Univerzita Karlova

Přírodovědecká fakulta

Studijní program: Chemie

Studijní obor: Medicinální chemie

Tereza Havlíková

Synthesis of selaginpulvilin X

Syntéza selaginpulvilinu X

Bakalářská práce

Vedoucí závěrečné práce: Dr. Lukáš Rýček, M.Sc.

Praha, 2024

Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 22.5.2024

Podpis

Abstrakt

Cílem této bakalářské práce je syntéza selaginpulvilinu X, který byl připraven v šesti krocích, a porovnání spekter izolované přírodní látky se spektry látky připravé. Během syntézy je využito Sonogashirova a Suzukiho couplingu, generace a adice organolithné sloučeniny na aldehyd, následné oxidace vzniklého alkoholu a finální deprotekce. Různé ochranné skupiny jsou také využity při přípravě derivátů selaginpulvilinu X.

Klíčová slova: Přírodní látky, totální syntéza, rod *Selaginella, Selaginellaceae* polyfenoly, selaginpulvilin X, Suzuki-Miyaura coupling, Sonogashira coupling

Abstract

This bachelor work aims to synthesize the natural product selaginpulvilin X using a six-step synthetic route. Subsequently, the spectra of the synthesized compound were compared with the spectra of the previously isolated compound from the genus Selaginella to verify the proposed structure of selaginpulvilin X. The total synthesis of natural product deploys Suzuki and Sonogashira coupling, generation, and addition of an organolithium compound to aldehyde, consecutive oxidation, and final deprotection. Various protective groups are also used in the preparation of selaginpulvilin X derivatives.

Keywords: Natural product, total synthesis, genus *Selaginella*, *Selaginellaceae* polyphenols, selaginpulvilin X, Suzuki-Miyaura coupling, Sonogashira coupling

Table of Contents

Used acronyms and abbreviations

Acetyl-coA – Acetyl coenzyme A

AMP – Adenosine monophosphate

AT – Apparent triplet

cAMP – Cyclic adenosine monophosphate

cGMP – Cyclic guanosine monophosphate

CNS – Central nervous system

COSY – Correlated Spectroscopy

DCM – Dichloromethane

DMF – Dimethylformamide

ESI – Electrospray ionization

EtOAc – Ethyl acetate

FDA – Food and Drug Administration R

FT-IR – Fourier-transform infrared spectroscopy

GMP – Guanosine monophosphate

Hex – *n*-hexane

HMBC – Heteronuclear multiple bond correlation

HR-ESI-MS –High-resolution electrospray ionization mass spectrometry

HRMS – High-resolution mass spectrometry

HSQC – Heteronuclear Single Quantum Coherence

IC_{50} – Half-maximal inhibitory concentration

IR – Infrared spectroscopy

LRMS – Low-resolution mass spectrometry

Malonyl-coA – Malonyl coenzyme A

MeOH – Methanol

MIC_{80} – Minimal concentration to inhibit 80%

MOM – Methoxymethyl ether

MS – Mass spectrometry

n-BuLi – *n*-butyllithium

ND – Not determined

NIS – N-Iodosuccinimide

NMR – Nuclear magnetic resonance

OH – Hydroxyl group

OMe – Methoxy group

PCC – Pyridinium chlorochromate

PDE – Phosphodiesterase

PDE4 – Phosphodiesterase-4

PTP1B – Protein tyrosine phosphatase 1B

RT – Room temperature

SI – selectivity index

SPhos – Dicyclohexyl(2′,6′-dimethoxy[1,1′-biphenyl]-2-yl)phosphane

TBAF –Tetra-*n*-butylammonium fluoride

TBS – *tert*-butyldimethylsilyl

t-BuLi – *tert*-butyllithium

THF – Tetrahydrofurane

TLC – Thin layer chromatography

TMS – Trimethylsilyl

UV – Ultraviolet

XPhos – Dicyclohexyl[2′,4′,6′-tris(propan-2-yl)[1,1′-biphenyl]-2-yl]phosphane

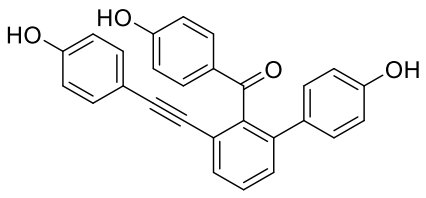
XPhosPdG2 –2nd Generation XPhos Precatalyst

1. Introduction

Natural medicine has been used since the dawn of humankind from various flowers to animal products. Modern medicine is today also interested in the medicine of our ancestors, as evidenced by approved drugs by the FDA. Almost 30 % of these substances are derived from natural products or are natural products themselves. Amongst the most notable, one can name morphine, taxol, or penicillin, all being natural products. The total synthesis of natural products is not only a way to access these compounds but also plays a crucial role in the development of novel chemical methodologies. It can also serve as a powerful tool for the determination of structural aspects and clarification of ambiguities often connected with the structure elucidation of isolated natural products. [1]

Genus Selaginella, some of the species being old almost 400 million years ago, have been historically and likely continue to be utilized in certain regions as herbal medicine. The primary focus of current research lies in their *Selaginellaceae* polyphenols, which exhibit various biological activities from cancerostatics to antimicrobiological activity.[2] Synthesizing various *Selaginellaceae* polyphenols also serves as a useful tool for developing new methodologies[3] and is being used for confirmation or rejection of their predicted structure, for example, in our laboratory we clarified structural discrepancies related to selagibenzophenone A and B.[4] Further, we have prepared selagibenzophenone C and confirmed its structure, by comparing analytical data of isolated and synthetic material.[5] We have also developed several biologically active derivatives of these natural products with selective antiproliferative properties.

In line with these findings, our current research focuses on the synthesis and structural confirmation of selaginpulvilin X (**1a**) (Figure 1) which was isolated in 2023 from *Selaginella pulvinata* by Zhu et al. [6]



1a

Figure 1 Structure of selaginpulvilin X (**1a**)

2. Theoretical part

2.1 Genus Selaginella

Genus Selaginella is an ancient group of lycophytes with the oldest fossils (Figure 2) dating back 350 million years ago. Approximately 700 species belong to this group, varying in their habitats, and therefore include tropical, drought-tolerant, and frost-tolerant species.[8] Selaginella has been used since ancient times as medicinal herbs for various ailments such as respiratory diseases, burns, hemorrhoids, and gastrointestinal hemorrhage.[9] Worth mentioning are species such as *Selaginella bryopteris* native in India which is used under the name Sanjeevani and was commonly used for the treatment of jaundice or burning urination, and *S. articulata* in Columbia used for the treatment of snakebites.[8,10] We can also mention one of the most known species *S. lepidophylla,* an ordinary house plant called the rose of Jericho.[8] The genus Selaginella is not only important for its pharmacology use but also serves as an important model in the evolution of vascular plants, as evidenced by the completion of the genome *S. moellendorffii* in 2011.[2,11]

Figure 2 Fossil of *Selaginella stachygynandroides*[7]

2.2 Extracts of Selaginella species and contained natural classes

The extracts of Selaginella species (e.g. *S. tamariscina* – also known as spike moss in English) have been used in traditional medicine and cosmetic applications (Figure 3).[9]

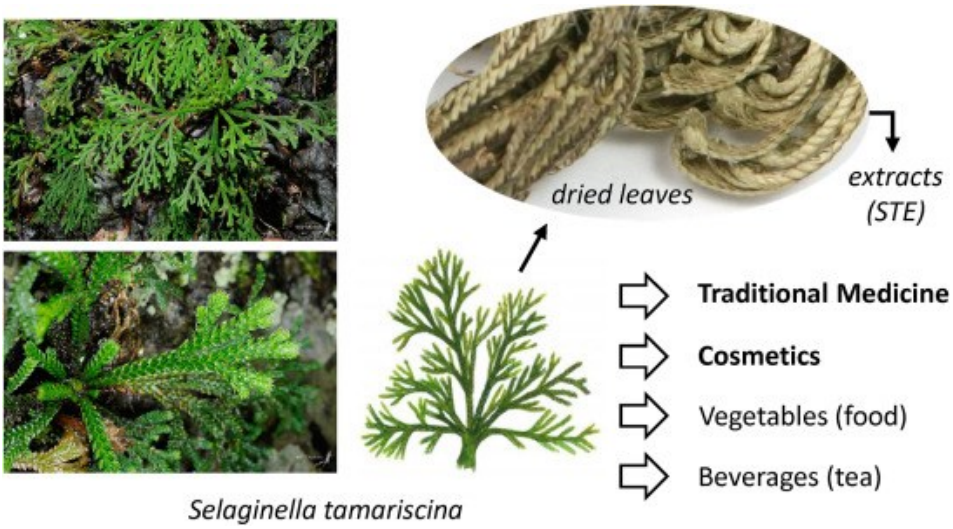


Figure 3 *S. tamariscina* and uses of its extracts[9]

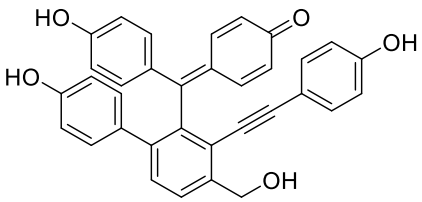
The most prevalent classes of natural products present in the extracts of Selaginella species were flavonoids, then lignans followed by phenols and selaginellins (herein referred to as *Selaginellaceae* polyphenols). Other classes of compounds present in these extracts included terpenoids, steroids, neolignans, alkaloids, quinones, and fatty acids. The experimental procedure can heavily influence the final contents of extracts. For example, Křížkovská et al. attempted to prepare extracts of 9 Selaginella species (*S. apoda*, *S. biformis*, *S. cupressina*, *S. delicatula*, *S. erythropus*, *S. myosuroides*, *S. uncinata*, *S. ramosii*) using MeOH and DCM for extraction. Interestingly, alkaloids were absent in DCM extract whereas they were present in MeOH extracts of some tested selaginella species.[12]

Furthermore, Křížkovská et. al investigated the biological activities of prepared extracts, namely cytotoxicity against cancer cell lines, anti-inflammatory activity, antioxidant activity, and inhibitory activity against acetylcholinesterase. Anti-inflammatory activity was also tested as the ability to inhibit nitrate oxide production. The activity was mainly observed in some MeOH extracts. This activity correlated with neolignans and lignans content. All tested extracts exhibited some antioxidant activity, consistent with the content of flavonoids, neolignans, lignans, and *Selaginellaceae* polyphenols. The inhibition of acetylcholinesterase was mainly observed in DCM extracts and inhibition activity was attributed to *Selaginellaceae* polyphenols. For example, the DCM extract of *S. myosuroides* had the highest activity and

its MeOH was the only MeOH extract capable of inhibition of acetylcholinesterase from all of the tested MeOH extracts. Additionally, all extracts had activity against cancer lines HepG2 and HeLa. All these findings suggest that biological activity depends on species, present compounds in extracts, and therefore even on the employed procedure of the extraction.[12]

2.3 *Selaginellaceae* polyphenols

The initial efforts to characterize biologically active compounds unique to this genus can be traced to the year 2007 when the first member of polyphenolic compounds, now known as Selaginellins, was found in the extract of *S. sinensis*, as a racemic mixture.[2,13]



2

Figure 4 Structure of selaginellin (**2**)

The discovery of selaginellin (**2**) (Figure 4) was a milestone in the *Selaginellaceae* polyphenol research, and since then, more than 60 naturally occurring *Selaginellaceae* polyphenols have been isolated, mostly from species *S. pulvinata* (Figure 5) and *S. tamariscina*.[2] Total synthesizes have already been carried out for some of them.

Figure 5 *S. pulvinata*[14]

The nomenclature within this group is often misleading, and there are cases where different compounds are given the same name, as was a case of selaginellin U and X, both labeled selaginellin U.[2,2,15] On the other hand, there are examples of the same compound being named

by two different names, as seen in case selagibenzophenone B which is also called selaphenin A.[16,17] Overall no attempt for the unification and simplification of the whole nomenclature of this group has not yet been made. *Selaginellaceae* polyphenols can be categorized into 5 main groups (Figure 6), based on their skeletons and biochemical pathway of synthesis. [2,18]

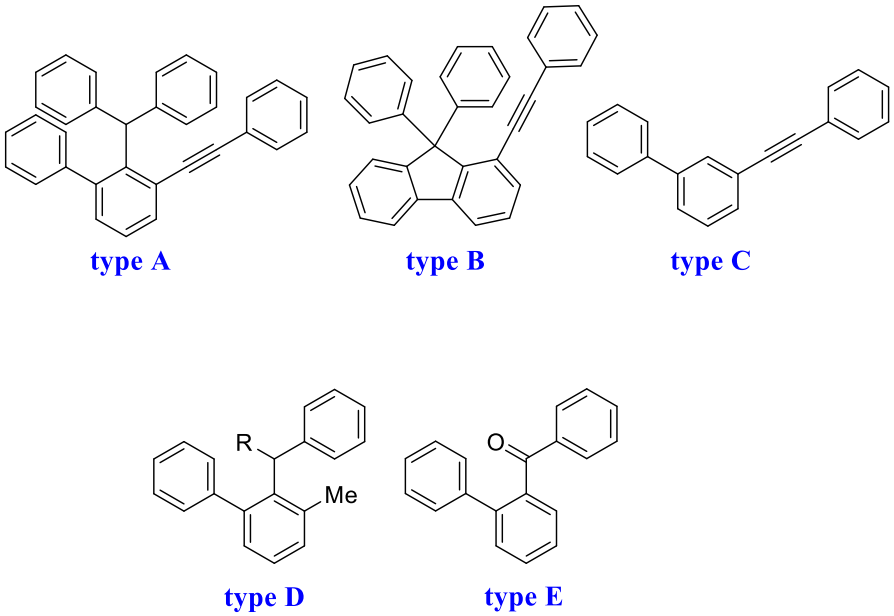
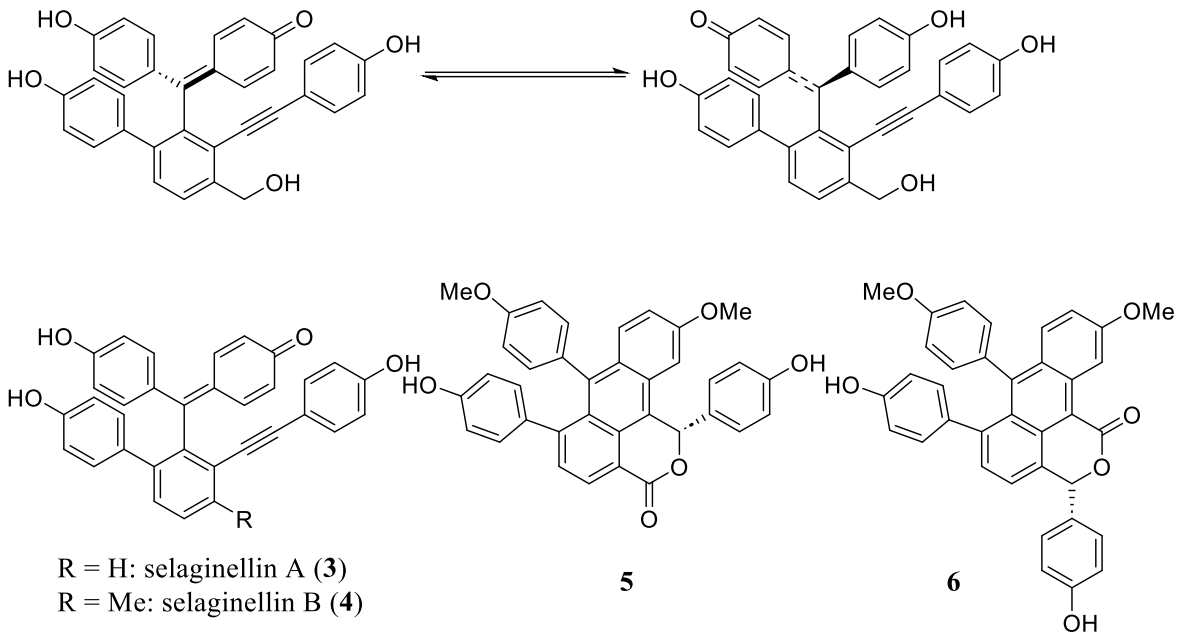


Figure 6 Classes of *Selaginellaceae* polyphenols

2.3.1 Type A skeleton, selaginellins

Group A contains 2,2-benzhydryl-3-phenylethynyl-1,1'-biphenyl skeleton or skeletons derived thereof. As previously mentioned, the oldest member of group A is selaginellin (**2**). Members of this group are usually named as selaginellins.



R = H: selaginellin A (**3**)
R = Me: selaginellin B (**4**)

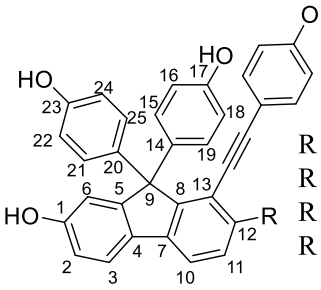
5 **6**

Figure 7 Tautomery of selaginellin, structure of selaginellin A (**3**), B (**4**), selariscin A (**5**) and B (**6**)

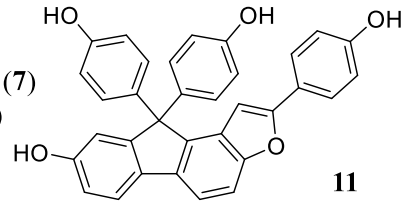
Some compounds within this group exhibit an interesting structural feature, phenol-quinone tautormery, as seen in the structure of (**2**). These compounds usually possess axial chirality and exist as racemic mixtures.[2] In the case of selaginellin, A (**3**) and B (**4**) (Figure 7), optically inactive forms were isolated.[19] However, unusual structures within this group have been reported.[2] For instance, dimeric diselaginellins A and B (**22**)[20] or lactone selaginellins selariscin A (**5**) and B (**6**) (Figure 7).[21]

2.3.2 Type B skeleton, selaginpulvilins

Type B compounds feature a 9,9-diphenyl-1-(phenylethynyl)9H-fluorene skeleton, with an arylalkynyl moiety in position C13. Members of this group are usually named selaginpulvilins.[2] The first discovered compounds of this class, Selaginpulvilin A to D (**7-10**), (Figure 8), were extracted from *S. pulvinata* in 2014.[22] The compounds differ in the substitution at the position C12. Several compounds were described that feature structural motifs derived from the reactivity of the C12 and alkyne moiety, including benzofurane isoselagintamarlin A (**11**) (Figure 8).[23]



R = CH_2OH: selaginpulvilin A (**7**)
R = CHO: selaginpulvilin B (**8**)
R = Me: selaginpulvilin C (**9**)
R = H: selaginpulvilin D (**10**)

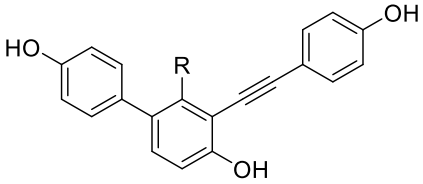


11

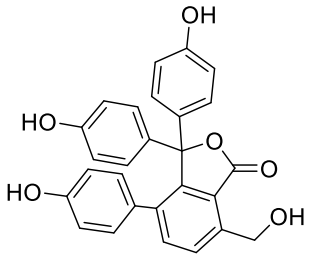
Figure 8 Structure of selaginpulvilin A-D (**7-10**) and isoselagintamarlin A (**11**)

2.3.3 Type C and D skeleton

Type C *Selaginellaceae* polyphenols are presumed to be intermediates of type A *Selaginellaceae* polyphenols with a C and/or a D ring-trimmed skeleton. The first discovered compounds of this class are selaginellin **K** (12) and **L** (13) (Figure 9). They were extracted from *S. tamariscina* in 2011.[2,24]



R = CHO: selaginellin K (**12**)
R = Me: selaginellin L (**13**)



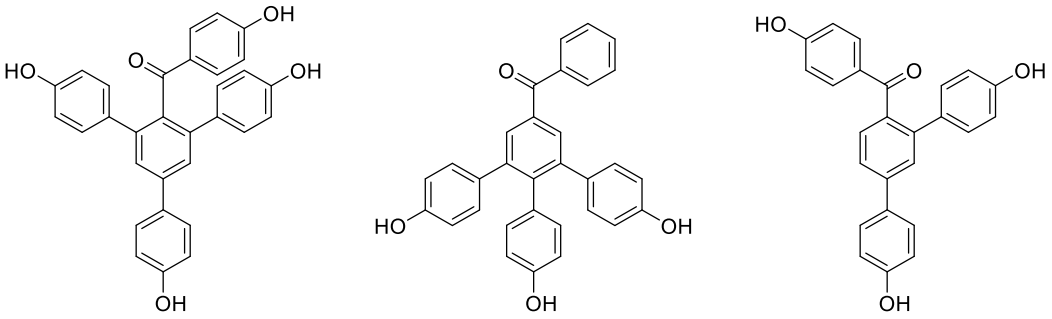
14

Figure 9 Structures of selaginellin K (**12**), L (**13**) and H (**14**)

Type D *Selaginellaceae* polyphenols are intermediates of type A selaginellin that do not contain an alkynylphenyl structure. In 2010, the first compound, selaginellin H (**14**), was discovered. This compound is notable for its γ-lactone ring (Figure 9).[2,25]

2.3.4 Type E skeleton, selagibenzophenones

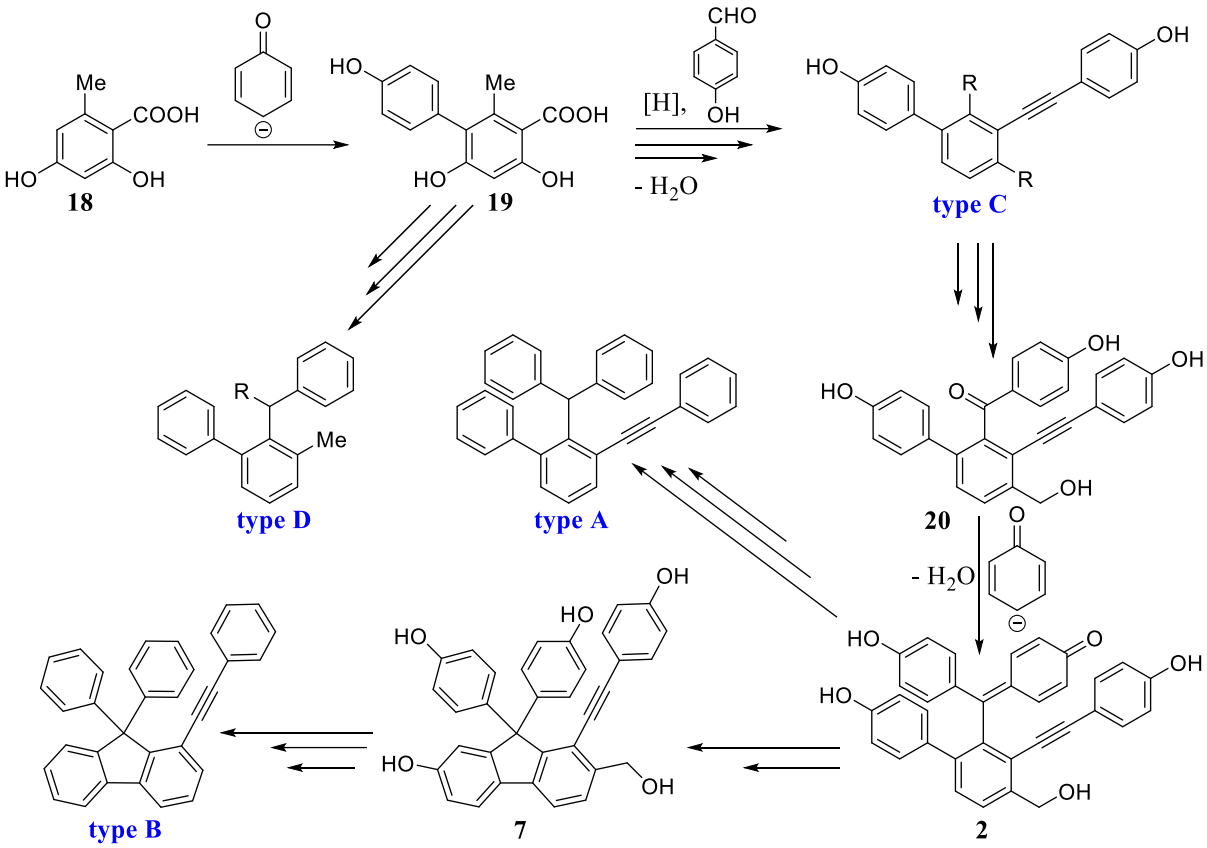
Type E *Selaginellaceae* polyphenols represent a novel group that contains an arylated benzophenone motif. The first member of this group, selagibenzophenone A (**15**) **(**Figure 10), was discovered in 2017 in *S. pulvinata* by Liu et al.[18] Later, selagibenzophenone C (**17**) (Figure 10), isolated by Chen et al[26] was recently synthesized in our group.[5] Two reports described the isolation and biological activity of selagibenzophenone B (**16**) (Figure 10) which differs in the substitution on the skeleton compared to selagibenzophenone A.[16,17] However, our research group proved, that the compound isolated and proposed to have a structure corresponding to **16** was wrongly assigned, and the authors, who claimed the isolation of selagibenzophenone B isolated previously known selagibenzophenone A.[4]



15 **16** (proposed structure, but proved by us to be incorrect) **17**

Figure 10 Structures of selagibenzophenone A (**15**), B (**16**) and C (**17**)

2.1 Biochemical synthesis of *Selaginellaceae* polyphenols



Scheme 1 Simplified scheme of probable biogenesis of various *Selaginellaceae* polyphenols

The whole biochemical synthesis pathway of every *Selaginellaceae* polyphenol is currently still unknown. The precursor for synthesis is believed to be orsellinic acid (**18**) (Scheme 1), which is formed from malonyl-CoA and acetyl-CoA. Orsellinic acid (**18**) is next modified through free radical coupling with 2,5-cyclohexadione. This formed intermediate (**19**) is crucial as it serves as an intermediate in the biogenesis. Compound **19** can undergo free radical coupling with 2,5-cyclohexadione again and additional oxidative steps to yield *Selaginellaceae* polyphenols of type D. Type C *Selaginellaceae* polyphenols are formed from **19** by reduction of the carboxylic group, followed by benzoin condensation with p-hydroxybenzaldehyde, dehydration, and additional oxidative steps. *Selaginellaceae* polyphenols of type A are formed from selaginellin S (**20**), which has the skeleton of type C. Initially, **2** is formed via coupling of **20** with 2,5-cyclohexadione followed by dehydration. Modification of **2** can be done via various redox reactions, dimerizations, intramolecular cyclizations, etc., resulting in different compounds. Type B *Selaginellaceae* polyphenols are formed from selaginpulvilin A (**7**).[2]

2.2 Biological activities of *Selaginellaceae* polyphenols

As previously mentioned, extracts of plants from the genus Selaginella have been used since ancient times as remedies for various health issues. Various *Selaginellaceae* polyphenols exhibit different biological activities depending on their structure. Currently, inhibition of phosphodiesterase-4 (PDE) and protein tyrosine phosphatase 1B (PTP1B) are main interests along with antimicrobial and cancerostatic activities.[2]

2.2.1 PDE4 inhibition

PDEs are a family of hydrolases that convert cAMP to AMP and cGMP to GMP, consequently reducing levels of these second messengers in the cell, whereas enzymes adenylyl cyclase and guanylyl cyclase increase levels of cAMP and cGMP, respectively. These processes regulate cell signaling pathways, including the formation of cytokines which can activate the cell's immunological response. Dysfunctions in cAMP signalization are associated with CNS disorders, inflammation, asthma, metabolic disorders, and some cancers. There are 11 isoforms of PDE, with PDE4 specifically converting cAMP to AMP (Figure 11).[2,27]

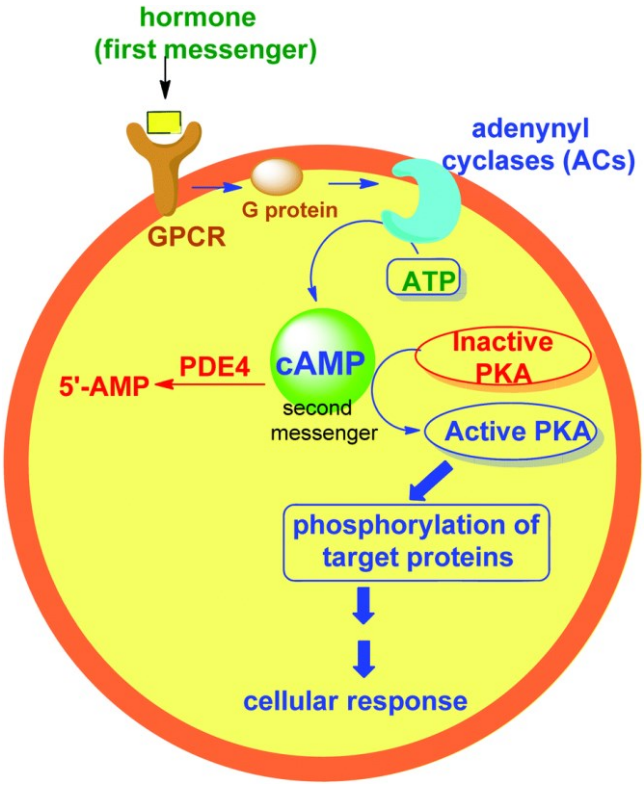


Figure 11 Role of PDE4 in cellular signalization[27]

Numerous *Selaginellaceae* polyphenols have been tested for inhibitory activity against PDE4 and therefore their IC_{50} were measured. Yin's group measured the IC_{50} values for some previously mentioned *Selaginellaceae* polyphenols as follows: **2** with a value of 0.97 µM, **3** with a value of 1.42 µM, **4** with a value of 1.25 µM, **7** with a value of 0.24 µM, **8** with a value of 0.11 µM, **9** with a value of 0.18 µM, **10** with a value of 0.26 µM, and **13** with a value of 7.42 µM (Table 1). [18,22]

Table 1 IC_{50} of some previously mentioned *Selaginellaceae* polyphenols

Compound	IC_{50} [µM]	Compound	IC_{50} [µM]
2	0.97 ± 0.10[22]	**8**	0.11 ± 0.02[22]
3	1.42 ± 0.10[22]	**9**	0.18 ± 0.02[22]
4	1.25 ± 0.04[18]	**10**	0.26 ± 0.05[22]
7	0.24 ± 0.03[22]	**13**	7.42 ± 0.31[18]

From structure-activity analysis has been observed that *Selaginellaceae* polyphenols containing a fluorene core, as depicted in Figure 12, generally exhibit lower IC_{50} values than their other counterparts. Methylation of this skeleton can also alter activity, as seen in cases of methylation of OH in positions 23 and 17. Additionally, the OH group at position 10 decreases activity.[2] We can highlight selaginpulvilin K (**21**) (Figure 12), which has an IC_{50} value of 0,011 µM and is therefore 50 times stronger than rolipram with an IC_{50} value of 0.54 µM which has been used as positive control in assays evaluating PDE4 inhibitory activities of *Selaginellaceae* polyphenols.[2,28]

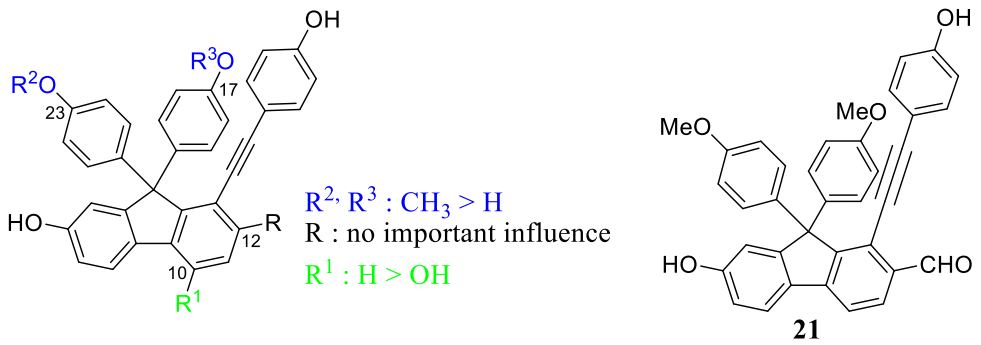


Figure 12 Structure-ativity analysis of selaginellins and structure of selaginpulvilin K (**21**)

2.2.2 PTP1B inhibition

PTP1B, a tyrosine phosphatase (Figure 13), is currently associated with type 2 diabetes, and obesity and is potentially implicated in various cancers. PTP1B acts as a negative regulator of insulin signaling by dephosphorylating insulin receptors. Studies have also shown that overexpression of PTP1B can lead to tumorigenesis in mice. The discovery of inhibitors of PTP1B was historically considered almost impossible in comparison to inhibitors of kinases due to possible bonding sites of PTP1B being too polar, limiting possible permeability for the inhibitor into the cell.[29] Woo's group discovered that some *Selaginellaceae* polyphenols act as inhibitors of PTP1B. We can mention compound **2** with a measured IC_{50} value of 15.9 μM.[30]

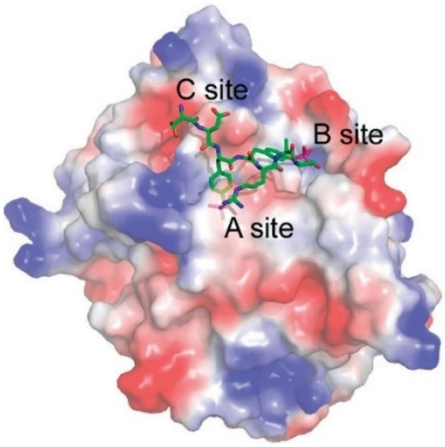


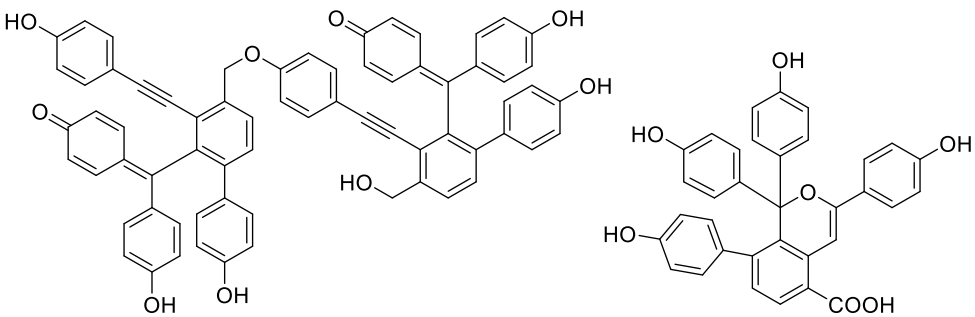
Figure 13 X-ray sructure of PTP1B with bonded insulin fragment[29]

2.2.3 Cytotoxicity

Certain *Selaginellaceae* polyphenols also demonstrate cytotoxic activity against various cancer cell lines. For example, diselaginellin B (**22**) (Figure 14), exhibits cytotoxic activity against the SMMC-7721 cell line, a hepatocarcinoma cell line, with a measured IC_{50} value of 9.0 μM. This selaginellin arrests the cell cycle in G1 phase and can reduce the formation of metastasis.[20] Additionally, selagintamarlin (**23**) (Figure 14) also exhibits cytotoxicity against several cell lines measured by IC_{50} values, including MGC-803 with a value of 8.73 μM, HepG2 (hepatoma) with a value of 10.32 μM, A549 (lung adenocarcinoma) with a value of 9.61 μM, NCI-H460 (lung carcinoma) with a value of 7.02 μM and SKOV-3 (ovarian carcinoma) with a value of 8.22 μM (Table 2).[31]

Table 2 Measured values of IC_{50} of **23** for various cell

Cell line	IC_{50} [μM]	Cell line	IC_{50} [μM]
MGC-803	8.73 ± 1.10	NCI-H460	7.02±1.20
HepG2	10.32±0.97	SKOV-3	8.22±1.26
A549	9.61±1.09		



22 **23**

Figure 14 Structure of diselaginellin B (**22**) and selagintamarlin (**23**)

Selagibenzophenone A (**15**) and various derivatives (Figure 15) were prepared by Lapinskaite et. al and these compounds were tested for cytotoxicity. Derivative **15a** showed an IC_{50} value of 17.7 µM against the HT-29 (colon cancer) cell line, with a high selectivity index (SI) of 8.2. On the other hand, derivative **15b** exhibited an IC_{50} value of 7.8 µM against the PC3 (prostate cancer) cell line, with an SI of 3.5.[32]

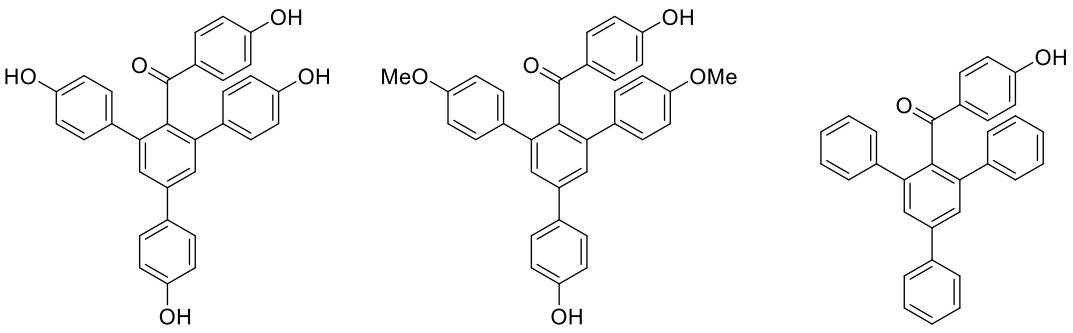


Figure 15 Structure of selagibenzophenone A **(15)** and its derivatives **15a** and **15b**

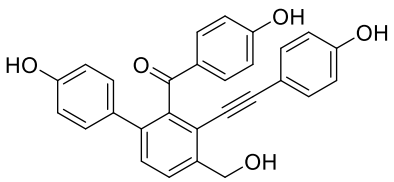
2.2.4 Microbiological activity and activity against fungi

Some *Selaginellaceae* polyphenols exhibit possible microbiological activity against multi-drug-resistant bacteria such as Staphylococcus aureus, while others can also possess antifungal properties against various fungi.[2] IC_{50} values for compounds **2-4** against S. aureus have been measured as 4.9, 1.2, and 1.2 µg/mL, respectively.[33] Furthermore, compounds **2-4** and others were also tested against various fungi.[2]

Table 3 Measured MIC_{80} for 2-4 against various fungi

	MIC_{80} [µg/ml]			
	C. albicans	**A.fumigatus**	**T.rubrum**	**T. mentagrophyte**
2	32	32	16	16
3	16	16	16	16
4	32	8	16	8
Fluconazole	0.25	100	2.0	4.0

The measured MIC_{80} values for **2** against including Candida albicans, Aspergillus fumigatus, Trichophyton rubrum, and Trichophyton mentagrophyte, are 32 µg/ml, 32 µg/ml, 16 µg/ml, and 16 µg/ml, respectively, for **3** those values are all 16 µg/ml, for **4** those values are 32 µg/ml, 8 µg/ml, 16 µg/ml and 8 µg/ml, respectively and values for the positive control Fluconazole are 0.25 µg/ml, 100 µg/ml, 2.0 µg/ml and 4.0 µg/ml, respectively (Table 3).[34] Additionally, selaginellin S (**20**) (Figure 16), exhibited promising results as an inhibitor of Hepatitis virus B replication and gene expression, as evidenced by IC_{50} values for surface antigen and e antigen, which are respectively 0.026 and 0.032 µg/mL.[35]

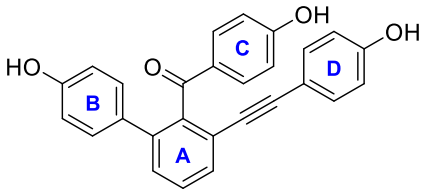


20

Figure 16 Structure of selaginellin S (**20**)

2.3 Selaginpulvilin X

The isolation of selaginpulvilin X (**1a**) from *S. pulvinata* was first reported in February 2023 by Zhu et al (Figure 17). The structure of (**1a**) was proposed, as depicted in Figure 17, bearing arylalkynyl (ring D) and aryl (ring B) moieties attached to the benzophenone (ring A and C) moiety. The structure was determined following measured ^{1}H, ^{13}C NMR spectra, COSY, HMBC, and HR-ESI-MS. The ability of inhibition of α-glucosidase was determined for **1a**. Selaginpulvilin X (**1a**) can also be qualified as a dehydroxyethyl derivative of **20**,[6] which was first isolated from *S. moellendorffii* in 2016.[35]

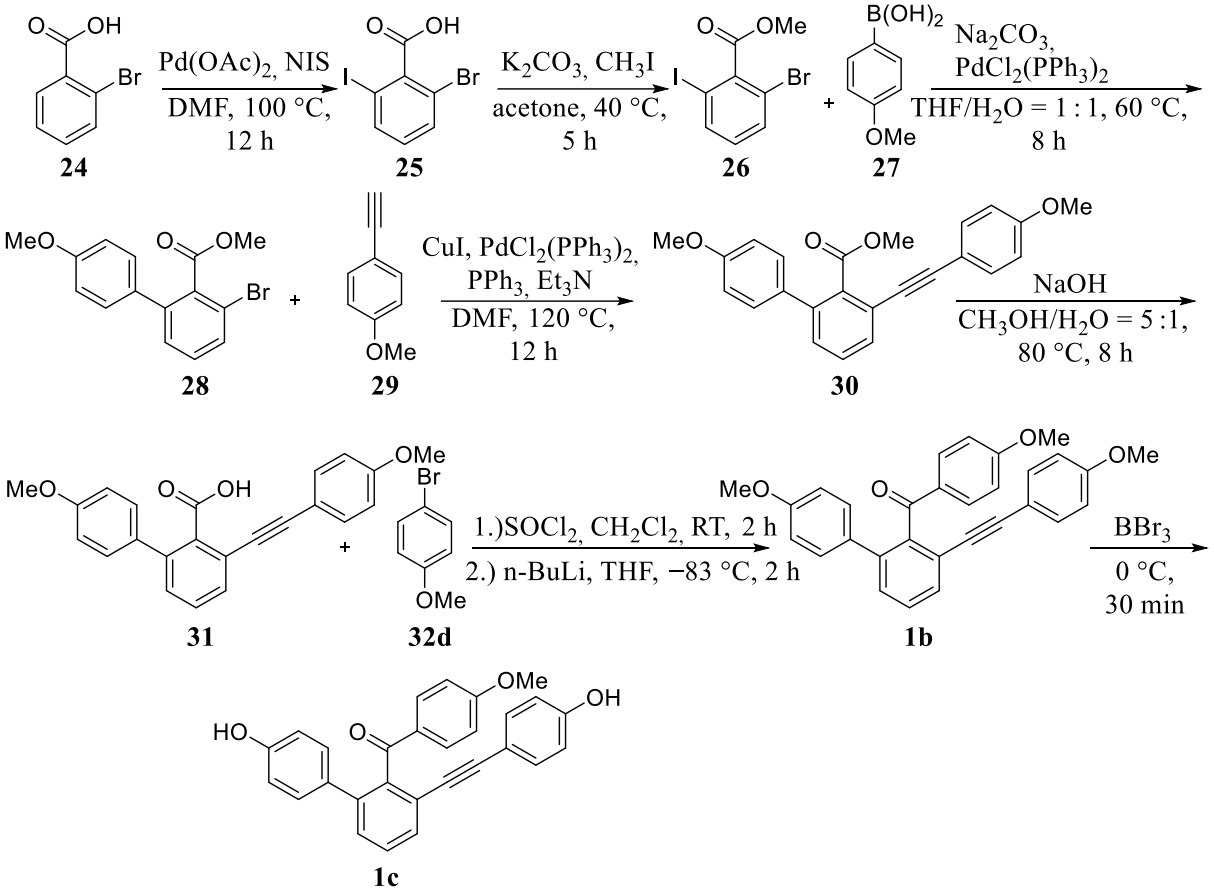


1a

Figure 17 Struture of selaginpulvilin X (**1a**) and used notation for its aromatic rings

2.4 Synthesis of derivatives of selaginpulvilin X

In 2019 Wang and his group synthesized selaginellin mimetics containing a type C skeleton. The group prepared among others methoxy derivatives (**1b**, **1c**) of in that time unknown **1a** (Scheme 2).[36]



Scheme 2 Synthesis of **1c**

Compound **24** was converted to **25** via iodization using $Pd(OAc)_2$ and NIS. The next step was esterification using K_2CO_3, CH_3I which yields **26**. This intermediate was converted to **28** via regioselective Suzuki coupling with 4-methoxyphenylboronic acid (**27**), $PdCl_2(PPh_3)_2$ as a catalyst, and Na_2CO_3 as a base. Then **28** was converted to **30** using Sonogashira's coupling with 4-methoxyphenylacetylene (**29**) and using catalyst system CuI and $PdCl_2(PPh_3)_2$, PPh_3 and Et_3N as base. Next, hydrolization of **30** was carried out using NaOH to form **31**. Derivative **1b** was prepared from **31**, which was converted to corresponding chloride using $SOCl_2$ and subsequently treated with aryl lithium species derived from bromide **32d**. Derivative **1b** was converted to **1c** via BBr_3 treatment. However, preparation of the fully demethylated compound **1a** failed using this method, therefore, leaving the synthesis of natural product incomplete.[36]

3. Goals

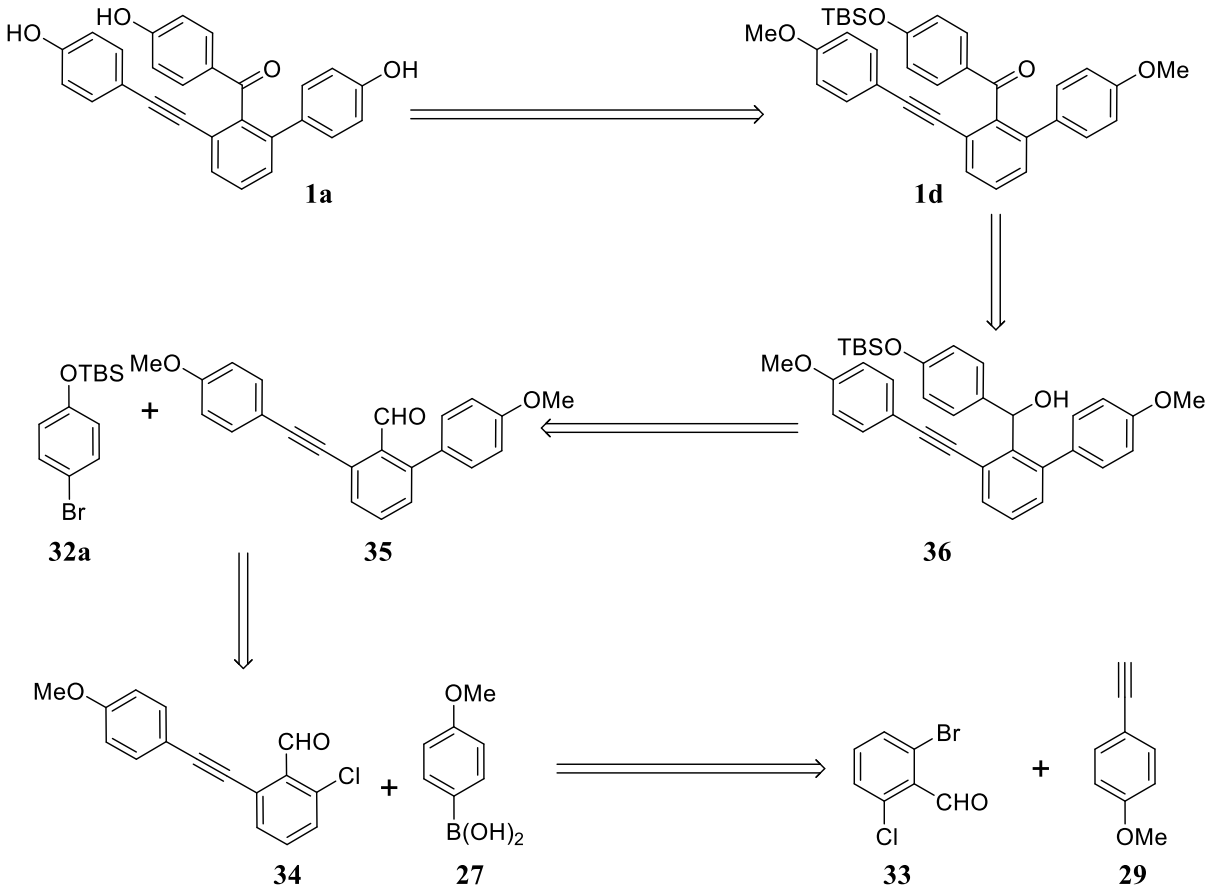
Our goals are:

1. Synthesis of pure selaginellinpulvilin X (**1a**)
2. Comparison of NMR data from isolated **1a** with our ^{1}H NMR spectra and ^{13}C NMR spectra of prepared **1a**
3. Confirmation of the proposed structure of **1a**

4. Discussion

The retrosynthetic analysis reveals (Scheme 3), that the natural compound **1a** can be derived from fully protected benzophenone **1d**, which can be further derived from alcohol **36**. Alcohol **36**, can be obtained by an addition of organometallic species to the aldehyde **35.** The synthesis of the aldehyde **35** relies on the two subsequent cross-couplings, namely Suzuki and Sonogashira couplings from starting material **33**.

It will be crucial to employ a cleavable protecting group, to avoid difficulties reported previously by Wang.[36] We envisioned using TBS as a suitable option for the phenol position at aromatic ring C of **1a**.

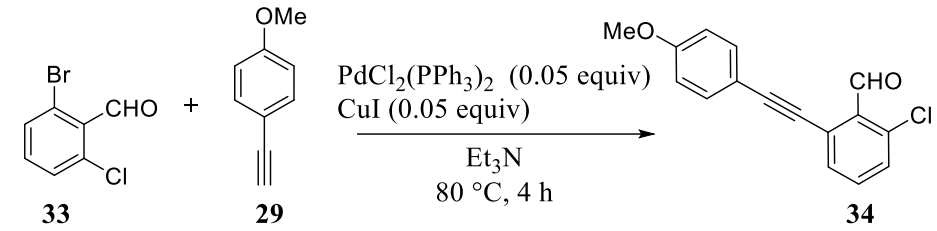


Scheme 3 Retrosynthetic analysis for preparation **1a**

4.1 Preparation of the carbon skeleton

4.1.1 Sonogashira cross-coupling

Compound **34** was prepared using 2-brom-6-chlorbenzaldehyde (**33**) and 4-methoxyphenylacetylene (**29**) as starting materials. The reaction was carried out in the presence of 0.05 equivalents of $PdCl_2(PPh_3)_2$ and CuI catalysts and Et_3N as a base (Scheme 4).



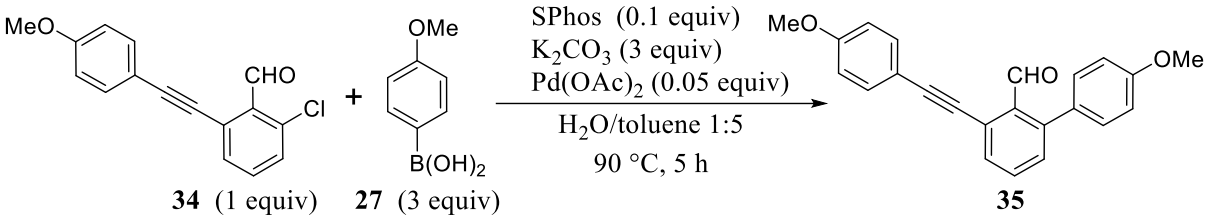
Scheme 4 Synthesis of **34**

The progress of the reaction was monitored by TLC analysis. In our initial attempt, we performed the reaction at 60°C for 20 hours. The analysis revealed a full consumption of the starting material, however a complex mixture of reaction products was obtained. Increasing the temperature to 80 °C allowed the shortening of the reaction time to 4 hours and product **34** was isolated in 59% yield.

Additional problems pose purification of **34** in repeated reactions. We attempted to purify the compound by column chromatography. However, using a system of hex/EtOAc proved to give an overall impure compound. Therefore, the impure product was subjected to the second round of column chromatography using the DCM/hex mobile phase. In the end, we were able to obtain the desired compound of sufficient purity. We have identified at least two side products from ^{1}H and ^{13}C NMR spectra, one being a dimeric product of used acetylene and the second being dehalogenated starting benzaldehyde.

It is noteworthy to mention, that the quality of the Et_3N plays a crucial role in the reaction course. Extended standing of the base prior to its use leads to a decrease in the yield and formation of undesired side-products. Therefore, for better reproducibility, the reaction should be performed using a freshly distilled Et_3N.

4.1.2 Suzuki cross-coupling



34 (1 equiv) **27** (3 equiv) **35**

Scheme 5 Synthesis of **35**

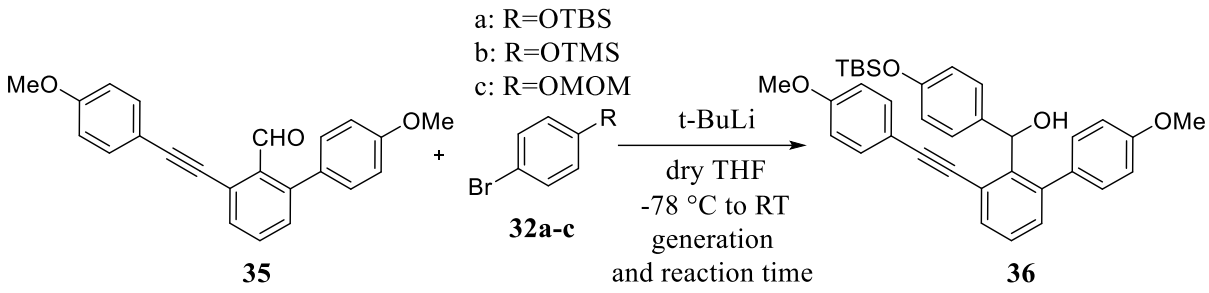
Aldehyde **35** was prepared by Suzuki cross-coupling of chloride **34** and 4-methoxyphenylboronic acid (**27**) (3 equiv) while using $Pd(OAc)_2$ as a catalyst, SPhos as a ligand, and K_2CO_3 (3 equiv) as a base while stirring and heating the reaction mixture to 90 °C for 5 hours (Scheme 5). The compound **35** was prepared with an 86% yield (Table 4, Entry 1).

Table 4 Reaction conditions of various Suzuki couplings used for formation of **35**

Entry	Catalyst (0.05 equiv)	Ligand (0.1 equiv)	Solvent	Yield
1	$Pd(OAc)_2$	SPhos	Toluene/H_2O (5:1)	86%
2	$Pd(OAc)_2$	XPhos	Toluene/H_2O (5:1)	<51%
3	$Pd(OAc)_2$	XPhos	Oxiran	ND
4	XPhosPdG2	XPhos	Toluene/H_2O (5:1)	53%

Unfortunately using SPhos as a ligand proved to be challenging and the reaction was not reproducible after using a new bottle of SPhos from a different vendor (Table 4, Entry 1). We also tried to recrystallize SPhos, but this did not lead to any improvement. Therefore we have tried using a different ligand, XPhos, while keeping other conditions the same. The conversion was complete after 1 hour and we were able to prepare **35** in <51% yield (Table 4, Entry 2), but purification of the reaction mixture proved to be difficult, as the formation of side products with identical retention factor complicated the column chromatography. Therefore we have changed the solvent to oxiran while maintaining all other conditions (Table 4, Entry 3). After 24 hours of reaction time, no product **35** formed which was observed via TLC analysis. Next, we used XPhosPdG2 as the catalyst while using Toluene/H_2O (5:1) as solvent and XPhos as ligand and we were able to obtain compound **35** in yields up to 53% (Table 4, Entry 4).

4.1.3 Generation and addition of organolithium species to aldehyde 35



Scheme 6 Preparation of **36**

For the preparation of compound **36** (Scheme 6)**,** we initially used 1.3 equivalents of *t*-BuLi and 1.4 equivalents of aryl bromide **32a**, bearing TBS as the protecting group (Table 5, Entry 1) for generation of the organolithium compound which proceeded at -78°C for 40 min. However, this did not lead to the formation of secondary alcohol and we observed no conversion of the starting material after 1 hour at RT. We further increased the amount of *t*-BuLi to 1.6 equivalents of *t*-BuLi, decreased generation time to 20 min, and prolonged reaction time to 24 hours while keeping the amount of aryl bromide **32a** unchanged (Table 5, Entry 2). This however did not lead to any improvement as no reaction took place.

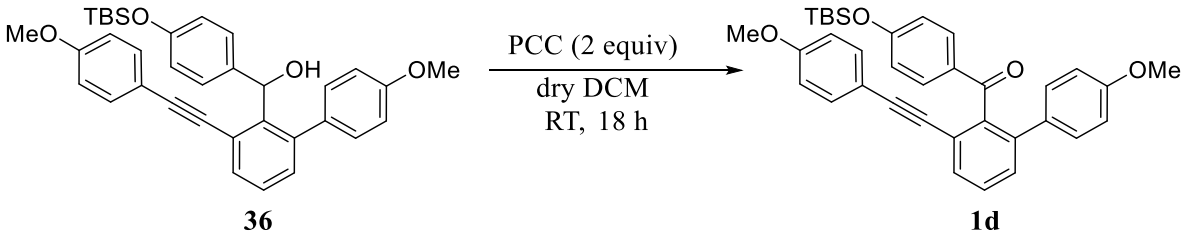
Table 5 Used equivalents and reaction times during optimization of alcohol **36** preparation

Entry	R	t-BuLi (equiv)	Bromide (equiv)	Generation time	Reaction time	Outcome
1	TBS	1.3	1.4	40 min	1 hour	No reaction
2	TBS	1.6	1.4	20 min	24 hours	No reaction
3	TMS	1.8	1.4	20 min	24 hours	No reaction
4	MOM	1.4	1.4	30 min	2 hours	No reaction
5	TBS	3.5	1.5	45 min	30 min	80%

We also attempted to use different protected bromophenols **32b**, and **32c**, with TMS and MOM protecting groups, respectively. The first attempt involved using TMS-protected bromophenol **32b** (1.4 equiv) and *t*-BuLi (1.8 equiv). The organolithium compound was left to generate for 20 min at a temperature of -78 °C, then benzaldehyde (1 equiv) was added and after 24 hours at RT no reaction took place (Table 5, Entry 3). The second attempt involved MOM-protected

bromophenol (**32c**) (1.4 equiv) and *t*-BuLi (1.4 equiv) but regrettably, no reaction took place after 30 min of generating organolithium compound at -78 °C followed by 2 hours of reaction time with an aldehyde (1 equiv) at RT (Table 5, Entry 4).

Finally, we returned to the original TBS protecting group and tried to use an excess of *t*-BuLi (3.5 equiv) which proved to be successful. Ultimately, we synthesized **36** from compound **35**. Firstly we prepared organolithium compound from **32a** (1.5 equiv) using *t*-BuLi (3.5 equiv) while stirring the mixture for 45 min at -78 °C, then we added the aldehyde **35** and stirred the mixture at RT for 30 minutes. In such a way, we were able to isolate alcohol **36** in a total 80% yield (Table 5, Entry 5).



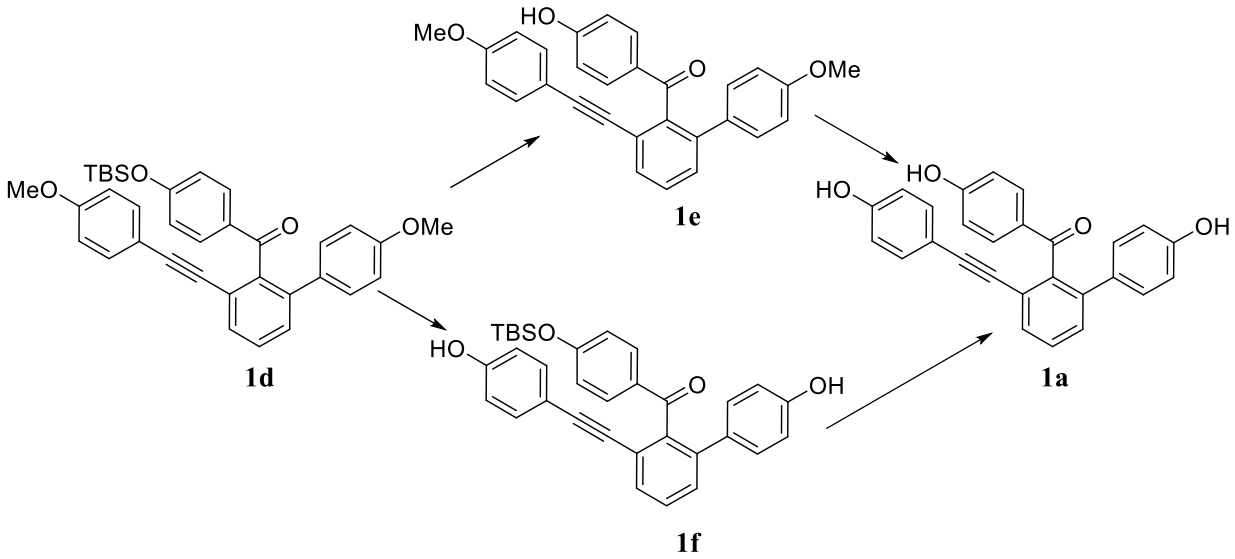
36 **1d**

Scheme 7 Preparation of derivative **1d**

Further, alcohol **36** was subjected to oxidation. We prepared ketone (**1d**) from compound **36** by its treatment with PCC (2 equiv) as an oxidation agent. The reaction was left to proceed for 18 hours at RT and the corresponding ketone **1d** was formed quantitatively (Scheme 7).

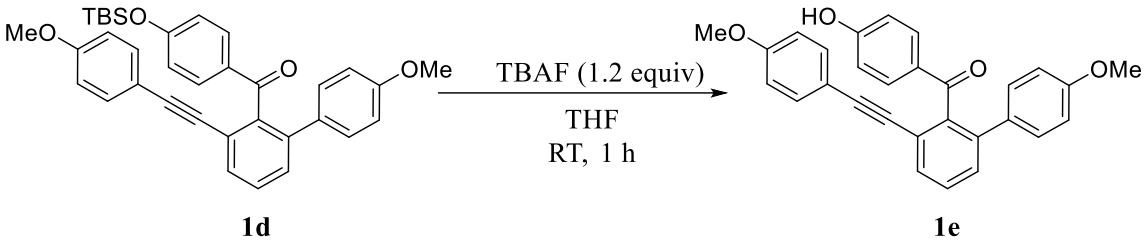
4.2 Deprotection of protective groups

Two routes for the deprotection of **1d** were envisioned. First relies on the initial deprotection of the TBS group and consecutive cleavage of methoxy groups from derivative **1e**. The second envisioned route commens with the methoxy deprotection, followed by the TBS group cleavage from derivative **1f** (Scheme 8).



Scheme 8 Routes for deprotection

We have attempted to prepare **1a** from **1d** via deprotection using BBr_3 (3.4 equiv) followed by TBS deprotection of the crude product using TBAF (1.2 equiv). In the ^{1}H NMR spectra of the crude reaction mixture, we were able to detect peaks belonging to desired product **1a**, but it was formed only as a minor product. Additionally, we have detected a major formation of unidentified side products. The ^{13}C NMR analysis revealed an absence of the alkyne triple bonds and the presence of signals with chemical shifts around 200 ppm. This suggests, that the electron-rich triple bond undergoes undesired side reactions, perhaps, the formation of a ketone from a triple bond took place as it is consistent with measured ^{13}C NMR data, where the peaks belonging to the ketone area were observed.



1d **1e**

Scheme 9 Preparation of **1e**

Next, we have explored the second envisioned route. We have successfully cleaved the TBS group at RT using TBAF (1.2 equiv) in THF and thus prepared derivative **1e** in 87% yield, however, the compound was contaminated with approximately 5 mol% of unknown impurity (Scheme 9). We have also attempted to use Olah's reagent (150 equiv) while using THF as the solvent and proceeding with the reaction at RT, but we detected the formation of side products via TLC analysis. Using such a large excess of Olah's reagent for deprotection also deemed the TBAF reaction superior. A crystal suitable for X-ray spectroscopy was grown from d_4-MeOH, and we confirmed the structure of **1e** by this method (Figure 18).

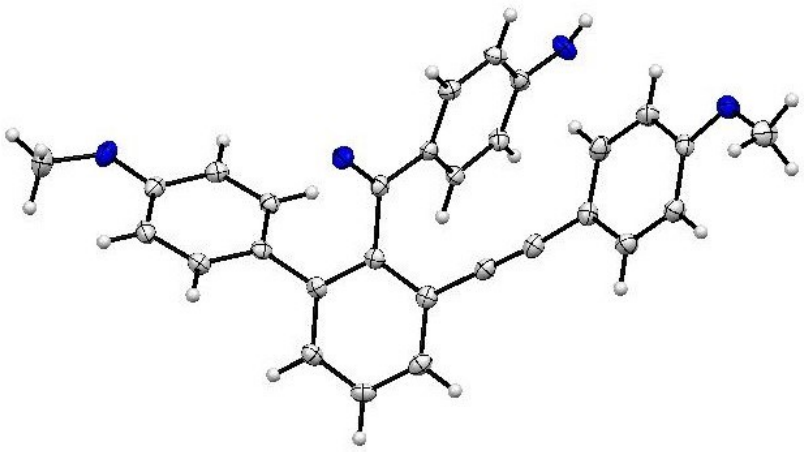


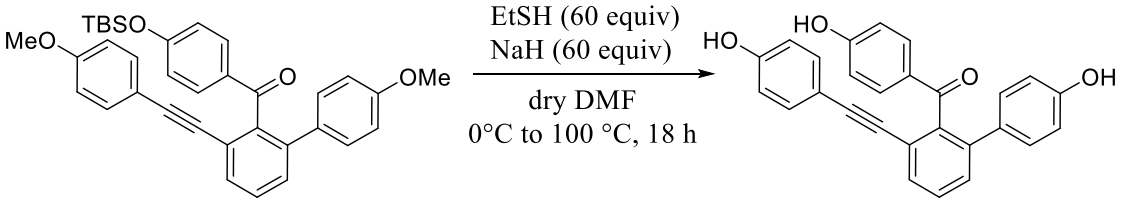
Figure 18 Structure of derivative **1e** determined by X-ray spectroscopy

In the next trial of deprotection, we attempted deprotection of **1e** of methoxy groups using BBr_3 (3.3 equiv) in dry DCM at RT for 1.5 hours which resulted in the detection of NMR ^1H peaks of the final product **1a** but in minority (Table 6, Entry 1). Next, we tried to decrease the amount of BBr_3 to (2.2 equiv) this reaction also produced **1a** in the minority, but many side products (Table 6, Entry 2) were formed. Therefore using BBr_3 was deemed unviable as many sideproducts are formed. Consequently, we have tried different approaches. Firstly we attempted to deprotect methoxy groups from **1e** via LiCl (6.7 equiv) in dry DMF in a microwave for 30 min at 160°C. No reaction occurred in this case (Table 6, Entry 3).

Table 6 Failed conditions for deprotection of **1e**

Entry	Solvent	Temperature	BBr_3 [equiv]	LiCl [equiv]	Reaction time	Yield of 1
1	Dry DCM	0 °C to RT	3.3	–	1.5 hours	Traces
2	Dry DCM	0 °C RT	2.2	–	1.5 hours	Traces
3	Dry DMF	160 °C	–	6.7	30 min	0 %

Next, we attempted to cleave off methoxy groups from **1d** with EtSNa which was generated at 0°C from EtSH (60 equiv) and NaH (60 equiv) for 1 hour in dry DMF. Then we added reactant **1d** and the reaction was left to proceed for 18 hours under reflux at 100 °C.[37] While using these conditions all protective groups were cleaved off and the desired final product **1a** was formed in 43 % yield (Scheme 10).



1d **1a**

Scheme 10 Deprotection of **1d** yielding **1a**

When we decreased the reaction time to 1 hour mixture of monomethoxyderivatives **1g** and **1h** (Figure 19) was formed in the ratio 100:6 in favor of derivative **1g** (determined from ^{1}H NMR). The structure of the major derivative **1g** was determined by HMBC analysis and HSQC analysis (Figure 19). We could observe the HMBC correlation between the carbon of the methoxy group with two protons of the aromatic ring. None of these protons correlated with the carbon signals of the triple bond. However, there was a correlation of a triple bond carbon to aromatic protons from different aromatic rings. This suggests, that alkyne moiety and methoxy moiety are attached to two different aromatic rings. This might be rationalized by a preferential nucleophilic attack of the thiolate at the electronically more deficient methoxy group, bearing relatively electronegative alkynyl moiety.

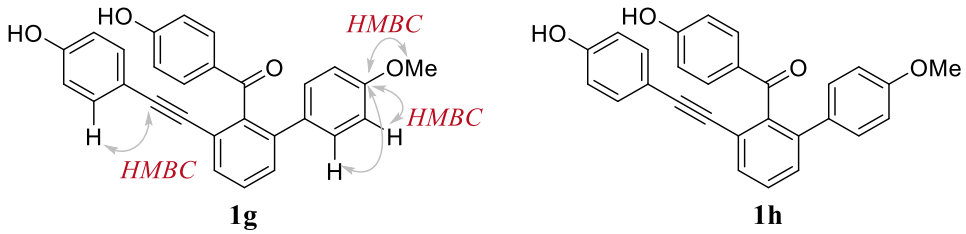
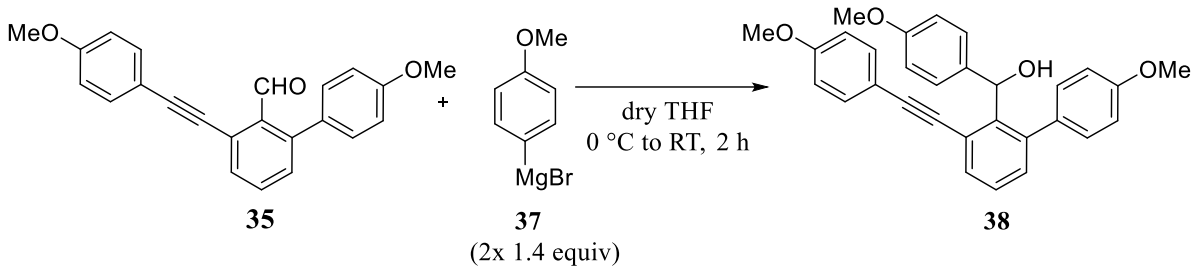


Figure 19 Derivative **1g** and coupling interactions detected via HMBC analysis and structure of derivative **1h**

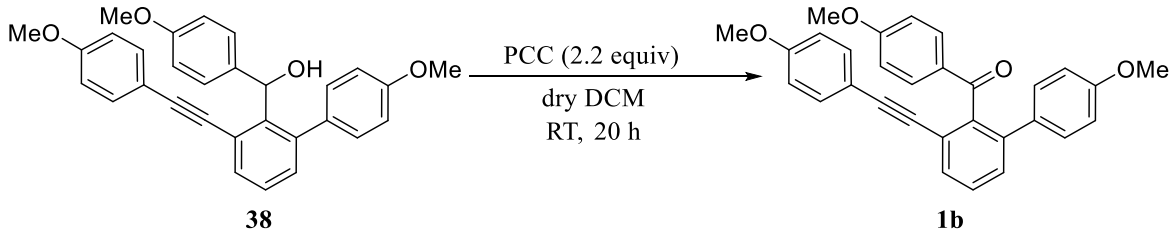
4.3 Synthesis of additional derivatives of selaginpulivllin X

We prepared fully methoxy-protected derivative **1b** from compound **35**. In the first step, **35** was converted to alcohol **38** using Grignard reagent **37** in two additions while the reaction mixture was stirred for 1 hour each time at RT (Scheme 11). The alcohol **38** was prepared in 58% yield.



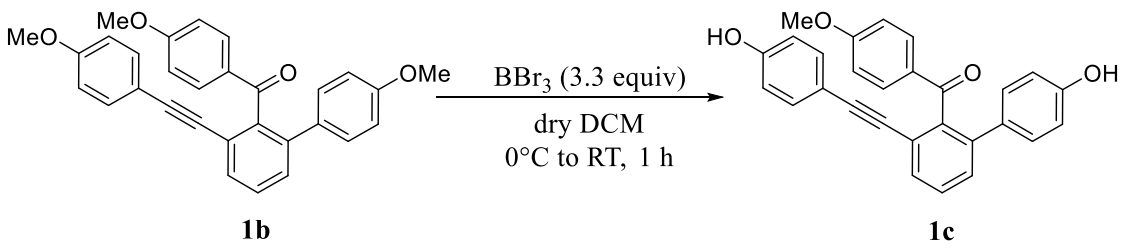
Scheme 11 Preparation of alcohol **38**

The alcohol **38** was then oxidized by its treatment with PCC (2.2 equiv). The reaction proceeded for 20 hours at RT. The desired derivative **1b** was prepared in quantitative yield (Scheme 12).



Scheme 12 Preparation of **1b**

To prepare other known derivatives of selaginpulvilin X (**1a**), we utilized a synthesis previously described by Wang's group.[36] This synthesis employs only OMe as a protective group and the OMe group at the aromatic ring C does not cleave using BBr_3. We were able to convert derivative **1b** to **1c** adding BBr_3 (3.3 equiv) dropwise at 0°C and leaving the reaction proceed for 1 hour at RT. We obtained derivative **1c** in 30% yield (Scheme 13).



Scheme 13 Preparation of **1c** derivative

4.4 Comparisons of analytical data of isolated Selaginpulvilin X (1a) and synthesized structure

In the case of ^{1}H spectra of the prepared compound **1a**, we detected all the reported signals of the isolated compound **1a** (Table 7).[6] We observed doublets with chemical shifts of 7.62, 7.10, 6.95, and 6.77 ppm. One doublet of doublet with a chemical shift of 7.39 ppm was present, and two multiplet areas with values of chemical shifts between 7.54 and 7.59 ppm and 6.66 ppm were observed.

Table 7 Values of ^{1}H chemical shifts for isolated and prepared selaginpulvilin X (**1a**)

^{1}H spectra of compound 1a in MeOH-d4 (600 MHz)	
Isolated [ppm] (intensity, multiplicity, J)	**Prepared [ppm] (intensity, multiplicity, J)**
7.61 (2H, d, J = 8.8 Hz)	7.62 (2H, d, J = 8.7 Hz)
7.52 – 7.50 (1H, m) 7.51 –7.48 (1H, m)	7.54 – 7.49 (2H, m)
7.38(1H, dd, J = 6.8, 2.1 Hz)	7.39 (1H, dd, J = 6.8, 2.2 Hz)
7.09 (2H, d, J = 8.6 Hz)	7.10 (2H, d, J = 8.7 Hz)
6.94 (2H, d, J = 8.5 Hz)	6.95 (2H, d, J = 8.7 Hz)
6.76 (2H, d, J = 8.8 Hz)	6.77 (2H, d, J = 8.7 Hz)
6.65 (2H, d, J = 8.5 Hz) 6.65 (2H, d, J = 8.6 Hz)	6.66 (4H, m)

In the case of ^{13}C spectra of the prepared compound **1a**, we were able to detect all the reported signals of isolated compound **1a** (Table 8).[6] Our measurement provided the spectra with a cleaner resolution compared to the original report, and therefore, we were able to differentiate a few carbons that were not differentiated in the previous work. For instance, we detected two signals, at 116.29 and 116.31 ppm, while in the original work only one signal at 116.3 ppm is reported for both carbons. Similarly, we could detect signal at 130.7, which was originally not reported by the authors, as it merged with other signal. In total, we detected 21 signals in the ^{13}C spectra which equals to the number of nonequivalent carbons present in selaginpulvilin X (**1a**).

Table 8 Values of ^{13}C chemical shifts for isolated and prepared selaginpulvilin X (**1a**)

^{13}C spectra of compound 1a in MeOH-d4 (400 MHz)	
Isolated [ppm]	**Prepared [ppm]**
199.1	199.1
164.3	164.2
159.3	159.3
158.2	158.2
142.6	142.7
141.7	141.7
133.9	133.9
133.7	133.7
132.3	132.3
131.2	131.2
130.9	130.9
Present but not reported	130.7
130.6	130.6
130.2	130.2
123.0	123.0
116.3	116.31
116.3	116.29
116.0	116.0
114.6	114.6
95.5	95.6
86.5	86.5

5. Experimental part

5.1 Used chemicals

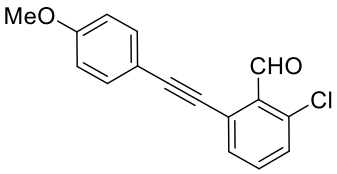
All used chemicals were bought from OrgChem, Sigma–Aldrich, Fluorochem, Acros Organics, PENTA Chemicals, Alfa Aesar, BLDpharm, Strem Chemicals, Tokyo Chemical Industry, and Lach:ner.

5.2 Used instruments

For TLC analysis a UV lamp with a wavelength of 254 nm and TLC plates with F254 bare silica from Silicycle were used. For preparative TLC glass backed TLC plates with 60A F254 silica gel from Silicycle were used. For column chromatography, silica gel 60A (0.040–0.063 mm) was used. All NMR spectra were measured on Bruker Avance III and Bruker Avance NEO 400 MHz spectrometers (400 MHz for ^{1}H and 100 MHz for ^{13}C) and Bruker Avance III 600 MHz spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C). MS were obtained on a VG-Analytical ZAB SEQ spectrometer. Melting points were measured on Kofler apparatus KB T300. IR spectra in the KBr mixture were measured on Thermo Scientific Nicolet AVATAR 370 FT-IR spectrometer.

5.3 Synthesis of compounds

5.3.1 2-chloro-6-((4-methoxyphenyl)ethynyl)benzaldehyde (34)



34

Aldehyde **33** (300 mg, 1.367 mmol, 1 equiv), CuI (13 mg, 0.068 mmol, 0.05 equiv) and $PdCl_2(PPh_3)_2$ (48 mg, 0.068 mmol, 0.05 equiv) were dissolved in Et_3N (10 ml) under an inert atmosphere. The reaction mixture was heated to 80° C. Alkyne **29** (194 mg, 190 µl 1.84 mmol, 1.03 equiv) was slowly added while stirring and the reaction mixture was left to react for 4 hours. After the reaction completion (TLC), the mixture was quenched with diluted HCl (10 ml, 2M), diluted with water (20 ml) and the mixture was extracted with DCM (3x30 ml). The organic phase was dried with $MgSO_4$ and purified with a chromatography column using 400 mL of EtOAC/hex (1:100) mixture, followed by 500 mL of EtOAC/hex (1:50) mixture as a mobile phase and silica gel as the stationary phase. The title compound was isolated as a pure yellow solid in 59% yield (220 mg).

m.p. (EtOAc): 54.1 °C

R_f (EtOAc/hex 1:10) = 0.26

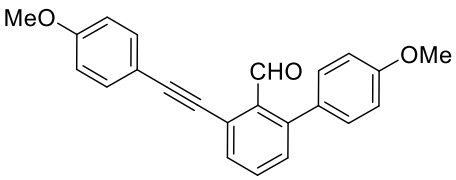
^{1}H NMR (400 MHz, $CDCl_3$) δ 10.68 (s, 1H), 7.57 – 7.49 (m, 3H), 7.45 – 7.37 (m, 2H), 6.90 (d, J = 8.8 Hz, 2H), 3.84 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 190.1, 160.5, 136.3, 133.6, 133.3, 132.8, 132.4, 130.6, 127.8, 114.5, 114.3, 97.5, 84.8, 55.5

HRMS (ESI) for $C_{16}H_{11}ClNaO_2$ calculated as 293.0340 and measured as 293.0340

IR (KBr) 2837, 2224, 2195, 1699, 1508, 1250, 1178, 1026, 791 cm^{-1}

5.3.2 4'-methoxy-3-((4-methoxyphenyl)ethynyl)-[1,1'-biphenyl]-2-carbaldehyde (35)



35

Compound **34** (100 mg, 0.370 mmol, 1 equiv), boronic acid **27** (153 mg, 1.007 mmol, 3 equiv), SPhos (15 mg, 0.037 mmol, 0.1 equiv), and $Pd(OAc)_2$ (4 mg, 0.017 mmol, 0.05 equiv) K_2CO_3 (159 mg, 1.150 mmol, 3 equiv) were dissolved in degassed H_2O/toluene 1:5 (8 ml) under an inert atmosphere. The mixture was stirred and heated to 90 °C under reflux for 5 hours. After the reaction completion (TLC) was the mixture diluted with water (20 ml)

and extracted with DCM (3x20 ml). The organic phase was then dried with $MgSO_4$ and purified with column chromatography using 1400 ml of EtOAc/hex mixture with a 1-6% gradient of EtOAc as a mobile phase and silica gel as a stationary phase. Compound **35** was prepared as an oily substance in 86% yield (109 mg).

R_f (EtOAc/hex 1:10) = 0.16

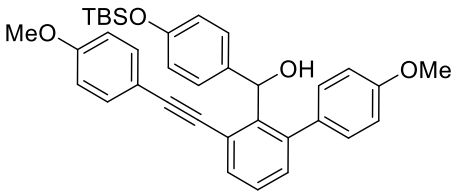
^{1}H NMR (400 MHz, $CDCl_3$) δ 10.22 (s, 1H), 7.66 – 7.62 (m, 1H), 7.59 (d, J = 8.9 Hz, 2H), 7.54 (at, J = 7.7 Hz, 1H), 7.34 (dd, J = 7.7, 1.3 Hz, 1H), 7.29 (d, J = 8.7 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.92 (d, J = 8.9 Hz, 2H), 3.89 (s, 3H), 3.86 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 192.2, 160.2, 159.7, 145.2, 134.6, 133.6, 132.7, 132.0 131.0, 130.9, 130.6, 124.6, 115.2, 114.2, 114.0, 95.3, 86.6, 55.5, 55.4

HRMS (ESI) for $C_{23}H_{18}NaO_3$ calculated as 365.1148 and measured as 365.1156

IR (KBr) 2835, 2200, 1695, 1512, 1248, 1176, 1030, 829 cm^{-1}

5.3.3 (4-((tert-butyldimethylsilyl)oxy)phenyl)(4'-methoxy-3-((4-methoxyphenyl)ethynyl)-[1,1'-biphenyl]-2-yl)methanol (36)



36

In a dry flask filled with an inert atmosphere bromide **32a** was dissolved (121 mg, 0.421 mmol, 1.5 equiv) in dry THF (2 ml). The flask was placed inside a dry ice/acetone bath and cooled to -78 °C. Then *t*-BuLi (590 µl, 1.00 mmol, 3.5 equiv) was slowly added and the mixture was stirred for 45 min. Then aldehyde **35** (98 mg, 0.286 mmol, 1 equiv) dissolved in dry THF (2 ml) was added. The reaction mixture was then left to warm up to RT and was left to react for 30 min. After the completion (TLC) was the reaction mixture quenched with a solution of saturated NH_4Cl (10 ml) and extracted with DCM (5x20 ml). The organic phase was dried with $MgSO_4$ and purified with column chromatography using 250 ml of hex/EtOAc (3%) mixture, followed by 600 ml of hex/EtOAc (5%) mixture and 500 ml of hex/EtOAc (7%) mixture as mobile phases and silica gel as stationary phase. Compound **36** was isolated as an amorphous yellow solid in 80% yield (126 mg).

R_f (EtOAc/hex 1:5) = 0.32

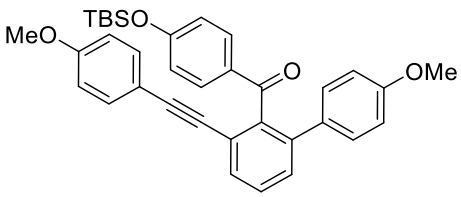
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (dd, J = 7.6, 1.5 Hz, 1H), 7.34 (at, J = 7.6 Hz, 1H), 7.29 – 7.25 (m, 1H), 7.17 (m, 4H), 7.09 (d, J = 8.8 Hz, 2H), 6.86 (d, J = 8.8 Hz, 2H), 6.78 (dd, J = 8.8, 2.1 Hz, 4H), 5.99 (d, J = 11.8 Hz, 1H), 3.81 (s, 3H), 3.79 (s, 3H), 0.97 (s, 9H), 0.19 – 0.05 (m, 6H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 160.0, 159.2, 154.6, 142.6, 142.4, 137.3, 133.0, 132.84, 132.82, 130.7, 130.5, 127.9, 127.3, 122.1, 119.7, 114.6, 114.1, 113.8, 96.3, 87.1, 72.6, 55.4, 25.8, 18.4, -4.26, -4.29

LRMS (ESI) for $C_{35}H_{38}NaO_4Si$ calculated as 573.2 and measured as 573.2

IR (KBr) 3541, 2954, 2929, 2202, 1510, 1250, 914, 833 cm^{-1}

5.3.4 (4-((tert-butyldimethylsilyl)oxy)phenyl)(4'-methoxy-3-((4-methoxyphenyl)ethynyl)-[1,1'-biphenyl]-2-yl)methanone (1d)



1d

PCC (99 mg, 0.459 mmol, 2 equiv) was dissolved in dry DCM (3 ml). Alcohol **36** (125 mg, 0.227 mmol, 1 equiv) dissolved in dry DCM (5 ml) was added and the reaction mixture was stirred for 18 hours. After the reaction was completed (TLC), the mixture was filtered through a silica plug using 200 ml of EtOAc/hexane (1:5) mixture as the mobile phase. Compound **1d** was prepared as a yellow amorphous paste in quantitative yield (125 mg).

R_f (EtOAc/hex 1:5) = 0.32

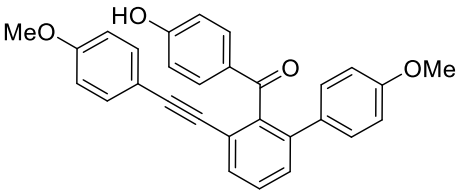
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, J = 8.6 Hz, 2H), 7.53 (dd, J = 7.7, 0.9 Hz, 1H), 7.45 (at, J = 7.7 Hz, 1H), 7.36 (dd, J = 7.7, 0.9 Hz, 1H), 7.21 (d, J = 8.7 Hz, 2H), 7.05 (d, J = 8.8 Hz, 2H), 6.87 – 6.65 (m, 6H), 3.77 (s, 3H), 3.74 (s, 3H), 0.95 (s, 9H), 0.18 (s, 6H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 196.6, 160.4, 159.8, 159.1, 141.7, 140.3, 133.0, 132.3, 132.1, 131.5, 130.4, 130.2, 129.8, 129.0, 121.8, 119.9, 115.1, 113.9, 113.8, 94.4, 86.4, 55.4, 55.3, 25.7, 18.3, -4.3

LRMS (ESI) for $C_{35}H_{36}NaO_4Si$ calculated as 571.2 and measured as 571.1

IR (KBr) 2954, 2929, 2204, 1666, 1595, 1510, 1254, 908, 833 cm^{-1}

5.3.5 (4-hydroxyphenyl)(4'-methoxy-3-((4-methoxyphenyl)ethynyl)-[1,1'-biphenyl]-2-yl)methanone (1e)



1e

Ketone **1d** (58 mg, 0.106 mmol, 1 equiv) was dissolved in a plastic flask in dry THF (5 ml). Then TBAF (130 µl, 0.130 mmol, 1.2 equiv) was added and the reaction mixture was stirred for 1 hour. After the reaction was completed (TLC), the reaction was quenched with a saturated solution of $NaHCO_3$ (4 ml) and acidified via a saturated solution of NH_4Cl (8 ml). The mixture was diluted with water (8 ml) and then extracted with DCM (3x20 ml). The organic phase was dried with $MgSO_4$ and purified with column chromatography using 250 ml of EtOAc/hex (1:3) mixture, followed by 150 ml of EtOAc/hex (1:1) mixture as mobile phase and silica gel as stationary phase. Compound **1e** appeared as a yellow amorphous solid and was obtained in an 87% yield (40 mg) with approximately 5% of unidentified impurity.

R_f (EtOAc/hex 1:1) = 0.54

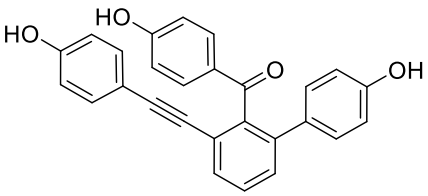
^{1}H NMR (400 MHz, MeOD) δ 7.63 (d, J = 8.8 Hz, 2H), 7.56 – 7.49 (m, 2H), 7.40 (dd, J = 7.1, 1.9 Hz, 1H), 7.20 (d, J = 8.7 Hz, 2H), 7.04 (d, J = 8.8 Hz, 2H), 6.83 – 6.74 (m, 6H), 3.75 (s, 3H), 3.72 (s, 3H)

^{13}C NMR (101 MHz, MeOD) δ 198.9, 164.4, 161.5, 160.7, 142.8, 141.5, 133.8, 133.7, 133.3, 131.2, 131.1, 130.8, 130.2, 122.9, 116.4, 116.1, 115.9, 115.0, 114.7, 95.2, 87.0, 55.75, 55.64

HRMS (ESI) for $C_{29}H_{22}NaO_4$ calculated as 457.1410 and measured as 457.1409

IR (KBr) 3410, 2528, 2202, 1647, 1595, 1512, 1250, 1030, 835 cm^{-1}

5.3.6 (4'-hydroxy-3-((4-hydroxyphenyl)ethynyl)-[1,1'-biphenyl]-2-yl)(4-hydroxyphenyl)methanone (1a)



1a

NaH (109 mg, 2.73 mmol, 60 equiv) was dissolved in dry DMF (5 ml) under an inert atmosphere in a dry flask. The reaction mixture was cooled to 0 °C via ice bath. Then EtSH (210 µl, 2.75 mmol, 60 equiv) was dropwise added. The mixture was then left to stir at 0 °C for 1 hour. Then ketone **1d** (25 mg, 0.0456 mmol, 1 equiv) dissolved in dry DMF was added and the mixture

was refluxed at 100 °C for 18 hours. After the reaction was completed (TLC), the reaction mixture was quenched using a saturated solution of NH_4Cl (6 ml), diluted with water (24 ml), and extracted with EtOAc (3x30 ml). Joined organic phases were washed with an aqueous solution of 5% LiCl (3x100 ml). The organic phase was dried with $MgSO_4$ and purified with column chromatography using 125 ml of EtOAc/hex (1:4) mixture, followed by 225 ml of EtOAc/hex (1:2) mixture and 150 ml of EtOAc/hex (1:1.6) mixture as mobile phases and silica gel as stationary phase. Compound **1a** in the appearance of a yellow oil was prepared in 43% yield (8 mg).

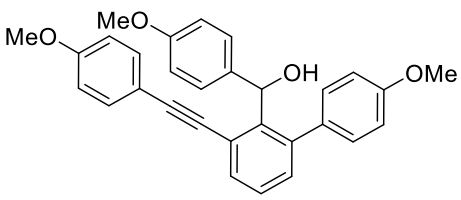
R_f (EtOAc/hex 2:1) = 0.52

^{1}H NMR (600 MHz, CDCl3) δ 7.62 (d, J = 8.7 Hz, 2H), 7.54 – 7.49 (m, 2H), 7.39 (dd, J = 6.8, 2.2 Hz, 1H), 7.10 (d, J = 8.7 Hz, 2H), 6.95 (d, J = 8.7 Hz, 2H), 6.77 (d, J = 8.7 Hz, 2H), 6.66 (m, 4H)

^{13}C NMR (151 MHz, $CDCl_3$) δ 199.1, 164.2, 159.3, 158.2, 142.7, 141.7, 133.9, 133.7, 132.3, 131.2, 130.9, 130.7, 130.6, 130.2, 123.0, 116.31, 116.29, 116.0, 114.6, 95.6, 86.5

The measured values correspond with those in the literature.[6]

5.3.7 (4'-methoxy-3-((4-methoxyphenyl)ethynyl)-[1,1'-biphenyl]-2-yl)(4-methoxyphenyl)methanol (38)



38

In a dry flask filled with an inert atmosphere crude aldehyde **35** was dissolved (251 mg, 0.733 mmol, 1 equiv) in dry THF (10 ml). The flask was placed inside an ice bath and cooled to 0 °C. Then 4-methoxyphenylmagnesium bromide (1.00 ml, 1.00 mmol, 1.4 equiv) was added dropwise. The reaction mixture was then left to warm up to RT and was left to react for 1 hour. Then the reaction mixture was again cooled to 0 ° C, 4-methoxyphenylmagnesium bromide (1.00 ml, 1.00 mmol, 1.4 equiv) was added and the mixture was warmed to RT and left to react for 1 hour. After the reaction was completed (TLC), the reaction mixture was quenched with a solution of saturated NH_4Cl (10 ml), diluted with water (10 ml), and extracted with EtOAc (3x20 ml). The organic phase was dried with $MgSO_4$ and purified with column chromatography using 500 ml of hex/EtOAc (5%), 900 ml of hex/EtOAc (7%), 500 ml of hex/EtOAc (10%), 500 ml of hex/EtOAc (12%) and 500 ml

of hex/EtOAc (14%) mixtures as mobile phases and silica gel as stationary phase. Compound **38** in the appearance of an amorphous yellow solid was prepared in 58% yield. (192 mg)

R_f (EtOAc/hex 1:5) =0.23

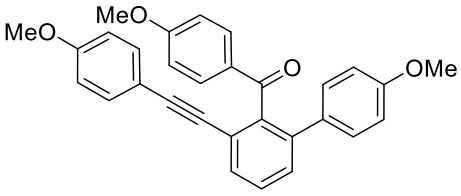
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (dd, J = 7.5, 1.5 Hz, 1H), 7.34 (at, J = 7.6 Hz, 1H), 7.29 –7.17 (m, 5H), 7.08 (d, J = 8.8 Hz, 2H), 6.86 (m, 4H), 6.79 (d, J = 8.8 Hz, 2H), 6.00 (d, J = 11.2 Hz, 1H), 3.81 (s, 3H), 3.79 (m, 6H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 160.0, 159.2, 158.6, 142.6, 142.4, 136.8, 132.97, 132.92 132.8, 130.7, 130.5, 127.9, 127.4, 122.0, 114.6, 114.1, 113.8, 113.5, 96.4, 87.1, 72.5, 55.4 (3C)

HRMS (ESI) for $C_{30}H_{26}NaO_4$ calculated as 473.1723 and measured as 473.1723

IR (KBr) 3543, 2835, 2202, 1606, 1510, 1248, 1174, 1032, 833 cm^{-1}

5.3.8 (4'-methoxy-3-((4-methoxyphenyl)ethynyl)-[1,1'-biphenyl]-2-yl)(4-methoxyphenyl)methanone (1b)



1b

PCC (107 mg, 0.496 mmol, 2.2 equiv) and alcohol **38** (100 mg, 0.222 mmol, 1 equiv) were dissolved in dry DCM (8 ml) and the reaction mixture was stirred for 20 hours. After the reaction completion (TLC), the mixture was filtered through a silica plug using 100 ml of EtOAc/hex (1:10) mixture, followed by 100 ml of EtOAc/hex (1:3) mixture and 200 ml of EtOAc/hex (1:1) mixture as mobile phases. Compound **1b** in the appearance of a yellow amorphous solid was prepared in quantitative yield (100 mg).

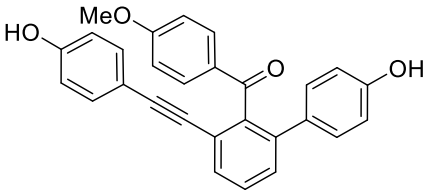
R_f (EtOAc/hex 1:5) = 0.16

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 8.9 Hz, 2H), 7.54 (dd, J = 7.6, 1.3 Hz, 1H), 7.45 (at, J = 7.6 Hz, 1H), 7.36 (dd, J = 7.6, 1.3 Hz, 1H), 7.23 (d, J = 8.7 Hz, 2H), 7.06 (d, J = 8.9z Hz, 2H), 6.83 (d, J = 8.9 Hz, 2H), 6.75 (m, 4H), 3.81 (s, 3H), 3.76 (s, 3H), 3.74 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 196.5, 163.6, 159.8, 159.1, 141.7, 140.1, 133.1, 132.3, 132.2, 131.0, 130.4, 130.2, 129.8, 128.9, 121.8, 115.1, 113.9, 113.8, 113.8, 94.4, 86.4, 55.5, 55.4, 55.3

The measured values correspond with those in the literature.[36]

5.3.9 (4'-hydroxy-3-((4-hydroxyphenyl)ethynyl)-[1,1'-biphenyl]-2-yl)(4-methoxyphenyl)methanone (1c)



1c

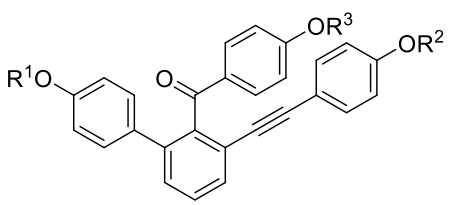
Ketone **1b** (60 mg, 0.134 mmol, 1 equiv) was dissolved in dry DCM (5 ml) under an inert atmosphere in a dry flask. The reaction mixture was cooled to 0° via ice bath and BBr_3 (440 µl, 0.440, mmol, 3.3 equiv) was added dropwise. The reaction mixture was then warmed to RT and stirred for 1 hour. After the reaction completion (TLC), the reaction mixture was quenched with a saturated solution of NH_4Cl (10 ml), diluted with water (20 ml) and extracted with EtOAc (3x20 ml). The organic phase was dried with $MgSO_4$ and then purified via preparative TLC using ETOAc/hex 1:2 and 1:1 mixtures as mobile phases and silica gel equipped preparative TLC plates. Compound **1c** in the appearance of a yellow amorphous solid was prepared in 30% yield (17 mg).

R_f (EtOAc/hex 2:1) = 0.60

^{1}H NMR (400 MHz, MeOD) δ 7.71 (d, J = 8.8 Hz, 2H), 7.50 – 7.42 (m, 3H), 7.33 – 7.27 (m, 2H), 7.04 (d, J = 8.6 Hz, 2H), 6.71 (m, 4H), 6.57 (d, J = 8.6 Hz, 2H), 4.22 (s, 2H), 3.73 (s, 3H).
^{13}C NMR (101 MHz, MeOD) δ 200.6, 198.0, 165.3, 163.8, 157.9, 141.7, 140.2, 134.5, 133.4, 133.1, 132.0, 131.7, 131.4, 130.9, 130.2, 129.7, 129.5, 116.1, 115.9, 114.5, 55.9, 43.2
The measured values correspond with those in the literature.[36]

6. Conclusion

We have successfully prepared selaginpulvilin X (**1a**), marking the first known synthesis of this natural compound. Initially, we synthesized the carbon skeleton **1d** with a TBS protective group at the phenol position of aromatic ring C, a departure from Wang's approach[36] which only deployed methoxy protective groups. Deprotection of **1e** using BBr_3 was ineffective as **1a** was formed in the minority. However, we have successfully cleaved off all protective groups in one step by employing in situ generated EtSNa, thereby converting **1d** to **1a**. Our analysis confirmed the proposed structure of selaginpulvilin X (**1a**) was correctly determined. Additionally, we prepared two pure methoxy derivatives **1b** and **1c** which had been previously reported by Wang. [36] Furthermore, access to a new derivative **1e**, was developed during deprotection trials of **1a**. These compounds (Figure 20) might serve as a good starting point for further investigation of the biological activity and should shine a light onto a basic structure-activity relationship.



1a-e

a: R^1=R^2=R^3=H
b: R^1=R^2=R^3=Me
c: R^1=R^2=H, R^3=Me
d: R^1=R^2=Me, R^3=TBS
e: R^1=R^2=Me, R^3=H

Figure 20 Structures of prepared compounds **1a-1e**

7. Acknowledgements

I am grateful to my supervisor Dr. Lukáš Rýček, M.Sc. for his exceptional guidance, support, and immense patience during my research. I sincerely thank my lab assistant Sundaravelu Nallappan, Ph. D. for his help and support in the lab. I would also like to thank our lab members Bc. Dominik Kunák and Miguel Mateus, M.Sc. for their support. Lastly, I would like to thank my family for their emotional support.

8. Literature

(1) Rýček, L. Total Synthesis of Natural Products: Case Studies in the Evaluation of New Synthetic Methods, Structural Elucidation and Drug Discovery. *Chem. Listy* **2022**, *116* (4), 204–214. https://doi.org/10.54779/chl20220204.

(2) Li, W.; Tang, G.-H.; Yin, S. Selaginellins from the Genus *Selaginella* : Isolation, Structure, Biological Activity, and Synthesis. *Nat. Prod. Rep.* **2021**, *38* (4), 822–842. https://doi.org/10.1039/D0NP00065E.

(3) Rycek, L.; Mateus, M.; Beytlerová, N.; Kotora, M. Catalytic Cyclotrimerization Pathway for Synthesis of Selaginpulvilins C and D: Scope and Limitations. *Org. Lett.* **2021**, *23* (12), 4511–4515. https://doi.org/10.1021/acs.orglett.1c00519.

(4) Lapinskaite, R.; Malatinec, Š.; Mateus, M.; Rycek, L. Cross-Coupling as a Key Step in the Synthesis and Structure Revision of the Natural Products Selagibenzophenones A and B. *Catalysts* **2021**, *11* (6), 708. https://doi.org/10.3390/catal11060708.

(5) Kunák, D.; Mateus, M.; Rycek, L. Synthesis and Structure Confirmation of Selagibenzophenone C. *European J. Org. Chem.* **2022**, *2022* (11), e202200014. https://doi.org/10.1002/ejoc.202200014.

(6) Zhu, Q.-F.; Luo, T.-T.; Chen, Q.; Gao, B.-B.; Zeng, A.-F.; Ao, J.-L.; Xu, G.-B.; Liao, S.-G.; He, X. Three New Selaginellin Derivatives from Selaginella Pulvinata and Their α-Glucosidase Inhibitory Activity. *Chem. Biodiver.* **2023**, *20* (4), e202300109. https://doi.org/10.1002/cbdv.202300109.

(7) Thomas, B. A Reinvestigation of Selaginella Species from the Asturian (Westphalian D) of the Zwickau Coalfield, Germany and Their Assignment to the New Sub-Genus Hexaphyllum. *Z. Dtsch. Ges. Geowiss.* **2005**, *156*, 403–414. https://doi.org/10.1127/1860-1804/2005/0156-0403.

(8) Banks, J. A. Selaginella and 400 Million Years of Separation. *Annu. Rev. Plant Biol.* **2009**, *60* (1), 223–238. https://doi.org/10.1146/annurev.arplant.59.032607.092851.

(9) Bailly, C. The Traditional and Modern Uses of *Selaginella Tamariscina* (P.Beauv.) Spring, in Medicine and Cosmetic: Applications and Bioactive Ingredients. *J. Ethnopharmacol.* **2021**, *280*, 114444. https://doi.org/10.1016/j.jep.2021.114444.

(10) Weng, J.-K.; Noel, J. Chemodiversity in Selaginella: A Reference System for Parallel and Convergent Metabolic Evolution in Terrestrial Plants. *Front. Plant Sci.* **2013**, *4*. https://doi.org/10.3389/fpls.2013.00119.

(11) Banks, J. A.; Nishiyama, T.; Hasebe, M.; Bowman, J. L.; Gribskov, M.; dePamphilis, C.; Albert, V. A.; Aono, N.; Aoyama, T.; Ambrose, B. A.; Ashton, N. W.; Axtell, M. J.; Barker, E.; Barker, M. S.; Bennetzen, J. L.; Bonawitz, N. D.; Chapple, C.; Cheng, C.; Correa, L. G. G.; Dacre, M.; DeBarry, J.; Dreyer, I.; Elias, M.; Engstrom, E. M.; Estelle, M.; Feng, L.; Finet, C.; Floyd, S. K.; Frommer, W. B.; Fujita, T.; Gramzow, L.; Gutensohn, M.; Harholt, J.; Hattori, M.; Heyl, A.; Hirai, T.; Hiwatashi, Y.; Ishikawa, M.; Iwata, M.; Karol, K. G.; Koehler, B.; Kolukisaoglu, U.; Kubo, M.; Kurata, T.; Lalonde, S.; Li, K.; Li, Y.; Litt, A.; Lyons, E.; Manning, G.; Maruyama, T.; Michael, T. P.; Mikami, K.; Miyazaki, S.; Morinaga, S.; Murata, T.; Mueller-Roeber, B.; Nelson, D. R.; Obara, M.; Oguri, Y.; Olmstead, R. G.; Onodera, N.; Petersen, B. L.; Pils, B.; Prigge, M.; Rensing, S. A.; Riaño-Pachón, D. M.; Roberts, A. W.; Sato, Y.; Scheller, H. V.; Schulz, B.; Schulz, C.; Shakirov, E. V.; Shibagaki, N.; Shinohara, N.; Shippen, D. E.; Sørensen, I.; Sotooka, R.; Sugimoto, N.; Sugita, M.; Sumikawa, N.; Tanurdzic, M.; Theissen, G.; Ulvskov, P.; Wakazuki, S.; Weng, J.-K.; Willats, W. W. G. T.; Wipf, D.; Wolf, P. G.; Yang, L.; Zimmer, A. D.; Zhu, Q.; Mitros, T.; Hellsten, U.; Loqué, D.; Otillar, R.; Salamov, A.; Schmutz, J.; Shapiro, H.; Lindquist, E.; Lucas, S.; Rokhsar, D.; Grigoriev, I. V. The Selaginella Genome Identifies

Genetic Changes Associated with the Evolution of Vascular Plants. *Science* **2011**, *332* (6032), 960–963. https://doi.org/10.1126/science.1203810.
(12) Křížkovská, B.; Kumar, R.; Kateřinařehořová; Sýkora, D.; Dobiasová, S.; Kučerová, D.; Tan, M.; Linis, V.; Oyong, G.; Ruml, T.; Lipov, J.; Viktorová, J. Comparison of Chemical Composition and Biological Activities of Eight Selaginella Species. *Pharmaceuticals* **2020**, *14*. https://doi.org/10.3390/ph14010016.
(13) Zhang, L.-P.; Liang, Y.-M.; Wei, X.-C.; Cheng, D.-L. A New Unusual Natural Pigment from Selaginella Sinensis and Its Noticeable Physicochemical Properties. *J. Org. Chem.* **2007**, *72* (10), 3921–3924. https://doi.org/10.1021/jo0701177.
(14) *Selaginella pulvinata (Hook. & Grev.) Maxim. | Plants of the World Online | Kew Science*. https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:77186579-1 (accessed 2024-04-29).
(15) Le, D. D.; Nguyen, D. H.; Zhao, B. T.; Seong, S. H.; Choi, J. S.; Kim, S. K.; Kim, J. A.; Min, B. S.; Woo, M. H. PTP1B Inhibitors from Selaginella Tamariscina (Beauv.) Spring and Their Kinetic Properties and Molecular Docking Simulation. *Bioorg. Chem.* **2017**, *72*, 273–281. https://doi.org/10.1016/j.bioorg.2017.05.001.
(16) Liu, R.; Zou, H.; Zou, Z.-X.; Cheng, F.; Yu, X.; Xu, P.-S.; Li, X.-M.; Li, D.; Xu, K.-P.; Tan, G.-S. Two New Anthraquinone Derivatives and One New Triarylbenzophenone Analog from Selaginella Tamariscina. *Natural Product Research* **2020**, *34* (19), 2709–2714. https://doi.org/10.1080/14786419.2018.1452008.
(17) Wang, C.-G.; Yao, W.-N.; Zhang, B.; Hua, J.; Liang, D.; Wang, H.-S. Lung Cancer and Matrix Metalloproteinases Inhibitors of Polyphenols from *Selaginella Tamariscina* with Suppression Activity of Migration. *Bioorg. Med. Chem. Lett.* **2018**, *28* (14), 2413–2417. https://doi.org/10.1016/j.bmcl.2018.06.024.
(18) Liu, X.; Tang, G.-H.; Weng, H.-Z.; Zhang, J.-S.; Xu, Y.-K.; Yin, S. A New Selaginellin Derivative and a New Triarylbenzophenone Analog from the Whole Plant of *Selaginella Pulvinata*. *J. Asian Nat. Prod. Res.* **2018**, *20* (12), 1123–1128. https://doi.org/10.1080/10286020.2017.1378646.
(19) Cheng, X.-L.; Ma, S.-C.; Yu, J.-D.; Yang, S.-Y.; Xiao, X.-Y.; Hu, J.-Y.; Lu, Y.; Shaw, P.-C.; But, P. P.-H.; Lin, R.-C. Selaginellin A and B, Two Novel Natural Pigments Isolated from Selaginella Tamariscina. *Chem. Pharm. Bull.* **2008**, *56* (7), 982–984. https://doi.org/10.1248/cpb.56.982.
(20) Cao, Y.; Zhao, M.; Zhu, Y.; Zhu, Z.-H.; Oberer, L.; Duan, J.-A. Diselaginellin B, an Unusual Dimeric Molecule from Selaginella Pulvinata, Inhibited Metastasis and Induced Apoptosis of SMMC-7721 Human Hepatocellular Carcinoma Cells. *J. Nat. Prod.* **2017**, *80* (12), 3151–3158. https://doi.org/10.1021/acs.jnatprod.7b00404.
(21) Woo, S.; Kang, K. B.; Kim, J.; Sung, S. H. Molecular Networking Reveals the Chemical Diversity of Selaginellin Derivatives, Natural Phosphodiesterase-4 Inhibitors from Selaginella Tamariscina. *J. Nat. Prod.* **2019**, *82* (7), 1820–1830. https://doi.org/10.1021/acs.jnatprod.9b00049.
(22) Liu, X.; Luo, H.-B.; Huang, Y.-Y.; Bao, J.-M.; Tang, G.-H.; Chen, Y.-Y.; Wang, J.; Yin, S. Selaginpulvilins A–D, New Phosphodiesterase-4 Inhibitors with an Unprecedented Skeleton from Selaginella Pulvinata. *Org. Lett.* **2014**, *16* (1), 282–285. https://doi.org/10.1021/ol403282f.
(23) Zhu, Q.-F.; Shao, L.-D.; Wu, X.-D.; Liu, J.-X.; Zhao, Q.-S. Isolation, Structural Assignment of Isoselagintamarlin A from Selaginella Tamariscina and Its Biomimetic Synthesis. *Nat. Products Bioprospect.* **2019**, *9* (1), 69–74. https://doi.org/10.1007/s13659-018-0195-5.
(24) Xu, K.-P.; Zou, H.; Li, F.-S.; Xiang, H.-L.; Zou, Z.-X.; Long, H.-P.; Li, J.; Luo, Y.-J.; Li, Y.-J.; Tan, G.-S. Two New Selaginellin Derivatives from *Selaginella Tamariscina*

(Beauv.) Spring. *J. Asian Nat. Prod. Res.* **2011**, *13* (4), 356–360. https://doi.org/10.1080/10286020.2011.558840.
(25) Cao, Y.; Chen, J.-J.; Tan, N.-H.; Wu, Y.-P.; Yang, J.; Wang, Q. Structure Determination of Selaginellins G and H from Selaginella Pulvinata by NMR Spectroscopy. *Magn. Reson. Chem.* **2010**, *48* (8), 656–659. https://doi.org/10.1002/mrc.2623.
(26) Chen, W.; Peng, Y.; Huang, W.; Zhou, L.; Quan, X.; Zhao, Q.; Zhang, D.; Sheng, X.; Luo, Y.; Zou, H. A New Diarylbenzophenone from Selaginella Tamariscina. *Rec. Nat. Prod.* **2020**, *14* (6), 421–426. https://doi.org/10.25135/rnp.182.20.03.1586.
(27) Sengupta, S.; Mehta, G. Natural Products as Modulators of the Cyclic-AMP Pathway: Evaluation and Synthesis of Lead Compounds. *Org. Biomol. Chem.* **2018**, *16* (35), 6372–6390. https://doi.org/10.1039/C8OB01388H.
(28) Huang, Y.; Liu, X.; Wu, D.; Tang, G.; Lai, Z.; Zheng, X.; Yin, S.; Luo, H.-B. The Discovery, Complex Crystal Structure, and Recognition Mechanism of a Novel Natural PDE4 Inhibitor from Selaginella Pulvinata. *Biochem. Pharmacol.* **2017**, *130*, 51–59. https://doi.org/10.1016/j.bcp.2017.01.016.
(29) Combs, A. P. Recent Advances in the Discovery of Competitive Protein Tyrosine Phosphatase 1B Inhibitors for the Treatment of Diabetes, Obesity, and Cancer. *J. Med. Chem.* **2010**, *53* (6), 2333–2344. https://doi.org/10.1021/jm901090b.
(30) Nguyen, P.-H.; Zhao, B.-T.; Ali, M. Y.; Choi, J.-S.; Rhyu, D.-Y.; Min, B.-S.; Woo, M.-H. Insulin-Mimetic Selaginellins from Selaginella Tamariscina with Protein Tyrosine Phosphatase 1B (PTP1B) Inhibitory Activity. *J. Nat. Prod.* **2015**, *78* (1), 34–42. https://doi.org/10.1021/np5005856.
(31) Yao, W.-N.; Huang, R.-Z.; Hua, J.; Zhang, B.; Wang, C.-G.; Liang, D.; Wang, H.-S. Selagintamarlin A: A Selaginellin Analogue Possessing a 1 *H* -2-Benzopyran Core from *Selaginella Tamariscina*. *ACS Omega* **2017**, *2* (5), 2178–2183. https://doi.org/10.1021/acsomega.7b00209.
(32) Lapinskaite, R.; Atalay, H. N.; Malatinec, Š.; Donmez, S.; Cinar, Z. O.; Schwarz, P. F.; Perhal, A. F.; Císařová, I.; Labanauskas, L.; Karpiński, T. M.; Dirsch, V. M.; Tumer, T. B.; Rycek, L. Synthesis of Selagibenzophenone A and Its Derivatives for Evaluation of Their Antiproliferative, RORγ Inverse Agonistic, and Antimicrobial Effect**. *ChemistrySelect* **2023**, *8* (7), e202204816. https://doi.org/10.1002/slct.202204816.
(33) Cao, Y.; Chen, J.-J.; Tan, N.-H.; Oberer, L.; Wagner, T.; Wu, Y.-P.; Zeng, G.-Z.; Yan, H.; Wang, Q. Antimicrobial Selaginellin Derivatives from *Selaginella Pulvinata*. *Bioorg. Med. Chem. Lett.* **2010**, *20* (8), 2456–2460. https://doi.org/10.1016/j.bmcl.2010.03.016.
(34) Cao, Y.; Yao, Y.; Huang, X.-J.; Oberer, L.; Wagner, T.; Guo, J.-M.; Gu, W.; Liu, W.-D.; Lv, G.-X.; Shen, Y.-N.; Duan, J.-A. Four New Selaginellin Derivatives from *Selaginella Pulvinata*: Mechanism of Racemization Process in Selaginellins with Quinone Methide. *Tetrahedron* **2015**, *71* (10), 1581–1587. https://doi.org/10.1016/j.tet.2015.01.017.
(35) Zhu, B.; Wang, T.-B.; Hou, L.-J.; Lv, H.-X.; Liu, A.-M.; Zeng, P.; Li, A.-H. A New Selaginellin from Selaginella Moellendorffii Inhibits Hepatitis B Virus Gene Expression and Replication. *Chem. Nat. Compd.* **2016**, *52* (4), 624–627. https://doi.org/10.1007/s10600-016-1725-1.
(36) Wang, S.; Liu, L.; Guo, X.; Li, G.; Wang, X.; Dong, H.; Li, Y.; Zhao, W. Synthesis of Novel Natural Product-like Diaryl Acetylenes as Hypoxia Inducible Factor-1 Inhibitors and Antiproliferative Agents. *RSC Adv.* **2019**, *9* (24), 13878–13886. https://doi.org/10.1039/C9RA02525A.
(37) Jan, N.-W.; Liu, H.-J.; Hsieh, M.-T.; Shia, K.-S. Total Synthesis of (–)-Isopisiferin: Confirmation of Absolute Configuration. *European J. Org. Chem.* **2010**, *2010* (22), 4271–4275. https://doi.org/10.1002/ejoc.201000449.