

A new approach towards the synthesis of Transtaganolides/Basiliolides

C-19 Terpenolides from Thapsia with SERCA-inhibiting Activity

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Chemical Engineering

Center of Synthesis and Analysis

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What we do in life, echoes in eternity...

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Abbreviations

ADP	–	Adenosine DiPhosphate
ATP	–	Adenosine TriPhosphate
CADD	–	Computer Aided Drug Design
DBTG	–	DeButanoylThapsiGargin
DHPR	–	Dihydropyridine Receptor
ER	–	Endoplasmatic Reticulum
IMPDA	–	IntraMolecular Pyrone Diels-Alder
P _i	–	Phosphate
PKA	–	Protein Kinase A
PLN	–	Phospholamban
PSA	–	Prostate-Specific Antigen
RyR	–	Ryanodine Receptor
SERCA	–	Sarco/Endoplasmatic Reticulum Ca ²⁺ -ATPase
SLN	–	Sarcolipin
ThGa	–	Thapsia Garganica L.
ThTr	–	Thapsia Transtagana Brot
ThVi	–	Thapsia Villosa var. Villosa L.
TG	–	TapsiGargin
TBDMS	–	TertButylDimethylSilyl protecting group
TMS	–	TriMethylSilyl protecting group
T/B	–	Transtaganolides/Basiliolides

Abstract

The Transtaganolides/Basiliolides (T/B) are C-19 terpenolides found in the plants *Thapsia Garganica* L., *Thapsia Transtagana* Brot and *Thapsia Villosa* var. *Villosa* L. from the Umbelliferae family. Reports suggest that these compounds have reversible SERCA-inhibiting properties and therefore are of great interest for the treatment of neurodegenerative disorders, such as Alzheimer's or Parkinson's disease.

The ground structure of the T/B is tetracyclic and has 6 contiguous stereocenters, suggesting a difficult and very challenging synthesis. This of course attracted synthetic chemists who saw a great challenge in finding a synthetic path for the total synthesis of these C-19 terpenolides. Several groups from around the world have been working towards the total synthesis, and most of them used an Ireland-Claisen rearrangement/intramolecular Diels-Alder reaction sequence as a key step. An intermediate with all carbon-carbon bonds from the T/B carbon skeleton has been obtained so far, however the total synthesis is yet to be completed as the introduction of a methyl ester needed for the last step proved very hard.²⁸

A new approach for synthesizing a key intermediate for the total synthesis of Transtaganolides/Basiliolides has been attempted in order to reduce the amount of steps. This reaction path includes an alkylation (addition of a terminal alkyne to an aldehyde), followed by a Tandem reaction (Stille like coupling followed by a cyclization reaction to get the important 2-pyranonone derivative).

The starting material for the alkylation reaction was made by TBDMS protection of 3-butyn-1-ol and the addition to the ethyl glyoxalate was catalyzed by the use of a rhodium-phosphine complex. This alkylation returned a secondary alcohol which was activated by mesylation. The last step (the Tandem reaction) is yet to be performed as time was insufficient.

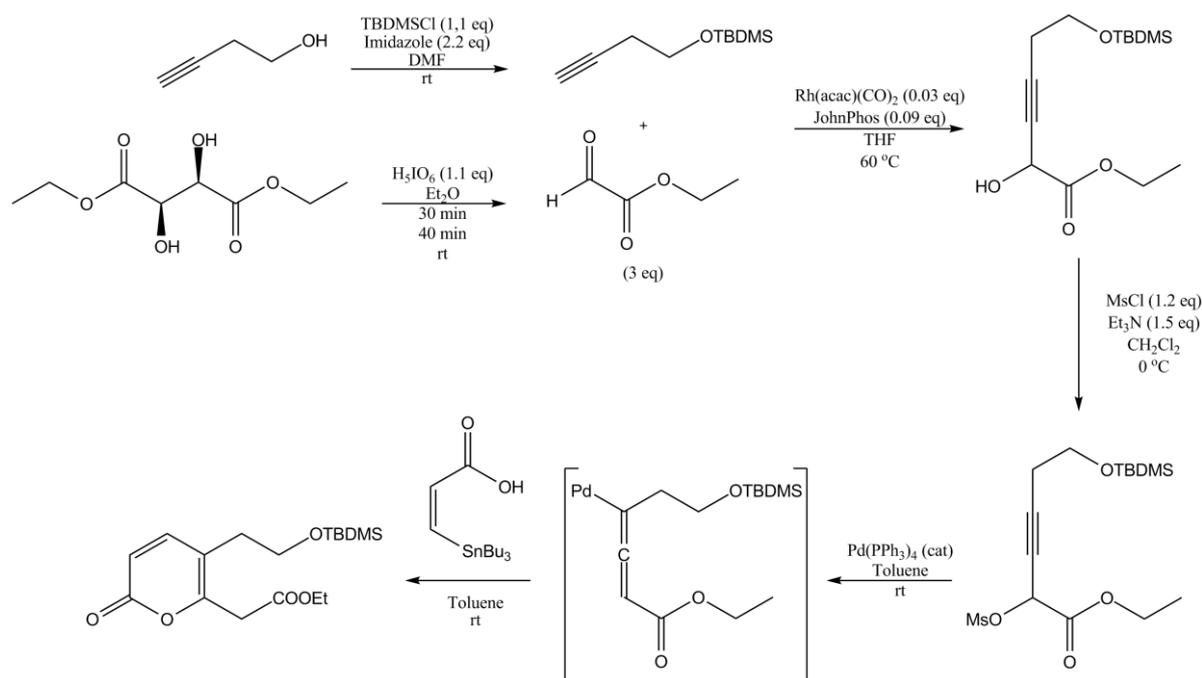


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1. Introduction

Nature has always been a source of inspiration for the human. From beautiful sights to dangerous creatures nature offers a great diversity of interesting things and phenomena we want to learn about and explore. One can easily state that nature, together with the aspiration in life and development, is the driving force of science. What we see in nature is what gives us the questions.

With this in mind it is interesting to mention that chemistry can explain very much of what happens in nature as life itself basically is chemistry in its purest form. In other words, chemistry, as all other sciences, derives from nature. The human has today managed to learn about chemistry in such a way that it can be used to mimic nature and produce compounds found in nature or even synthesize new compounds with unique properties.

This thesis will focus on the concept of natural product synthesis. This means that a substance found in nature (e.g. isolated from a plant by extraction) first is analysed and identified and then is synthesised in a chemical laboratory. Retrosynthesis is used as a tool to get a route for the total synthesis of the targeted substance and also, if it is of interest, modification of the substance (based on e.g. QSAR) is performed to obtain analogues with the exact properties needed.

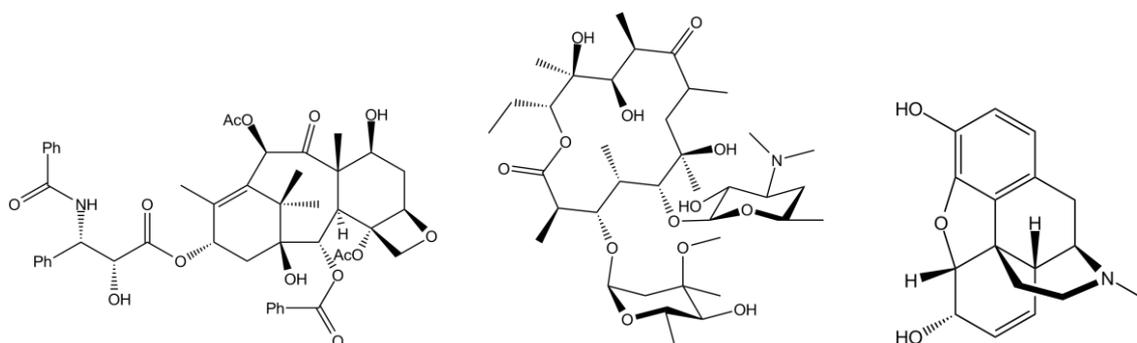


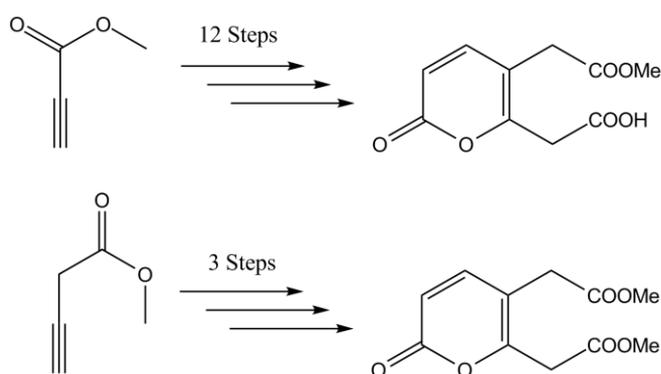
Figure 1. From left to right; Structures of the natural compounds paclitaxel, erythromycin and morphine

Even after the introduction of combinatorial chemistry in which artificial molecules with the exact properties needed are easily made by synthesis following the use of CADD (Computer Aided Drug Design), natural product synthesis stands firm and has a wide use. As both disease and disorders occur and originates from nature it is just right to search for the cure in nature (how many times don't we hear that "nature heals itself"?). Today we find numerous examples of natural products used in the pharmaceutical industry, such as paclitaxel (anticancer drug), erythromycin (antibiotic) and morphine (analgesic), see Figure 1. After all chemistry started in nature and this is also where this thesis begins...

1.1 Purpose

The purpose of this 20 week long Master of Science Thesis (Advanced Level, 30 hp – ECTS credits) was to find a new approach toward the synthesis of Transtaganolides/Basiliolides in order to apply theoretical and practical knowledge within the areas Chemical Engineering, Organic Chemistry and Natural Drug Synthesis obtained during the 4.5 year Chemical Engineering education at Lund Institute of Technology (2006-2011).

In previous work an important intermediate in the total synthesis of Transtaganolides/Basiliolides (T/B) has been made in 12 steps. By the introduction of our new approach we hope to make way for an easier path towards the total synthesis of (T/B) and primarily be able to obtain a very important intermediate from which the skeleton of these compounds can be done in 3 steps instead of 12 steps as before (Scheme 1), thus saving time, money and hopefully increase the total yield.



Scheme 1. The main purpose of this Master of Science Thesis is to examine the possibility of a simpler and more efficient synthesis of the important 2-pyranone intermediate

1.2 Background

1.2.1 *Thapsia Garganica* L., *Thapsia Transtagana* Brot and *Thapsia Villosa* var. *Villosa* L.

The plants *Thapsia Garganica* L. (ThGa), *Thapsia Transtagana* Brot (ThTr) and *Thapsia Villosa* var. *Villosa* L. (ThVi) derive from the genus *Thapsia* of the family Umbelliferae/Apiaceae belonging to the Laserpitieae tribe.¹ One of the first to study this genus was the famous Greek physician, pharmacologist and botanist Pedanius Dioscorides. According to him the name of the genus *Thapsia* originates from that it was first discovered in the island of Thapsos, Greece. ThGa was easily found in the promontory of Gargano, hence its species name of *Garganica*.²

These plants are regarded as medicinal plants and ThGa was included in the French pharmacopoeia (1937 edition). The resin from the roots of ThGa was named Bou néfa (meaning father of health) by Arabs in northern Africa who primarily used them in medicine.³ ThTr was regarded as synonymous to ThGa but differences will be discussed.

ThGa grows in rocky locations, fields and sunny slopes in the Mediterranean (southern Europe, from Spain to Greece and northern Africa)⁴, while ThTr grows in the southwest of the Iberian Peninsula and northwestern Marocco⁵ and ThVi⁶ grows in the western Mediterranean area. These herbaceous perennial plants (meaning that they flower in periods of the year followed by that the leaves and stems die in the end of the growing season for them to grow in the next coming season again as they are plants that live for more than two years) cannot grow in the shade and predominantly vegetates in mountainous zones. ThGa has a very characteristic look (see Figure 2).



Figure 2. From left to right; picture of ThGa before flowering, the whole ThGa and the hermaphrodite flowers, picture of the fruit and picture showing the glabrous lamina (without any stiff, short hair) of ThGa

ThGa is in flower between July and August and its flowers are hermaphrodites, meaning that they are self-fertile, although pollination can also be made by insects. Although ThTr was previously mistaken to be the same plant as ThGa (even classified as synonymous in *Flora Europea*) it differs in many ways as it is somewhat bigger, contains different chemical compounds and has slightly different appearance (see Figure 3). On the other hand ThVi was also included as a unique plant in the *Flora Europea*.



Figure 3. From left to right; picture of the hermaphrodite flowers of ThTr, the whole ThTr, picture of the fruit and picture showing the hispidulous lamina (covered with stiff, short hair) of ThTr

ThGa normally becomes 54-110 cm tall, while the bigger ThTr becomes 140-180 cm.¹ The leaf morphology of these plants is usually the same, but ThTr has sparsely hispidulous and generally broader lamina whilst the ThGa has glabrous lamina.^{1,3}



Figure 4. From left to right; picture of the hermaphrodite flowers of ThVi, the whole ThVi, picture of the fruit and picture showing the hispidulous lamina (covered with stiff, short hair) of ThVi

ThVi has hispidulous lamina as ThTr, but they are rather bigger and more triangular/deltoid shaped than the ones of ThTr or ThGa that often are rhomboid. Another difference is that while ThTr and ThGa have pale green coloured lower surfaces of the lamina, these are sometimes greyish in ThVi.¹

1.2.2 Transtaganolides/Basiliolides – Secondary Metabolites found in *Thapsia* plants

Except from having very fascinating appearance ThGa, ThTr and ThVi contain many interesting secondary metabolites that can be extracted mainly from their roots^{3,4,5,6} or ripe fruits.³ Some of these compounds are of great scientific interest as they possess features making them potential candidates as active ingredients in future medicinal drugs. Thapsigargin, guaianolides and the C-19 terpenolides (the Transtaganolides/Basiliolides, a class of tetracyclic C-19 lactones) are the most common and interesting compounds^{3,4,5} and in this thesis focus will be on the four C-19 terpenolides found in ThGa, ThTr and ThVi, see Figure 5.

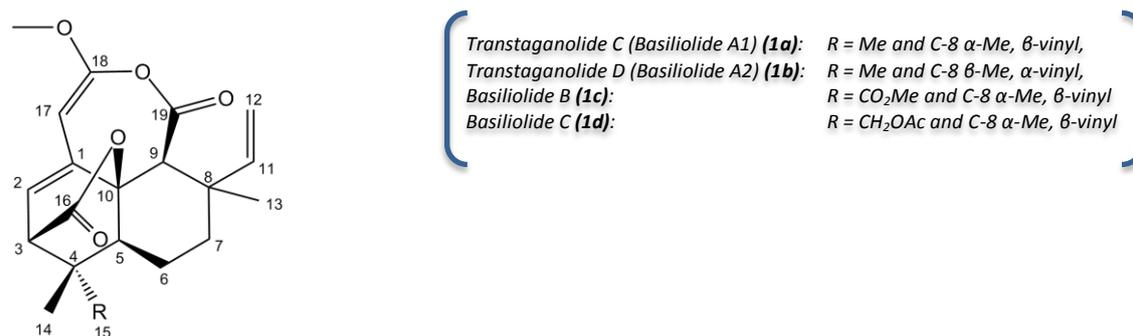


Figure 5. Structures of the C-19 terpenolides extracted from *Thapsia Garganica*, *Thapsia Transtaganana* and *Thapsia Villosa*

Basiliolides B, C and Transtaganolides C and D have been isolated and characterized from ThGa² while Transtaganolides C and D were recovered from ThTr⁵ and ThVi.⁶

Basiliolides B and C can be seen as analogues of Transtaganolides C (Basiliolides A1) as the only difference between them is that they have different substituents on the position 15 (indicated as R in Figure 5). Changes on this particular position have shown having a critical role in the biological activity as e.g. very differing NFAT activation in Jurkat cells.⁷ The most interesting effect of these C-19 terpenolides is their inhibiting activity of sarco/endoplasmatic reticulum Ca²⁺-ATPases (SERCA).^{4,5}

1.2.3 Sarco/endoplasmatic reticulum Ca²⁺-ATPase, SERCA

External and internal membranes can establish communication by interaction between voltage-gated Ca²⁺ channels in the exterior membranes, Dihydropyridine Receptors (DHPRs), and the Ca²⁺-release channels of the SR, Ryanodine Receptors (RyRs).⁸

The signaling used for the DHPRs and RyRs to interact with each other differs between skeletal and cardiac muscle cells even though the key structural element allowing the interaction between them is their vicinity in both cell types. Calcium induced calcium release occurs in cardiac muscle cells as depolarization is followed by an influx of Ca²⁺ through DHPRs which in turn triggers the opening of RyRs. In skeletal muscle cells it is instead a mechanical coupling, meaning that Ca²⁺ is not needed for RyRs to open as there are direct links between RyRs and DHPRs.⁸

The extracellular [Ca²⁺] (~ 10⁻³M) is much higher than the [Ca²⁺] in the cytosol (~ 10⁻⁷M), which means that when nerve impulses trigger the release of calcium ions (Ca²⁺) from the SR through RyRs into the cytosol to stimulate muscle contraction this will have a large effect on the

[Ca²⁺] in the cytosol. This subsequently means that the transport of Ca²⁺, from the cell's cytosol (intracellular fluid) to the lumen of the SR, by SERCA is very important and that the gradient must be steep in order to maintain the low cytoplasmic [Ca²⁺] of resting muscles.

The SERCA is a calcium adenosine triphosphatase (Ca²⁺ ATPase) that is a member of a family of P-type cation pumps (P-ATPases). In other terms it is an ATP-powered protein, embedded in the membrane of the sarcoplasmic reticulum (SR) in muscle cells, requiring ATP hydrolysis in order to function as an active cation pump that establishes ion concentration gradients across cell and organelle membranes.⁹

There are different SERCA genes encoding different isoforms. The ones interesting in this case are the SERCA1, which are exclusively expressed in skeletal muscle, and SERCA2, mainly present in cardiac muscle. The activity of SERCAs is regulated by naturally occurring muscle regulators with SERCA inhibiting effects in the body such as phospholamban (PLN) and sarcolipin (SLN).

In its dephosphorylated monomeric form, PLN mainly interacts with SERCA2a (can also interact with other SERCAs) and thereby decrease the affinity of SERCA2a for Ca²⁺, thus inhibiting its Ca²⁺-transport. This SERCA-inhibiting effect from PLN is subsequently relieved following protein kinase A (PKA) mediated phosphorylation, hence providing the understanding that PLN is a reversible SERCA-inhibitor in the body regulating the muscle contraction/relaxation.¹⁰ SLN can be seen as a functional PLN homologue, with the same reversible SERCA-inhibiting effect, that mainly interacts with SERCA1a.

To understand the mechanism behind the SERCA pump activity it is important to know that the cytoplasmic face of this Ca²⁺ ATPase is built by three main domains: the activator domain (A), nucleotide-binding domain (N) and the phosphorylation domain (P). In addition it also has an active site and aspartic acid and the head of this P-type ATPase has 10 transmembrane α -helices, of which four form transmembrane Ca²⁺-binding sites or cavities (Figure 6).

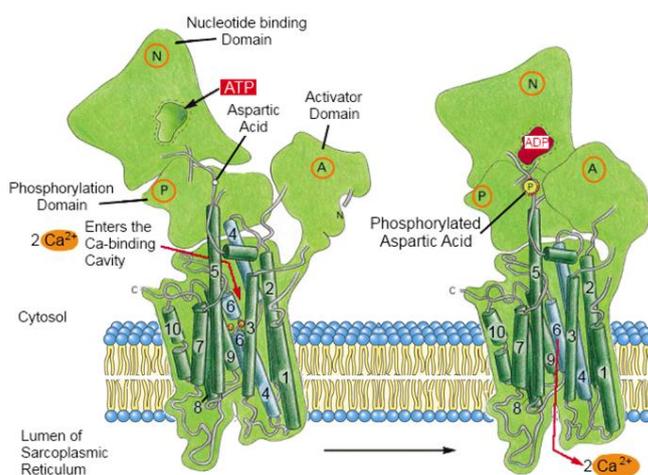


Figure 6. Mechanism of the SERCA pump activity and structures of the conformations with unphosphorylated and Ca²⁺-bound state (left conformation, E₁, which is based on X-ray crystallographic structure⁹) respectively phosphorylated and Ca²⁺-free state (right conformation, E₂, which is based on lower-resolution structures determined by electron microscopy¹¹)

When the SERCA is in unphosphorylated low energy state, conformation E_1 , the helices 4 and 6 are disrupted. This leads to the formation of a Ca^{2+} -cavity, on the cytosolic side of the membrane, which binds 2 Ca^{2+} tightly. This stabilizes E_1 and allows ATP to bind to an active site on the same side of the membrane. ATP is hydrolysed to ADP + P_i at which point the P-domain forms a covalent bond with the released phosphate (P_i), inducing changes in conformation ($E_1 \rightarrow E_2$, from low to higher energy state) to facilitate the nearing of the N- and P domains and subsequently a $\sim 90^\circ$ rotation of the A domain. This rotation rearranges the transmembrane α -helices so that the disruptions in helices 4 and 6 are eliminated, thereby closing the Ca^{2+} -cavities and releasing Ca^{2+} into the lumen of SR. Dissociation of Ca^{2+} promotes the hydrolysis of E_2 and catalyze the reformation of ATP ($ADP + P_i \rightarrow ATP$) and so conformational recovery to E_1 will occur and helices 4 and 6 will be disrupted again for the formation of the Ca^{2+} -cavities, meaning that the cycle has been completed (See Figure 6 for illustration of this described mechanism).¹¹ It is here important to note that this mechanism is only explained with two out of four helices that bind Ca^{2+} in order to simplify.

1.2.4 Thapsigargin – a SERCA-inhibitor with anticancer properties

Thapsigargin (TG) is the main constituent of the ThGa⁴, ThTr⁵. It is a sesquiterpene lactone working as an irreversible inhibitor of the SERCAs.¹²

A study in which the enzymatic ATP hydrolysis was measured during treatment with TG revealed that TG inhibited the microsomal SERCA with a half maximal inhibitory concentration (IC_{50}) value of ≈ 30 nM. This inhibition showed to be specific for Ca^{2+} -stimulated ATPase activity, as basal Mg^{2+} -ATPase in the membrane fraction was unaffected. Furthermore, it was proved that this inhibitory effect was restricted to the microsomal form of the enzyme.⁸

The SERCAs, as mentioned in section 1.2.3, transport Ca^{2+} from the cell's cytosol to the lumen of the SR. TG inhibits SERCAs from this transport and cause a net transfer of Ca^{2+} from the endoplasmic reticulum (ER) into the cytosol instead, hence the cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) is increased and apoptosis via ER stress is induced.⁸

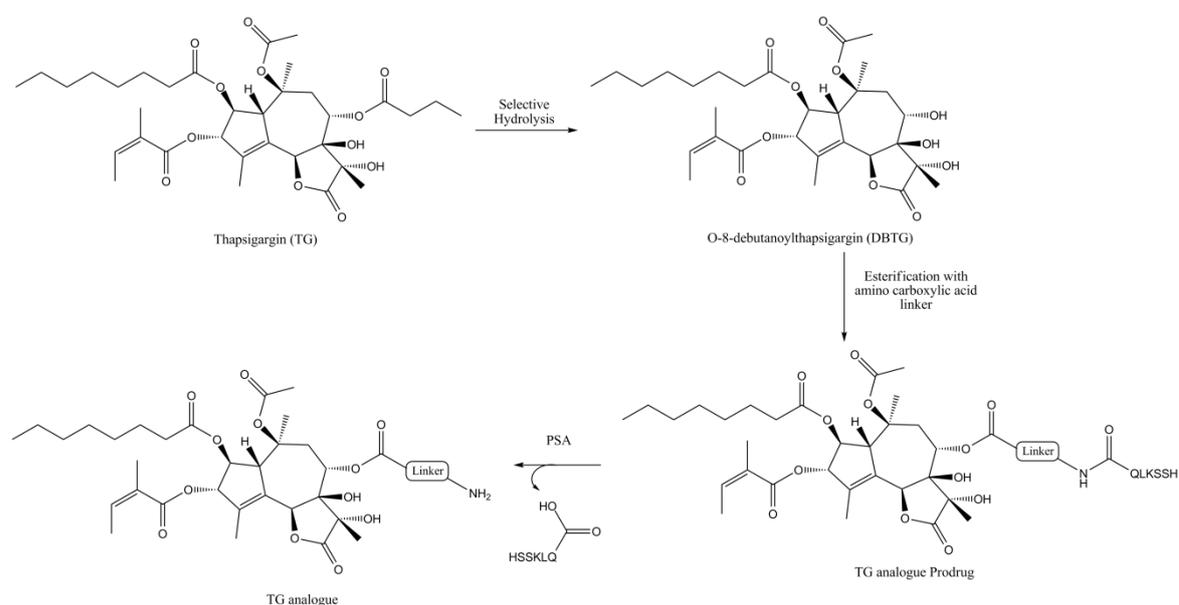
This identifies the SERCA pump as a therapeutic target for activating apoptosis of androgen-independent prostatic cancer cells and so, many studies on TG and its anticancer activity has been made.^{7,12,13}

In a very interesting study by Y. Furuya¹⁴ treatment of androgen-independent prostate cancer cells of both rat and human origin with TG was evaluated. They showed that the SERCA inhibiting effect on these cancer cells results in an increase of 300-400 % of the $[Ca^{2+}]_i$ within minutes of exposure. This increase also showed to be sustained due to a secondary influx of extracellular Ca^{2+} through RyR. This contributed to morphological changes, in the form of cell rounding, as well as biochemical changes of the cells within 6-12 h. Androgen-independent prostatic cancer cells stopped progression through the cell cycle within 24 h of exposure with a median effective concentration value of 31 nM TG, and irreversibly lost their multiplying ability. During the next 24-48h the cells undergo double-strand DNA fragmentation followed by plasma membrane integrity loss and fragmentation into apoptotic bodies. Another interesting aspect is that there is no acidification in the intracellular pH during this process.¹⁴

TG however has an immense problem in that it is a compound that will not only be toxic to the unwanted cancer cells, but also to the host cells, making it very difficult to administer systemically. Therefore C. M. Jacobsen¹³ modified the TG in an attempt to get a more specific effect.

Prostatic cancer cells secrete prostate-specific antigens (PSAs) in a unique manner and this fact was considered interesting for the modification of TG to get a tumor-specific cytotoxicity.

A PSA substrate (only enzymatically active in the extracellular fluid of prostatic cancer cells) with the sequence His-Ser-Ser-Lys-Leu-Gln (HSSKLQ) was introduced by first converting TG to O-8-debutanoylthapsigargin (DBTG) followed by esterification of DBTG in the O-8 position with an amino carboxylic acid linker. The resulting TG analogue prodrug showed able to be hydrolyzable by enzymatically active PSA only in the extracellular fluid of prostatic cancer cells, accordingly making the TG analogue specifically cytotoxic to prostatic cancer cells (Scheme 2).¹³



Scheme 2. TG undergoes selective hydrolysis at the O-8 position to yield the DBTG, which is in turn esterified with an amino carboxylic acid linker to introduce the PSA substrate HSSKLQ and get the analogue prodrug. PSA releases the active TG enabling the cancer-specific cytotoxicity

The results observed after the making of several analogues were interesting with one analogue being considerably potent. Generally more lipophilic analogues had higher potency.¹³

1.2.5 SERCA-inhibiting effects and biological activity of Transtaganolides/Basiliolides

The structures of the Transtaganolides/Basiliolides (T/B) are significantly differing to the TG, but still they behave as biological analogues by also possessing potent SERCA-inhibiting properties.⁴ This makes the biological activity of these C-19 terpenolides interesting to investigate and it is in fact also something that has been made.

It was found that Basiliolide A1 had the ability to inhibit SERCA activity and cause a transfer of Ca^{2+} from the ER to the cytosol (alike TG).^{7,15} However further investigation (with Jurkat cells) showed that unlike the irreversible SERCA-inhibiting effect from TG that also induces apoptosis, the effect from Basiliolide A1 was only sufficient to increase $[\text{Ca}^{2+}]_i$, so that gene expression was

activated. Basiliolide A1 was not able to induce apoptosis, suggesting that this compound targets the Ca^{2+} ER stores in a mechanism different from that of TG.⁷

In contrast to TG, the T/B appear to inhibit SERCA reversibly, thus not leading to apoptosis but rather associates the effect with cell homeostasis.¹⁵ Noncytotoxic compounds mobilizing Ca^{2+} by targeting the ER are of great interest for the treatment of neurodegenerative disorders, such as Alzheimer's or Parkinson's disease.^{7,15}

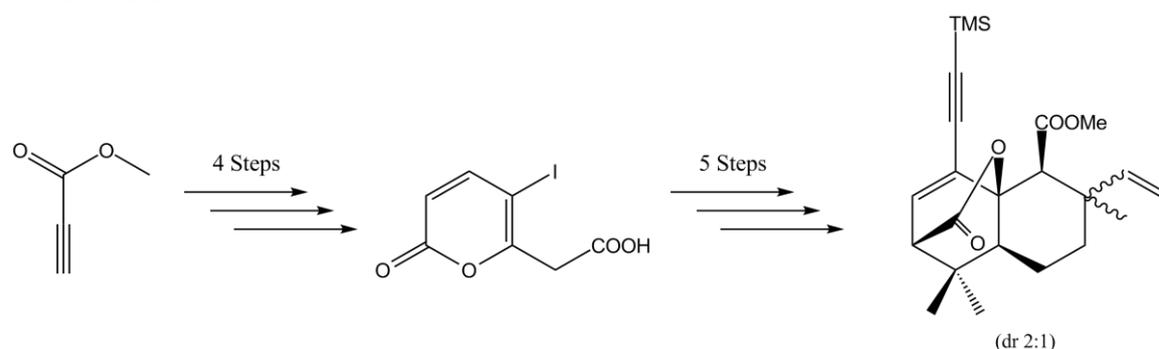
As mentioned before changes on the position 15 in T/B plays a critical role in the biological activity.⁷ Carmen Navarrete¹³ showed this during an investigation of the NFAT activation by T/B in Jurkat cells. This is however an area that should be further investigated in a way close to how analogue studies have been made on TG.

It would be very interesting to isolate T/B from plants and then modify these to get analogues and perform a study of the biological activity. By involving QSAR in a study such as this, one can perhaps get the properties needed for these compounds to act as substances specific against neurodegenerative disorders or even obtain an irreversible SERCA-inhibitory effect. This type of study would also be important as the groups working independently towards the total synthesis of T/B maybe would become more aware of what effects these compounds can provide.

1.2.6 Previous work towards the synthesis of Transtaganolides/Basiliolides

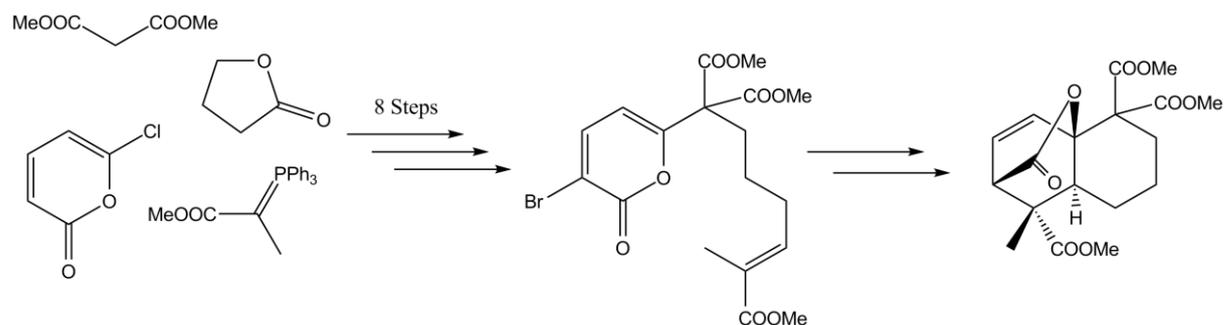
After T/B were found in ThGa, ThTr and ThVi a wide interest was spread amongst chemists. By observing the structure (Figure 5) it becomes clear that it is tetracyclic and has 6 contiguous stereocenters, and this suggests a difficult and very challenging synthesis. This of course attracted synthetic chemists who saw a great challenge in finding a synthetic path for the total synthesis of these C-19 terpenolides. In this section previous work will be presented

In a biomimetic synthesis of T/B, Sterner¹⁶ involved an Ireland-Claisen rearrangement/intramolecular Diels-Alder reaction sequence followed by a coupling with a TMS alkyne to become the first to obtain an intermediate with all carbon-carbon bonds from the T/B carbon skeleton.¹⁶



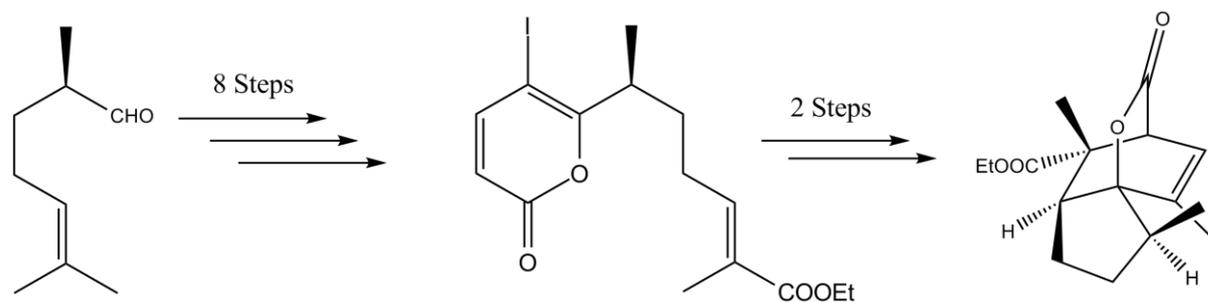
Scheme 3. Scheme including the starting material, 2-pyrone and carbon skeleton of T/B from the biomimetic synthesis towards the T/B by Sterner¹⁶

In a similar approach to Sterner¹⁶, Stoltz¹⁷ also used an intramolecular pyrone Diels–Alder (IMPDA) step to obtain an intermediate to Basiliolide B. First off, the IMPDA product was only obtained in bad yields. An attempt to optimize the reaction by temperature increase only yielded a decarboxylated product, lacking the pyrone, and without any observation of the wanted product. This however was solved by bromine substitution at the 3-position of the pyrone ring prior to the key IMPDA step. In a subsequent step the bromine was removed in order to obtain a key intermediate in the synthesis of Basiliolide B.¹⁷



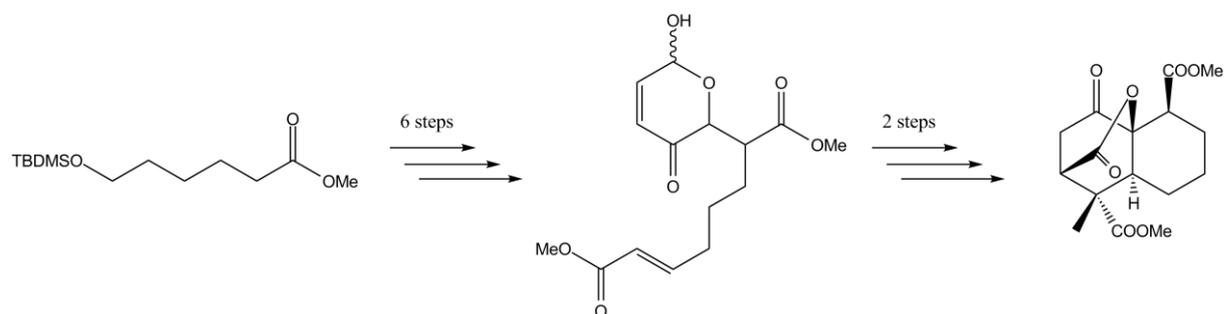
Scheme 4. The progress towards the synthesis of T/B by Stoltz¹⁷

Dudley¹⁵ presented a well-developed diastereoselective IMPDA reaction from which a tricyclic intermediate was obtained. Prior to this IMPDA reaction an iodopyrone (the IMPDA substrate) was made through iodocyclization of an enyne obtained after a series of reactions following a sonogashira coupling with an iodide.¹⁵ Interestingly this reminds of the biomimetic approach by Sterner¹⁶ (who worked independently approximately at the same time) that also begins with a sonogashira coupling with the exact same iodide compound and gets a similar iodopyrone (iodide at the 5-position of the pyrone ring) after a series of reactions to then perform the final step (which then actually differs to the one of Dudley¹⁵ giving an intermediate very close to the actual target molecule).^{15,16} Unfortunately the final substance made by Dudley¹⁵ can only be considered as a model substance as it lacks many vital parts and has a 5-membered ring instead of the strived 6-membered ring.



Scheme 5. Scheme showing the approach towards Basiliolide B by Dudley¹⁵

With chemists from both Europe and The United States of America working with T/B we only lack a candidate from Asia to be able to really consider this as a worldwide research. Chi-Sing Lee¹⁸ and co-workers used Base-Catalyzed Diels-Alder Reactions in an approach towards the Basiliolide B.

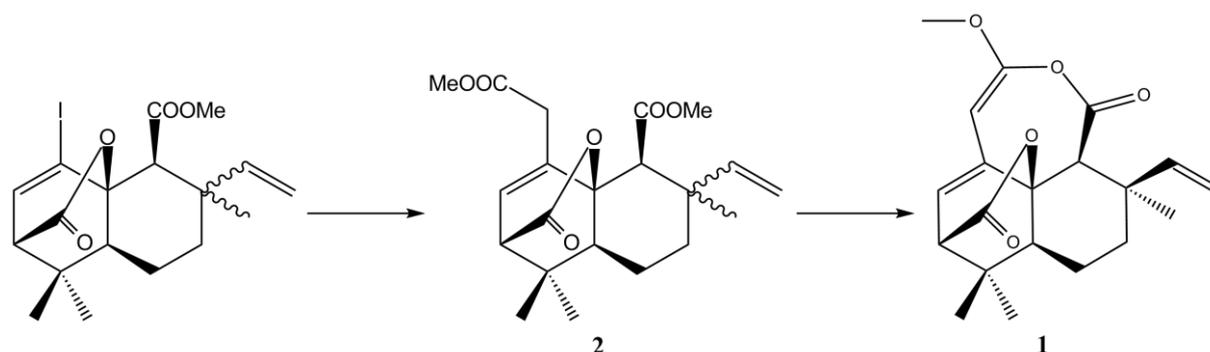


Scheme 6. Approach by Chi-Sing Lee¹⁸ giving a Diels-Alder product with the tricyclic core of Basiliolide B

1.2.7 Problems in previous work

The oxepine ring in T/B is likely to be unstable and thus most of the previous work was designed so that this unique structural feature would be constructed in the final stages of the synthesis.

When looking at the original retrosynthesis of Sterner¹⁶ we understand that a methyl ester should have been introduced in place of the iodide and as a last step a selective O-acylation would yield the oxepine ring. However the introduction of the methyl ester proved very hard (Scheme 7) and preparation of the carbon skeleton of T/B by introduction of the two remaining carbons was instead completed after a Sonogashira coupling with TMS alkyne (Scheme 3).¹⁶ Sadly the subsequent oxidation of the alkyne failed.



Scheme 7. Introduction of the methyl ester to get (2) that would allow the last selective O-acylation step to give the oxepine ring (1). The introduction of the methyl ester unfortunately proved being very hard and so the retrosynthesis was redesigned

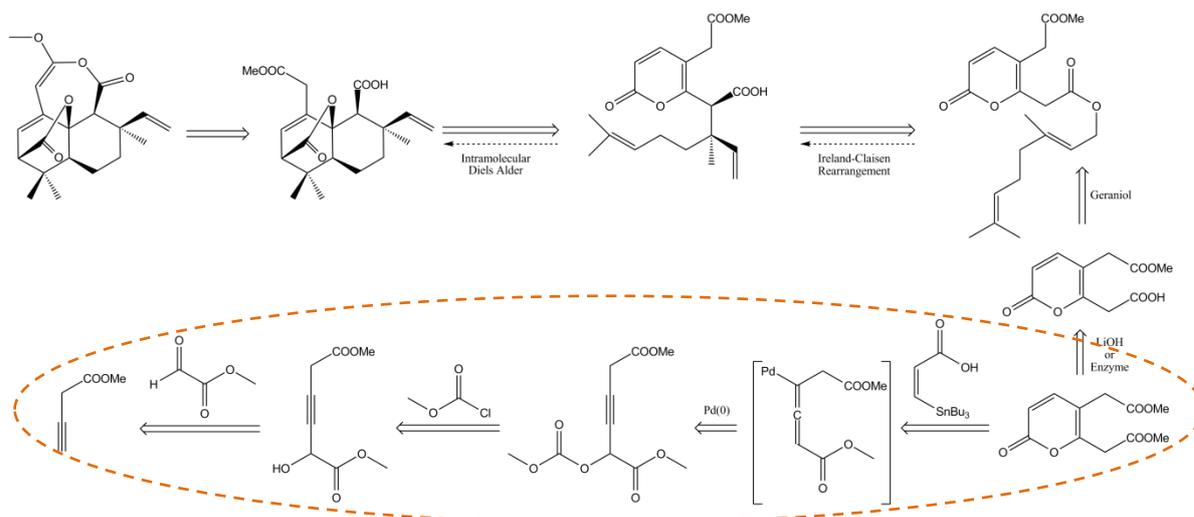
As a result the retrosynthesis was redesigned (unpublished work by Rikard Larsson, Olov Sterner and Martin Johansson) so that the methyl ester would be introduced in the early stages of the synthesis prior to the Ireland-Claisen rearrangement/intramolecular Diels-Alder reaction sequence (synthesis with 12 steps in Scheme 1).

This thesis is based upon the unpublished work by Rikard Larsson and co-workers and the main goal is to make the same important intermediate in 3 steps instead of 12 (synthesis with 3 steps in Scheme 1). With this new approach we hope being able to improve yields and time efficiency as well as minimizing the costs and environmental impact and in the same time introduce a method in which gram-scale production is possible.

2. Thesis

2.1 Retrosynthetic analysis

In order to find a suitable synthesis path to obtain the target molecule of this Master of Science Thesis a retrosynthetic analysis was made (Scheme 8):



Scheme 8. Retrosynthetic analysis of the target molecule, the marked area is the one involved in this thesis

Hence the synthesis would include an alkylation in which the terminal alkyne is added to methyl glyoxylate. The secondary alcohol on the so obtained alkyne would then be activated via the introduction of a carbonate in order for it to act as a good leaving group in the last palladium catalyzed Tandem reaction with tributylstannylacrylic acid including a reaction similar to a Stille coupling followed by a ring closure. To provide an understanding of that this is a route also towards the total synthesis the entire retrosynthesis is available in Scheme 8.

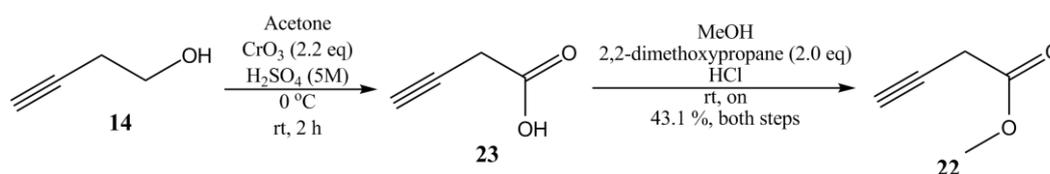
It is of importance to note that Scheme 8 shows only the original retrosynthetic analysis and that the actual synthesis path was slightly modified during the laboratory work.

2.2 Synthesis of the target molecule – Step A – Alkylation

Prior to the first synthesis step (addition of alkyne to an aldehyde) the starting material had to be synthesized as it was not commercially available. This was however rather simple.

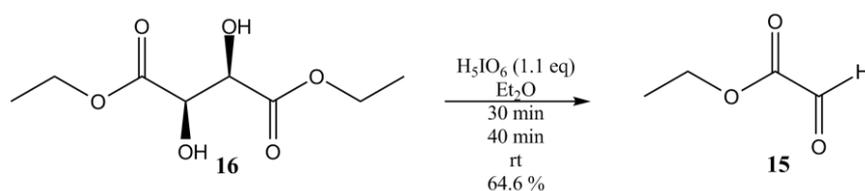
Jones oxidation under reverse and slow addition conditions of the commercially available 3-butyn-1-ol (**14**) followed by esterification in acidic methanol of the crude acetylenic carboxylic acid (3-butynoic acid, **23**) gave the acetylenic ester (methyl 3-butynoate, **22**) (Synthesis is outlined in Scheme 9).¹⁹

The Jones oxidation is very easy to follow as the solution with Jones reagent containing H_2CrO_4 , Cr(VI) , gives a very strong red colour and after the dropwise addition of the alcohol Cr(III) will be obtained, which gives a dark green colour.

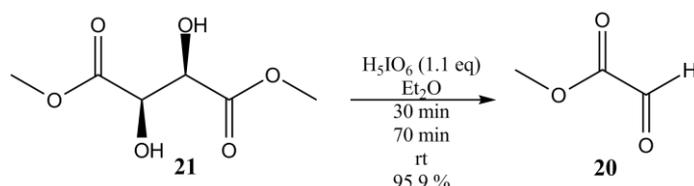


Scheme 9. Jones oxidation of 3-butyn-1-ol followed by esterification in acidic methanol to obtain methyl 3-butynoate (22)

Ethyl glyoxalate (15) is commercially available as a 50 % solution in anhydrous toluene, although before this was ordered both methyl- and ethyl glyoxalate were synthesized by oxidative cleavage of the corresponding tartrates, (+)-Dimethyl L-tartrate (21) respectively (-)-Diethyl D-tartrate (16), with periodic acid in diethyl ether (Scheme 10 and Scheme 11):²⁰

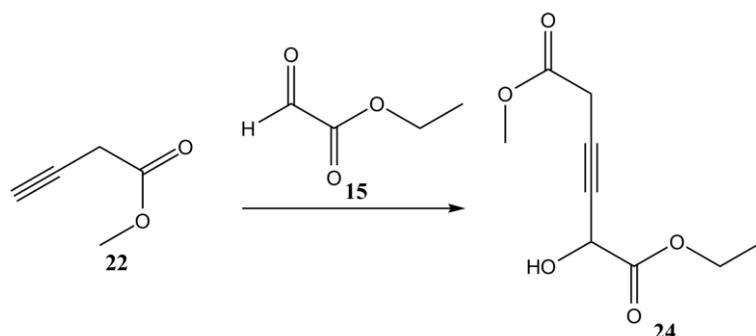


Scheme 10. Oxidation of (-)-Diethyl D-tartrate (16) using periodic acid to obtain the ethyl glyoxalate (15)



Scheme 11. Oxidation of (+)-Dimethyl L-tartrate (21) using periodic acid to obtain the methyl glyoxalate (20)

This oxidative cleavage was performed with fast reaction times and high yields and so with both starting material and reagent available attempts for the alkylation could be initiated. The fact that many reports showed the success of similar alkylation made the task seem easy, however this proved not to be the case. Scheme 12 shows the general alkylation reaction, while reaction conditions of the different attempts are presented in Table 1.



Scheme 12. Addition of the methyl 3-butynoate (22) to the ethyl glyoxalate (15) to get the alkyne product (24), reaction conditions not included as this scheme demonstrates only the general alkylation

Alkynylation attempt	Catalyst/Reagent	Solvent	Ligand	Temperature	Time	Reaction
A ₁	ZnCl ₂ /Et ₃ N	Toluene	No	rt	on	No
A ₂	Zn(OTf) ₂ /Et ₃ N	Toluene	No	rt	on	No
A ₃	Zn(OTf) ₂ /Et ₃ N	Toluene	Yes	rt	on	No
A ₄	LDA	Toluene	No	-15 °C → -78	2 h	No

Table 1. Summary of the alkynylations in which addition of the methyl 3-butynoate to the ethyl glyoxalate was attempted without success

One of the most common methods to alkynylate aldehydes is the addition of alkynylmetals to the aldehydes. However, the high reactivity and strong basicity of the alkynylmetals (n-BuLi e.g.) can cause undesired side effects, prompting us to find another way for this synthesis step.

Reports suggested that the addition of alkynes to aldehydes would be successfully promoted by the use of a lewis acid (such as ZnCl₂ or the stronger Zn(OTf)₂) in combination with a base (Et₃N), see A₁ respectively A₂ in Table 1.²¹

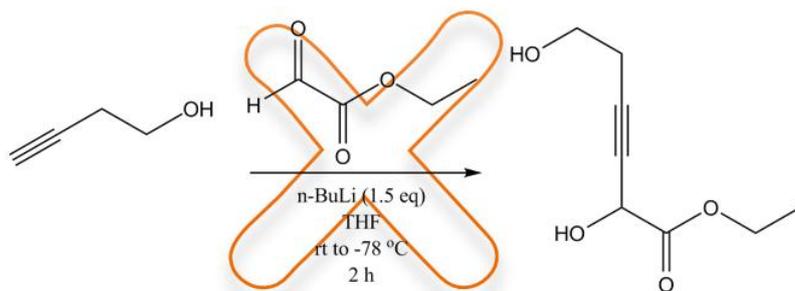
Attempts were initiated with the inexpensive ZnCl₂ without success and thus the same reaction was tried with the more expensive Zn(OTf)₂ as lewis acid instead as reports suggested that this would be a more effective catalyst for the alkynylations.²¹ Also this attempt with the stronger lewis acid (Zinc Triflate) failed. Reports suggest that the use of Zinc Triflate as a lewis acid gives inconsistent results as different commercial sources provide different qualities. This reaction did not succeed in our case, but further investigation was needed.

Observing the alkynylation product (**24**) it becomes clear that a stereocenter is present. Interestingly we found reports suggesting that alkynylations in which chiral tertiary carbon centers were constructed, could only be performed successfully in the presence of a ligand (using a chiral ligand will also make this method highly enantioselective).²² Mechanistic studies had shown the formation of a zinc alkynylide intermediate in the course of the alkynylations. This subsequently means that the alkynylation is an enantioselective addition of zinc alkynylide to the aldehyde. A chiral amino alcohol based ligand can therefore catalyze this reaction as the nucleophilicity of the zinc alkynylide will be increased with higher electron density.²² With this in mind A₂ was performed with N,N-dimethylethanol-amine as a N-O ligand (A₃ in Table 1). Unfortunately also this reaction failed. The failure of these reactions forced us to look for a possible explanation. It seemed as if the electronwithdrawing effect from the methyl ester on the terminal alkyne was the main reason for these reactions not to work and for the starting material to be degraded. This would also explain why there is no starting material present after the reaction and the NMR's are inconclusive.

As these alkynylations failed, a last attempt in which LDA was supposed to work as a sterically hindered base in an acid-base reaction to perform the alkynylation (The high reactivity and strong basicity of alkynylmetals, n-BuLi in this case, may cause undesired side reactions²¹ and instead LDA was used as it is less likely to attack the carbonyl on the methyl 3-butynoate). Sadly also this

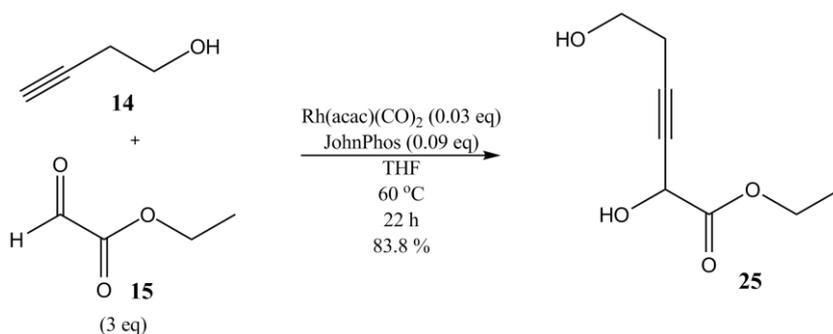
failed and so our attention was instead turned towards performing the alkylation directly with the 3-butyn-1-ol (**14**) as starting material instead of with the methyl 3-butynoate (**22**).

Using the basic organometallic n-BuLi with 3-butyn-1-ol as starting material would give an alkynyllithium which would then be added to the aldehyde. However Scheme 13 shows that this specific reaction did not work (a possible explanation would be that the n-butyl attacks instead of just getting the alkynyllithium needed for the reaction to work).



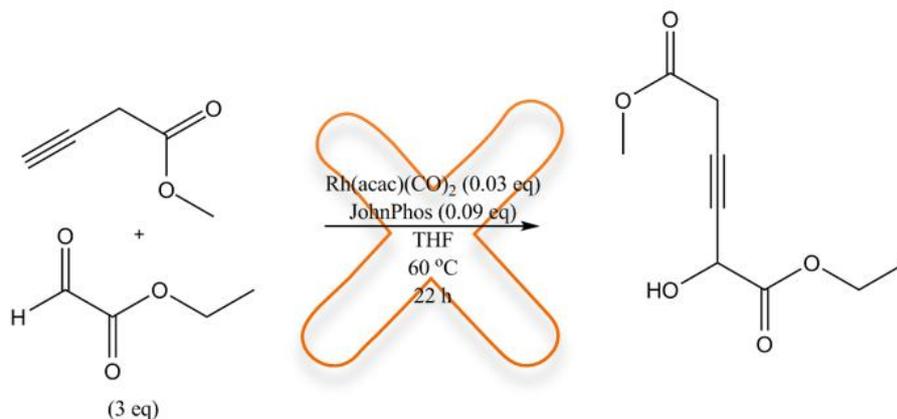
Scheme 13. Alkylation by addition of the alkynyllithium to the ethyl glyoxalate, did not succeed.

With the failure of using n-BuLi (Scheme 13) work was continued with 3-butyn-1-ol (**14**) instead of the corresponding methyl ester, methyl 3-butynoate (**22**). The reaction was carried out by using dicarbonylacetato rhodium(I) and the bulky, electron rich JohnPhos-ligand. This gave a rhodium-phosphine complex able to catalyze the reaction under relatively mild conditions (60 °C over 22 h). As this alkylation required a high concentration of ligand, 3 equivalents of JohnPhos to that of the rhodium catalyst were used. This reaction finally gave a successful alkylation, see Scheme 14:²³



Scheme 14. Addition of 3-butyn-1-ol (14) to ethyl glyoxalate (15) catalyzed by a rhodium-phosphine complex obtained from the JohnPhos ligand and dicarbonylacetato rhodium(I) to get alkylation product (25)

After finally finding the reaction conditions for the alkyne alkylation it was tempting to see whether or not the same reaction conditions would work with the corresponding methyl ester of 3-butyn-1-ol (**22**) (Scheme 15).



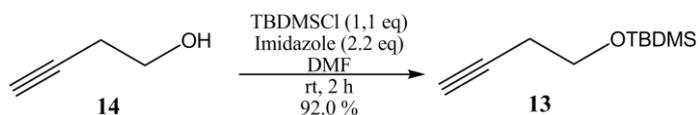
Scheme 15. Addition of methyl 3-butynoate to ethyl glyoxylate catalyzed by a rhodium-phosphine complex obtained from the JohnPhos ligand and dicarbonylacetonato rhodium(I), the cross indicates that the reaction did not succeed

It was previously mentioned that electronwithdrawing effects might explain the failed alkyne alkylation with the methyl ester. Therefore the same reaction (Scheme 15) was also carried out with the less electron rich S-Phos ligand instead of the JohnPhos, although without any success. ¹H-NMR indicated presence of the alkyne alkylation product in both cases, however full evidence could not be obtained by other spectra. This reaction is still interesting to investigate as with this step not working we are forced to introduce two new steps (protection and deprotection of the primary alcohol), losing time and decreasing the total yield. Focus was put on getting further in the synthesis, but in future work this step should be studied further.

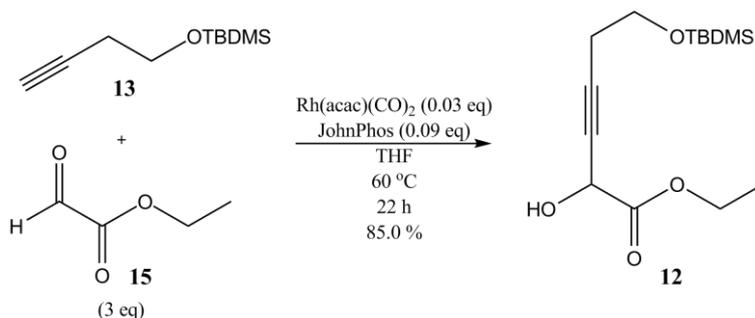
The inability of performing the alkyne alkylation in presence of the methyl ester prompted us to redesign the synthesis path so that the methyl ester would be introduced by e.g. Jones oxidation followed by esterification after performing the Tandem reaction (Stille coupling followed by a cyclisation reaction). Subsequently this means that the primary alcohol from 3-butyn-1-ol will be present in the next coming reactions.

By observing Step B with the goal to further optimize the synthesis path we obtain the knowledge that the secondary alcohol on (**25**) is activated in order to act as a leaving group following the attack from palladium and get a cumulative double bond. It is although of importance to note that an eventual coupling with methyl chloroformate to get the carbonate will affect both the secondary and the primary alcohol and so a selective protection of the primary alcohol, followed by activation of the secondary alcohol would be necessary.

Noting that the selectivity issue could cause bad yields and loss of material during separation etc. we opted to protect the 3-butyn-1-ol with a tert-butyl dimethylsiloxy, TBDMS, protecting group (Scheme 16) prior to the alkylation (Scheme 17).²⁴



Scheme 16. Protection of 3-butyn-1-ol (14) with TBDMS to get (13)

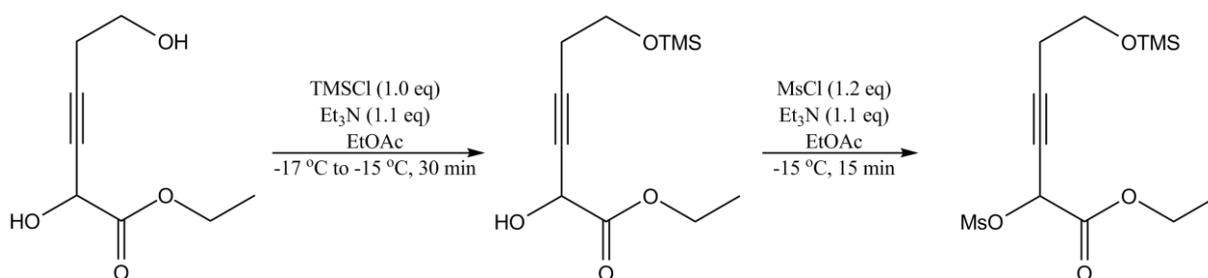


Scheme 17. Addition of the TBDMS protected 3-butyn-1-ol (13) to ethyl glyoxylate (15) to obtain alkylation product (12)

This direct protection of the primary alcohol was simple and fast, and after work up the yield was very high. The following alkylation proceeded with ease and yields were good.

Another option to this reaction would be to follow a different, interesting path. Instead of using TBDMSCl for the protection of the primary alcohol trimethylsilyl chloride (TMSCl) is used.²⁵ In this way the alkylation can be performed with 3-butyn-1-ol to get (25) and thus obtain both a primary and a secondary alcohol. According to literature²⁵ the selectivity of the subsequent protection with TMSCl could be very high and the product with only the primary alcohol protected could be synthesized, possibly in high yields. This selective protection is followed by activation of the secondary alcohol via mesylation as illustrated in Scheme 18.²⁵ This means that it will work as a good leaving group for a cumulative double bond to be formed in the attack from Palladium.

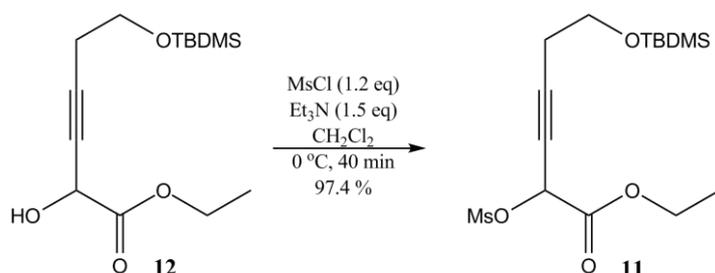
The two main advantages with this method is that TMSCl costs less than one third of the price of TBDMSCl and that the total yield of this reaction type can exceed 90%.²⁵ Although it is important to note that the TMS protecting group is rather sensitive to hydrolysis and the limited time for this project prompted us only to perform the TBDMS protection.



Scheme 18. Selective TMS protection of the primary alcohol followed by mesylation of the secondary alcohol, this suggested reaction (which has actually not been performed), in which the second step can be performed in situ after the first is completed, has been reported giving over 91 % yield in total for other compounds.²⁵

2.3 Synthesis of the target molecule – Step B – Activation of the secondary alcohol

In Step B we opted to activate the secondary alcohol by mesylation of the secondary alcohol on **(12)** with methanesulfonyl chloride (MsCl) to get a good leaving group for the next step. Originally this was supposed to be done by the introduction of a carbonate. But the protection prior to the activation persuaded us to mesylate instead, as this was reported working very well in the presence of TBDMS-ethers.²⁶



Scheme 19. Mesylation of the secondary alcohol on the TBDMS protected alkyne product (12) from step A to get (11)

Alternatives other than carbonation or mesylation for the activation of the secondary alcohol would be the use of toluenesulfonyl chloride or benzenesulfonyl chloride.²⁶ But reports suggested that the activation with these electrophiles failed even after prolonged reaction times.²⁶ By instead using MsCl as an electrophile the desired product was obtained with very short reaction times, easy work up and high yields, as was the outcome in our case (Scheme 19). We also believe that carbonation would be as effective.

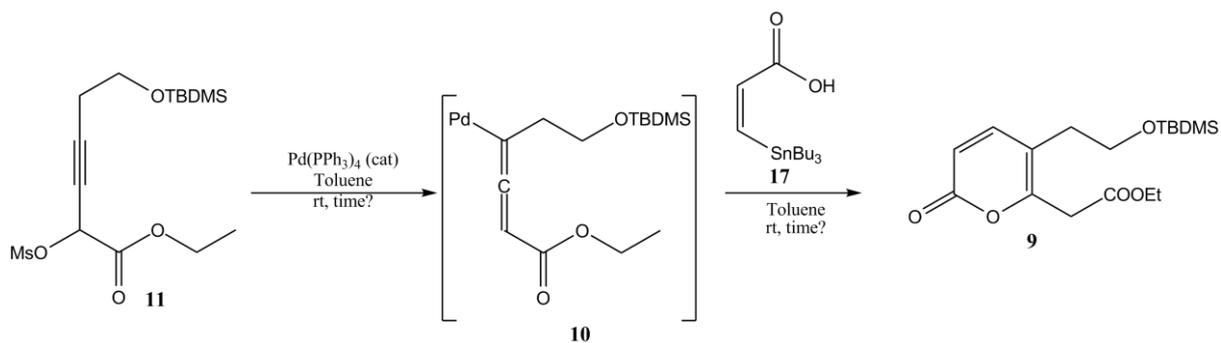
A very important aspect is that activation of the secondary alcohol by mesylation will also protect this alcohol in case that one would like to deprotect the TBDMS group and perform the oxidation followed by esterification of the primary alcohol already after step B and before the Tandem reaction. Reports also suggest that deprotection of a TMS-ether in presence of a mesylate give high yields. This could be interesting to have in mind if the TMS-ether is used instead of TBDMS and deprotecting of the primary alcohol is performed already at this point.

We are in this case fortunate to have a free choice of when to deprotect, oxidate and esterificate the primary alcohol. This will prove useful in the final step of this synthesis.

2.4 Synthesis of the target molecule – Step C – Tandem reaction

Step C was the supposed last step according to the original retrosynthesis (Scheme 8). It is a Tandem reaction (cascade reaction):

- The alkyne is converted to a cumulative double bond via attack from a palladium(0) catalyst with the mesylate acting as a leaving group, is followed by
- an intermolecular reaction with (Z)-3-(tributylstannyl)acrylic acid similar to a Stille coupling, and finally
- the desired 2-pyranone ring is obtained via an intramolecular cyclization reaction.

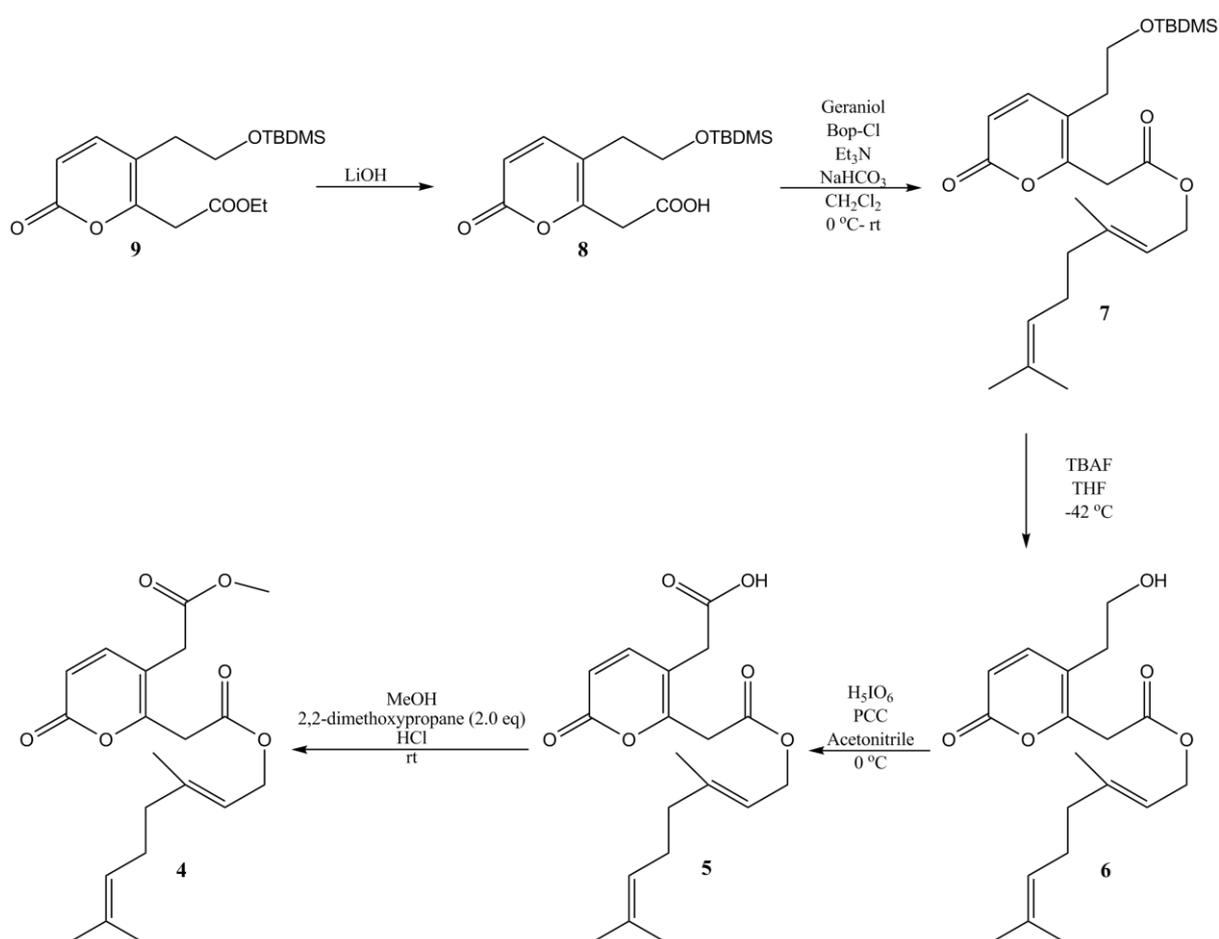


Scheme 20. Tandem reaction of a intermolecular Stille coupling followed by an intramolecular cyclization reaction to obtain the desired 2-pyranone

This reaction was unfortunately never tried as time was insufficient. However it is very interesting to see whether or not this reaction works.

2.5 Synthesis of the target molecule – Last steps

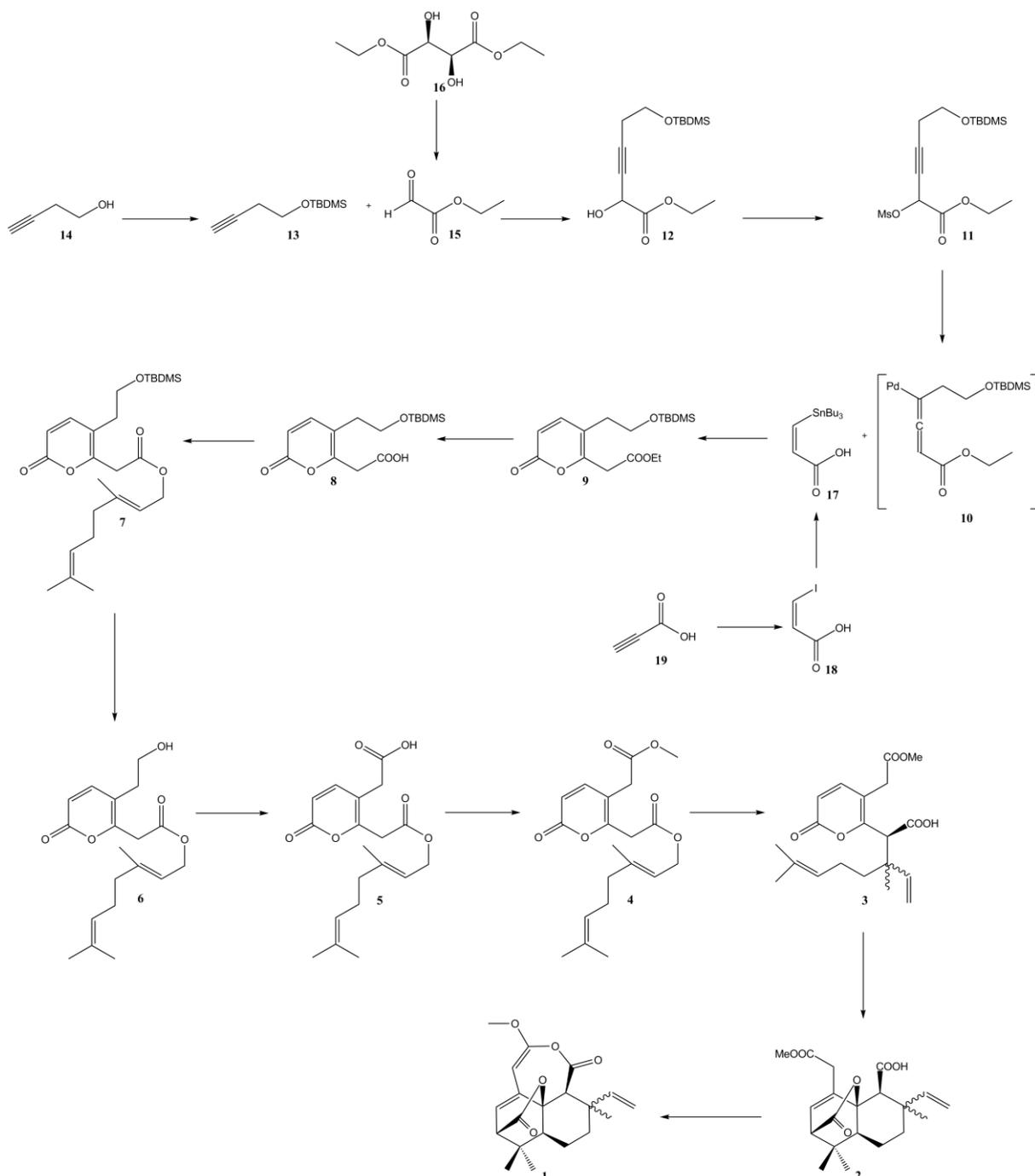
Another very interesting approach is to make a slight modification to the synthesis path. In the beginning we wanted to introduce a methyl ester, but now instead we have a TBDMS protected primary alcohol. With both an ethyl ester and a methyl ester present we would have to introduce a selective deprotection of the ethyl ester prior to the geranylation (See Scheme 8). But as we now only have one ester it can be deprotected to get the corresponding carboxylic acid and this can be geranylated. Following this reaction a deprotection of the TBDMS would be performed and after this the obtained alcohol would be oxidized by Periodic acid and PCC in acetonitrile to a carboxylic acid (other methods that can also be used for the oxidation is Jones oxidation or Collins reagent) that in turn would undergo esterification to the corresponding methyl ester (Scheme 21).



Scheme 21. Deprotection followed by oxidation and esterification gives the key intermediate towards the synthesis of T/B which is the targeted molecule of this Master of Science Thesis (reaction conditions are based on similar reactions)

3. Results and discussion

Prior to the first step 3-butyn-1-ol (**14**) is protected by TBDMS to give (**13**) (92 % yield) and an oxidative cleavage of Diethyl D-Tartrate (**16**) gives the ethyl glyoxalate (**15**) (64 %). The first step which is the rhodium-phosphine complex catalyzed alkylation (addition of alkyne (**13**) to aldehyde (**15**)) gives (**12**) (85 %). Mesylation of alkylation product (**12**) gives the mesylate (**11**) (97 %). These are the steps done and successful so far. For future work we present our total synthesis path and explain the steps.



Scheme 22. Total synthesis map of this Master of Science Thesis

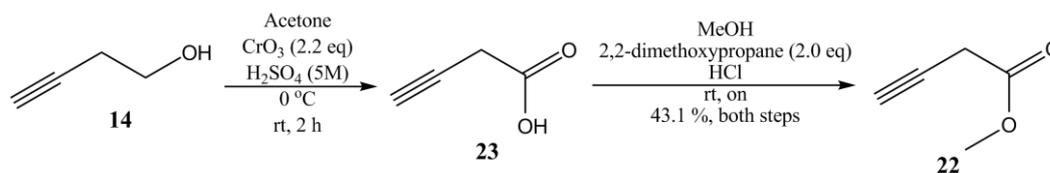
By the use of a Palladium(0) catalyst the cummen **(10)** is obtained from **(11)** via attack on the alkyne. In a subsequent intermolecular Stille like reaction with (Z)-3-(tributylstannyl)acrylic acid followed by an intramolecular cyclization reaction yields the desired 2-pyranone **(9)**. The ethylester in **(9)** is then deprotected to its corresponding carboxylic acid **(8)** for the geranylation to give **(7)**.

The TBDMS protected primary alcohol on **(7)** is deprotected to give **(6)** then oxidized to **(5)** and then undergoes esterification to the corresponding methyl ester **(4)**. **(4)** can then give **(3)** and subsequently **(2)** by an Ireland-Claisen rearrangement/intramolecular Diels-Alder reaction sequence. The final step would be the introduction of the oxepine ring **(1)** via a selective O-acylation (this gives Transtaganolide C and D).

4. Experimental section

All experiments and work with chemicals was carried out in approved laboratory fume hoods at the Center of Analysis and Synthesis (CAS) in Lund Institute of Technology (LTH) in a period of 20 weeks between 14/10-2010 and 11/3-2011.

Materials were obtained from Sigma Aldrich® or Acros Organics and were used without further purification unless otherwise noted. All moisture and air-sensitive reactions were carried out under an atmosphere of dry nitrogen using oven-dried glassware. NMR spectra were recorded on a Bruker Avance II at 400 MHz (^1H) and chemical shifts are relative to the residual peak of the deuterated solvent. All flash chromatography was performed on 60 Å 35-70 μm Matrex silica gel. TLC analyses were made on silica Gel 60 F₂₅₄ (Merck) plates and visualised with a) UV-light and b) vanillin/sulphuric acid or potassium permanganate and heating.

Methyl 3-butynoate (22):

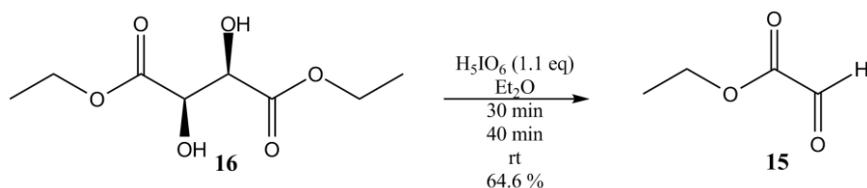
7.609 g CrO_3 (76 mmol) was dissolved in 95 ml 5 M H_2SO_4 and the so obtained red solution was cooled to 0°C .

2.87 ml 3-butyn-1-ol (**14**) (38 mmol) diluted in 38 ml Acetone was added dropwise over 30 min. After the addition was complete the reaction mix turned dark green and was allowed to warm to room temperature and was stirred under an atmosphere of N_2 .

After 1.5 h the reaction mix was separated between 100 ml H_2O and 100 ml EtOAc. The water phase was extracted 5 times with EtOAc. The pooled organic phases were washed twice with 100 ml brine, dried over Na_2SO_4 , filtered and evaporated to give 3.47 g of crude 3-butynoic acid (**23**).

The crude was dissolved in 24.0 ml MeOH and to the solution was added 3.1 ml 2,2-dimethoxypropane and a drop of HCl. The solution was stirred at room temperature under an atmosphere of N_2 . After stirring overnight the reaction mix was evaporated to near dryness, diluted with 50 ml EtOAc, washed with 50 ml saturated (aq.) NaHCO_3 , dried over Na_2SO_4 , filtered and evaporated to give 1.603 g methyl 3-butynoate (16 mmol, 43.1 % over two steps) (**22**).

NMR data was consistent to previous reports.¹⁹

Ethyl Glyoxalate (15):

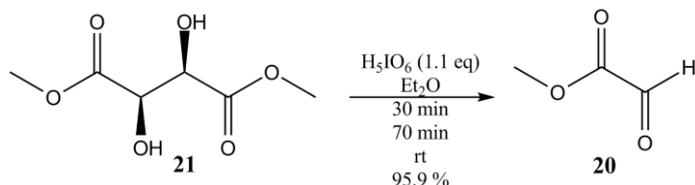
To a stirred suspension of 0.082 ml Diethyl D-Tartrate (**16**) (0.24 mmol) in 2 ml Et_2O was added 0.122 g H_5IO_6 (0.27 mmol) in small portions over a period of 30 min at room temperature.

After the addition was complete the reaction mix turned white and unclear and was allowed to stir under an atmosphere of N_2 .

After 40 min the solution turned clear and the white solid separated out during the addition of H_5IO_6 was allowed to settle and the reaction mix was poured through a folded filter.

The white solid was washed with 1 ml Et_2O and the solution was poured through the filter. The filter was washed with 0.5 ml Et_2O and the ethereal phases were combined and dried over Na_2SO_4 , filtered and evaporated and put under vacuum for 5 min to give 0.032 g of pure ethyl glyoxalate (**15**) (0.31 mmol, 64.6 %).

NMR data was consistent to previous reports.²⁷

Methyl Glyoxylate (20):

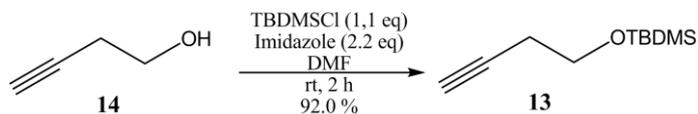
To a stirred suspension of 2.000 g Dimethyl L-Tartrate (**21**) (0.0112 mol) in 50 ml Et_2O was added 2.82 g H_5IO_6 (0.0123 mol) in small portions over a period of 30 min at room temperature.

After the addition was complete the reaction mix turned white and unclear and was allowed to stir under an atmosphere of N_2 .

After 70 min the solution turned clear and the white solid separated out during the addition of H_5IO_6 was allowed to settle and the reaction mix was poured through a folded filter.

The white solid was washed with 25 ml Et_2O and the solution was poured through the filter. The filter was washed with 13 ml Et_2O and the ethereal phases were combined and dried over Na_2SO_4 , filtered and evaporated and put under vacuum for 5 min to give 0.948 g of pure methyl glyoxylate (**20**) (0.0107 mol, 95.9 %).

NMR data was consistent to previous reports.²⁷

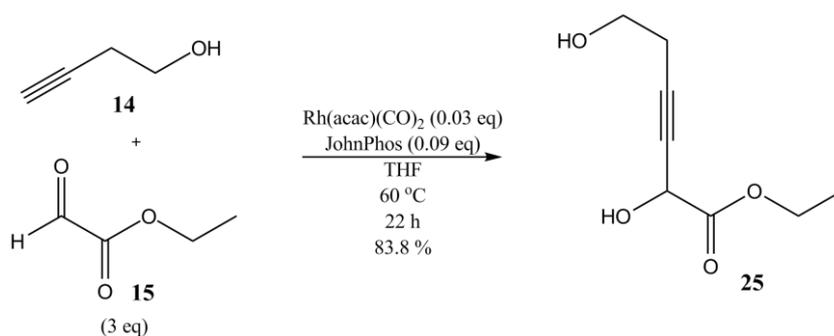
(but-3-ynoxy)(tert-butyl)dimethylsilane (13):

2.308 g Imidazole (0.0339 mol) was dissolved in 8.0 ml DMF and to this solution was added 1.00 ml freshly distilled 3-butyn-1-ol (**14**) (0.0154 mol) at room temperature and stirring under an atmosphere of N₂. The solution was cooled to 0 °C and 2.554 g TBDMS-Cl (0.0169 mol) in 6.0 ml DMF was added.

After 1 h the reaction mix was allowed to warm to room temperature.

After an additional hour the reaction mix was quenched with 50 ml H₂O. The water phase was extracted with 50 ml of 3:1 Hexane:EtOAc 3 times. The pooled organic phases were dried over Na₂SO₄, filtered and evaporated and put under vacuum over night to give 2.613 g of the desired TBDMS-ether (**13**) (0.0142 mol, 92.0 %).

NMR data was consistent to previous reports.²⁰

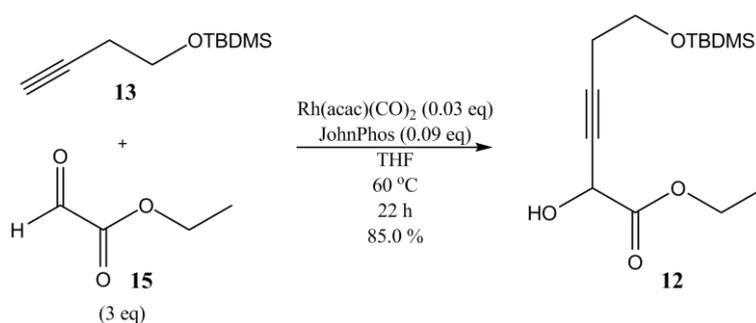
Ethyl 2,6-dihydroxyhex-3-ynoate (25):

0.9 ml THF, 0.424 ml Ethyl glyoxylate (**15**) (2.14 mmol) (50 % solution in Toluene) and 0.054 ml freshly distilled 3-butyn-1-ol (**14**) (0.71 mmol) was added through syringe to catalytic amounts of JohnPhos ligand and dicarbonylacetonato rhodium(I) at room temperature and was stirred under an atmosphere of N_2 .

The reaction mix was put in a preheated oil bath at 60 °C and was stirred under an atmosphere of N_2 . After 22 h the reaction mix was cooled to room temperature, pre-absorbed on silica gel and purified by silica gel chromatography using Petroleum ether/EtOAc (1:1) as eluent to get 0.076 g of the alkyne product (**25**) (0.60 mmol, 83.8 %).

$^1\text{H NMR}$ (400 MHz, CDCl_3): δ 4.82 (bs, 1H), 4.28 (qd, $J_1=14.3$, $J_2=7.1$, $J_3=1.5$, 2H), 3.72 (t, $J=6.2$, 2H), 2.90 (b, 1H), 2.48 (td, $J_1=6.1$, $J_2=2.2$, 2H), 1.31 (t, $J=7.2$, 2H)

$^{13}\text{C NMR}$ (100 MHz, CDCl_3): δ 170.63, 92.61, 83.78, 62.84, 61.76, 60.75, 23.18, 14.17

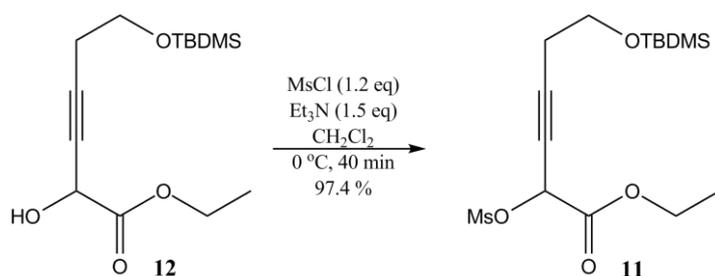
Ethyl 6-(tert-butyldimethylsilyloxy)-2-hydroxyhex-3-ynoate (12):

0.9 ml THF, 0.161 ml Ethyl glyoxylate (0.81 mmol) (50 % solution in Toluene) and 0.056 ml TBDMS protected 3-butyne-1-ol (**13**) (0.27 mmol) was added through syringe to catalytic amounts of JohnPhos ligand and dicarbonylacetonato rhodium(I) at room temperature and was stirred under an atmosphere of N_2 .

The reaction mix was put in a preheated oil bath at 60 °C and was stirred under an atmosphere of N_2 . After 23 h the reaction mix was cooled to room temperature, pre-absorbed on silica gel and purified by silica gel chromatography using Petroleum ether/EtOAc (4:1) as eluent to get 0.066 g of the alkyne product (**12**) (0.23 mmol, 85.0 %).

$^1\text{H NMR}$ (400 MHz, CDCl_3): δ 4.80 (dt, $J_1=7.4$, $J_2=7.1$, 1H), 4.30 (m, $J_1=6.6$, $J_2=3.6$, $J_3=27.9$, 2H), 3.72 (t, $J=7.1$, 2H), 2.95 (d, $J=7.4$, 1H), 2.44 (td, $J_1=7.1$, $J_2=2.2$, 2H), 1.33 (t, $J=7.2$, 3H), 0.89 (s, 9H), 0.06 (s, 6H)

$^{13}\text{C NMR}$ (100 MHz, CDCl_3): δ 83.81, 62.86, 61.71, 61.60, 25.99, 23.26, 18.45, 14.18, -5.18 (Carbonyl carbon and one of the alkyne (sp-hybridized) carbons missing in spectra)

Ethyl 6-(tert-butyldimethylsilyloxy)-2-(methylsulfonyloxy)hex-3-ynoate (11):

0.025 g alkyne (**12**) (0.087 mmol) was diluted in 0.44 ml CH₂Cl₂ to give a 0.2 M solution. The solution was cooled to 0 °C and to it was added 0.018 ml Et₃N (0.131 mmol) and 0.0081 ml MsCl (0.105 mmol) at room temperature and it was stirred under an atmosphere of N₂.

After 40 min the reaction mix was diluted with 5 ml CH₂Cl₂. The organic phase was washed with 4 ml H₂O, 4 ml saturated Copper(II) sulfate solution and 4 ml brine and then dried over Na₂SO₄, filtered and evaporated and put under vacuum to give 0.031 g (0.085 mmol, 97.4 %) of the mesylated product.

¹H NMR (400 MHz, CDCl₃): δ 6.32 (t, J=7.3, 1H), 4.27 (qd, J₁=14.3, J₂=7.1, J₃=2.7, 2H), 3.78 (td, J₁=6.3, J₂=1.3, 2H), 3.68 (s, 3H), 2.51 (q, J₁=13.5, J₂=6.4, 2H), 1.30 (t, J=7.1, 3H), 0.89 (s, 9H), 0.06 (s, 6H)

¹³C NMR (100 MHz, CDCl₃): δ 202.82, 162.54, 115.73, 105.60, 62.29, 61.61, 39.29, 32.58, 25.99, 18.40, 14.27, -5.26

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