CHARLES UNIVERSITY

FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ

Department of Inorganic and Organic Chemistry

UNIVERSITY OF LIMOGES

FACULTY OF PHARMACY

Laboratory of Natural Substances Chemistry

DIPLOMA THESIS

Synthesis and characterisation of flavonoids as potential anticancer agents

Supervisors:

prof. RNDr. Milan Pour, Ph.D.

Dr. Christelle Pouget

Hradec Králové 2016 **Lukáš Novotný**

DECLARATION

I declare that this thesis is my original work. All used literature and sources are listed in the list of used literature at the end of the thesis and are properly cited. This work has not been used to obtain equal or different degree.

Hradec Králové 2016

Signature:

Acknowledgement

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Abstrakt

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Školitelé: prof. RNDr. Milan Pour, Ph.D., Dr. Christelle Pouget
Student: Lukáš Novotný
Název diplomové práce: Syntéza a charakterizace flavonoidů jako potenciálních protinádorových látek

Nádorová onemocnění představují ročně s více jak 3 miliony nových případů a 1,7 milionu úmrtí druhou nejčastější příčinu úmrtí v Evropě. Hledání nových protinádorově působících látek je jednou z nejdůležitějších cest pro zlepšení léčby a zvládání této nemoci. V současné době okolo 50 % používaných protinádorových léčiv jsou přírodní látky nebo sloučeniny z přírodních látek odvozené. Flavonoidy, deriváty 2-fenyl-1,4-benzopyronu, jsou známy pro své antioxidační, protizánětlivé, vaskuloprotektivní, antimikrobní a protinádorové účinky. Tento projekt se zaměřuje na flavonoidy s otevřeným kruhem C, zvané chalkony. Zahrnuje syntézu dvou originálních látek, bichalkonu a chalkonu navázaného na polyamin, za účelem budoucího testování jejich potenciální antiproliferativní aktivity na nádorových buněčných koloniích. Deriváty chalkonů byly získány pomocí Claisenovy–Schmidtovy kondenzace a Suzukiho reakce. Různé syntetické přístupy jsou komentovány. V současné době jsou obě získané sloučeniny předmětem biologického hodnocení.

Abstract

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Consultants: prof. RNDr. Milan Pour, Ph.D., Dr. Christelle Pouget
Student: Lukáš Novotný
Title of Thesis: Synthesis and characterisation of flavonoids as potential anticancer agents

With more than 3 million new cases and 1.7 million deaths each year, cancer represents the second most important cause of morbidity in Europe. Search for new anticancer agents is one of the most important ways to improve treatment and management of cancer. At present, around 50 % of drugs against cancer are natural compounds or have a natural origin. Flavonoids, 2-phenyl-1,4-benzopyrones derivatives, are known for their antioxidant, anti-inflammatory, vasculoprotective, antimicrobial and anticancer properties. This project focuses on flavonoids with open C ring, named chalcones. The synthesis of two original molecules is described: one bichalcone and a combined chalcone-polyamine, prepared for the purpose of screening antiproliferative effect on cancer cell lines. The chalcone derivatives were made using Claisen-Schmidt condensation and Suzuki reaction. Different synthetic approaches are discussed. Presently, the two products are being subjected to biological evaluation.

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LIST OF ABBREVIATIONS

Boc	*tert*-butyloxycarbonyl protecting group
CA-4	combretastatin A-4
calcd	calculated
d	doublet
dd	doublet of doublets
DEGBG	diethylglyoxal bis(guanylhydrazone)
DMF	dimethylformamide
DNA	deoxyribonucleic acid
ED_{50}	median effective dose
eq.	equivalent
exp.	experiment
Hex	hexane
IC_{50}	half maximal inhibitory concentration
IR	infrared spectroscopy
L	ligand
LCSN	Laboratory of Natural Substances Chemistry
m	multiplet
MNs	malignant neoplasms
MS	mass spectroscopy
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	microwawe
NMR	nuclear magnetic resonance
Nu	nucleophile
ODC	ornithine decarboxylase
PAO	polyamine oxydase
PPTS	pyridinium p-toluenesulfonate
PTSA	p-toluenesulfonic acid
R	radical

r.t.	room temperature
RNA	ribonucleic acid
s	singlet
SAME-DC	*S*-adenosylmethionine decarboxylase
t	triplet
THF	tetrahydrofuran
TLC	thin layer chromatography
Tol	toluene
UV	ultraviolet
X	halogen

1. INTRODUCTION

1.1 Cancer

1.1.1 **Definition and epidemiology**

Cancer is the name given to a group of diseases involving abnormal and uncontrolled cell growth and multiplication. The life of cells is normally regulated by a multitude of control mechanisms. They lead to the death of old or damaged cells by apoptosis or phagocytosis and to the formation of new cells when it is needed. In a cancer process, the cells become either resistant to the control mechanisms and differentiate and multiply abnormally, or these mechanisms do not function properly. The outcome of the process usually is the formation of masses of tissues, called solid tumors. The tumors can progressively invade surrounding tissues. In addition, some cancer cells can break off and travel to distant places in the body to form new tumors there. This process is called metastasis and is the major cause of death by cancer.[1, 2]

As regards molecular level, a cancer cell is a cell which has been affected by certain modifications. The following factors can be listed among the reasons: sustaining proliferative signaling, evading growth suppressors, activating invasion and metastasis, enabling replicative immortality, inducing angiogenesis and resisting cell death. These modifications are the result of the genetic load of the cell and of external factors (physical carcinogens, such as ultraviolet and ionizing radiation, chemical carcinogens, biological carcinogens).[1, 2, 3]

Cancer is the second main reason of death in the Czech Republic after cardiovascular diseases.[4] In 2014, 27 600 people died because of cancer which amounts to 26.1 % of deaths in that year.[4] The statistics concerning the European Union is similar.[5] The mortality by cancer has remained unchanged in the Czech Republic since 2004, unlike the European Union, where the mortality has decreased.[4, 5] Concerning the incidence, the number of new cases in the Czech Republic between 1985 and 2011 doubled and considerably grew up in the European Union. The discrepancy between the trends of mortality and incidence can be explained by the improvement of the diagnostics and therapy over the last decades *(Fig. 1).*[6] These data are in agreement with the unfavourable prognosis of the World Health Organization which predicts a 70% increase in new cancer cases expected over the next two decades worldwide.[3]

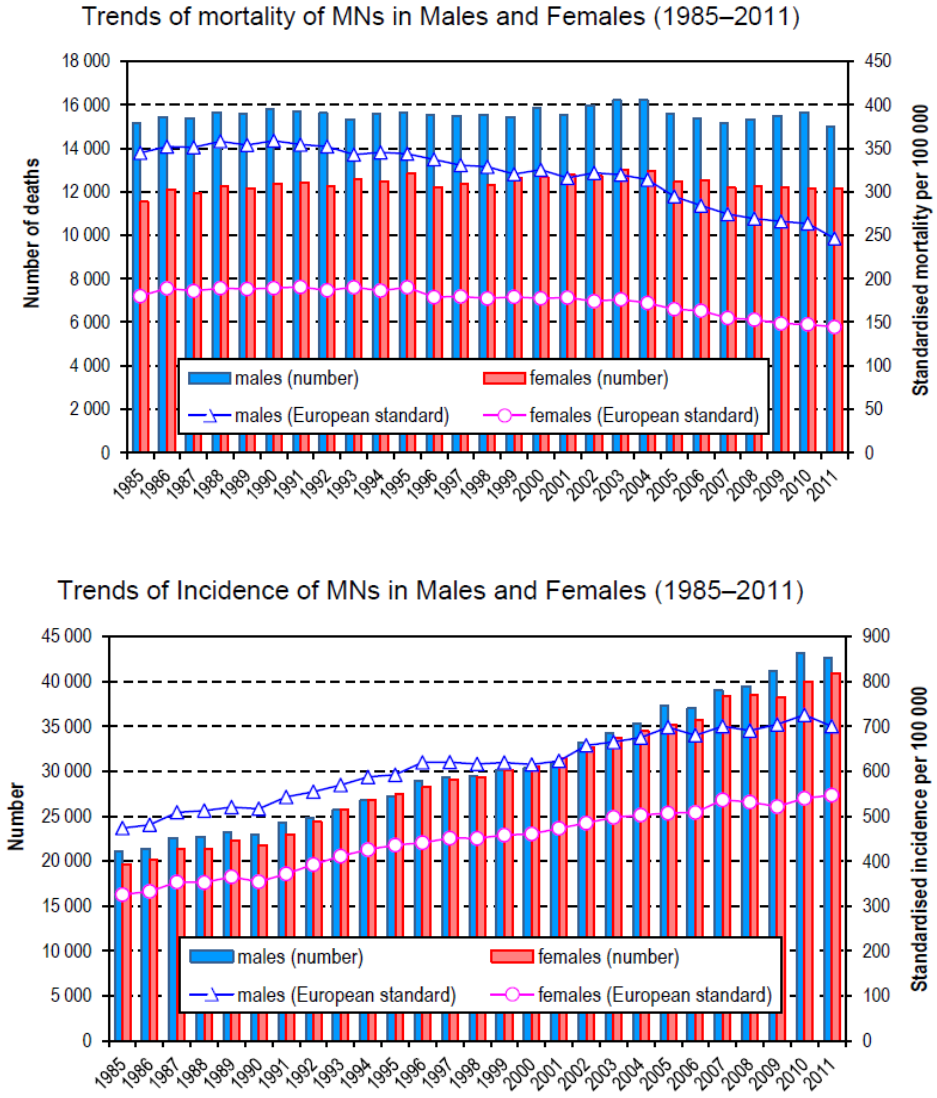


Fig. 1: Trends of mortality and incidence of MNs in the Czech Republic and the European Union

1.1.2 **Cancer treatment**

As regards cancer treatment, a number of options exists. Surgery, chemotherapy and radiation therapy are traditionally and widely used. Immunotherapy, stem cells transplants, photodynamic therapy or targeted therapy are among the more recent, promising therapies, generally with fewer side effects.[7]

Targeted therapy is a special type of chemotherapy that takes advantage of small differences between normal and cancer cells. It is sometimes used alone, but most often also in combination with other procedures.[7]

Chemotherapy includes the use of chemical drugs. Anticancer drugs can be divided into several groups on the basis of their mechanism of action. Alkylating agents directly damage DNA and disable cell proliferation. Antimetabolites interfere with DNA and RNA replication through providing „false" building blocks. Cytotoxic antibiotics act by altering the DNA inside cancer cells. Topoisomerase inhibitors interfere with enzymes called topoisomerases, which help to separate the strands of DNA so that they cannot be copied during the S phase. Mitotic inhibitors work by stopping mitosis in the G2/M phase. The last two groups include a number of plant alkaloids and other compounds derived from nature.[8]

1.1.3 **Natural compounds in cancer therapy**

A number of natural molecules have served as anticancer drugs or their prototypes. At present, circa 50 % of drugs against cancer are natural compounds or have a natural origin.[9]

1.1.3.1 Tubulin binding drugs

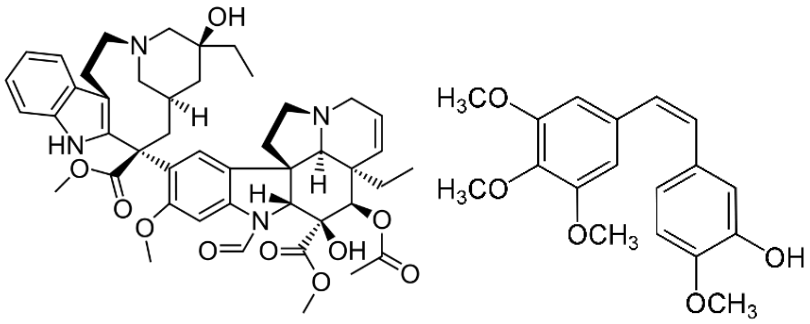
One of the targets of anticancer drugs is the microtubule system. Microtubules are key components of the cytoskeleton of eucaryotic cells which have various cellular functions, such as intracellular transport. They play a crucial role in cell division by being involved in the movement and attachment of the chromosomes during various stages of mitosis. [10, 11]

The drugs can be divided into several groups according to their chemical structure.

Vinca alkaloids are a group of compounds obtained from the Madagascar periwinkle plant *Cataranthus roseus.* There are four major vinca alcaloids in clinical use: vincristine (**1**, *Fig. 2*), vinblastine, vinorelbine, vindesine, and a semisynthetic derivative vinflunine. The vinca alkaloids inhibit the polymerization of tubulin into microtubules, which results in the G2/M arrest within the cell cycle and eventually in cell death.[12]

Combretastatin A-4 (CA-4) (**2**, *Fig. 2*) is a polyphenol present in the bark of *Combretum caffrum*. Similar to vinca alcaloids, it belongs to a group of tubulin polymerization interfering drugs. This substance differs from vinca alcaloids in its binding site on the tubulin protein. For

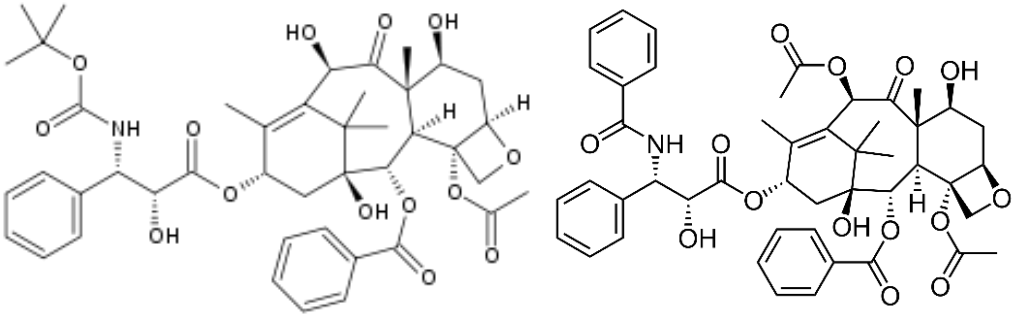
CA-4, the corresponding domain is the colchicine binding site. CA-4 phosphate, more soluble and bioavailable form of CA-4, is currently under clinical trials.[13]



*vincristine **1*** *combretastatin A-4 **2***

*Fig. 2: Structures of vincristine **1** and combretastatin A-4 **2***

Paclitaxel (**3**, *Fig. 3*) and docetaxel (**4**, Fig. 3) represent the taxane family of drugs originally isolated from *Taxus brevifolia*. Cabazitaxel is another, semisynthetic drug of this group. Similar to previous examples, tubulin is the target of taxane compounds. However, in this case, the molecules act by attaching to tubulin within existing microtubules. As a result, the polymer is stabilized and depolymerization to tubulin becomes impossible.[14]



*paclitaxel **3*** *docetaxel **4***

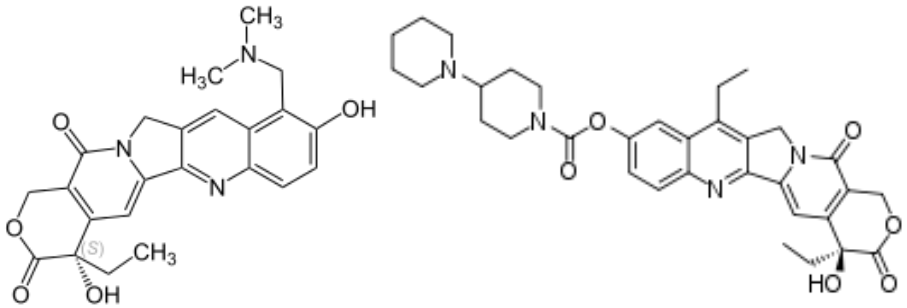
*Fig. 3: Structures of paclitaxel **3** and docetaxel **4***

1.1.3.2 Topoisomerase inhibitors

Beside the microtubule system, topoisomerase I and topoisomerase II may be targets of certain natural compounds used in cancer therapy. These are referred to as topoisomerase inhibitors. They inhibit either topoisomerase I, an enzyme which controls the structure of DNA by cutting one of the two strands of double-stranded DNA, breaking the strand and religating it, or topoisomerase II, which controls the structure of DNA by cutting and religating the two

strands of DNA. These alkaloids form a ternary complex with DNA and topoisomerase, and prevent religation of the DNA strand(s). This disruption prevents DNA replication and leads to cell death. [15]

Camptothecin is a quinoline alcaloid isolated from the bark and stem of *Campthoteca iluminata.* Two analogues with fewer side effects and better bioavailability have been approved for the treatment of cancer: topotecan (**5**) and irinotecan (**6**) (*Fig. 4*). Both inhibit topoisomerase I. [15]

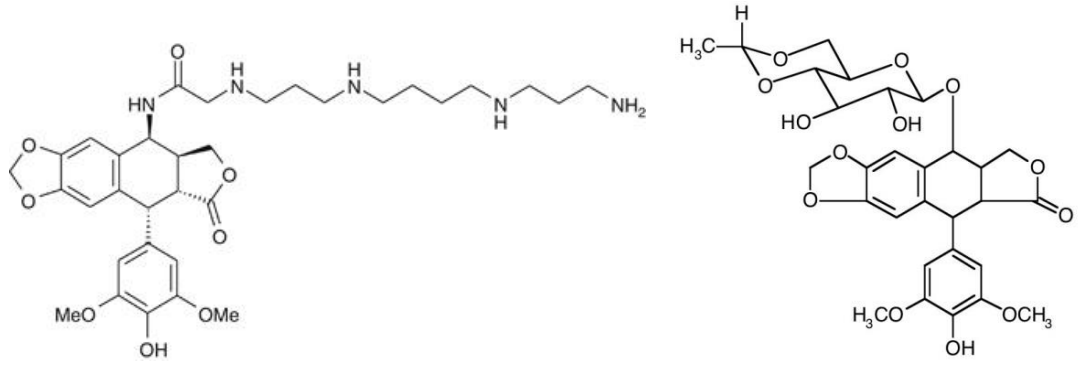


*topotecan **5*** *irinotecan **6***

*Fig. 4: Structures of topotecan **5** and irinotecan **6***

Podophyllin is a non-alkaloid lignan extracted from the rhizomes of *Podophyllum* species such as *Podophyllum hexandrum*, whose structures paved the way towards two semisynthetic derivatives with fewer side effects: etoposide and teniposide. Unlike podophyllin which acts via tubulin binding, etoposide (**7**, *Fig. 5*) and teniposide are topoisomerase II inhibitors.[15, 16]

F14512 (**8**, *Fig. 5*) is a new topoisomerase II inhibitor which is currently in clinical trials in patients with acute myeloid leukemia.[17] It is a derivative of etoposide containing a polyamine moiety. It has been shown that polyamines linked to an active compound have the ability to target the compound into tumor, to improve the bioavailability of the compound, and therefore to increase topomerase II inhibition.[18]

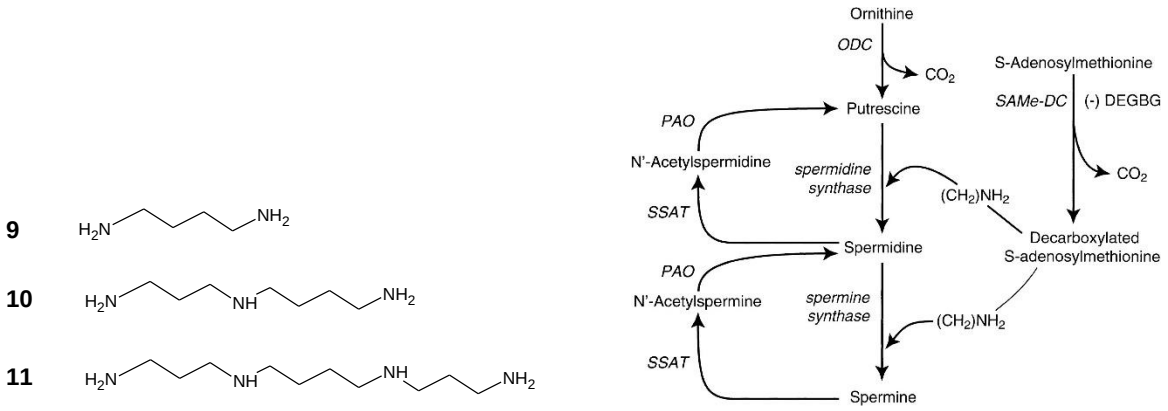


*etoposide **7*** *F14512 **8***

Fig. 5: Structures of etoposide **7** *and F14512* **8**

1.2 Polyamines

Polyamines are small molecules having one hydrocarbon aliphatic chain and at least two amino groups. They play multiple roles in mammalian physiology, especially in the proliferation and development of cells. The compounds are ubiquitous in the liver of poultry, fermented soybeans, wheat germs and mushrooms. Apart from diet, polyamine level is maintained via microbial production and *de novo* synthesis in the cells. Polyamines synthesized in mammalian cells are putrescine **9**, spermine **10** and spermidine **11**. Endogenously, putrescine is formed from ornithine. The latter is, in turn, formed from arginine by the action of ornithine decarboxylase (ODC) which is a highly regulated step in maintaining polyamine level. Putrescine is converted to spermidine by spermidine synthesis, while spermidine is further converted to spermine by spermine synthase. The synthesis of polyamines declines with age. The structure of major polyamines and their synthesis is depicted in *Scheme 1*.[19, 20, 21]



Scheme 1: Structures of putrescine **9**, *spermidine* **10**, *spermine* **11,** *and scheme of their biosynthesis*

Polyamines are positively charged at physiological pH. Electrostatic interactions through cationic amino groups are therefore dominant. For this reason, polyamines may act as ligands at multiple sites on DNA, RNA, proteins, phospholipids and nucleotide triphosphates. It has been proven that lack of polyamines causes a retardation or inhibition of cell growth. Supply of exogenous polyamines can restore the growth of these cells. The cells have a polyamine transporter system on their membrane which enables the intake of exogenous polyamines, and which thus influences polyamine level in the cells. This system is highly active in rapidly proliferating cells, for example in the cells of foetus, and particularly in tumor cells because endogenous polyamine synthesis, even though enhanced, is insufficient.[20, 22]

Besides the effects on growth and proliferation, polyamines activate angiogenesis in tumor cells, and have a role in the process of metastasis. These effects can be explained by the influence of cationic amino groups on the conformation of DNA, and thus on the expression of certain genes or stabilisation of DNA related to resistance against apoptosis.[23]

For all these reasons, research on polyamines has considerably intensified in recent years. The main area of research is the relationship between polyamines and cancer.[22] Several approaches are possible. One approach could be based on an effort to decrease the concentration of polyamines in tumor cells. Another approach is based on the possibility of using the hyperactivation of the transport system. As the transport system is not specific enough, it can be the target of drugs having a polyamine moiety linked to an active compound. Using this principle, the anticancer drug can be transported specifically to tumor cells.[24] By way of example, the compound F14512 **8** (see the previous section 1.1.3.2) acts via this mechanism.[17]

1.3 Flavonoids

1.3.1 **General information**

Flavonoids are the most commonly distributed group of plant phenolic compounds having a 4*H*-benzo[b]pyran structure.[25] More specifically, flavonoids are compounds derived from 2-phenyl-chromen-4-one, whereas the substances derived from 3-phenylchromen-4-one are termed isoflavonoids *(Fig. 6).*[26]

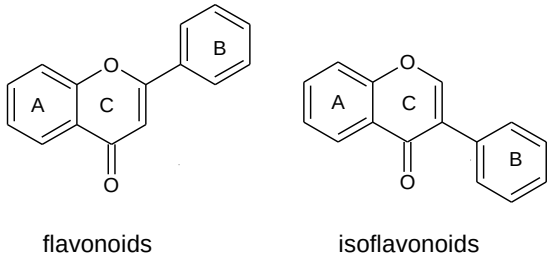


Fig. 6: Basic structures of flavonoids and isoflavonoids

Flavonoids can be divided into several groups depending on the degree of oxidation and unsaturation of the C ring. Substitutions of the core structure (flavones) include hydroxylation in position 3 (flavonols), reduction of 2(3) carbon-carbon double bond (flavanones), sometimes simultaneously complemented with hydroxylation in position 3 (flavanonols). Hydroxylation of the skeleton without oxo group in various positions (flavan-3-ols, flavan-4-ols, flavan-3,4-diols) or a cationic form (anthocyanidins) can also occur. Finally, the flavonoids with open C ring are named chalcones *(Fig. 7)*[25, 26]

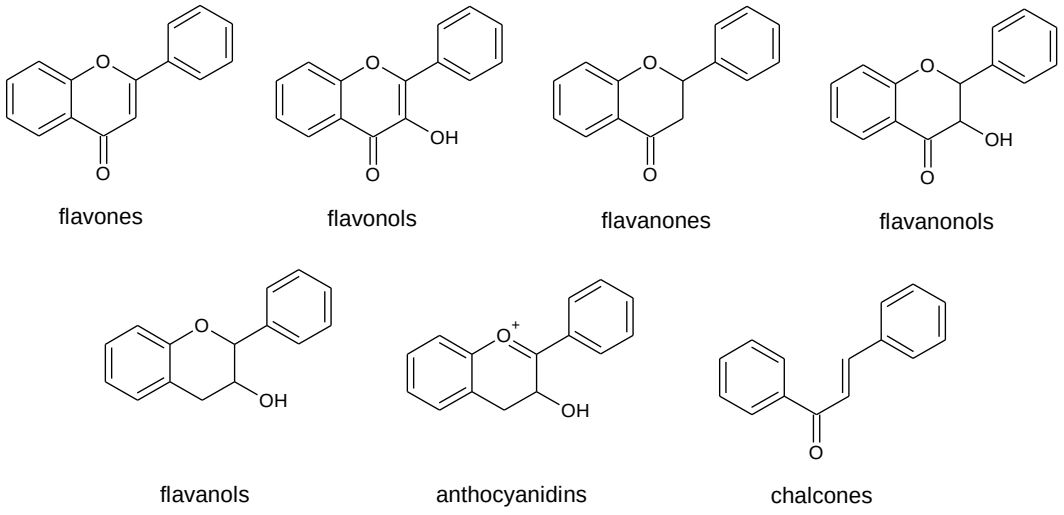


Fig. 7: Basic structures of different groups of flavonoids

Flavonoids are widely spread in Nature as the major coloring components of flowering plants.[25] More than 6 000 different compounds have been identified.[27] They are an integral part of human and animal diet consumed in the form of fruit, vegetables and drinks such as wine and tea.[25]

As regards the physico-chemical properties, flavonoids are reducing agents, which results in limited stability. The compounds are weakly acidic with pKa between 8 and 10. For this reason, they are not stable under alcaline conditions. Because of the double bonds and aromatic ring, flavonoids emit fluorescence upon exposure to UV light. The substances are also

able to form chelate complexes with metals. Finally, low water solubility of flavonoid aglycones have essential influence on their bioavailability. Hence, the development of semi-synthetic, more soluble forms has been a challenge for modern research.[28, 29]

Due to their structure and physico-chemical properties, flavonoids possess the ability to modulate the activity of some enzymes, hormones and neurotransmiters, and to modify the behaviour of many cell systems. As a consequence, they exhibit a broad variety of biological activities.[30]

1.3.2 **Biological properties**

Undoubtedly, the capacity of flavonoids to act as antioxidants is their best described property. The arrangement of functional groups influences mechanisms of antioxidant activity based on radical scavenging and metal ion chelation ability *(Fig. 8).* The B ring hydroxyl donates hydrogen to hydroxy, peroxy and peroxynitrite radicals, stabilizing them with concomitant formation of relatively stable flavonoid radicals.[25]

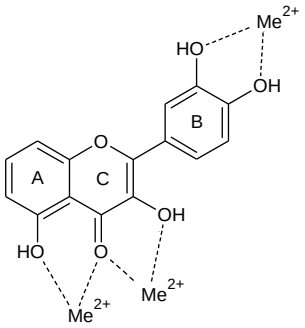


Fig. 8: Binding sites of flavonoids for the chelation of metal ions

Secondly, flavonoids have anti-inflammatory effects. Besides their ability to inhibit reactive oxygen and nitrogen compounds, they suppress enzymes such as cyclooxygenase and lipoxygenase, which are responsible for the production of pro-inflammatory metabolites of arachidonic acid.[31]

Furthermore, flavonoids are known for their vasculoprotective effects which are caused, among other factors, by their capacity to increase the concentration of the potent vasodilatator nitric oxide by inhibiting its degradation and stimulating nitric oxide synthase. Recent papers have shown curative efficiency of flavonoids in the treatment of endothelial dysfunction which is a cause of circulatory diseases.[32]

Moreover, flavonoids have been shown to be effective antimicrobial agents against a wide array of bacterias, fungi and viruses.[25]

Finally, flavonoids have a part in the prevention and treatment of cancer.[25]

1.3.3 **Flavonoids and cancer**

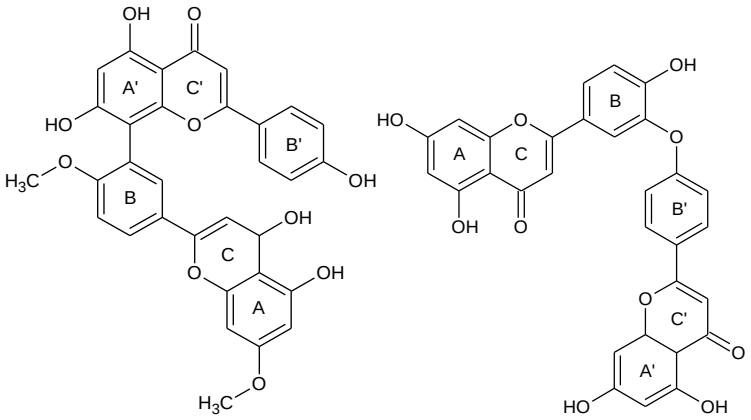
Flavonoids may have a role in the treatment of hormone-dependent cancers. Estrogens have an important role in the proliferation of tumor cells. Two mechanisms of action exist. The first mechanism is based on an effect on the estrogen receptors of the tumor cells. In this case, the substance has the role of a receptor antagonist acting upon hormone receptors. A molecule binds strongly to a hormone receptor, but does not activate this receptor and makes it unresponsive to the hormone. The second mechanism is based on the effect on aromatase, an enzyme which converts androgens to estrogens. A substance binds to the enzyme at the same site as testosteron. As a result, less testosterone is bound to aromatase, and, consequently, less testosterone can be converted to estradiol.[33, 34]

In addition, flavonoids can also affect all other forms of cancer. One of their anticancer mechanisms is based on their antimitotic ability. Similar to some other natural compounds used in cancer treatment, they have the ability to inhibit the formation of the mitotic spindle by causing the inhibition of the polymerization of tubulin into microtubules, resulting in the G2/M arrest. Like combretastatins, some flavonoids bind to tubulin at the colchicine domain.[21, 35]

1.3.4 **Biflavonoids and bichalcones**

Biflavonoids are flavonoid dimers, which vary in the oxidation and unsaturation degrees of the monomers and in the interflavonyl linkage. The linkage may involve rings A, B, C at various positions through carbon-carbon or ether bridges bonds. Therefore, the compounds can be classified by indicating the rings involved (AA, AB, BB, etc.). Biflavonoids are widely distributed in plants. Most of them contain a linkage between two A-rings (AA type) or between an A-ring of one and the B-ring of another flavonoid moiety (AB type). Two examples of structures can be shown: the first known biflavonoid ginkgetin (**12**, *Fig. 9*), isolated by Furukawa in 1929 from *Ginkgo biloba*, representing the AB type with a carbon-carbon linkage,

and ochnaflavone (**13**, *Fig. 9*), isolated from various species of *Garcinia*, representing the AA type with an ether bridge.[36]



*ginkgetin **12*** *ochnaflavone **13***

Fig. 9: Structures of ginkgetin **12** *and ochnaflavone* **13**

Similar to flavonoids, biflavonoids may have many biological properties (antimicrobial, antioxidant, antiallergenic, anticancer, etc.), sometimes enhanced in comparison with the corresponding monomers. This also concerns synthetic biflavonoids. Due to a considerable number of structures of biflavonoids, that have been unexplored, a renewed interest in their biological activities is logical.[36]

Unlike the wide number of natural biflavonoids, natural bichalcones are rare and less studied class. It is possible to mention rhuschalcones I-VI (**14**, *Fig. 10*), six bichalcones isolated from *Rhus pyroides*. All the rhuschalcones exhibit varying degrees of cytotoxic activity on some cell lines. As a group, they show more activity especially on the colon cancer cell lines HT-29 (ED_{50}: 3 µg/ml for rhuschalcones I, III) and HCT-116 (ED_{50}: 5 µg/ml for rhuschalcones I, III, VI).[37]

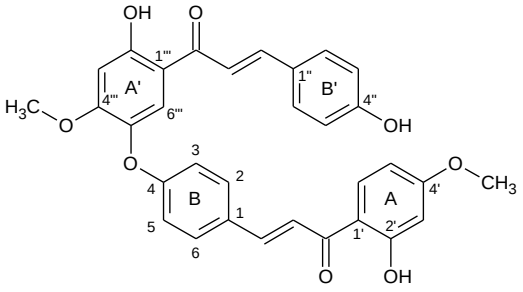


*Fig. 10: Structure of rhuschalcone I **14***

As regards the synthesis of biflavonoids (bichalcones), literature reports include a plethora of conditions, usually based on aldol (Claisen-Schmidt) and Ullmann condensations

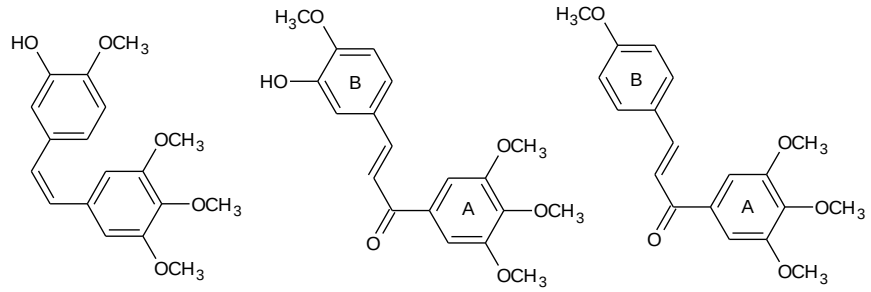
of suitable precursors. The strategies are based on the synthesis of biaryl compounds followed by condensation resulting in a biflavonoid structure, or preparation of a flavonoid monomer followed by coupling of two molecules.[36, 38] However, following the studies on structurally similar molecules, a different approach using Suzuki coupling can also be proposed.[39, 40] Application of the Claisen-Schmidt condensation is attractive, since the reaction generates *(E)*-isomer from the commercially available substituted benzaldehydes and acetophenones.[41]

1.4 LCSN Research

The LCSN Research on anticancer properties of chalcones follows the earlier promising results obtained with these compounds.

A large number of chalcones were synthesized and screened on the K562 myelogenous leukemia cell line for cell growth inhibitory properties. The most active substance was chalcone **15** (*Fig. 11*). The study has demonstrated the importance of the three methoxy groups attached to the A-ring for antiproliferative activities and suitable physicochemical properties. Additionally, a mechanism of action based on the inhibition of polymerisation of tubulin into microtubules has been demonstrated.[41]

In addition, chalcone **16** *(Fig. 11)* inhibits cell growth and motility, and induces cell cycle arrest and apoptosis of human ovarian cells. The chalcone was tested on A2780 (IC_{50}: 1.3 μmol/l after 48h exposure), A2780/CDDP and SKOV3 cell lines. Compared to the biological tests of its mother compound, 1,3-diphenylprop-2-en-1-one, this substance exhibited better water solubility and much stronger effect. The tests also showed that this chalcone did not interrupt tubulin polymerization. Therefore, two structurally similar molecules can act by multiple mechanisms.[38]



combretastatin A-4 ***2*** *chalcone* ***15*** *chalcone* ***16***

Fig. 11: Structures of combretastatin A-4 ***2****, 3-hydroxy-4,3',4',5'-tetramethoxychalcone* ***15*** *and 4,3',4',5'-tetramethoxychalcone* ***16***

Within LCSN research, the last two chalcones were synthesized and tested on different cell lines (MTT assay). The resulting IC_{50} values, concentrations of the compounds that are required for 50% inhibition *in vitro*, reach 0.87 µg/ml after 48h exposure for chalcone **15** (HCT-116 cell line) and 6.4 µg/ml for chalcone **16** (HT-29 cell line) *(Fig. 12)*. The criteria to categorize the cytotoxic activity of the substances are as follows: $IC_{50} \leq 20$ µg/ml = highly active, IC_{50} 21 - 200 µg/ml = moderately active, IC_{50} 201 - 500 µg/ml = weakly active and $IC_{50} > 501$ µg/ml = inactive.[42] These results opened a new area in anticancer drug research.

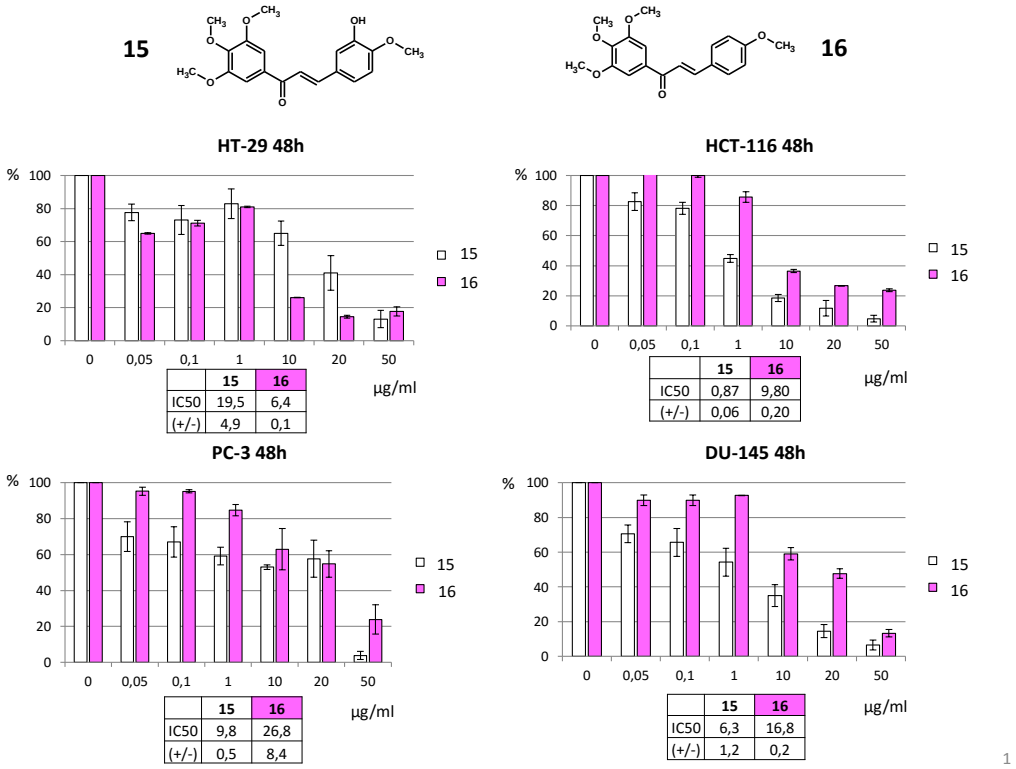


*Fig. 12 Biological evaluation of chalcones **15** and **16***

The chalcones **15** and **16** were also used as lead molecules in the design of new antiproliferative agents. For example, they were coupled to polyamine moieties through different linkers. The new derivatives always possess the structure of trimethoxylated A-ring, but vary in the substitution of the B-ring, in the nature of the polyamine and in its connection to the molecule of the chalcone *(Fig. 13)*.

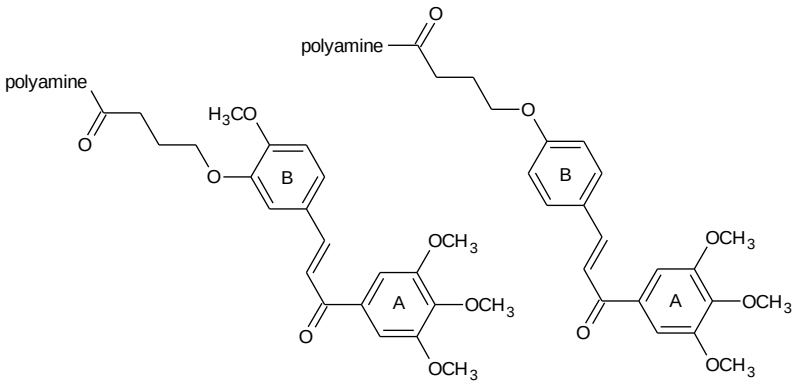


Fig. 13: Some of the chalcones prepared within LCSN research

1.5 Aims of the work

The aim of this work was to synthesize and characterise two new chalcones.

Firstly, we decided to couple two molecules of chalcone **16** and to obtain the spectral characteristics of the resultant bichalcone (**17**, *Fig. 14*).

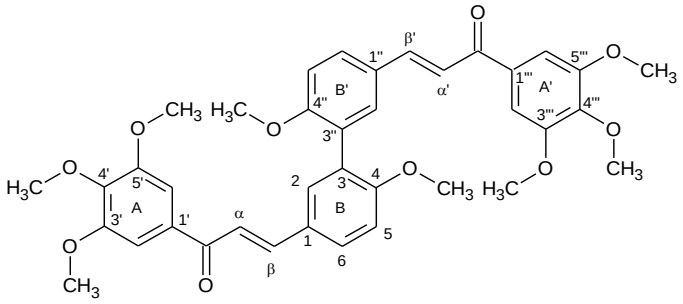
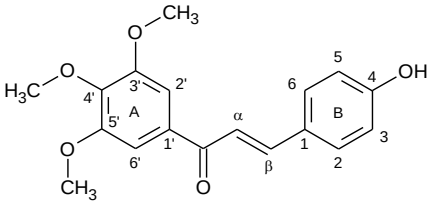
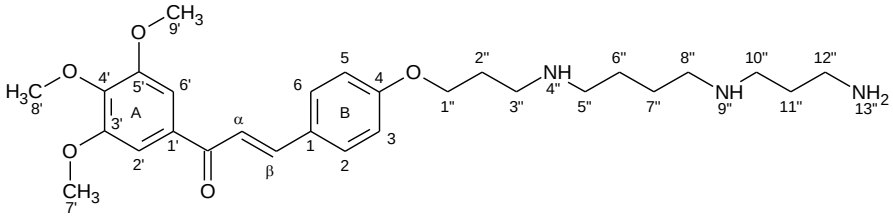


*Fig. 14: Structure of 4,3',4',5',4'',3''',4''',5'''-octamethoxy-3-3''-bichalcone **17***

Secondly, we focused on the synthesis and characterisation of chalcone **18,** and its conjugate with spermidine **19** *(Fig. 15).* Chalcone **18** is a demethoxylated positional isomer of the previously prepared chalcone **15**.



18



19

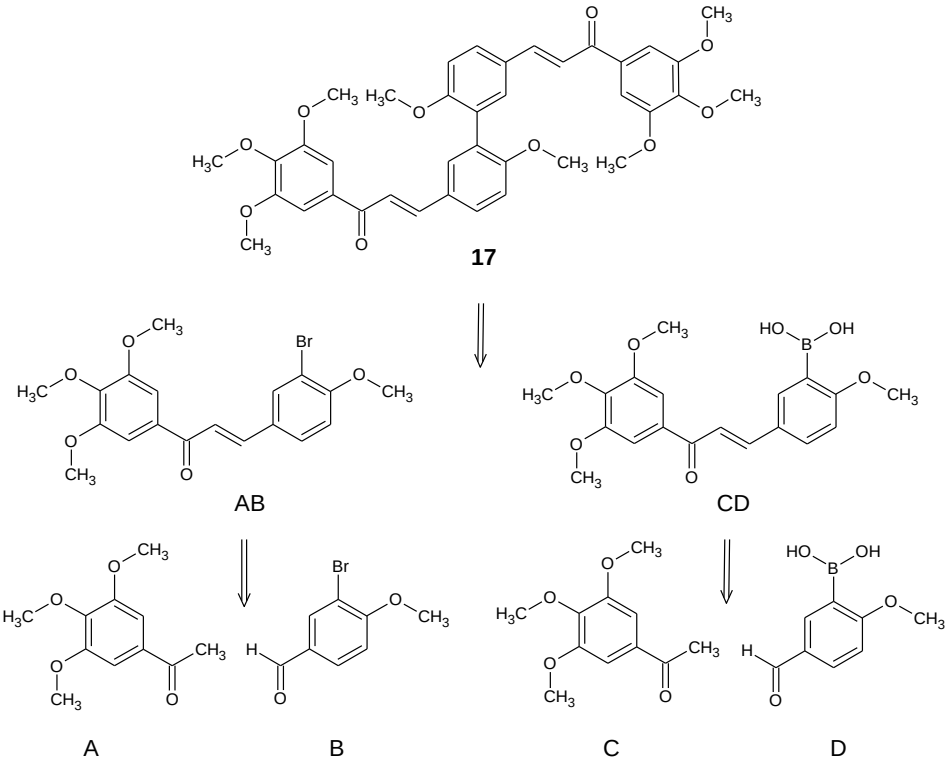
Fig. 15: Structures of 4-hydroxy-3',4',5'-trimethoxychalcone ***18*** *and 4-(4'',9'',13''-triazatridecyl)-3',4',5'-trimethoxychalcone* ***19***

2. RESULTS AND DISCUSSION

2.1 Synthesis and characterisation of bichalcone 17

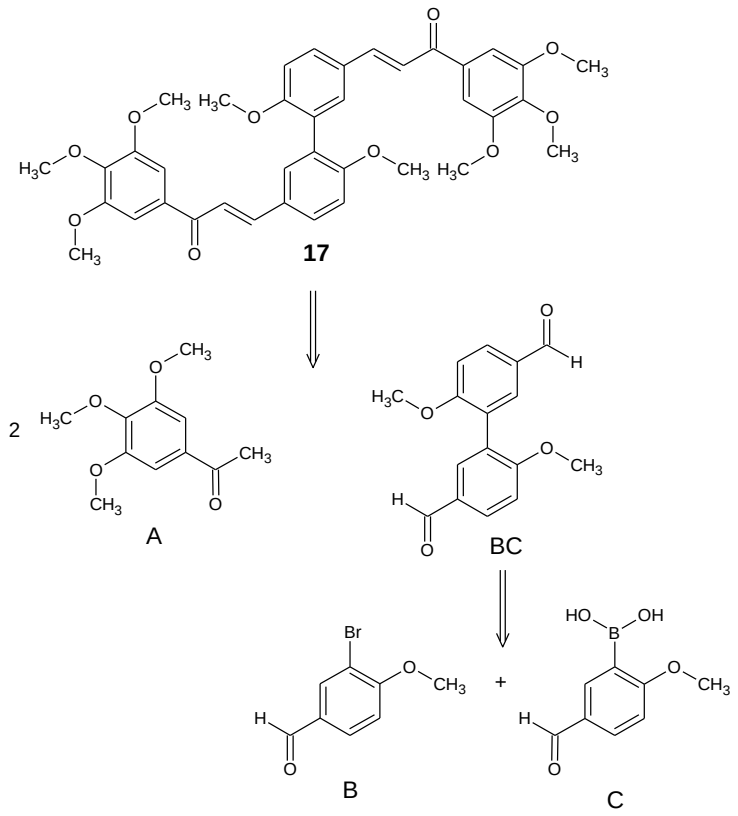
2.1.1 **Plans of synthesis**

Two different approaches were followed in order to obtain bichalcone **17**. Using the first of them, Suzuki coupling was chosen to couple two monomers. The two building blocks should have been, in turn, prepared by aldol condensation. The retrosynthetic strategy is outlined in *Scheme 2*.



*Scheme 2: Retrosynthetic strategy towards bichalcone **17***

The second plan was based on a Suzuki reaction with substituted benzaldehydes as the coupling partners. The resultant biphenyl derivative would subsequently be subjected to an aldol condensation with a substituted acetophenone. The strategy is depicted in *Scheme 3.*

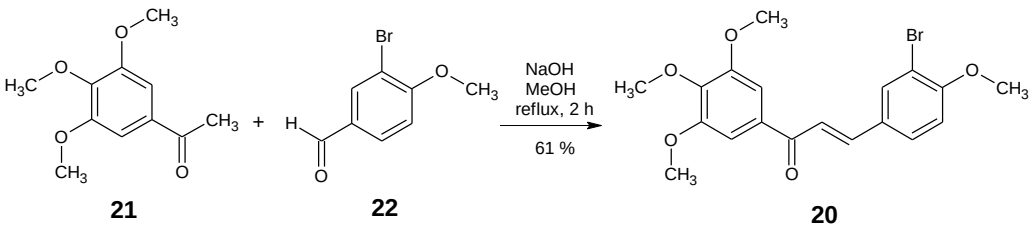


*Scheme 3: Alternative retrosynthetic strategy leading to bichalcone **17***

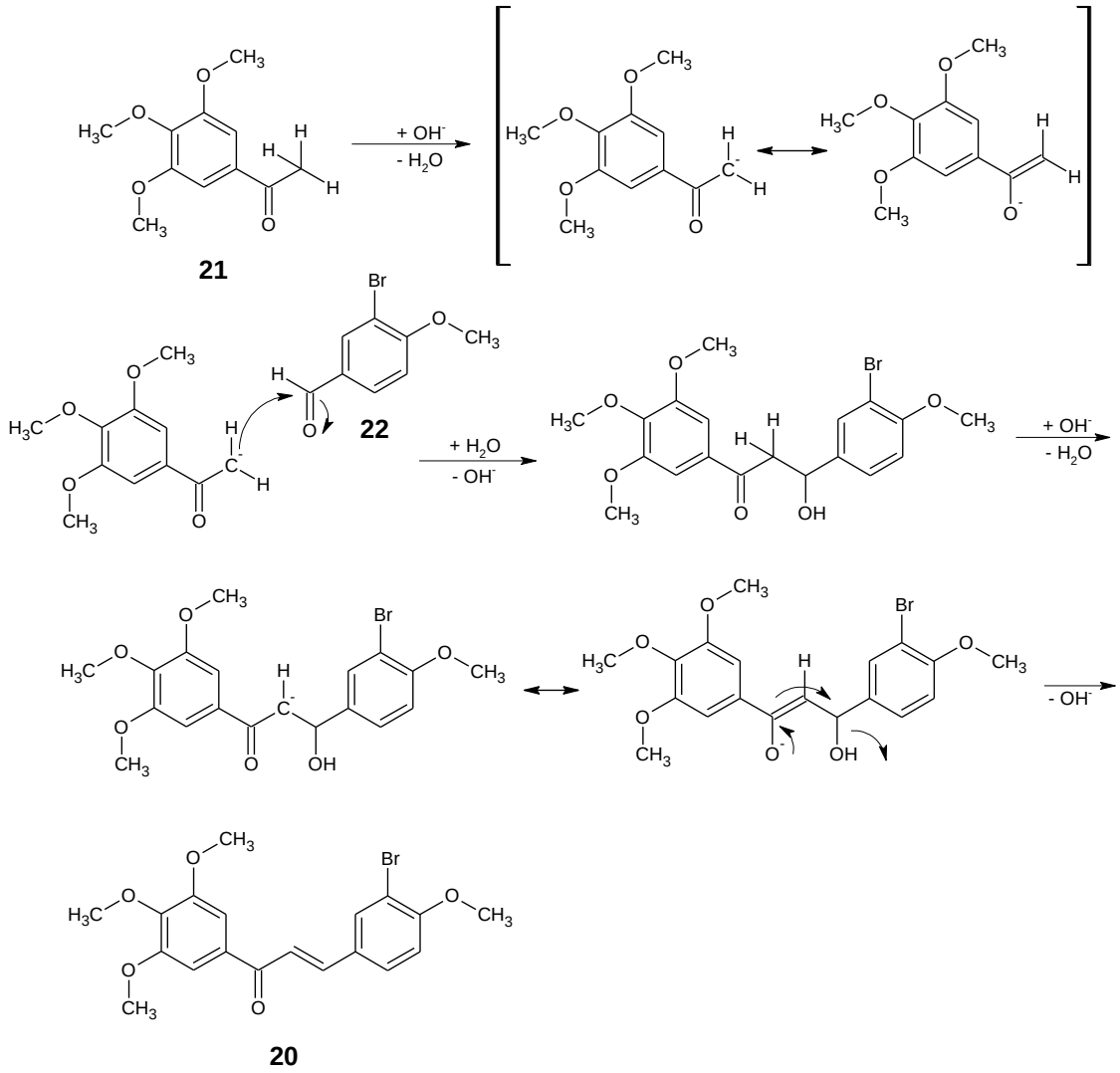
2.1.2 **Synthesis of bichalcone 17 by Suzuki coupling**

2.1.2.1 Preparation of bromo chalcone **20**

The preparation of bromo chalcone **20** is based on a base-catalyzed Claisen-Schmidt condensation between acetophenone **21** and benzaldehyde **22**. The reaction mechanism includes the formation of an enolate from acetophenone **21**, and subsequent addition of the enolate to benzaldehyde **22** followed by dehydratation *(Schemes 4 and 5).*



*Scheme 4: Synthesis of 3-bromo-4,3',4',5'-tetramethoxychalcone **20***

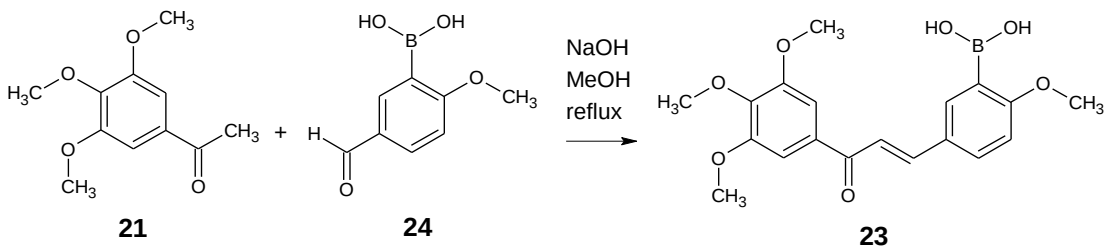


Scheme 5: Mechanism of the Claisen-Schmidt condensation

The product **20** was obtained in 61% yield.

2.1.2.2 Preparation of chalconyl boronic acid **23**

The chalconyl boronic acid **23** was formed by Claisen-Schmidt condensation from acetophenone **21** and boronic acid **24**. The reaction is outlined in *Tab 1*.



exp.	**21** (eq.)	**24** (eq.)	time (h)	mobile phase	yield
1.	1.0	1.2	2.0	Tol:EtOAc 8:2 (1x)	53 %, impure
2.	1.0	1.2	1.0	Tol:EtOAc 8:2 (1x) + Tol:EtOAc 6:4 (1x)	86 %, impure
3.	1.0	0.8	3.5	Hex:EtOAc 8:2 (2x) + Tol:EtOAc 7:3 (2x)	50 %, pure

*Tab. 1: Synthesis of 4,3',4',5'-tetramethoxychalcon-3-ylboronic acid **23***

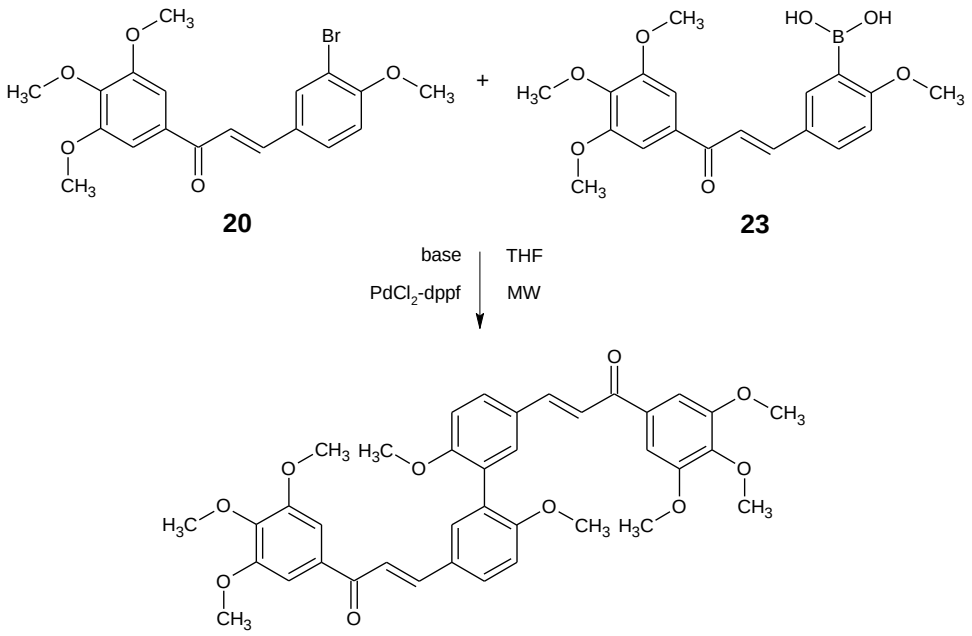
The product **23** was initially obtained in 86% yield, its purity, however, was insufficient, since traces of acid **24** were detectable in the product despite extensive purification. The presence of this impurity complicated the next step of the synthesis, the Suzuki coupling between the two chalcones.

To exclude the presence of acid boronic **24** in the product, we reduced its initial quantity so that it became the limiting reagent (experiment 3, *Tab. 1*). Consequently, the resulting chalconyl boronic acid **23** was obtained in a considerably better purity (higher than 98 %, as judged by ^{1}H NMR), adequate for the next step of the synthesis.

2.1.2.3 Synthesis of bichalcone **17** by Suzuki coupling

The final step of the proposed synthesis of the bichalcone **17** was a coupling between the building blocks **20** and **23**. For this purpose, Suzuki coupling was applied (*Tab. 2*).

For syntheses of structurally similar compounds by Suzuki coupling, literature mentions use of $PdCl_2$-dppf or $Pd(PPh_3)_4$ in 0.1 eq. as catalysts, K_2CO_3 or NaOH in 3 eq. as bases, and THF as solvent.[40]



exp.	**20** (eq.)	**23** (eq.)	base (3 eq.)	$PdCl_2$-dppf (eq.)	time (min)	mobile phase	result
1.	1.0	1.1	K_2CO_3	0.1	50	Hex:EtOAc 6:4 (1x)	< 3 %, impure
2.	1.0	1.1	NaOH	0.1	20	$CHCl_3$:EtOAc 19:1 (2x)	< 3 %, impure
3.	1.0	2.0	NaOH	0.2	20	$CHCl_3$:EtOAc 19:1 (2x)	19 %, impure
4.	1.0	1.1	NaOH	0.2	50	$CHCl_3$:EtOAc 19:1 (2x)	41 %, pure

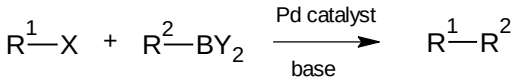
*Tab. 2: Preparation of 4,3',4',5',4'',3''',4''',5'''-octamethoxy-3-3''-bichalcone **17***

In our first two experiments, using impure chalconyl boronic acid **23**, we obtained only a very small (< 3 %) amount of the desired bichalcone **17**. By changing the reaction conditions (amounts of chalconyl boronic acid **23** and the catalyst), we improved the yield of the bichalcone **17**, but did not achieve an adequate purity (exp. 3, *Tab. 2*).

Since we suspected that insufficient purity of acid **23** was the reason for the low yield (see the previous section 2.1.2.2), we attempted the proces again with material of better purity (exp. 4, *Tab. 2*).

Finally, the bichalcone **17** was obtained in 41% yield and in adequate purity (impurities in the order of several per cent by the ^{1}H NMR).

The Suzuki reaction is a Pd-catalyzed coupling reaction in which the coupling partners are an organoboron species and a halide (*Scheme 6*).

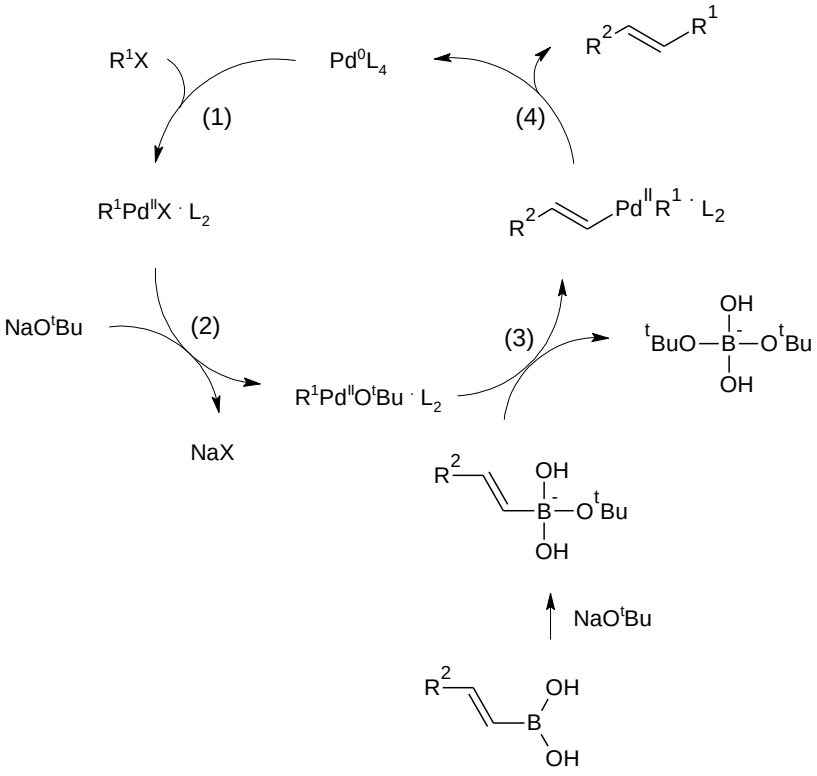


In our case with the following substituents:

R^1, R^2: 5-formyl-2-methoxyphenyl
X: -Br
Y: -OH

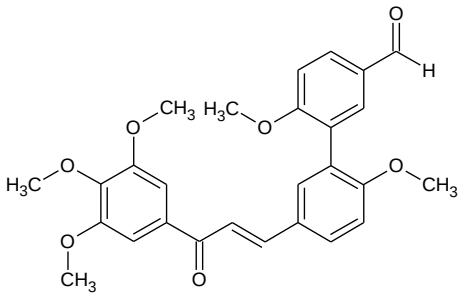
Scheme 6: General scheme of the Suzuki reaction

The course of the reaction is composed of several parts *(Scheme 7)*. The first step is oxidative addition of the palladium catalyst to the organohalide to form an organopalladium species. In this part, palladium is oxidized from palladium(0) to palladium(II) (1). Subsequently, the base present attacks palladium and exchanges with the halide. This step is known as metathetic exchange (2). The resultant organo-organoxo-palladium intermediate is subject to transmetallation providing diorgano-palladium compound (3). Finally, the palladium(II) complex eliminates the product R-R' and regenerates the palladium(0) catalyst (4).[43]



Scheme 7: Catalytic cycle of Suzuki reaction

In experiments 1 - 3 *(Tab. 2)*, the major isolated product was chalcone **25**, arising from the reaction of chalcone **20** and boronic acid **24** *(Fig. 16).*



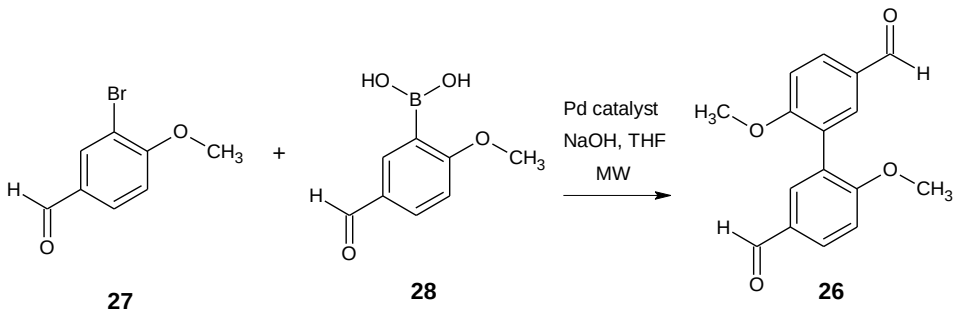
25

*Fig. 16: 3-(5-formyl-2-methoxyfenyl)-4,3',4',5'-tetramethoxychalcone **25***

2.1.3 **Synthesis of bichalcone 17 from a biphenyl derivative 26**

2.1.3.1 Preparation of biphenyl **26**

Biphenyl **26** had to be prepared first. Suzuki reaction, in which the two substituted benzaldehydes **27** and **28** served as the coupling partners, was applied. The reaction is depicted in *Tab. 3.*



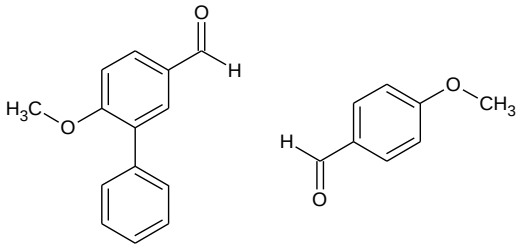
27 **28** **26**

exp.	**27** (eq.)	**28** (eq.)	catalyst (eq.)	time (min)	mobile phase	yield (%)
1.	1.0	2.0	$PdCl_2$-dppf 0.1	30	Hex:EtOAc 7:3 (2x)	55
2.	1.0	3.0	$PdCl_2$-dppf 0.1	20	CHCl3:EtOAc 19:1 (2x)	63
3.	1.0	2.5	$PdCl_2$-dppf 0.2	10	Hex:EtOAc 7:3 (3x)	78
4.	10	2.5	$Pd(PPh_3)_4$ 0.2	20	Hex:EtOAc 6:4 (1x)	50

*Tab. 3: Preparation of 6,6'-dimethoxybiphenyl-3,3'-dicarbaldehyde **26***

The results in *Tab. 3* show that $PdCl_2$-dppf appears to be a somewhat better catalyst for this reaction than $Pd(PPh_3)_4$. The product was obtained in 78% yield.

In addition to the desired substance **26**, some degradation products were isolated. Two of them were identified by NMR spectra (**29, 30**, *Fig. 17).*

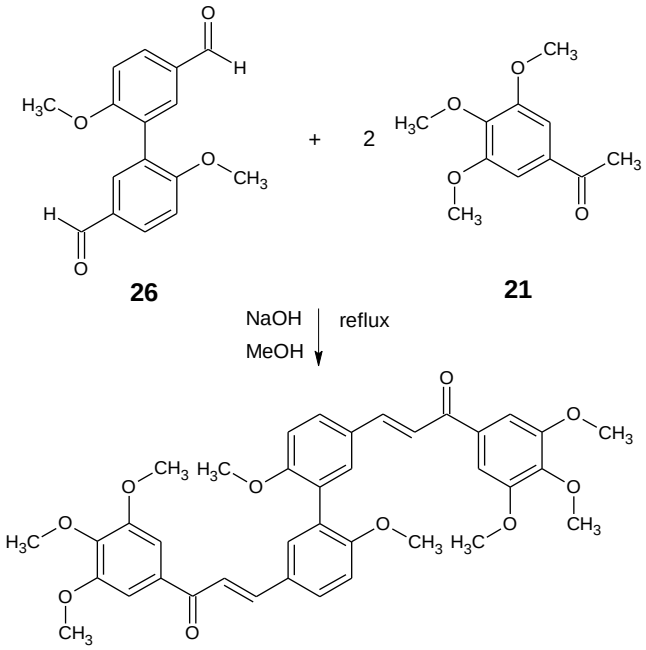


29 **30**

*Fig. 17: 6-methoxybiphenyl-3-carbaldehyde **29** and 4-methoxybenzaldehyde **30***

2.1.3.2 Preparation of bichalcone **17** by the Claisen-Schmidt condensation

The second step of the synthesis of the bichalcone **17** was the Claisen-Schmidt condensation between biphenyl **26** and the acetophenone **21**. Two experiments were performed. The individual reaction conditions and the results are summarised in *Tab. 4.*



26 **21**

17

exp.	**26** (eq.)	**21** (eq.)	time (h)	mobile phase	yield (%)
1.	1.0	2.0	4	x	x
2.	1.0	4.0	5	$CHCl_3$:EtOAc 19:1 (2x)	51[a]/15[b]

[a] after one purification
[b] after two purifications

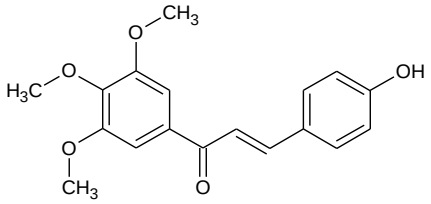
*Tab. 4: Preparation of 4,3',4',5',4'',3''',4''',5'''-octamethoxy-3-3''-bichalcone **17***

During the first experiment, the formation of the desired bichalcone **17** was observed according to TLC, but a degradation occurred at the end.

The second attempt was more successfull, a certain amount of **17** was isolated. Its purity, however, was insufficient, since traces of reagents were detectable in the product despite extensive purifications.

2.2 Synthesis and characterisation of chalcone 19

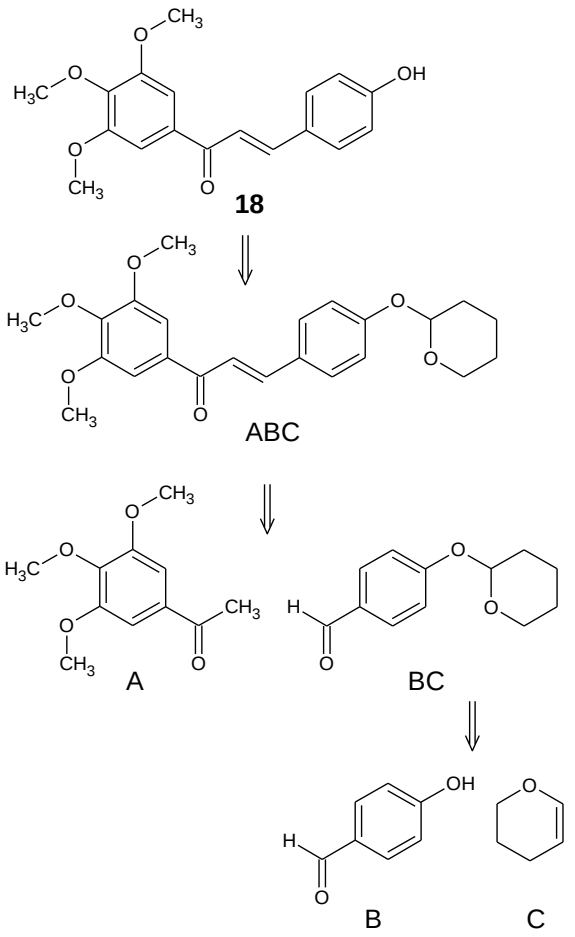
2.2.1 **Preparation of chalcone 18**



18

*Fig. 18: Structure of 4-hydroxy-3',4',5'-trimethoxychalcone **18***

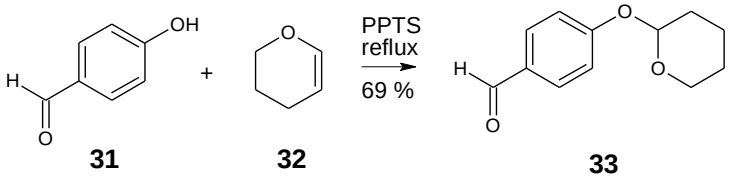
A retrosynthetic strategy for chalcone **18** is outlined in *Scheme 8*.



*Scheme 8: Retrosynthetic strategy of chalcone **18***

2.2.1.1 Protection of hydroxy group by acetal formation

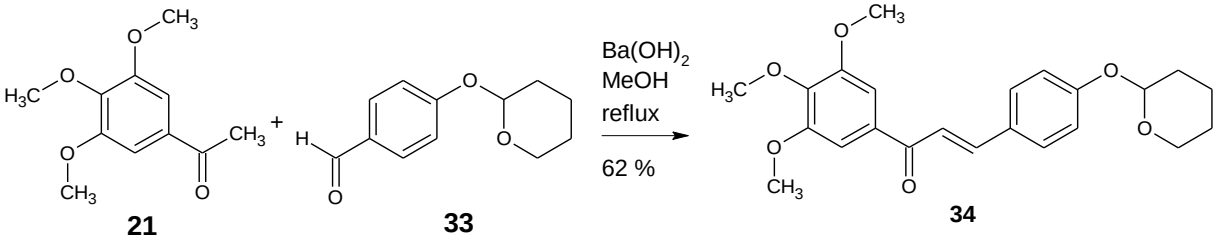
The first step in the synthesis of the intended chalcone was the protection of the hydroxy group of benzaldehyde **31** to prevent its deprotonation in the subsequent Claisen-Schmidt condensation. For this purpose, acetal formation was used. To this end, benzaldehyde (**31**) was treated with dihydropyrane **32** in the presence of PPTS at reflux to give protected compound **33** in 69% yield (*Scheme 9*).



Scheme 9: Synthesis of 4-(tetrahydro-2H-pyran-2-yloxy)benzaldehyde **33**

2.2.1.2 Synthesis of chalcone **34**

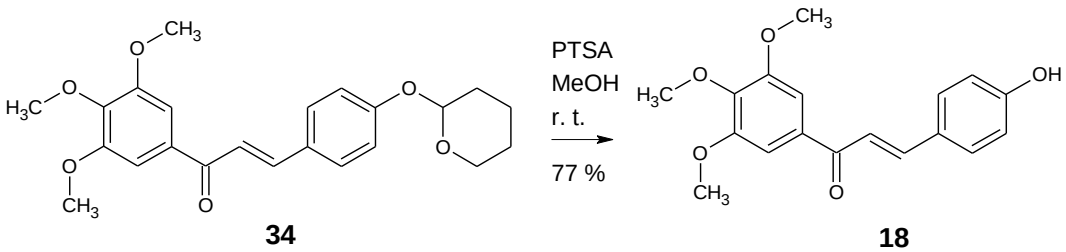
The next step was the Claisen-Schmidt condensation between benzaldehyde **33** and acetophenone **21** induced by $Ba(OH)_2$. The product was obtained in a favourable 62% yield (*Scheme 10*).



*Scheme 10: Preparation of 3',4',5'-trimethoxy-4-(tetrahydro-2H-pyran-2-yloxy)chalcone **34***

2.2.1.3 Deprotection of hydroxy group

In order to remove the acetal protective group, chalcone **34** obtained in the preceding step was exposed to PTSA (10 %) in MeOH for 3.5 hours to give chalcone **18** in 77% yield (*Scheme 11*).

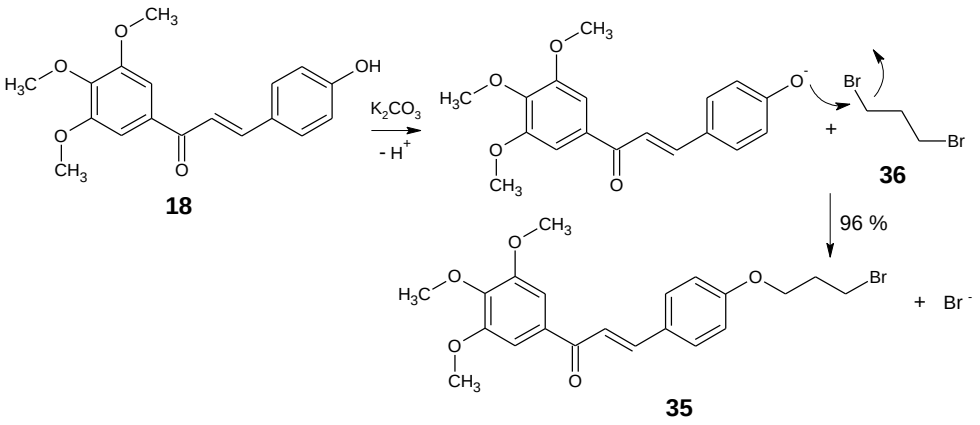


*Scheme 11: Preparation of 4-hydroxy-3',4',5'-trimethoxychalcone **18***

In summary, the preparation of the building block **18** via the proposed strategy was feasible. Total yield of the product was 33 % over 3 steps.

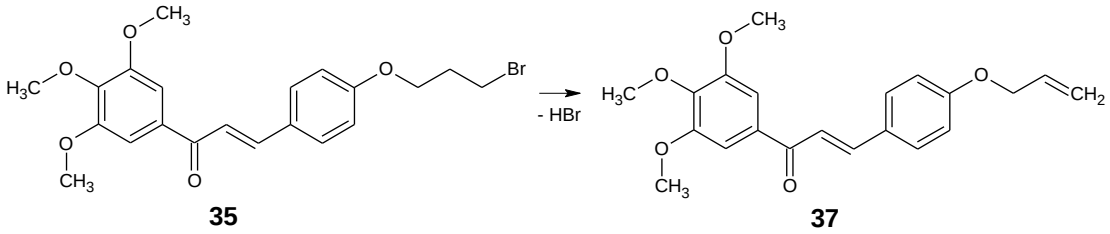
2.2.2 **Synthesis of bromo chalcone 35**

In order to install the linker for the attachment of a polyamine, bromo chalcone **35** was prepared via Williamson synthesis. Chalcone **18** was therefore treated with K_2CO_3, and the ensuing enolate was then reacted with 1,3-dibromopropane **36** *(Scheme 12).*



*Scheme 12: Synthesis of 4-(3-bromopropoxy)-3',4',5'-trimethoxychalcone **35***

Besides the desired substance **35**, alkene **37** was formed by elimination *(Scheme 13).*



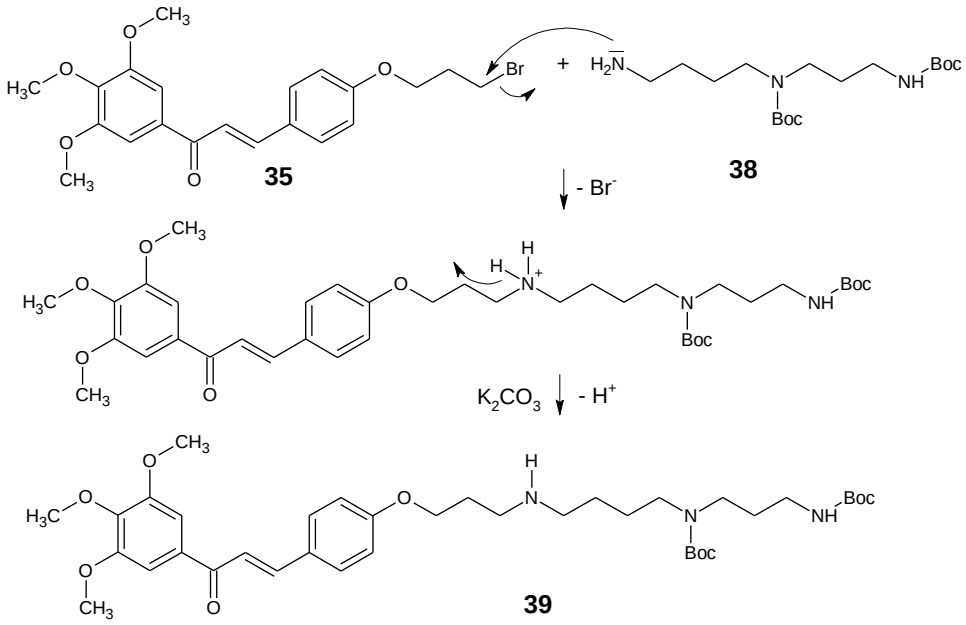
*Scheme 13: Degradation of 4-(3-bromopropoxy)-3',4',5'-trimethoxychalcone **35** to 4-allyloxy-3',4',5'-trimethoxychalcone **37***

Hovewer, the yield of **35** remained high (96 %).

2.2.3 **Coupling with spermidine 38**

The next step of synthesis, reaction between the alkyl halide **35** and spermidine **38**, was aliphatic nucleophilic substitution. Amine **38** acted as a nucleophile and attacked the electrophilic carbon of the alkyl halide **35** displacing the bromide and forming a new C-N bond. Subsequently, the present base deprotonated the ammonium center. As a result, the alkylation product **39** was obtained.

Two experiments were performed. The second attempt resulted in the formation of the coupled product **39** (*Tab. 5*). The yield was, however, small (18 %).



exp.	**35** (eq.)	**38** (eq.)	solvent	time	mobile phase	yield (%)
1.	1.0	4.0	DMF 10 ml	6 h, reflux	$CHCl_3$:MeOH 97.5:2.5 (1x)	x
2.	1.0	1.1	acetonitrile 5 ml	40 min, MW heating	CHCl3:MeOH: NH4OH 94:5:1 (1x)	18

*Tab. 5: Synthesis of 4-(9,13-bis-Boc-4,9,13-triazatridecyl)-3',4',5'-trimethoxychalcone **19***

3. CONCLUSION

The main purpose of the work, syntheses of two chalcones derivatives, bichalcone **17** and coupled chalcone **19**, was achieved.

The bichalcone **17** was prepared in sufficient amount and purity for the subsequent biological tests. However, the reaction conditions can still be more optimised in order to increase the yield. Of the two synthetic pathways explored, the first one using the coupling between two chalcones (**20**, **23)** seems to be more convenient. Using the second approach, we were unable to obtain the product in desirable purity.

All the products were characterised by ^{1}H NMR, ^{13}C NMR and MS spectra.

At present, the biological evaluation of the prepared substance is in progress.

As regards the synthesis of the chalcone coupled with a polyamine moiety **19**, an optimisation has to be performed in order to increase the yield and also the purity of the product. Moreover, the molecule has to be deprotected to remove the Boc groups.

4. EXPERIMENTAL PART

4.1 General methods

All reagents and solvents were commercial products (Alfa Aesar, Sigma Aldrich, Fischer, Acros, Carlo Erba) and were used as received. THF was predried prior to use.

Reactions were monitored by thin layer chromatography (TLC) on 0.2 mm E. Merck silica gel plates (60 F_{254}). Products were detected by UV lampe (254 and 366 nm). The detection of amine functional groups was performed by the ninhydrin test.

Products were isolated using TLC silica gel glass preparative plates (20 cm x 20 cm) uniformly coated with 0.5 mm layer of silica gel (Silica gel 60, Merck 1.09385.2500), dried and activated in laboratory oven with 120 °C for 12 hours.

Flash column chromatography was carried out using CombiFlash Companion, serial number 205G20190, and GraceResolv 24g Silica cartridges.

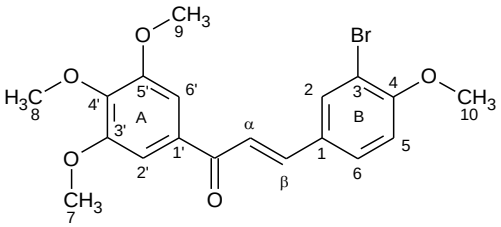
NMR spectra were recorded on Bruker DPX-400 NMR spectrometer (500.15 MHz for ^{1}H NMR and 125.78 MHz for ^{13}C NMR). Chemical shifts (δ) were quoted in parts per million (ppm) downfield from trimethylsilane (TMS). Coupling constants (*J*) were quoted in Hertz (Hz). $CDCl_3$ was used as solvent.

Mass spectra (MS) were determinated by electrospray ionisation (ESI-TOF) on Bruker maXis Impact spectrometer at Mass Spectroscopy Platform of Institute of Organic and Analytical Chemistry of the University of Orleans.

The melting points of products were not determinated as a considerable part of products had an oleaginous or amorphous character.

4.2 Synthesis

3-bromo-4,3',4',5'-tetramethoxychalcone (20)



20

Chemical formula: $C_{19}H_{19}BrO_5$

Molecular weight: 407.255 g/mol

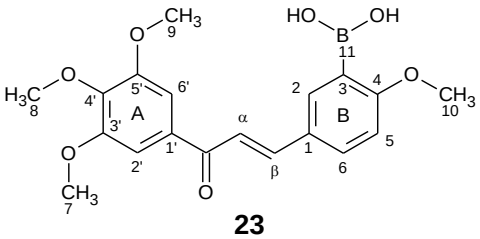
3,4,5-trimethoxyacetophenone (**21**, 100 mg, 0.476 mmol) and NaOH (95.1 mg, 2.378 mmol, 5 eq.) were added to MeOH (8 ml) and H_2O (1 ml). The mixture was stirred at r.t. for 30 min and 3-bromo-4-methoxybenzaldehyde (**22**, 122.8 mg, 0.571 mmol, 1.2 eq.) was then added. The resultant mixture was heated under reflux for 2 hours. The reaction mixture was monitored by TLC. When TLC indicated the completion of the reaction, the solvent was evaporated and 20 ml of H_2O were added to the residue. The mixture was acidified by 3M HCl solution to pH 6, extracted three times by CH_2Cl_2 (20 ml each). The combined organic portions were dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by chromatography (Tol:EtOAc 90:10) to give **20** as a light yellow amorphous solid (117 mg, 0.287 mmol, 61 %).

^{1}H NMR (500.15 MHz, CD_3OD) δ 8.05 (1H, d, *J* = 2.0 Hz, H-2), 7.70 (1H, dd, *J* = 2.0 Hz, *J* = 8.5 Hz, H-6), 7.70 (1H, d, *J* = 15.5 Hz, H-β), 7.66 (1H, d, *J* = 15.5 Hz, H-α), 7.40 (2H, s, H-2', H-6'), 7.08 (1H, d, *J* = 8.5 Hz, H-5), 3.94 (9H, s, H-7, H-8, H-9), 3.86 (3H, s, H-10)

^{13}C NMR (125.78 MHz, CD_3OD) δ 189.4 (C=O), 157.9 (C-4), 153.2 (C-3', C-5'), 143.1 (C-β), 142.5 (C-4'), 133.4 (C-1'), 132.8 (C-2), 129.9 (C-6), 129.0 (C-1), 120.2 (C-α), 111.9 (C-5), 111.8 (C-3), 106.1 (C-2', C-6'), 59.8 (C-8), 55.5 (C-10), 55.5 (C-7, C-9)

HRMS (ESI): m/z calcd for $C_{19}H_{20}BrO_5$ ($M+H^+$) 407.0489, found 407.0487

4,3',4',5'-tetramethoxychalcon-3-ylboronic acid (23)



23

Chemical formula: $C_{19}H_{21}BO_7$

Molecular weight: 372.177 g/mol

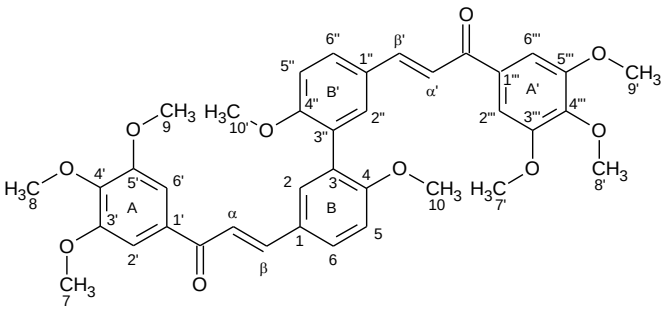
3,4,5-trimethoxyacetophenone (**21**, 200 mg, 0.951 mmol) and NaOH (190.2, 7.76 mmol, 5 eq.) were dissolved in MeOH (16 ml) and H_2O (1 ml). The mixture was stirred at r.t. for 30 min and 5-formyl-2-methoxybenzeneboronic acid (**24**, 205.4 mg, 1.142 mmol, 1.2 eq.) was then added. The resulting mixture was heated under reflux for 2 hours. The reaction mixture was monitored by TLC. When TLC indicated the completion of the reaction, the solvent was evaporated and 20 ml of H_2O were added to the residue. The mixture was acidified by 3M HCl solution to pH 6, extracted three times by CH_2Cl_2 (20 ml each). The combined organic portions were dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by chromatography (Tol:EtOAc 60:40) to give **(23)** as a light yellow amorphous solid (185 mg, 0,497 mmol, 53 %).

^{1}H NMR (500.15 MHz, $CDCl_3$) δ 8.20 (1H, d, *J* = 2.0 Hz, H-2), 7.81 (1H, d, *J* = 16.0 Hz, H-β), 7.71 (1H, dd, *J* = 2.5 Hz, *J* = 8.5 Hz, H-6), 7.42 (1H, d, *J* = 15.5 Hz, H-α), 7.28 (2H, s, H-2', H-6'), 6.97 (1H, d, *J* = 8.5 Hz, H-5), 5.98 (2H, s, H-11), 3.98 (3H, s, H-10), 3.95 (6H, s, H-7, H-9), 3.94 (3H, s, H-8)

^{13}C NMR (125.78 MHz, $CDCl_3$) δ 189.4 (C=O), 166.2 (C-4), 153.2 (C-3', C-5'), 144.3 (Cβ), 142.5 (C-4'), 137.0 (C-2), 133.8 (C-6), 133.8 (C-1'), 128.1 (C-1), 124.7 (C-3), 120.3 (Cα), 110.5 (C-5), 106.2 (C-2', C-6'), 61.0 (C-8), 56.5 (C-7, C-9), 55.9 (C-10)

HRMS (ESI): m/z calcd for $C_{19}H_{22}BO_7$ ($M+H^+$) 373.1456, found 373.1459

4,3’,4’,5’,4’’,3’’’,4’’’,5’’’-octamethoxy-3-3’’-bichalcone (17)



17

Chemical formula: $C_{38}H_{38}O_{10}$

Molecular weight: 654.70 g/mol

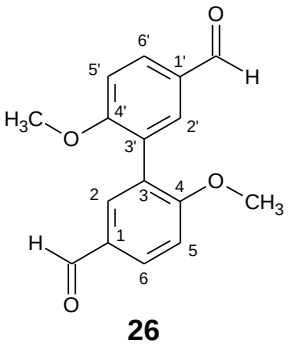
20 (100 mg, 0.245 mmol), **23** (100 mg, 2.69 mmol, 1.1 eq.), $PdCl_2$-dppf (19.6 mg, 0.027 mmol, 0.2 eq.) and 3M NaOH water solution (134 μl, 0.403 mmol, 3 eq.) were added to THF (6 ml). The reaction mixture was stirred using MW heating (120 °*C*, 150 W, 5x10 min), being monitored by TLC. When TLC indicated the completion of the reaction, the solvents were evaporated. 20 ml of H_2O were added to the residue. The mixture was then acidified by 3M HCl solution to pH 6, extracted 4 times by CH_2Cl_2 (20 ml each). The combined organic portions were dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by chromatography ($CHCl_3$:EtOAc 19:1)(2x) to give **17** as a yellow oil (64.8 mg, 0.099 mmol, 41 %).

^{1}H NMR (500.15 MHz, $CDCl_3$) δ 7.84 (2H, d, *J* = 15.5, H-β, H-β’), 7.66 (2H, dd, *J* = 2.0, *J* = 8.5, H-6, H-6’’), 7.59 (2H, d, *J* = 2.0, H-2, H-2’’), 7.38 (2H, d, *J* = 15.5, H-α, H-α’), 7.26 (4H, s, H-2’, H-6’, H-2’’’, H-6’’’), 7.04 (2H, d, *J* = 8.5, H-5, H-5’’), 3.94 (12H, s, H-7, H-9, H-7’, H-9’), 3.93 (6H, s, H-8, H-8’), 3.85 (6H, s, H-10, H-10’)

^{13}C NMR (125.78 MHz, $CDCl_3$) δ 189.3 (C=O), 159.2 (C-4, C-4’’), 153.2 (C-3’, C-5’, C-3’’’, C-5’’’), 144.5 (Cβ, Cβ’), 142.5 (C-4’, C-4’’’), 133.8 (C-1’, C-1’’’), 131.1 (C-2, C-2’’), 130.5 (C-6, C-6’’), 127.8 (C-1/C-3, C-1’’, C-3’’), 127.5 (C-1/C-3, C-1’’/C-3’’), 119.8 (Cα, Cα’), 111.1 (C-5, C-5’’), 106.2 (C-2’, C-6’, C-2’’’, C-6’’’), 61.0 (C-8, C-8’), 56.5 (C-7, C-9, C-7’, C-9’), 55.9 (C-10, C-10’)

HRMS (ESI): m/z calcd for $C_{38}H_{39}O_{10}$ ($M+H^+$) 655.2538, found 655.2540

6,6'-dimethoxybiphenyl-3,3'-dicarbaldehyde (26)



26

Chemical formula: $C_{16}H_{14}O_4$

Molecular weight: 270.28 g/mol

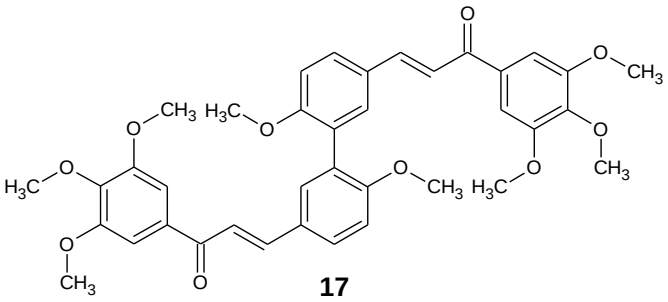
3-bromo-4-methoxybenzaldehyde (**27**, 50 mg, 0.233 mmol), 5-formyl-2-methoxybenzeneboronic acid (**28**, 104.6 mg, 0.581 mol, 2.5 eq.), $PdCl_2$-dppf (34 mg, 0.0465 mmol, 0.2 eq.), 3M NaOH water solution (233 µl, 0.698 mmol, 3 eq.) were added to THF (3 ml). The reaction mixture was stirred using MW heating (120 °*C*, 150 W, 10 min), being controlled by TLC. When TLC indicated the completion of the reaction, the solvent was evaporated. 20 ml of H_2O and 5 ml of CH_2Cl_2 were added to the residue. The mixture was then acidified by 3M HCl solution to pH 6 and extracted three times by CH_2Cl_2 (20 ml each). The combined organic portions were dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by chromatography (Hex:EtOAc 70:30) to give **26** as a white amorphous solid (49 mg, 0.18 mmol, 78 %).

^{1}H NMR (500.15 MHz, $CDCl_3$) δ 9.93 (2H, s, CHO), 7.92 (2H, dd, *J* = 2.5 Hz, *J* = 9.0 Hz, H-6, H-6'), 7.78 (2H, d, *J* = 2.5 Hz, H-2, H-2'), 7.10 (2H, d, *J* = 8.5 Hz, H-5, H-5'), 3.87 (6H, s, OCH_3)

^{13}C NMR (125.78 MHz, $CDCl_3$) δ 190.8 (C=O), 162.0 (C-4, C-4'), 133.1 (C-6, C-6'), 131.9 (C-2, C-2'), 129.7 (C-1, C-1'), 127.2 (C-3, C-3'), 111.0 (C-5, C-5'), 56.7 (OCH_3)

HRMS (ESI): m/z calcd for $C_{16}H_{15}O_4$ ($M+H^+$) 271.0965, found 271.0964

4,3',4',5',4'',3''',4''',5'''-octamethoxy-3-3''-bichalcone (17)



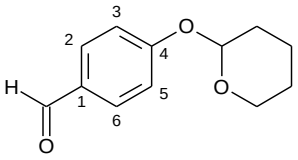
17

Chemical formula: $C_{38}H_{38}O_{10}$

Molecular weight: 654.70 g/mol

3,4,5-trimethoxyacetophenone (**21**, 258 mg, 1.23 mmol, 4 eq.) and 3M NaOH water solution (513 µl, 1.54 mmol, 5 eq.) were dissolved in MeOH (20 ml) and left stirred 20 min at r.t. **26** (83 mg, 0.307 mmol) was then added and the mixture was heated under reflux, being monitored by TLC. After 5 hours, the solvent was evaporated. 20 ml of H_2O was added to the residue. The mixture was acidified by 3M HCl solution to pH 6 and filtrated. The resulting mixture was then extracted three times by CH_2Cl_2 (20 ml each). The combined organic portions were dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by chromatography ($CHCl_3$:EtOAc 19:1) to give **17** as a yellow oil (29 mg, 0.045 mmol, 15 %).

4-(tetrahydro-2H-pyran-2-yloxy)benzaldehyde (33)



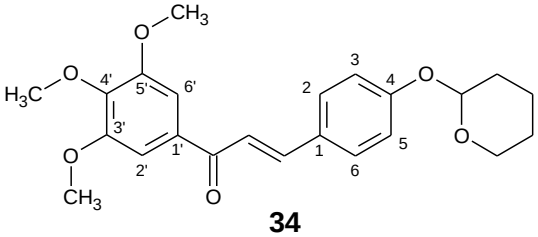
33

Chemical formula: $C_{12}H_{14}O_3$

Molecular weight: 206.24 g/mol

4-hydroxybenzaldehyde (**31**, 1000 mg, 8.19 mmol) and PPTS (51 mg, 0.205 mmol, 0.025 eq.) were dissolved in CH_2Cl_2 (12 ml) and left stirred at r.t. for 30 min. 3,4-dihydro-2*H*-pyrane (**32**, 2.24 ml, 2.73 mmol, 3 eq.) was then added. The reaction mixture was stirred at r.t. for 24 hours. When TLC indicated the completion of the reaction, the mixture was diluted by CH_2Cl_2 (8 ml) and washed three times by 0,3M solution of NaOH (20 ml each). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to give **33** as a white amorphous solid (1177 mg, 5.71 mmol, 69 %)**.**

3',4',5'-trimethoxy-4-(tetrahydro-2*H*-pyran-2-yloxy)chalcone (34)



34

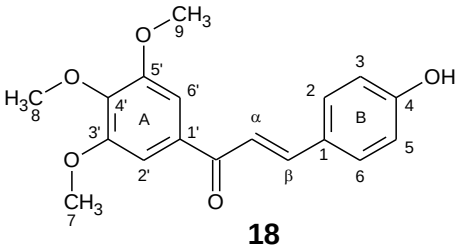
Chemical formula: $C_{23}H_{26}O_6$

Molecular weight: 398.45 g/mol

3,4,5-trimethoxyacetophenone (**21**, 1000 mg, 4.76 mmol) and $Ba(OH)_2$ (4 068 mg, 23.79 mmol, 5 eq.) were dissolved in MeOH (15 ml) and left stirred at r.t. for 30 min. **33** (1 177 mg, 5.71 mmol, 1.2 eq.) was then added and the reaction mixture was heated under reflux for 30 min. When TLC indicated the completion of the reaction, the solvent was evaporated. 20 ml of H_2O was added to the residue. The resulting mixture was then acidified by 3M HCl to pH 6 and extracted three times by CH_2Cl_2 (20 ml each). The combined organic portions were dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by flash chromatography on

silica gel (CH_2Cl_2) to give **34** as a light yellow oil (1 175 mg, 2.95 mmol, 62 %) and a small amount of **18** as a light yellow oil (25 mg, 0.08 mmol, 0.02 %).

4-hydroxy-3',4',5'-trimethoxychalcone (18)



18

Chemical formula: $C_{18}H_{18}O_5$

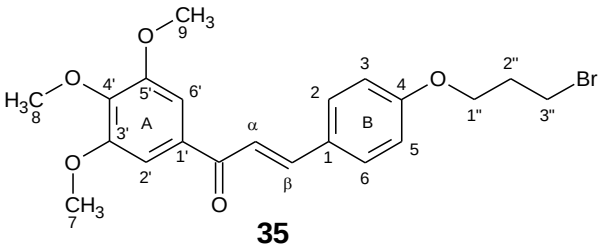
Molecular weight: 314.33 g/mol

34 (810 mg, 2.03 mmol) and PTSA (39 mg, 0.203 mmol, 0.1 eq.) were dissolved in MeOH (6 ml) and left stirred at r.t. The reaction was monitored by TLC. After 3.5 hours, when TLC indicated the completion of the reaction, the solvent was evaporated. 20 ml of H_2O were then added to the residue. The resulting mixture was extracted three times by EtOAc (20 ml each). The combined organic portions were dried over anhydrous Na_2SO_4 and the solvent was evaporated to give **18** as a light yellow oil (493 mg, 1.57 mmol, 77 %).

^{1}H NMR (500.15 MHz, $CDCl_3$) δ 7.79 (1H, d, *J* = 15.6 Hz, H-β), 7.57 (2H, d, *J* = 8.6, H-2, H-6), 7.36 (1H, d, *J* = 15.6, H-α), 7.27 (2H, s, H-2', H-6'), 6.90 (2H, d, *J* = 8.6, H-3, H-5), 5.85 (1H, s, OH), 3.95 (6H, s, H-7, H-9), 3.94 (3H, s, H-8)

^{13}C NMR (125.78 MHz, $CDCl_3$) δ 189.5 (C=O), 158.1 (C-4), 153.1 (C-3', C-5'), 144.8 (C-β), 142.4 (C-4'), 133.8 (C-1'), 130.5 (C-2, C-6), 127.7 (C-1), 119.4 (C-α), 116.0 (C-3, C-5), 106.1 (C-2', C-6'), 61.0 (C-8), 56.4 (C-7, C-9)

4-(3-bromopropoxy)-3',4',5'-trimethoxychalcone (35)



35

Chemical formula: $C_{12}H_{23}BrO_5$

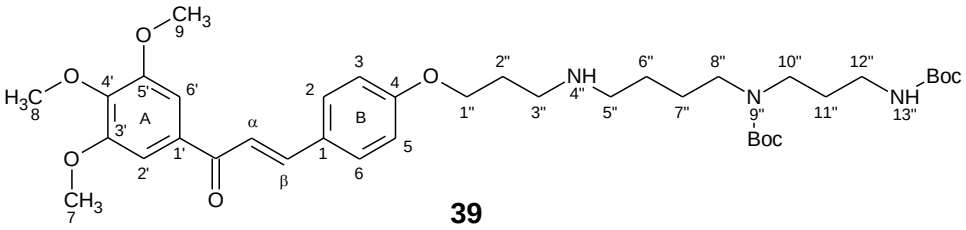
Molecular weight: 435.308 g/mol

18 (100 mg, 0.318 mmol) and K_2CO_3 (87.9 mg, 0.636 mmol, 2 eq.) were added to DMF (10 ml) and left stirred for 10 min. 1,3-dibromopropane (**36**, 323 µl, 3.18 mmol, 10 eq.) was then added. The resulting reaction mixture was stirred at r.t. for 1.5 hours, being monitored by TLC. When TLC indicated the completion of the reaction, the solvent was evaporated, H_2O (20 ml) and CH_2Cl_2 (5 ml) were added to the residue. The mixture was extracted three times by CH_2Cl_2 (20 ml each). The combined organic portions were dried over anhydrous Na_2SO_4 and the solvent was evaporated. The residue was purified by chromatography (Hex:EtOAc 7:3) to give **35** as a light yellow oil (132 mg, 0.305 mmol, 96 %).

^{1}H NMR (500.15 MHz, $CDCl_3$) δ 7.79 (1H, d, *J* = 15.5, H-β), 7.61 (2H, d, *J* = 9.0, H-2, H-6), 7.36 (1H, d, *J* = 16.0, H-α), 7.27 (2H, s, H-2', H-6'), 6.95 (2H, d, *J* = 8.5, H-3, H-5), 4.17 (2H, t, H-1''), 3.95 (6H, s, H-7, H-9), 3.94 (3H, s, H-8), 3.61 (2H, t, H-3''), 2.37-2.32 (2H, m, H-2'')

^{13}C NMR (125.78 MHz, $CDCl_3$) δ 189.3 (C=O), 160.8 (C-4), 153.2 (C-3', C-5'), 144.5 (C-β), 142.4 (C-4'), 133.8 (C-1'), 130.3 (C-2, C-6), 127.9 (C-1), 119.6 (C-α), 115.0 (C-3, C-5), 106.1 (C-2', C-6'), 65.5 (C-1''), 60.1 (C-8), 56.4 (C-7, C-9), 32.2 (C-3''), 29.7 (C-2'').

4-(9,13-bis-Boc-4,9,13-triazatridecyl)-3',4',5'-trimethoxychalcone (39)



39

Chemical formula: $C_{38}H_{57}N_3O_3$

Molecular weight: 699.874 g/mol

35 (43.5 mg, 0.10 mmol), N^1,N^4-bis-Boc-spermidine (**38**, 38 mg, 0.11 mmol, 1.1 eq.) and K_2CO_3 were added to acetonitrile (5 ml). The reaction mixture was stirred using MW heating (90 °*C*, 120 W, 20x2 min), being monitored by TLC. When TLC indicated the completion of the reaction, the solvent was evaporated, H_2O (20 ml) was added to the residue. The mixture was then acidified by 3M HCl to pH 6 and extracted three times by CH_2Cl_2 (20 ml each). The combined organic portions were dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by chromatography [CHCl3:MeOH:NH4OH 94:5:1 (1x)] to give **39** as a light yellow oil (12 mg, 0.0171 mmol, 18 %).

^{1}H NMR (500.15 MHz, $CDCl_3$) δ 7.79 (1H, d, *J* = 15.5, H-β), 7.59 (2H, d, *J* = 8.5, H-2, H-6), 7.36 (1H, d, *J* = 15.5, H-α), 7.27 (2H, d, *J* = 3.5, H-2', H-6'), 6.92 (2H, d, *J* = 8.5, H-3, H-5), 4.66 (1H, s, NH), 4.10 (2H, t, H-1''), 3.95 (6H, s, H-7, H-9), 3.94 (3H, s, H-8), 3.23-3.09 (6H, m, C-8'', C-10'', C-12''), 2.94 (2H, t, C-3''), 2.79 (2H, m, C-5''), 2.09 (2H, pseudoquintuplet, C-2'', 1.65 (2H, pseudoquintuplet, C-11''), 1.57 (2H, m, C-6'', C-7''), 1.45 (9H, s, Boc CH_3), 1.43 (9H, s, Boc CH_3)

^{13}C NMR (125.78 MHz, $CDCl_3$) δ 189.3 (C=O), 160.9 (C-4), 153.2 (C-3', C-5'), 156.1 (Boc C=O), 144.5 (C-β), 142.4 (C-4'), 133.8 (C-1'), 130.3 (C-2, C-6), 127.8 (C-1), 119.5 (C-α), 114.9 (C-3, C-5), 106.1 (C-2', C-6'), 79.8 (Boc quaternary C), 79.1 (Boc quaternary C), 66.1 (C-1''), 61.0 (C-8), 56.4 (C-7, C-9), 48.8 (C-5''), 46.7 (C-8''/C-10''/C-12''), 46.1 (C-3''),

44.0 (C-8’’/C-10’’/C-12’’), 37.6 (C-8’’/C-10’’/C-12’’), 28.6 (C-11’’), 28.5 (Boc CH_3), 28.3 (C-2’’), 26.2 (C-6’’/C-7’’), 25.6 (C-6’’/C-7’’)

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