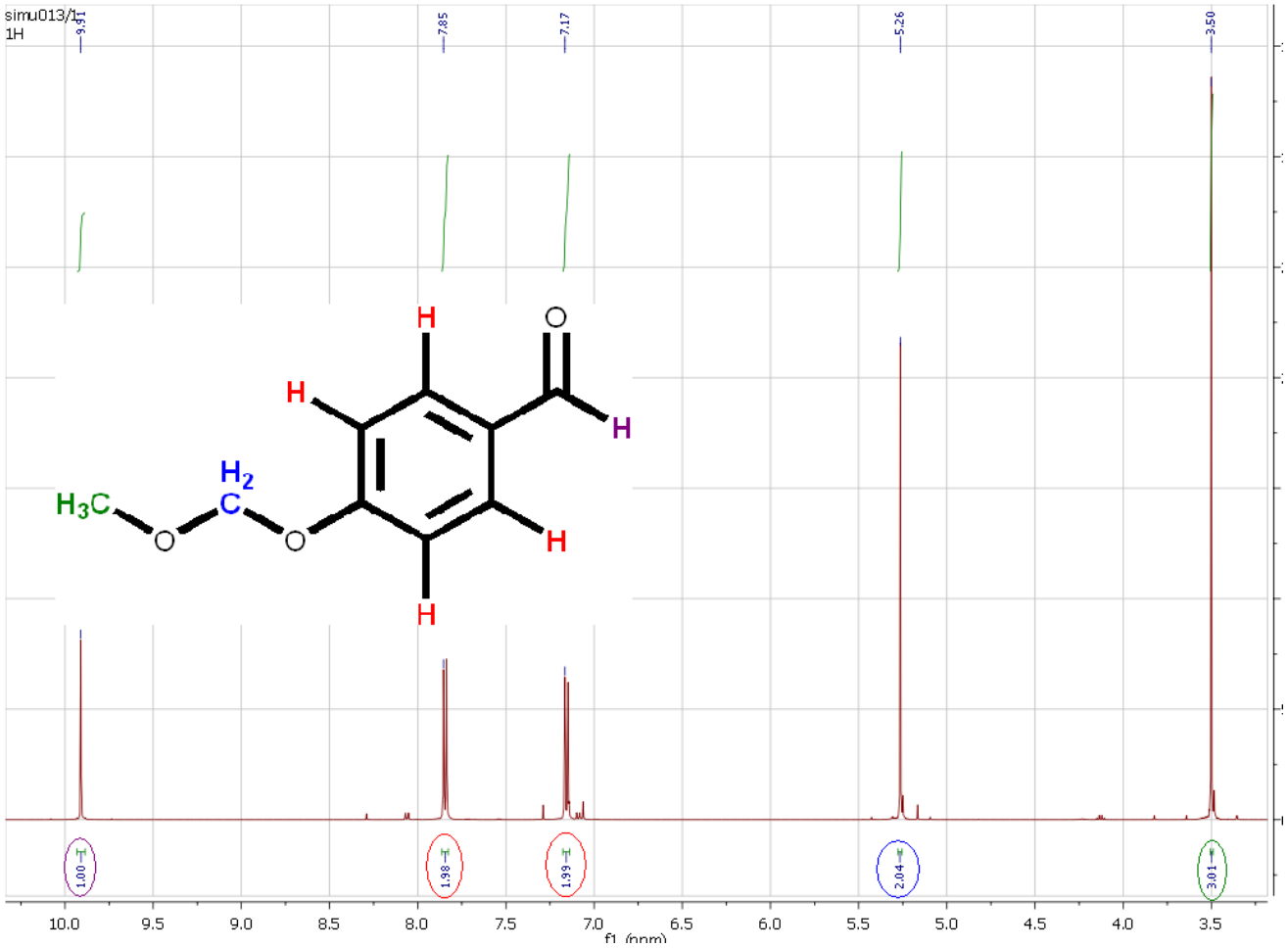


Fig. 32: ^{1}H-NMR spectrum of 4-methoxymethoxybenzaldehyde in $CDCl_3$

^{1}H-NMR-shifts of 4-methoxymethoxybenzaldehyde:

δ [ppm]	Intensity	Type of signal	J Coupling constant [Hz]	Type of hydrogen
9.91	1 H	Singlet	-	Aldehyde
7.85	2 H	Multiplet	8.75	H-C2/H-C6
7.17	2 H	Multiplet	8.75	H-C3/H-C5
5.26	2 H	Singlet	-	Methylene
3.50	3 H	Singlet	-	Methyl

Table 3: 1H-NMR shifts and coupling constants of 4-methoxymethoxybenzaldehyde in $CDCl_3$

The ^{1}H-NMR spectrum of 4-Methoxymethoxybenzaldehyde shown in figure 32 is rather similar to that of 3,4-bis(methoxymethoxy)benzaldehyde. Again, we find the proton of the carbonyl function around ~9.91 ppm. Interestingly, the aromatic protons are not only showing a triplet but a multiplet (figure 33). This is due to the magnetic differences between the protons which are chemically equal in terms of symmetry. The H-C2 is coupling through ^{3}J H-C3 but through ^{5}J to H-C5, so these coupling constants differ from each other. This leads to a spectrum of a higher order[43]. The H-C3 = H-C5 is around 7.17 ppm while the protons of C2 and C6 are shifted more into the downfield at around 7.85 ppm. This is probably due to the electron withdrawing effect of the carbonyl moiety.

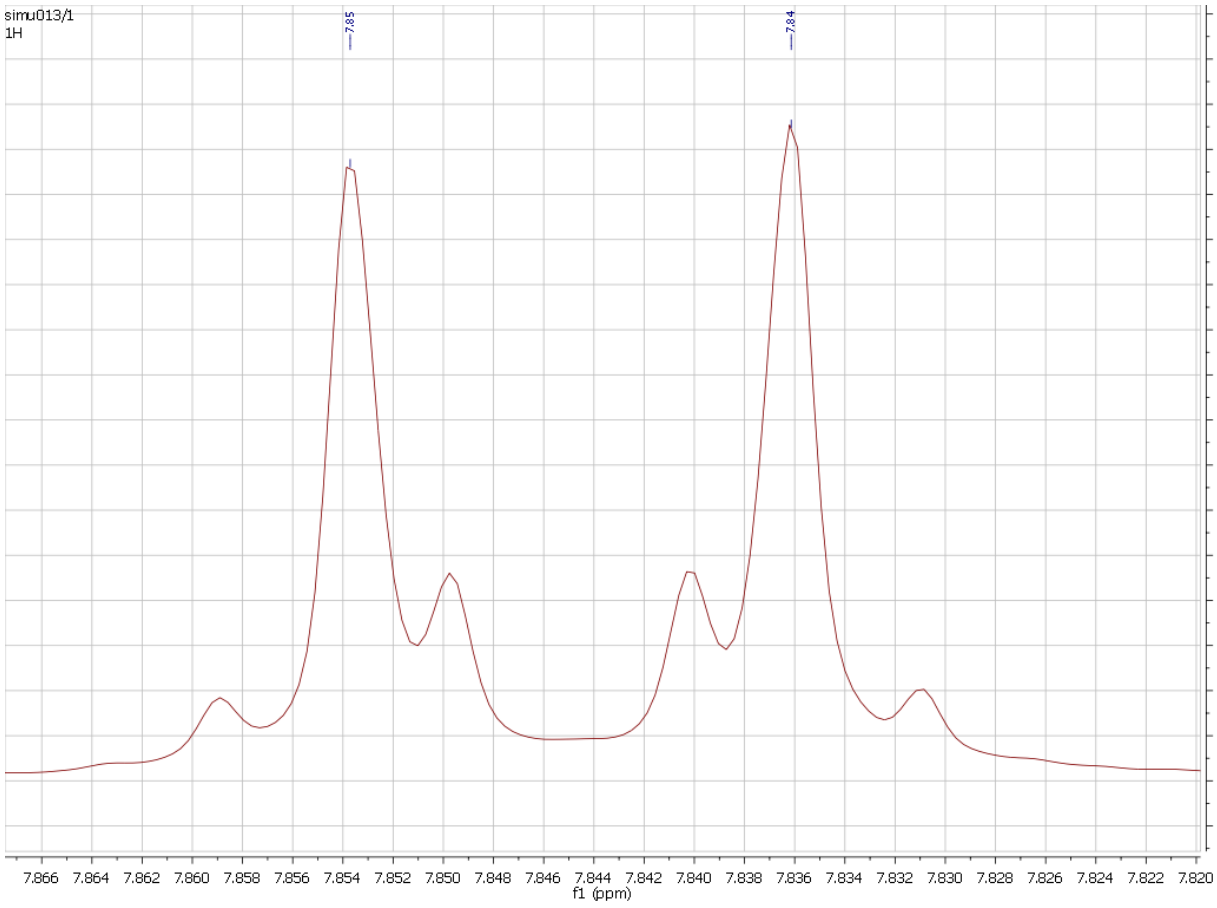


Fig. 33: ^{1}H-NMR spectrum of 4-methoxymethoxybenzaldehyde, zoom into aromatic hydrogens in $CDCl_3$

The ^{1}H-NMR of 2'-Hydroxy-4',6'-di(methoxymethoxy)acetophenone is shown in figure 34. Far in the downfield at 13.37 ppm, there is the proton of the 2'-hydroxy-group, which was not protected in the reaction process as already described in figure 11. The two aromatic protons are not completely identical, though only 0.01 ppm shifted from each other. The coupling constant is ^{4}J = 2.36 Hz. The protection groups can be found with 2 x 2 methylene protons and 2 x 3 methyl protons in the high field. The methyl protons have the highest electron density, they are at 2.68 ppm.

2'-Hydroxy-4'methoxymethoxyacetophenone possesses a ^{1}H-NMR spectrum (figure 35) consisting of 3 aromatic protons which have the same coupling pattern as in 3,4-bis(methoxymethoxy)benzaldehyde, so the H-C'5 shows a doublet of doublets in the spectrum because of its long range ^{4}J = 2.44 Hz coupling with H-C'3 and its short range ^{3}J = 8.84 Hz with H-C'6.

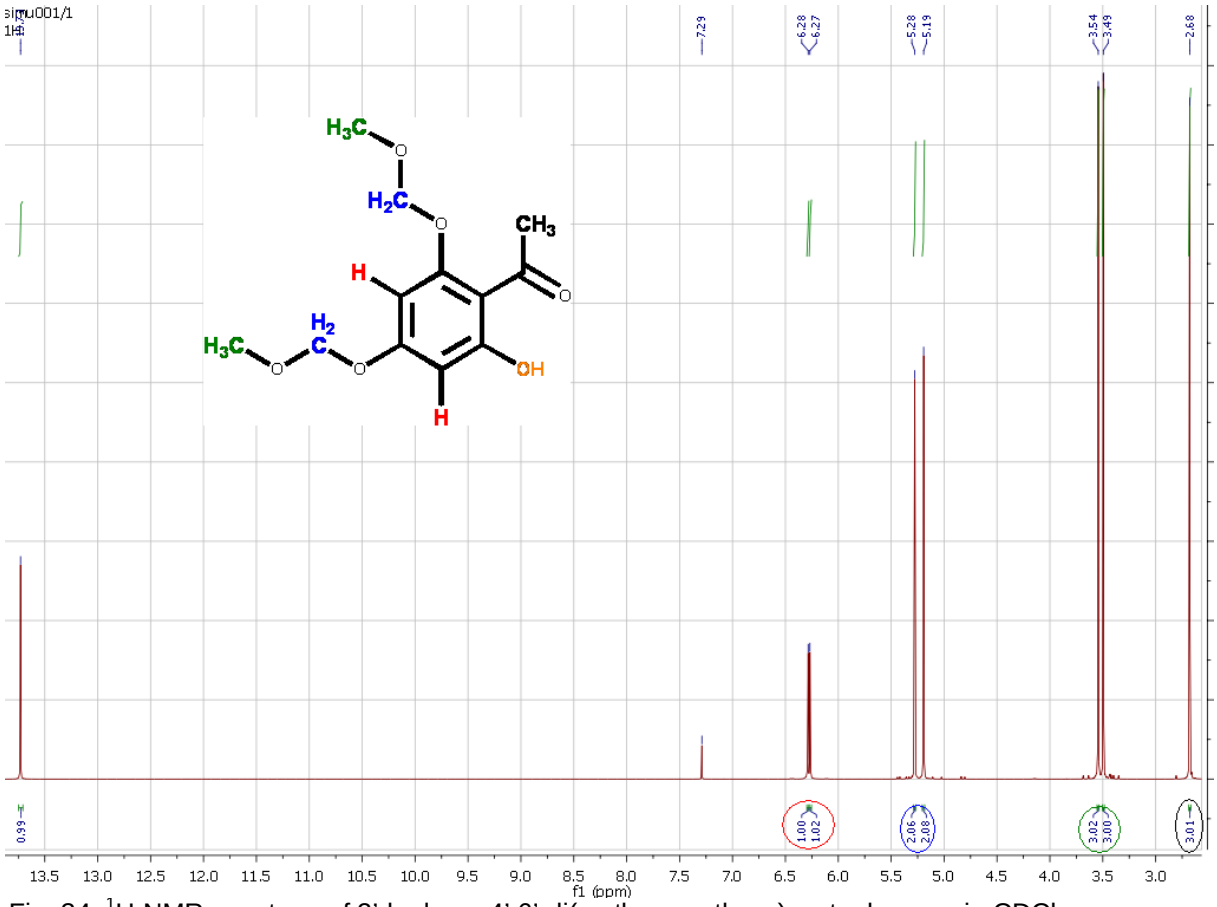


Fig. 34: ^{1}H-NMR spectrum of 2'-hydroxy-4',6'-di(methoxymethoxy)acetophenone in $CDCl_3$

^{1}H-NMR-shifts of 2'-hydroxy-4',6'-di(methoxymethoxy)acetophenone:

δ [ppm]	Intensity	Type of signal	Coupling constant [Hz]	Type of hydrogen
13.73	1 H	Singlet	-	Hydroxyl
6.28	1 H	Doublet	2.36	H-C'3/H-C'5
6.27	1 H	Doublet	2.36	H-C'3/H-C'5
5.28	2 H	Singlet	-	Methylene
5.19	2 H	Singlet	-	Methylene
3.54	3 H	Singlet	-	Methyl
3.49	3 H	Singlet	-	Methyl
2.68	3 H	Singlet	-	Methyl

Table 4: ^{1}H-NMR shifts and coupling constants of 2'-Hydroxy-4',6'Di(methoxymethoxy)acetophenone in $CDCl_3$

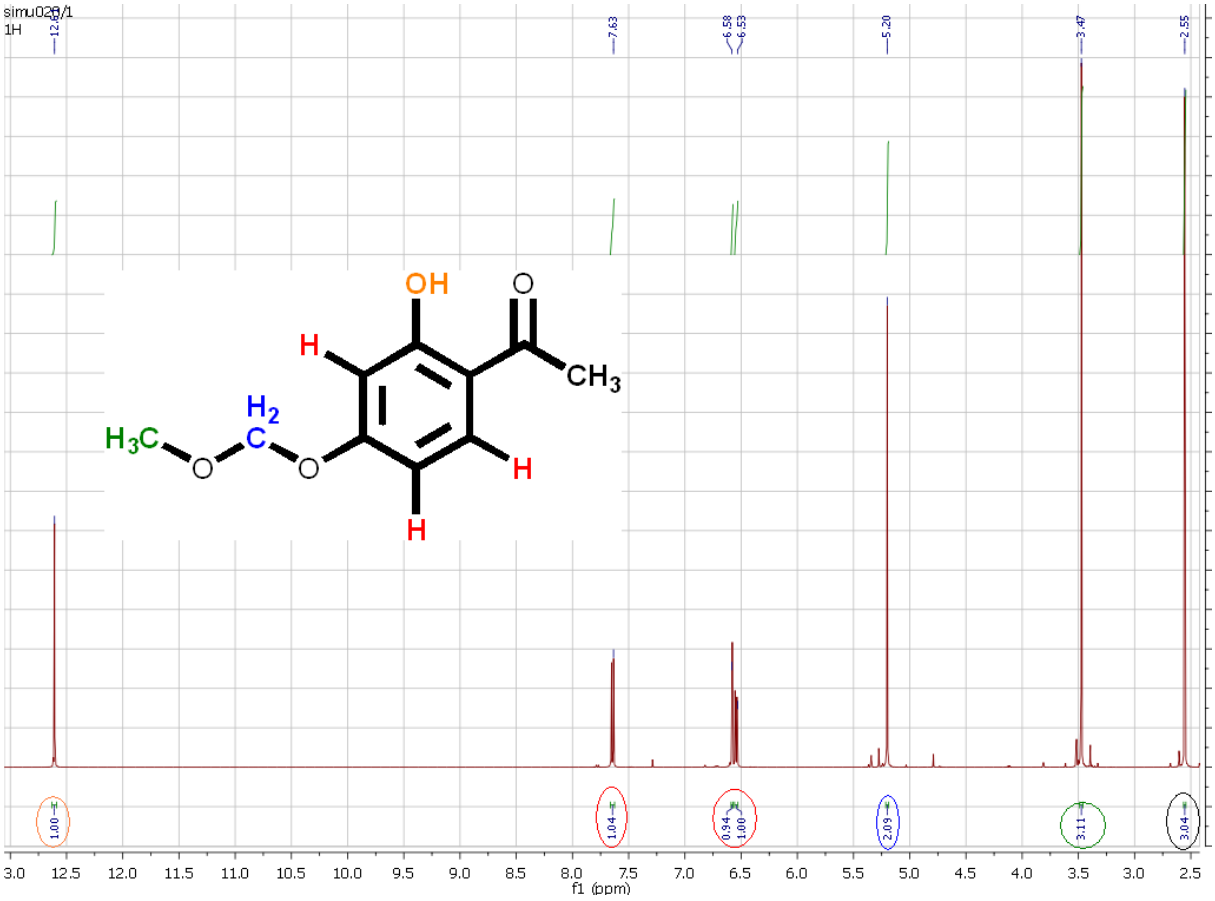


Fig. 35: ^{1}H-NMR spectrum of 2'-hydroxy-4'-methoxymethoxyacetophenone in $CDCl_3$

^{1}H-NMR-shifts of 2'-hydroxy-4'-methoxymethoxyacetophenone

δ [ppm]	Intensity	Type of signal	Coupling constant [Hz]	Type of hydrogen

12.61	1 H	Singlet	-	Hydroxyl
7.63	1 H	Doublet	8.84	H-C6
6.58	1 H	Doublet	2.44	H-C3
6.53	1 H	Doublet of doublets	8.84 / 2.44	H-C5
5.20	2 H	Singlet	-	Methylene
3.47	3 H	Singlet	-	Methyl
2.55	3 H	Singlet	-	Methyl

Table 5: ^{1}H-NMR shifts and coupling constants of 2'-Hydroxy-4'-methoxymethoxyacetophenone

4.2.1.3 Discussion

The protection step provides yields between 50-80 %. The major issue in this step is the purification, which is complicated by the fact that the products show similar retardation factor values ($\mathbf{R_f}$ values) in liquid column chromatography. This is worst for the 3.4-bis(methoxymethoxy)benzaldehyde because the singly protected benzaldehyde is partially formed as well and its $\mathbf{R_f}$ value is highly similar to that of 3,4-bis-(methoxymethoxy)benzaldehyde. However, the protection of the acetophenone leads to by-products limiting the yields of the desired product to ~65 %. So, the introduction of the MOM protection group only generates moderate yields and the purification is difficult.

4.2.2 Spectroscopic analysis of the protected chalcones

4.2.2.1 Reaction scheme

The now obtained materials were used for the Claisen-Schmidt-Condensation (Fig. 36). Starting with always one acetophenone and one benzaldehyde, four different coupling products can be formed from these starting materials.

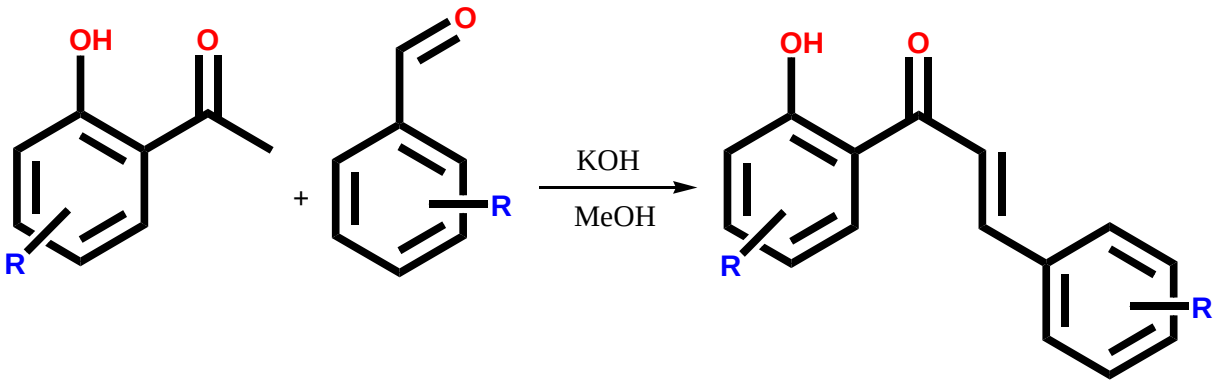


Fig. 36: General procedure of chalcone synthesis

The four possible products are 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone, 2'-hydroxy-4,4',6'-tri(methoxymethoxy)chalcone, 2'-hydroxy-3,4,4'-tri(methoxymethoxy)-

chalcone and 2'-hydroxy-4,4'-di(methoxymethoxy)chalcone. The synthesis was performed in alkaline methanolic solution and the obtained products were purified via recrystallization in hexane/ethyl acetate or via liquid column chromatography with hexane/ethyl acetate (7:3). This procedure was performed for 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone and 2'-hydroxy-3,4,4'-tri(methoxymethoxy)chalcone, but for 2'-hydroxy-4,4',6'-tri(methoxymethoxy)chalcone the direct synthesis from the corresponding flavonoid naringenin produced a higher yield while generating lower costs. The purification of 2'-hydroxy-4,4'-di(methoxymethoxy)chalcone had not been completed by the end of this paper.

4.2.2.2 ^{1}H-NMR spectra

The ^{1}H-NMR spectrum of 2'-Hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone (figure 37) shows the aromatic protons of the chalcone around 6.2-7.6 ppm. Hα and Hβ are at 7.8 ppm, they have a high coupling constant of 15.5 Hz, which is a strong hint to a trans-conformation of the double bond as trans coupling constants are usually bigger than cis coupling constants[44]. The proton of the hydroxyl group is not shown in the spectrum for reasons of clarity. The aromatic protons corresponding to the B-ring (the former benzaldehyde) are shifted in the area between 7.19 ppm to 7.53 ppm, the protons of the A-ring (the former acetophenone) are between 6.29 ppm to 6.34 ppm. The four protection groups have two singlet signals, one consists of the two protons of the methylene group and one consists of the three protons of the methyl group.

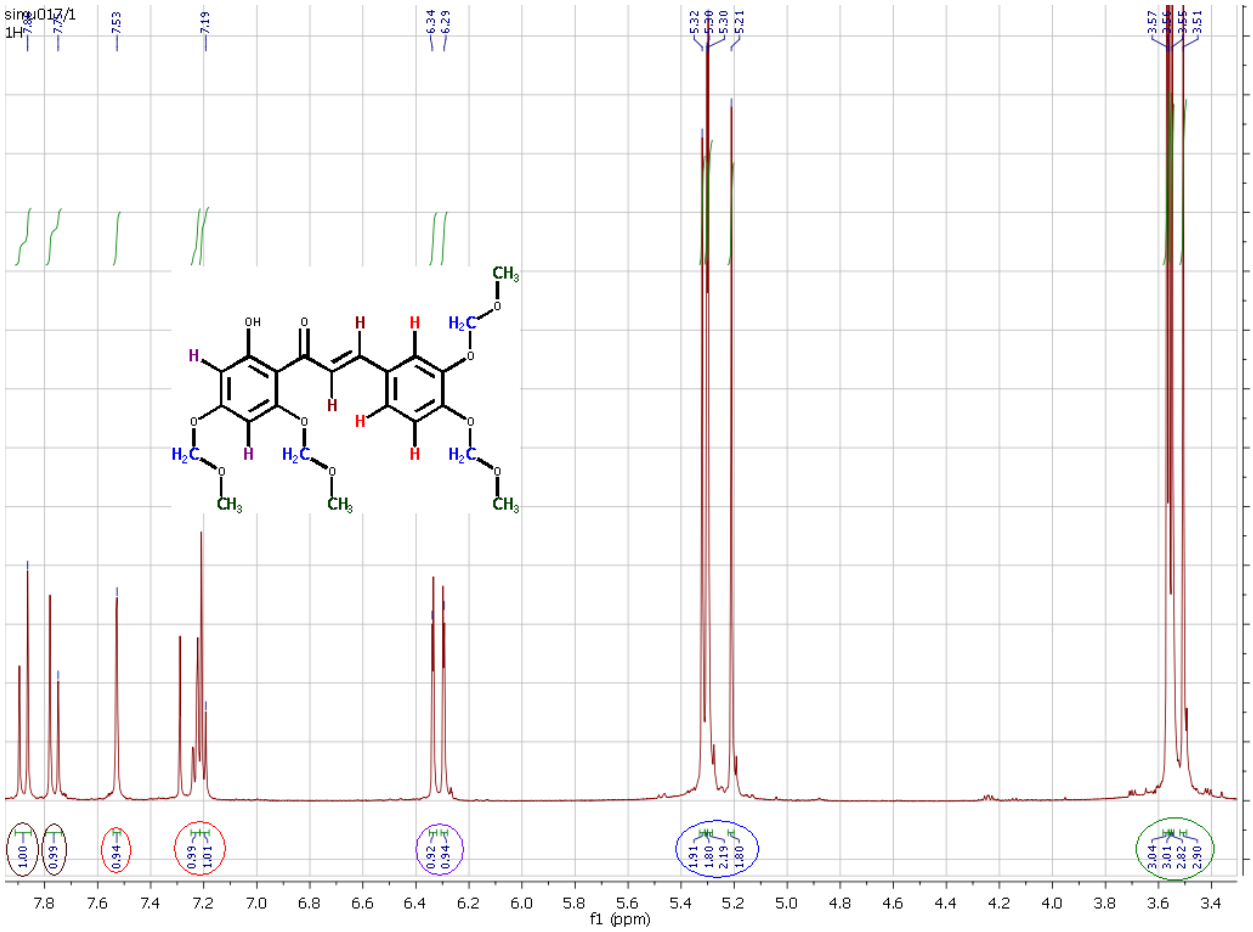


Fig. 37: ^{1}H-NMR spectrum of 2'-Hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone in $CDCl_3$

^{1}H-NMR shifts of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxychalcone:

δ [ppm]	Intensity	Type of signal	Coupling constant [Hz]	Type of hydrogen
13.93	1 H	Singlet	-	Hydroxyl
7.86	1 H	Doublet	15.54	Hβ
7.73	1 H	Doublet	15.54	Hα
7.53	1 H	Doublet	1.56	H-C2
7.24	1 H	Doublet of doublets	8.47 / 1.56	H-C6
7.19	1 H	Doublet	8.47	H-C5
6.34	1 H	Doublet	2.22	H-C'3/H-C'5
6.29	1 H	Doublet	2.22	H-C'3/H-C'5
5.20 – 5.33	4 x 2 H	Singlet	-	Methylen
3.49 – 3.58	4 x 3 H	Singlet	-	Methyl

Table 6: ^{1}H-NMR shifts and coupling constants of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone in $CDCl_3$

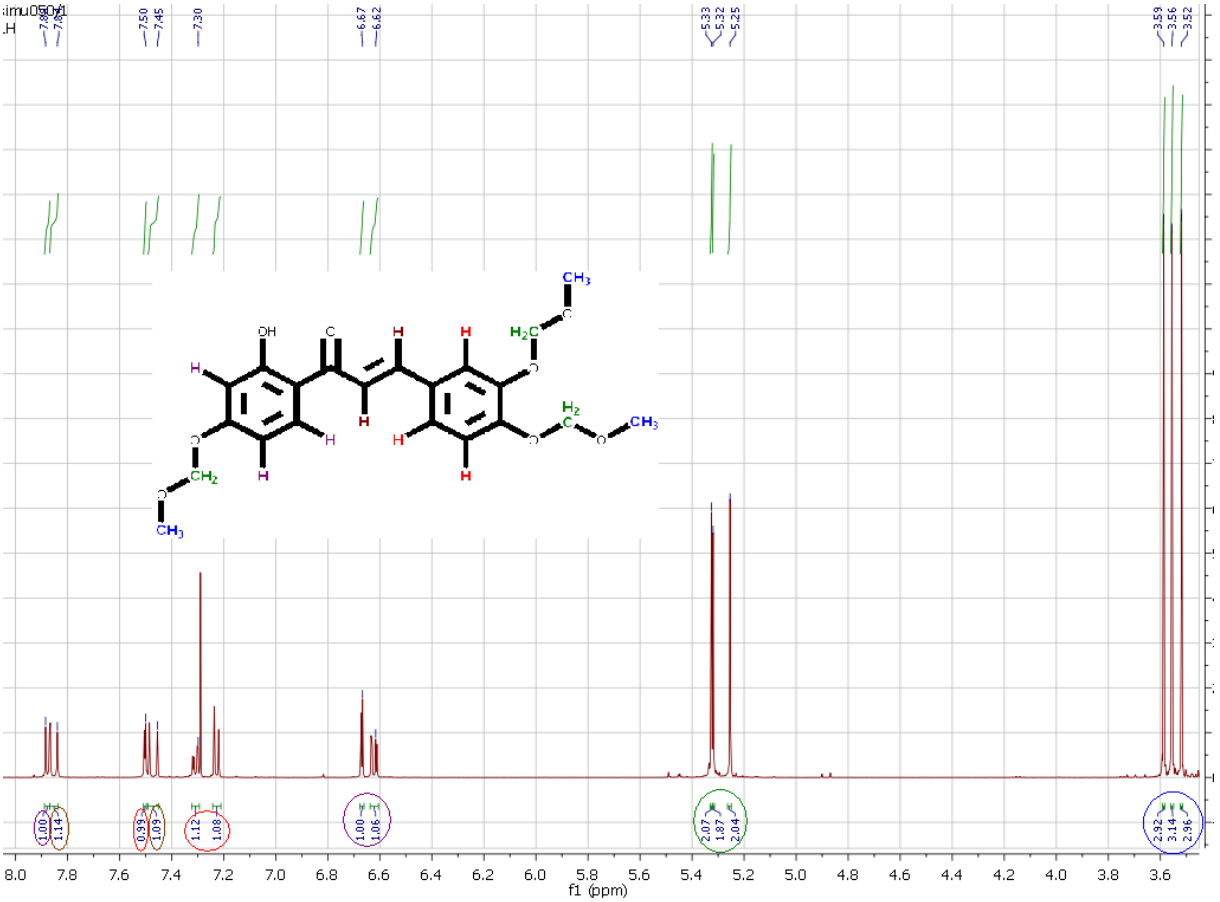


Fig. 38: ^{1}H-NMR spectrum of 2'-hydroxy-3,4,4'-tri(methoxymethoxy)chalcone in $CDCl_3$

^{1}H-NMR shifts of 2'-hydroxy-3,4,4'-tri(methoxymethoxy)chalcone:

δ [ppm]	Intensity	Type of signal	Coupling constant [Hz]	Type of hydrogen
13.35	1 H	Singlet	-	Hydroxyl
7.88	1 H	Doublet	9.02	H-C'6
7.84	1 H	Doublet	15.35	Hβ
7.50	1 H	Doublet	2.02	H-C2
7.45	1 H	Doublet	15.35	Hα
7.30	1 H	Doublet of doublets	8.45 / 2.02	H-C6
7.22	1 H	Doublet	8.45	H-C5
6.67	1 H	Doublet	2.42	H-C'3
6.62	1 H	Doublet of doublets	9.02 / 2.42	H-C'5
5.20 – 5.33	3 x 2 H	Singlet	-	Methylen
3.49 – 3.58	3 x 3 H	Singlet	-	Methyl

Table 7: ^{1}H-NMR shifts and coupling constants of 2'-hydroxy-3,4,4'-tri(methoxymethoxy)chalcone in $CDCl_3$

2'-Hydroxy-3,4,4'-tri(methoxymethoxy)chalcon exhibits a close to similar spectrum (fig. 33) as the tetra(methoxymethoxy)chalcone does. Instead of the four protection groups of the tetra(methoxymethoxy)chalcone, the spectrum shows only three (3 x 2 methylen protons and 3 x 3 methyl protons) and therefore possesses an additional aromatic proton. This proton can be found around 7.88 ppm, thus it has the lowest electron density of all aromatic protons. This is due to the withdrawing effect of the carbonyl function direct in ortho position to H-C6. The additional proton leads to a doublet of doublets for the H-C5.

4.2.2.3 Discussion

The Claisen-Schmidt-Condensation in alkaline solution produces yields of 55 % - 75 %. For the coupling reaction, these yields are reasonable. The purification by recrystallization works well; the critical step is the washing of the product with sufficient water directly after acidification of the alkaline solution, because otherwise acetic acid is formed after extraction, which leads to the formation of by-products (deprotection, polymerization). The purification via column chromatography works less well, but has to be performed for 2'-hydroxy-4,4'-di(methoxymethoxy)chalcone, as this product does not crystallize.

4.2.3 Spectroscopic analysis of the polyhydroxychalcones

4,2,3,1 Reaction scheme

2',3,4,4',6'-Pentahydroxychalcone and 2',3,4,4'.tetrahydroxychalcone were synthesized from the protected chalcones via deprotection step. 2',4,4',6'-tetrahydroxychalcone was obtained from its corresponding flavone, which is naringenin. This can be easily done, because grapefruits possess a high amount of naringenin and thus, it can be purchased cheaply (see section 4.2.4).

2',3,4,4',6'-Pentahydroxychalcone is the compound with the highest interest for this group as it is the most common substrate for aureusidin synthase. Thus, the characterization of this compound was done in more detail than for the other chalcones.

4.1.3.2 NMR spectra

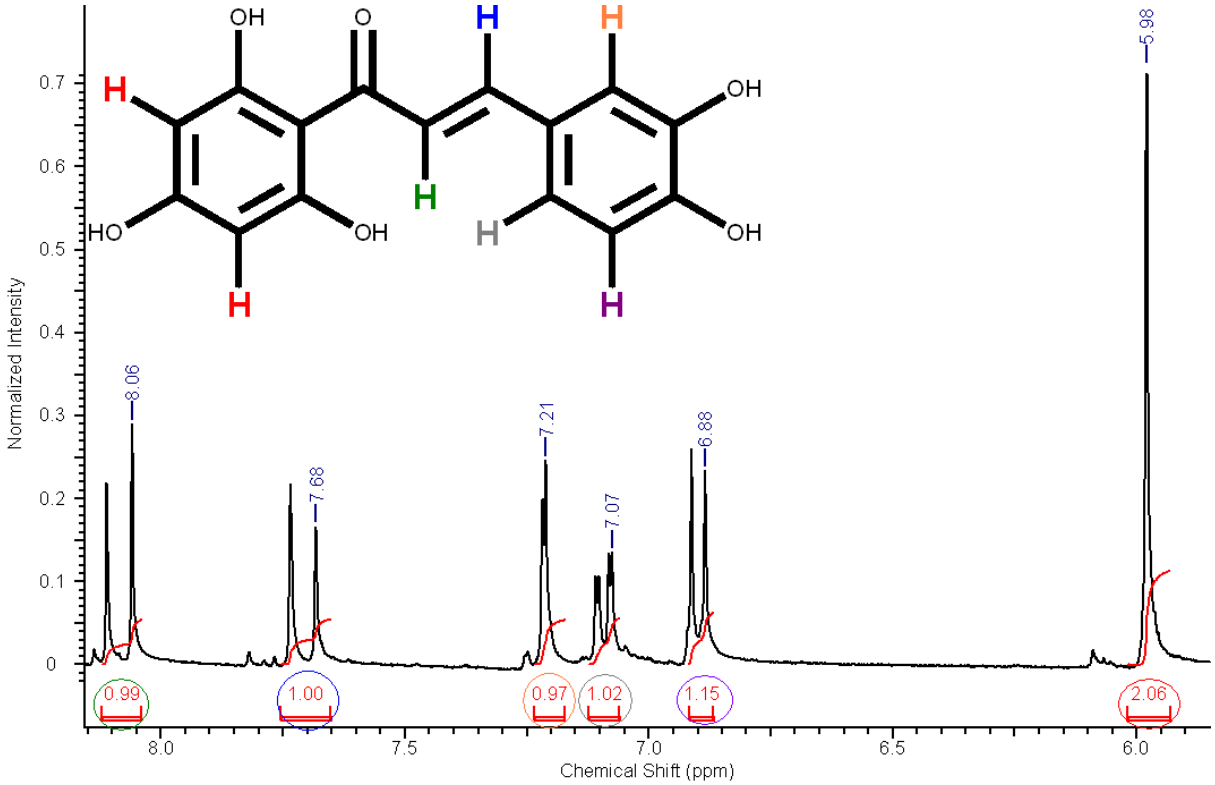


Fig. 39: ^{1}H-NMR spectrum of 2',3,4,4',6'-pentahydroxychalcone in d_6_acetone

^{1}H-NMR shifts of 2',3,4,4',6'-pentahydroxychalcone:

δ [ppm]	Intensity	Type of signal	Coupling constant [Hz]	Type of hydrogen
8.06	1 H	Doublet	15.55	Hβ
7.68	1 H	Doublet	15.55	Hα
7.21	1 H	Doublet	1.94	H-C2
7.08	1 H	Doublet of doublets	8.20 / 1.94	H-C6
6.88	1 H	Doublet	8.20	H-C5
5.98	2 H	Singlet	-	H-C'3/H-C'5

Table 8: ^{1}H-NMR shifts and coupling constants of 2',3,4,4',6'-Pentahydroxychalcone in d_6_acetone

The spectrum of 2',3,4,4',6'-pentahydroxychalcone shows the Hβ and Hα in the downfield. The three aromatic protons of the B-ring show their typical coupling pattern with a doublet of doublets for H-C6, which is coupling to the meta-proton H-C1 with a low constant and to the ortho-proton H-C5 with a higher constant. The two protons of the A-ring are identical; they form one singlet at 5.98 ppm.

These results are confirmed by the literature[8]. However, none of these publications has assigned the carbon atoms in a ^{13}C-NMR spectrum. To determine the chemical shifts in the ^{13}C-NMR spectrum, an *HSQC* (*heteronuclear single-quantum correlation*) experiment was

performed. This technique of 2D-NMR provides information about the coupling of primary, secondary and tertiary carbons with their protons. 2',3,4,4',6'-pentahydroxychalcone only possesses tertiary and quaternary carbon atoms. Nevertheless, an HSQC can provide a high amount of information enabling to solve the ^{13}C-NMR spectrum.

On the ordinate, all those carbon signals which are bound directly to a hydrogen atom and thus, couple with it are displayed. There are six signals corresponding to seven carbons, because C'3 and C'5 in ring A are similar. The intensity of the signal of C'3/C'5 is stronger than that of the other carbons. On the abscissa is the ^{1}H-NMR spectrum pointed out. The straight lines connect the ^{1}H-NMR peaks with their corresponding ^{13}C-NMR signals. It is worth noting that the Cβ clearly possesses the lowest electron density of tertiary carbons while the Hβ has a higher electron density than Hα. The fact that the Cβ has such a low electron density is due to the α-β-unsaturated carbonyl system which is partially similar to a Michael-acceptor system (fig. 41).

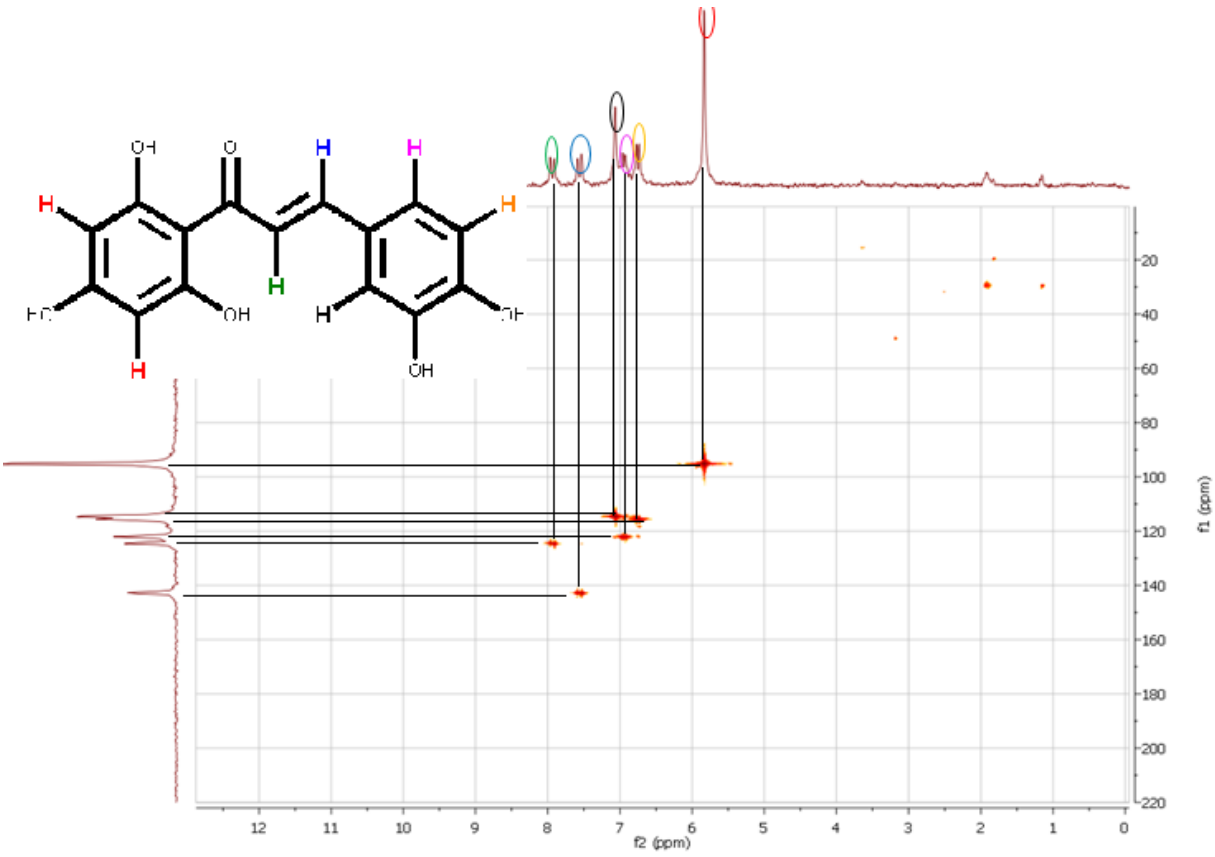


Fig. 40: ^{1}H-^{13}C-HSQC-NMR of 2',3,4,4',6'-Pentahydroxychalcone in d_6_acetone

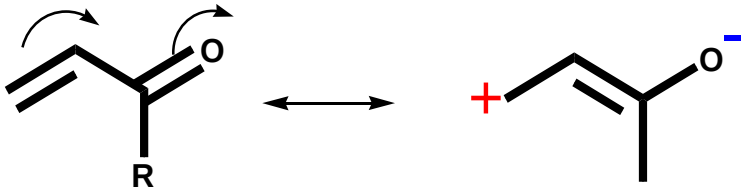
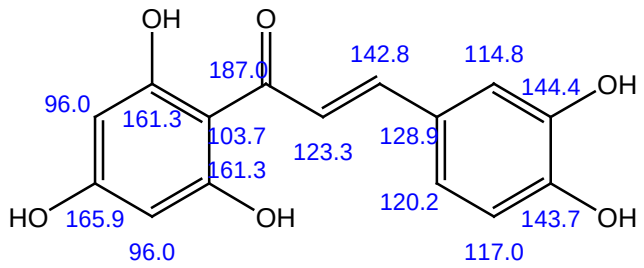


Fig. 41: Typical Michael-acceptor system with a low electron density on Cβ

The ^{13}C-NMR spectrum remains complicated as the five hydroxyl-groups are difficult to distinguish. Therefore, a ChemDraw-calculation of the electron density was performed. This can be a useful tool to obtain some specific information but should never be seen as solving the whole spectrum. In this specific example the information that can be obtained with the ChemDraw calculations is mainly the difference in the electron density of the carbon atoms connected to a hydroxyl group.

ChemNMR C-13 Estimation



Estimation Quality: blue = good, magenta = medium, red = rough

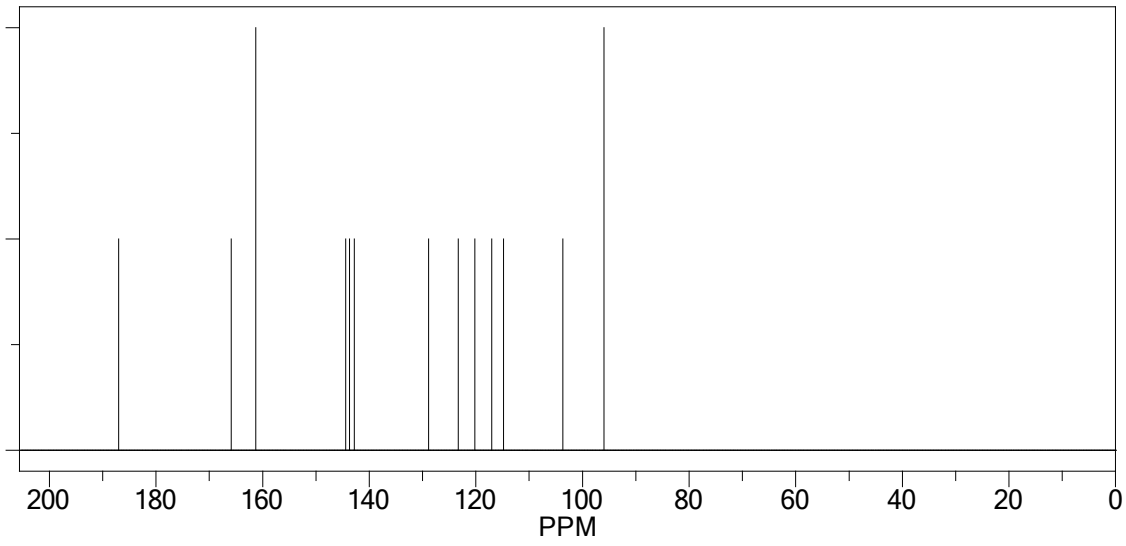


Fig. 42: ^{13}C-NMR shifts calculated by ChemDraw Ultra

Comparing the carbon atoms on hydroxyl groups, we can see a higher electron density for the carbon atoms in the B-ring than for those in the A-ring. This is due to the carbonyl function close to the A-ring which is withdrawing the electrons from the ring. Interestingly, the electron density on the C'1, C'3 and C'5 is high in comparison to C'2, C'4 and C'6. When put

in relation to the mesomeric stabilization, it can be seen that the electron density only decreases in ortho and para position to the carbonyl function. This is the reason why carbonyl benzenes are meta-directing electrophilic substitution reactions, as the electron density is not decreased in meta-position.

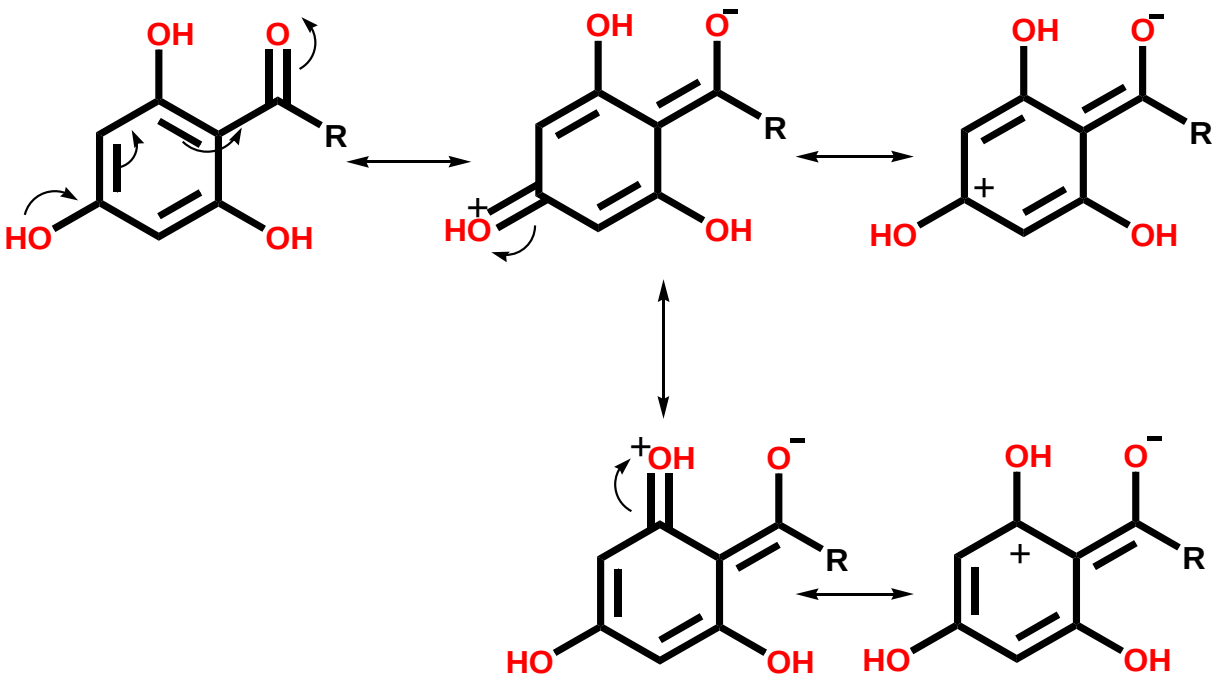


Fig. 43: Mesomeric effects on Ring-A of 2',3,4,4',6'-pentahydroxychalcone

With all this information, it is now possible to solve the ^{13}C-NMR spectrum (Fig. 44). The carbon of the carbonyl function is shifted the most to the down field. The carbons with hydroxyl groups are around 140-170 ppm, all other aromatic carbons are around 95-130 ppm. The carbons of the double bond are at 125.41 ppm and 143.68 ppm.

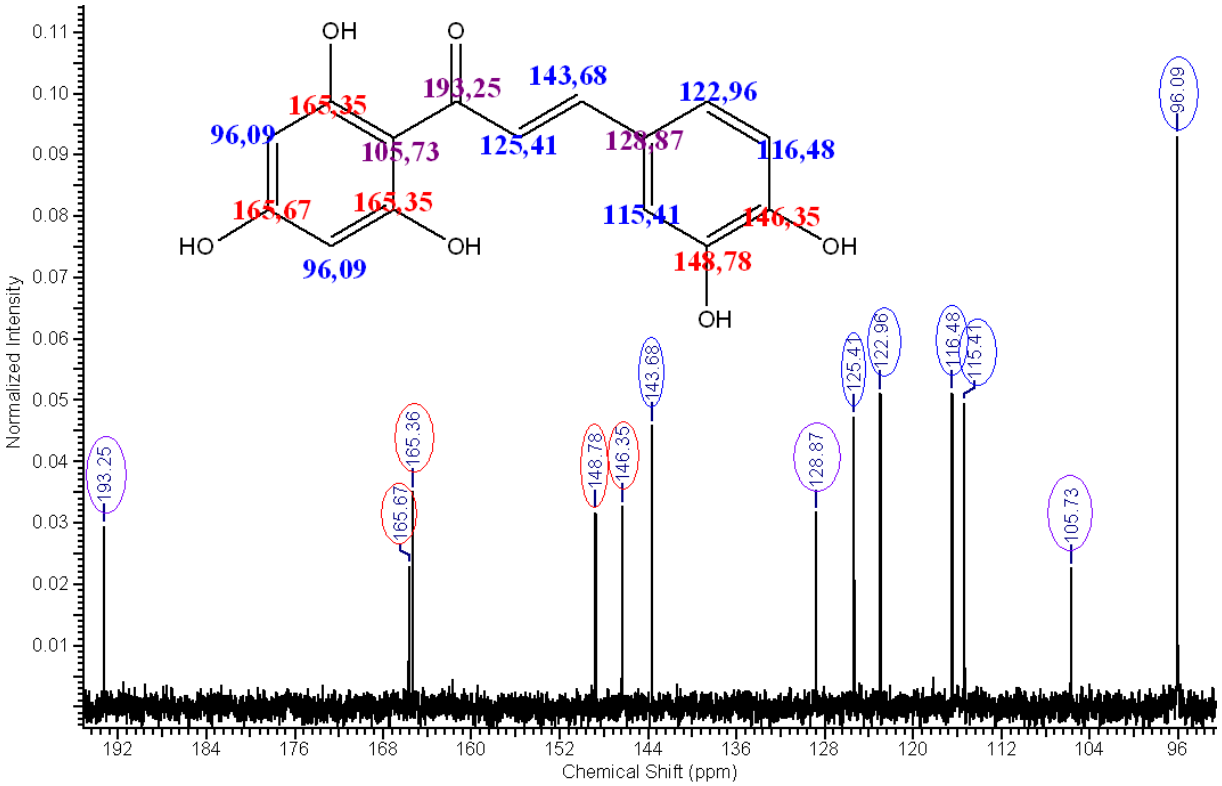


Fig. 44: ^{13}C-NMR of 2',3,4,4',6'-Pentahydroxychalcone in d_6_acetone

^{13}C-NMR shifts of 2',3,4,4',6'-pentahydroxychalcone:

δ [ppm]	Carbon	δ [ppm]	Carbon
96.09	C'3, C'5	143.68	Cβ
105.73	C'1	146.35	C4
115.41	C2	148.78	C3
116.48	C5	165.35	C'2, C'6
122.96	C6	165.67	C'4
125.41	Cα	193.25	C=O

Table 9: ^{13}C-NMR shifts of 2',3,4,4',6'-Pentahydroxychalcone in d_6_acetone

2',3,4,4'-Tetrahydroxychalcone (Butein. Fig. 45) shows a ^{1}H-NMR spectrum with all protons on ring A and on ring B being different. This leads to a doublet of doublets for H5' and H6. The H-C'6 is shifted most to the downfield. The reason is the mesomeric effect that was already described in the case of the ^{13}C spectrum of 2',3,4,4',6'-pentahydroxychalcone (see also fig. 43).

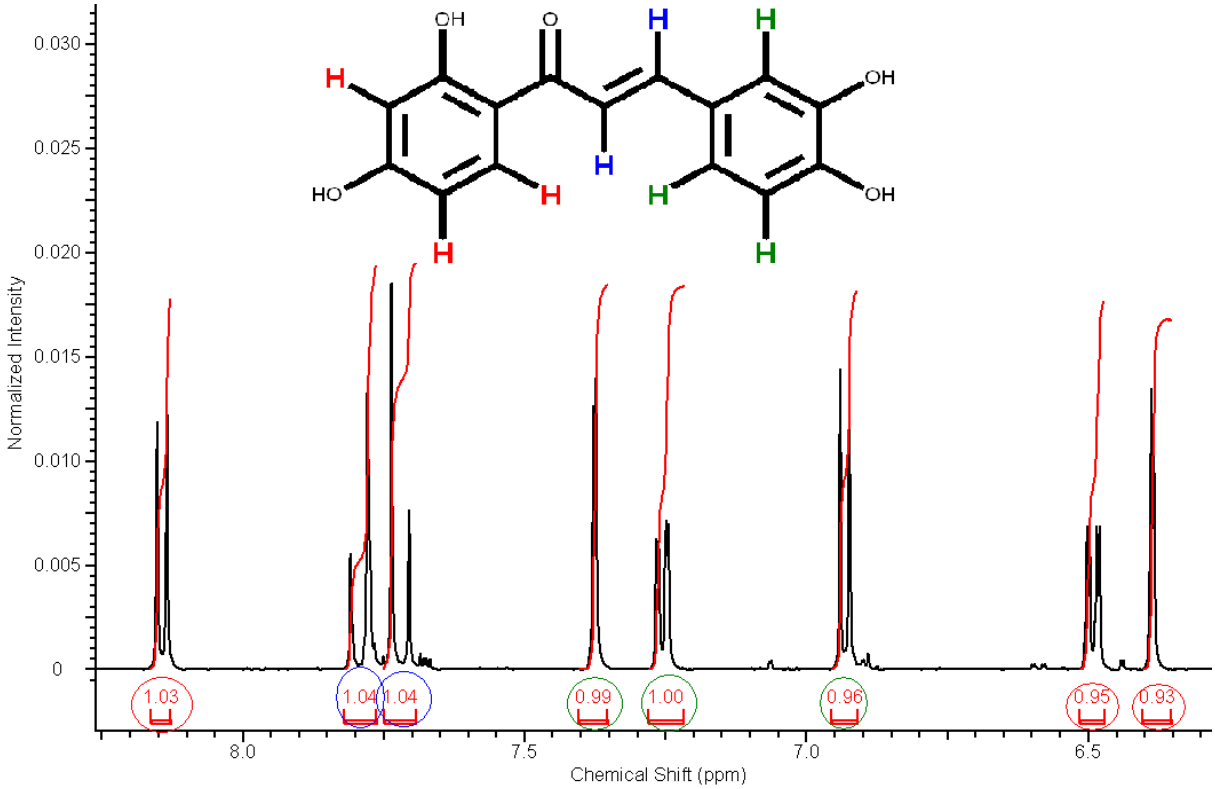


Fig. 45: ^{1}H-NMR of 2',3,4,4'-tetrahydroxychalcone in d_6_acetone

^{1}H-NMR shifts of 2',3,4,4'-tetrahydroxychalcone:

δ [ppm]	Intensity	Type of signal	Coupling constant [Hz]	Type of hydrogen
8.15	1 H	Doublet	8.96	H-C'6
7.80	1 H	Doublet	15.45	Hβ
7.72	1 H	Doublet	15.45	Hα
7.36	1 H	Doublet	2.05	H-C2
7.25	1 H	Doublet of doublets	8.20 / 2.05	H-C6
6.93	1 H	Doublet	8.2	H-C5
6.49	1 H	Doublet of doublets	8.96 / 2.40	H-C'5
6.38	1 H	Doublet	2.40	H-C'3

Table 10: 1H-NMR shifts of 2',3,4,4'-tetrahydroxychalcone (compared to Lit.[45])

4.2.3.3 Discussion

This last step of the polyhydroxychalcone synthesis leads to a complex mixture of products and the purification step is complicated with only few really pure fractions after liquid column chromatography. One reason for these side-reactions may be the formaldehyde which is formed in this deprotection step (Fig. 46). Formaldehyde is known to polymerize easily with all kinds of substrates[46].

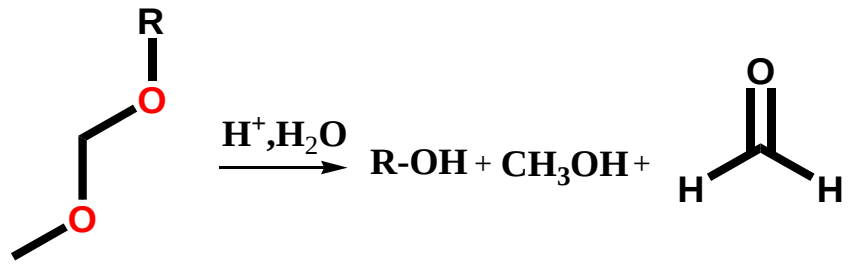


Fig. 46: Scheme of the cleavage of methoxymethyl ether in acidic conditions

Because of the low yields in this last step of polyhydroxychalcone synthesis, other synthetic approaches were investigated on 2',3,4,4',6'-pentahydroxychalcone to find a route of synthesis leading to higher yields and easier purification (see section 4.3).

4.2.4 Synthesis of 2',4,4',6'-tetrahydroxychalcone

4.2.4.1 Reaction scheme

2',4,4',6'-Tetrahydroxychalcone was synthesized by a ring-opening of its corresponding flavones naringenin (fig. 47)[47].

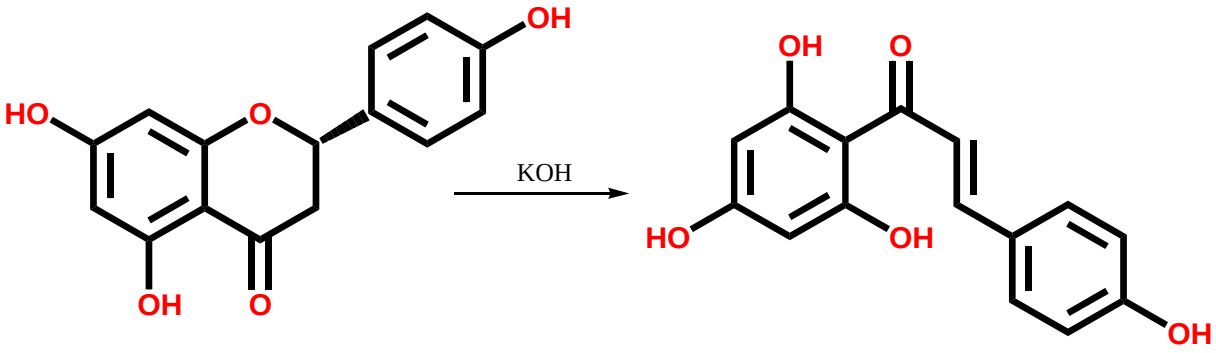


Fig. 47: Reaction scheme of ring opening of naringenin

4.2.4.2 1H-NMR

2',4,4',6'-Tetrahydroxychalcone (THC, Fig. 48) differs from PHC in the lack of the vicinale hydroxyl groups on ring B. This leads to a simpler ^{1}H-NMR spectrum, as the molecule is more symmetric.

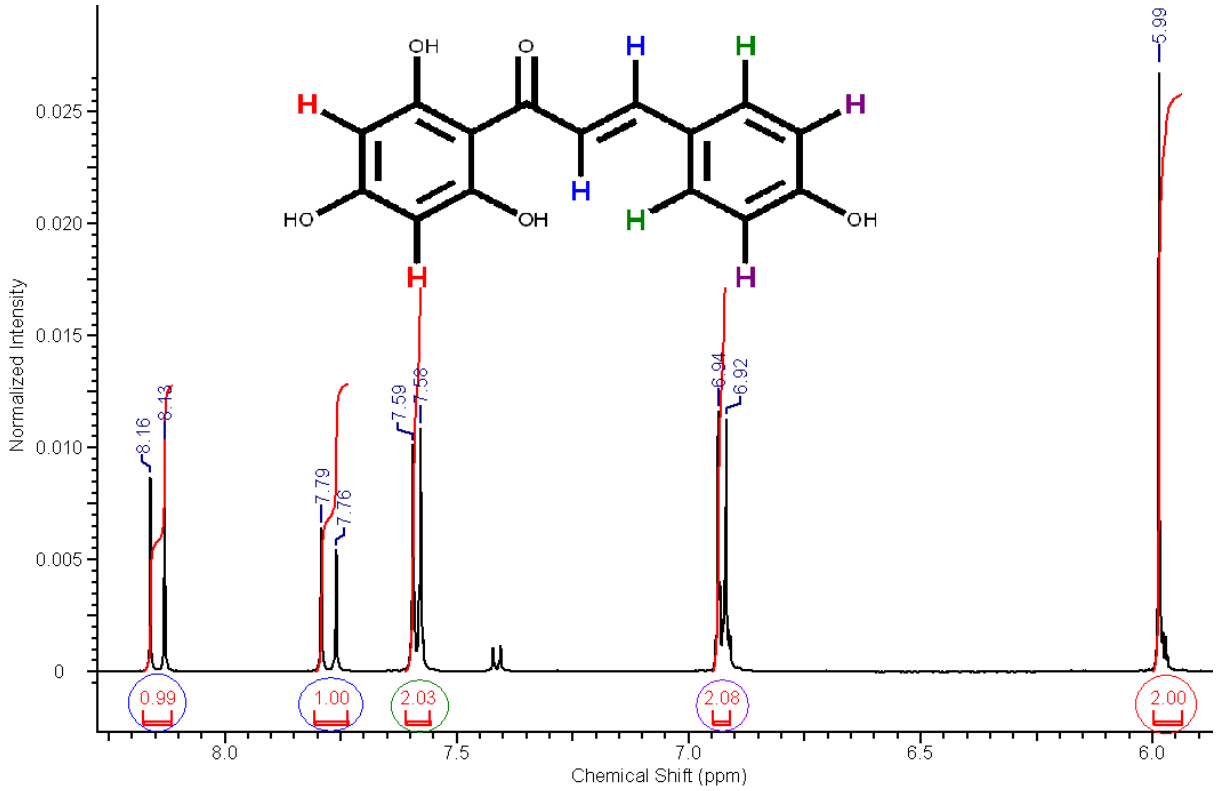


Fig. 48: ^{1}H-NMR of 2',4,4',6'-tetrahydroxychalcone in d_6_acetone

^{1}H-NMR shifts of 2',4,4',6'-tetrahydroxychalcone:

δ [ppm]	Intensity	Type of signal	Coupling constant [Hz]	Type of hydrogen
8.15	1 H	Doublet	15.45	Hβ
7.78	1 H	Doublet	15.45	Hα
7.58	2 H	Multiplet	8.67	H-C2, H-C6
6.93	2 H	Multiplet	8.51	H-C3, H-C5
5.99	2 H	Singlet	-	H-C'3, H-C'5

Table 11: ^{1}H-NMR shifts of 2',4,4',6'-tetrahydroxychalcone in d_6_acetone

The protons of the double bond are shifted most into the downfield. The six aromatic protons exhibit only three different peaks. This is due to the symmetry in both ring systems, which results in H-C'3 = H-C'5 in ring A, and H-C2 = H-C6, H-C3 = H-C5 in ring B. H-C2 and H-C3 are multiplets as the coupling of H-C2 with H-C3 is not the same as with H-C5.

4.3 Other synthetic approaches for 2',3,4,4',6-Pentahydroxychalcone

There are mainly three possible approaches to improve the synthesis of polyhydroxychalcones:

(1) Milder condition for the deprotection of MOM ether, leading to fewer by-products

(2) Establishing a direct way of polyhydroxychalcone synthesis to spare protection and deprotection
(3) Introducing a different protection group which can be cleaved under mild conditions

4.3.1 MOM ether cleavage

For the first approach, a mild way to cleave MOM ether selectively from phenolic hydroxyl groups was investigated[48]. This is done by using silica-supported sodium hydrogen sulfate as catalyst (Fig. 49).

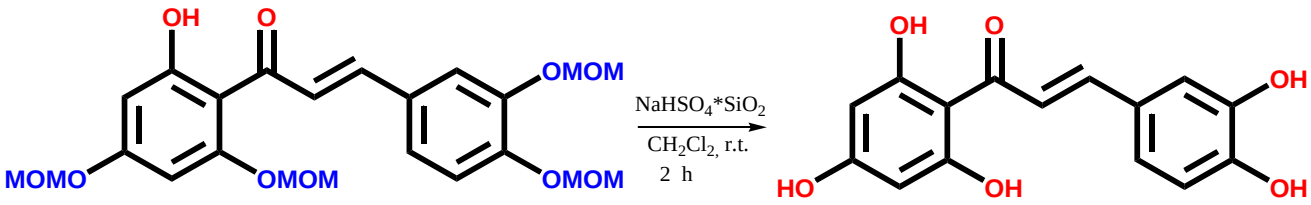


Fig. 49: Scheme of deprotection for 2'-Hydroxy-3,4,4',6'-Tetrakis(methoxymethoxy)

The heterogeneous catalyst was prepared by adding SiO_2 for column chromatography to an aqueous solution of $NaHSO_4$ and subsequently heating the mixture until a white precipitate was obtained. The solid was dried for 48 h at 120°C in an oven[49]. After the reaction mixture stirred for 2 h, the product obtained was directly purified via column chromatography (CH_2Cl_2 / MeOH: 95 / 5). All fractions collected were directly measured on an UV/Vis photometer to detect 2',3,4,4',6'-pentahydroxychalcone (PHC). However, none of the fractions showed the specific band of PHC at 378 nm and the ^{1}H-NMR spectrum of the collected fractions showed no deprotected pentahydroxychalcone. Thus, this method of deprotection seems to be inapplicable for polyhydroxychalcones.

4.3.2 Direct synthesis of pentahydroxychalcone

4.3.2.1 Reaction scheme

The second approach is to find a direct way to synthesize polyhydroxychalcones starting from the unprotected 2',4',6-Trihydroxyacetophenone and 3,4-Dihydroxybenzaldehyde. There are some papers describing methods to synthesize chalcones possessing one or two phenolic hydroxyl groups [6, 12]. One of the approaches is based on the in-situ formation of HCl by using $SOCl_2$ in ethanol. However, thionyl chloride is a highly reactive material and its reaction with ethanol is strongly exothermic[50]. Thus, an approach using another strong acid as catalyst was investigated. For this purpose, 2',4',6'-trihydroxyacetophenone and 3,4-dihydroxybenzaldehyde were dissolved in acetic acid (conc.) and stirred for 24 hours.

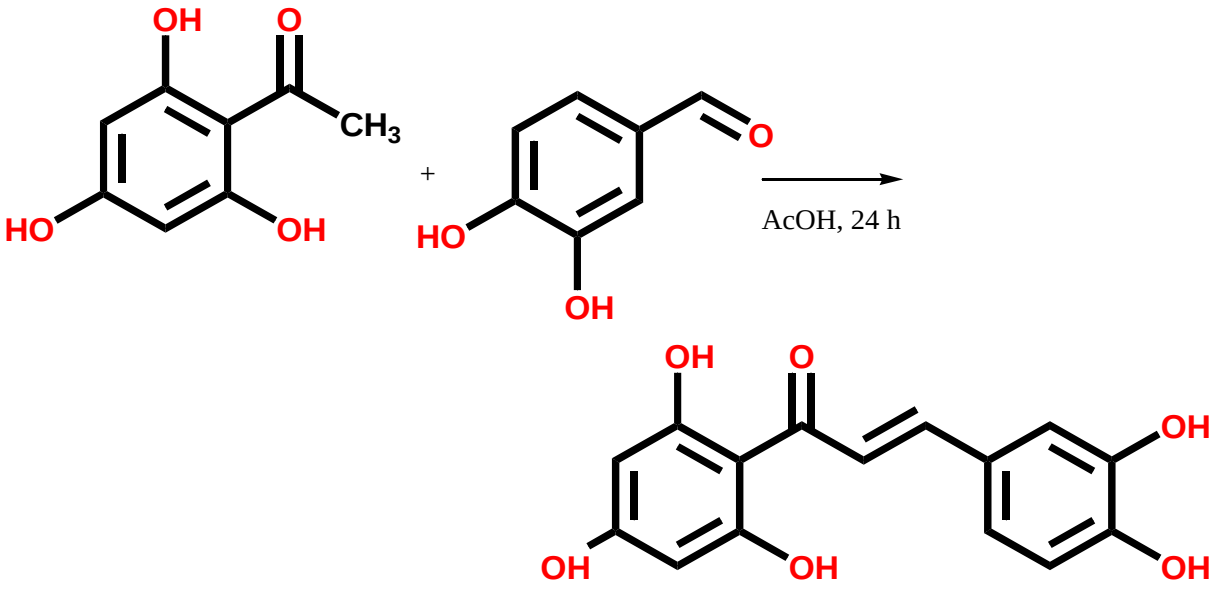


Fig. 50: Reaction scheme for the acidic direct coupling of 3,4-benzaldehyde and 2,4,6-trihydroxyacetophenone

4.3.2.2 ^{1}H-NMR spectrum

The results of this coupling reaction are shown in figure 49.

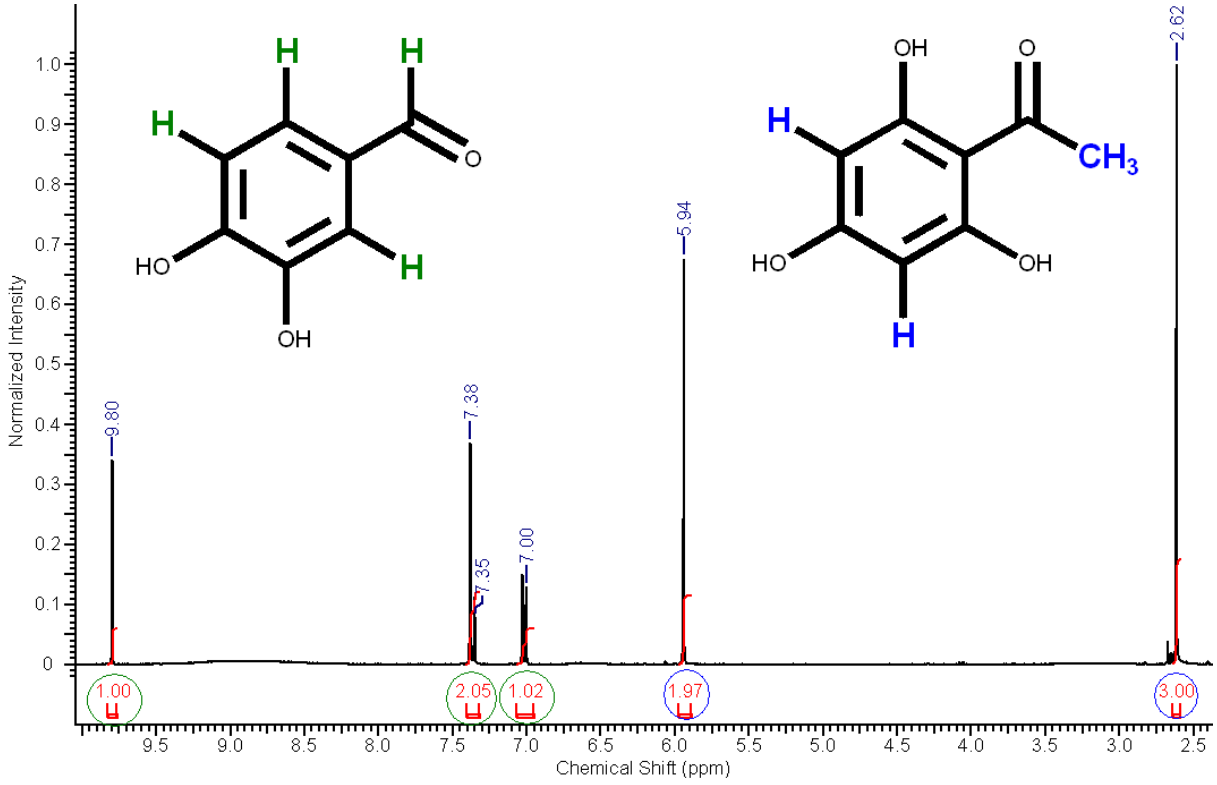


Fig. 49: ^{1}H-NMR of 3,4-dihydroxybenzaldehyde and 2',4',6'-trihydroxyacetophenone after acidic coupling in d_6_acetone

^{1}H-NMR shifts of 3,4-dihydroxybenzaldehyde and 2',4',6'-trihydroxyacetophenone:

δ [ppm]	Intensity	Type of signal	Coupling constant [Hz]	Type of hydrogen
9.80	1 H	Singlet	-	Carbonyl (BA)
7.38	1 H	Doublet	2.20	H-C2 (BA)
7.35	1 H	Doublet of doublets	7.78 / 2.20	H-C6 (BA)
7.00	1 H	Doublet	7.78	H-C5 (BA)
5.94	2 H	Singlet	-	H-C'3/H-C'5 (AP)
2.62	3 H	Singlet	-	Methyl (AP)

Table 12 : ^{1}H-NMR shifts of 3,4-dihydroxybenzaldehyde and 2',4',6'-trihydroxyacetophenone after acidic coupling in d_6_acetone

The ^{1}H-NMR spectrum shows the two starting materials next to each other. The lack of the double bond shows that the coupling did not occur. The coupling did not even occur to a small extent, suggesting that this approach is not employable to polyhydroxychalcone synthesis.

4.3.2.3 Discussion

It is not completely clear why an acidic aldol-condensation should be less obstructed by electron donating groups on the aromatic system (see Fig. 50) than the alkaline aldol-condensation as described by Petrov et al.

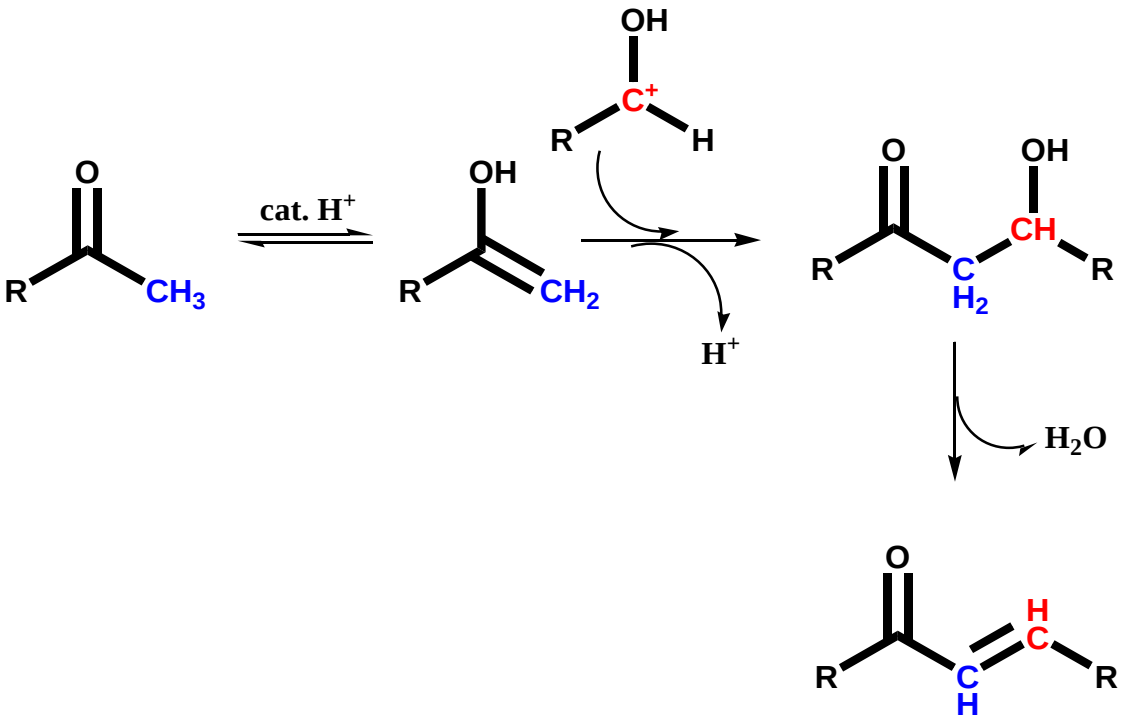


Fig. 50: Mechanism of acidic Claisen-Schmidt-Condensation

The acidic Claisen-Schmidt-Condensation should have one major advantage and one major disadvantage compared to the basic Claisen-Schmidt-Condensation seen from an electronic

point of view when having electron donating hydroxyl groups on the aromatic systems. The major advantage is that the negative effects of the electron donating groups on the pKa value of the acetophenone are becoming irrelevant, as there is no deprotonation step forming the enolate from the acetophenone. The pKa value of acetophenone is negatively influenced in the case of basic Claisen-Schmidt-Condensation which means that the enolate is less likely to be formed due to the electron donating groups. This is because electrons are pushed into an area of high electron density (the anion formed after deprotonation). This becomes irrelevant as the acidic Claisen-Schmidt-Condensation does not involve the formation of an enolate moiety. In the case of the benzaldehyde, the electronic situation is more complicated. It is true that the electron donating mesomeric effects of the hydroxyl groups are lower when they are protonated compared to their unprotonated relatives. However, they are still +M-substituent and so they lead to two opposite effects for the reactivity of the benzaldehyde. The first effect is that the benzaldehyde should be protonated more easily in the first place, as the cation is stabilized by the +M-effect. The second effect is obviously that the benzaldehyde is becoming a less strong electrophile because of this effect. This is even more important than in the case of the alkaline Claisen-Schmidt-Condensation, because the enol is a much weaker nucleophile than the enolate. The benzaldehyde has to be a really strong electrophile to be able to react with such a weak nucleophile as the enol is.

4.3.3 Indirect synthesis of pentahydroxychalcone by introduction of liable protecting group

4.3.3.1 Reaction scheme

The third possible approach includes the introduction of other, more liable protection groups. Silyl ethers have the great advantages that they can be cleaved selectively under mild conditions with fluoride ions. This is due to the strong affinity of silicon to fluoride ions with their high electro negativity. The Si-F bond possesses an energy of 142 kcal/mole compared to 112 kcal/mole for Si-O[51]. So, the cleavage of Si-O-protection and the formation of Si-F are thermodynamically favored. The protection itself is usually performed in alkaline conditions. Here, the synthesis was performed with imidazole as the base, leading to a nucleophilic substitution of chloride by the hydroxyl group of the benzaldehyde or acetophenone.

It is important to note that the chosen protecting group, tert-butyl-dimethyl-silyl-ether, is liable under strong alkaline reaction conditions[52]. Obviously, this leads to a problem as the coupling of the acetophenone and the benzaldehyde is performed in alkaline methanolic solution with a pH value of around 12-13. Therefore, a different coupling reagent is introduced according to Barron et. al.[53] Lithium bis(trimethylsilyl)amide is a strong non-nucleophilic base, thus it should not cleave the protecting groups and still be able to catalyze the reaction. The reaction scheme is shown in figure 51.

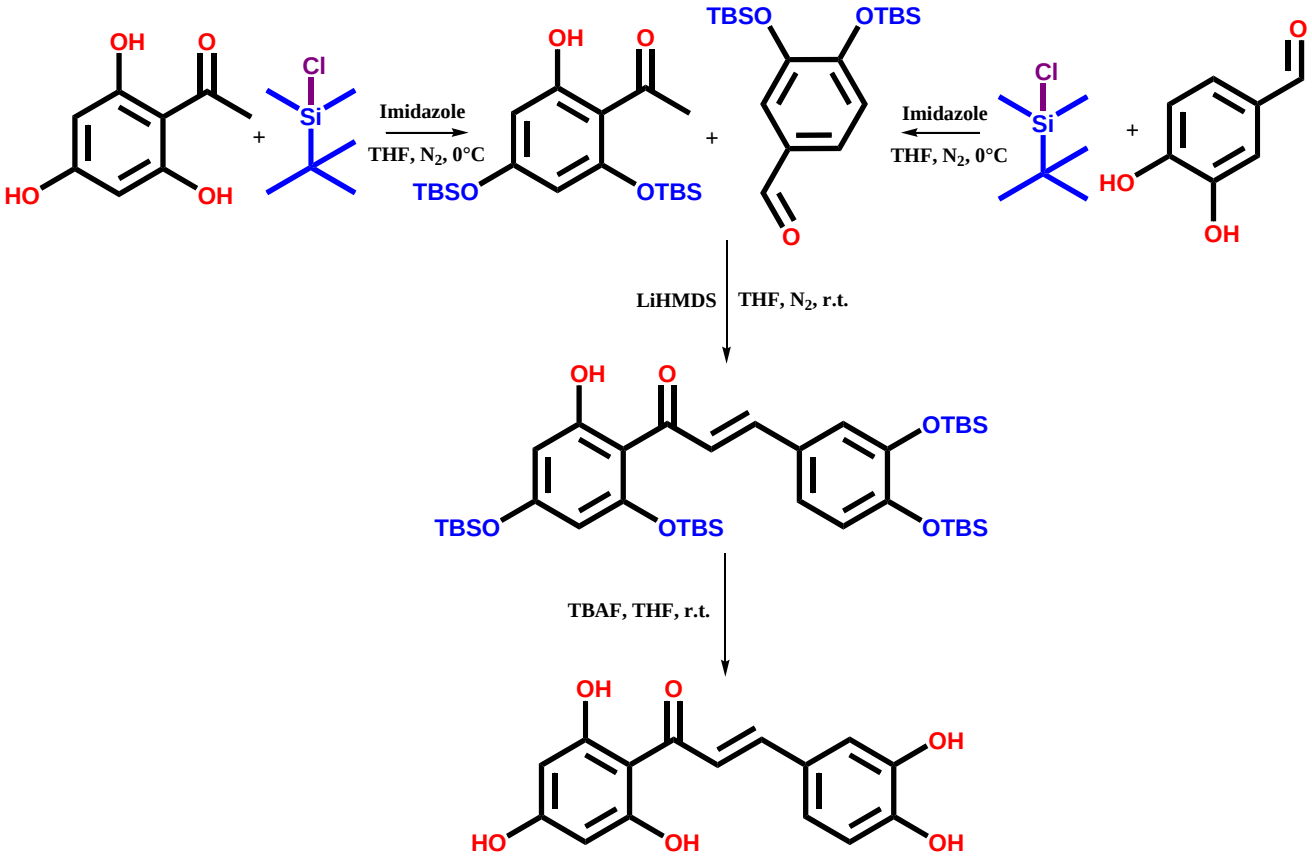


Fig. 51: Synthesis of 2',3,4,4',6'-pentahydroxychalcone via tert-butyldimethylsilyl ether protection and LiHMDS as coupling base

4.3.3.2 Spectroscopic analysis of protection

The protected 3,4-dihydroxybenzaldehyde and 2',4',6'-trihydroxycetophenone were purified via column chromatography and analyzed via ^{1}H-NMR.

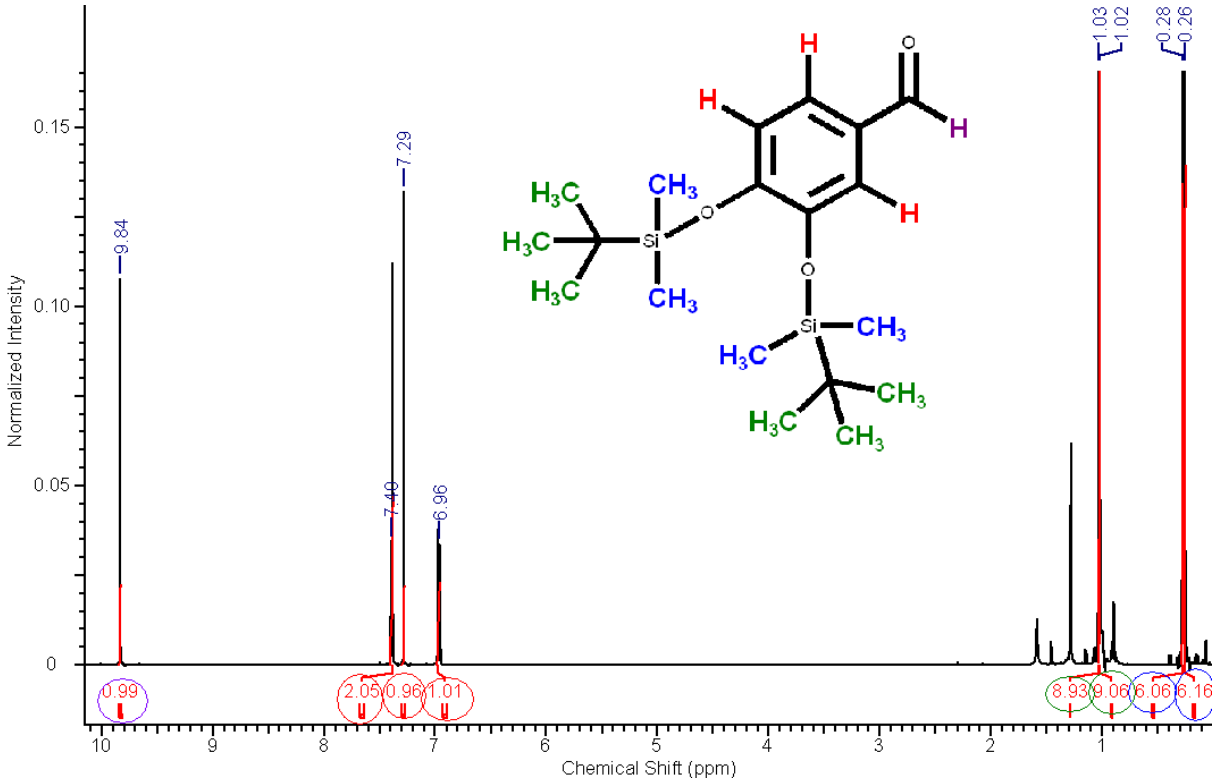


Fig. 52: ^{1}H-NMR of 3,4-O,O-bis(tert-butyldimethylsilyloxy)benzaldehyde in $CDCl_3$

1H-NMR shifts of 3,4-O,O-bis(tert.-butyldimethylsilyloxy)benzaldehyde

δ [ppm]	Intensity	Type of signal	Coupling constant [Hz]	Type of hydrogen
9.84	1 H	Singlet	-	Carbonyl hydrogen
7.40	1 H	Doublet of doublets	8.04 / 2.05	H-C6
7.38	1 H	Doublet	2.05	H-C2
6.96	1 H	Doublet	8.04	H-C5
1.03	9 H	Singlet	-	Methyl on tert.-butyl
1.02	9 H	Singlet	-	Methyl on tert.-butyl
0.28	6 H	Singlet	-	Methyl on silicon
0.26	6 H	Singlet	-	Methyl on silicon

Table 13: ^{1}H-NMR shifts of 3,4-O,O-Bis(tert-butyldimethylsilyloxy)benzaldehyde in $CDCl_3$

The spectrum of 3,4-O,O-bis(tert-butyldimethylsilyloxy)benzaldehyde (Fig. 52) is showing the 12 protons of the methyl groups connected to silicon very close to the TMS-NMR-standard (tetramethylsilan) at around 0.26 – 0.28 ppm. The 18 protons of the methyl groups on the two quaternary carbons of the protecting groups exhibit singlets at 1.03 ppm. The aromatic

protons of the benzaldehyde do not show the same structure as the MOM-protected benzaldehyde protons do. They show three doublets of doublets, indicating that the coupling in para-position of C-H5 and C-H2 is possible, even though with a small coupling constant. However, the aromatic protons are complicated to resolve as the peaks of C-H2 and C-H6 are overlapping. The carbonyl hydrogen exhibits a singlet at 9.84 ppm.

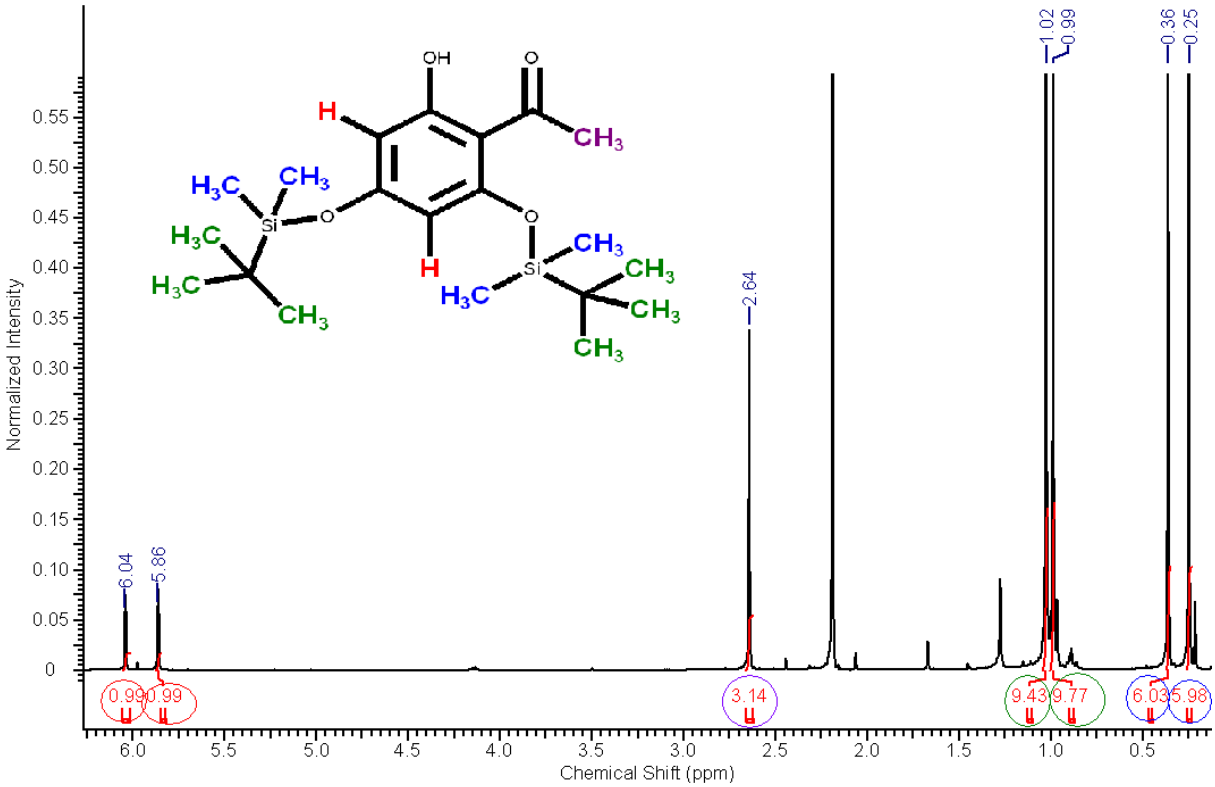


Fig. 53: ^{1}H-NMR of 2'-Hydroxy-4',6'-O,O-di(tert-butyldimethylsilyloxy)acetophenone in $CDCl_3$

^{1}H-NMR shifts of 2'-hydroxy-4',6'-O,O-di(tert-butyldimethylsilyloxy)acetophenone:

δ [ppm]	Intensity	Type of signal	Coupling constant [Hz]	Type of hydrogen
13.52	1 H	Singlet	-	Hydroxyl hydrogene
6.04	1 H	Doublet	2.4	H-C'5
5.86	1 H	Doublet	2.4	H-C'3
2.64	3 H	Singlet	-	Carbonyl methyl group
1.02	9 H	Singlet	-	t-butyl methyl
0.99	9 H	Singlet	-	t-butyl methyl
0.36	6 H	Singlet	-	Silicon attached methyl
0.25	6 H	Singlet	-	Silicon attached

				methyl

Table 14: ^{1}H-NMR shifts of 2'-Hydroxy-4',6'-O,O-di(tert-butyldimethylsilyloxy)acetophenone in $CDCl_3$

The spectrum of 2'-Hydroxy-4',6'-O,O-di(tert-butyldimethylsilyloxy)acetophenone (fig. 53) displays a singlet for the hydroxyl hydrogen at 13.52 ppm, the two aromatic protons forming a doublet are found in between 5.86 and 6.04 ppm. The three methyl protons at the carbonyl function exhibit a singlet at 2.64 ppm and the methyl groups of the protecting groups can be found between 0.25 and 1.02 ppm.

4.3.3.3 Discussion of protection step

The protection step resulted in moderate yields for both products, in the case of the benzaldehyde the desired product was obtained with a yield of 65%, in the case of the acetophenone 84% (compare: 63% for MOM-protection) of the product were obtained. However, the purification was a lot easier than for the MOM-protected reagents and thus, solvents, silica gel and time were saved by this method. Moreover, tBDMS is less toxic than the highly mutagenic MOM protection group[54]. So, the protection of 2',4',6'-trihydroxyacetophenone and 3,4-dihydroxybenzaldehyde with tBDMS is advantageous to the protection with MOM ethers.

4.3.3.4 Spectroscopic analysis of the coupling step

The second step in this synthesis is the coupling of the benzaldehyde with the acetophenone with LiHMDS as coupling reagent in dry THF. The step is performed in dry THF under nitrogen. The spectrum of the obtained purified products is shown in figures 54 and 55. The purification was performed via liquid chromatography with hexane / ethyl acetate (95 / 5).

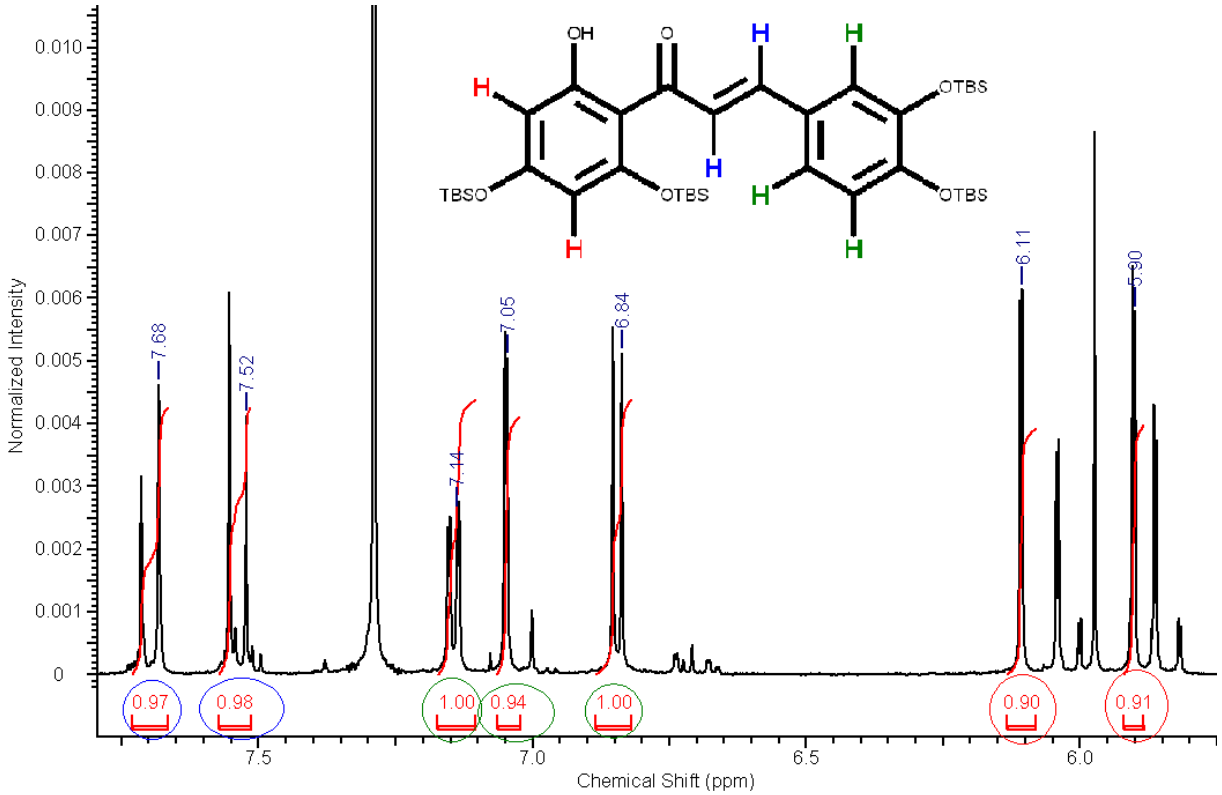


Fig. 54: ^{1}H-NMR of aromatic protons of 2'-hydroxy-3,4,4',6'-O,O,O,O-tetra(tert-butyldimethyl-silyloxy)chalcone in $CDCl_3$

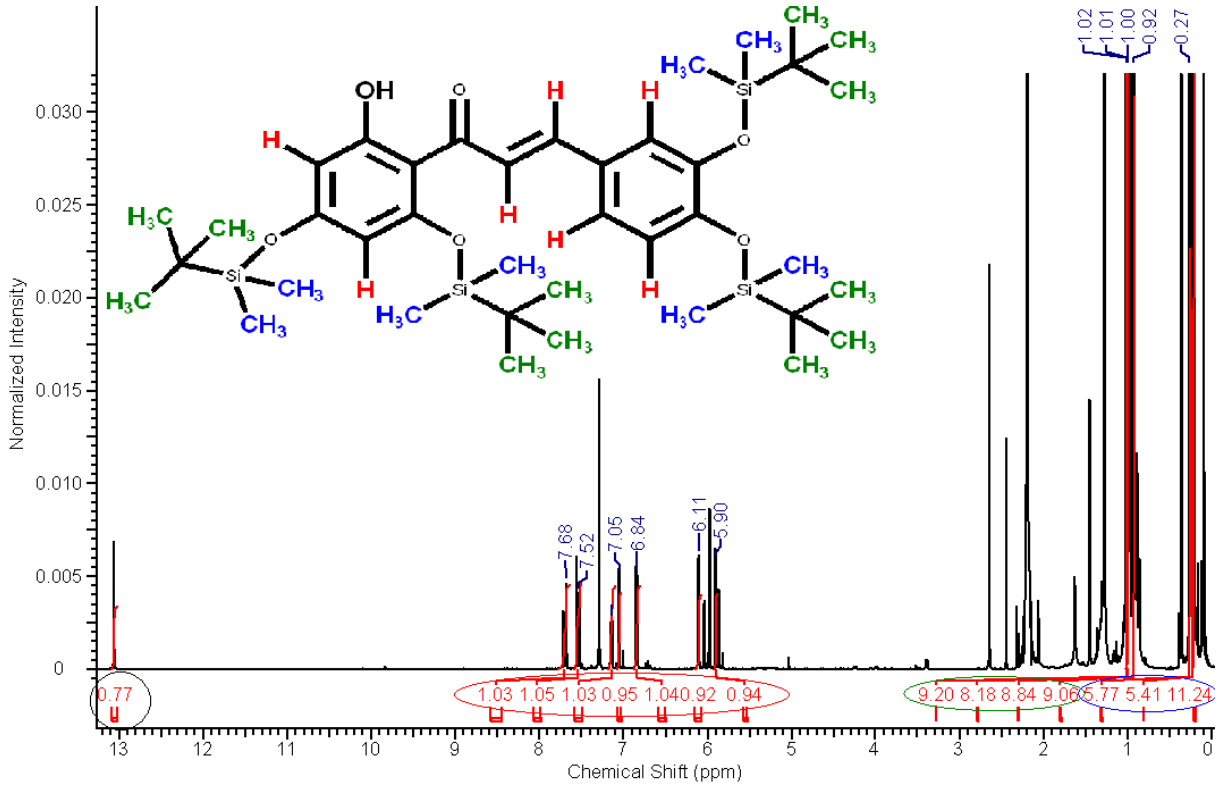


Fig. 55: 1H-NMR of 2'-hydroxy-3,4,4',6'-O,O,O,O-tetra(tert-butyldimethylsilyloxy)chalcone in $CDCl_3$

^{1}H-NMR shifts of 2'-hydroxy-3,4,4',6'-O,O,O,O-tetra(tert.-butyldimethylsilyloxy)chalcone:

δ [ppm]	Intensity	Type of signal	Coupling constant [Hz]	Type of hydrogen
13.06	1 H	Singlet	-	
7.68	1 H	Doublet	15.61	Hα/β
7.52	1 H	Doublet	15.61	Hα/β
7.13	1 H	Doublet of doublets	8.35 / 2.05	H-C6
7.05	1 H	Doublet	2.05	H-C2
6.84	1 H	Doublet	8.35	H-C5
6.11	1 H	Doublet	2.21	H-C'3/H-C'5
5.90	1 H	Doublet	2.21	H-C'3/H-C'5
0.92 – 1.03	4 x 9 H	Singlet	-	Methyl on tert-butyl
0.21 – 0.25	4 x 6 H	Singlet	-	Methyl on silicon

Table 15: 1H-NMR shifts of 2'-hydroxy-3,4,4',6'-O,O,O,O-tetra(tert-butyldimethylsilyloxy)chalcone in $CDCl_3$

As can be seen in fig. 54 and fig. 55, the coupling was successful and the double bond with the characteristic 15.6 Hz coupling constant can be found around 7.52-7.68 ppm. The other aromatic protons are all in the region between 5.90-7.13 ppm and all integrals fit well to the expectations. The methyl-groups on the tert-butyl rest and the silicon atom are shifted to the low field, where they are around 0.92-1.03 ppm and 0.21-0.25 ppm. However, there are also the peaks of the acetophenone in the mixture. They can be found in the aromatic region close to 6 ppm and in the low field for the protection groups. They can be distinguished because of the lower concentration of the acetophenone in the mixture, which leads to lower integrals for the peaks.

4.3.3.5 Discussion

The coupling reaction worked in low yields and the purification was very challenging and did not succeed totally as can be seen in fig 55. As the acetophenone is the starting material which is more difficult to remove by column chromatography, working with an excess of benzaldehyde could be a reasonable approach. Moreover, the LiHMDS used was a powder not dried before reaction which means that the use of dry LiHMDS in a dry THF solution could be a way to improve the yields strongly. The reaction worked already a lot better when LiHMDS was used in excess, which is a hint to certain humidity in the coupling reagent. LiHMDS is sensitive to water because it decomposes. This affects the reaction strongly, as LiHMDS is then not able to catalyze the reaction any longer. However, Barron et al.

described the coupling reaction to work with a yield of 25 – 40 %. This is not competitive enough to the alkaline catalyzed aldol condensation. Adjustments of the reaction conditions could lead to higher yields. The best reaction conditions still need to be investigated, but the potential of the reaction is high, especially because the protection step is working very well. Nevertheless, the deprotection step has to be tested as well. This was not finished in this paper because of the difficulties in the purification of the protected coupling product. Barron et al. claimed the deprotection to work quantitatively, suggesting the deprotection to be a less challenging step than the coupling. In any case, this has to be investigated in further studies.

4.4 Synthesis of polyhydroxychalcone metal complexes

After analyzing the crystal structure of a chalcone and synthesizing the polyhydroxychalcones with different methods, the last aim of this paper was to investigate the complexation reaction of polyhydroxychalcones with different metal ions.

The coordination of metal ions by 2'-hydroxy-chalcones was proposed to work through the 2'-hydroxy-carbonyl-functionality (fig. 56)[55].

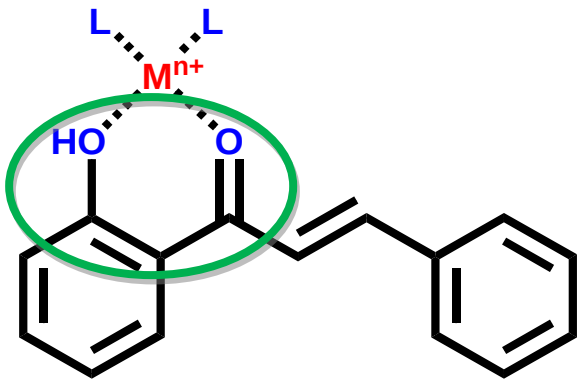


Fig. 56: 2'-hydroxy-carbonyl-functionality possibly responsible for metal ion coordination

The synthetic approaches for the metal complexes of the compounds discussed here were mainly established with the 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone. The reasons for this choice were multifarious: First, the 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone is not sensitive to oxidation as the 3,4-dihydroxygroups are protected Second, the complexation would be clearly performed by the 2'-hydroxy-carbonyl moiety and not by the 3,4-vicinale hydroxyl groups in the B-ring, offering results that could be interpreted easily. Moreover, the final step of polyhydroxychalcone synthesis, the deprotection step which provides poor yields, is superfluous. This offers the possibility to first set up the optimal reaction conditions for efficient complexation before utilizing the less disposable pentahydroxychalcone.

Two different synthetic approaches were examined. They are described in sections 7.3.12 and 7.3.13. The solids obtained were analyzed via mass spectrometry, as shown in figure 57 in the case of copper complexation.

4.4.1 Complexation with 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone

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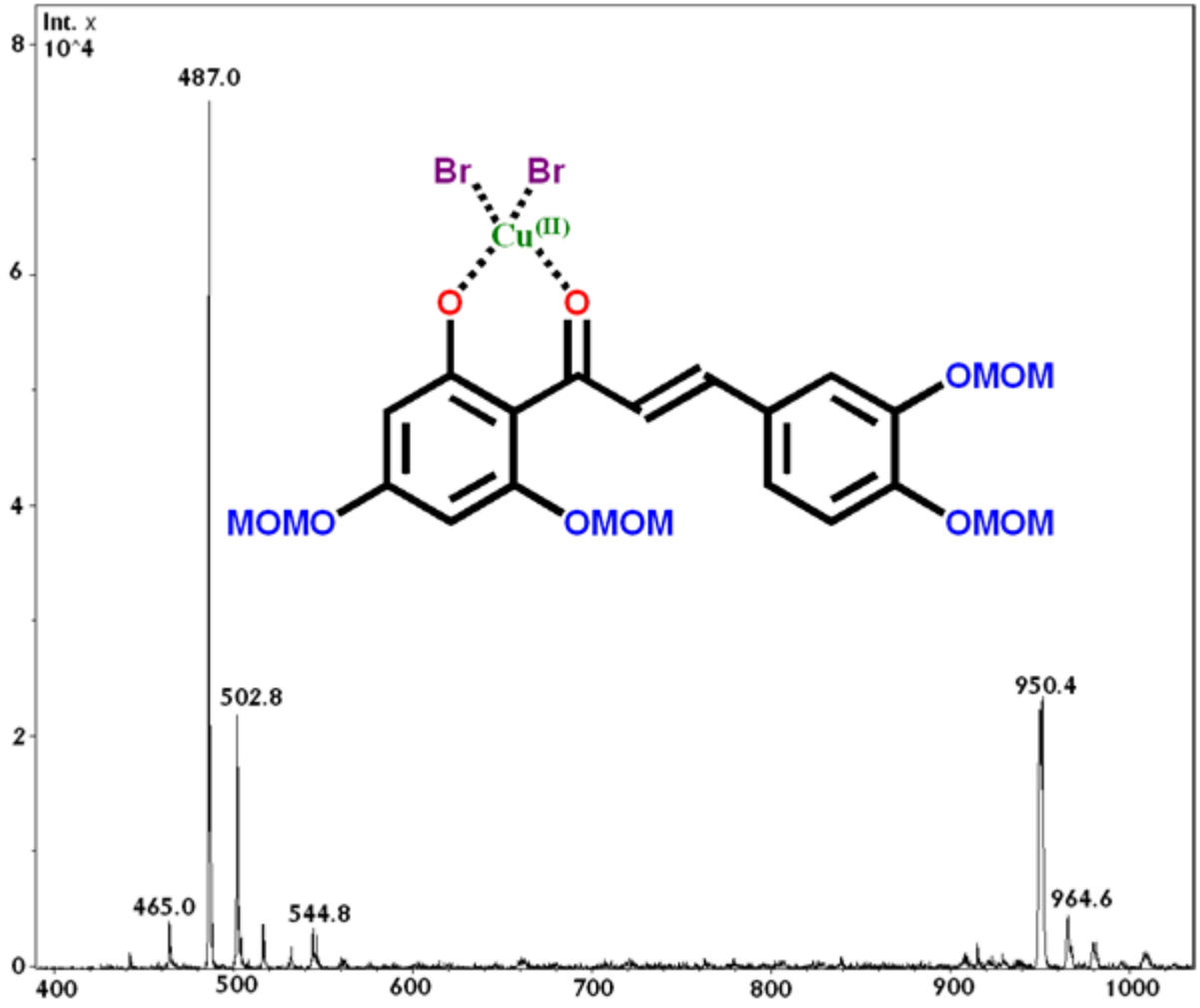


Fig. 57: ESI-mass spectrum (+) of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone complexated with copper(II) with a possible structure of the complex shown

Peaks obtained by mass spectrometry of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone complexated with copper(II):

Compound	Peak	Calculated mass
Ligand + H^+	465.0 m/z	465,18 m/z
Ligand + Na+	487.0 m/z	487,16 m/z
Ligand + K+	502.8 m/z	503,13 m/z
Ligand$^+$ + H^+ + Br^-	544.8 m/z	544.9 m/z
2 Ligand + Na^+	950.4 m/z	951,33 m/z

2 Ligand + 1 K^+	964.6 m/z	969.09 m/z

Table 16: ESI(+)-MS-Peaks for copper with 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone

None of the peaks shows a complex of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone with copper. The same applies to the mass spectrum for the complexation with nickel (Fig. 58).

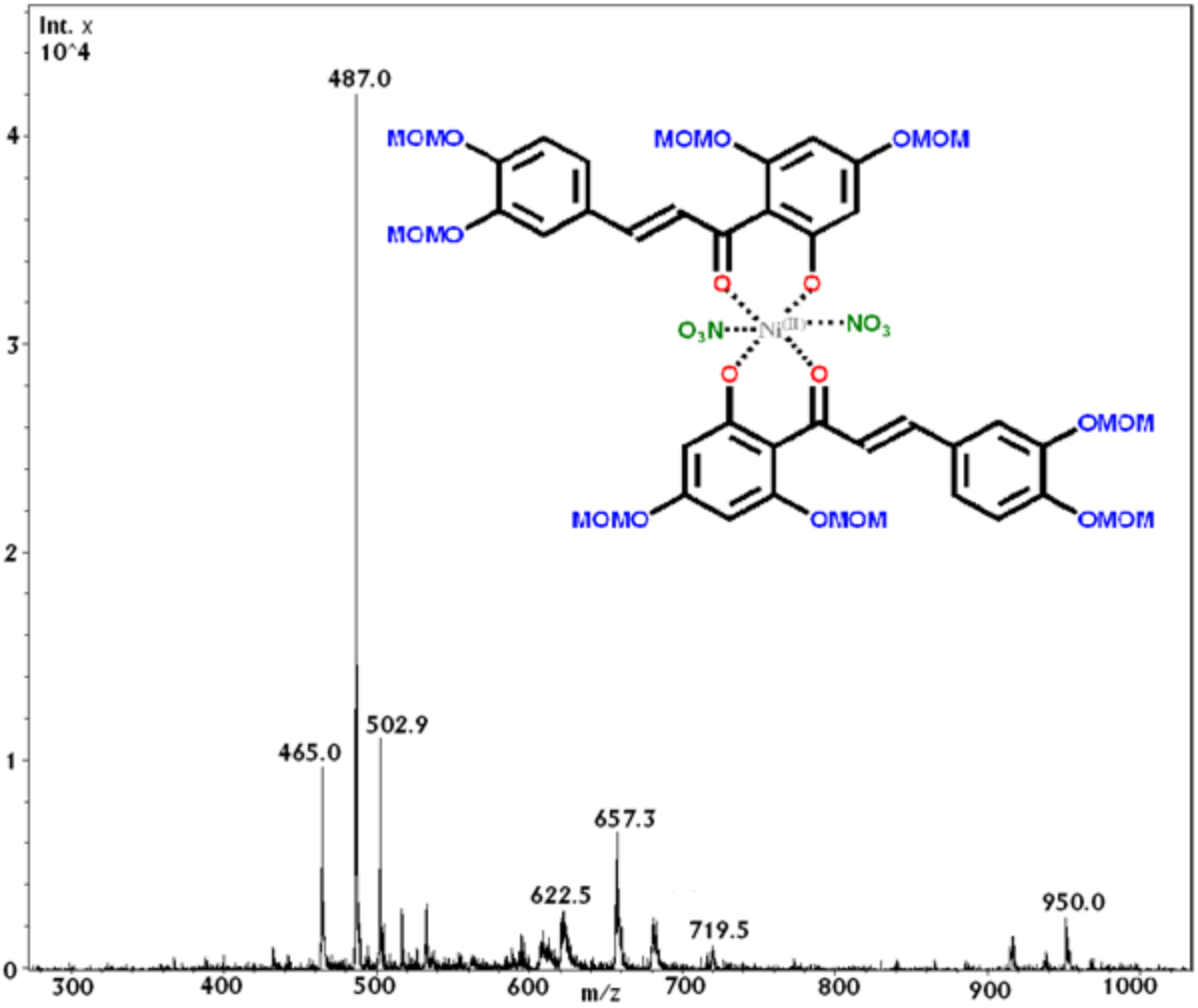


Fig. 58: ESI-mass spectrum (+) of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone complexated with nickel(II) and its possible structure

Peaks obtained by mass spectrometry of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone complexated with nickel(II):

Compound	Peak	Calculated mass
Ligand + H^+	465.0 m/z	465,18 m/z
Ligand + Na^+	487.0 m/z	487,16 m/z
Ligand + K^+	502.9 m/z	503,13 m/z

4 Ligand + 3 H^+ / z = 3	622.5 m/z	619.9 m/z
4 Ligand + 3 K^+ / z = 3	657.3 m/z	657.6 m/z
3 Ligand + 2 Na^+ / z = 2	719.5	719.5 m/z
2 Ligand + Na+	950.0	951 m/z

Table 17: ESI(+) MS peaks of nickel-2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone complex

All the peaks contribute to the ligand, while there are no hints to a formed complex. However, the complex might be too labile to be detected by mass spectrometry as the molecules are always ionized in mass spectrometry. The electron spray ionization (ESI) technique, which was applied in this experimental approach, is one of the gentlest ionization methods, normally leading to no fragmentation of the organic molecules at all[56]. However, complexes are compounds with electronic interactions, not with covalent bonds. So, they can dissociate much more easily than molecules with covalent bonds, especially when their complexation constant is low. Hence, other methods for detecting a complexation were applied, one of them being infrared spectroscopy. Infrared spectroscopy is based on the direct absorption of infrared radiation by molecular entities leading to a spectrum with specific peaks for specific functionalities in the molecule.

The spectra of the polyhydroxychalcone and its metal complexes show the same peak pattern in the fingerprint area (1500 – 400 cm^{-1}). The only difference in this area comes from the different concentrations of the solutions. The copper (blue line) solution was less concentrated than the solutions of the zinc complex and the ligand itself. Therefore, the peaks corresponding to the solvent acetonitrile (2260-2200 cm^{-1} -C≡N stretching (v), 1450 cm^{-1} twisting H-C≡N (v)) are stronger in the case of the copper complex. Around 3400-3200 a broad band can be found for the two complexes but not for the ligand alone. This peak indicates that water molecules are coordinated by the metal ions (-OH stretching (s) broad band around 3600-3200 cm^{-1} for complexated water compared to a defined peak at 3710 cm^{-1} for free water in solution[57]). However, the coordination of metal ions by the chalcone through the 2'-hydroxyl-carbonyl functionality should lead to a shift in the C=O asymmetrical stretching at 1650 cm^{-1}. That is not the case; the peak is at the same wave length for all three spectra.

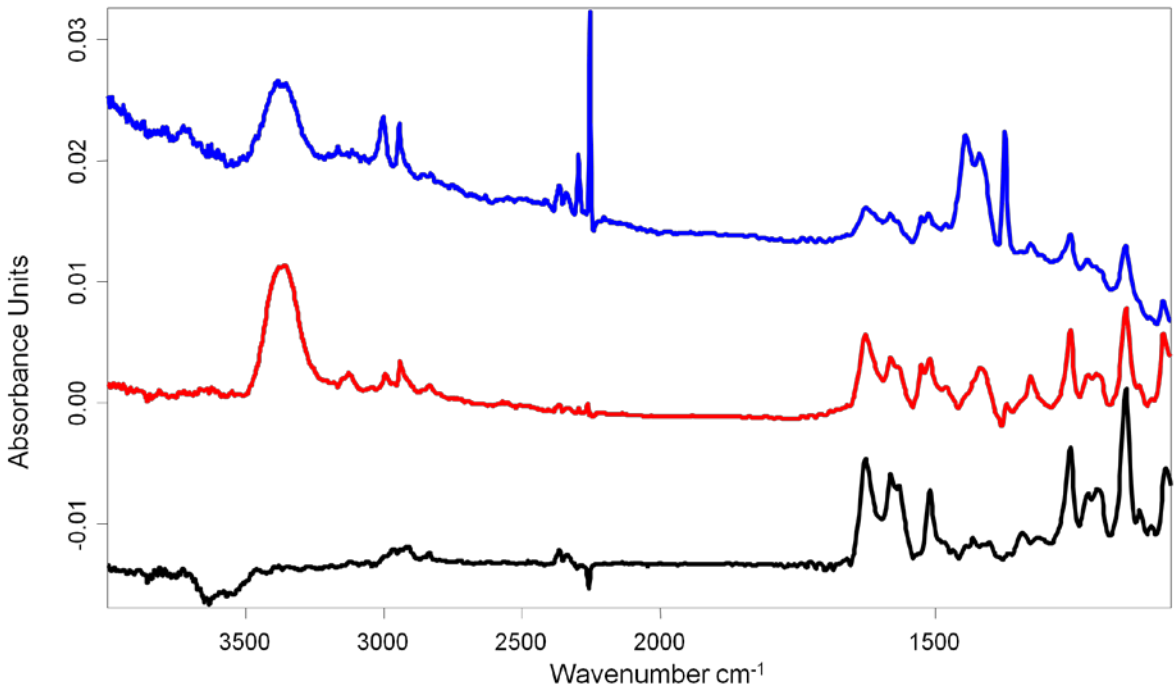


Fig. 59: Infrared spectra of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone (black line) and its copper (blue) and zinc (red) complexes dissolved in MeCN

Thus, the results of the infrared spectroscopy support the results that were obtained by mass spectrometry. No complexes of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone were isolated with the experimental approaches presented so far.

There are two possible reasons for this result. The first is the total lack of coordination of the metal ions by the ligand, which would mean that 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone is not able to bind zinc, nickel, cobalt, copper or iron at all. The second possible reason is that the complexation constant of this process is too low to result in a compound that can be isolated. In that case, coordination should be observable in UV/Vis spectroscopy.

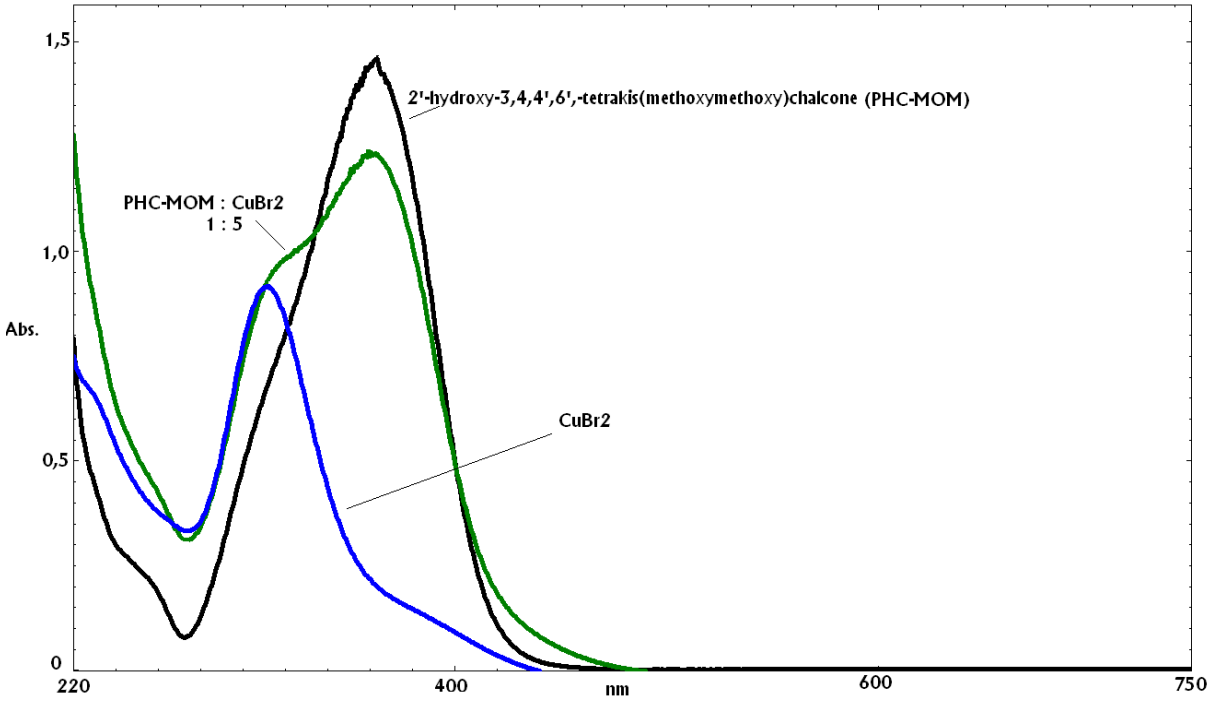


Fig. 60: UV/Vis-spectrum of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone complexated with $CuBr_2$ in methanol/acetonitrile (V/V = 1/1). Ligand (black line), metal salt (blue line) and ligand with metal salt in 1:5 ratio (green line).

For the complexation of copper(II)-bromide with 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone (fig. 60), the UV/Vis spectrum shows neither a shift of the ligand band nor the formation of a completely new band. Thus, there are no indications of a complexation, One would expect to see band shifts because of the ligand to metal charge-transfer. This result is confirmed a study of the behavior of other metal ions with 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone.

Nickel behaves in a similar manner to copper when added to 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone (fig. 61). No band shifts or new band formations can be observed.

The conclusion from these results is that the 2'-hydroxy-carbonyl-functionality in 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone is not a complexation area for the transition metals zinc, copper, and nickel. Thus, the question is to know if polyhydroxychalcones can perform complexations of these transition metals with their 3,4-vicinale-hydroxyl groups in the B-ring. To find out, UV/Vis-spectra of three different polyhydroxychalcones without any protection groups were performed with the metal ions.

4.4.2 Complexation with PHC, Butein and THC

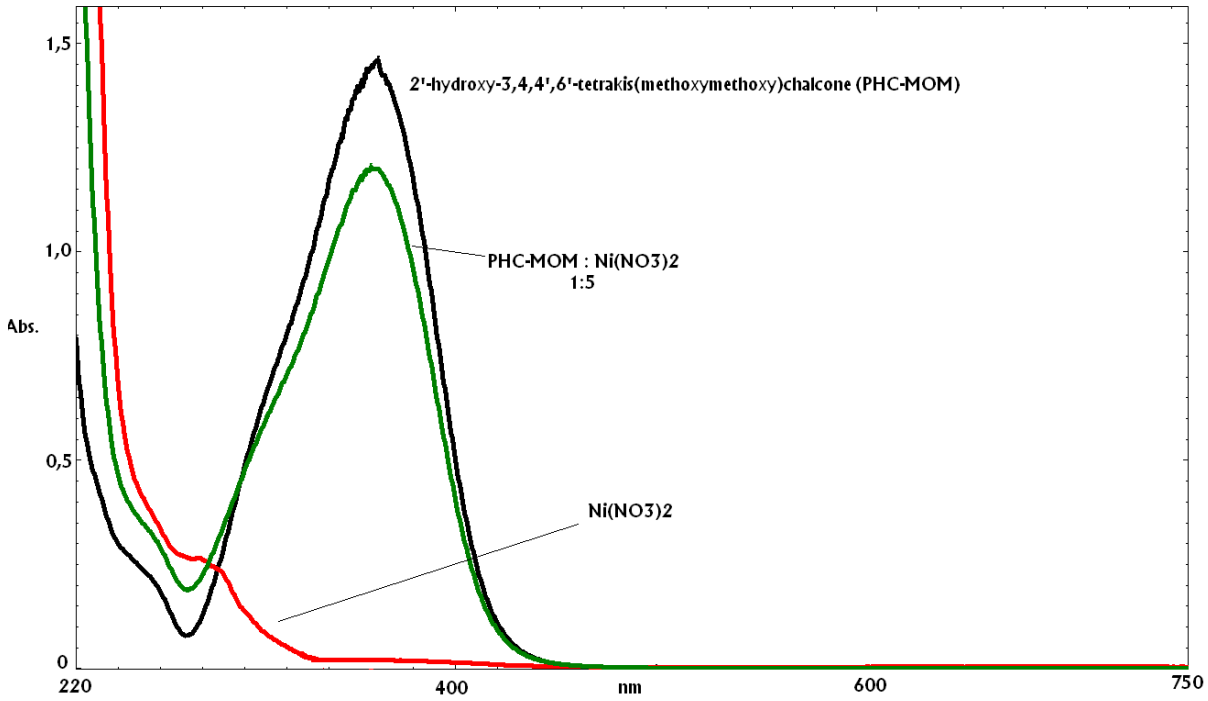


Fig. 61: UV/Vis-spectrum of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone complexated with $Ni(NO_3)_2$ in methanol/acetonitrile (V/V = 1/1). Ligand (black line), metal salt (blue line) and ligand with metal salt in 1:5 ratio (green line).

First, 2',3,4,4',6'-pentahydroxychalcone was treated with copper(II) bromide, nickel(II) nitrate and cobalt(II) chloride.

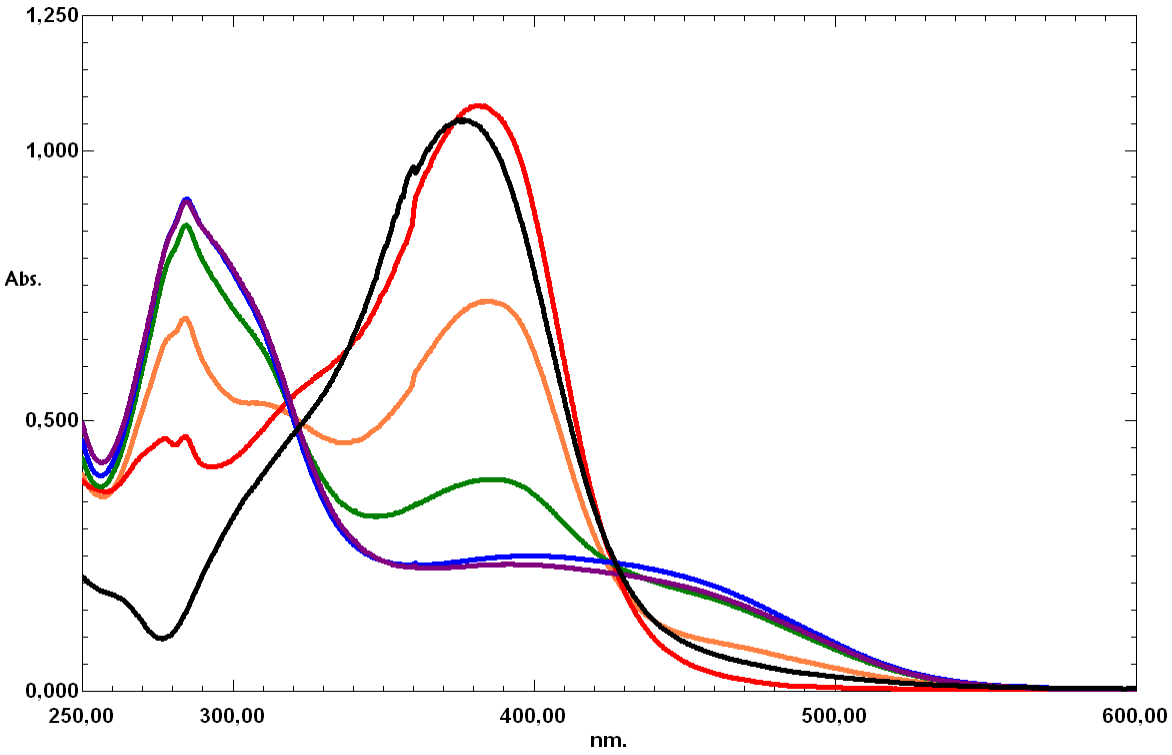


Fig. 62: UV/Vis-spectrum of 2',3,4,4',6'-pentrahydroxychalcone (PHC) complexated with $CuBr_2$ in methanol/acetonitrile (V/V = 1/1). Ligand (black line), Ligand – $CuBr_2$ (1:1, red line), PHC – $CuBr_2$ (1:2, orange line), PHC – $CuBr_2$ (1:3, green line), PHC – $CuBr_2$ (1:4, blue line), PHC – $CuBr_2$ (1:5, purple line)

2',3,4,4',6'-Pentahydroxychalcone (PHC) treated with copper(II)bromide leads to a spectrum (Fig. 62) with a strong decrease of the PHC band at 374 nm when $CuBr_2$ is added. Furthermore, the band is broadened, resulting in a broad band from 340 nm – 500 nm. A new peak can be found around 280 nm, showing a different pattern than the metal salt alone (compare Fig. 60). These results are in line with the results obtained from the complexation of PHC with cobalt (II) chloride (Fig. 63).

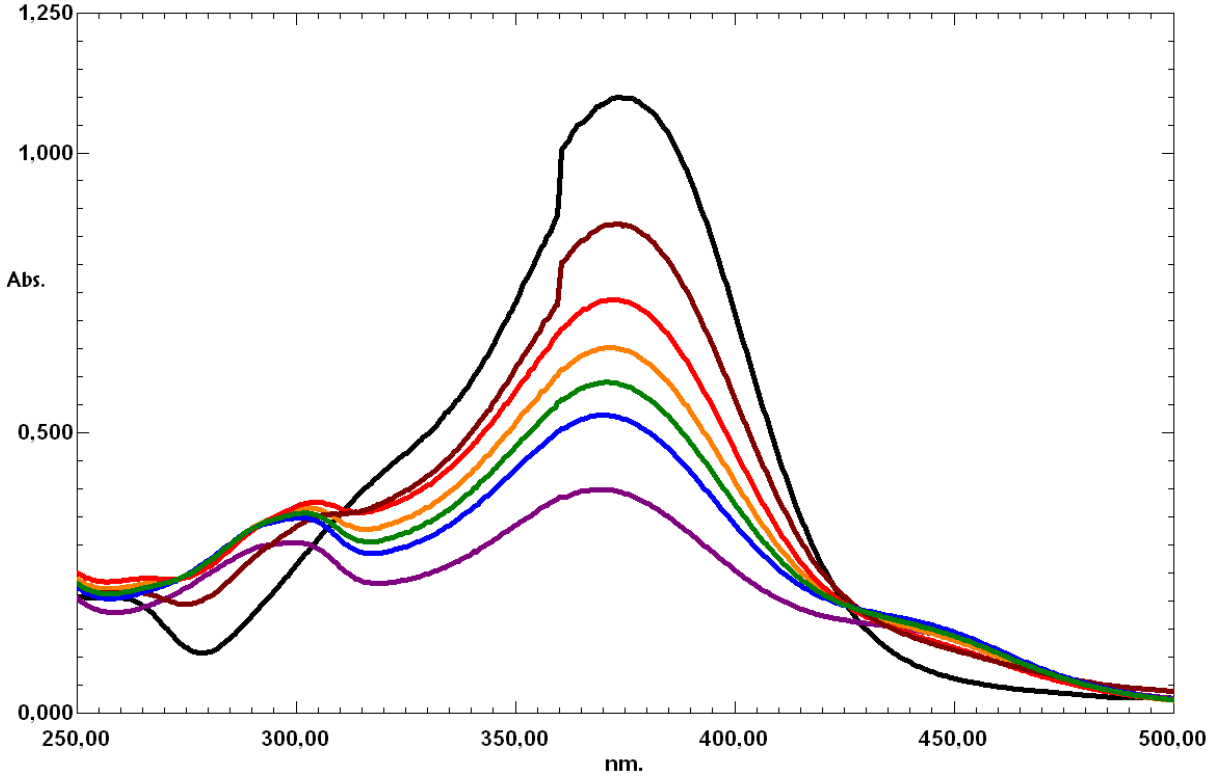


Fig. 63: UV/Vis-spectrum of 2',3,4,4',6'-pentrahydroxychalcone (PHC) complexated with $CoCl_2$ in methanol/acetonitrile (V/V = 1/1). Ligand (black line), PHC – $CoCl_2$ (1:1, brown line), PHC – $CoCl_2$ (1:2, red line), PHC – $CoCl_2$ (1:3, orange line), PHC – $CoCl_2$ (1:4, green line), PHC – $CoCl_2$ (1:5, blue line), PHC – $CoCl_2$ (1:10, purple line)

Cobalt (II) chloride leads to the same strong decrease in the PHC band at 374 nm as copper does. The broad band in the range 340-500 nm can be found, as well. However, this band looks more like two discrete bands rather than just one broad band. Still, the results suggest that the cobalt acts in a similar manner on PHC as copper does.

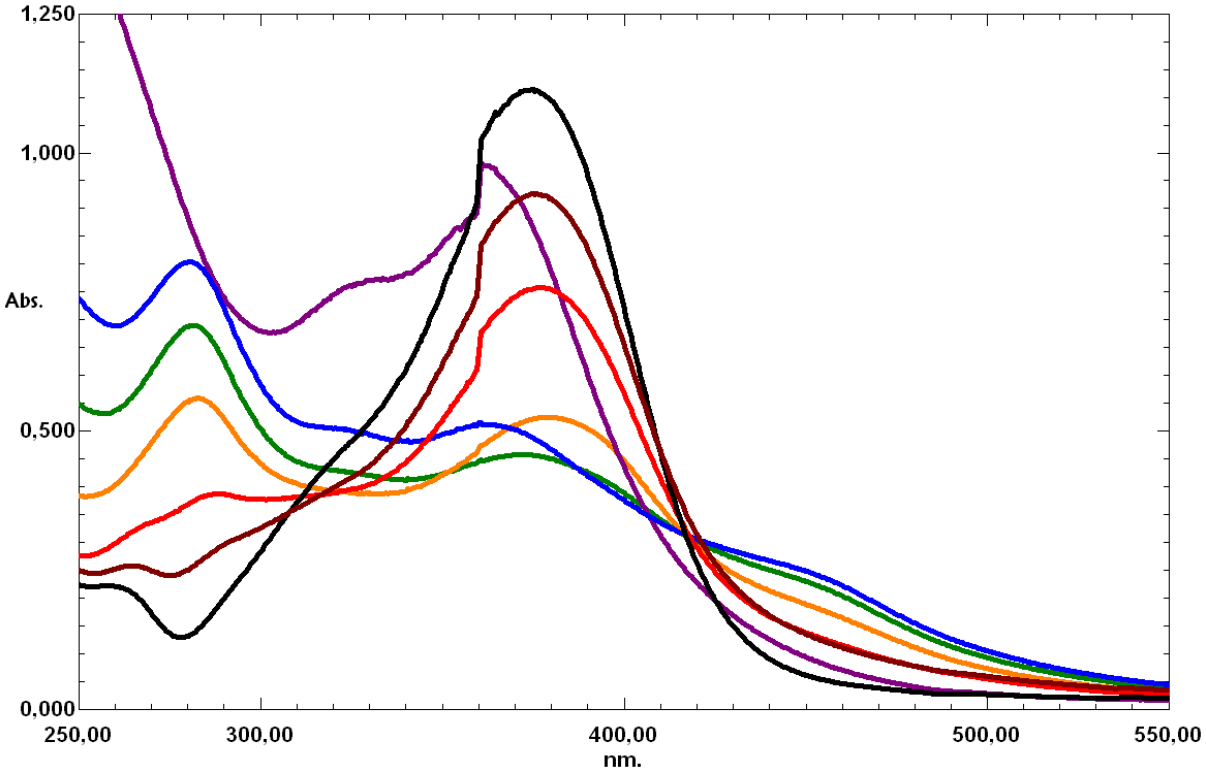


Fig. 64: UV/Vis-spectrum of 2',3,4,4',6'-pentrahydroxychalcone (PHC) complexated with $FeCl_3$ in methanol/acetonitrile (V/V = 1/1). Ligand (black line), PHC – $FeCl_3$ (1:1, brown line), PHC – $FeCl_3$ (1:2, red line), PHC – $FeCl_3$ (1:3, orange line), PHC – $FeCl_3$ (1:4, green line), PHC – $FeCl_3$ (1:5, blue line), $FeCl_3$ (purple line)

PHC treated with iron(III)chloride leads to spectrum (fig. 64) with the same band broadening as with cobalt(II) and copper(II). It is not clear if it is one broad band from 360-500 nm as in copper(II) or more like two distinct bands as in cobalt(II). The bands of $FeCl_3$ make this interpretation difficult. In any case, iron(III), cobalt(II) and copper(II) seem to act in similar manners with PHC. Interestingly, this is not the case for nickel (II) nitrate (Fig. 65).

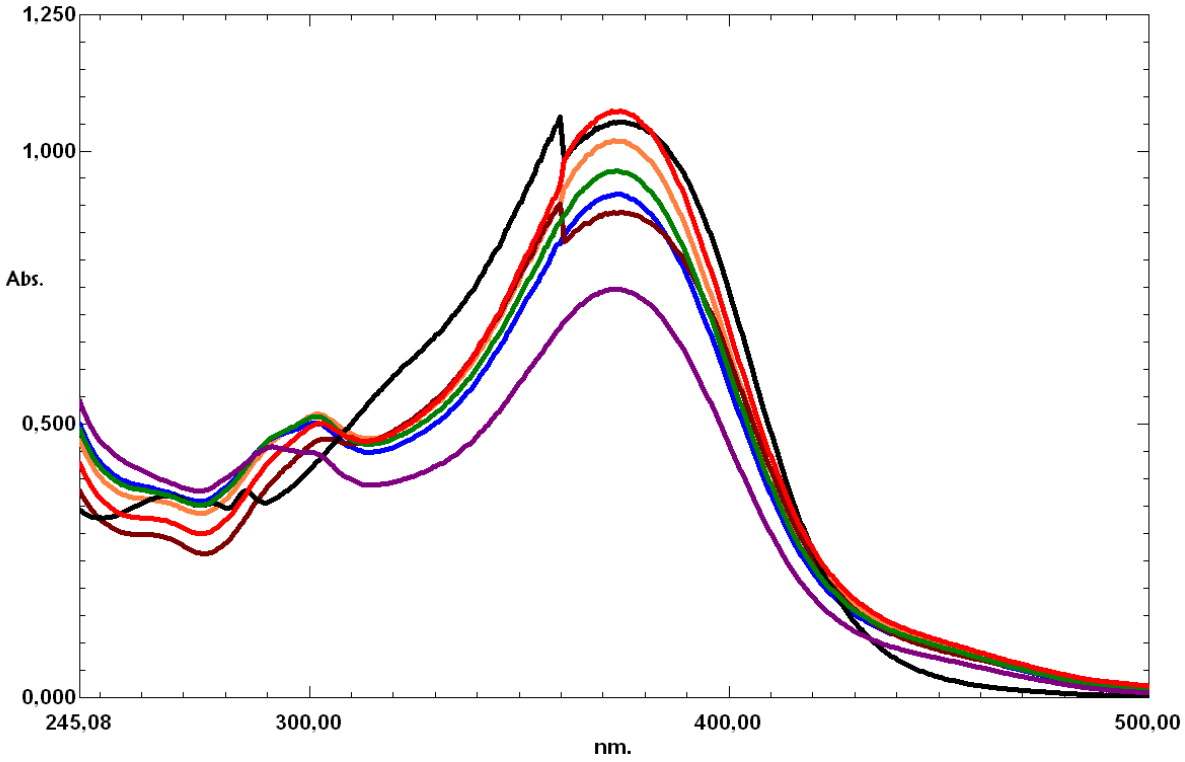


Fig. 65: UV/Vis-spectrum of 2',3,4,4',6'-pentrahydroxychalcone (PHC) complexated with $Ni(NO_3)_2$ in methanol/acetonitrile (V/V = 1/1). Ligand (black line), Ligand – $Ni(NO_3)_2$ (1:1, brown line), PHC – $Ni(NO_3)_2$ (1:2, red line), PHC – $Ni(NO_3)_2$ (1:3, orange line), PHC – $Ni(NO_3)_2$ (1:4, green line), PHC – $Ni(NO_3)_2$ (1:5, blue line), PHC – $Ni(NO_3)_2$ (1:10, purple line)

As can be seen in the spectrum of nickel (II) nitrate, there is no such broadening of the band. The band decrease of PHC is just due to the dilution by the additional solution that is added when $Ni(NO_3)_2$ is added. So, it seems like nickel is acting in a different manner than copper, iron and cobalt do. Neither a ligand-metal-charge transfer nor anything similar can be observed. However, in the case of complexation there should be a shift or formation of a new band as nickel is a classical transition metal with an [Ar] $3d^8$ electron configuration. Electrons from the ligand could be used to fill the d-electrons to $3d^{10}$.

Interestingly, nickel (II) has a lower redox potential than copper(II), cobalt (II) and iron (III)[58]. That means that it does not oxidize other compounds as easily as the other metal ions do. The oxidation process was already shown in figure 15 when the radical scavenging properties of polyhydroxychalcones were discussed. The polyhydroxychalcones that are able to perform radical scavenging best possess the 3,4-hydroxyl moiety in the B-ring[8]. If the observed spectrochemical shifts in fig. 62-64 were due to radical scavenging, the oxidation should be possible best for chalcones with the 3,4-hydroxyl groups and a lot worse for a polyhydroxychalcone without these functionalities like 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone or 2',4,4',6'-tetrahydroxychalcone (THC). However, the

same bands as for the PHC should be found for Butein (2',3,4,4'-tetrahydroxychalcone). The reaction of Butein with copper(II) bromide is shown in figure 66.

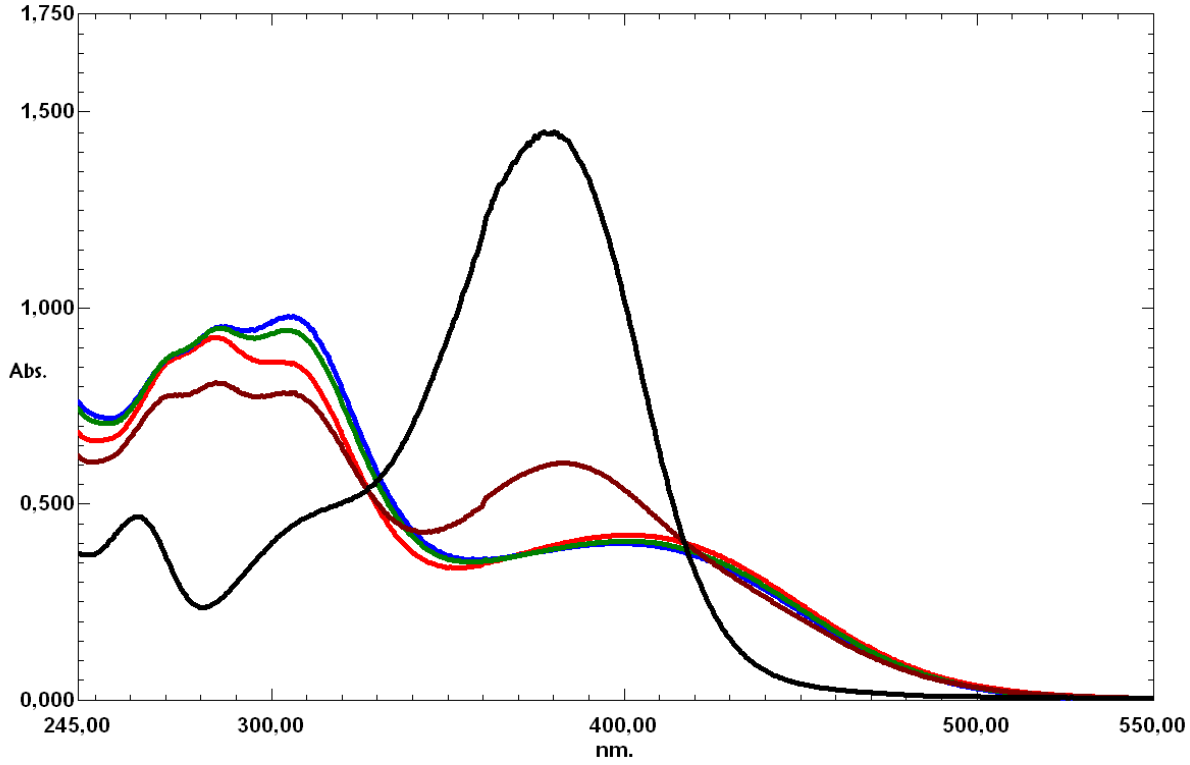


Fig. 66: UV/Vis-spectrum of 2',3,4,4'-tetrahydroxychalcone (Butein) complexated with $CuBr_2$ in methanol/acetonitrile (V/V = 1/1). Ligand (black line), Ligand – $CuBr_2$ (1:1,5; brown line), PHC – $CuBr_2$ (1:2, red line), PHC – $CuBr_2$ (1:4, green line), PHC – $CuBr_2$ (1:5, blue line)

The spectrum behaves in a very similar manner to figure 62 and 63. Obviously a new compound is formed that exhibits a broad band from 340-500 nm instead of the Butein with its peak at 378 nm. Even small amounts of copper (brown line is 1.5 times $CuBr_2$ to Butein) lead to this change. However, the spectrum does not change much when more $CuBr_2$ is added, suggesting that the product can be obtained with a small amount of copper (II) bromide.

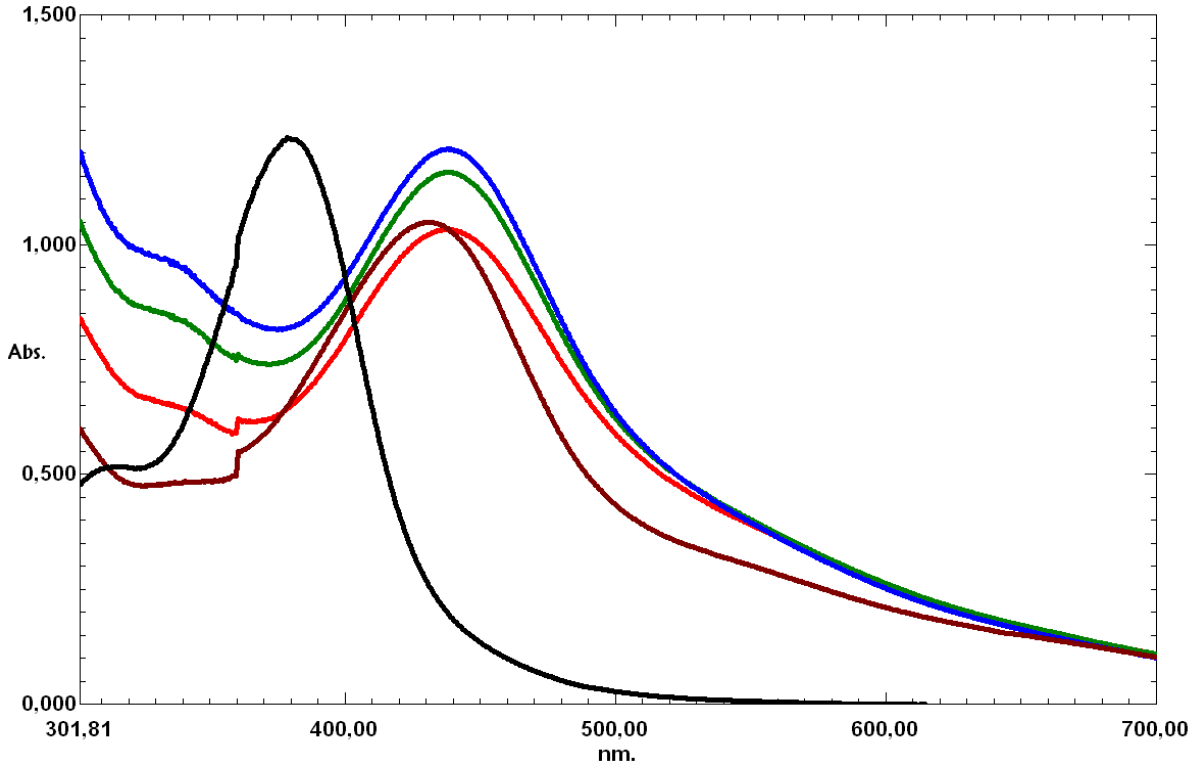


Fig. 67: UV/Vis-spectrum of 2',3,4,4'-tetrahydroxychalcone (Butein) complexated with $FeCl_3$ in methanol/acetonitrile (V/V = 1/1). Ligand (black line), Ligand – $FeCl_3$ (1:1, brown line), PHC – $FeCl_3$ (1:2, red line), PHC – $FeCl_3$ (1:4, green line), PHC – $FeCl_3$ (1:5, blue line)

Iron (III) chloride reacts with Butein by a shift of the main peak from 378 nm to 437 nm and by a broadening of this band up to more than 700 nm. However, this behavior is different to that of copper (II) towards Butein. A clear peak can be defined, which is not the case for the copper with Butein reaction. This suggests the possibility that iron acts in a different manner on polyhydroxychalcones than copper does.

In contrast to PHC, Butein reacts in the same way with nickel and cobalt. This can be concluded from figures 68 and 69. Both metal ions seem inert to Butein while cobalt led to a band broadening of PHC.

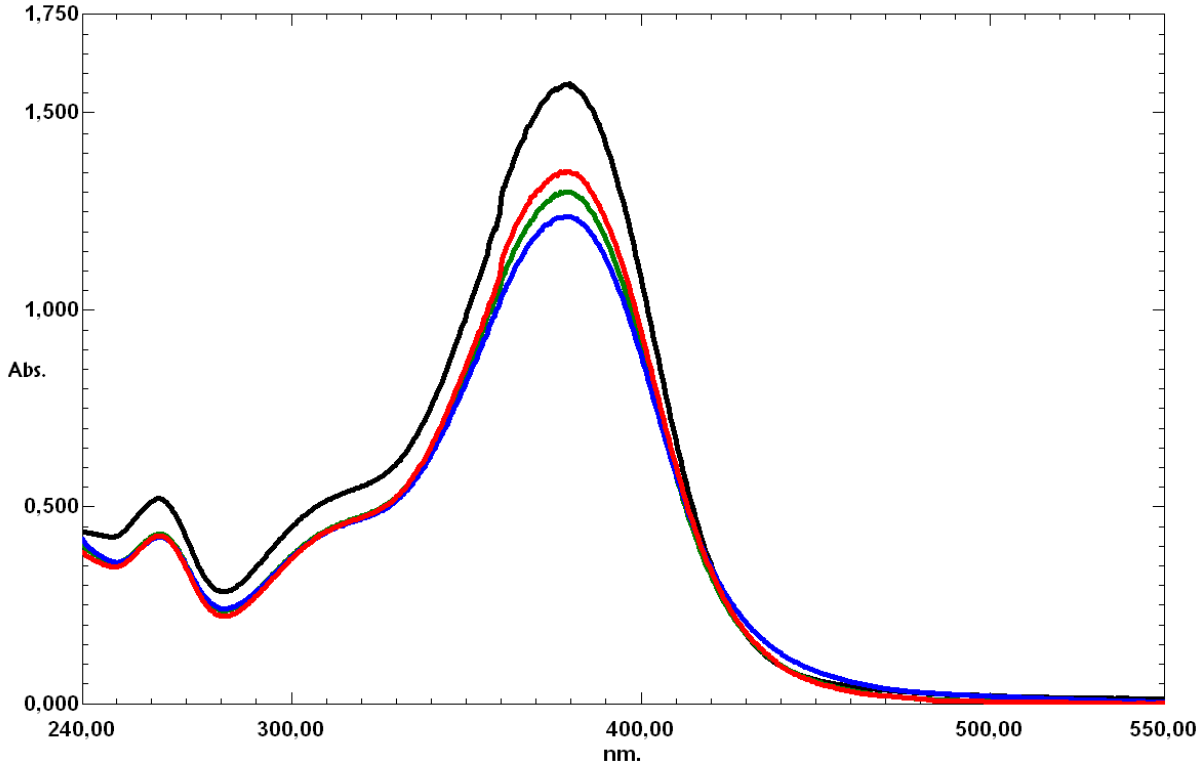


Fig. 68: UV/Vis-spectrum of 2',3,4,4'-tetrahydroxychalcone (Butein) complexated with $Ni(NO_3)_2$ in methanol/acetonitrile (V/V = 1/1). Ligand (black line), Ligand – $Ni(NO_3)_2$ (1:1, red line), PHC – $Ni(NO_3)_2$ (1:2, green line), PHC –$Ni(NO_3)_2$ (1:4, blue line)

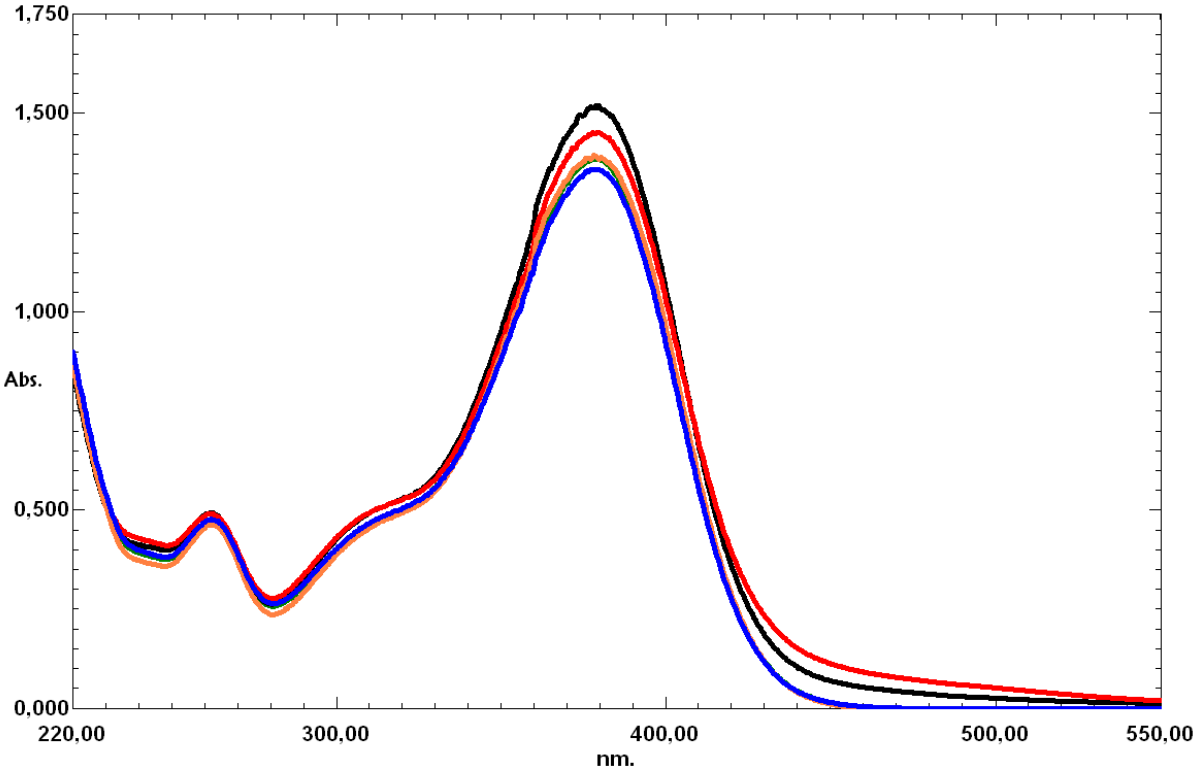


Fig. 69: UV/Vis-spectrum of 2',3,4,4'-tetrahydroxychalcone (Butein) complexated with $CoCl_2$ in methanol/acetonitrile (V/V = 1/1). Ligand (black line), Ligand – $CoCl_2$ (2:1, red line), PHC – $CoCl_2$ (1:2, orange line), PHC – $CoCl_2$ (1:4, green line), PHC - $CoCl_2$ (1:5, blue line)

2',4,4',6'-Tetrahydroxychalcone (THC) possesses only one hydroxyl-group in the B-ring. The absence of the 3,4-vicinale hydroxyl groups could prevent electron trapping. However, this would influence the probability of complexation, as well. The spectra of THC with different metal ions show for iron, cobalt and nickel the expected lack of any band shifts or broadening. By contrast copper, is in some way active on THC (fig. 70).

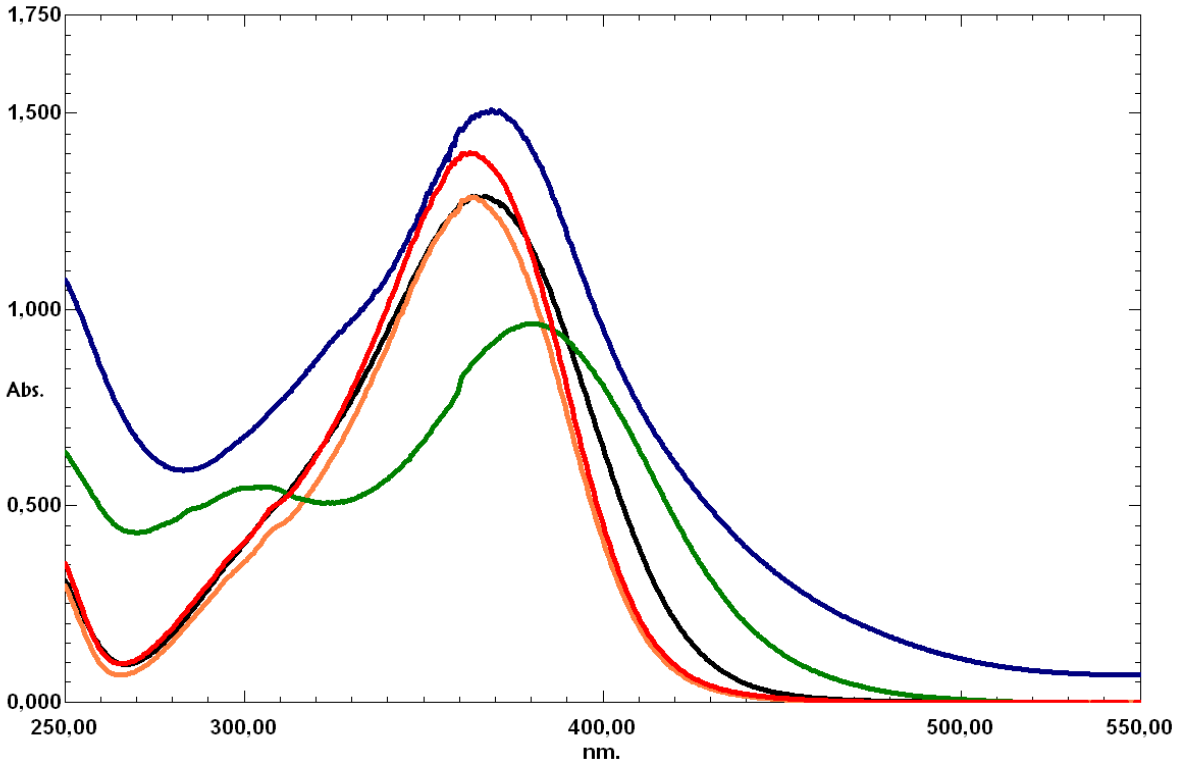


Fig. 70: UV/Vis-spectrum of 2',4,4',6'-tetrahydroxychalcone (THC) (black line) complexated with $FeCl_3$ (THC 1 : 4 $FeCl_3$; blue line); $Ni(NO_3)_2$ (THC 1 : 4 $Ni(NO_3)_2$; orange line); $CoCl_2$ (THC 1 : 4 $CoCl_2$; red line); $CuBr_2$ (THC 1 : 4 $CuBr_2$; green line)

To better understand what happened in these complexation reactions that were observed by UV/Vis spectroscopy, PHC, Butein and THC were converted with $CuBr_2$ and then, the products obtained were isolated and analyzed via ^{1}H-NMR spectroscopy. If the chalcones were oxidized in the manner described in figure 15, the products isolated for NMR spectroscopy should be aurones or quinones.

The ^{1}H-NMR spectra of PHC (fig. 71), Butein (fig. 72) and THC (fig.74) treated with $CuBr_2$ are shown below. Butein was also treated with $FeCl_3$ (fig. 75) to detect possible different reaction pathways, depending on the metal ion.

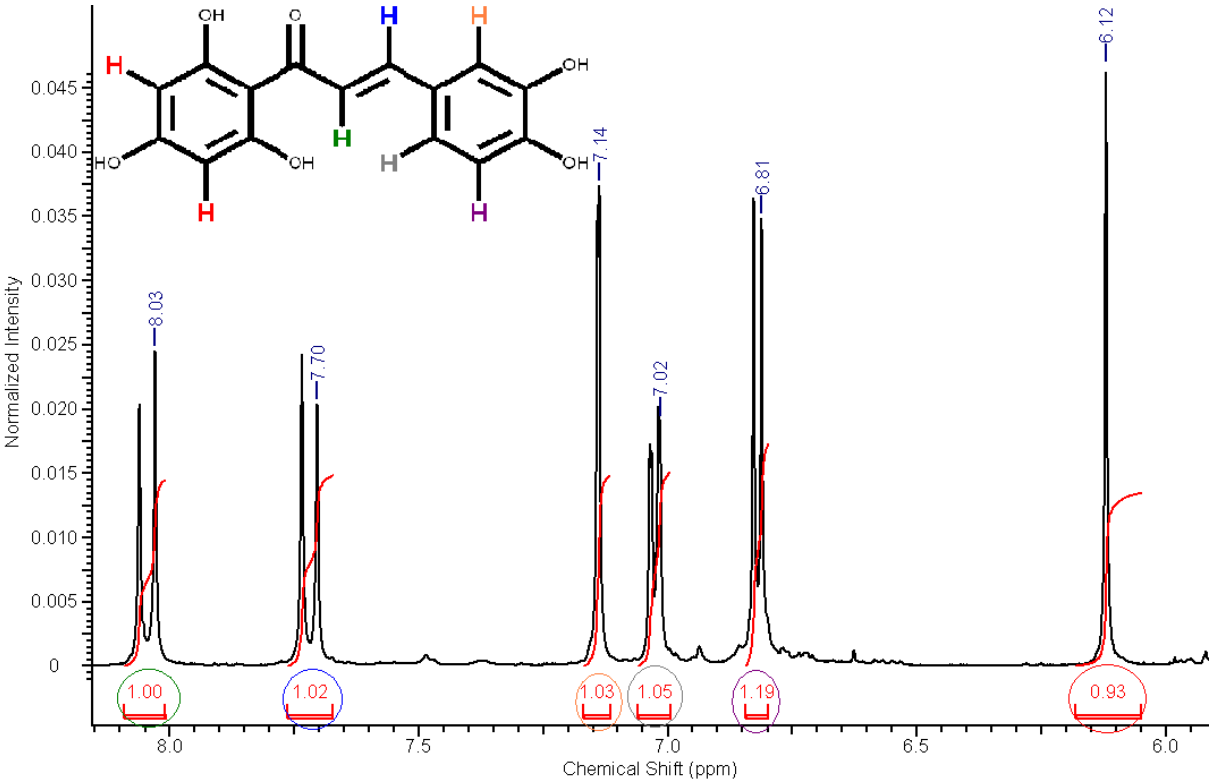


Fig. 71: ^{1}H-NMR spectrum of 2',3,4,4',6'-pentahydroxychalcone (PHC) after treating with $CuBr_2$ in d_4_methanol

The spectrum shows the same peaks as the spectrum of untreated PHC (fig. 39). No cyclization product has been formed as the double bond is still observable with a very well fitting integral of 1. All shifts may be due to the solvent, which is methanol instead of the acetone which was used for previous spectra. However, the integral of the proton on ring A is only 1 instead of 2. This is a strong divergence from the spectrum of untreated PHC. The missing integral and the corresponding missing proton are not found at any other position in the spectrum. However, the spectrum clearly possesses the protons of a double bond, which means that no aureusidin is formed. Aureusidin would have only one proton on a double bond. This proton could not couple to another proton at all and would exhibit a singlet signal in ^{1}H-NMR spectroscopy.

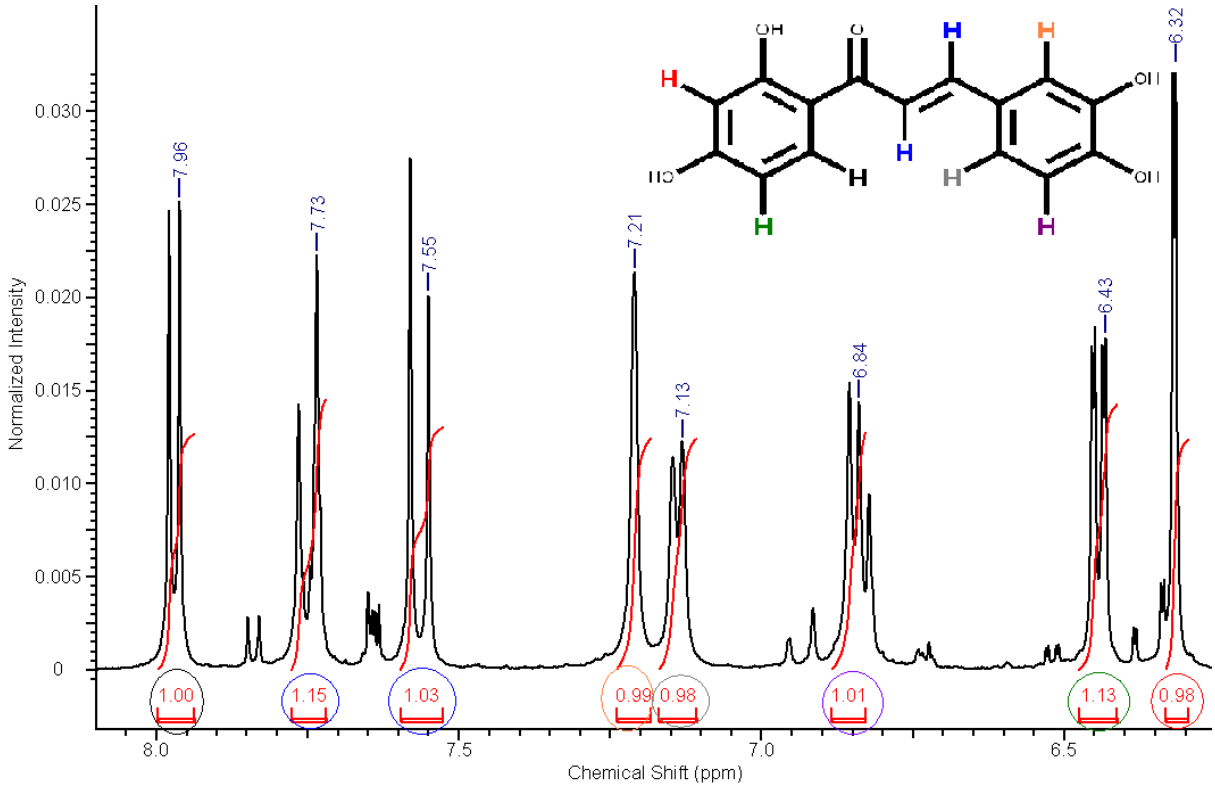


Fig. 72: ^{1}H-NMR spectrum of 2',3,4,4'-tetrahydroxychalcone (Butein) after treating with $CuBr_2$ in d_4_methanol

A part from the major peaks that can be clearly attributed to Butein, there are some other minor peaks in the aromatic proton region, indicating that some by-products could have been formed. However, Butein is clearly the major product in this spectrum. Interestingly, the coupling pattern on ring B has changed. The H-C2 shows a singlet, which means that this proton is not coupling with H-C6. In accordance with this, H-C6 only shows a doublet, which is the coupling to H-C5. The shifts of these atoms compared to the untreated Butein only differ slightly, which could be attributed to the different solvents used. The differences in the coupling pattern may be contributed to the solvent as well[59], but the results from Butein treated with iron(III) make this improbable (see fig. 75). A structure that could have close to the same shifts like Butein but a different coupling pattern may be the analogue catechol (fig. 72). However, this molecule would be expected to react further to the aurone. Moreover, the effect is not found for the pentahydroxychalcone which would be expected if the molecule in fig. 72 is formed.

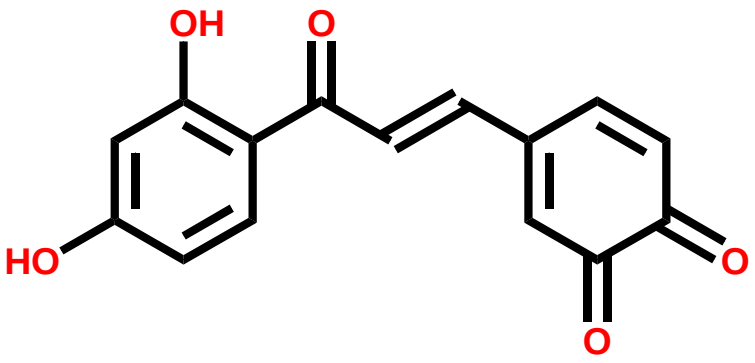


Fig. 73: Possible oxidation product of Butein

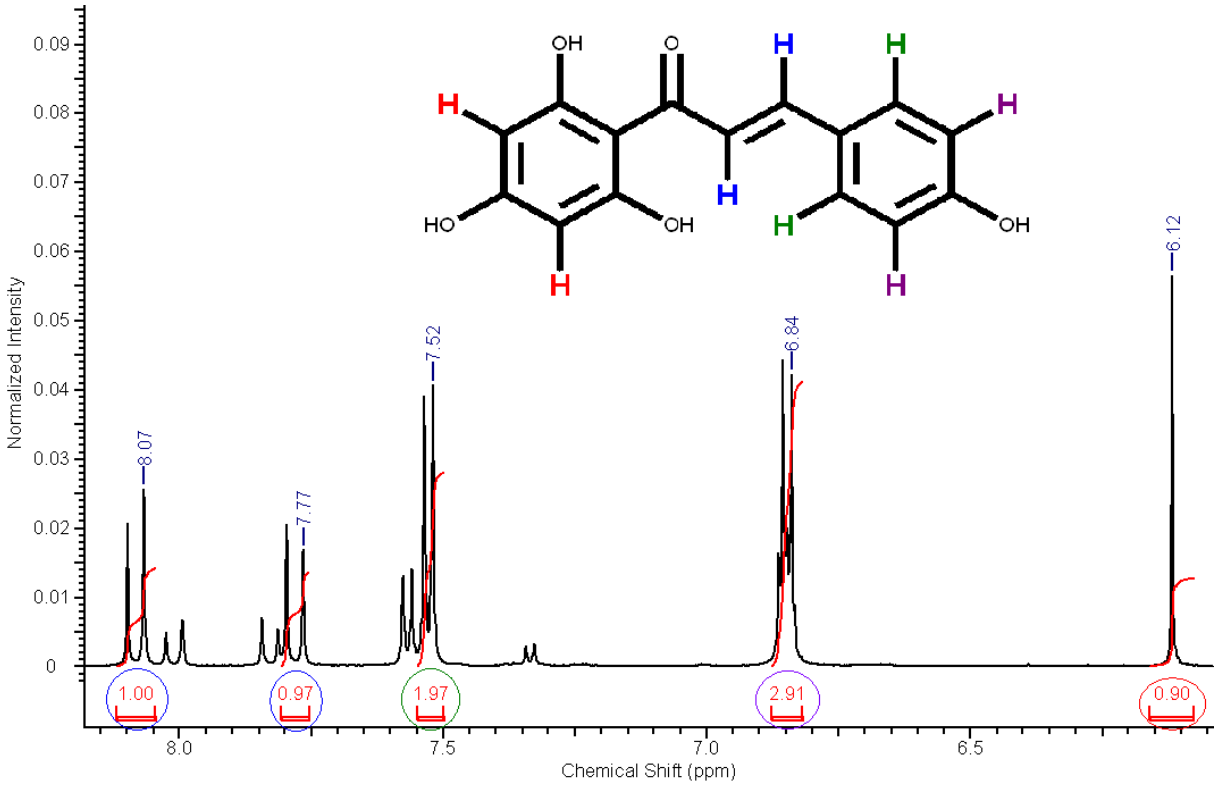


Fig. 74: ^{1}H-NMR spectrum of 2',4,4',6'-tetrahydroxychalcone (THC) after treating with $CuBr_2$ in d_4_methanol

The spectrum of THC after the reaction with copper(II) (fig. 74) shows the doublets of the double bond and the two multiplets corresponding to the protons of the B-ring. The integral of the H-C2/H-C6 on ring B is 2. The integral of H-C3/H-C5 is almost 3, but this may be due to a by-product that can also be seen in the form of very similar peaks next to the double bond protons and the H-C2/H-C6 protons. However, the integral of the protons on ring A is 1. So, this is the same characteristic as seen in for PHC Fig. 71. It may be that the second proton can be found in the peak of H-C3/H-C5. However, one would then expect the peak of the A ring to possess a coupling constant, but the peak exhibits a singlet. This means that there are no interactions with a chemically different proton.

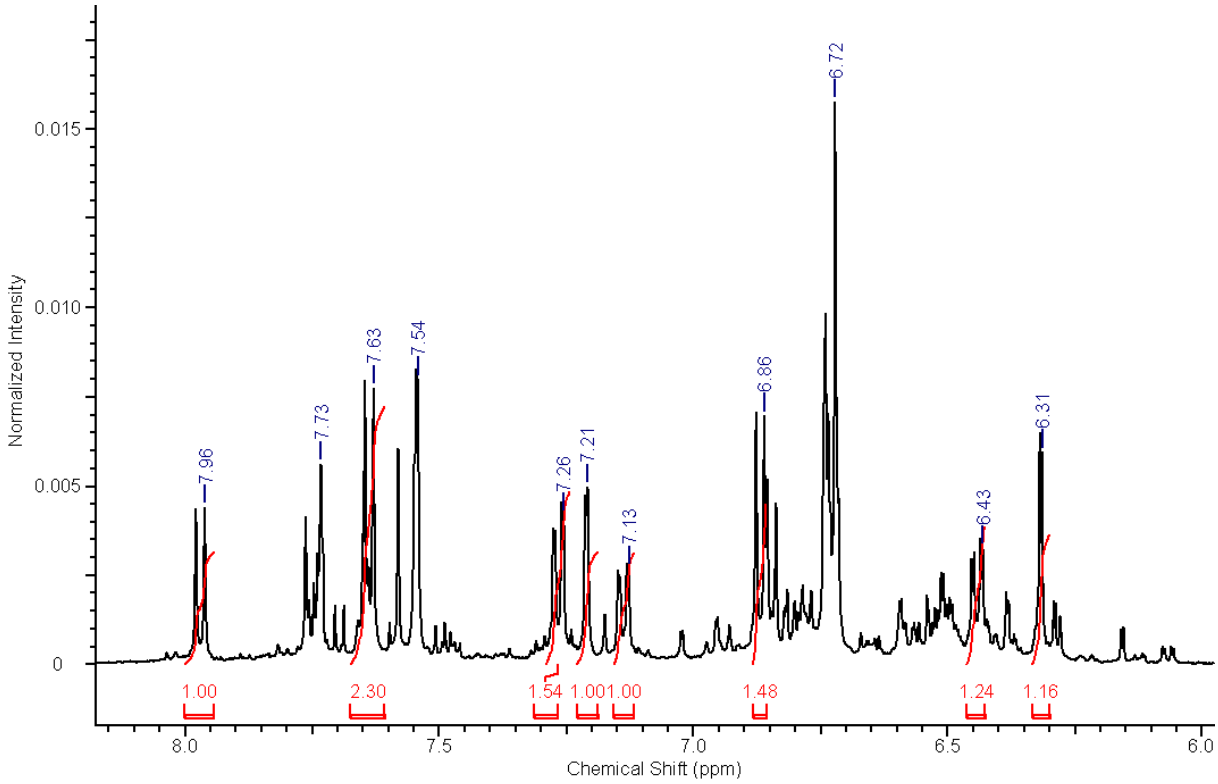


Fig. 75: ^{1}H-NMR spectrum of 2',3,4,4'-tetrahydroxychalcone (Butein) after being treated with $FeCl_3$ in d_4_methanol

The ^{1}H-NMR spectrum of Butein shows a mixture of many different products that were formed by treating Butein with iron(III) ions. The double bond coupling is not in the spectrum, so that Butein reacted completely to the newly formed mixture of products. It is not only one product that was formed (like the corresponding aurone sulfuretin) but a complex mixture of products. This means that some sort of decomposition of the chalcone must have taken place.

However, the coupling pattern of the ring B (the peaks of ring B can be found at 6.8 ppm, 7.2 ppm and 7.3 ppm) is showing a doublet to doublet of doublets to doublet pattern again. This is so important because it shows that the above mentioned changes in the coupling patterns of ring B (fig. 72) are probably not due to the solvent methanol as this spectrum was recorded in methanol as well. Nevertheless, this cannot be taken as a proof of the formation and stabilization of an oxidized product such as in fig. 73, either.

5 Conclusion

5.1 Synthesis and characterization of polyhydroxychalcones

In this study 2',3,4,4',6'-Pentahydroxychalcone, 2',4,4',6'-tetrahydroxychalcone and 2',3,4,4'-tetrahydroxychalcone were synthesized. It was shown that protection of the benzaldehydes and acetophenones with methoxymethylbromide is a first barrier in the synthesis of polyhydroxychalcones. The introduction of the protection group works with only moderate yields and the purification via liquid column chromatography is challenging and time-intense. The two benzaldehyde 3,4-bis(methoxymethoxy)benzaldehyde and 4-methoxymethoxybenzaldehyde as well as the two acetophenones 2'-hydroxy-4',6'-di(methoxymethoxy)acetophenone and 2'-hydroxy-4'-methoxymethoxyacetophenone were obtained and characterized in this paper.

The coupling reaction of the benzaldehydes and the acetophenones was performed in alkaline conditions and the produced protected chalcones were purified via recrystallization. 2'-Hydroxy-3,4,4'4,6'-tetrakis(methoxymethoxy)chalcone and 2'-hydroxy-3,4,4'-tri(methoxymethoxy)chalcone were synthesized by this method and characterized via ^{1}H-NMR. The coupling step worked well for these two compounds. However, for the other two compounds which could have been formed out of the benzaldehydes and acetophenones obtained, (2'-hydroxy-4,4',6'-tri(methoxymethoxy)chalcone ,and 2'-hydroxy-4,4'-di(methoxymethoxy)chalcone), the coupling worked less well and the purification via column chromatography did not directly lead to the purified compounds. In the case of the 2',4,4',6'-tetrahydroxychalcone (THC), this problem was solved by directly synthesizing the compound out of naringenin. This reaction worked with reasonable yields. The trihydroxychalcone (Isoliquiritigenin) was not obtained in the course of this study.

The deprotection step of the obtained protected chalcones led to the polyhydroxychalcones PHC and Butein with several by-products. The yield for PHC was low (21%), even significantly lower than the yields described in literature. This may be due to the strong interaction of the hydroxyl-groups of PHC with the silica gel in the column. However, the yields for the deprotection of Butein reached a satisfactory 55%.

Two conclusions can be drawn from this recapitulation of the results. First, the synthesis of polyhydroxychalcones by the use of alkaline coupling with MOM as protection group generally works. Second, the synthetic approach has several disadvantages. The yields are rather low, MOM is highly carcinogenic, the purification after the protection and the deprotection are complicated and the coupling does not work to the same extent for all chalcones.

Of the alternative approaches that were investigated in this study, the approach using tBDMS as protection group and LiHMDS as coupling reagent was the most promising. Neither the alternative deprotection with $NaHSO_4*SiO_2$ as catalyst nor the acidic one-step coupling of 2',4',6'-trihydroxyacetophenone with 3,'4-dihydroxybenzaldehyde led to the polyhydroxychalcone. For the three step synthesis with LiHMDS as coupling reagent, the first step worked well while the actual coupling still needs to be adjusted to optimize the reaction conditions. However, the results look promising. This may be a very competitive way to synthesize polyhydroxychalcones compared to the alkaline coupling with MOM as protecting group.

5.2 Synthesis and characterization of metal ion complexes of polyhydroxychalcones

The complexation assays of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone did not lead to any metal ion complexes of this chalcone. This was the result of the analysis via mass spectrometry, infrared spectroscopy and UV/Vis-spectroscopy. However, it is not completely clear why the coordination of metal ions is not performed by this chalcone. There are two different possible explanations. The first one is that the 2'-hydroxy-carbonyl-functional unit of chalcones is by nature unable to coordinate metal ions like copper, nickel, cobalt, zinc and iron. This is the conclusion that the experiments strongly indicate. However, it would contradict the results other groups have obtained in the field of chalcone coordination chemistry, as described in section 2.2.3. The second possible explanation is that the protecting groups somehow prevent the binding of metal ions on the 2'-hydroxy-carbonyl function of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone. The formation of stable dimers (shown in section 4.1) may be the reason why the coordination of metal ions is not energetically favorable anymore. However, it would be necessary to analyze the complexation behavior of 2'-hydroxychalcones with no protection groups and no other coordination sides to clarify this question. In any case, it was clearly demonstrated that 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone does not coordinate transition metal ions like copper, nickel, cobalt, or zinc.

For the polyhydroxychalcones PHC, THC and Butein the interpretation of the results is more complicated. Results from UV/Vis spectroscopy show that PHC and Butein are sensitive to copper(II), iron(III) and in the case of PHC also to cobalt(II). However, none of the polyhydroxychalcones show band shifts when treated with nickel (II). Most nickel(II) complexes possess strong colors, the d8 configuration of nickel(II) is sensitive to ligand-metal-electron-charge-transfers, one would expect the process of complexation for nickel to be detectable by UV/Vis spectroscopy. Interestingly, nickel(II) has the lowest redox potential of all metal ions studied here. That means that nickel(II) is more unlikely to be reduced to nickel(I) or nickel(0) then copper(II) to copper(I), for example. As polyhydroxychalcones with

a 3,4-dihydroxy function on the B ring are known to exhibit strong radical scavenging properties[9], such a process was expected to be responsible for the observed shifts in UV/Vis-spectroscopy. This would have explained that PHC and Butein show shifts with all metals except nickel(II) (and in case of Butein also Co(II)), but not with nickel(II), which has the lowest redox potential. It would also explain the observed UV/Vis spectra of THC with the metal ions as this compound lacks the 3,4-dihydroxy groups on ring B and has only a 4-hydroxy group instead. THC shows only a shift for copper(II), while showing no shifts in UV/Vis spectroscopy for iron(III), cobalt(II), and nickel(II). So, ^{1}H-NMR spectra of the three polyhydroxychalcones after treatment with copper were measured.

In all three cases, these ^{1}H-NMR spectra showed the chalcone and not the expected oxidation product, which would have been the corresponding aurone. In the case of Butein, a change in the coupling pattern of the B-ring was observed which could maybe be explained with the formation of the quinone system (also see fig. 73). However, such a result would be expected for PHC, as well. Here, the coupling constants on ring B only changed to a very small degree, the coupling constants are almost the same as in the PHC spectrum in section 4.2.3. The difference may be explained by the different solvents used for the spectrum.

Moreover, it remains unclear why the quinone system would be stable. One would expect the quinone system to be further processed into the corresponding aurone, especially as the spectra were recorded 12 hours after the reaction with copper.

THC and PHC showed an unexpected pattern on ring A, as the integral of the H-C'3/H-C'5 showed only one proton. At this point, there is no good explanation for this result. All possible decompositions of the chalcones would be expected to happen on the double bond, which is completely intact in the spectra.

For iron(III) with Butein, such a decomposition and complex mixture of compounds can be found. So, iron(III) reacts in a different manner with Butein than copper(II) does. Iron(III) is a little more sensitive to redox reactions than copper(II) is ($Fe^{3+} + e^{-} <-> Fe^{2+}$ 0,77 V; $Cu^{2+} + 2\ e^{-} <-> Cu$ 0,34 V[58]). However, understanding how iron(III) and copper(II) react with polyhydroxychalcones and why they do remains a challenge for future research. In this paper, it was demonstrated that polyhydroxychalcones show band shifts in UV/Vis spectra with several transition metal ions.

6 Future research and error analysis

6.1 Future research on the synthesis of polyhydroxychalcones

In this paper, it was shown that there is room to improvement in the synthesis of polyhydroxychalcones. For the deprotection step of the MOM-ether, it may be useful to purify the product by HPLC (high-performance liquid chromatography), which could lead to a better removal of by-products. However, the major focus may lie on establishing the best reaction conditions for the synthesis with the tBDMS protection group. Therefore, the reaction temperature, reaction time, and the dryness of the coupling step will have to be adjusted. Moreover, other coupling agents than LiHMDS could be tested, for example LDA (lithium diisopropylamide).

A completely different synthetic approach for polyhydroxychalcones may be to start from the cinnamic acid residue to lead to the corresponding polyhydroxychalcone via Friedel-Crafts-acelyation (fig. 76). Especially for symmetric A-ring systems, this may be a very promising approach.

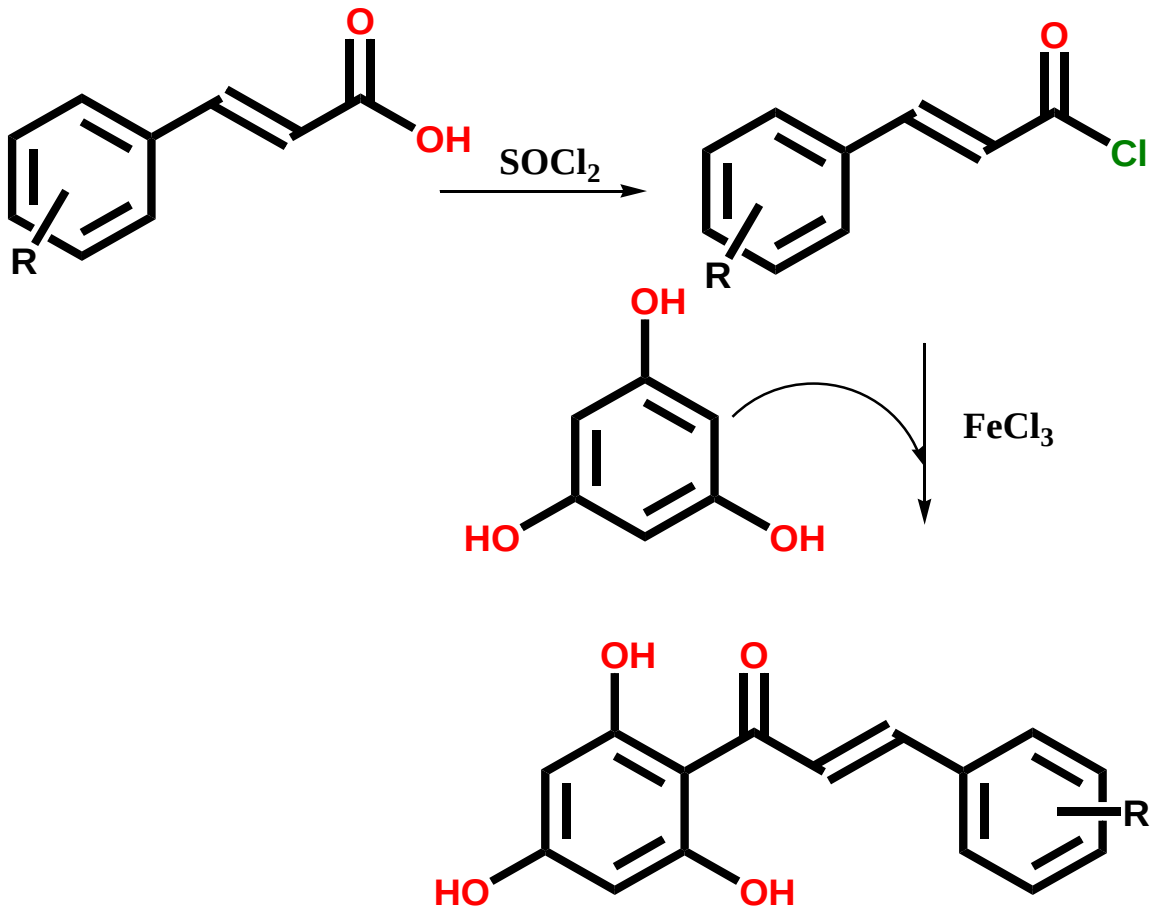


Fig. 76: Possible synthetic approach for polyhydroxychalcones by starting with cinnamic acid analoga via Friedel-Crafts-Acylation

The reaction mechanism behind a Friedel-Crafts-Acylation is the electrophilic aromatic substitution. 1',3',5'-Trihydroxybenzene should be ortho/para-derigative in an electrophilic aromatic substitution because hydroxyl-groups are ortho/para-derigative and activating in this type of reaction.

6.2 Outlook metal ion complexation of polyhydroxychalcones

Several questions in the complexation of polyhydroxychalcones have remained unanswered in this paper. The ^{1}H-NMR spectra that were recorded after treatment with copper showed no formation of aurones. The whole reaction process of metal ions with different polyhydroxychalcones has not yet been totally clarified. To get better insights into the reaction mechanisms in-situ-NMR and in-situ-IR, measurements could be performed. Reaction assays with the deprotected polyhydroxychalcones and transition metal ions could result in the isolation of metal complexes. However, for all of these approaches, the challenge of synthesizing polyhydroxychalcones in sufficient amounts has to be met first.

6.3 Error analysis

The final step in the synthesis of 2',3,4,4',6'-pentahydroxychalcone in the classical 3-step-synthesis provided yields significantly lower than those described in literature. The reason may be the interaction of PHC with the silica gel of the purification via flash liquid chromatography. The hydroxyl groups are likely to interact intensely with the SiO_2. This problem may be solved by using reversed phase liquid chromatography.

The coupling step using LiHMDS as coupling agent for the tBDMS protected acetophenone and benzaldehyde offered only low yields. The LiHMDS used for this reaction was a powder that was not as dry as the LiHMDS in THF solution would be. So, it may be that the yields can be increased significantly by using dry LiHMDS.

The NMR-experiments for the metal complexation were performed at the very end of this study. They have not been repeated so far. It has to be checked if the results obtained are robust by repeating the experimental procedure several times.

7 Experimental section

7.1 Facilities

7.1.1 Nuclear magnetic resonance spectroscopy

The NMR spectra were recorded in the group of Prof. Galanski at the University of Vienna or in the group of Dr. Belle in Grenoble. The facilities for NMR in the department of Inorganic Chemistry of the University of Vienna consist of two *BRUKER* 500 MHz superconducting actively shielded magnets (UltraShieldPlus)[60]. The spectra were treated with ACD/Labs 12.0 freeware NMR processor. In Grenoble, the facilities consist of one Avance 400 MHz FT-NMR and one Avance 300 MHz FT-NMR, both from *BRUKER*[61].

7.1.2 Infrared spectroscopy

The infrared spectra were recorded with an infrared spectrometer *BRUKER* IFS 25[62] in the department of Biophysical Chemistry at the University of Vienna. OPUS 6.5 by *BRUKER* was used to treat the infrared spectra.

7.1.3 Ultraviolet-visible spectrophotometry

The UV/Vis spectra were recorded with a *SHIMADZU* 1800 UV spectrophotometer. The spectra were treated with *SHIMADZU* UVProbe 2.33. The measurements were performed at the department of Biophysical Chemistry at the University of Vienna.

7.1.4 Mass spectrometry

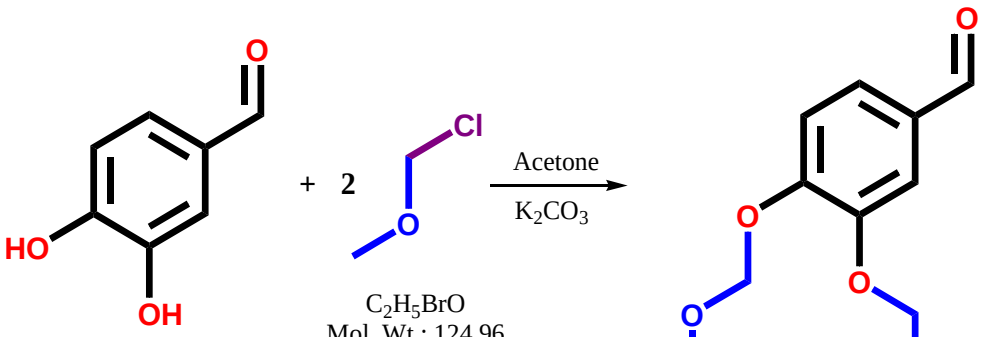
The mass spectra were recorded with an ion trap mass spectrometer BRUKER esquire 3000 with an orthogonal ESI source. These measurements were performed at the department of Inorganic Chemistry at the University of Vienna[63].

7.1.5 X-ray structure analysis

The X-ray structure analysis of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone was performed at the department of molecular chemistry in Grenoble. The facilities there for X-ray structure analysis consist of a *MACH3 ENRAF NONIUS* diffractometer and a *KAPPA CCD NONIUS* diffractometer. The data obtained were treated with Mercury 2.4 from CCDC.

7.2 Synthesis

7.2.1 3,4-Bis(methoxymethoxy)benzaldehyde



$C_7H_6O_3$
Mol. Wt.: 138,12

C_2H_5BrO
Mol. Wt.: 124,96

$C_{11}H_{14}O_5$
Mol. Wt.: 226,23

Substance	**Molecular mass**	**Amount of substance**	**Mol / equivalents**	**Purchased from**
3,4-Dihydroxy-benzaldehyde	138.12 g/mol	5.52 g	40 mmol / 1 eq.	Merck
Chloromethyl methyl ether	80.51 g/mol d = 1.06 g/mL	7.4 mL	99.16 mmol / 2.5 eq.	Sigma Aldrich
Potassium carbonate	138.21 g/mol	38.5 g	278 mmol / 7 eq.	Fluka
Acetone		500 mL		Dried over molecular sieve

5.52 g of 3,4-Dihydroxybenzaldehyde were added to 38.5 g of K_2CO_3 in a dried three-neck round bottom flask with 500 mL of Acetone (dried over molecular sieve). 7.4 mL of chloromethyl methyl ether were added dropwise to the suspension and the mixture was refluxed for 4 hours.

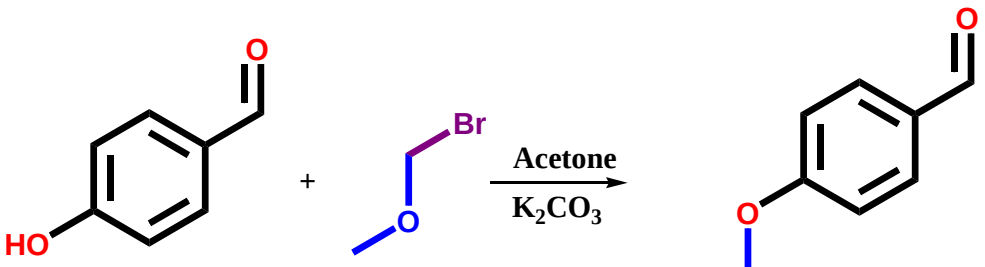
The suspension was cooled to room temperature; filtered, washed thoroughly with acetone and subsequently, the filtrate was evaporated and dried under vacuum.

The yellow oil obtained was purified by flash column chromatography with hexane / ethyl acetate (7 / 3). This purification step was repeated (2 x) for the collected mixed fractions.

A white solid was obtained.

Yield: 6.1 g (26.7 mmol) → 67.4 %

7.2.2 4-Methoxymethoxybenzaldehyde



$C_7H_6O_2$
Mol. Wt.: 122,12

C_2H_5BrO
Mol. Wt.: 124,96

$C_9H_{10}O_3$
Mol. Wt.: 166,17

Substance	**Molecular mass**	**Amount of substance**	**Mol / equivalents**	**Purchased from**
4-Hydroxy-benzaldehyde	122.12 g/mol	4.89 g	40 mmol / 1 eq.	Sigma Aldrich
Bromomethyl methyl ether	80.51 g/mol	4.1 mL	65 mmol / 1.6 eq.	Sigma Aldrich
Potassium carbonate	138.21 g/mol	40 g	290 mmol / 7.2 eq.	Fluka
Acetone		500 mL		Dried over molecular sieve

4.89 g of 4-Hydroxybenzaldehyde were added to 40 g of K_2CO_3 in a dried three-neck round bottom flask with 500 mL of Acetone (dried over molecular sieve). 4.1 mL of bromomethyl methyl ether were added dropwise to the suspension and the mixture was refluxed for 4 hours.

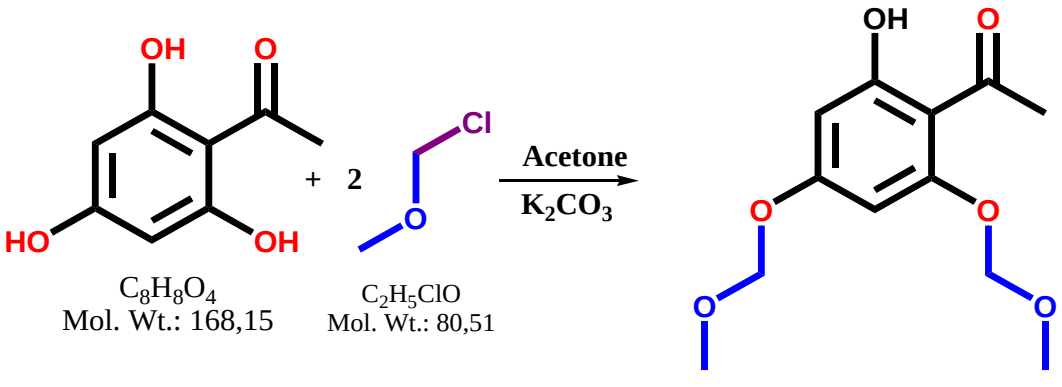
The suspension was cooled to room temperature; filtered, washed thoroughly with acetone and subsequently, the filtrate was evaporated and dried under vacuum.

The yellow oil obtained was purified by flash column chromatography with hexane / ethyl acetate (7 / 3).

A white oil was obtained.

Yield: 2.74 g (16.4 mmol) → 41.2 %

7.2.3 2'-Hydroxy-4',6'-Di(methoxymethoxy)acetophenone



$C_8H_8O_4$
Mol. Wt.: 168,15

C_2H_5ClO
Mol. Wt.: 80,51

$C_{12}H_{16}O_6$
Mol. Wt.: 256,25

Substance	**Molecular mass**	**Amount of substance**	**Mol / equivalents**	**Purchased from**
2',4',6'-Trihydroxy-acetophenone monohydrate	186.05 g/mol	7.4 g	40 mmol / 1 eq.	Molekula
Chloromethyl methyl ether	80.51 g/mol d = 1.06 g/mL	7.4 mL	99.16 mmol / 2.5 eq.	Sigma Aldrich
Potassium carbonate	138.21 g/mol	38.5 g	278 mmol / 7 eq.	Fluka
Acetone		500 mL		Dried over molecular sieve

7.4 g of 2',4',6'-Trihydroxy were added to 38.5 g of K_2CO_3 in a dried three-neck round bottom flask with 500 mL of Acetone (dried over molecular sieve). 7.4 mL of chloromethyl methyl ether were added dropwise to the suspension and the mixture was refluxed for 4 hours.

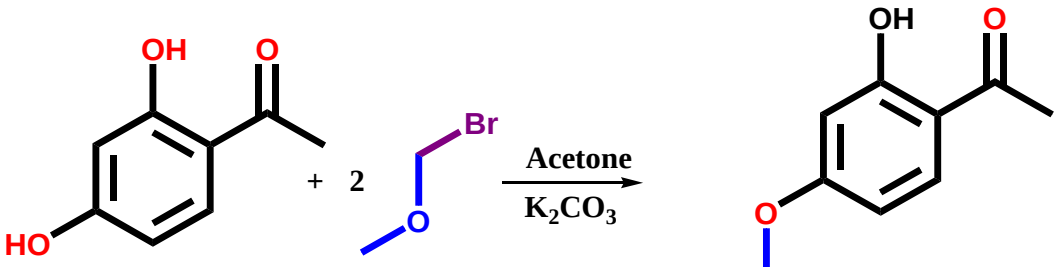
The suspension was cooled to room temperature; filtered, washed thoroughly with acetone and subsequently, the filtrate was evaporated and dried under vacuum.

The red oil obtained was purified by flash column chromatography with hexane / ethyl acetate (7 / 3).

A white/yellow solid was obtained.

Yield: 6.07 g (23.7 mmol) → 59.3 %

7.2.4 2'-Hydroxy-4'-methoxymethoxyacetophenone



$C_8H_8O_3$
Mol. Wt.: 152,15

C_2H_5BrO
Mol. Wt.: 124,96

$C_{10}H_{12}O_4$
Mol. Wt.: 196,20

Substance	Molecular mass	Amount of substance	Mol / equivalents	Purchased from
2',4'-Dihydroxy-acetophenone	152.15 g/mol	6.1 g	40 mmol / 1 eq.	Sigma Aldrich
Bromomethyl methyl ether	80.51 g/mol	4.1 mL	65 mmol / 1.6 eq.	Sigma Aldrich
Potassium carbonate	138.21 g/mol	38.5 g	278 mmol / 7 eq.	Fluka
Acetone		500 mL		Dried over molecular sieve

6.1 g of 2',4'-Dihydroxyacetophenone were added to 38.5 g of K_2CO_3 in a dried three-neck round bottom flask with 500 mL of Acetone (dried over molecular sieve). 4.1 mL of bromomethyl methyl ether were added dropwise to the suspension and the mixture was refluxed for 4 hours.

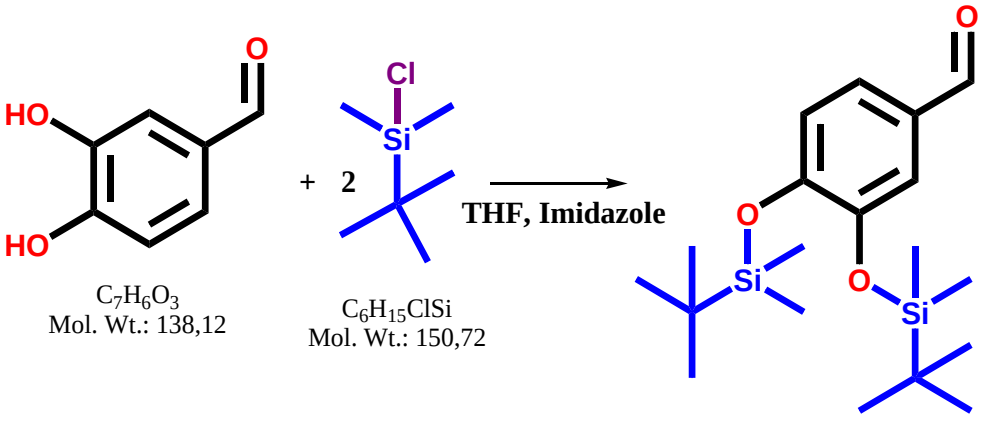
The suspension was cooled to room temperature; filtered, washed thoroughly with acetone and subsequently, the filtrate was evaporated and dried under vacuum.

The red oil obtained was purified by flash column chromatography with hexane / ethyl acetate (7 / 3).

A white solid was obtained.

Yield: 5.03 g (25.7 mmol) → 64.2%

7.2.5 3,4-O,O-Bis(tert.-butyldimethylsilyloxy)benzaldehyde



$C_7H_6O_3$
Mol. Wt.: 138,12

$C_6H_{15}ClSi$
Mol. Wt.: 150,72

$C_{19}H_{34}O_3Si_2$
Mol. Wt.: 366,64

Substance	**Molecular mass**	**Amount of substance**	**Mol / equivalents**	**Purchased from**
3,4-Dihydroxy-benzaldehyde	138. 12 g/mol	2.76 g	20 mmol / 1 eq.	Merck
Tert-butyldimethyl-silylchloride	150.72 g/mol	5.5 g	36.7 mmol / 1.8 eq.	Molekula
Imidazole	68.08 g/mol	2.73 g	40 mmol / 2 eq.	Merck
Tetrahydrofuran		100 mL		Dried over molecular sieve

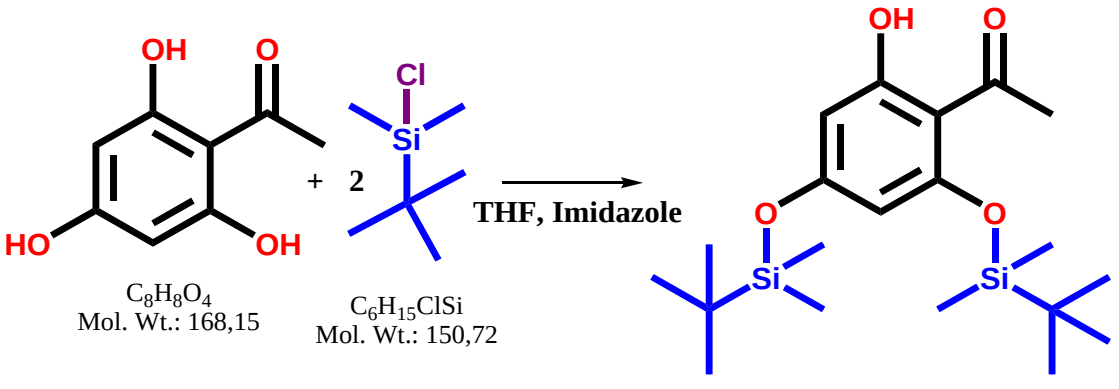
2,.76 g of 3,4-Dihydroxybenzaldehyde were dissolved in 100 mL of THF under N_2-atmosphere at 0°C. 5.5 g of t-BDMS and 2.73 g of imidazole were added sequentially. The reaction mixture stirred h at room temperature for 24.

Subsequently, the reaction mixture was quenched with 40 mL of saturated NH_4Cl solution and extracted 3 x with EtOAc. The organic layer was washed with H_2O (2 x) and brine solution (2 x). Next, the organic layer was dried over Na_2SO_4, filtered, washed with EtOAc and the solvents were evaporated on a rotavapor to dryness. The product obtained was purified by flash column chromatography with hexane / ethyl acetate (9.5 / 0.5) as eluent.

A white solid was obtained.

Yield: 4.7 g (12.8 mmol) → 64.2%

7.2.6 2'-Hydroxy-4',6'-O,O-Di(tert.-butyldimethylsilyloxy)acetophenone



$C_8H_8O_4$
Mol. Wt.: 168,15

$C_6H_{15}ClSi$
Mol. Wt.: 150,72

$C_{20}H_{36}O_4Si_2$
Mol. Wt.: 396,67

Substance	Molecular mass	Amount of substance	Mol / equivalents	Purchased from
2',4',6'-Trihydroxy-acetophenone	186.05 g/mol	3.72 g	20 mmol / 1 eq.	Molekula
Tert-butyldimethyl-silylchloride	150.72 g/mol	9 g	60 mmol / 3 eq.	Molekula
Imidazole	68.08 g/mol	4.31 g	63 mmol / 3.2 eq.	Merck
Tetrahydrofuran		100 mL		Dried over molecular sieve

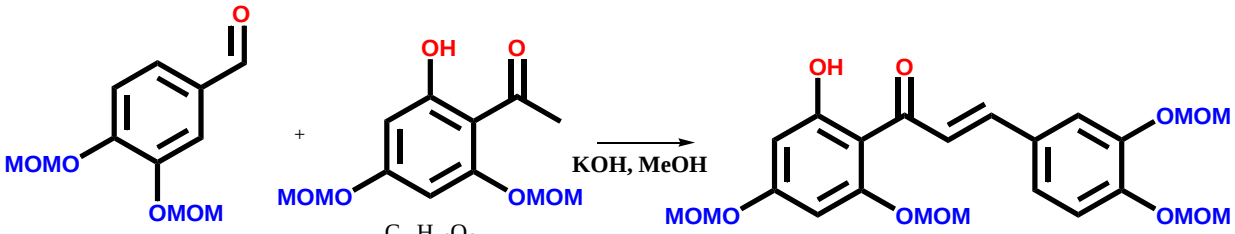
2.76 g of 3,4-Dihydroxybenzaldehyde were dissolved in 100 mL of THF under N_2-atmosphere at 0°C. 9 g of t-BDMS and 4.31 g of imidazole were added sequentially. The reaction mixture stirred for 24 h at room temperature.

Subsequently, the reaction mixture was quenched with 40 mL saturated NH_4Cl solution and extracted 3 x with EtOAc. The organic layer was washed with H_2O (2 x) and brine solution (2 x). Next, the organic layer was dried over Na_2SO_4, filtered, washed with EtOAc and the solvents were evaporated on a rotavapor to dryness. The product obtained was purified by flash column chromatography with hexane / ethyl acetate (9.5 / 0.5) as eluent.

A white/yellow solid was obtained.

Yield: 6.6 g (16.6 mmol) → 83.1%

7.2.7 2'-Hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone



$C_{11}H_{14}O_5$
Mol. Wt.: 226,23

$C_{12}H_{16}O_6$
Mol. Wt.: 256,25

$C_{23}H_{28}O_{10}$
Mol. Wt.: 464,46

Substance	**Molecular mass**	**Amount of substance**	**Mol / equivalents**	**Purchased from**
3,4-Bis(methoxy-methoxy)-benzaldehyde	226.23 g/mol	1.244 g	5.5 mmol / 1 eq.	-
2'-Hydroxy-4',6'-di(methoxymethoxy)-acetophenone	256.25 g/mol	1.409 g	5.5 mmol / 1 eq.	-
KOH (20%)		5 mL		Merck
Ethanol		15 mL		Merck

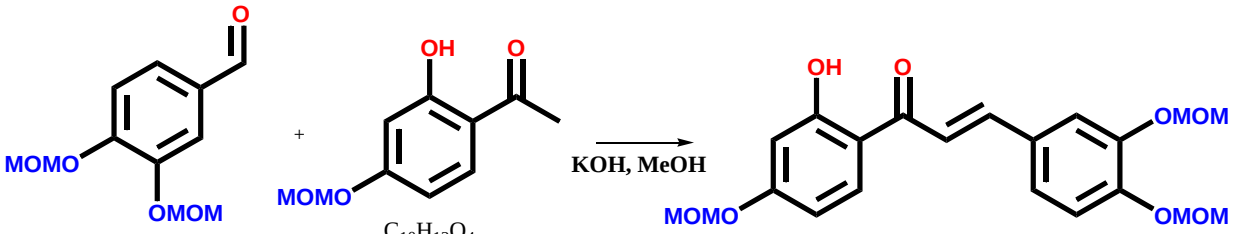
1.244 g of 3,4-Bis(methoxymethoxy)benzaldehyde and 1.409 g of 2'-Hydroxy-4',6'-di(methoxymethoxy)acetophenone were dissolved in 15 mL of ethanol. Next, 5 of mL KOH (20% aqueous solution) were added and the mixture stirred at room temperature for 72 h.

Subsequently, the mixture was cooled to 0°C in an ice bath and 20 mL 4 N HCl were added. A yellow precipitate was formed, filtered, washed with 4 N HCl and with an excess of water and dried under vacuum.

After recrystallization in EtOAc / hexane a yellow solid was obtained.

Yield: 1.91 g (4.11 mmol) → 74.7 %

7.2.8 2'-Hydroxy-3,4,4'-tri(methoxymethoxy)chalcone



$C_{11}H_{14}O_5$
Mol. Wt.: 226,23

$C_{10}H_{12}O_4$
Mol. Wt.: 196,20

$C_{21}H_{24}O_8$
Mol. Wt.: 404,41

Substance	**Molecular mass**	**Amount of substance**	**Mol / equivalents**	**Purchased from**
3,4-Bis(methoxy-methoxy)-benzaldehyde	226.23 g/mol	679 mg	3 mmol / 1 eq.	-
2'-Hydroxy-4'-methoxymethoxy-acetophenone	196.2 g/mol	588 g	3 mmol / 1 eq.	-
KOH (20%)		3 mL		Merck
Ethanol		9 mL		Merck

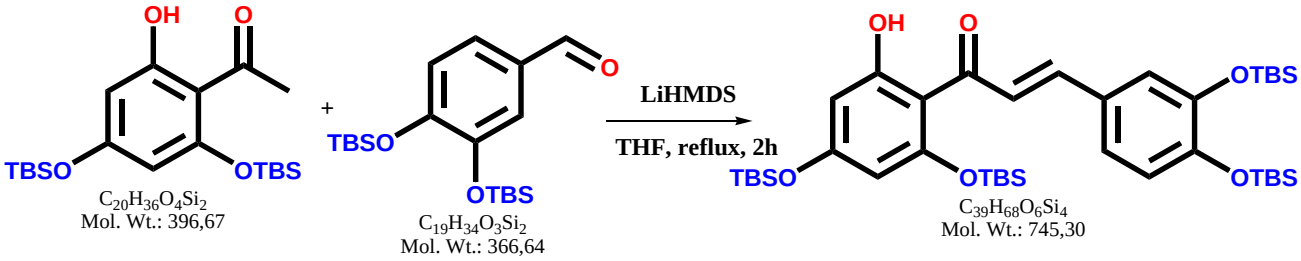
679 mg of 3,4-Bis(methoxymethoxy)benzaldehyde and 588 mg of 2'-Hydroxy-4'-methoxymethoxyacetophenone were dissolved in 9 mL of ethanol. Next, 3 mL of KOH (20% aqueous solution) were added and the mixture stirred at room temperature for 72 h.

Subsequently, the mixture was cooled to 0°C in an ice bath and 20 mL of 4 N HCl were added. A yellow precipitate was formed, filtered, washed with 4 N HCl and with an excess of water and dried under vacuum.

After recrystallization in EtOAc / hexane a yellow solid was obtained.

Yield: 550 mg (1.36 mmol) → 45.3 %

7.2.9 2'-Hydroxy-3,4,4',6'-O,O,O,O-tetra(tert-butyldimethylsilyloxy)chalcone



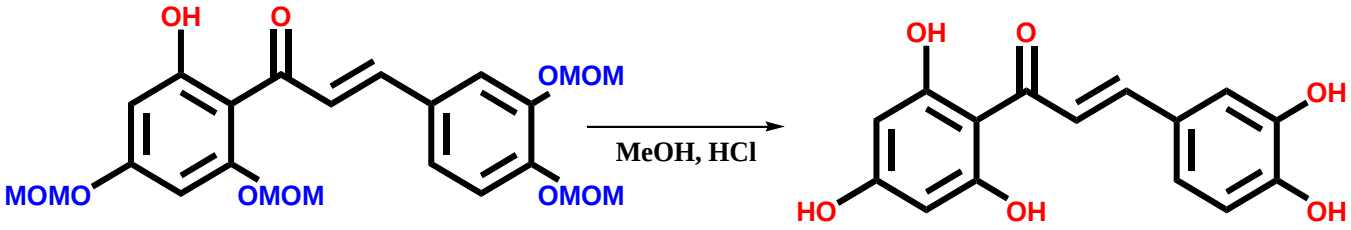
Substance	Molecular mass	Amount of substance	Mol / equivalents	Purchased from
2'-Hydroxy-4',6'-O,O-Di(tert.-butyldimethylsilyloxy)-acetophenone	396.67 g/mol	1.2 g	3 mmol	-
3,4-O,O-Bis(tert.-butyldimethylsilyloxy)-benzaldehyde	366.64 g/mol	1.1 g	3 mmol	-
LiHMDS	167.33 g/mol	1.51 g	9 mmol	Sigma Aldrich
Tetrahydrofuran		20 mL		Sigma Aldrich

1.2 g of 2'-hydroxy-4',6'-O,O-di(tert.-butyldimethylsilyloxy)acetophenone and 1.1 g of 3,4-O,O-bis(tert-butyldimethylsilyloxy)benzaldehyde were suspended in 20 mL tetrahydrofuran. Next, 1.51 g of LiHMDS were added sequentially. The mixture refluxed for 2 hours and was then cooled to room temperature. Next, 50 mL hydrochloric acid (0.1%) were added and the mixture stirred for another 15 minutes. Then, the product was extracted with ethyl acetate (3 x) and the organic layer was dried over Na_2SO_4. The product was filtered, the sodium sulfate washed with ethyl acetate and the solvent evaporated on a rotavapor. The product obtained was dried on a vacuum pump.

The yellow oil was purified by flash column chromatography with hexane/ethyl acetate (9.5/0.5).

A yellow oil was obtained.

7.2.10 2',3,4,4',6'-Pentahydroxychalcone



$C_{23}H_{28}O_{10}$
Mol. Wt.: 464,46

$C_{15}H_{12}O_6$
Mol. Wt.: 288,25

Substance	**Molecular mass**	**Amount of substance**	**Mol / equivalents**	**Purchased from**
2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)-chalcone	464.46 g/mol	191 mg	0.41 mmol	-
Hydrochloric acid (10 % aqueous solution)		3.3 mL		Sigma Aldrich
Methanol		8 mL		Sigma Aldrich

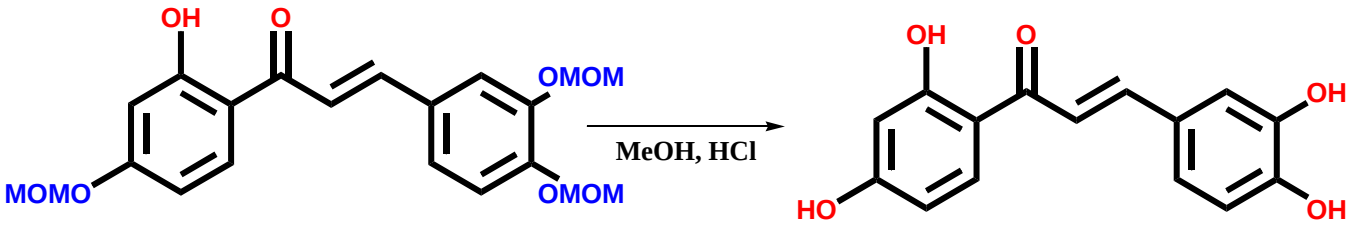
191 mg of 2'-Hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone were dissolved in 8 mL of methanol. 3.3 mL of 10% HCl (aqueous solution) were added and the mixture refluxed for 15 minutes. Then, the solution was diluted with water and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4, filtered, washed with EtOAc and evaporated.

The product was purified by flash column chromatography with CH_2Cl_2 / MeOH (9.5 / 0.5) and the fractions were detected by UV/Vis-photometry.

An orange solid was obtained.

Yield: 25 mg (0.087 mmol) → 21.2 %

7.2.11 2',3,4,4'-Tetrahydroxychalcone



$C_{21}H_{24}O_8$
Mol. Wt.: 404,41

$C_{15}H_{12}O_5$
Mol. Wt.: 272,25

Substance	**Molecular mass**	**Amount of substance**	**Mol / equivalents**	**Purchased from**
2'-hydroxy-3,4,4'-tri(methoxymethoxy)-chalcone	404.41 g/mol	400 mg	0.99 mmol	-
Hydrochloric acid (10 %)		3.3 mL		Sigma Aldrich
Methanol		8 mL		Sigma Aldrich

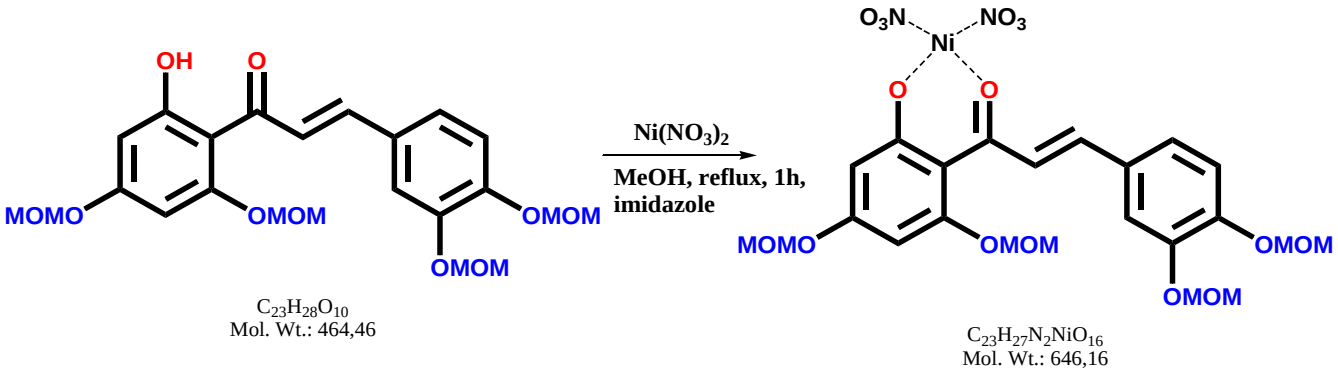
400 mg of 2'-Hydroxy-3,4,4'-tri(methoxymethoxy)chalcone were dissolved in 20 mL of methanol. 6.9 mL of 10% HCl (aqueous solution) were added and the mixture refluxed for 15 minutes. Then, the solution was diluted with water and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4, filtered, washed with EtOAc and evaporated.

The product was purified by flash column chromatography with CH_2Cl_2 / MeOH (9.5 / 0.5) and the fractions were detected by UV/Vis-photometry.

An orange solid was obtained.

Yield: 148.2 mg (0.54 mmol) → 55 %

7.2.12 2'-Hydroxy-3,4,4',6'-Tetrakis(methoxymethoxy)chalcone-metal-complex



$C_{23}H_{28}O_{10}$
Mol. Wt.: 464,46

$C_{23}H_{27}N_2NiO_{16}$
Mol. Wt.: 646,16

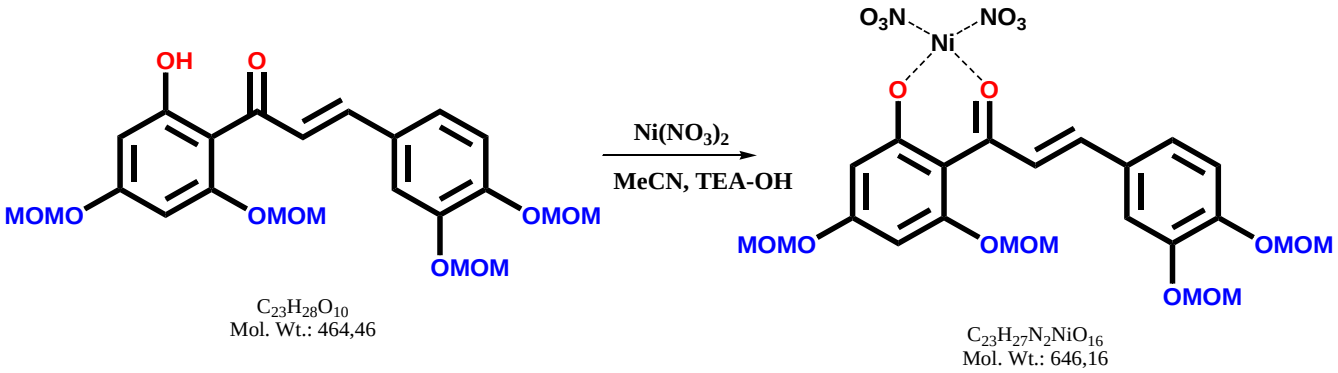
Substance	**Molecular mass**	**Amount of substance**	**Mol / equivalents**	**Purchased from**
2'-hydroxy-3,4,4',6'-tetrakismethoxymethoxychalcone	464.46 g/mol	233 mg	0.5 mmol	-
$Ni(NO_3)_2$ * Hexahydrate	290.79 g/mol	72.71 mg	0.25 mmol	Merck
Methanol		30 mL		Sigma Aldrich
Imidazole	68.08 g/mol			Merck

233 mg of 2'hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone were suspended in 15 mL of MeOH. The mixture was refluxed, 15 mL of a solution of 72.71 mg $Ni(NO_3)_2$ in MeOH were added and the pH value was adjusted to 10 with imidazole. The mixture refluxed for 1 h, and then it was cooled to room temperature, the formed solid was filtered, washed with cold MeOH and dried under vacuum.

The yellow solid was analyzed via ESI-mass spectrometry.

The same approach was used for $CuBr_2$, $FeCl_3$, $ZnCl_2$ and $CoCl_2$.

7.2.13 2'-Hydroxy-3,4,4',6'-Tetrakis(methoxymethoxy)chalcone-metal-complex



$C_{23}H_{28}O_{10}$
Mol. Wt.: 464,46

$C_{23}H_{27}N_2NiO_{16}$
Mol. Wt.: 646,16

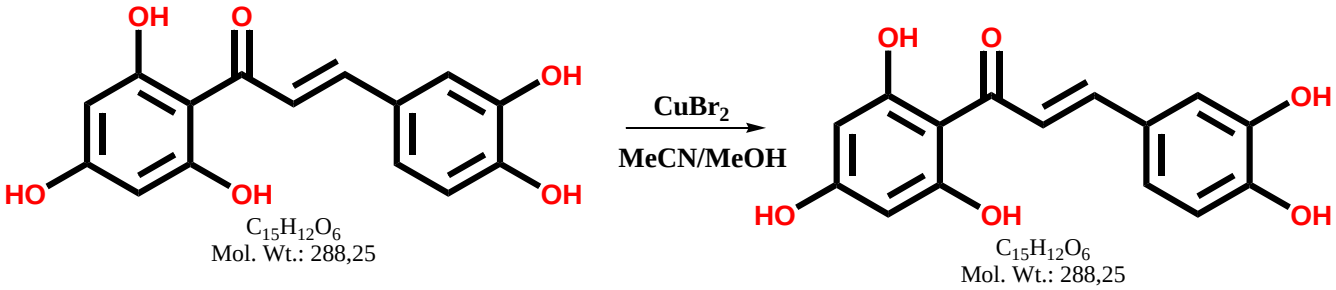
Substance	**Molecular mass**	**Amount of substance**	**Mol / equivalents**	**Purchased from**
2'-hydroxy-3,4,4',6'-tetrakismethoxymethoxychalcone	464.46 g/mol	50 mg	0.1 mmol	-
$Ni(ClO_4)_2$ * Hexahydrate	365.69 g/mol	18,3 mg	0.05 mmol	Merck
Acetonitrile		8 mL		Sigma Aldrich
Tetraethylammonium hydroxide	147.26 g/mol	14.3 µL	0.1 mmol	Fluka

50 mg of 2'hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone were dissolved in 6 mL MeCN and 14.3 µL of tetraethylammoniumhydroxide were added. The color of the solution changed from yellow to red. Now, 18.3 mg $Ni(ClO_4)_2$ dissolved in 2 mL MeCN were added and the mixture stirred at room temperature for 1 h. The solvent was partially evaporated on a rotavpor and the mixture was cooled to 4°C over night.

The yellow solid was analyzed via ESI-mass spectrometry.

The same approach was used for $Cu(OTf)_2$, $Cu(ClO_4)_2$, $FeCl_3$, $Zn(ClO_4)_2$.

7.2.14 2',3,4,4',6'-Pentahydroxychalcone treatment with $CuBr_2$



$C_{15}H_{12}O_6$
Mol. Wt.: 288,25

$C_{15}H_{12}O_6$
Mol. Wt.: 288,25

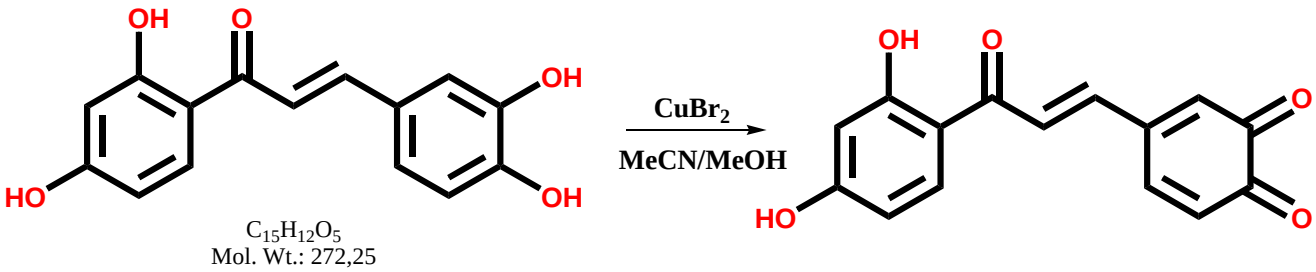
Substance	**Molecular mass**	**Amount of substance**	**Mol / equivalents**	**Purchased from**
2',3,4,4',6'-Pentahydroxychalcone	288.25 g/mol	10 mg	0.035 mmol	-
$CuBr_2$	223.35 g/mol	22.3 mg	0.1 mmol	Merck
Methanol		5 mL		Sigma Aldrich
Ethyl acetate		5 mL		VWR

10 mg of 2',3,4,4',6'-pentahydroxychalcone were dissolved in 5 mL ethyl acetate. Then, 5 mL of a solution consisting of 22.3 mg $CuBr_2$ in methanol were added and the mixture stirred for 30 minutes.

Next, water was added to the solution and the organic layer was extracted. For this purpose, NaCl was added to improve the phase separation. The organic layer was dried over Na_2SO_4, filtered, washed with EtOAc and evaporated to dryness.

The solid obtained was directly analyzed via ^{1}H-NMR spectroscopy.

7.2.15 2',3,4,4'-Tetrahydroxychalcone treatment with $CuBr_2$



$C_{15}H_{12}O_5$
Mol. Wt.: 272,25

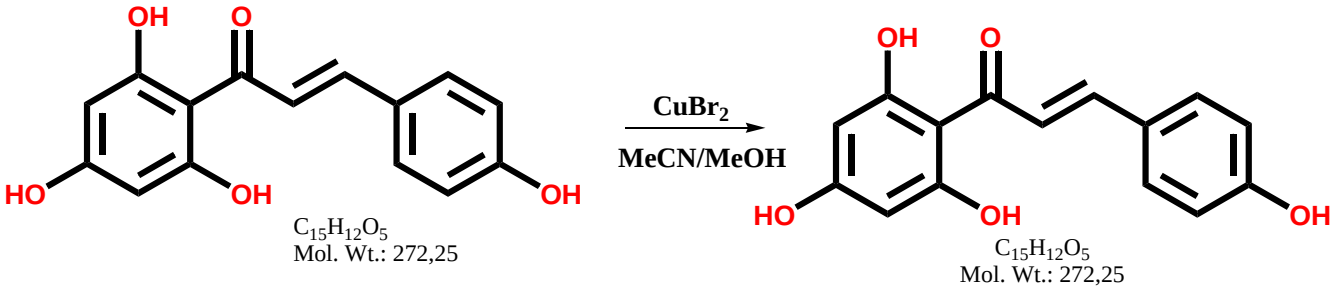
Substance	**Molecular mass**	**Amount of substance**	**Mol / equivalents**	**Purchased from**
2',3,4,4'-Tetrahydroxychalcone	272.25 g/mol	10 mg	0.037 mmol	
$CuBr_2$	223.35 g/mol	22.3 mg	0.1 mmol	Merck
Methanol		5 mL		Sigma Aldrich
Ethyl acetate		5 mL		VWR

10 mg of 2',3,4,4'-tetrahydroxychalcone were dissolved in 5 mL ethyl acetate. Then, 5 mL of a solution consisting of 22.3 mg $CuBr_2$ in methanol were added and the mixture stirred for 30 minutes.

Next, water was added to the solution and the organic layer was extracted. For this purpose, NaCl was added to improve the phase separation. The organic layer was dried over Na_2SO_4, filtered, washed with EtOAc and evaporated to dryness.

The solid obtained was directly analyzed via ^{1}H-NMR spectroscopy.

7.2.16 2',4,4',6'-Tetrahydroxychalcone treatment with $CuBr_2$



$C_{15}H_{12}O_5$
Mol. Wt.: 272,25

$C_{15}H_{12}O_5$
Mol. Wt.: 272,25

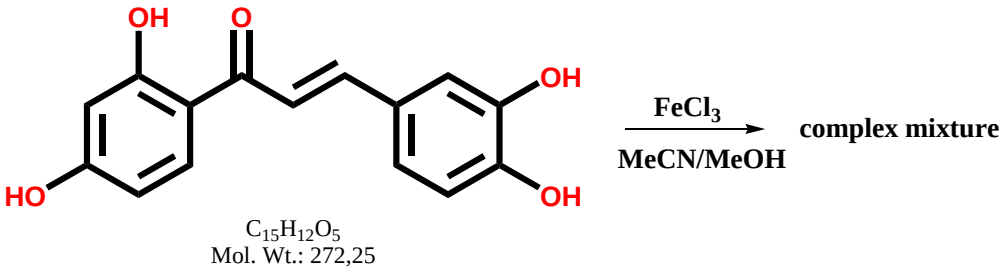
Substance	**Molecular mass**	**Amount of substance**	**Mol / equivalents**	**Purchased from**
2',3,4,4'-Tetrahydroxychalcone	272.25 g/mol	10 mg	0.037 mmol	
$CuBr_2$	223.35 g/mol	22.3 mg	0.1 mmol	Merck
Methanol		5 mL		Sigma Aldrich
Ethyl acetate		5 mL		VWR

10 mg of 2',4,4',6'-tetrahydroxychalcone were dissolved in 5 mL ethyl acetate. Then, 5 mL of a solution consisting of 22.3 mg $CuBr_2$ in methanol were added and the mixture stirred for 30 minutes.

Next, water was added to the solution and the organic layer was extracted. For this purpose, NaCl was added to improve the phase separation. The organic layer was dried over Na_2SO_4, filtered, washed with EtOAc and evaporated to dryness.

The solid obtained was directly analyzed via ^{1}H-NMR spectroscopy.

7.2.17 2',3,4,4'-Tetrahydroxychalcone treatment with $FeCl_3$



$C_{15}H_{12}O_5$
Mol. Wt.: 272,25

Substance	Molecular mass	Amount of substance	Mol / equivalents	Purchased from
2',3,4,4'-Tetrahydroxychalcone	272.25 g/mol	10 mg	0.037 mmol	
$FeCl_3$ * 6 H_2O	270.29 g/mol	27.0 mg	0.1 mmol	Sigma Aldrich
Methanol		5 mL		Sigma Aldrich
Ethyl acetate		5 mL		VRW

10 mg of 2',3,4,4'-tetrahydroxychalcone were dissolved in 5 mL ethyl acetate. Then, 5 mL of a solution consisting of 27.0 mg $CuBr_2$ in methanol were added and the mixture stirred for 30 minutes.

Next, water was added to the solution and the organic layer was extracted. For this purpose, NaCl was added to improve the phase separation. The organic layer was dried over Na_2SO_4, filtered, washed with EtOAc and evaporated to dryness.

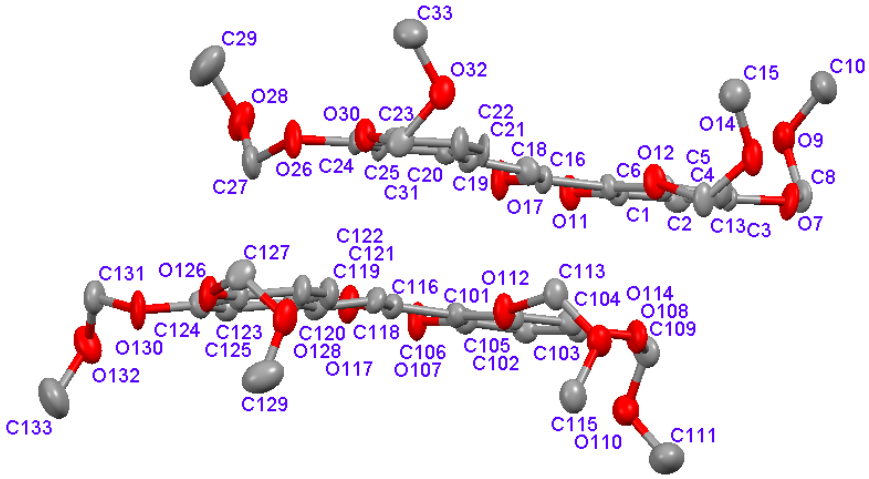
The solid obtained was directly analyzed via ^{1}H-NMR spectroscopy.

8 Abbreviations

4CL	4-Coumarate:CoA ligase
AP	Acetophenone
AS	Aureusidin synthase
BA	Benzaldehyde
Butein	2',3,4',4'-Hydroxychalcone
C4H	4-Hydroxylase

CHI	Chalcone Isomerase
CS	Chalcone synthase
d	doublet
dd	doublet of doublets
ESI	Electrospray ionization
HSQC	Heteronuclear Single Quantum Coherence
IR	Infrared
LDA	Lithium diisopropyldiamide
LiHMDS	Lithium bis(trimethylsilyl)amide
m	multiplet
MeOH	Methanol
MOM-Cl	Methyl chloromethyl ether
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
PAL	Phenylalanine ammonia lyase
PHC	2',3,4,4',6'-Pentahydroxychalcone
s	singlet
tBDMS-Cl	Tert-butyldimethylsilyl chloride
THC	2',3,4',6'-Tetrahydroxychalcone
UV/Vis	Ultraviolet/Visible

9 Appendix



Structural parameters of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone

Position of atoms in the crystal structure of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone

Num.	Label	Xfrac+ESD	Yfrac+ESD	Zfrac+ESD
1	C23	-0.7918(7)	-0.1420(4)	-0.7177(5)
2	C24	-0.6505(8)	-0.1400(4)	-0.7569(4)
3	C20	-0.5208(8)	-0.1423(4)	-0.6088(5)
4	C13	-0.5489(7)	-0.1909(5)	-0.2333(5)
5	H13F	-0.6429	-0.2020	-0.2712
6	H13G	-0.5154	-0.2390	-0.2033
7	C25	-0.5172(7)	-0.1399(4)	-0.7032(4)
8	H25	-0.4220	-0.1381	-0.7304
9	C3	-0.0918(8)	-0.1697(4)	-0.1363(4)
10	C1	-0.0190(7)	-0.1650(4)	-0.2881(4)
11	C4	-0.2482(8)	-0.1702(4)	-0.1662(5)
12	H4	-0.3253	-0.1724	-0.1239
13	C16	-0.2006(8)	-0.1591(4)	-0.4225(5)
14	C21	-0.6606(8)	-0.1446(4)	-0.5713(4)
15	H21	-0.6641	-0.1471	-0.5074
16	C18	-0.3555(8)	-0.1556(4)	-0.4660(5)
17	H18A	-0.4074	-0.2037	-0.4510
18	H18B	-0.4098	-0.1139	-0.4363
19	C31	-1.0631(8)	-0.1549(4)	-0.7457(5)
20	H31A	-1.1375	-0.1625	-0.7976
21	H31B	-1.0603	-0.2023	-0.7092
22	C6	-0.1723(7)	-0.1640(4)	-0.3232(4)
23	C19	-0.3778(8)	-0.1454(4)	-0.5552(4)
24	H19A	-0.3182	-0.1862	-0.5830
25	H19B	-0.3252	-0.0970	-0.5681
26	C22	-0.7962(8)	-0.1435(4)	-0.6238(5)
27	H22	-0.8912	-0.1438	-0.5961
28	C8	0.0854(8)	-0.1560(4)	-0.0075(5)
29	H8A	0.0887	-0.1623	0.0590
30	H8B	0.1547	-0.1943	-0.0322
31	C2	0.0198(8)	-0.1668(4)	-0.1960(5)
32	H2	0.1239	-0.1660	-0.1748
33	C27	-0.5261(9)	-0.1640(5)	-0.8924(5)

34	H27A	-0.4849	-0.2101	-0.8610
35	H27B	-0.5564	-0.1786	-0.9555
36	C15	-0.6236(10)	-0.0654(5)	-0.2010(6)
37	H15A	-0.6618	-0.0344	-0.1522
38	H15B	-0.7033	-0.0705	-0.2498
39	H15C	-0.5339	-0.0407	-0.2244
40	C33	-1.1349(11)	-0.0254(5)	-0.7441(7)
41	H33A	-1.1955	0.0104	-0.7102
42	H33B	-1.1888	-0.0368	-0.8023
43	H33C	-1.0356	-0.0025	-0.7545
44	C10	0.0635(13)	-0.0234(5)	0.0190(6)
45	H10C	0.0741	-0.0331	0.0843
46	H10D	-0.0450	-0.0213	-0.0005
47	H10E	0.1120	0.0253	0.0056
48	C29	-0.4502(13)	-0.0451(6)	-0.9497(7)
49	H29A	-0.5389	-0.0190	-0.9269
50	H29B	-0.4745	-0.0623	-1.0118
51	H29C	-0.3635	-0.0099	-0.9487
52	C5	-0.2863(7)	-0.1673(4)	-0.2576(4)
53	O12	-0.4338(5)	-0.1662(3)	-0.2903(3)
54	O32	-1.1125(5)	-0.0950(3)	-0.6929(3)
55	O7	-0.0692(6)	-0.1694(3)	-0.0447(3)
56	O30	-0.9174(5)	-0.1422(3)	-0.7784(3)
57	O9	0.1355(6)	-0.0837(3)	-0.0281(3)
58	O17	-0.0899(6)	-0.1569(3)	-0.4711(3)
59	O11	0.0959(5)	-0.1617(3)	-0.3442(3)
60	H11	0.1252	-0.2061	-0.3556
61	O14	-0.5830(5)	-0.1383(3)	-0.1676(3)
62	O26	-0.6570(5)	-0.1396(3)	-0.8496(3)
63	O28	-0.4119(6)	-0.1097(4)	-0.8936(4)
64	C123	-1.0465(7)	-0.3494(4)	-0.9758(5)
65	C105	-0.5462(7)	-0.3415(4)	-0.5090(5)
66	C113	-0.8100(8)	-0.3235(4)	-0.4808(5)
67	H11A	-0.7792	-0.2767	-0.4472
68	H11B	-0.9052	-0.3118	-0.5173
69	C120	-0.7809(8)	-0.3439(4)	-0.8621(5)
70	C101	-0.2799(8)	-0.3388(4)	-0.5422(5)
71	C124	-0.9035(8)	-0.3557(4)	-1.0129(5)
72	C103	-0.3517(8)	-0.3470(4)	-0.3885(5)
73	C116	-0.4667(8)	-0.3313(4)	-0.6741(5)
74	C104	-0.5069(8)	-0.3450(4)	-0.4188(5)
75	H104	-0.5839	-0.3461	-0.3763
76	C118	-0.6204(8)	-0.3310(4)	-0.7190(5)
77	H11C	-0.6811	-0.3702	-0.6891
78	H11D	-0.6671	-0.2809	-0.7066
79	C106	-0.4334(8)	-0.3378(4)	-0.5762(5)
80	C109	-0.1715(9)	-0.3631(5)	-0.2622(5)
81	H10A	-0.1059	-0.3233	-0.2871
82	H10B	-0.1672	-0.3569	-0.1956
83	C119	-0.6371(8)	-0.3429(4)	-0.8072(5)
84	H11E	-0.5883	-0.3928	-0.8177
85	H11F	-0.5729	-0.3041	-0.8351
86	C125	-0.7737(8)	-0.3541(4)	-0.9564(5)
87	H125	-0.6771	-0.3600	-0.9812
88	C102	-0.2389(8)	-0.3452(4)	-0.4492(5)
89	H102	-0.1346	-0.3484	-0.4286
90	C127	-1.3150(8)	-0.3346(5)	-1.0082(5)
91	H12A	-1.3100	-0.2840	-0.9785

92	H12B	-1.3862	-0.3305	-1.0623
93	C115	-0.8855(9)	-0.4505(5)	-0.4619(5)
94	H11G	-0.9286	-0.4843	-0.4173
95	H11H	-0.7964	-0.4749	-0.4864
96	H11I	-0.9623	-0.4405	-0.5111
97	C121	-0.9213(9)	-0.3372(4)	-0.8272(5)
98	H121	-0.9287	-0.3308	-0.7639
99	C122	-1.0543(8)	-0.3398(4)	-0.8842(5)
100	H122	-1.1509	-0.3349	-0.8592
101	C131	-0.7791(9)	-0.3460(5)	-1.1510(5)
102	H13A	-0.8105	-0.3388	-1.2157
103	H13B	-0.7384	-0.2967	-1.1275
104	C129	-1.3890(11)	-0.4608(6)	-0.9819(8)
105	H12C	-1.4451	-0.4914	-0.9395
106	H12D	-1.2881	-0.4833	-0.9883
107	H12E	-1.4455	-0.4600	-1.0409
108	C111	-0.1811(14)	-0.4931(6)	-0.2304(7)
109	H11J	-0.1640	-0.4816	-0.1659
110	H11K	-0.1333	-0.5420	-0.2437
111	H11L	-0.2910	-0.4961	-0.2460
112	C133	-0.7003(14)	-0.4677(6)	-1.1965(7)
113	H13C	-0.7749	-0.4984	-1.1657
114	H13D	-0.6074	-0.4977	-1.2028
115	H13E	-0.7434	-0.4530	-1.2565
116	O126	-1.1705(5)	-0.3531(3)	-1.0359(3)
117	O112	-0.6946(5)	-0.3421(3)	-0.5404(3)
118	O108	-0.3264(6)	-0.3518(3)	-0.2974(3)
119	O114	-0.8403(5)	-0.3799(3)	-0.4194(3)
120	O110	-0.1152(6)	-0.4337(3)	-0.2829(4)
121	O128	-1.3726(6)	-0.3869(4)	-0.9493(4)
122	O117	-0.3547(6)	-0.3262(3)	-0.7232(3)
123	O107	-0.1656(5)	-0.3361(3)	-0.5976(3)
124	H107	-0.2011	-0.3377	-0.6515
125	O130	-0.9093(5)	-0.3653(3)	-1.1039(3)
126	O132	-0.6640(6)	-0.3998(3)	-1.1440(4)

Bond length in crystal structure of 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone

Num.	Atom1	Atom2	Length
1	C23	C24	1.40(1)
2	C23	C22	1.39(1)
3	C23	O30	1.382(8)
4	C24	C25	1.377(9)
5	C24	O26	1.371(7)
6	C20	C25	1.40(1)
7	C20	C21	1.38(1)
8	C20	C19	1.45(1)
9	C13	H13F	0.990(6)
10	C13	H13G	0.990(8)
11	C13	O12	1.422(8)
12	C13	O14	1.386(9)
13	C25	H25	0.950(6)
14	C3	C4	1.42(1)
15	C3	C2	1.36(1)
16	C3	O7	1.360(7)
17	C1	C6	1.415(9)
18	C1	C2	1.387(9)

19	C1	O11	1.346(8)
20	C4	H4	0.950(7)
21	C4	C5	1.377(9)
22	C16	C18	1.47(1)
23	C16	C6	1.480(9)
24	C16	O17	1.244(9)
25	C21	H21	0.950(6)
26	C21	C22	1.38(1)
27	C18	H18A	0.990(7)
28	C18	H18B	0.990(7)
29	C18	C19	1.337(9)
30	C31	H31A	0.989(7)
31	C31	H31B	0.991(7)
32	C31	O32	1.393(9)
33	C31	O30	1.412(9)
34	C6	C5	1.437(9)
35	C19	H19A	0.990(7)
36	C19	H19B	0.990(7)
37	C22	H22	0.951(7)
38	C8	H8A	0.990(7)
39	C8	H8B	0.990(7)
40	C8	O7	1.455(9)
41	C8	O9	1.381(9)
42	C2	H2	0.949(7)
43	C27	H27A	0.990(8)
44	C27	H27B	0.991(7)
45	C27	O26	1.411(9)
46	C27	O28	1.38(1)
47	C15	H15A	0.979(9)
48	C15	H15B	0.981(9)
49	C15	H15C	0.980(9)
50	C15	O14	1.41(1)
51	C33	H33A	0.98(1)
52	C33	H33B	0.98(1)
53	C33	H33C	0.98(1)
54	C33	O32	1.44(1)
55	C10	H10C	0.981(9)
56	C10	H10D	0.98(1)
57	C10	H10E	0.979(9)
58	C10	O9	1.43(1)
59	C29	H29A	0.98(1)
60	C29	H29B	0.98(1)
61	C29	H29C	0.98(1)
62	C29	O28	1.43(1)
63	C5	O12	1.357(7)
64	O11	H11	0.840(5)
65	C123	C124	1.40(1)
66	C123	C122	1.37(1)
67	C123	O126	1.368(8)
68	C105	C104	1.36(1)
69	C105	C106	1.45(1)
70	C105	O112	1.358(8)
71	C113	H11A	0.989(7)
72	C113	H11B	0.990(7)
73	C113	O112	1.420(9)
74	C113	O114	1.380(9)
75	C120	C119	1.46(1)
76	C120	C125	1.41(1)

77	C120	C121	1.37(1)
78	C101	C106	1.41(1)
79	C101	C102	1.41(1)
80	C101	O107	1.334(9)
81	C124	C125	1.37(1)
82	C124	O130	1.357(9)
83	C103	C104	1.41(1)
84	C103	C102	1.38(1)
85	C103	O108	1.358(9)
86	C116	C118	1.47(1)
87	C116	C106	1.47(1)
88	C116	O117	1.260(9)
89	C104	H104	0.951(7)
90	C118	H11C	0.990(7)
91	C118	H11D	0.991(7)
92	C118	C119	1.32(1)
93	C109	H10A	0.990(8)
94	C109	H10B	0.991(7)
95	C109	O108	1.442(9)
96	C109	O110	1.37(1)
97	C119	H11E	0.990(7)
98	C119	H11F	0.989(7)
99	C125	H125	0.950(7)
100	C102	H102	0.950(7)
101	C127	H12A	0.990(9)
102	C127	H12B	0.990(7)
103	C127	O126	1.395(9)
104	C127	O128	1.38(1)
105	C115	H11G	0.980(8)
106	C115	H11H	0.980(8)
107	C115	H11I	0.980(7)
108	C115	O114	1.43(1)
109	C121	H121	0.950(7)
110	C121	C122	1.40(1)
111	C122	H122	0.949(7)
112	C131	H13A	0.990(7)
113	C131	H13B	0.990(8)
114	C131	O130	1.415(9)
115	C131	O132	1.38(1)
116	C129	H12C	0.98(1)
117	C129	H12D	0.98(1)
118	C129	H12E	0.98(1)
119	C129	O128	1.39(1)
120	C111	H11J	0.98(1)
121	C111	H11K	0.98(1)
122	C111	H11L	0.98(1)
123	C111	O110	1.44(1)
124	C133	H13C	0.98(1)
125	C133	H13D	0.98(1)
126	C133	H13E	0.98(1)
127	C133	O132	1.45(1)
128	O107	H107	0.841(4)

Angles in crystal structure of 2’-hydroxy-3,4,4’,6’-tetrakis(methoxymethoxy)chalcone

Num.	Atom1	Atom2	Atom3	Angle
1	C24	C23	C22	119.4(6)
2	C24	C23	O30	115.0(6)

3	C22	C23	O30	125.6(6)
4	C23	C24	C25	120.3(6)
5	C23	C24	O26	115.4(6)
6	C25	C24	O26	124.3(6)
7	C25	C20	C21	118.4(6)
8	C25	C20	C19	118.6(6)
9	C21	C20	C19	122.9(6)
10	H13F	C13	H13G	107.7(7)
11	H13F	C13	O12	108.7(6)
12	H13F	C13	O14	108.8(6)
13	H13G	C13	O12	108.8(6)
14	H13G	C13	O14	108.8(6)
15	O12	C13	O14	113.9(6)
16	C24	C25	C20	120.6(6)
17	C24	C25	H25	119.7(6)
18	C20	C25	H25	119.7(6)
19	C4	C3	C2	121.3(6)
20	C4	C3	O7	113.2(6)
21	C2	C3	O7	125.5(6)
22	C6	C1	C2	122.4(6)
23	C6	C1	O11	120.3(6)
24	C2	C1	O11	117.3(6)
25	C3	C4	H4	120.6(7)
26	C3	C4	C5	118.8(6)
27	H4	C4	C5	120.6(7)
28	C18	C16	C6	122.3(6)
29	C18	C16	O17	118.6(6)
30	C6	C16	O17	119.0(6)
31	C20	C21	H21	119.0(7)
32	C20	C21	C22	122.1(6)
33	H21	C21	C22	118.9(7)
34	C16	C18	H18A	107.0(6)
35	C16	C18	H18B	107.0(6)
36	C16	C18	C19	121.1(6)
37	H18A	C18	H18B	106.8(7)
38	H18A	C18	C19	107.0(6)
39	H18B	C18	C19	107.1(6)
40	H31A	C31	H31B	107.8(7)
41	H31A	C31	O32	108.9(6)
42	H31A	C31	O30	109.0(6)
43	H31B	C31	O32	108.9(6)
44	H31B	C31	O30	109.0(6)
45	O32	C31	O30	113.1(6)
46	C1	C6	C16	117.8(6)
47	C1	C6	C5	115.9(6)
48	C16	C6	C5	126.3(6)
49	C20	C19	C18	128.3(6)
50	C20	C19	H19A	105.2(6)
51	C20	C19	H19B	105.2(6)
52	C18	C19	H19A	105.3(6)
53	C18	C19	H19B	105.2(6)
54	H19A	C19	H19B	105.9(6)
55	C23	C22	C21	119.2(6)
56	C23	C22	H22	120.4(7)
57	C21	C22	H22	120.4(7)
58	H8A	C8	H8B	108.0(7)
59	H8A	C8	O7	109.4(6)
60	H8A	C8	O9	109.3(6)

61	H8B	C8	O7	109.3(6)
62	H8B	C8	O9	109.2(6)
63	O7	C8	O9	111.5(6)
64	C3	C2	C1	119.7(6)
65	C3	C2	H2	120.2(7)
66	C1	C2	H2	120.1(7)
67	H27A	C27	H27B	107.7(7)
68	H27A	C27	O26	108.7(7)
69	H27A	C27	O28	108.8(7)
70	H27B	C27	O26	108.7(7)
71	H27B	C27	O28	108.7(7)
72	O26	C27	O28	114.1(6)
73	H15A	C15	H15B	109.5(8)
74	H15A	C15	H15C	109.5(8)
75	H15A	C15	O14	109.5(7)
76	H15B	C15	H15C	109.5(8)
77	H15B	C15	O14	109.4(7)
78	H15C	C15	O14	109.4(7)
79	H33A	C33	H33B	109.4(9)
80	H33A	C33	H33C	109.5(9)
81	H33A	C33	O32	109.5(8)
82	H33B	C33	H33C	109.5(9)
83	H33B	C33	O32	109.5(8)
84	H33C	C33	O32	109.5(8)
85	H10C	C10	H10D	109.4(9)
86	H10C	C10	H10E	109.5(9)
87	H10C	C10	O9	109.5(8)
88	H10D	C10	H10E	109.5(9)
89	H10D	C10	O9	109.5(8)
90	H10E	C10	O9	109.5(8)
91	H29A	C29	H29B	109(1)
92	H29A	C29	H29C	110(1)
93	H29A	C29	O28	109.5(9)
94	H29B	C29	H29C	110(1)
95	H29B	C29	O28	109.4(9)
96	H29C	C29	O28	109.4(9)
97	C4	C5	C6	121.9(6)
98	C4	C5	O12	121.6(6)
99	C6	C5	O12	116.5(5)
100	C13	O12	C5	118.7(5)
101	C31	O32	C33	112.3(6)
102	C3	O7	C8	117.2(5)
103	C23	O30	C31	118.8(5)
104	C8	O9	C10	114.4(6)
105	C1	O11	H11	109.5(5)
106	C13	O14	C15	114.7(6)
107	C24	O26	C27	117.4(5)
108	C27	O28	C29	113.9(7)
109	C124	C123	C122	119.6(6)
110	C124	C123	O126	116.0(6)
111	C122	C123	O126	124.4(6)
112	C104	C105	C106	122.2(6)
113	C104	C105	O112	121.3(6)
114	C106	C105	O112	116.5(6)
115	H11A	C113	H11B	107.5(7)
116	H11A	C113	O112	108.6(6)
117	H11A	C113	O114	108.7(6)
118	H11B	C113	O112	108.6(6)

119	H11B	C113	O114	108.6(6)
120	O112	C113	O114	114.7(6)
121	C119	C120	C125	117.7(6)
122	C119	C120	C121	123.8(7)
123	C125	C120	C121	118.5(7)
124	C106	C101	C102	122.3(7)
125	C106	C101	O107	121.2(6)
126	C102	C101	O107	116.5(6)
127	C123	C124	C125	119.3(7)
128	C123	C124	O130	114.6(6)
129	C125	C124	O130	126.1(6)
130	C104	C103	C102	120.7(7)
131	C104	C103	O108	114.7(6)
132	C102	C103	O108	124.6(6)
133	C118	C116	C106	124.9(6)
134	C118	C116	O117	117.8(6)
135	C106	C116	O117	117.3(6)
136	C105	C104	C103	119.9(7)
137	C105	C104	H104	120.1(7)
138	C103	C104	H104	120.0(7)
139	C116	C118	H11C	107.4(6)
140	C116	C118	H11D	107.4(6)
141	C116	C118	C119	119.5(7)
142	H11C	C118	H11D	107.0(7)
143	H11C	C118	C119	107.5(7)
144	H11D	C118	C119	107.4(7)
145	C105	C106	C101	115.6(6)
146	C105	C106	C116	125.4(6)
147	C101	C106	C116	119.0(6)
148	H10A	C109	H10B	107.7(7)
149	H10A	C109	O108	109.0(7)
150	H10A	C109	O110	109.1(7)
151	H10B	C109	O108	109.0(7)
152	H10B	C109	O110	109.1(7)
153	O108	C109	O110	112.8(6)
154	C120	C119	C118	126.4(7)
155	C120	C119	H11E	105.7(6)
156	C120	C119	H11F	105.7(6)
157	C118	C119	H11E	105.7(7)
158	C118	C119	H11F	105.7(7)
159	H11E	C119	H11F	106.2(7)
160	C120	C125	C124	121.4(7)
161	C120	C125	H125	119.2(7)
162	C124	C125	H125	119.4(7)
163	C101	C102	C103	119.2(7)
164	C101	C102	H102	120.4(7)
165	C103	C102	H102	120.4(7)
166	H12A	C127	H12B	107.7(7)
167	H12A	C127	O126	108.8(7)
168	H12A	C127	O128	108.8(7)
169	H12B	C127	O126	108.8(7)
170	H12B	C127	O128	108.8(7)
171	O126	C127	O128	113.9(6)
172	H11G	C115	H11H	109.5(8)
173	H11G	C115	H11I	109.5(8)
174	H11G	C115	O114	109.5(7)
175	H11H	C115	H11I	109.4(8)
176	H11H	C115	O114	109.4(7)

177	H11I	C115	O114	109.5(7)
178	C120	C121	H121	119.8(7)
179	C120	C121	C122	120.5(7)
180	H121	C121	C122	119.7(7)
181	C123	C122	C121	120.8(7)
182	C123	C122	H122	119.6(7)
183	C121	C122	H122	119.6(7)
184	H13A	C131	H13B	107.7(7)
185	H13A	C131	O130	108.7(7)
186	H13A	C131	O132	108.7(7)
187	H13B	C131	O130	108.7(7)
188	H13B	C131	O132	108.7(7)
189	O130	C131	O132	114.1(6)
190	H12C	C129	H12D	109(1)
191	H12C	C129	H12E	109(1)
192	H12C	C129	O128	109.5(9)
193	H12D	C129	H12E	109(1)
194	H12D	C129	O128	109.5(9)
195	H12E	C129	O128	109.5(9)
196	H11J	C111	H11K	109(1)
197	H11J	C111	H11L	109(1)
198	H11J	C111	O110	109.5(9)
199	H11K	C111	H11L	109(1)
200	H11K	C111	O110	109.5(9)
201	H11L	C111	O110	109.4(9)
202	H13C	C133	H13D	109(1)
203	H13C	C133	H13E	109(1)
204	H13C	C133	O132	109.5(9)
205	H13D	C133	H13E	110(1)
206	H13D	C133	O132	109.5(9)
207	H13E	C133	O132	109.4(9)
208	C123	O126	C127	120.1(5)
209	C105	O112	C113	119.4(5)
210	C103	O108	C109	117.7(6)
211	C113	O114	C115	112.8(5)
212	C109	O110	C111	111.6(7)
213	C127	O128	C129	115.7(7)
214	C101	O107	H107	109.4(5)
215	C124	O130	C131	118.4(6)
216	C131	O132	C133	112.5(7)

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