

Proposal of 14- Step Total Synthesis Route of Dihydrolucilactaene, a Potent Antimalarial Compound

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Abstract

Malarial disease has been under heated discussion. The Osada group has extracted and purified potent antimalarial compounds, including dihydrolucilactaene and its derivatives. In this work, a 14-step total synthetic route of Dihydrolucilactaene, the compound with the highest pharmaceutical value among its derivatives, is designed and presented after considering and combining four partially feasible but hindered approaches. The complex molecule is first split into lactam-containing and long unsaturated fragments. The long unsaturated carbon chain can be effectively synthesized by two subsequent aldol condensations and the enabling Wittig reaction. Dicarbonyl synthesis and a base-mediated intramolecular nucleophilic attack can synthesize the lactam fragment. The final connection between two fragments are conducted through an enolate attack of electrophilic nitrile followed by hydrolysis. Three protection groups, named trimethyl silyl, acetal, and N-Boc, facilitate the synthesis in the last step. This research successfully developed a 14-step efficient total synthetic route of dihydrolucilactaene with commercially available building blocks and catalysts. The proposed total synthesis route consists of developed and straightforward synthetic methodologies and therefore is expected to be efficient and have a high overall yield.

Keywords: Total Synthesis, Organic Chemistry, Antimalarial, Dihydrolucilactaene

1. INTRODUCTION

The malaria disease has greatly influenced public health for decades worldwide due to its long lifespan and simple physiological mechanism. According to World Malaria Report 2021 conducted by the World Health Organization, there were 241 million cases of malaria causing 627,000 deaths in 2020 over the globe [1]. Among continents and main regions, in Africa especially in sub-Saharan Africa, cases take about 95% of all cases worldwide, which is estimated to result in losses of US\$12 billion a year [1]. People have been looking for target drugs or drug-lead candidates for malaria treatment. One example of early treatment is chloroquine, a synthetic 4-aminoquinoline derivative that exhibited better tolerability and side effects discovered in 1934[2].

Along the decades of adoption of chloroquine to treat malaria, drug resistance toward *P. falciparum* appeared in the late 1950s and early 1960s, and then became a global issue. In recent studies of causes of chloroquine resistance (CQR), gene PFCRT, localizing to the digestive vacuole membrane, was identified as a candidate

gene for CQR due to the point mutations in a different transmembrane domain in PFCRT [3-5]. The evolution of drug resistance parasites leads to a severe threat to malaria control because of the increased malaria morbidity and mortality. With technological advancements, new and more efficient drugs are developed; Dihydrolucilactaene is one excellent example. It is a secondary metabolism regulator and a potent antimalaria medicine identified and purified from *Fusarium* by the Osada group at RIKEN Center for Sustainable Resource Science [6]. It can inhibit the growth of parasites within the human body. Therefore, the importance of producing such medicine in abundance is prioritized, and it is made possible with the help of retrosynthesis. However, no viable total synthesis path of dihydrolucilactaene has been achieved since the discovery of its structure. Although dihydrolucilactaene has multiple derivatives, including Lucilactaene and NG391, dihydrolucilactaene shows the highest antimalarial activity among those three, whose structures are shown in Figure 1 [6]. Herein, a 14-step total synthesis route of dihydrolucilactaene for its highest pharmaceutical value is proposed.

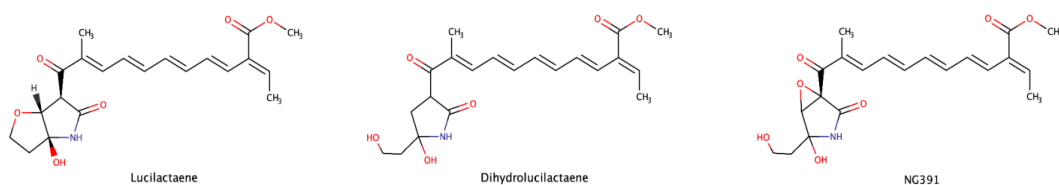


Figure 1. The chemical structure of Lucilactaene, Dihydrolucilactaene, and NG391 [Owner-Draw].

Lucilactaene has an additional tetrahydrofuran group formed by the two alcohol groups and attached to the lactam group in Dihydrolucilactaene. NG391 has an additional epoxide group attached to the lactam group in Dihydrolucilactaene. In addition and most importantly, all stereogenic centers in dihydrolucilactaene have unspecified stereoisomeric configurations, compared to Lucilactaene and NG391, which have.

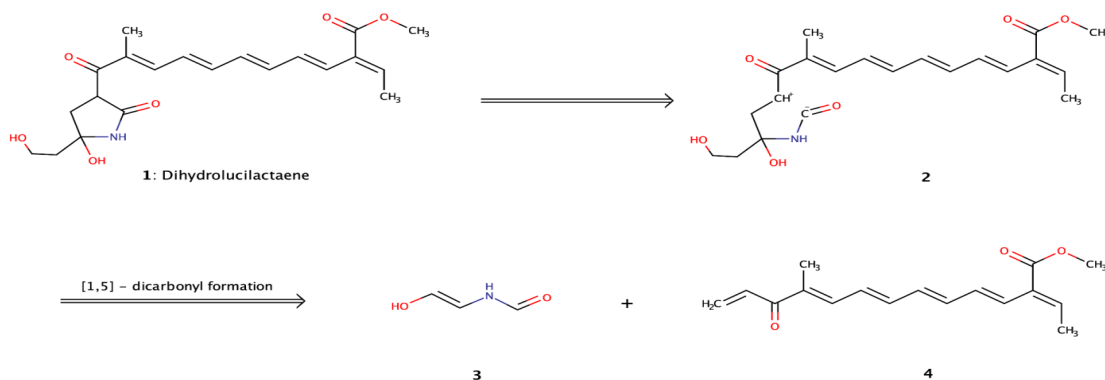
2. HINDERED APPROACHES

Four hindered approaches and their mechanisms are presented in the following paragraphs. All approaches are partially feasible and stopped by certain reasons including intermediate-instability.

2.1 Hindered Approach 1

While looking at the process of retrosynthesis of dihydrolucilactaene, a different perspective raised to our attention. Instead of disconnecting the ring and the carbon

chain first, deoxygenation could be applied to break two bonds within the ring structure and achieve a similar goal of having smaller and more obtainable functional groups. It is possible to first put the molecule in an acidic condition and protonate the hydroxyl group on carbon 4, making it an H₂O leaving group. Then use a dicarbonyl formation pattern to inverse the bond between carbon 3 and carbon 4 [1, 5]. Then the bond between alpha carbon 2 and the carbonyl carbon is tackled to the right, using the dioxygenation patterns [1, 3]. However, the retrosynthesis idea might have worked, but the forward reaction would be problematic. There are underlying problems when forward synthesis is considered. First of all, if the two carbon groups shown in Figure 2. were attached to each other, the second carbon on the top carbon chain will be slightly more delta-negative, the connection will not be ideal and resonance structures will be dominant. Furthermore, adding the -OH group back in is more difficult if the ring structure is closed first.



Note: The approach starts with a bond dissociation at the carbonyl position in the lactam ring followed by a-dicarbonyl formation [1,5].

Figure 2. Hindered Retrosynthetic Analysis 1 [Owner-Draw].

2.2 Hindered Approach 2

Figure 3 shows the second approach of the retrosynthetic analysis. The second approach starts with a critical disconnection that splits the whole structure into two halves: the lactam half and the carbon chain half. Then, the lactam undergoes a ring-opening and transforms into Compound 7. However, this approach is inherently unfeasible for two reasons. First of all, compound 7 is very unstable and therefore very easy to undergo a

reversible reaction to generate compound pair 9. The disconnection is spontaneous because the left part of compound 7 is an amide, which is a good leaving group due to resonance between the electronegative oxygen and nitrogen atoms. The other reason is that the carbon chain compound 5 has two functional groups with similar functionality on two ends: an aldehyde group and an ester group. The similarity creates difficulty to distinguish them in future steps.

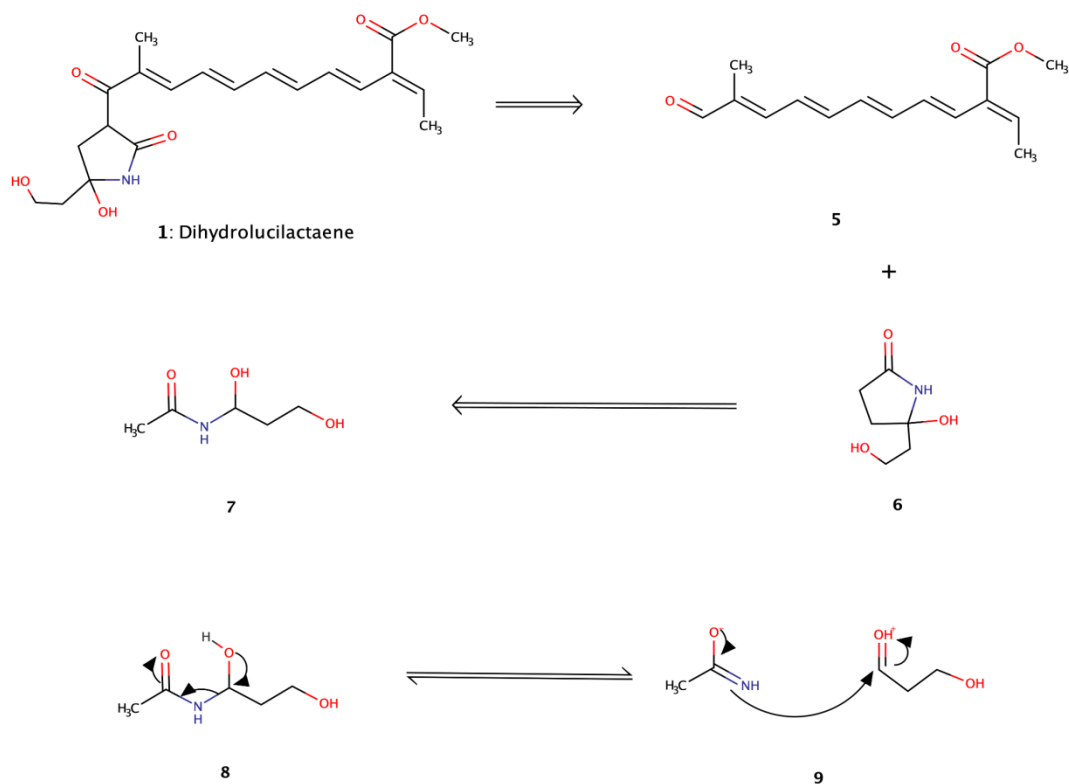


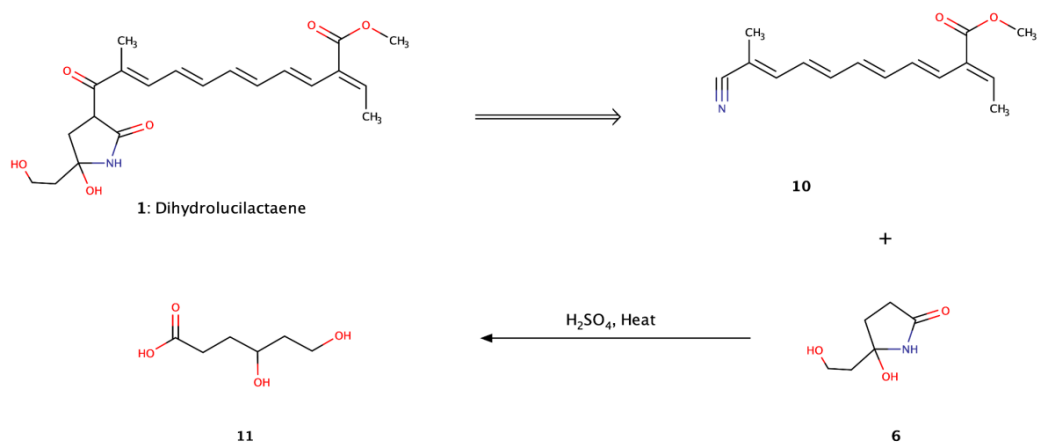
Figure 3. Hindered Retrosynthetic Analysis 2 [Owner-Draw].

This approach starts with a disconnection at the alpha position of the lactam ring. The ring is then opened to form an anhydride-like structure. Due to the instability of this structure (compound 8), this approach is hindered.

2.3 Hindered Approach 3

In this approach, the dihydrolucilactaene is disconnected into compound 5 and compound 6, shown in Figure 4. Subsequently, Compound 6 undergoes reflux with

sulphuric acid to break the ring structure. However, both steps are not feasible for the following reasons. Firstly, the carbonyl groups on both ends of dihydrolucilactaene have similar functionality, so the chances to be attacked by the hydrogen cyanide ions are the same. Therefore, the structure cannot be disconnected in this way. Secondly, compound 11 would not be produced because the ring structure is more stable and the nitrogen in compound 6 would not be removed.



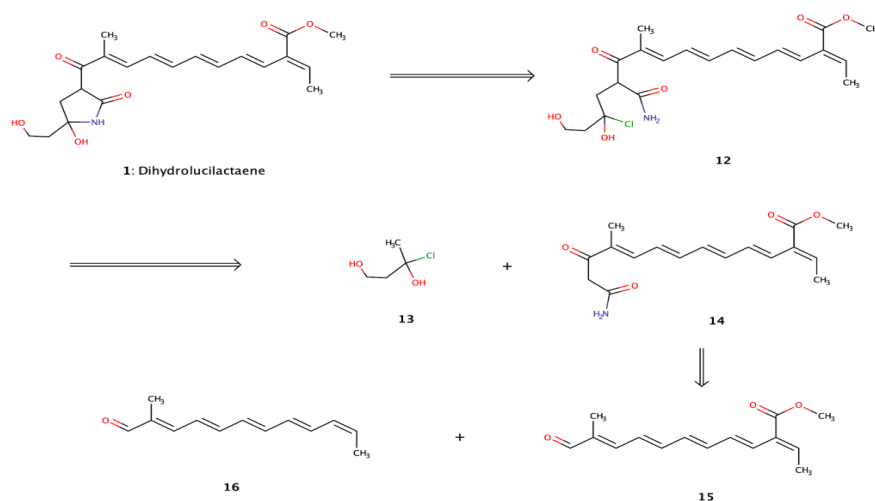
Note: This approach uses nitrile group to disconnect the same bond disconnected in hindered approach 2.

Figure 4. Hindered Retrosynthetic Analysis 3 [Owner-Draw].

2.4 Hindered Approach 4

The last approach toward the retrosynthesis of Dihydrolucilactaene is shown in Figure 5. Dihydrolucilactaene comprises a long intricate carbon chain and a five-member ring on one side. Foremost, a method to untie the five-member ring is found. Observing the ketone and the nitrogen bonded to it, we came up with the idea of untying the amide bond. In order to do so, a good leaving group must attach to the carbonyl to enable a nucleophilic attack of nitrogen. For the long complex carbon chain, we mainly focus on the ester attached to it, searching for a way to remove the ester so that it

only remains a pure carbon chain. Considering a methyl chloroformate, the chloride may attach to the hydrogen on the carbon. Hydrogen chloride forms so that the ester group is able to bond to the long carbon chain. However, the problem with this retrosynthesis route is the long carbon chain treatment. Even though the complex chain is partially simplified by removing an ester functional group attached to it, the long chain is not ultimately divided into some succinct parts. What we aim for is to directly break the carbon chain at the central carbon atom, which results in the breakdown of the whole long chain into two molecules with only five to six carbons within them.



Note: This approach starts with disconnecting the carbon-nitrogen bond within the lactam ring. Yet, the retrosynthetic synthon compound 13 is very unstable.

Figure 5. Hindered Retrosynthetic Analysis 4 [Owner-Draw].

3. Retrosynthetic Analysis

Combining the inspiration from the previously hindered retrosynthetic analysis, our group organized a finalized pathway. The proposed total synthesis path of Dihydrolucilactaene is presented in Figure 6. To start with, the structure of Dihydrolucilactaene consists of a long unsaturated carbon chain linked to the alpha position of a lactam. The two distinctive patterns provide an intuitive disconnecting method in which the lactam's alpha-connection to the long unsaturated carbon chain is first disconnected. This disconnection manner is adopted from hindered approach 2. To further combine our thought by inserting a nitrile group mentioned above with this disconnection, a functional group interconversion of the carbonyl group located at the left end of the carbon chain fragment is first applied to make an imine. In this case, the synthons of this disconnection will be a nitrile group connected to the carbon chain (compound 10) and a lactam enolate, which is synthetically equivalent to

compound 6. This step was from hindered synthetic path 3, and the necessity of the interconversion falls into the difference in functionality between a carbonyl group and a nitrile. If a carbonyl instead of a nitrile is on the left end of compound 10, the left end and the right end would have very similar functionalities that minimize regioselectivity. To further perform retrosynthetic analysis on the lactam fragment, we noticed that the adjacent carbon to the nitrogen atom is in ketone/aldehyde oxidation level, as it connects to an alcohol group and an amide group. Therefore, it is fairly straightforward to utilize this carbon to perform a ring-closing reaction on compound 18 by using the nitrogen atom to attack a carbonyl carbon. Subsequently, compound 18 can be synthesized by dicarbonyl formation, which is essentially an enolate attack on an electron-deficient carbon with an attached halide leaving group [1,4,7].

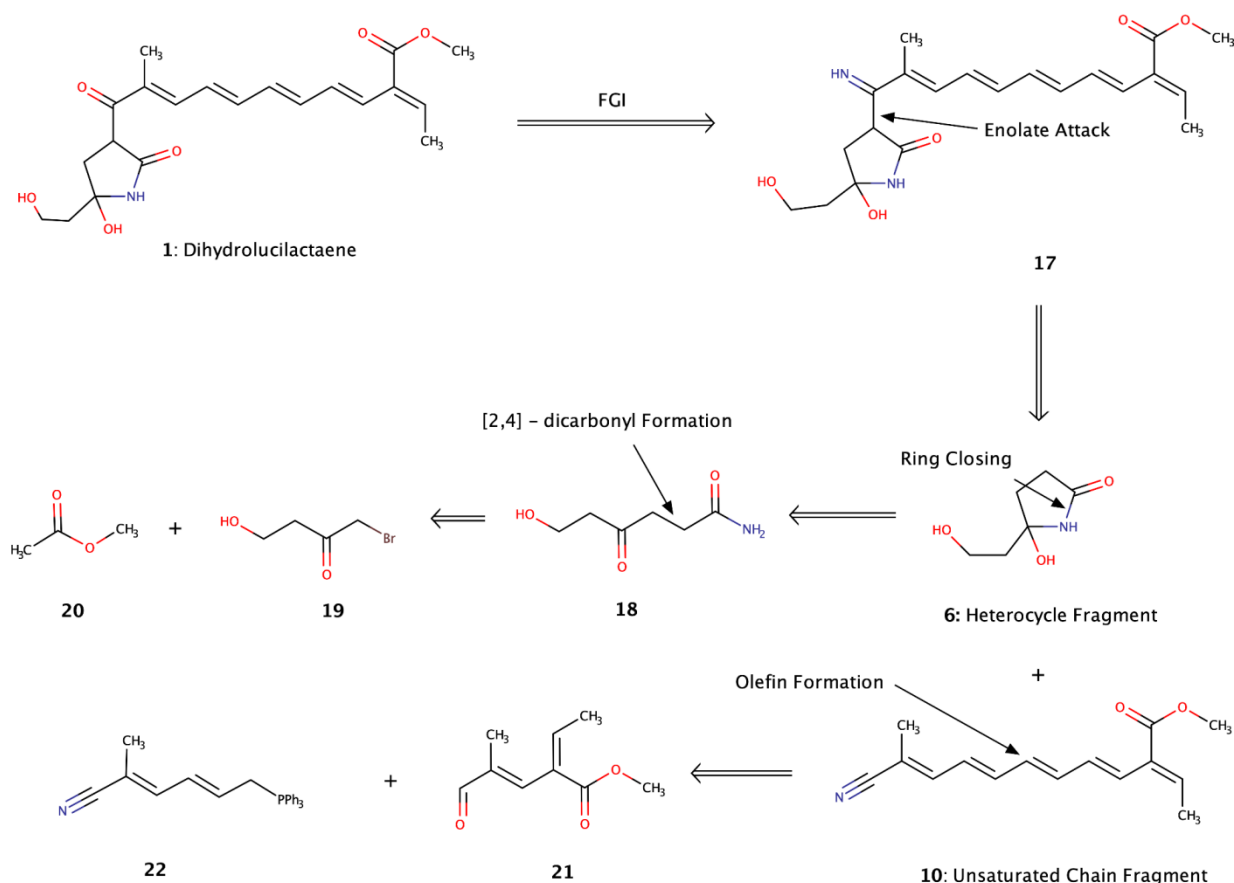


Figure 6. Retrosynthetic Analysis of Dihydrolucilactaene. PPh₃=triphenylphosphine [Owner-Draw].

For the unsaturated carbon chain fragment, a method that maximizes convergency to split the long chain into two parts with similar sizes is implemented. The enabling step will be done with the Wittig reaction to make a carbon-carbon double bond. One point to notice is that in order to perform the Wittig reaction, a triphenylphosphine ylide and an aldehyde are needed. However, the ylide and aldehyde functional groups can be placed in either fragment. Here our group chose to insert the triphenylphosphine in the nitrile-carrying fragment since the nitrile end can stabilize the ylide structure through the pi-system, pushing the reaction to the (E)-alkene, which is our desired product.

3.1 Forward Synthesis of the Lactam Fragment

Based on the previously designed retrosynthesis, the forward total synthesis path of dihydrolucilactaene can be derived. The synthesis schemes are presented in Figure

7-11. In step a in Figure 7, a hydroxyl functional group exists and should be protected because a strong base Lithium diisopropylamide (LDA) is coming later on, and the proton on the alcohol group would be subject to deprotonation and therefore hinder the reaction. Since compound 23 is a concise chemical that has relatively small steric hindrance, protection of the alcohol group by Trimethyl Silyl (TMS) group[8,9] could be done first. The chloride atom in TMSCl serves as a leaving group that leaves after the nucleophilic attack of the alcohol group the electrophilic silicon atom within the TMS group. In order to activate the nucleophilicity of the alcohol group, N, N-Diisopropylethylamine (DiPEA) acts as a base to deprotonate the alcohol proton. Note that DiPEA or other non-nucleophilic bases must be used here, as the base must not compete with the alcohol to conduct a nucleophilic attack.

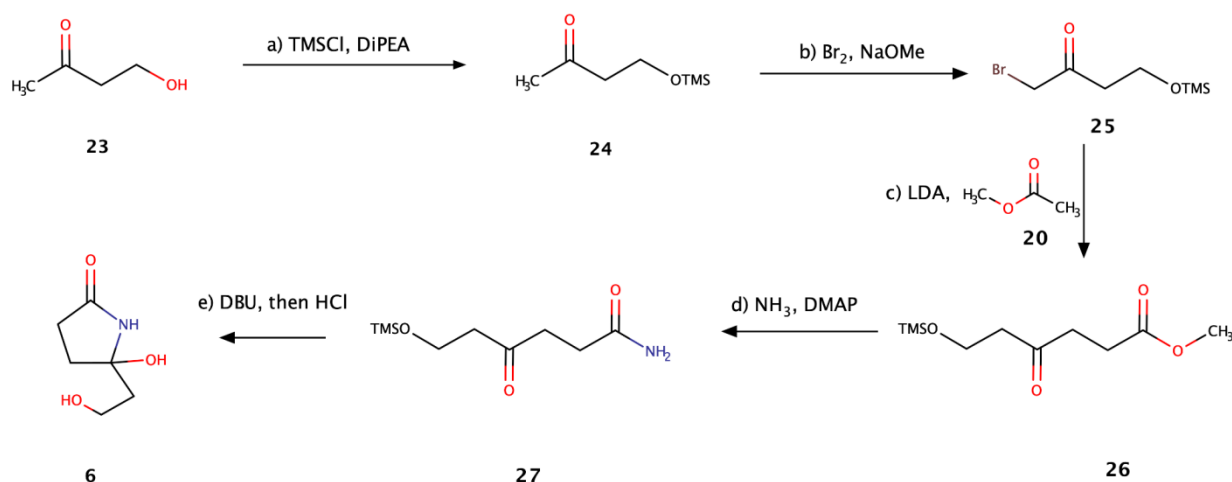


Figure 7. Forward synthesis path of the lactam fragment [Owner-Draw].

TMS=trimethyl silyl. Compound 23 is first protected with TMS to avoid deprotonation by base in following steps. The reaction ends with intramolecular nucleophilic attack (reaction e) and work up which removes the TMS group.

In step b), we designed the reaction to add bromine to the alpha position of the acyl group. In particular, base-mediated alpha-bromination is used, as acid needs to be avoided because the silicon ether in compound 24 will lose the alcohol protection groups in acid conditions. However, step b) inherently lacks control because the alpha positions of a carbonyl are very easy to undergo multi-halogenation under basic condition[8]. This can be partially overcome by putting a stoichiometric amount of bromine.

After the bromination reaction, LDA and methyl ethanoate (compound 20) is implemented to form compound 26 through aldol addition. As a non-nucleophilic strong base, lithium diisopropylamide (LDA) can deprotonate alpha hydrogen from the methyl formate[10]. Note that in this step, LDA needs to protonate compound 20, not compound 25, to avoid cross-Aldol addition[8]. To do so, compound 20 and LDA need to be first placed in the apparatus, and compound 25 needs to be added dropwise to conduct concentration control.

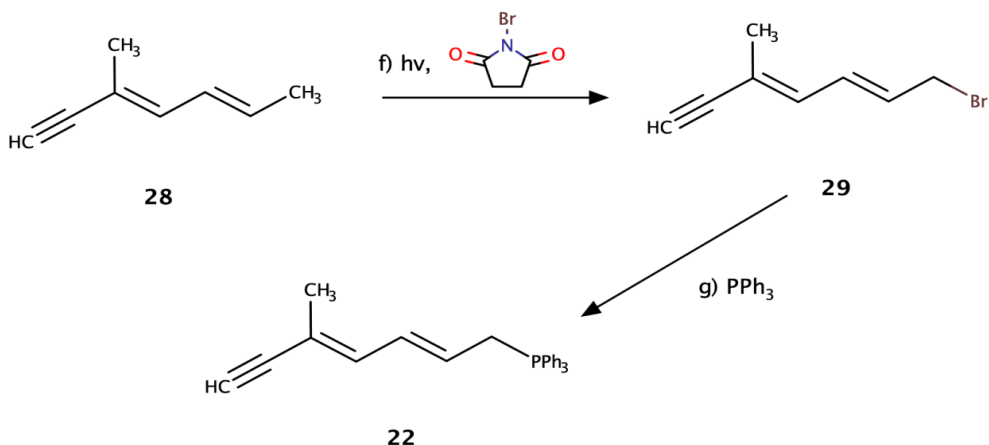
In step d), aiming for the formation of compound 6, the ester with a methyl group is substituted by an amino group, via the reaction between compound 26 and ammonia in which an amide forms in compound

27. 4-dimethylaminopyridine (DMAP) is a potent nucleophilic catalyst for the transformation from an ester bond to an amide bond in step d) [11]. DMAP is used because the nucleophilicity of the nitrogen in an amide is attenuated due to resonance. With a nucleophilic catalyst, the reaction would happen at a much faster speed and with a higher yield.

In step e), the amide nitrogen is first deprotonated by a non-nucleophilic base DBU. After deprotonation, the nitrogen can undergo a ring-closing intramolecular nucleophilic attack to form the lactam ring.

4. Forward Synthesis Of The Unsaturated Chain Fragment

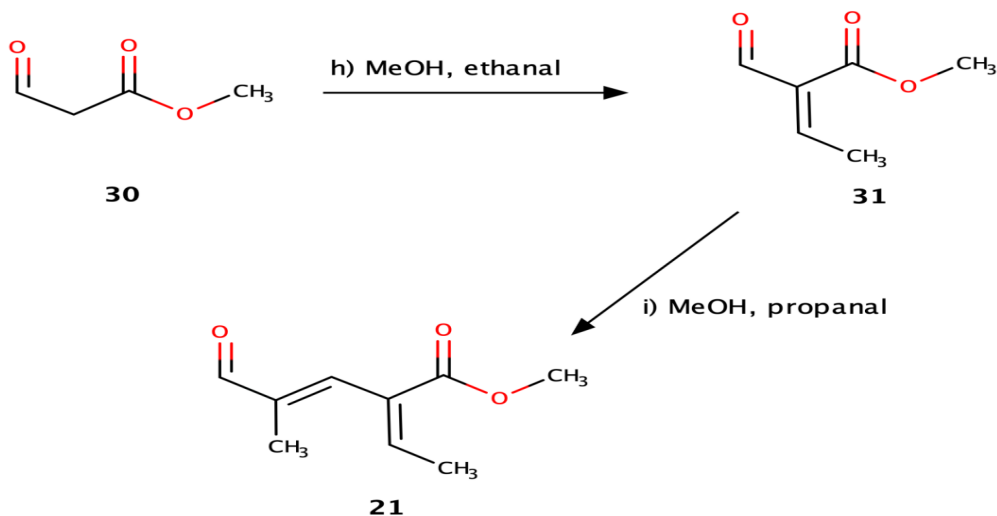
In step f), N-bromosuccinimide (NBS) is used as the bromination agent to substitute the hydrogen of the allylic carbon of compound 28 in the presence of light [8,12]. The hydrogen bonded with allylic carbon shares the free radical with the free radicals of bromide produced in the initiation step to produce hydrogen bromide and free radicals of compound 6 at allylic carbon [8]. Then, the free radical on compound 28 is reactive enough to react with the free radical of bromide produced in initiation to produce compound 29 shown in Figure 8. A drawback of this reaction is that compound 28 has two allylic positions, both subject to bromination. Thus, further separation of the reaction product must be done to obtain the pure desired product.



Note: The enabling step is reaction f, which inserts a bromine atom to the allylic position via photocatalysis.

Figure 8. The first part of forward synthesis path of the unsaturated chain fragment [Owner-Draw].

After step f), triphenylphosphine is used to convert the primary halide produced in step f to compound 22 as shown in Figure 8. The phosphate on PPh₃ has an electron lone pair that is ready to attack the carbon that bonded to bromide.



Note: Compound 21 can be synthesized by successive aldol condensation.

Figure 9. The second part of forward synthesis path of the unsaturated chain fragment [Owner-Draw].

Subsequently, in step h) shown in Figure 9, methanol and ethanal are used to add a branch to compound 30 via aldol condensation [13]. The process starts with the negatively charged methoxide group dissociated from MeOH deprotonating the carbon at the alpha position to make an enolate. Then, the enolate attacks the central carbon of the ethanal. The carbon-to-oxygen double bond of ethanal is now unstable due to the extra electrons shared by compound 30, therefore, electrons at the double bond flow to oxygen, which makes oxygen negatively charged. The negatively charged oxygen quickly picks up a proton

in methanol to retain the alcohol group. Subsequently, the remaining alpha-hydrogen on compound 30 is removed by the methoxide group again to induce the elimination of the alcohol group [8,14]. During elimination, the alcohol group from the previous aldehyde quickly picks up another proton to become a positively charged hydro group and leaves. Note that heat is not necessary in these aldol condensation reactions because the products are stabilized by resonance and therefore thermodynamically favored.

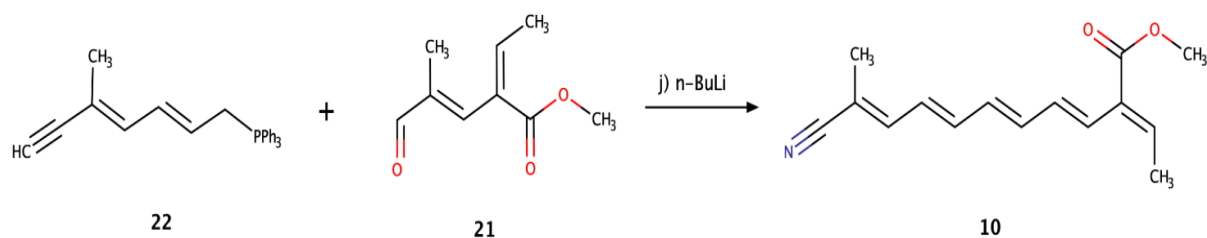


Figure 10. The third part of forward synthesis path of the unsaturated chain fragment [Owner-Draw].

The same mechanism and reaction condition can be extended to step I), where a propanal is used instead of an ethanal.

Step j) is to connect compound 21 and compound 22 to assemble compound 10, the long unsaturated chain fragment of dihydrolycilaene. This step is enabled by the famous Wittig reaction. *n*-butyllithium will initiate the olefin formation by deprotonating the carbon next to the triphenylphosphine group in compound 22, leaving the carbon with a negative charge and forming a ylide [15]. Then the carbon will be attracted to the slightly positive carbonyl carbon in compound 21, forming a four-membered ring intermediate. Since the ylide of compound 22 is very much stabilized by the nitrile group at the end through resonance, the intermediate formation in the Wittig reaction will be reversible. Therefore, the thermodynamically favored (*E*)-alkene will form instead of the (*Z*)-alkene.

4.1 Connection of the Two Fragments

To finalize the forward synthesis of dihydrolycilaene, LDA is used to deprotonate the α -hydrogen of the lactam fragment to make an enolate. However, before the deprotonation, protection of the two alcohol groups and the amide group is necessary, as the protons on the oxygen in alcohol and on the nitrogen in amide are subject to deprotonation under basic conditions. To conduct protection, acetone is first inserted under trace acid to convert the dialcohol to a ketal. To protect the amide group, a Boc group is inserted under basic addition [16]. After protection, the two fragments are connected by an enolate attack on the nitrile group. The product of the attack is an imine, which needs to be further converted to a carbonyl. The conversion can be carried out under acidic hydrolysis in which the water first attacks the imine carbon, and the amine group leaves after the protonation from acid. Notice that under acidic conditions, the ketal and the Boc protecting group are concurrently deprotected. This step is an efficient step that combines the functional group interconversion and two deprotections.

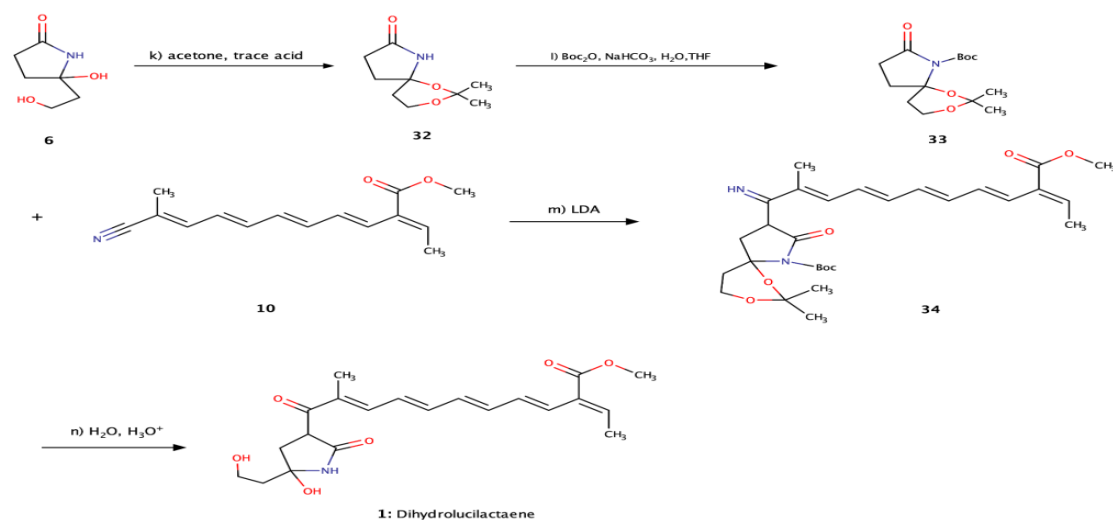


Figure 11. The connection of the lactam and unsaturated chain fragments to form the final product [Owner-Draw].

Upon protection of the two alcohol groups, two previously synthesized fragments (compound

6 and compound 7) can be connected through enolate attack on electrophilic nitrile. Deprotection and the

hydrolysis of imine are done simultaneously by acid work up in reaction n.

5. Conclusion

The retrosynthetic analysis of Dihydrolucilactaene, a potent antimalarial compound, was successfully derived, and a 14-step synthetic route was proposed. The total synthesis route contains elemental yet efficient reactions that maximize convergency. With two enabling steps, the LDA-driven enolate attack, and the Wittig addition, the desired product can be synthesized quickly. Yet, the synthetic route still has a weak spot: the alpha-bromination in reaction b) may result in multi-bromination. Further investigation could be done to overcome the problem and improve overall yield. Further experiments need to be conducted to verify the feasibility of this synthetic route.

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